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Abstracts

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Metabolism and Transport

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Effect of alanylglutamine infusion on tumor growth and cellular immune response

The prolonged use of currently available TPN solution leads to mucosal atrophy of the small intesine, presumably because of less glutamine (Gln) in the solution due to poor solubility and lack of stability. Recently, Gln-containing dipeptide, Ala-Gln, has been able to safely and efficiently utilize as a source of free Gln to prevent atrophy of the mucosa. Gln appears to be not only a nutrient for mucosal metabolism as a major oxidative fuel, but also an important substrate for tumor growth. Since the rate of tumor growth may be stimulated by Gln-enriched solution, we investigated the effect of Ala-Gln on subcutaneously inoculated Yoshida sarcoma growth in Donryu rats maintained on TPN and compared to that of rats on chow. In addition, Gln is preferentially utilized for the provision of fuels in proliferating lymphocytes and macrophages, and then we evaluated cellular immune responses to the administration of TPN rich in Ala-Gln in tumor-bearing rats.

The present results showed that intravenous supply of Ala-Gln as a 30% Gln within amino acid solution has conserved host nitrogen balance and did not accelerate tumor growth as measured by changes in weight, volume and thymidine incorporation in tumor mass. The tumor-bearing rats were associated with an increase in the activities of natural killer cell and alveolar macrophages. We suggested that not-stimulating effect of Ala-Gln on tumor growth was due to maintenance of cellular immune response at control level or some immune-enhancing effect of Gln liberated from Ala-Gln on tumor-bearing rats.

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Effect of amino-carbonyl reaction products on the digestive enzymes and the decomposition of nitrite

In order to study the effect of amino-carbonyl reaction products on the organism we investigated their effects on the digestive enzyme activities and also the nitrite decomposing activities as one of the inhibitory factors to nitrosoamine formation.

I. Digestive enzyme activities. 1. Trypsin: The reaction products between Phe or Trp and carbohydrate inhibit the activity but in the case of Met, Gly, Lys, His did not inhibit. 2. Carboxypeptidase A: His, Lys, Gly, Met inhibit the activity in this order. 3. Pepsin: Arg only inhibit the activity among investigated amino acids (His, Lys, Met, Gly, Leu, Cal, Glu). But in the case of addition of $NaHCO_3$, Arg Val, Lys, Leu, Met Gly, His, Glu inhibit in this order. 4. α-Amylase: The rate of inhibition was very small in every case. The inhibition order was His, Met, Gly, Arg in the case of $NaHCO_3$ coexistence but the order of Arg and Gly was reversed in the absence of $NaHCO_3$.

II. Decomposition of nitrite. 1. Amino-carbonyl reaction products decomposed nitrite and these activities changed according to the composition of the reaction mixtures. 2. Above mentioned activities could not be attributed only to the reducing power of the reaction products. 3. The duration of heating the mixtures of amino acids and carbohydates increased the coloring, the reducing power and nitrite-decomposing activity, but the plateau existed in the last case. 4. Most of nitrite-decomposing products could be dialysed through a semipermeable membrane. We also investigated the fraction of amino-carbonyl reaction products by Sephadex G-50 chromatography. 5. In the case of addition $NaHCO_3$ for the reaction acceleration, the nitrite decomposing activity of Arg decreased, the activity of Met did not change and the activities of Ala, Hyp, Ser, Pro increased. His did not decompose nitrite in this case as His alone.

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Electrophoretic polyamines transport in rat liver mitochondria

The naturally occuring polyamines, ubiquitous organic polycations, are essential in the control of cellular growth, in differentiation processes and in several cellular functions.

Besides these effects, spermine has a protective action on both mitochondrial function and mitochondrial membrane barrier permeability.

Polyamines homeostasis and adjustment of intracellular concentrations to physiological requirements are regulated by synthetic and catabolic reactions. However, "de novo" biosynthesis can be substituted by polyamine uptake from the environment.

Specific polyamines transport systems have been demonstrated in prokaryotes and in eukaryotes and in many aspects resemble aminoacids uptake systems. In general, polyamines transport across the plasma membrane is energy and temperature dependent, and saturable, suggesting that it is a carrier-mediated transport.

It has also been demonstrated [1] that spermine may be transported across the inner mitochondrial membrane in both rat liver and heart mitochondria. This transport, which requires membrane energization and a high transmembrane electrical potential, could take place by a "leak" pathway or by a specific channel.

In this work we have comparatively studied in rat liver mitochondria the transport of the single polyamines (cadaverine, putrescine, spermidine and spermine) by demonstrating the existence of an electrophoretic transport mechanism. However, in spite of this mechanism, the distribution ratio of polyamines across the inner mitochondrial membrane does not reach the nernstian equilibrium suggesting the existence of an efflux pathway also.

1. Toninello A, et al (1988) J Biol Chem 263: 19407–19411

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Glutamine increases the steady-state levels of mRNA for type I and III procollagens and fibronectin in cultured dermal fibroblasts

Increasing the concentration of glutamine from 0 to 10 mM in the culture medium of confluent human dermal fibroblasts induced a dose-dependent variation in collagen synthesis. Concomitantly the synthesis of several non-collagen proteins were either increased or inhibited, as demonstrated through the process of polyacrylamide gel electrophoresis. The steady state levels of α 1(I) procollagen mRNA, α 1(III) procollagen mRNA and fibronectin mRNA, analyzed by dot-blot hybridization, were found to be increased. Analogues of glutamine such as acivicin, 6-diazo-5-oxo-L-norleucine and homoglutamine did not replace glutamine. The role of membrane transport systems for amino acids was assessed by using 2-(methylamino)-isobutyric acid, β_2-aminobicyclo-(2.2.1)heptane-2-carboxylic acid, L-butionine-SR-sulfoximine and hippurate. The glutamine effect on collagen synthesis was not found to be dependent on either the activity of the amino acid transport system A or on the β-glutamyl-transpeptidase pathway. However, the inhibition of the amino acid transport system L by β_2-aminobicyclo-(2.2.1)heptane-2-carboxylic acid prevented the stimulating effect of glutamine on collagen synthesis.

In conjunction with the stimulation of collagen synthesis, glutamine induced a decrease in the intracellular level of cyclic AMP.

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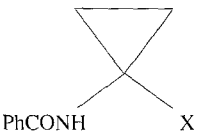
The inhibition of peptidases by cyclopropane-containing amino acid derivatives and aminoacid analogues

In 1987, we reported that some peptides containing 1-aminocyclo-propane-1-carboxylic acid are time-dependent irreversible inhibitors of carboxypeptidase A (*J. Chem. Soc., Chem. Commun.*, 1987, 480–482). This paper describes investigations concerning the generality of this reaction and studies of its mechanism. Structural analogues of amino acids and peptides and representatives of each of the main classes of peptidase have been studied. Thus 1-substituted-1-benzamidocyclopropyl ketones, secondary alcohols and sulphones were synthesized and tested as enzyme inhibitors. Although several compounds had weak competitive effects on chymotrypsin and pepsin, time-dependent inhibition was observed only with carboxypeptidase A. The kinetics of this reaction did not follow the first order processes normally associated with time-dependent inhibition but showed subsstrate activation. This unusual behaviour will be discussed in the context of the structures of the inhibitors and their possible binding modes to carboxypeptidase A.



X=COPro
=$COCH_3$, $COCH_2Ph$
=$CH(OH)CH_3$, $CH(OH)CH_2Ph$
=$COCH_2SOCH_3$, $COCH_3$, $COCH_2SO_2Ph$

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The anabolic effects of amino acids on muscle wound healing in diabetics rats

In order to determine the effects of various anabolic amino acids on the Anterior Leg Muscle (ALM) wound healing in normal as well as diabetic rats, adult male Sprague-Dawley rats (200 gms; 10 animals/group) were injected with streptozotocin (STZ) (65 mg/kg) 30 days prior to the experiment and their blood sugars checked at this time. Eight groups (control-saline, control-glycine, control-leucine, control-methionine, diabetic-saline, diabetic-glycine, diabetic-leucine, diabetic-methionine) of rats were either injected subcutaneously daily with saline or one of the anabolic amino acids (150 mg/kg body weight) in saline starting 2 days presurgical to 5 days post-surgical wound induction in the ALM

(6 mm Baker biopsy punch under anaesthesia) at which time all rats were sacrificed. We observed that these amino acids had no significant effect on the various parameters measured among the 4 groups of normal control rats. In diabetic rats we observed that methionine significantly increased ($p < 0.02$) the percentage of wound healing in the ALM (90.8 ± 1.7 versus 76.2 ± 4.8) and decreased ($p < 0.02$) the ALM wound volume (mm^3) (18.5 ± 4.4 versus 45.2 ± 8.0) when compared with diabetic-saline injected rats on day 5-post-surgery. However, no significant differences were observed in the muscle tensile strength (gms force). Methionine also significantly increased ($p < 0.05$) the ALM total protein when compared to the leucine- and glycine-diabetic injected groups. Our data suggest that anabolic amino acids, like methionine, can noticeably improve muscle wound healing, especially in a diseased state such as diabetes.

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Synergistic effects of amino acids on lactation

Our laboratory has reported that 7,12-Dimethylbenzanthracene (DMBA), a carcinogenic aromatic hydrocarbon, administered to pregnant rats caused a significant decrease in mammary gland RNA (synthesis) and DNA (growth) contents on day 1 of lactation. In order to determine the effects of reproductive amino acids on DMBA primed mammary gland tissue, female adult virgin Long Evans rats (12 animals/group) were intraperitoneally injected daily with 1 mg DMBA in 0.2 ml sesame oil on days 8 through 12 of pregnancy. On days 8 through 17 of gestation these rats were subcutaneously injected with 150 mg/kg body weight of either L-tryptophan, L-arginine, or L-glycine in 1 ml saline solution. On day 1 of lactation we observed that L-tryptophan and L-glycine significantly increase the depressed DMBA control values for total RNA (mg), RNA (mg/100 g B.W.), RNA (mg/100 mg M.G.), and RNA/DNA to that of sesame oil controls. L-glycine significantly increased both the total DNA and DNA (mg/100 g B.W.) to that of sesame oil control levels, whereas, L-tryptophan had a positive effect only on the latter parameter. However, L-arginine had no stimulatory effect on the RNA and DNA values. Our data suggests that L-glycine, which increase growth hormone release, and L-tryptophan, which enhances and mimicks prolactin secretion, negate the harmful effects of DMBA by stimulating mammary gland growth (DNA) and secretion (RNA). The clinical benefits to the human female may be significant, especially if these amino acids, which have reproductive hormonal effects, are administered in combination with different steroids.

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What kind of peptide bonds being weaker for non-specific chemical treatment?

Substrate specificity of enzymes suggests a delicate difference in a specific bond of peptides to be cleaved or ligated.

Amides are the common chemical bonds in peptides and proteins, and proteolytic enzymes can discriminate some particular nature of amide bonds, to which amino acid residues are largely contributed.

In the present study, non-specific chemical treatment of casein molecules was attempted to examine what kind of amide bonds are more susceptible or resistant to the mildest hydrolytic conditions. Casein (alpha-casein), 1 g, was dissolved in 2 N HCl, and heated by means of a microwave (2450 MHz) for 20 min, or in an oven at 100° for appropriate duration (less than 5 h). Elution patterns of the hydrolyzed fractions from a Dowex 50 W 2 X column were reproducible in multiple runs by both methods. Capacity of hydrolysis of the peptides by both heating methods in the presence of 2 N HCl was found to be equivalent. Amino terminal (N-terminal) analysis (by Edman's sequential method) and carboxy terminal (C-terminal) analysis (by thiohydantoin sequential method) suggested some tendency of weaker peptide bond to be hydrolyzed under milder and limited hydrolytic conditions: thus, when the concerned amino acid residues are assigned to be acidic (A), basic (B), and neutral (N), the most resistant peptide bond against acid hydrolysis seemed to be -A-B-, and others, such as -A-A-, -B-B-, -N-N-, -A-N- and -B-N-, are hydrolyzed more or less similarly.

During the purification or other courses of peptide treatments, therefore, peptides should be carefully considered for artifact modification of the bonds.

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L-Cysteine metabolism via 3-mercaptopyruvate pathway and sulfate formation in rat liver mitochondria

Sulfate is the main final product of L-cysteine metabolism in mammals. There are two types in metabolic pathways of the cysteine sulfur to sulfate. The first type is the one in which cysteine sulfur is oxidized before removal from the carbon skeleton and proceeds through L-cysteine sulfinic acid as an intermediate. This type constitutes the main pathway of L-cysteine metabolism as the oxidation pathway. In the second type, the cysteine sulfur is removed from the carbon skeleton before oxidation and finally oxidized to sulfate. The 3-mercaptopyruvate pathway (or transamination pathway) belongs to this type and proceeds through 3-mercaptopyruvate as an intermediate. We report here that, in rat liver mitochondria, L-cysteine is metabolized through the 3-mercaptopyruvate pathway to form sulfate.

Sulfate formation was only about 0.1 μmol when mitochondria from one g of liver was incubated with 10 mM L-cysteine at 37 °C for 60 min. Addition of 10 mM 2-oxogluterate or 10 mM GSH increased the sulfate formation 3 to 4 times. The addition of both 2-oxoglutarate and GSH resulted in a 20-fold increase in the formation of sulfate. When 3-mercaptopyruvate, the transamination product of L-cysteine, was incubated with rat liver mitochondria, sulfate, thiosulfate and sulfite were formed, and the addition of GSH to the reaction mixture accelerated the sulfate formation. These results indicate that sulfate formation in the present system proceeded through the 3-mercaptopyruvate pathway.

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Protein kinetics using a flooding dose technique with the stable isotope 15 N, 13 C valine

In order to reduce the disparity in precursor pool specific activity, tissue fractional rates of protein synthesis (ks) can be determined using labeled amino acid single injection procedures in conjunction with a raised, unlabeled amino acid perfusate. This approach is particularly useful in small mammals (< 20 kg). However, in large animals, these flooding dose procedures are complicated by technical considerations as well as concern over potential pharmacological effects. In the present study, sixteen, seven month old lambs weighing on average 57 kg were used to study the effects of a continuous vascular infusion of valine. The sheep were prepared with bilateral jugular vein catheters and infused with a sterile solution of L-valine in 0,9% NaCl at one of the following rates; 0.0, 6.9, 13.9, or 20.8 mmol per hour for 9 hours. A stable isotope of 13 C 15 N or 2 H valine (99% enriched) was added to the perfusate for the determination of ks values by GCMS analysis. High performance liquid chromatography was used to analyse amino acid concentrations in serial plasma samples and tissues collected post-mortem. In five animals infused at 20.8 mmol per hour valine significantly raised the pre-infusion plasma levels from 0.245 ± 0.009 (SE) mM to 5.046 ± 1.319 mM ($p < 0.01$). Valine concentration in the skeletal muscle intracellular free pool approached plasma levels (3.356 ± 0.103 mM per gram tissue). However, reductions ($P < 0.05$) in plasma levels of tyrosine, tryptophan and lysine (20–60%) and an increase in isoleucine (62%) were noted. At the 20.8 mmol per hour infusion rate, ks values for skeletal muscle ranged from 8%/day to 14%/day calculated from intracellular and plasma relative abundance of 15 N/14 N valine respectively. The data suggested that a continuous infusion valine flooding dose may be suitable in large animals, however, the effects of attendant changes in plasma levels of selective amino acids need to be examined.

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Influence of amino acids on proteolytic enzymes: a possible regulation of protein breakdown

A direct relationship between the qualitative amino acid intake and a decrease in protein degradation has been shown in clinical and experimental studies among different tissues. Several mechanisms have been proposed, including oxoacids, urea, ammonia or oligopeptides. Previous studies suggested that an intralysosomal regulatory action of amino acids on endoprotease activity could exist.

The aim of this study was to demonstrate that some proteinogenic amino acids, at the levels we found in hepatocyte of fasted rat, could inhibit the activity "in vitro" activity of two of the most important endoproteases (cathepsins B and D).

Methods: Determinations of cathepsins B and D activities were done by Barret and Anson's methods using Bz-Arg-NNap and human haemoglobin as substrates, respectively. We tested enzyme catalytic concentrations in the response of each one of the twenty amino acids at the intracellular levels described in rat hepatocystes.

Results: We found a non-competitive inhibition pattern for cathepsin B in the presence of Leu, Val and Gln and a similar pattern for cathepsin D in the presence of Gly with statistically ($p < 0.001$, Student's T and Mann-Withney U tests) significant differences with regard to controls.

AA tested	without AA	with AA	
Leu	1 699 ± 35	1 136 ± 43	cathepsin
Val	1 688 ± 32	1 250 ± 79	B
Gln	1 704 ± 133	1 388 ± 109	(nmol/s.l)
Gly	100 ± 5	56 ± 6	cathepsin D (relat. units)

These results suggest a possible regulatory effect of some proteinogenic amino acids on protein breakdown.

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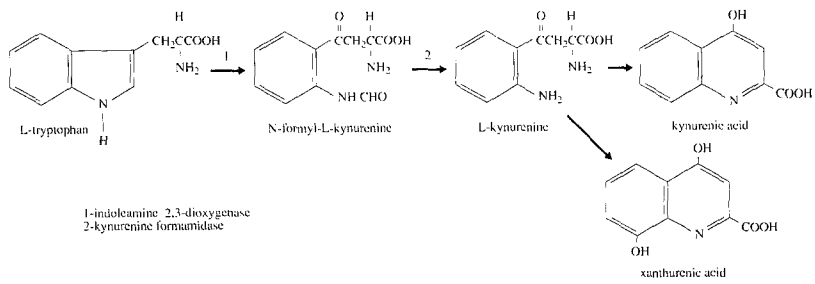
Increased plasma L-kynurenine pool compensates for reduced hepatic L-kynurenine formation caused by kynurenine formamidase inhibition by organophosphorous acid triesters

Diazinon [0,0-diethyl-0-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate], the model organophosphorous acid triester (OP), alters mice L-tryptophan metabolism in two respects: First, it inhibits liver kynurenine formamidase (KFase) (aryl-formylamine amidohydrolase, EC 3.5.1.9). Structure-activity studies both in vitro/in vivo revealed that liver KFase was suceptible to inhibition by numerous OP's. The most potent KFase inhibitors were phenyl saligenin cyclic



phosphates, 0,0-dialkyl-0-substituted pyrimidylphosphates, and 0,0-dialkyl-0-crotonamide phosphates. Inhibition of KFase in liver slices prepared from diazinon-treated mice resulted in reduced biosynthesis of L-kynurenine and a concomitant accumulation of N-formyl-L-kynurenine.

Second, L-kynurenine was increased five-fold in the plasma following in vivo diazinon administration. Consequently, the higher plasma L-kynurenine concentration resulted in increased urinary excretion of xanthurenic and kynurenic acid. These findings, paradoxical to the conclusions of kynurenine formamidase inhibition, suggest that induction of L-tryptophan oxygenation may be the compensation mechanism in response to the OP-induced inhibition of N-formyl-L-kynurenine hydrolysis. Comparative analysis of plasma, urine and brain quinolinic acid and L-kynurenine after administration of L-tryptophan, hydrocortisone and lipopolysaccharide, respectively, provides indirect evidence about the possible involvement of indoleamine, 2,3-dioxygenase in nonhepatic tissues in this process.

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Amino acid substrate dependency of the O_2 consumption/O_2 transport relationship in sepsis

O_2 consumption and O_2 transport patterns were analyzed in 82 severely ill septic patients undergoing total parenteral nutrition with different doses of amino acids, glucose and fat.

O_2 consumption and O_2 transport (VO_2, O_2T, ml/Kg/min) were determined simultaneously, together with a large series of hemodynamic, respiratory and metabolic variables in 245 measurements. Amino acid infusion rate (AAIR, g/Kg/day) ranged from 0.0 to more than 2.0, with a mean ± SD = 1.1 ± 0.6; glucose and fat infusion rates (GIR, FIR, g/Kg/day) were 5.8 ± 2.7 and 1.1 ± 0.4. Ten patients were receiving supplemental doses of branched chain amino acids (BCAA) during the measurements.

VO_2 for all measurements was 4.15 ± 1.25 (mean ± SD); O_2T was 15.18 ± 6.97. Regression analysis showed that VO_2 was directly and significantly related to both, O_2T and AAIR, which explained together 50% of the VO_2 variability: $VO_2 = 1.84$ (log O_2T) + 0.64 (AAIR) − 1.33 [$r^2 = 0.5$, $p < 0.001$]. AAD accounted for 16% of the totally explained variability. At any given O_2T, VO_2 increased by 922 ml/g AAIR, which corresponded to a thermogenic effect of amino acids of about 100%. This effect was observed also at low O_2T (independently from the cause: low flow, hypoxemia and anemia), and was more evident at high O_2T when the O_2T-dependency of VO_2 became less prominent, or during high-dose BCAA support. There was a similar, less relevant effect of FIR, and no effect of GIR, on the VO_2/O_2T relationship. Increases in VO_2, at any given O_2T, were also directly related to body temperature ($p < 0.01$) and unrelated to changes in hemoglobin-O_2 affinity, vascular tone, pulmonary gas exchanges and acid-base balance variables.

These data indicate that in sepsis there is a substrate dependency of VO_2, and that the abnormal septic VO_2/O_2T relationship may be modified by increasing AAIR and BCAA doses, thus restoring impaired energy metabolism by the provision of readily oxidizable substrates.

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Dependency of pulmonary O_2 exchange patterns on plasma amino acid levels in sepsis

The interdependence between pulmonary O_2 exchange and plasma amino acid (AA) levels was assessed in 168 measurements performed in 16 critically ill septic patients. Blood samples for AA-grams were taken simultaneously with the determination of arterial and central venous O_2 tensions (PaO_2, PvO_2), O_2 saturations (SaO_2, SvO_2), alveolar-arterial O_2 gradient (A-aDO_2), respiratory index (RI), pulmonary venous admixture (Qva/Qt) and additional variables.

Independently from pulmonary edema, infection, atelectasia or embolism, PaO_2 and SaO_2 were lower in cases with low plasma branched chain AA (BCAA) and high aromatic AA (AAA). For any given PvO_2 of SvO_2, PaO_2 was directly related to BCAA and inversely related to AAA ($p < 0.01$ for all). RI and Qva/Qt increased with decreasing PaO_2; PvO_2 and levels of BCAA and AAA explained 34% of the variability of RI ($r^2 = 0.34$, $p < 0.001$). Plasma phenylalanine (Phe, μmol/L) and leucine (Leu, μmol/L) explained by themselves as much as 18% of the variability of Qva/Qt:

$$Qva/Qt = 7.708\ (Phe/Leu) + 39.395,$$
$$r^2 = 0.181\ \ p < 0.001.$$

In sepsis the adequacy of pulmonary O_2 exchange is in part related to AA levels. In addition to implying a parallel evolution of metabolic and respiratory abnormalities, these data may also imply an effect of AA or of their byproducts on pulmonary vasculature, and thus the possibility of modulating Qva/Qt abnormalities through the administration of modified AA formulae.

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Amino acid uptake in plasma membrane vesicles isolated from proliferating tumor cells and tissues

Alteration of ion and nutrient transport appear to be one of the main aspects of cell growth. There is a general agreement that amino acid transport rates increase in response to proliferation, however the changes of amino acid transport in transformed cells have not been clearly defined [1–2].

Recently the system A transporter has been postulated to be a target of protooncogene action, thus regulating the cellular growth [3].

We have investigated the characteristics of the transport agency (-ies) involved in alanine transfer across the plasma membrane in a very undifferentiated rat ascites hepatoma cell line (Yoshida AH-130) and in hyperplastic diethylnitrosamine (DENA)-induced liver foci and/or preneoplastic nodules [4], using purified preparations of plasma membrane vesicles.

Actually, the transport experiments performed with vesiculated plasma membrane fragments make it possible to assay the transport activity separated from the effects of intracellular metabolism and compartmentalization. This allows the possibility of presetting the experimental conditions

and permits the use of physiological substrates instead of non-metabolizable analogs.

In Yoshida ascites hepatoma cells the Na^+-dependent time courses of alanine uptake into the vesicles and the kinetic as well as inhibition and countertransport experiments showed differences as compared to normal hepatocytes. In these cells alanine transport was also related with the phase of cellular growth (exponential-stationary) [5].

Kinetic analyses of Na^+-dependent alanine uptake during the rat DENA hepatocarcinogenesis showed that this amino acid transport correlated consistently with the rate of cell proliferation while transformation did not alter appreciably the alanine transport. An increased affinity of the transporter for alanine during the induction of hyperplastic and preneoplastic nodules has been observed, suggesting the appearance of an altered transport system under these conditions.

1. Weber MJ, Evans PK, Johnson MA, McNair TF, Nakamura DK, Salter DW (1984) Federation Proc 43: 107–112
2. Christensen HN (1990) Physiol Rev 70: 43–77
3. Saier Jr MH, Daniels GA, Boerner P, Lin J (1988) J Membrane Biol 104: 1–20
4. Solt DB, Medline A, Farber E (1977) Am J Pathol 88: 595–618
5. Leonardi MG, Comolli R, Ciordana B (1990) Cancer Biochem Biophys 11: 289–303

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Effect of the cationic polypeptide polylysine on neutral amino acid transport in isolated brain microvessels

The blood-brain barrier, which regulates the restricted movements of nutrients between the blood and the brain compartment, is anatomically formed by the endothelical cells of brain microvessels. Characteristic of this endothelium is the presence of tight junctions between the cells and the poor formation of vesicles or channels. In most body districts, microvessels possess negatively charged areas where neither vesicles nor channels are formed, and which are known to be specific targets for interaction with highly cationic proteins. Since the blood-brain barrier permeability is reportedly influenced by interaction with polyactions, we have tested the effect of various lysine homopolymers (Mr ranging from 2 to 180 kDA), on the uptake of neutral amino acids by isolated bovine brain capillaries. There was, upon addition of polylysine with Mr > 22 kDa, an increased uptake of α-methylaminoisobutyrate, reportedly transported by the Na^+-dependent A-system, the most relevant stimulation being obtained upon addition of the 68 kDa polymer. This effect, which could be essentially ascribed to an increased affinity of the amino acid for its carrier (with a decrease of the Km value from 265 to 169 μM), depended on the presence of Na^+ ions in the incubation medium and was abolished by phloretine. As the polylysine size was increased beyond 68 kDa, there was however a progressive inhibition. The uptake of hydrophobic amino acids via the Na^+-independent L-system was instead adversely affected by all polymers exceeding 22 kDa, the level of inhibition being directly related to the polylysine size. A non-specific damage to endothelial cell membranes could however be demonstrated only upon addition of the highest molecular weight polymer (180 kDa), which caused a 30% increase of passive dye efflux from carboxyfluorescein-diacetate preloaded microvessels.

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Source of amino acids for charging tRNA: implications for measurements of protein synthetic rates

Estimates of protein synthetic rates using radioisotopes require accurate measurements of the specific activity of the label in protein and precursor pools over time. Although the extracellular (EC and (IC) pools of amino acids are easiest to sample, the tRNA pool is the true precursor. To determine the relationship between pools, chicken macrophages were incubated in either 0.23 mM leucine and 14.5 μCi ^{3}H-leu, or 2.3 mM leu plus 145.0 μCi ^{3}H-leu. At both leu levels, the tRNA specific activity (SA_t) reached a plateau quickly but did not equilibrate with either the EC or IC specific activity within 30 min and remained closer to that of protein. In a second experiment, proteins in chicken macrophages were labeled with ^{3}H-leu for 2 days. When the prelabeled cells were incubated in 0.23 mM, or 2.3 mM non-isotopic leu, SA_t reached a plateau within 2 min and remained closer to that in protein than IC or EC pools for at least 60 min. In a third experiment, chicks were injected ip with 300 μCi ^{3}H-Leu in cold Leu (100 mmoles). In general, SA_t remained below that in IC or plasma in liver and pectoral muscle for at least 30 min. We hypothesize that amino acids are channelled from protein degradation to tRNA without mixing with IC amino acids. When protein synthetic rates are estimated by label incorporation, use of EC or IC specific activity values result in a marked underestimation.

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A new model of in-vivo-measurement of branched chain amino acids' metabolism with ^{13}C in malignant human colon tumors

and

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In-vivo-measurement of BCAA metabolism with ^{13}C-leucine in malignant human colon tumors: first results

In former studies could be shown that in the tumor vessels of the malignant human colon tumor there was a significant arterial/venous-difference of branched chain amino acids (BCAA). With the common methods it was unable to decide what the tumor really did with the BCAA (f. e. retention/metabolisation).

By using the ^{13}C-leucine-tracer-technique it is possible to get quantitative results of the whole-body protein-oxydation, -turnover, -synthesis, -breakdown and -retention.

To get quantitative results in BCAA-metabolism of the tumor itself it was necessary to develop a modification of the

^{13}C-leucine-tracer-technique: The primed ^{13}C-leucine-infusion was started with the beginning of the operation. After dissecting the tumor blood samples had been taken from the tumor-artery and the tumor-vene. Simultaneously the blood flow was measured in the tumor-artery. Additional to the results of the (whole-body) ^{13}C-leucine-tracer-technique we measured the ^{13}C-leucine and ^{13}C-keto-isocaproate-(KIC)-concentration in the tumor-arterial blood and in the tumor-venous blood. Also the $H^{13}CO_3$- was measured in the blood samples of the tumor-vene. The weight of the tumor had been also measured.

With these parameters it is possible to get quantitative results of the BCAA-metabolism of the malignant human colon tumor itself. For the samples are in work, first results can be expected in june 1991.

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Glycine and urea metabolism during normal and diabetic pregnancies

Glycine-N and urea kinetics were determined postabsorptively during late gestation in six normal pregnant subjects and two subjects with pregnancy complicated by insulin dependent diabetes mellitus (IDDM) using continuous infusions of [^{15}N]glycine and [^{13}C]urea with appropriate priming. The study was repeated in the same women at 4 months postpartum. Stable isotope enrichment was measured by gas chromatography – mass spectrometry. A steady state enrichment was found for glycine and urea in both groups of subjects; glycine and urea turnover were quantified by isotope tracer dilution.

1. During normal pregnancy, the glycine-N flux was unchanged relative to the postpartum period (2.23 ± 0.32 vs 2.20 ± 0.29 mg N/kg/hr; mean ± SD) but elevated flux (2.78 ± 0.27 mg N/kg/hr) was found in mothers with diabetes relative to normal mothers which decreased postpartum (2.24 ± 0.01 mg N(kg/hr).

2. Urea turnover (as reflected in the flux measurements) was depressed during normal pregnancy (7.00 ± 0.25 mg/kg/hr) compared to postpartum values (13.42 ± 1.62 mg/kg/hr; $p < 0.001$); the same phenomenon occurred in IDDM women but to a lesser extent (7.82 ± 0.49 vs 10.51 ± 2.10 mg/kg/hr, pregnant and non-pregnant state respectively).

3. No relationship was found between amino acid flux and estimates of fetal weight determined at test and subsequent birthweight in normal subjects.

We conclude that, postabsorptively in both non-diabetic and diabetic pregnant subjects, the lower plasma and urinary urea seen was a consequence of decreased urea production wich may be important in conserving nitrogen and the higher glycine-N flux shown in diabetic pregnant subjects would suggest that glycine metabolism was altered in diabetic pregnancy possibly to satisfy fetoplacental metabolic needs.

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Plasma amino acids during insulin-dependent diabetic and normal pregnancies and their relationship to estimates of fetal weight

The concentrations of amino acids, cholesterol, triglycerides and insulin-like growth factor 1 (IGF 1) were determined serially in the maternal plasma of 14 insulin-dependent diabetic (IDDM) and 9 normal mothers during pregnancy and again at 12 weeks post-partum. The decrease in amino acids during gestation (compared with post-partum values) was seen predominantly in 5 out of 23 amino acids, the decreased values having occurred during early pregnancy in both normal and diabetic subjects. IDDM mothers had significantly higher levels of the amino acids proline, arginine and the aromatic group (phenylalanine, tyrosine and tryptophan) but lower levels of two of the gluconeogenic amino acids, glutamine and aspartate, relative to the non-diabetic mothers during gestation. Total amino acid values were similar in the two groups post-partum; individually arginine, histidine and the aromatic amino acids were significantly higher and glutamine levels lower in diabetic compared with non-diabetic women. Cholesterol and triglycerides levels were progressively increased in both patient groups as gestation advanced, values of triglycerides being higher at 34 weeks gestation in the diabetic pregnant patients relative to normal control mothers; maternal plasma IGF 1 was higher in normal pregnant subjects compared with their diabetic counterparts.

No correlations were obtained between estimated fetal weight and cholesterol, triglycerides or IGF 1. Significant negative correlations were found at 34 weeks gestation between some amino acids and sonar estimates of fetal weight supporting the possibility that maternal plasma amino acid levels may influence fetal growth.

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Blood alanine and lactate behaviour during oral glucose tolerance test in non-insulin-dependent diabetes mellitus

Conflicting evidences have been reported on the metabolic fate of glucose following an oral ingestion. Some authors sustain that the increase in glucogenic substrates (lactate and pyruvate) during glucose loading must be accounted for by splanchnic (gut or liver) generation, whereas other authors sustain that peripheral, not splanchnic, tissues play the predominant role in the disposal of an oral glucose load.

With these discrepancies in mind, we measured the metabolic pattern of glucogenic precursors such as alanine, predominantly produced by peripheral tissue (muscle), and lactate after an oral glucose load in ten normal subjects and in eighteen subjects with non-insulin-dependent diabetes mellitus. Both in normals and in diabetics not significant changes in blood alanine were observed whereas a significant increase in blood lactate was observed at 60, 90 and 120 min. Although a similar behaviour in blood alanine and lactate was found between normals and diabetics, in the latter ones significantly higher levels of alanine and lactate were found at each time.

Frome these observations we conclude:

1) when glucose is ingested under post-absorptive conditions, since blood alanine levels do not change, splanchnic, not peripheral, tissues play the predominant role in glucose disposal

2) after an oral glucose load, the pattern of gluconeogenic precursors is similar in healthy controls and in diabetic subjects

3) the main cause of fasting and post-prandial hyperglycemia in diabetic subjects may be due to an overproduction of alanine as well as lactate.

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The use of glutathione analogues as probes to the active-site of glutathione dependent enzymes

Glutathione (α-glutamylcysteinylglycine) is a tripeptide which exists in the two forms, reduced (GSH) and oxidised (GSSG). This tripeptide is one of the most abundant non-protein thiols present in the cells of plants, mammals and microorganisms in the comparatively high concentration of 0.5–10 mM in the reduced form.

In addition to maintaining cells in the reduced state this tripeptide participates in a significant variety of enzymatic reactions as a coenzyme, cosubstrate and as part of the substrate architecture.

In this talk I will describe the synthesis and use of glutathione analogues as probes to the active-site of glutathione dependent enzymes and in some cases identify the structural homology which exists between different glutathione recognition sites.

1. D'Silva C, Seddon AP, Douglas KT (1981) JCS Perkin 1/12: 3029
2. D'Silva C, Douglas KT (1983) Photobiochem Photbiophys 5: 151
3. D'Silva C, Timari AA, Douglas KT (1982) Biochem J 207: 329
4. D'Silva C (1986) Febs Lett 202: 240
5. D'Silva C (1990) Biochem J (in press)
6. D.Silva C (1990) Biochem J (in press)

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Kinetic studies of the PLP-serine schiff-bases

The interconversion of pairs of amino acids and α-ketoacids by transaminases has been viewed in the context of two half-reactions. In the first one the Pyridoxal 5'-phosphate (PLP) form of the enzyme binds to the α-aminoacidate ion forming an aldimine Schiff-base. The activation of an α proton of the amino acid part causes hydrolysis release the α-keto acid and Pyridoxamine 5'-phosphate (PMP). The second half-reaction is the reverse reaction.

In model reactions the transamination process not always is observed. However, the labilization of an α proton of an amino acid can lead to different reactions (dealdolation, elimination, transamination...). This process may also cause the elimination of a good leaving group from the β position.

This panel focuses on the transamination process of α-amino acid and PLP. It presents fully kinetic study of such process between serine and PLP.

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Effects of various metabolites of essential amino acids on growth of tumor cells

In mammals, essential amino acids can be related in the diet by their metabolites, with varying degrees of efficiency, for example, homocysteine for methionine and many α-keto acids for the corresponding essential amino acids. Among these metabolites, homocysteine has been reported not to show an activity as a substitute for methionine in maintaining the growth of some tumor cells. However, there is no study about the other metabolites. The present study was conducted to clarify whether various metabolites were used in vitro as substitutes for the corresponding essential amino acids in tumor cells or not.

Materials and methods: Six kinds of tumor cells from animals and human subjects were used, AH 130, L 1210, U 397, A 375, A 549 and Hela cells.

Six metabolites of essential amino acids were examined, α-ketoisocaproate, acetyllysine, phenylpyruvate, phenyllactate, indolepyruvate and indolelactate.

Growth of tumor cells in a medium was evaluated by MTT assay after 72 hr-cultivation at 37 °C and 5% CO_2.

Conclusion: 1. All the tumor cells did not grow in a medium devoid of an essential amino acid.

2. Addition of α-ketoisocaproate, acetyllysine, phenylpyruvate and indolepyruvate to a medium devoid of a corresponding essential amino acid enabled the tumor cells to restore the growth depression, respectively.

3. Addition of phenyllactate and indolelactate could not restore the growth depression of tumor cells cultured in a medium devoid of phenylalanine and tryptophan, respectively, indicating that the tumor cells could not convert the metabolites to the pyruvate-forms.

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Glutaminase and glutamine synthetase activities in various hepatoma cells

Glutamine catabolism is of considerable interest because of its link to neoplastic transformation. Glutaminase and glutamine synthetase activities in rat, chicken and human hepatoma cells were determined.

Phosphate-dependent and phosphate-independent glutaminase activities were markedly high in various hepatoma cells compared to those in normal liver tissues. Enhanced expression of these enzyme activites were also detectable in human cirrhotic liver, and in the liver of rats during 3'-methyl-4-dimethylaminoazobenzene-induced hepatocarcinogenesis. Oxypolarographic tests showed that glutamine oxidation was prominent in mitochondria of these hepatoma cells.

Marked decrease in glutamine synthetase activity was observed in most of the hepatoma cells thus far examined. In the liver of rats fed with the chemical carcinogen, glutamine synthetase activity decreased only in the neoplastic nodules and tumors.

From the present findings we concluded that the imbalance in the glutamine metabolism in the tumor cells is independent of the nature of carcinogenic agent and the species.

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Regulation of insulin release by amino acids

The stimulation of both proinsulin biosynthesis and insulin release by amino acids such as L-leucine, its nonmetabolized analogue 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid, L-norvaline, L-isoleucine, L-glutamine and L-asparagine is currently considered within the framework of the fuel concept for nutrient-induced insulin secretion. Thus, the changes in 0_2 uptake for ATP generation rate evoked by these amino acids in islet cells and attributable to their own catabolism or the modified catabolism of endogenous nutrients, as modulated for instance by activation of glutamate dehydrogenase, are commensurate with the biosynthetic and secretory response of the islet B-cell. Such is also the case for various combinations of these and other nutrient secretagogues. However, this fuel concept does not apply to L-arginine, which is the amino acid most commonly used as an insulin secretagogue. Moreover, comparison of the islet cell metabolic, ionic and secretory responses of L-arginine, L-ornithine, L-homoarginine, 4-amino-1-guanylpiperidine-4-carboxylic acid, L-lysine and L-histidine, as well as the effect of 2-aminoisobutyric acid and D,L-α-dufluoromethylornithine thereupon, failed to support any of the alternative hypotheses so far considered to account for the insulinotropic action of L-arginine. Such hypotheses include the accumulation of cationic amino acids in islet cells leading to plasma membrane depolarization, the *de novo* generation of polyamines, the capacity of the amino acids or their metabolites to act as a substrate for transglutaminase, the biosynthesis of nitric oxide, a change in intracellular pH and the stimulation of cyclic AMP net production. The mechanism for the insulinotropic action of cationic amino acids thus remains a largely unsettled topic.

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L-alanine transport by plasma membrane vesicles islated from Ehrlich cells

Plasma membrane vesicles isolated from Ehrlich ascitesl tumor cells by 2-phase compartmentation efficiently transport L-alanine. Upon the imposition of an inwardly-directed Na^+-gradient, L-alanine is mainly transported by Na^+-dependent transmport systems. In experiments with 100 mM NaCl outside, and zero NaCl inside the vesicles, Na^+-dependent transport of 0.1 mM L-alanine reaches a maximum at 30 s; afterwards there is a decline in the transport rate owing to the dissipation of the Na^+-gradient. The partial inhibition of Na^+-dependent L-alanine transport by methylaminoisobutyric acid reveals the implication of both systems A and ASC in this transport. Kinetics of both Na^+-dependent systems A and ASC are michaelian, showing K_m values of 0.1, and 0.4 mM, respectively.

Basal Ganglia

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Anticataleptic potencies of glutamate-antagonists

Dopamine and glutamate (via the NMDA-subtype of glutamate receptors) exert opposite effects on akinesia and rigidity (catalepsy) in the rat, an animal model of Parkinson's disease. Therefore, not only dopamine agonists, as currently used, but also NMDA-antagonists may be predicted to have a therapeutic potency in the treatment of Parkinson's disease. Here, the effects of several NMDA-antagonists were tested in rats in which catalepsy was induced by pretreatment with haloperidol (0.5 mg/kg i.p.).

Non competitive NMDA-antagonists: Catalepsy measured on a horizontal bar, a podium and a vertical grid, was dose dependently antagonized by dizocilpine (0.08, 0.16, 0.33 mg/kg i.p.) and by memantine (5, 10 mg/kg i.p.). Both drugs have stimulatory effects on locomotion in an open field. In a movement initiation task, dizocilpine and memantine shortened reaction time but only dizocilpine increased initial acceleration.

Competitive NMDA-antagonists: Catalepsy was antagonized by CCP (1.5, 3 mg/kg), CGP 37849 (2, 4, 8 mg/kg i.p.) and CGP 35991 (10 mg/kg i.p.). However, locomotion was not increased. In the movement initiation task only reaction time was restored but not acceleration.

Antagonists at the allosteric glycine binding site of the NMDA receptor: D-cycloserine a partial glycine agonist per se had no effect. However it enhanced the effects of dizocilpine when coadministered. At the cellular level this may be explained by an increase int the opening rate of the NMDA-receptor gated ion channel were dizocilpone binds use dependently. In contrast, the effects of CGP 37849 were abolished, possibly because D-cycloserine converts the NMDA binding site in tis agonist preferring state.

Conclusion: Antagonists at the NMDA receptor may have a therapeutic potential in the treatment of Parkinson's disease. Their effect can be specifically manipulated at the glycine binding site.

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Further contribution to the analysis of corticostriatal glutamatergic and nigrostriatal dopaminergic interactions within the striatal network

An emerging concept is that cellular interactions between glutamatergic corticostriatal and dopaminergic (DA) nigro-

striatal neuronal pathways consist in a functional antagonism. Indeed, data from behavioural pharmacology emphasize the activatory action of excitatory amino-acid (EAA) antagonists and of dopaminergic agonists on locomotor activity, whereas EAA agonists and dopamimergic antagonists have depressor effects. Such cellular functional actions will be cooperative since cortical ablation in the rat was shown to potentiate the activatory effect of DA agonists and to antagonize the depressor effect of neuroleptics. To further analyze such mechanisms, we studied the effect of EAA agonists and antagonists on spontaneos locomotor activity and on amphetamine-timulated behaviour and the effects of cortical ablation on striatal DA parameters. Cortical ablation was shown to potentiate the effect of amphetamine and to decrease the action of haloperidol on the DA/DOPAC striatal concentrations whereas it does not affect basal DA/DOPAC levels suggesting that glutamate action depends on DA activity. Interestingly, the EAA agonists glutamate, AMPA and quisqualate were shown to facilitate the striatal DA release monitored by in vivo voltammetric methods whereas NMDA depressed it in an APV sensitive manner, suggesting that EAA have dual opposite action over DA transmission. To further characterize the EAA receptors putatively located at presynaptic level on the DA nerve terminals, we finally use ^{3}H-AMPA and ^{3}H-CPP binding methods to label putative AMPA and NMDA EAA receptor subtypes following 6-OHDA induced lesion of the DA neurons. Such as lesion does not decrease the binding of these two ligands. Interestingly, the number of ^{3}H-CPP binding sites was shown to increase by about 30% suggesting receptor-receptor interactions between EAA and DA receptors previously suggested on the basis of studies of EAA action on D 1 receptor activation. Thus, functional antagonism between corticostriatal glutamaterigc and nigrostriatal dopaminergic activities will be supported by both presynaptic and postsynaptic integrative mechanisms.

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Excitatory amino acidergic pathways and binding sites in the basal ganglia

We review the organization of excitatory amino acidergic (EAAergic) pathways involving the basal ganglia. The organization of neocortical, allocortical, and subcortical striatal afferens is reviewed. The organization of subthalamic nucleus projections is reviewed, as is the status of possibly EAAergic afferents from intralaminar thalamic nuclei and the pedunculopontine nucleus.

Receptor autoradiography was used to determine the distribution of EAA binding sites in rat basal ganglia. NMDA, AMPA, metabotropic (MET), kainate (KA), and non-NMDA, non-KA, non-quisqulate (NNKQ) glutamate binding sites have a high density in the striatum with a higher density of NMDA and AMPA binding sites in the ventral striatum. KA binding sited had greater density in the lateral striatum. Other basal ganglia nuclei had lower densities of binding sites with heterogeneous distribution of binding sites. AMPA and MET binding sites had high relative density in the globus pallidus, ventral pallidum and subthalamic nucleus. MET and NNKQ binding sites had a high relative density in the nigra. NMDA receptors had uniformly low density in all non-striatal nuclei. EAA receptors changes in Huntington's disease and Parkinson's disease are discussed.

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Blockade of NMDA receptors exerts opposite effects on dopaminergic D-1 and D-2 mediated turning behavior in the 6-OHDA model of Parkinson

Neurons containing glutamate and dopamine largely project on striatal neurons where they exert a control of the activity of efferent neurons mediating motor behavior.

In rats bearing unilateral lesions of the dopaminergic nigro-striatal neurons with 6-hydroxydopamine (6-OHDA), we have observed that blockade of N-methyl-D-aspartate (NMDA) receptors by MK-801 strongly potentiates the contralateral turning induced by L-DOPA (Morelli et al., 1990, Eurp. J. Pharm. 182, 611). The potentiation induced by MK-801 took place selectively towards D-1 mediated responses, since SKF 38393 and CY 208–243 turning were potentiated, while LY 171555 turning was depressed. Administration of the D-1 antagonist SCH 23390 blocked MK-801-induced potentiation of L-DOPA turning, confirming the D-1 nature of the observed effects.

Expression of the early gene c-fos in the caudate-putamen (CPu) is known to be activated by stimulation of supersensitive D-1 receptors. The study of the expression of c-fos in the CPu homolateral and contralateral to the 6-OHDA lesion, revealed an increase of c-fos in the dorso-lateral aspect of the lesioned CPu after combined administration of MK-801 and SKF 38393.

Studies using local cerebral glucose utilization are in progress in order to identify the efferent areas were neuronal transmission is modified following combined administration of MK-801 and SKF 38393.

Blockade of NMDA receptors, therefore, acts synergistically with D-1 and antagonistically with D-2 receptors stimulation in the 6-OHDA model of turning, suggesting that different glutamatergic pathways are involved in the mediation of D-1 and D-2 responses.

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Functional role of presynaptic potassium channels in the regulation of glutamate release from rat brain synaptosomes

Evidence is available that presynaptic potassium channels sensitive to Dendrotoxin (DTX) play an important role in modulating the release of exoitatory amino acids (EAA) in vivo and in vitro. Blockade of Ia-like K^{+}-currents in synaptosomes by Dendrotoxin (DTX), induces a Ca^{+2}-dependent and dose-dependent release of glutamate (GLU), this effect being modulated by drugs that activate K^{+} conductances in other preparations. Interestingly, intracerebral administration of DTX or a short exposure of synaptosomes to nanomolar concentrations of DTX also causes a long-lasting decrease in the size of the releasable pool of GLU in

response to a subsequent depolarising stimulus. However, this effect shows a marked brain region specificity. Synaptosomes prepared from cerebellum are highly sensitive to DTX, followed by the striatum and the cerebral cortex. In contrast, GLU release from hippocampal synaptosomes is almost unaffected by DTX. In conclusion, presynaptic K^+ channels sensitive to DTX seem to exert a specific role in controlling the releasable pool size of glutamate in some populations of excitatory nerve endings.

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Modulation mechanisms of glutamine release from glutamatergic neurons in striatum

The action mechanisms and functions of excitatory amino acids in the brain have become recently the focus of attention of neuropharmacologists, neurophysiologists, and neurochemists. The lively interest of this substances in research is explained by the fact that pathogenesis of a number of brain diseases appears to be associated with disturbances in their metabolism, release, or effect on the central neurons. In particular, there is evidence that degeneration of the central neurons can be caused by a non-controlled increase of the extracellular level of glutamate in the brain.

In my report data are presented on the mechanisms of presynaptic modulation of glutamate release in rat striatum obtained by the local superfusion method. It has been shown that dopamine, acethylcholine and meth-enkephalin modulate differently the glutamate release. Two mechanisms of such presynaptic modulation were revealed: presynaptic inhibition and the homeostatic principle. The role of cyclic nucleotides, Ca^{2+}, and Na^+ in the mechanisms of presynaptic modulation of glutamate release is discussed.

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Interactions between monoamine-, amino acid-, peptide- and cholinergic systems in the basal ganglia of the rat: studies with in vivo microdialysis

Two major afferent systems into the mammalian neostriatum are the mesostriatal dopamine (DA) and the corticostriatal glutamate (Glu) pathways. These two pathways converge onto intrinsic and projecting neuronal systems, such as acetylcholine (ACh) and GABA, respectively. In addition, DA and Glu can directly interact with each other via axo-axonic links. In vivo microdialysis studies have shown that extracellular levels of DA, Glu, ACh and GABA can be simultaneously monitored in rat striatum under basal and local K^+-depolarizing conditions and following selective stimulations of both the mesostriatal and the corticostriatal pathways. The neuronal origin of striatal extracellular levels of DA, Glu, ACh and GABA was studied using selective uptake and enzymatic inhibition, as well as following specific lesions. The Ca^{2+}-dependence of the K^+ evoked increases in the extracellular levels of all the above substances was also studied.

Under basal conditions (perfusion medium = modified CSF; halothane-anaesthesia), striatal extracellular DA concentration was of the order of 10^{-8} M, Glu was 10^{-6} M, and GABA was 10^{-7} M. ACh could be only detected following inhibition of ACh-esterase. Following perfusion with neostigmine (10^{-6}M), striatal ACh concentration was in the order of 10^{-7} M. Perfusion with KCl (10^{-1} M) increased DA and GABA levels by > 10 fold, while ACh levels were increased by > 5 fold. However, under the same conditions, Glu levels were increased by less than 2 fold, suggesting that the KCl evoked GLu is rapidly taken up by neuronal and glia elements. Thus, the role of uptake mechanisms in maintaining steady-state and stimulated extracellular Glu levels was studied. When dihydrokainate (DHKA) (10^{-3} M), a selective Glu uptake blocker was included in the perfusion medium, striatal Glu levels were increased by approximately 5 fold following K^+-depolarization. Furthermore, it was found that the K^+-evoked increases in striatal extracellular DA, Glu, ACh and GABA levels were inhibited when the perfusion medium was replaced with a Ca^{2+} free perfusion medium containing either EDTA or EGTA (2×10^{-3} M). Chemical stimulation of mesostriatal and corticostriatal pathways at the cell body level selectively modified extracellular DA and Glu levels measured at the terminal fields in the ipsilateral striatum (changes in striatal GABA and ACh levels were also detected). In the nigra, GABA and opioid agonists exerted an inhibition, while tachykinins exerted a stimulation of striatal DA release. However, cortical stimulation produced both an increase and a decrease in striatal DA, ACh or GABA release depending upon which Glu receptors were stimulated, as well as upon the involvement of local mono- and polysynaptic loops. The effect of specific lesions of mesostriatal, nucleus basalis-cortical or corticostriatal pathways on cortical and striatal DA, Glu, ACh and GABA levels showed that while pathways could selectively be impaired by the specific lesions, changes in the downstream neurotransmitter system could be also detected.

In conclusion, we show that in vivo microdialysis is an appropriate technique for studying the functional anatomy of the basal ganglia, as discrete neuronal circuitries can specifically be analyzed.

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Glutamate and cholecystokinin (CCK) release in the cortex and striatum of the rat: an in vivo mycrodialysis study

Glutamate (Glu) is considered to be a major neurotransmitter in cotrico-cortical and the cortico-striatal pathways of the rat. Furthermore, neurons containing CCK mRNA are found in all layers of the neocortex, with a clear concentration in layers II/III and V/VI [1]. Indeed, it has recently been reported that many of the pyramidal projecting neurons of the neocortex express the CCK gene in adult and developing rats [2]. In the present study, we show for the first time that extracellular Glu and CCK levels can be simultaneously detected and monitored in perfusate samples collected with in vivo microdialysis from cortex and striatum of halothane-anaesthetized rats.

Two microdialysis probes (CMA 10, Carnegie Medicin AB, Sweden) were stereotaxically implanted, one into the

frontoparietal cortex (inserted with a 40° angle from vertical in the coronal plane) and the other into the ipsilateral striatum (inserted vertically) of male Sprague-Dawley rats (b.w. 500 g). The microdialysis probes were perfused with a Krebs medium including 2 g/L BSA and 0.3 g/L Bacitracin. Perfusate samples were taken from cortex and striatum under basal and KCl-stimulated conditions and assyed for Glu and aspartate (Asp) with HPLC coupled to a fluorescence spectrophotometer (following o-phtaldialdehyde and 2-mercaptoethanol derivatization), or assayed for CCK with a sensitive radioimmunoassay (RIA) and CCK-antiserum raised in rabbit.

Under basal conditions, cortical extracellular Clu concentration was of the order of 2×10^{-6} M, Asp was 5×10^{-7} M and CCK 2×10^{-11} M, while in the striatum Glu was 10^{-6} M, Asp 10^{-7} M and CCK 2.5×10^{-11} M. Perfusion with KCl (10^{-1} M) increased Glu and Asp levels by less than 2 fold in both cortex and striatum, while CCK levels were increased by > 10 fold in the cortex and by > 50 fold in the striatum. A Glu uptake blocker was included in the perfusion medium in order to further investigate the neuronal dependence of extracellular levels of Glu and Asp. Following perfusion with dihydrokainate (DHKA) (10^{-3} M), Glu and Asp levels in both brain areas were increased by approximately 3 fold following K^+-depolarization, suggesting a neuronal origin for both Glu and Asp. However, it was found that the K^+-evoked increase in Glu, but not Asp was inhibited by perfusing with a Ca^{2+} free medium (including 2×10^{-3} M EGTA), suggesting vesicular and nonvesicular releasing mechanisms for Glu and Asp respectively.

In conclusion, we show that extracellular levels of the excitatory amino acids Glu and Asp, and the peptide CCK can be simultaneously monitored in the cortex and striatum of rats by using in vivo microdialysis. Their neuronal origin can be characterized, and perhaps, in the future, it may be possible to determine if Glu and CCK are released from the same corticostriatal neurons.

1. Lindefors et al (1990) Exp Brain Res 80: 489–500
2. Burgunder, Young (1990) J Comp Neurol 300: 26–46

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Disruption of the performance of a successive discrimination by the local application of kynurenic acid into the nucleus accumbens of the rat

Male Sprague-Dawley rats, trained to perform a visual discrimination, were administered kynurenic acid, an antagonist of excitatory amino acid receptors, 4.7 µg bilaterally into the nucleus accumbens. The performance of the visual discrimination was impaired 15, but not 360 min after administration. In addition, inter-trial crosses in the test apparatus were markedly increased by the treatment, whereas no effects on motor activity were noted when the animals were observed in an open field. The abnormal behavior produced by kynurenic acid has previously been observed after administration of high doses of compounds likde d-amphetamine and L-DOPA, and generally discriminative behavior has been shown to be highly dependent on normal impulse mediated release of dopamine in brain. The present results show that this behavior also is dependent on an intact excitatory amino acid neurotransmission. In further support of this contention we recently showed that systemic administration of another excitatory amino acid antagonist, phenyclidine, also disrupts the performance of the discriminative behavior.

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Interactions between central glutamatergic, catecholaminergic and cholinergic systems with regard to psychomotor functions

Recent animals experiments suggest that dopamine plays a less crucial role than formerly supposed for the regulation of psychomotor functions. This is illustrated by the finding that even in the virtually complete absence of brain dopamine, a pronounced behavioural activation is produced in mice following suppression of glutamatergic neurotransmission: We have found that the selective non-competitive NMDA receptor antagonist MK-801 causes a marked locomotor stimulation in mice depleted of monoaminergic stores by means of reserpine and α-methyl-p-tyrosine pretreatment. Moreover, we have demonstrated a pronounced synergism in monoamine-depleted mice when MK-801 in a low dose, which doest not affect motor activity *per se*, is combined with a high dose of the α-adrenergic agonist clonidine or the muscarinic antagonist atropine. A synergistic effect with regard to motor activity is also observed when MK-801 is combined with a low (sub-threshold) dose of the dopaminergic agonist apomorphine.

Supporting the conclusion that NMDA receptors are involved in the locomotor stimulatory effects observed following MK-801 treatment in monoamine-depleted mice are recent findings showing that also the non-competitive NMDA antagonists phencyclidine (PCP; given alone or in combination with clonidine) and ketamine (in combination with clonidine) promote locomotion. The doses needed to induce behavioural activation with these agents in monoamine-depleted mice are about ten (PCP) and fity (ketamine) times higher than with MK-801. Moreover, we have recently observed that high doses of the *competitive* NMDA antagonist SDZ EAA 494 also induce locomotor stimulation in monoamine-depleted mice; if combined with clonidine this compound causes a pronounced increase in locomotion already in low doses. The gross appearance of the animals receiving SDZ EAA 494 is virtually indistinguishable from that of monoamine-depleted mice receiving MK-801.

From our findings it can be inferred that: (1) DA is not indispensable for initiation and generation of locomotion, (2) central glutamatergic systems exert a powerful inhibitory influence on locomotion, and (3) central glutamatergic and catecholaminergic systems are functionally opposed with regard to locomotion – possibly this antagnonistic interaction takes palce within the striatum, in analogy to the presumed cholinergic/dopaminergic antagonism within this structure.

Our findings may have implications for future treatment strategies in Parkinson's disease. Considering the powerful synergism between MK-801 and clonidine/apomorphine/atropine an interesting clinical approach might be to combine an NMDA antagonist with a catecholaminergic agonist or a muscarinic antagonist, in order to achieve an optimal therapeutic effect and a minimum of side effects.

Cross Linking

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Natural enzyme induced cross-linking of proteins

The cross-linking of protein macromolecules to form stable supramolecular-aggregates capable of acting as protective and supporting structures is a common feature of organisms coping with the stress of life. Such cross-links must be carefully controlled since non-optimal cross-linking could lead to malfunction or even death of the organism. Their formation is therefore highly regulated by specific enzymes cross-linking the molecules at specific sites in the molecule to provide the functional properties required.

Cross-linking through Lysine-aldehydes. The connective tissue proteins collagen and elastin are both polymerised by a copper containing enzyme, Lysyl oxidase, which oxidatively deaminates the e-amino groups of specific lysines. The subsequent reactions of the lysyl aldehydes are different depending on the group's environment thereby providing elastin with its elastic properties and collagen with its inextensible fibres. The chemistry of the cross-links becomes more complex as these structures change with age, this second phase of cross-linking occurring spontaneously rather than enzymatically

Cross-linking through the aromatic residues. The di-Tyrosine cross-links originally identified in resilin have now been reported to the present in many tissues. They are formed by the action of peroxidases to form a carbon-carbon bond between two or three tyrosines.

Cross-linking through quinone groups. The quinone cross-links in mussel are formed through the action of catechol oxidases.

Cross-linking through iso-peptide bonds. The -glutamyl-lysine bonds characteristic of the insoluble protein keratin and the fibrin clot are formed through the action of a calcium requiring enzymes transglutamidase to form a iso-peptide cross-link.

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Protein folding/refolding analysis by mass spectrometry: identification of disulphide bonded intermediates

The analysis of protein folding/refolding is a problem of considerable interest in the field of protein chemistry, both in theoretical studies and in practical applications.

We have previously introduced a new technique for the analysis of disulphide bridges in proteins, and suggested that the method would be applicable to the dynamic analysis of protein folding. This method is based upon determination of the mass of disulphide linked peptides by the direct analysis of unfractionated or partially purified protein digests by high-field fast-atom bombardment mass spectrometry (FAB/MS).

The results described in the present communication demonstrate that this MS method allows sampling of the folding/refolding experiment on a time-course basis, and give rapid definition of the exact nature of the disulphide bridges formed at any particular time, via a definite assignment of disulphide-linked peptides according to their unique mass values.

Since insulin was chosen as the reference protein to be analyzed in the static mode, we first addressed our attention to this protein in refolding studies. With the use of an appropriate thiol concentration, scrambling of the native disulphide bonds in bovine insulin occurs. The disruption of native and the formation of new disulphide bonds can be monitored as described above, and interestingly, B-chain dimers containing CysB 7-CysB 7 and CysB 7-CysB 19 bonds are detected.

The refolding pathway of reduced and denatured RNase A was further studied. When re-oxidation was carried out in the presence of 8 M urea all four native and five "nonnative" disulphide bridges were identified. The occurrence of such a limited number of disulphide bridges compared to the 28 theoretically possible cysteine couplings shows that the formation of disulphide bridges does not occur at random, even when re-oxidation takes place under denaturing conditions. When the re-oxidation was performed under non-denaturing conditions, the formation of some well-defined native and "non-native" S-S bridges was observed at early stages of the refolding process. Under appropriate conditions, all four native disulphide bridges could be identified at later stages of refolding.

This procedure can then be effective in the actual definition of the intermediates along the refolding pathways of S-S containing proteins.

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Change in elastin structure in human aortic connective tissue diseases

Biochemical pathogenesis of aortic connective tissue diseases (Marfan's syndrome, dissecting aneurysm or aortic aneurysm) was examined by estimating the elastin content in the aorta and the cross-linking components (isodesmosine, cysteine, cysteine and histidinoalanine). The elastin content in the aorta (mg/mg dry tissue) and elastin-specific isodesmosine (nmol/mg dry tissue) obtained from the extract of the aortic sample decreased in comparison with the control sample obtained from regugitation. Ratio of cysteine residues (Cys/Cys-Cys) in the elastin fraction in disease increased more than that in the control. Content of histidinoalanine (formed between acid structural protein residues and elastin or collagen, and the cross-linking may be responsible for the aging of human aorta) was found to be decreased. It may be suggested that elastin is maintained in its native shape by inter- and intra-molecular cross-link bridgings, and it is readily denatured by various disease conditions. After elastin was solubilized by elastase, immuno-reactive elastin content in those aortic diseases was found to be increased in human connective tissue. Serum elastin-hydrolyzing and elastase-like

activities tend to increase more than that in the control. These findings may suggest that the change in the structure of elastin would make more susceptible to elastase and other proteolytic enzymes. These findings correlate well with the histopathogenic changes observed in the aorta, demonstrating fragmentation and loss of elastic fibers in disease states.

Pharmacology

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The effect of alpha-alkyl-amino acids on triglyceride levels in the mouse

Alpha-alkyl-amino acid analogues (n = 1) were administered orally in a mean dosage of 30 mg7Kg body weight/day for a period of 6 weeks to 6 week olf female mice (OH 1 mice). The amino acid analogues (AAA) were applied in solid food (mouse chow Altramin 1321 ff).

The aim of the study was to incorporate the AAA into connective tissue proteins. Testing the serum parameters triglycerides, GOT, GPT, LDH, CK, Glucose, protein, albumin, alkaline phosphatase and BUN as routine parameters of the study protocol we found that two of the AAA were potent effectors on serum triglycerides. One of the analogues decreasing serum triglycerides increased glucose levels in serum significantly and was not followed. PE-ABA did not change the routine parameters given above. PE-ABA treated animals showed mean triglyceride levels of 63.2 mg/100 ml ($\pm$21.1) in contrast to the untreated group with 115.1 mg/100 ml ($\pm$47.6). Statistical calculations showed a significant difference ($p < 0.02$).

Not toxic effects could be detected.

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Boron analogues of amino acids and peptides

We have been extensively involved in the synthesis of isoelectronic and isostructuralboron analogues of the α-amino acids. These have ranged from simple glycine analogues such as H_3NBH_2COOH and Me_2NHBH_2COOH to the more complex analogues of alanine, phenylalanine, leucine, etc. These have been incorporated into peptides containing boroamino acids and normal amino acids.

A diverse variety of analogues, including precursors and derivatives have expressed potent pharmacological activity, including anticancer, antiinflammatory, analgesic, hypolipidemic, and antiosteoporosis activity in animal model studies and in vitro cell cultures.

Some dipeptides show particular promise for use in Boron Neutron Capture Therapy.

Acute toxicity studies of some lead compounds have shown them to be free of toxicity at 1, 2, or 5X their therapeutic dose in rodents.

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The effects of beta-alanyl-melphalan in cancer physiology and chemotherapy

The targeting of proven anticancer drugs specifically to cancer cells would provide a unique opportunity to restrict neoplasms without damaging the cancer patient. The present research utilizes the phenomenon of illicit transport, i.e. the coupling of normally impermeant metabolites to permeant metabolites, in targeting the drug melphalan to mouse Ehrlich ascites tumor cells. The dipeptide beta-alanyl-melphalan was synthesized and tested in vitro for toxicity towards mouse Ehrlich ascites tumor cells, mouse liver cells, and mouse 3 T 3 embryonic cells. The peptide was shown to be greater than 85% pure and was significantly more stable at 37 °C than melphalan, exhibiting a half-life in solution of 607.71 minutes. The half-life of melphalan under similar conditions was 105.21 minutes. In in vitro toxicity assays, melphalan was shown to be toxic to all three cell systems studied, whereas beta-alanyl-melphalan was toxic only towards the Ehrlich ascites tumor cells and the 3 T 3 fibroblast cells. In vivo chemotherapy assays, using Ehrlich ascites tumor cells injected into the abdominal cavity of mice, revealed that melphalan, at concentrations of 5 and 10 mg/kg, was an effective anticancer drug providing for T/C rations of 179 and 193 respectively. The dipeptide, beta-alanyl melphalan, was also an effective anticancer drug, exhibiting reduced toxicity towards the tumor bearing animal when compared to the parent drug melphalan, providing for T/C rations of 152 at a drug concentration of 40 mg/kg.

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Plasma amino acid patterns in chronic fatigue syndrome: Effects of treatment with specific essential amino acid supplementation

In an open trial, fasting plasma amino acid levels were tested in 30 subjects (22 female, 8 male) exhibiting the symptoms of chronic fatigue syndrome (CFS). An amino acid supplement consisting of 10 essential amino acids, pyridoxal-5-phosphate and aketoglutaric acid was formulated according to test result levels, i.e. higher amounts of amino acids were given in the formula if that amino acid were low relative to other amino acids. This procedure generated a unique formulation of amino acids and synergists presumably matched to measured biochemical need of the subject. Subjects were asked to take 15 grams of this supplement per day

for 90 days. A post trial interview assessed any change in symptoms. Responses were graded as no change, 25%, 50%, 75% and 100% symptom reduction. Post-trial plasma amino acid levels were taken. All subjects showed significant deviation in amino acid levels from normal ranges indicating possible impairment TCA cycle function. These levels showed improvement after supplementation. Sixteen (53%) of subjects experienced at least 50% improvement in symptoms, six (20%) experienced 100% improvement in symptoms following the trial. These results indicate amino acid metabolism may be affecting cellular energy production and/or immune system function and that these functions may be improved with appropriate supplementation. The failure rates in the trial indicates the multivariate etiology of CSF where amino acid metabolism may not be involved. Further research on organic and metabolism in CSF patients is onging and may elucidate the mechanisms of amino acid supplementation efficacy.

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Central depressor effects of amino acids in conscious normotensive and two-kidney, one-clip renovascular hypertensive rats

The circulatory effects of intracisternal injection of amino acids were investigated in conscious nomotensive control (NCR) and two-kidney, one-clip renovascular hypertensive rats (RHR). Arterial pressure was observed by means of an indwelling catheter connected to a pressure transducer. Heart rate was counted from arterial pulsation. The injection of glycine, GABA, taurine, serine, alanine, and sarcosine into a chronically implanted intracisternal cannula decreased blood pressure by an average of 16–40 mmHg in NCR and by an average of 32–55 mmHg in RHR. Both absolute and percent changes of depressor effects by GABA, taurine, serine, and alanine were larger in RHR than in NCR. All these amino acids also showed similar bradycardiac effects in both NCR and RHR, when compared in absolute values. The percent change of bradycardia induced by taurine and sarcosine was larger in RHR than in NCR. However, the degree of bardycardia by serine was larger in NCR than in RHR. These results suggest the possibilities that serine, alanine and sarcosine in addition to glycine, GABA, and taurine, which are present in brain, play important roles in blood pressure control in conscious normotensive rats via central neural mechanisms and that development of hypertension in renovascular hypertensive rats may change a setting point in central mechanism related to the peripherally cardiovascular tone.

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Amino acids as immunoregulators with special regard to aids

Stimulated human and murine lymphocyte population, T cell clones and T cell tumors were found to have generally a weak membrane transport activity for cystine, but a strong transport activy for cysteine. The plasma cysteine concentration, however, is extremely low in comparison with other protein forming amino acids. Complementary laboratory experiments suggest strongly that the cysteine supply has an important regulatory function in T cell mediated immune responses. Cysteine was found to inhibit the transcription factor NF-κB and to inhibit the expression of NF-κB dependent genes. However, other important lymphocyte functions including the rate of IL-2 dependent DNA synthesis and the activation of cytotoxic T cells are positively regulated by cysteine and strongly influenced by small variations of the extracellular cysteine level even in the presence of relatively high and approximately physiological concentrations of cystine. Macrophages function as a "cysteine pump" for T cell. In double chamber experiments, macrophages were shown to increase the intracellular glutathione level and the proliferative activity of activated lymphocytes across a porous membrane. Macrophages are known to play an important role as antigen presenting cells for T cells and come into close contact with these cells in the course of T cell activation. The function as a "cysteine pump" is based on the fact that macrophages take up more cystine than they need a release after intracellular reduction the excess cysteine into the extracellular space. This process is suppressed by elevated extracellular glutamate concentrations, which inhibit competitively the influx of cystine into the macrophages. Markedly elevated plasma glutamate levels have been found in several pathological conditions including advanced malignancies. A combination of hyperglutamataemia, hypocystinaemia and hypocysteinaemia has been identified in HIV-1 infected patients. Taken together, our studies suggest strongly that the cysteine supply is impaired directly or indirectly in several pathological conditions that are associated with immunodeficiencies including the acquired immunodeficiency syndrome (AIDS). Cystine or cysteine derivatives may, therefore, be considered for the treatment of patients with HIV-1 infection.

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Lysin amide derivatives repair stress conditioned disregulation like substance P. Structure-activity-relationship

The previous findings that the N-terminal tetrapeptide of substance P (Arg-Pro-Lys-Pro = SP 1–4) and especially the dipeptide regions SP 3–4 are responsible for the neurotropic SP-effects, prompted us to investigate further structural modifications of the Lys-Pro-dipeptide.

The effects were preferently estimated in normalization of hypertension and in reversal of endogenous opioid dependence (gut dependence) in rats, chronically stressed by immobilization.

Lys-Pro, Lys-pyrrolidide, several other lysin amides and their N^{α} and/or N^{ε}-acetylated derivatives have been synthesized by different routes in high yields.

After intraperitoneal application some of the amides show nearly the same effects against stress induced hypertension like SP 1–11, SP 1–4, SP 3–4 in a dose of 0,2 μMol/kg. The effects on the gut dependence response are obviously influenced by the lipophilicity of the peptide. SP 1–4 and SP 3–4 are inactive.

Lysinpyrrolidides are orally active in both tests when administered in a dose of 1 mg/kg. One of the orally applied amides shows the SP-effect of normalizing sleep-disturbances as revealed by the electroencephalographic analysis of sleep stages in rats chronically stressed by indetermination conflicts. these results offer a new approach in avoiding or repairing disregulations induced by permanent stress.

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A comparison of neurotrophic activities of nerve growth factor and triap, a small molecular weight compound

Nerve growth factor (NGF) has become the prototype macromolecular neurotrophic factor. NGF is necessary for the normal development, maintenance, and survival of not only sensory and sympathetic neurons in the peripheral nervous system but also cholinergic fibers in the central nervous system. Unfortunately therapeutic application of such a large protein molecule is limited and the need for small neurotrophic molecules which may be orally active and readily penetrate the blood brain barrier becomes obvious. One such compound, Triap (1,1,2 tricyano-2-amino-1-propene), possesses a wide variety of biological and biochemical properties. Early studies of this compound showed that it increases lipid, protein, and RNA in neuronal tissue with a concomitant decrease in RNA content of the associated oligodendrocytes. Further studies showed this compound to augment neuromuscular transmission and hasten peripheral nerve regeneration in both dogs and rabbits. Similarly to NGF, Triap also induces neurite formation in chick spinal ganglia as well as supporting survival of dissociated chick sympathetic neurons in culture. These studies showed Triap to be as effective as NGF but at much lower concentrations (10^{-12} M). Recently our laboratory demonstrated Triap to induce neurite sprouting in the PC12 cell line and also potentiate NGF's morphological and enzymatic effects on these cells. In vitro studies of primary neuronal culture have shown Triap to support survival of CNS derived cholinergic neurons and increase choline acetyltransferase activity. The neurotrophic properties of this compound along with its small molecular weight and potency make it an ideal candiate for its investigation as a therapeutic compound for a number of neurological diseases as well as a useful tool in exploring neuronal death and regeneration.

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Formation of beta-hydroxyhistamine from beta-hydroxyhistidine and its pharmacological action on mammals

The formation of β-hydroxyhistamine (β-HO-HA) from β-hydroxyhistidine (β-HO-His) was examined in organs of rats pretreated with aminoguanidine, an inhibitor of diamine oxidase. β-HO-HA was detected in all the organs of the rats injected intiaperitoneally with DL-threo-and erythro-β-HO-His, and the highest concentration was observed in the stomach. The enzyme involved in this formation was partially purified from stomach, and characterized possibly as L-histidine decarboxylase by the properties of the enzyme reaction. Effects of R- and S-β-HO-HA on histamine (HA) receptors were also examined using the crude membrane fraction of the brain homogenate and the primary cultured neurons of rats, and the gastric mucosa and the cardiac ventricle of guinea pigs. Both R-and S-β-HO-HA were bound to HA receptor (H_2 receptor) in the same extent as HA. The accumulation of cAMP in the materials by the stimulation of the amines was studied, and R- and S-HO-HA were proved to act agonistically to the H_2 receptor.

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Pharmacological effects of amino acids and dipeptides on the female reproductive system

In order to study the pharmacological effects of those amino acids and dipeptides on the female reproductive system, immature female CF-1 mice (9–12 g; 12 animals/group) or immature female Charles River COBS-CD rats (23 days old; 11 animals/group) were given 6 subcutaneous injections of various amino acids or dipeptides in saline solution over 3 days (X 3, X 2, X 1) at different concentrations. Saline injected animals served as controls. All animals were autopsied on day 4. When compared with controls, ovarian and uterine weights of L-tryptophan injected groups were significantly higher. L-isoleucine and 7-aza-tryptophan, a protein inhibitor, had uterotropic effects, whereas, an antigonadotropic effect was found with L-arginine. In Pregnant Mare Serum (PMS) – Human-Chorionic Gonadotropin (HCG) primed immature rats a significant increase in ovarian weight by L-tyrosine and L-tryptophan was observed while L-phenylalanine caused a significant increase in the uterine weight when compared to saline injected controls. No teratogenicity of various gestational parameters was found with comparable doses of these various amino acids. With dipeptides, a significant increase in both the ovarian weight by L-tryptophan-L-isoleucine and the uterine weight by L-tryptophan-L-phenylalanine was observed. However, an antigonadotropic effects was found with L-phenylalanine-L-isoleucine. These results suggest that some amino acids and dipeptides have a gonadotropic-like and/or steroidal-like activity similar to that caused by gonadotropic hormone.

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Effects of nicotine on amino acids and urea levels in plasma after daily repeated administration to sheep

In this study the effects of nicotine on plasma amino acids and urea levels after daily repeated administration to sheep were investigated. The experiments were performed in merino sheep (n = 2, 36 kbw). Daily, through a permanent catheter inserted into the arteria, nicotine (500 µg) was administered. Blood samples were obtained hourly up to 10 th after nicotine application. The amino acids were assayed on supernatan plasma fraction following protein precipitation with sulphosalicilic acid. The samples obtained were subjected

to amino acids analyser. The results showed a short increase of the concentrations of THR, GLY, VAL, ILE and LEU in the second hours after nicotine administration. However, this effect was present only in the first five days of experiment approximately. Then the levels of THR, VAL, ILE and LEU, but also of MET, ORN and HIS from day to day decreased. Comparing the urea levels before and after nicotine administration, increase in the second hour after nicotine up to 100% in the day was observed. After the ninth day of experiment, the urea levels from day to day decreased up to basal level. The significance of the amino acids is related to the protein synthesis, and also to their function as neurotransmitter. It seems possible that many nicotine effects may be related to the nicotine-induced amino acids alteration. In this study observed increase of the urea levels in the day, and the decrease of CIT, OTN and ARG from day to day may be related to the urea formation. Moreover, the decreases of many amino acids and urea from day to day after the first days of experiment, can be related to tolerance development as previously observed by others. In conclusion the present results indicate that nicotine altered the plasma amino acids and urea levels in vivo. This can be related, at least in part, to the nicotine effect on liver, kidney, muscle, brain, but also on the secretion of hormones.

Exercise

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Can branched chain aminoacids treatment change the athletes exercise performances?

Muscular tissue Branched Chain Amino Acid (BCAA) metabolism, in relation to energy request during muscular exercise, is well known, particularly when it is linked to their raised oxidation.

Aim of present work is to investigate the effects of a chronic treatment in biochemical traditional markers (substrates and hormones) as well as in exercise performance.

The athletes (n = 14) were well trained; a first group was submitted to a chronic treatment (n = 7) of BCAA (oral intake was 2 g/Kg/die) and a second group, as controls (n = 7), assumed an oral placebo.

All subjects performed a muscular exercise test (40 km/h, cycle race, per 90 min). This test was made before and after one month of the treatment, during this period of treatment the athletes followed a well standardized diet. Samples of blood were drawn before, at the end and during the recovery (60 min). Measurements of concentrations of FFA, KB, BCAA, free carnitine (C) and acetyl-carnitine (AC) were performed.

Results: plasma BCAA levels do not demonstrate variation either before or after the exercise performance, or between the two groups (Table 1). The biochemical markers, substrates and hormones, KB, FFA, Lactate, free carnitine, Insulin and Growth Hormone plasma levels do not demonstrate significant differences from the patterns present in literature. Only plasma levels of acetyl-carnitine were significant different ($p < 0.05$) between the two groups at the end of the exercise. Conconi's test demonstrated a significant difference in the exercise performance of the two groups ($p < 0.01$).

In conclusion our results seem to confirm that a BCAA treatment is able to influence the exercise performance studied with training markers, both with acetyl-carnitine as well as with a physiological marker such as Conconi's test.

Other biochemical parameters such as ammonium and plasma BCAA profiles do not seem to be in agreement during the test with other Authors. This latter observation could be caused by the quantity of work load, and training state of the athletes.

Table 1

Marker/Time	BCAA treated			Controls		
	0′	end	recovery	0′	end	recovery
Val (μmol(l)	365 (50)	421 (60)	327 (39)	366 (37)	370 (53)	383 (40)
Ileu (μmol/l)	93 (12)	91 (11)	68 (9)	87 (11)	87 (16)	71 (8)
Leu (μmol/l)	159 (21)	153 (20)	136 (8)	169 (21)	162 (19)	131 (13)
AC (μmol/l)	1.8 (0.6)	11.5 (1.1)*	14.5 (1.3)	3.4 (1.0)	18.0 (1.3)*	14.8 (3.1)
C (μmol/l)	37 (2)	35 (2)	32 (2)	34 (2)	32 (1)	32 (1)

(mean and (st. err.))
(*) $p < 0.05$

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Effects of arginine-glutamate supplementation on amino-acid profile during a cycle exercise in man

During endurance exercise, the utilisation and degradation of muscle proteins are increased.

Can exercise be compared to a stress and can the organism benefit by an amino-acid (AA) supplementation?

Effects of glutamate-arginine (GLU-ARG) supplementation were evaluated according to the patterns of AA and the detoxication capabilities during cycling.

Three top level cyclists were submitted to 6 trials on a motor braked cycle. After receiving placebo or 20 g GLU-ARG, they were given 1/2 h for warming and then went through three tests: rest, cycling during 2 h at 60% VO_2max and cycling during 1 hour at 80% VO_2max.

Plasma concentration of AA, urea, creatinin, lactate, ammonia and CK were determined during and after exercise and compared to pre-exercise values (PEV).

With placebo, during the exercises, most AA were reduced (20–40%) except for hydroxy-proline (20–60% increased) with no return to PEV before 10 hours.

With GLU-ARG, at rest, most AA increased (< 15%) in 1/2 h and then decreased (25–80% PEV) within 2 h before re-ascending. They did not return to PEV before 10 h. Arginine was very high, 250% 1 h and 150% 10 h after intake. During the exercises and 1 h after, all AA except arginine and onithine decreased (40–60%). Then they increased up to PEV within 10 h. Arginine increased 150% and decreased back to PEV within 2 h. Ornithine profile was close to arginine (+ 60%). Urea, lactate and ammonia increased more during intense exercise. Only ammonia was higher under placebo. CK did not change.

The exercises modified the pattern of AA with a higher decrease under GLU-ARG. AA patterns did not change with intensity of exercise. Under GLU-ARG supplementation, AA were higher 10 h after exercise.

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Effect of saline solution on skeletal muscle branched chain and aromatic amino acids during the early posttraumatic period

Branched-chain amino acids (BCAA) (valine, leucine, isoleucine) could be degraded, aromatic amino acids (AAA) (phenylalanine, tyrosine) could not, while alanine and glutamine are synthesized in the skeletal muscles. In the present work we investigated these amino acids in the injured and uninjured musculature as well as effects of saline administration (which has beneficial effects on fluid loss compenstaion, electrolyte status and survival after injury) on muscle and extracellular amino acids during the early period following severe injury. Examinations were done 30, 60, 180 minutes after 128-minute bilateral hindlimb ischemia in Wistar rats. Immediately after ischemic period traumatized animals were treated with saline in the 5% amount of b.w. (E group, n = 18) or not (C group, n = 18). Intact animals (n = 6) were used as controls.

Several times increased contents of BCAA and AAA in directly injured muscles (m. femoralis), BCAA/AAA molar ratio within control limits, increased liberation of amino acids suggested increased net protein degradation at the site of injury in C group. In uninjured (m. biceps brachi) muscles of the same group BCAA, particularly leucine and isoleucine contents decreased without alteration in AAA, molar BCAA/AAA ratio decreased, alanine and glutamine increased. Results indicate intensified catabolism of amino acids in the uninjured musculature. Saline treatment (group E) increased alanine and glutamine liberation from the injured region, but has not significantly influenced amino acid profile in directly injured muscles. However, BCAA, alanine and glutamine in free amino acid pool of uninjured muscles were normalized after treatment indicating beneficial effects on muscle amino acid metabolism.

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Phosphocreatine, exercise, protein synthesis, and insulin

The metabolic effects of insulin and of exercise are similar. Energy transfer in striated muscle is through the Creatine Phosphate Shuttle. Isozymes of creatine phosphokinase (CPK) are attached near the myosin ATPase sites and the ATP translocase of the mitochondria. Myofibrillar ATPase converts ATP to ADP. ATP is regenerated by the attached CPK, converting creatine phosphate to creatine. Creatine difusses to mitochondrial CPK where it is converted to phosphocreatine from nascent ATP, and the phosphocreatine returns to the myobibril. This process serves a two-fold function. First, it supplies energy to the myofibril, and second, creatine stimulates the mitochondria to regenerate ATP (acceptor effect).

The acceptor role of creatine is similar to the mitochondrial effect of insulin. A parsimonious paradigm in which insulin attaches hexokinase to mitochondria, providing an acceptor stimulation, was proposed and much supporting data have been developed. Insulin stimulates increased mitochondrial Krebs cycle activity in isolated cells or tissues, reaching a maximum sustained rate within 45 to 60 seconds. This is accompanied by increased incorporation of146 substrate counts into protein, apparently due to the increased delivery of ATP from mitochondria to microsomes.

Creatine phosphate can stimulate microsomal protein synthesis more effectively than ATP. Creatine liberation, which occurs during exercise, causes increased flux of creatine phosphate to the microsome. Exercise is therefore, equivalent to insulin stimulation of protein synthesis, both causing increased delivery of phosphate energy to the microsome.

Inborn Errors

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Glutamine-valine index. An aid in the detection of urea-cycle disorders

Glutamine, a non-toxic carrier of ammonia is an important aminoacid to measure in conditions of hyperammonaemia. Symptomatic patients with deficiencies in enzymes of the ureacycle regularly show increased plasma levels of glutamine where this is accurately measured. Plasma glutamine tends to remain elevated even when blood ammonia level has been reduced to normal by a low protein intake.

Measurement of ammonia, aminoacids, and urinary orotic acid following a protein load is the best available method for the detection of suspected carriers of ornithine transcarbamylase (OCT) deficiency, and the mild forms of carbamoyl phosphate synthetase (CPS) deficiency.

The changes in plasma glutamine following a protein load have not been reliable in the detection of asymptomatic patients with OCT deficiency due to the wide range in reference values. Discrimination was improved by relating the change in glutamine concentration to the corresponding change in valine following protein load. The resulting "index" corrects to a large extent for variations in amount of protein and rates of absorption, correlates well with orotic acid excretion and is able to differentiate obligate carriers of OCT deficiency from normals.

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Development of methodology by reverse-phase high performance liquid chromatography applicable to the differential diagnosis of the aminoacidopathies

The ability to separate und quantitate amino acids in physiological fluids is essential for the differential diagnosis of the aminoacidopathies. Recent studies have demonstrated that the derivatizing agent o-phthaldialdehyde (OPA) when mixed with 2-mercaptoethanol (MCE) in alkaline buffer is particularly suitable for automatic amino acid analysis by reverse-phase HPLC.

The purpose of the present study was (i) to develop and validate HPLC methodology for the accurate and precise determination of the amino acids as OPA/MCE derivatives in physiological fluids; and (ii) to explore the feasibility of the method in the screening of the aminoacidopathies.

Two different elution systems (I and II) were used. Systems I and II were applied respectively to the analysis of plasma and urine samples on the same column: Novapack C_{18} 5 µM, 15 × 3.9 mm. The chromatographic separation has been achieved by a stepwise elution gradient wich was longer for system II. Automatic derivatization was performed using a Waters Wisp 710 sampler and fluorimetric detection (λex = 340 nm; λem = 425 nm) was used.

The resolution achieved was excellent: 25 amino acids in 37 min and 35 amino acids in 44 min for systems I and II, respectively. The overall precision of the procedure was evaluated. Results from consecutive injections indiacted that the average relative standard deviation ($\bar{s}$ %) of the retention times was ± 0.865% (in the standard) and 0.882% (in the plasma sample) and that of the measured peak heights was ± 6.55% (in the standard). When the results were normalized to an internal standard (4-aminobutyric acid) the $\bar{s}$ % was calculated to be ± 4.24%. The $\bar{s}$ % of the calculated concentration in plasma samples was ± 5.03%. Response was linear in the concentration range of 25–500 pmol (0.992 R 1.00). Limitations of this procedure include secondary amines can not be derivatized and some sulfur amino acids exhibit very low fluorescence. Applications of the chromatography system to the differential diagnosis of aminoacidopathies have been used with success in our laboratory and these data will be presented.

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Differential diagnosis of (inherited) amino acid metabolism or transport disorders

Disorders of amino acid metabolism or transport are most clearly expressed in urine. Nevertheless the interpretation of abnormalities in urinary amino acid excretion remains difficult. An increase or decrease of almost every amino acid in urine can be due to various etiology. To differentiate between primary and secondary aminoacidopathies systematic laboratory investigation is necessary.

Early diagnosis of disorders of amino acid metabolism or transport is very important, because most of them can treated, leading to the prevention of (further) clinical abnormalities. In those disorders, which cannot be treated, early diagnosis in an index-patient may prevent the birth of other silblings by means of genetic counseling and prenatal diagnosis.

Primary aminoacidopathies can be due to genetically determined transport disorders and enzyme deficiencies in amino acid metabolism or degradation. Secondary aminoacidopathies are the result of abnormal or deficient nutrition, intestinal dysfunction, organ pathology or other metabolic diseases like organic acidurias.

A survey of amino acid metabolism or transport abnormalities will be given, illustrated with metabolic pathways and a number of characteristic abnormal amino acid chromatograms in urine.

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A new generation of low-concentration conjugates in propionic acidaemia

It is well known that benzoic acid in the human is converted to the glycine conjugate, hippuric acid and excreted

as such in the urine. Like benzoyl-CoA, there are a number of CoA-derivatives in Propionic acidaemia that accumulate and seem to be metabolically acitve intermediates. The CoA-derivative is either hydrolysed and excreted as the free acid or further metabolised to a number of other organic acids and acid-conjugates. In Propionic acidaemia, it is suspected that a small proportion of the propionic acid is conjugated to glycine by the action of glycine-N-acylase (EC 2.3.2.13), to result in propionylglycine. We have decided to investigate the possibility that other amino acids may also conjugate to some of the accumulating CoA-derivatives in propionic acidaemia. Up till now we have detected a number of previously unknown conjugates associated with this pathological condition. First in line was propionylalanine, a conjugate that we have detected in the urine of a patient with Propionic acidaemia. This specific compound was detected in urine samples that were collected at a time when the patient received small doses of L-alanine as part of the treatment programme. Next in line was propionylglutamic acid, a conjugate that occur in much lower concentrations than the alanine analog, but still important from the biochemical point of view. Finally, we have identified N-acetylaspartic acid and propionylaspartic acid in another case affected by Propionic acidaemia. The identity of all the conjugates were confirmed by synthesis of the authentic compound, gaschromatography, mass spectrometry, infrared spectroscopy and ^{1}H, ^{13}C Nuclear Magnetic Resonance.

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Amino acid and organic acid profiles in the Fanconi syndrome associated with different inborn errors of metabolism

The renal Fanconi syndrome is characterized by a generalized renal tubular dysfunction leading to impaired net proximal reabsorption of amino acids, glucose, phosphate and other molecules and therefore increased urinary excretion of all these solutes.

This syndrome can be either idiopathic, associated with various inborn errors of metabolism or acquired from several toxic agents.

We studied amino acid and organic acid profiles in: Idiopathic Fanconi (2 patients); Cystinosis (4 patients); Tyrosinemia type I (6 patients); Galactosemia (3 patients) and Succinate: cytocrome C reductase deficiency (1 patient).

Quantitative analyses of amino acids in plasma and urine were performed by automatic amino acid analyzer and the more recent samples by reverse phase HPLC as phenylisothiocianate derivatives. Urine organic acids were analyzed as their TMS-derivatives by GC-MS.

In idiopathic Fanconi and cystinosis, a severe generalized nonspecific hyperaminoaciduria occurs. There is over ten fold the normal amino acid excretion (Val; Gln; Arg; Orn; Lys; Thr and Pro are the more increased). Amino acid and organic acid profiles do not differentiate both diseases.

In Tyrosinemia type I there is a specific generalized hyperaminoaciduria, being methionine and tyrosine among those amino acid more increased, although the presence of δ-aminolevulinate and succinylacetone are the specific indicators of this disease.

The pattern of urine amino acids in succinate: citocrome C reductase deficiency and in galactose-1-P uridyltransferase deficiency patients is similar to that of tyrosinemia type I. However, the high level of lactate in the first case and the absence of δ-aminolevulinate and succinylacetone in both diseases establish the differential diagnosis.

It could be concluded that, combined analyses of amino and organic acids are useful in establishing the differential diagnosis of inherited disorders associated with the Fanconi syndrome.

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One year experience with HPCL amino acid analysis in biologic fluids of patients with inborn errors of intermediary metabolism

HPCL amino acid studies have been performed in biologic samples of 276 children suspected of having an inborn error of intermediary metabolism during one year period. In 9 further children, who were already diagnosed, amino acids were quantified owing to a crisis or as treatment control. Organic acid and enzymatic studies were performed to achieve or complete the diagnosis.

Amino acids were analysed as phenylisothiocyanate derivatives by reverse phase HPCL, organic acids as trimethylsilyl derivatives by GC-MS, and enzymatic studies were performed by radiometric and spectrophotometric techniques.

62 patients showed abnormal amino acid profiles:

1) 17 aminoacidopathies: 3 urea cycle enzyme deficiences (1 CPS, 1 OCT, 1 citrullinemia), 3 defects in aromatic amino acid pathway (2 PKU from the neonatal screening, 1 tyrosinemia type I), and 11 defects in sulphur amino acid pathway or transport (3 homocystinurias, 1 cystinosis and 7 cystinurialysinurias);

2) 4 organic acidurias from the branched chain amino acid pathways (1 MSUD, 2 PCC and 1 MMA).

3) 5 deficiencies of the energy metabolism (2 PDH, 1 SCR, 1 COX and 1 glycogenosis)

4) 27 patients with secondary abnormal profiles.

5) 9 diagnosed patients for regular control studies (1 argininsuccinic aciduria, 2 homocystinurias, 1 tyrosinemia type I, 2 lactic acidemias, 1 MSUD, 1 PKU and 1 cystinurialysinuria).

We conclude that amino acid quantification is indispensible for the diagnosis of aminoacidopathies, and is an interesting attention call for that of organic acidurias and defects of energy metabolism. Moreover, it is always necessary for treatment control. HPLC is a good method for amino acid quantification in plasma, CSF, leucocytes and some special determinations which require high sensitivity, like palsma protein-bound homocysteine in heterozigous subjects. Urine analysis involve some interpretation problems which can be overcome by pre-treatment of the urine samples with Set-Pack columns.

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Indian childhood cirrhosis as a possible defect of cysteine catabolism: identification of the stored material as copper sulfide

Indian childhood cirrhosis (ICC) is a copper storage disorder characterized by rapidly progressive liver disease, normal serum ceruloplasmin, and death in infancy or early childhood. Hepatocytes of affected patients accumulate electron-dense aggregates known to contain copper and sulfur. We investigated a 29-month old American boy who satisfied all the clinical, biochemical, and histologic criteria for ICC. Subcellular fractionation of a piece of the patient's open liver biopsy was achieved by differential centrifugation. Electron microscopy revealed prominent electron-dense granules in the heavy fractions (250 × **g**, 600 × **g**, and 10,000 × **g**), consistent with the presence of copper in these fractions. Strong acid treatment of the 250 **g** fraction released abundant hydrogen sulfide (315 nmol/690 mg liver wet weight), quantitated using an alkaline zinc acetate trap. (Some sulfide was also present in the 600 × **g** fraction). The identification of the copper-sulfide in the cellular aggregates suggests that ICC may represent a disorder of sulfur metabolism rather than of copper metabolism. In particular, the formation of sulfate from cysteine may be impaired, with accumulation of volatile hydrogen sulfide which is trapped in vivo by binding to copper. Moreover, electron microscopic studies revealing fibrillar whorls and crystalloids in ICC fibroblasts indicate that these cultured cells can be used to investigate further the basic metabolic defect in this genetic disorder.

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Changes of phenylalanine tolerance and some other important metabolic markers in pregnancies of mothers suffering from hyperphenylalaninemias

Pathologically increased phenylalanine (Phe) in maternal blood has a detrimental effect on the early development of the foetus. It causes the growth retardation, skeletal, cardiac or brain malformation. In most cases microcephaly and mental retardation were found.

Thanks to the systematically organized facultative screening for hyperphenylalaninaemia among healthy pregnant women during last 15 years the prematrimonial genetic prevention as well as the introducing of lowphenylalanine dietetic treatment and strong metabolic monitoring of all detected patients have been performed.

In 14 pregnancies from 8 hyperphenylalaninaemic patients following metabolic parameters have been followed: Phe and Tyr in maternal blodd, biopterin and neopterin in maternal urine, Phe-tolerance according to Güttler et al. in natural food, nutritional markers (prealbumin, albumin, transferrin, haemoglobin), vitamins, trace elements, Phe, Tyr and organic acids in maternal milk.

12 children born after dietetic treatment introduced before conception and during the whole pregnancy have the psychomotric development within normal limits.

The increased Phe-tolerance in the second half of pregnancy oscillated from 20–200%. Phe levels in umbilical blood didn't correlate with maternal blood as well as with amniotic fluid values at the beginning of the labor.

Neurochemistry

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Hippocampal synaptic plasticity induced by excitatory amino acids

The hippocampus is widely used in investigations of different forms of synaptic plasticity, including long-term potentiation and kindling. Receptors for excitatory amino acids (EAAs) play a prominent role in these phenomena. Recently, it has been demonstrated that exposure of hippocampal slices to EAAs and related agonists produces biphasic effects on excitatory synaptic transmission: initial blockade of synaptic responses is followed by a delayed recovery. These phenomena occur only in young animals. The recovered responses demonstrate altered pharmacological properties: they acquire sensitivity to N-methyl-D-aspartate (NMDA) antagonists during L-glutamate exposure and lose sensitivity to both NMDA and non-NMDA antagonists under L-aspartate. These changes persist for many hours. It was suggested that this form of hippocampal plasticity may involve transitions between distinct states of synaptic functioning. It was found that the synaptic transmission recorded under EAAs loses sensitivity to ω-conotoxin and adenosine. In pyramidal neurones it was found that N-type voltage-sensitive Ca^{2+} channels are effectively blocked by NMDA antagonists and that adenoise protects the neurons from this block. It is concluded that EAA-induced synaptic plasticity includes changes in N-type Ca^{2+} channel functioning.

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Interaction between excitatory and amino acid receptors and Ca channels in the neuronal membrane

The modulation of voltage-dependent calcium channels by various neurotransmitters has been demonstrated in may neurones. Because of the critical role of Ca^{2+} in transmitter release and, more generally, in transmembrane signaling, this modulation has important functional implications. Hippocampal neurones are known to possess low threshold (T-type) Ca^{2+} channels and both L- and N-type high voltage-activated Ca^{2+} channels. Recently ω-conotoxin and adenosine have been shown to block N-type Ca^{2+} channels selectively. Both of these substances block excitatory synaptic transmission in the hippocampus, while dihydropyridines, which selectively block L-type channels, are ineffective. Excitatory synaptic transmission in the hippocampus displays a number of plasticity phenomena that are initiated by Ca^{2+} entry via ionic channels operated by N-methyl-D-aspartate (NMDA) receptors. Here we report that NMDA receptor angonists selectively and effectively depress the N-type Ca^{2+} channels. The inhibitory effect is eliminated by the competitive NMDA antagonist D-2-amino-5-phosphonovalerate, does not require Ca^{2+} entry into the cell and is most probably receptor-mediated. This phenomenon may provide a negative feed-back between the liberation of excitatory transmitter and entry of Ca^{2+} into the cell. It may be of importance both in presynaptic inhibition and in the regulation of synaptic plasticity.

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Activation and desensitization of NMDA receptors in hippocampal neurons

Ionic currents evoked by rapid application of N-methyl-D-aspartate (NMDA) agonists with glycine (Gly) were studied in isolated voltage-clamped hippocampal neurons (whole-cell mode). Applications of saturating Gly (10 μM) on the background of Asp in different concentrations elicited responses with peak amplitudes smaller than those due to the simultaneous applications of Asp and Gly. This suggests that the fraction of Asp-bound NMDA receptors loses the ability to be activated by Gly. This steady-state desensitization was not accompanied by a change in the receptor affinity for Asp (K_d = 51 μM). The steady-state desensitization was prevented by competitive Gly antagonist kynurenate, but not by Mg^{2+} ions. This desensitization may effect the Gly site of NMDA receptors.Steady-state desensitization of NMDA receptors under a concerted action of Asp and Gly was studied using preincubation in : (a) various concentrations of Asp and sturating Gly (10 μM), and (b) various concentration of Gly and saturating Asp (5 mM). In the both procedures preincubation was followed by the applications of Asp and Gly in saturating concentrations, evoking test responses. The K_d values for the dependences of test responses on concentration of co-agonist chosen for preincubation were 8.7 μM for Asp (a) and 120 nM for Gly (b), significantly lower than those obtained from the dose-response experiments, with the difference more pronounced for Asp. Thus, when both co-agonists are applied, a distinct steady-state desensitization of NMDA receptor is observed, which is accompanied by an apparent increase in affinity of receptors for both coagonists.

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Highly potent selective N-methyl-D-aspartate and non-selective glutamate antagonisits from the venom of spider

A group of toxins extracted from the venom of spider was studied on acutely isolated perfused hippocampal neurons of rat by means of the concentration clamp technique. The toxins A and NPS187 appeared to be non-selective antagonists of excitatory amino acid receptors, while B, NPS020 and NPS022 proved to be selective N-methyl-D-aspartate (NMDA) antagonists. When applied in concentrations 5–500 μM, the non-selective antagonists blocked the ionic currents elicited by rapid applications of kainate, demonstrating profound use- and voltage-dependence of action at the non-NMDA receptor. The other toxins in concentrations below 10 μM left the responses to KA unchanged. However, all the toxins in concentrations between 0.1 nM and 1 μM blocked the responses to applications of NMDA or L-aspartate. The action of B, NPS022 and NPS187 was clearly use-dependent, while the others toxins were active after the preincubation in toxin alone. The action of all substances except for NPS020 on NMDA receptors was voltage-dependent, being less pronounced with an icrease in holding potential from −100 to −30 mV. The action of all antagonists except for NPS020 could be completely or partially reversed only after the washout in the presence of NMDA agonist, more effective at positive membrane potentials form 0 to +20 mV. The results imply that spider toxins B and NPS022 are most potent and selective NMDA antagonists which preferentially interact with open NMDA-activated ionic channels. The molecules of toxins may be "trapped" by the NMDA receptor-channel complex when the ionic channel opens, like the molecules of phencyclidine or MK-801.

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A novel selective NMDA agonist, N-phthalamoyl-L-glutamic acid (PhGA)

Ionic currents elicited by N-phthalamoyl-L-glutamic acid (PhGA) were investigated on freshly isolated hippocampal neurons by means of whole-cell voltage clamp and using the concentration clamp technique. PhGA elicited desensitizing

inward currents in Mg^{2+}-free salines only in the presence of glycine (Gly). The dose-response relationship for PhGA was close to a Langmuir isotherm with K_d = 3.7 mM and saturating level 0.75 of that of L-aspartate (L-Asp). PhGA-activated currents were blocked by Mg^{2+} ions, competitive, N-methyl-D-aspartate (NMDA) antagonist D-2-amino-phosphonovalerate and competitive Gly antagonist kynurenate. Their reversal potential was identical to that for L-Asp-activated currents, suggesting involvement of the same channels. Complete cross-desensitization was obtained between the receptor responses to PhGA and L-Asp. Thus, PhGA is a selective NMDA agonist, though the affinity of NMDA receptors for PhGA and maximal effect produced by this agonist are lower than those for Asp and NMDA. The PhGA molecule contains three carboxylic and no amino group, in contrast to other glutamate agonists, possessing a basic group. We conclude that PhGA is a new selective "suberacidic" agonist of the NMDA type of glutamate receptors. The action of PhGA as NMDA agonist puts new compounds interacting with the NMDA receptor which can contribute to the search for pharmacological agents acting on the CNS.

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NMDA receptors are probably involved in pressure-induced decrease in long-term potentiation of hippocampal cells responses

When man and animals are exposed to high pressure (80 atm), they manifest a disturbances of neurologic functions like tremor, myoclonia, convulsions and EEG changes refered to as the high pressure nervous syndrome (SNHP). On the other hand, divers showed performance decrements and deficiency in mnesic tasks. Studies of basic mechanisms of learning and memory under pressure are necessary to understand the changes observed in divers memory. The limbic system is the nervous structure principally involved in the memory processes. This structure includes the hippocampus adopted these later years for a study of long-term potentiation (LTP) which is known to be the neuronal substrate of learning and memory. We have studied the effects of high pressure on LTP in GA 1 cells of the rat hippocampal slices. These cells are activated by glutamatergic afferents fibers; their activity is regulated by NMDA receptors excitation and GABA receptors inhibition. We recorded simultaneously the field excitatory postsynaptic potential (EPSP) at the dendritic layer and the population spike (PS) at the somatic layer of CA 1 cells after stimulation of the commissural/Schaffer afferents. Under 1 atm, the tetanization (2 sec duration) of afferent fibers potentiates EPSP (100%) and PS (300%). Then, CA 1 cells intrinsic excitability was increased without potentiation in NMDA depolarizing effect on these cells. Under 80 atm, no potentiation of EPSP and PS was observed. Moreover, on the contrary of tetanus-induced effects, the pressure-induced hyperexcitability of CA 1 cells is accompanied by hyperactivity in NMDA mechanisms and a hypoactivity in the synaptic activity. The LTP of potentials seems to be facilitated by a modest depolarization of cells. Then, we suggest that high pressure induced a high depolarizing level in CA 1 cells which is principally induced by an exageration in NMDA excitatory mechanisms. This potentiation in NMDA activity under pressure could have an antagonist effect on the tetanus-induced LTP. Alternatively, pressure-induced decrease in synaptic response could be also involved in the blockade of LTP in CA 1 cells. We conclude that NMDA receptors are the principal molecules influenced by the allosteric change induced by high pressure in cell membrane. More studies are needed to determine a therapeutic treatment for HPNS which occur in divers. (grant IFREMER).

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Neuroexcitatory aminoacids: analogs and kainic acid

The excitatory aminoacids (EAA) L-glutamate (L-Glu) and L-Aspartate (L-Asp) appear to be the major excitatory neurotransmitters in the vertebrate central nervous system.

Selective electrophysiological responses to agonists have been used to define the four main receptors termed:

- N-methyl, D-Aspartate (NMDA).
- Kainate (KA)
- Quisqualate)QA)
- L-2 Amino 4-phophonobutanoic acid (L-AP 4)

There is a great pharmacological and also therapeutic interest in specific agonists and antagonists for subtypes of EAA receptors.

We will report our work concerning the efficient synthesis of analogs (subtituted on α-carbon and phosphonic analogs) of kainic acid.

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Differences in GABA-ergic action on neocortical and hippocampal seizure development observed from bicuculline-induced effects

To investigate how GABA-ergic function affects seizure development, effects of a GABA antagonist, bicuculline, on neocortical and hippocampal kindling were examined. Chronic experiments were performed on 15 adult male rabbits 2 weeks after operation. These rabbits were divided into three kindling groups of 5 rabbits each: visual cortical, motor cortical and hippocampal kindling groups. Kindling-inducing stimulations consisted of stimulus trains repeated at 5-min interstimulus intervals to produce so-called "rapid kindling". The stimulus parameters consisted of monopolar square pulses of 1–5 msec duration, 60 Hz, 1–5 mA and 1 sec in total duration. The changes in after-discharge (AD) durations induced by each of 15 trial of stimulus trains per session were compared before and 30 min after i.p. injection of bicuculline solution (2 mg/kg) in each kindling group. In the visual and motor cortical kindling groups, the ADs were more often misfired or shorter in duration after bicuculline injection than before the injection. The bicuculline-induced misfire or shortening of the ADs was more marked in the visual cortical

kindling group than in the motor cortical kindling group. In contrast, the hippocampal kindling group showed marked bicuculline-induced AD development, with the ADs being less frequently misfired and more markedly prolonged in duration after bicuculline injection than before the injection. These results suggest that GABA-ergic function plays different roles in neocortical and hippocampal seizure development, exhibiting inhibitory and facilitative actions, respectively.

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Effect of aspartame on L-[^{3}H]-glutamate binding to rat brain synaptic membranes

Recently consumption of aspartame (L-aspartyl-L-phenylalanine methyl ester), a low-calorie sweetener, has been reported to be responsible for neurological and behavioral disturbances in sensitive individuals. There is controversy whether prolonged exposure of aspartame can elicit neurotoxicity via activation excitatory amino acid receptors by the primary interaction with aspartame.

Using ligand-receptor binding methods, we found that aspartame inhibited L-[^{3}H]-glutamate binding to its N-methyl-D-aspartate (NMDA)-sensitive synaptic membrane receptors in concentration dependent manner. L-Aspartate, a major metabolite of aspartame, inhibited the binding more stronger than aspartame, while the other metabolites, L-phenylalanine and methanol had no effect at the same concentration. Aspartame caused a significant change in the affinities of the binding without altering V_{max} values of the binding, suggesting the inhibition is competitive. These results obtained suggest that aspartame may act as an agonist of L-glutamate on the NMDA-sensitive glutamate binding sites and the primary interaction may play a central role in mediating the potentiation of hippocampal excitability previously reported by Fountain et al. However, these in vitro findings, of course, cannot be directly regarded as convincing evidence for the neurological adverse effects of aspartame as reported so for, since aspartame is rapidly hydrolysed in the gut. Based on the foregoing, there is need for additional study on the safety of aspartame as a food additive.

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Synthetic antagonists of mammalian and invertebrate glutamate receptors: monoacylated spermines

Monoacylated spermines have been used at micromolar concentrations to demonstrate antagonism of L-glutamate receptors in cortical wedge preparations from rats. These acylated polyamines were initally screened using hte metathoracic retractor unguis nerve-muscle of the female locust *Schistocerca gregaria*. Antagonism of the quisqualate-sensitive glutamate receptors of this preparation was inferred from the depression of the neurally-evoked twitsch contraction and the tractile response to L-glutamate of the retractor unguis muscle. Experiments were performed on the rat cortical wedge preparation described by Harrison and Simmonds [1]. Control responses to NMDA and AMPA (2.5 and 5.0 µm) were obtained in Krebs bicarbonate buffer (magnesium-free), gassed with 95% oxygen/5% carbon dioxide and containing tetrodotoxin (100 nM) to suppress epileptiform discharges. Tissues were then incubated with philanthotoxin-343 (PhTX-343) (10 µM) for 20 min, washed and exposed to repeated additions of NMDA and AMPA. Unlike in the control experiments, the amino acid responses progressively declined with successive applications of agonist to 40% and 20% of control levels, respectively, by the third application.

A less marked antagonism was obtained using N-(4-hydroxyphenylpropanoyl)-spermine at 1, 10, and 100 µM. These concentrations of toxin caused significant antagonism of the AMPA response (respectively: 23%, 13%, and 19% at the third application of AMPA). However, the synthetic analogue N-(4-hydroxyphenylacetyl)-spermine was virtually inactive over a similar concentration range against AMPA. Neither of the toxin analogues, at low concentration range against AMPA. Neither of the toxin analogues, at low concentrations (1 and 10 µM), had any effect on responses to NMDA, but significant antagonism of the NMDA response was obtained in the presence of 100 µM of these toxins; the responses were reduced by 55% and 92% respectively with the phenylpropanyol and phenylacetyl analogues. After washout of the toxin analogues, no recovery was seen with two subsequent additions of NMDA. The quinoxaline DNQX (1 µM) reversibly reduced AMPA responses by 51%.

Antagonism of AMPA-sensitive glutamate receptors by synthetic PhTX-343 and by N-(4-hydroxyphenylpropanoyl)-spermine appears to be use-dependent. Antagonism of the NMDA response may be due to an action at the "polyamine site" on the glutamate receptor complex [2], it is unclear whether both PhTX-343 and spermine bind at the same site and the functional interactions with the receptors remain to be elucidated.

1. Harrison NL, Simmonds MA (1985) Br J Pharmacol 84: 381–391
2. Carter C, Rivy J-P, Scatton B (1989) Eur J Pharmacol 164: 611–612

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Synthetic approaches to argiotoxins-622 and 636: potent glutamate receptor antagonists from the venoms of *argiope* and *araneus*

Argiotoxin-636 (ArgTX-636) [1] is an important member of a new class of polyamine-derived neurotoxins found in spider and wasp venoms. These compounds are antagonists of quisqualate sensitive L-glutamate receptors (quis-GluR) which mediate the neurotransmitter action of excitatory

amino acids in arthropods [2]. ArgTX-636 contains the unusually substituted aromatic chromophore, 2,4-dihydroxyphenylacetic acid [3]. We report approaches to the synthesis of ArgTX-636, a potent, non-competitive antagonist of the well characterized quis-GluR found on female locust (*Schistocerca gregaria*) muscle.

The preferred route was convergent: the chromophore-asparagine and polyamine-arginine fragments were synthesized separately. 2,4-Dihydroxyphenylacetic acid was prepared by the Kindler modification of the Willgerodt reaction on the corresponding acetophenone. The acid was shown to be very air-sensitive in solution (in water and in common organic solvents), but stable under an atmosphere of anhydrous nitrogen. The phenols were conveniently protected as the corresponding O-benzyl ethers.

The protected acid may be coupled to Asn *via* its N-hydroxysuccinimide ester in a mixed organic-aqueous solvent system. The activation of acylated Asn may be accomplished, without recourse to protecting groups, by using the p-nitrophenyl ester, formed at $< 0\,^{\circ}C$. This procedure avoids any dehydration of the amide functional group of the corresponding nitrile. It was envisaged, in the proposed route, that the 533 polyamine could be selectively acylated with a protected Arg residue prior to acylation with the activated chromophore-Asn described above. ArgTX-636 provides new leads for insecticides, pharmaceuticals, and selective pharmacological tools.

We thank the UK SERC for a studentship (to A.J.M.), the British Technology Group for generous financial support, and Dr. T.W. Smith for his interest in our work.

1. Adams ME, Carney RL, Enderlin FW Fu ET, Jarema MA, Li JP, Miller CA, Schooley DA, Shapiro MJ, Venema VJ (1987) Biochem Biophys Res Commun 148: 678–683
2. Jackson H, Usherwood PNR (1988) Trends Neurosci 11: 278–282
3. Blagbrough IS, Bycroft BW, Mather AJ, Usherwood PNR (1989) J Pharm Pharmacol [Suppl] 41: 96 P

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Structure activity studies in a series of monoacylated spermines and guanidines. Reversible antagonists of glutamate receptors

Spider venom toxins have recently been identified as potent, reversible, non-competitive antagonists of quisqualate-sensitive L-glutamate receptors (QUIS-GluR). Our synthetic analogues which are less polar then the L-arginine-containing spider toxins, and should, therefore, traverse lipid membrane barriers [1], were shown to be active antagonists of QUIS-GluR on the female locus [2] *Schistocerca gregaria* retractor unguis nerve-muscle preparation.

In a typical procedure for the synthesis of a monoacylated spermine, a solution of n-heptanoic acid (200 mg), DCC (375 mg), and NHS (200 mg) in dichloromethane (5 ml) was stirred at 25 °C for 2 h. The urea precipitate was removed by filtration and spermine (466 mg, 1.5 equiv.) was added in one portion. After a further 4 h, the solution was concentrated and the residue was then purified by column chromatography over silica gel (eluant 4 : 4 : 1 CH_2Cl_2 : MeOH : NH_4OH) to yield a pale yellow oil (120 mg, 25%). Chromatography, over silica gel, of these polar compounds was satisfactorily accomplished using 4 : 2 : 1 CH_2Cl_2 : MeOH : NH_4OH (R_F typically 0.2).

N-(4-Hydroxyphenylacetyl)-spermine was guanadinylated using 2-methyl-2-thiopseudourea. The corresponding 4-hydroxyphenylpropanoyl analogue was converted into the acyclic and also into the cyclic guanidines (with 2-methylthio-2-imidazoline) (ethanol, 79 °C, 18 h). The potency of these analogues as glutamate receptor antagonists was essentially equivalent to that obtained with the analogues which terminated in a primary amine. An aromatic residue was present in those analogues which were more potent.

Patterns are beginning to emerge with respect to the relative importance of the lipophilic or chromophoric residue, the terminal primary amino or guanidino functionality, the length of the polyamine chain, and the number of positive charges at physiological pH in determining potency of the acylated toxins. Our results have demonstrated that an aromatic residue, though not always hydroxylated, is present in the more active analogues.

We thank the British Technology Group for generous financial support, and Dr. T. W. Smith for his interest in our work.

1. Seymour PA, Mena EE (1989) Soc Neurosci Abs 15: 463.24
2. Blagbrough IS, Bruce M, Bycroft BW, Mather AJ, Usherwood PNR (1990) Pestic Sci 30: 397–403

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Structure-activity relationships in excitatory amino acid receptor antagonism

Acylated polyamine toxins, obtained from spider venoms, have been identified as potent, but reversible non-competitive antagonists of the quisqualate-sensitive L-glutamate receptor (quis-GluR) of locust skeletal muscle. Structure-activity relationship studies using the female locust (*Schistocerca gregaria*) retractor unguis nerve-muscle preparation have demonstrated that the aromatic residue is not necessarily, 2,4-dihydroxyphenylacetyl, but may be a hydroxylated benzoic or phenylpropanoic acid derivative. These natural products have been isolated from serveral spider species, including: *Argiope, Araneus, Agelenopsis, Hebestatis, Nephila,* and the tarantula species *Aphnonopelma* and *Harpactirella* [1].

The highly basic polyamine residue, frequently spermine or an analogous polyamine, will be essentially fully protonated at physiological pH. Also, at this pH, the aromatic functional group will be a phenol not a phenoxide. Guanadinoxy moieties, as found in canacanine and other insect repellants and also found in compounds which are toxic to invertebrates and in active mammalian hypoglycaemic agents, may possess advantages over terminal guanidino or amino functional groups; further work is needed to confirm this result. An aromatic residue, not necessarily hydroxylated, is present in the more active analogues of the spider toxins. The polyamine requires at least three basic centres for activity at sub-millimolar concentrations. Novel polyamines have re-

cently been isolated from certain organisms and from vertebrates.

A pattern is emerging with respect to the relative positions of the phenolic residue and the terminal primary amine, the length of the polyamine chain and the pK_a's of these groups. These and additional data are being employed to design and develop synthetic antagonists which may contain an aromatic binding site and are of lower overall polarity than the natural venoms. These compounds have potential as selective pharmaceutical agents and pesticides. Furthermore, this selectivity has been demonstrated by the in vivo nematocidal activity of Argiotoxin-636 which did not compete with glutamate for binding to the mammalian glutamate receptor [2].

The author acknowledges generous financial support from the British Technology Group, and thanks Professor B. W. Bycroft, Professor P. N. R. Usherwood, and Dr. T. W. Smith for their continuing interest in these studies.

1. Skinner WS, et al (1990) Toxicon 28: 541–546
2. Schaeffer JM, et al (1989) Pesticide Biochem Physiol 35: 26–32

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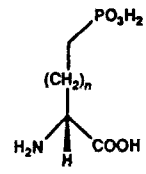
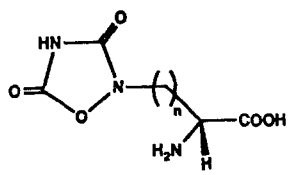
Quisqualic acid analogues as potential NMDA antagonists

Despite the existence of several receptors responsible for glutamateric neurotransmission, the NMDA receptor remains the most clearly understood. The investigation of its characteristics and physiological properties has been facilitated in the main by the availability of specific antagonists for this receptor. Classical NMDA antagonists e.g. AP 5 (1, n = 2) and AP 7 (1, n = 4) are structurally similar in that they are diacidic amino acids of the D-configuration with a 4 or 6 carbon chain separating the two acidic functional groups. The potential therapeutic value of this class of compounds has been demonstrated by their anticonvulsant activity [1] and their neuroprotective action in models of ischaemia [2].



(1)



(2)

Following our continuing investigations into the pharmacological activity of synthetic quisqualic acid analogues, D-homo (2, n = 2) and D-bishomoquisqualic acid (2, n = 3) were synthesized as potential NMDA antagonists. These compounds exhibit all the structural requirements necessary for NMDA antagonism.

These novel D-amino acids were tested at one concentration in guinea pig cortex. The assay measures the reversal of NMDA inhibition of carbachol stimulated accumulation of ^{3}H-labelled inositol phosphates [3]. D-Homoquisqualic acid was found to be inactive, but D-bishomoquisqualic acid exhibited a statistically significant level of NMDA antagonism. Structural refinement of this promising compound may lead to a new series of potentially useful NMDA antagonists.

We thank the UK SERC for a studentship (to N. H.).

1. Dingledine R, McBain CJ, McNamara JO (1990) Trends Pharmacol Sci 11: 334–348
2. Lodge D, Collingridge GL (1990) Trends Pharmacol Sci 11: 22–24
3. Godfrey PP, Wilkins CJ, Tyler W, Watson SP (1988) Br J Pharmacol 95: 131–138

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Excitatory amino acid neurotoxicity: a model of its involvment in Alzheimer disease

Greenamyre [1] outlines the evidence that excitatory amino acids (EAA) are involved in the neuropathogenesis of Alzheimer Disease. The ability of EAA to produce excitotoxic lesions forms the basis of this postulation. Excitotoxicity is induced from overstimulation of post-synaptic glutamate receptors. However, Cowburn [2] submits that, in common with other neurotransmitter systems, glutamatergic neurones are lost in Alzheimer Disease. This loss is suggested by a reduction in presynaptic glutamate uptake site density and a decreased glutamate uptake rate. This remains the only assessment of glutamatergic neuropathology in Alzheimer Disease because EAA immunocytochemistry can only be performed on perfusion-fixed tissue [3].

We propose that the observed loss of uptake sites and the reduced uptake rate, does not portray neuronal loss. Rather, it leads to an icrease in extracellular glutamate, creating excitotoxic conditions.

Employing the serotonergic neurotransmitter system, we have mimicked the data that Cowburn used to postulate denervation, without destroying synaptic endings. Fenfluramine (a 5-HT neurotoxin) or phenoxybenzamine (a non-competitive 5-HT uptake site antagonist) were administered i.p. to rats. A parallel decrease in both [^{14}C]5-HT uptake rate (Vm) (without change in affinity (Km)), and [^{3}H]paroxetine binding to 5-HT uptake sites (B_{max}) was found in rat brain synaptosomes prepared from either experimental group when compared with control.

Uptake time courses also revealed a parallel reduction in equilibrium [^{14}C]5-HT content (E_{max}) in both experimental groups. However the functional significance of this remains unclear. Expressing E_{max}/B_{max} revealed a difference between experimental groups, and may be useful in deliniating glutamatergic pathology in post-mortem samples of Alzheimer brain tissue.

1. Greenamyre JZ, Young AB (1989) Neurobiology Aging 10: 593–602
2. Cowburn RF, Hardy JA, Roberts PJ (1990) Biochem Soc Transact 18: 390–392
3. Ottersen OP, Storm-Mathisen J, Bramham C, Torp R, Laake J, Gundersen V (1990) Progress Brain Res 83: 99–114

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Pharmacological characterization of excitatory amino acid receptors on cultured rat cerebellar granule cells

The pharmacological properties of glutamate sensitive receptors on cultured cerebellar granule cells were studied using the whole-cell patch clamp technique. Granule cell-enriched cultures were obtained from cerebella of P 7–8 Wistar rats. Cells were grown in serum containing media in the presence of factors known to promote cell survival and maturation (see [1]). Superfusion with glutamate (Glu, 50 μM) or its analogues kainate (KA, 50 μM), quisqualate (QA, 10 μM), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA, 10 μM) and N-methyl-D-aspartate (NMDA, 140 μM) induced inward currents. The NMDA-induced current was very small and was greatly enhanced when the cells were simultaneously exposed to glycine (Gly, 10 μM). In addition, Gly augmented the response to Glu.

KA-, QA- and AMPA-induced responses were completely blocked by 6,7-dichloroquinoxaline-2,3-dione (DNQX, 25 μM) but remained almost unaffected by DL-2-amino-5-phosphonovaleriate (APV, 100 μM) and 7-chlorokynurenate (7-CK, 10 μM). Responses induced by NMDA/Gly were blocked by APV and substantially reduced by 7-CK but were not altered by strychnine (10 μM). In addition Mg^{2+} blocked the response to NMDA/Gly in a voltage dependent way. Responses to Glu were completely blocked by DNQX but remained unaffected by APV. All antagonists reduced the inward current evoked by Glu/Gly.

This study shows that cultured cerebellar granule cells contain "conventional" types NMDA and non-NMDA receptors. In addition, it is demonstrated that Glu apparently activated non-NMDA receptors and that the presence of Gly is required for the simultaneous activation of NMDA receptors.

1. Balázs R, Hack N (1990) Adv Exp Med Biol 268: 221

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Spermine blocks Ca^{++}-activated K^+-channels in GH 3 pituitary tumor cells

Polyamines such as putrescine, spermidine and spermine are polyvalent cations which are ubiquitously present within cells. A remarkable increase of the polyamine concentration is found during cell replication, differentiation or normal and malignant growth where their intracellular concentration can rise to millimolar amounts. In nerve cells and other excitable cells polyamines are involved in the regulation of synaptic activity, regeneration and survival of cells. Electrical stimulation or electroconvulsive shock induced epilepsy has been shown to cause an increase of internal polyamines. Only a few studies have investigated the effects of polyamines on membrane ion transport and single ion channel activity [1, 2].

In this study we demonstrate the effects of spermine on Ca^{++}-activated and K^+-channels from rat pituitary tumor cells (GH 3). Applied to inside-out patches spermine was found to reduce the single channel amplitude and open probability of channels, whereas the closing probability increased in a dose dependent manner. The effects were reversible on removal of spermine. The results suggest that spermine acts as a blocker at Ca^{++}-activated K^+-channels. Reduction of the single channel amplitude indicates that spermine acts as a fact blocker direct on the channel. The reduction of the open-probability further indicates that spermine may act at a second site where it competes with calcium at the calciumsensor of the channel.

1. Drouin H, Hermann A (1990) In: Water and ions in biomolecular systems. Birkhäuser, pp 213–220
2. Weiger T, Hermann A (1991) Pflügers Arch 418: R 6

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Does ATP control the activation of NMDA-gated cationic channel?

Binding of MK-801, the potent noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor was investigated in rat brain membranes exposed to ATP.

According to recent evidence, (+)[^{3}H]-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (^{3}H-MK-801) can be used as a marker for the open state of the NMDA-associated cationic channel [Yoneda et al. (1989) Brain Res 499: 305–314].

Binding site is maximally accessible in channels fully activated by 100 μM L-glutamate and 30 μM glycine. The activation kinetic of the cationic channel can be modulated by several sites on the receptor complex where ligands can bind [Dingeldine et al. (1988) CRC Crit Rev Neurobiol 4: 1–96].

Recently it was demonstrated that guanine nucleotides modulate the activation of the NMDA-receptor complex in brain membranes treated with Triton [Yondea et al. (1990) Neurosci Res 9: 114–125]. GTP competively inhibits the binding of ^{3}H-glutamate and ^{3}H-MK-801 whereas ATP seems to be without effect.

In the present study it was shown that ATP inhibits ^{3}H-MK-801 binding in a concentration dependant manner (IC_{50} = 0.57 mM). ADP and a,β-methylene-ATP are less potent than ATP (IC_{50} = 1.1 mM, respectively IC_{30} = 0.50 mM).

ATP potently inhibits the activation by L-glutamate and glycine of the ^{3}H-MK-801 binding to brain membranes. Inhibition by 1 mM ATP was more pronounced in membranes preincubated (for 10 min) with ATP than in membranes previously incubated with one or other of the amino acids.

The present results suggest that ATP may control the activation of the NMDA-gated cationic channel, probably by competing the glutamate activation.

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Platelet levels of aspartate, glutamate, glutamine and gamma-aminobutyric acid in patients with stroke and normal subjects

Abnormal high levels of glutamate/aspartate are supposed to be involved in cell damage following a cerebral

	group	n	m/f	mean age + SD (years)	time (weeks)	*ASP*	*GLT*	*GLN*	*GABA*
stroke	A	11	9/ 2	65.1 + 7.2	4– 8	1.34 + 0.34	4.68 + 1.41	1.53 + 0.44**	0.12 + 0.07**
	B	23	15/ 8	56.8 + 17.7	9–22	1.94 + 0.84	5.38 + 1.79	1.90 + 0.69*	0.13 + 0.07**
	A + B	34	24/10	59.5 + 15.5	4–22	1.74 + 0.77	5.14 + 1.68	1.78 + 0.64**	0.16 + 0.05**
contr		24	16/ 8	57.8 + 13.3		1.88 + 0.72	5.38 + 1.42	2.34 + 0.75	0.29 + 0.14

m male; *f* female
statistics: Kolmogorov-Smirnow test, nonparametric two sample test; stroke versus controls
* $p < 0.05$; ** $p < 0.001$

ischaemic stroke event. Platelets are involved in stroke and they contain neurotransmitters like aspartate (ASP), glutamate (GLT) glutamine (GLN) and gamma-aminobutyric acid (GABA). Therefore we have investigated the content of the excitatory amino acids GLT and ASP and the GLT metabolites GLN and GABA in platelets from patients with ischaemic stroke (n = 34) and compared the results with those of age and sex matched healthy controls (n = 24). The group of patients was subdivided into three groups depending on the time between the insult and the time point of biochemical investigation (A: 4–8, B: 9–22, A + B: 4–22 weeks). All patients were under medication. Platelet separation and thrombolyzation were performed according to Mangano et al. 1982. For determination of the amino acids HPLC-method after OPT-derivatisation of the amino acids by Lenda et al. 1980 was used. The results are shown in the table below (platelet content calculated to nmole amino acid /10^8 blatelets + SD).

It was found that the platelet GLN and GABA levels in all stroke groups were significantly reduced as compared to the control group. Furthermore the values of platelet ASP and platelet GLT were markedly decreased in group A but the difference was statistically not significant. The decreased platelet amino acid concentrations in stroke can be related to the time interval between the ischaemic insult and the time point of biochemical investigation because the values in A were lower than in B. Taken together it may be assumed that the courses of amino acids in platelets represent a marker of the modified cerebral amino acids following brain damage and heal up after the stroke event. On the other hand platelet amino acids may be an alternative source of neurotransmitters necessary in neuronal cell damage and/or reconstruction.

1. Lenda K, Svenneby G (1980) Rapid high-performance liquid chromatographic determination of amino acids in synaptosomal extracts. J Chromatogr 198: 516–519
2. Mangano RM, Schwarcz R (1982) Huntingtons disease; glutamate and aspartate metabolism in blood platelets. J Neurol Sci 53: 489–500

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Comparison of long-lasting alterations of amino acids in whole brain areas and synaptosomes following a single or repeated audiogenic seizures

Genetically determined audiogenic seizures in 2 sublines of Rb mice with various convulsive patterns (Rb 1 clonic and tonic, Rb 2 clonic seizures), and the availability of a Rb 3 subline which do not display convulsions provide a tool to explore: 1) the long-lasting alterations of amino acid neurotransmitters (AAN) in whole brain areas and synaptosomes following a single or repeated seizures (twice a day during 15 days), 2) the effect of an acoustic stimulation which does not induce seizures in Rb 3. The levels of aspartate (Asp), glutamate (Glu), taurine (Tau), GABA, glycine (Gly), as well as some of their precursors, glutamine (Gln), serine (Ser), were determined in olfactory bulbs (OB), amygdala (A), hippocampus (Hi), inferior colliculus (IC), cerebellum (C) and pons-medulla (P), in whole brain areas (WBA) and in the corresponding synaptosomal fractions (SF), 16–18 hours after the last seizure, according J. Neurochem., 45, 879–889, 1985 and J. Chromatog. 341, 11–21, 1985. Statistical analysis was performed by two-way ANOVA. A singel audiogenic seizure induces scarce long-lasting changes in AAN in WBA and SF. Following repeated seizures, alterations of AAN levels were more frequent in SF than in the corresponding WBA; similar changes were recorded only in P, for Asp (Rb 2: −20%), and Ser (Rb 1: −30%). Opposite changes of AAN contents in SF compared to WBA, were observed in Rb 1, in P for Glu (WBA: −30%, SF: +25%), in Hi for GABA (WBA: −20%, SF: +20%) and in OB for Ser (WBA: +30%, SF: −40%). The data recorded suggest the existence of several pools, only one synaptosomal being directly involved in neurotransmission. The reported observations will be discussed.

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Quinolinic acid: its neuropharmacology and developmental involvement in degeneration of hippocampal neurons

Quinolinic acid (QUIN), a glutamate agonist acting at NMDA receptor, caused a neuronal loss 48 h after intracerebroventricular injection (2 μmoles) followed by a massive bilateral neurodegeneration in the hippocampal formation of 30-day-old rats. In contrast, QUIN was almost ineffective in 12-day-old animals. Ketamine (50 mg/kg i.p.), but not kynurenic acid (KYNA, 6 μmoles i.c.v.), caused almost complete protection against QUIN neurotoxicity. In primary hippocampal cultures of 3-5-day-old rats QUIN (5 or 10 mM) led to neuronal degeneration which was prominent after 24 h of continous exposure. Lower doses than 1 mM did not alter

the neuronal morphology until 7–10 days of QUIN exposure. Glutamate antagonists acting at the NMDA receptor site (D-APV; CPP), at glycine modulatory site (KYNA; 7-Cl-KYNA) and/or at the PCP channel site (MK-801) exhibited partial or complete protection against QUIN toxicity. Moreover, at 5 mM QUIN, a 10 min stimulation of K^+-depolarized hippocampal neurons increased the release of GABA and taurine more than 5-times while 50 mM KCl alone the release of both amino acids was less than 2-fold in comparison to controls (2 mM KCl). The results suggest that the QUIN-induced release of GABA may be responsible for the depletion of the inhibitory neurotransmitter in QUIN-lesioned brain, and that swelling accompanied by an increase in the release of taurine may be early component of QUIN neurotoxicity.

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Hypothermia during transient cerebral ischaemia protects the highly susceptible somatostatin containing neurons in the rat dentate hilus from free zinc accumulation and cell necrosis

We have previously shown that somatostatin containing neurons in the rat dentate hilus are highly suceptible to ischaemic brain damage. Within 48 hours after ischaemia they demonstrate fast ischaemic cell death and simultaneously we have shown that necrotic cells in dentate hilus accumulate free zinc in their cytosol. We have now demonstrated directly that the somatostatin containing cells in dentate hilus also accumulate zinc during maturation of fast ischaemic cell death. Since it is known that hypothermia ameliorates ischaemic brain damage, we furthermore studied whether hypothermia (29 ° Celsius) protected the highly susceptible somatostation containing neurons in dentate hilus from ischaemic cell death and zinc accumulation. It was demonstrated that hypothermia during transient cerebral ischaemia prevented cell necrosis and neuronal zinc accumulation in dentate hilus. Hypothermia possibly prevents excitotoxic glutamate release and concomitantly translocation of zinc from the glutamatergic mossy fiber terminals to the vulnerable somatostatin containing cells during ischaemia. We find it less likely that zinc is released from intracellular binding sites during ischaemic cell death, because neurons located outside the mossy fiber layer do not show zinc accumulation during necrosis.

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Methylmercury uptake and efflux from rat primary astrocyte cultures

To better delineate the relationship between astrocytes and methylmercury (MeHg) neurotoxicity, MeHg uptake and efflux were studied in neonatal rat brain primary astrocyte cultures. Uptake of [^{203}Hg]-MeHg exhibited the kinetic criteria of a specific transport system when added to the media as the L-cysteine conjugate. Saturation kinetics, substrate specificity, and trans-stimulation were demonstrated in the presence of L-cysteine. Cysteine-mediated uptake of MeHg was inhibited by L-methionine, and 2-aminobicyclo-[2, 2, 1]-heptane-2-carboxylic acid (BCH). 2-methyl-aminoisobutyric acid (MeAIB) was ineffective in inhibiting the uptake of MeHg-cysteine conjugates. Astrocytic loading with L-glutamate was moderately effective in trans-stimulating the uptake of MeHg-cysteine conjugates, while in the absence of L-cysteine, uptake of [^{203}Hg]-MeHg in L-glutamate loaded cells was unchanged. Net efflux of MeHg from astrocytes was most rapid during the first 5 min. The percentage of [^{203}Hg]-MeHgCl retained was independent of the loading period (30 min to 4 h), but the total amount of intracellular ^{203}Hg that was available for efflux gradually decreased as the loading period increased. MeHg efflux was unchanged when astrocytes were incubated i hypotonic buffer, suggesting that swollen astrocytes remain impervious to MeHg and/or its adducts. Trans-stimulation of [^{203}Hg]-MeHg efflux was detected when astrocytes were incubated with "cold" MeHg and graded L-cysteine concentrations. Thus, MeHg influx and efflux are apparently specific for the MeHg-L-cysteine conjugate and represent transport by the neutral amino acid System L.

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Induction of indoleamine-2,3-dioxygenase activity and increased quinolinic acid in brain following transient ischaemia in the gerbil

Previously we have reported that delayed increases in the levels of the NMDA receptor agonist, quinolinic acid (QUIN), occur in brain following transient ischaemia in the gerbil [J Cerebr Blood Flow Metabol 10: 660–667 (1990)]. These changes are marked particularly in regions which showed the most extensive brain damage (striatum and hippocampus). In the present study, the activity of indoleamine-2,3-dioxygenase (IDO), the first and rate limiting enzyme of kynurenine pathway from L-tryptophan to QUIN was measured during the post ischaemic phase in gerbils. Marked increased regional brain IDO activities occured after a delay of 2 or more days, but not in early post ischaemic periods. At 4 days following a 10 minute bilateral carotid artery occlusion, IDO was increased in striatum, hippocampus, cerebral cortex and thalamus, 563%, 1628%, 361%, and 443%, respectively, as compared to sham operated animals, but not in cerebellum. Parallel increases in regional brain QUIN content were also observed. Four days after a 5 min occlusion, IDO activities and QUIN concentrations were increased 545% and 442%, respectively, only in the hippocampus as compared to sham operated animals. These increases in brain IDO activity provide a mechanism to accelerate formation of QUIN within the central nervous system. Because of the

association between activation of IDO with cytokines such as interferon-γ and tumor necrosis factor, we hypothesize that the increased kynurenine pathway metabolism following ischaemia reflects immune stimulation within the brain, perhaps within macrophage infiltrates, secondary to brain damage. The role of increased QUIN and other neuroactive kynurenines in the post-ischaemic period to brain damage and recovery remains to be determined.

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Modulation by glycine on vascular and behavioural effects of NMDA: in vivo experiments

Our previous investigations have shown the EAA action on the cardiovascular system. Both physiological and pathologic actions have been seen. NMDA and KA receptors play a primary role in the cardiovascular effects exerted by EAA.

Our aim has been to evaluate if glycine, an allosteric modulator of NMDA receptor, is involved in the vascular and behavioural effects induced by the activation of CNS NMDA receptors.

Research has been carried out on anaesthetized (urethane 1 g/kg ip) and freely-moving male Sprague Dawley rats (220–280 g).

Icv NMDA (0.01 to 1 mcg/rat in the 3rd ventricle) determined a significant and dose-dependent increase in arterial blood pressure in freely-moving and anaesthetized rats.

In freely-moving rats the hypertension was more intense and associated with behavioural modifications (jumping, rearing, teething, dyspnoea, exophthalmus).

Glycine pretreatments (1–10 mcg/rat ivc), after about 10 minutes of latency, significantly and in dose-dependent manner increased NMDA-hypertension (from anout 50 to 180%) and blocked the NMDA behavioural effects. Alone glycine did not cause any arterial blood pressure modifications while it induced a slight sedation.

HA-966 (an antagonist of the glycine site on NMDA receptor) administration (1–10 mcg/rat icv 5 min before glycine) significantly and in a dose-dependent manner antagonized glycine potentiation of NMDA-hypertension (from 30 to 100%; n = 5). Alone HA-966 neither modified arterial blood pressure and behaviour, nor antagonized NMDA hypertension. In conclusion, our investigations confirm NMDA receptors involvement in cardiovascular function and they demonstrate that in vivo glycine positively modulates NMDA receptor.

In vivo modifications of glycine on NMDA vascular and behavioural effects (potentation of hypertension and inhibition of behavioural effects) suggest that glycine may have different action sites which modulate the vascular and behavioural effects of NMDA.

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Cysteic acid receptors on rat hippocampal CA 1 neurons

Cysteic acid is an endogenous substance which is known to both activate excitatory amino acid receptors and, in excess, to cause excitotoxic damage. We have investigated the distribution and pharmacologic sensitivity of cysteic responses on pyramidal neurons in the CA 1 region of rat hippocampal brain slices. Slices (450 μm thickness) were cut perpendicular to the long axis of the hippocampus of a 200–250 gm rat, and were submerged in the recording chamber. Intra- or extracellular recordins were made of responses to ionophoretic application of agonists (10 mM in 150 mM NaCl) and bath application of antagonists. Responses to ionophoresis of cysteic acid were obtained from application to both the apical and basal dendritic trees, although the basal dendrites were consistently more responsive. The sensitivity to cysteic acid was considerably less than that to quisqualat in the apical dendritic trees, but only slightly less in the basal dendrites. However cysteic acid was about as potent as NMDA on both dendritic trees. In order to determine the receptor type(s) activated by cysteic acid we applied CNQX, which blocks the quiqualate/kainate receptors, and APV, a specific antagonists for the NMDA receptor. CNQX (2×10^{-5} M), which essentially totally blocked the response to quisqualate, reduced the cysteic acid response by about 50%. APV (5×10^{-5} M), which depressed NMDA responses to about 25% of control, also depressed cysteic acid responses to about 50% of control. These results are most consistent with the conclusion that cysteic acid is an endogenous compound which can activate both NMDA and non-NMDA receptors.

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Alimentary and environmentally-related excitatory amino acids may reduce neurotoxicity through a synergic mechanism

Several kinds of food contain high amounts of common excitatory amino acids (EAAs), such as aspartate and glutamate. Occasionally, rare EAAs may be present, as it occurred with domoic acid (DA) in canadian cultured mussels. We determined the EAA content of cultured mussels and used rat cerebellar neurons in primary culture as an experimental system where to characterize the effects of DA when associated to other EAAs present in food. 50% neurotoxicity by DA-containing canadian toxic mussel extract (TE) occurred at a lower concentration of DA within the extract (~ 1 μM), as compared to purified DA (~ 7.5 μM). A control mussel extract (CE) was significantly less neurotoxic than TE ($Tox._{50\%}$ ~ 30 μM vs ~ 8.5 μM of total EAA content). DA neurotoxicity was antagonized by NON-NMDA excitatory amino acid receptor antagonists, such as CNQX. However, full protection from TE neurotoxicity was achieved by using a combination of NMDA and NON-NMDA receptor antagonists such as APV + CNQX or MK-801 + CNQX, while neurotoxicity by CE was fully antagonized by NMDA receptor antagonists used alone. TE neurotoxicity curve was reproduced by adding subtoxic concentration of DA to CE. The synergistic interaction between subtoxic concentrations of DA and other EAAs acting at the NMDA receptor was confirmed by using purified EAAs and a synergism factor (SF) was defined. We suggest that SF may have been relevant in the occurrence of the neurological problems reported in

humans who ingested "toxic mussels". In this respect, the presence of D-EAAs in mussel tissue and their possible neurotoxicological relevance in the Canadian episode of intoxication will be discussed.

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Neurosecretory and trophic action on fetal neuroplast induced by an amino acid mixture

The effects of a synthetically obtained mixture of amino acids (FACE) were investigated on the trophic and neurosecretory activity of in vitro cultures of fetal rat neuronal cells. The addition of 10 M FACE to the culture medium significantly increased cell DNA content. Secretions of IR-SRIF, IR-VIP, and IR-GRF were also augmented in different proportions by the presence of FACE. Time studies demonstrated that IR-SRIF was significantly increased after 48 ($P < 0.05$) and 72 ($P < 0.01$) hr of exposure to FACE, and IR-VIP secretion was potentiated after only 24 hr of culture. Dose-response experiments with 10 to 10 M FACE indicated that concentrations of 10 and 10 M significantly increased both somatostatin released to the medium and cell content of IR-SRIF. FACE concentrations as low as 10 M augmented the secretion of IR-GRF, and there was a dose-response correlation between 10 and 10 M FACE. The release and cell content of IR-VIP were also increased by FACE, with a dose-response relation at concentrations of 10 to 10 M. It can thus be concluded that FACE has a powerful effect on the multiplication and survival of fetal cerebrocortical cells and is also an important potentiator of IR-SRIF, IR-VIP, and IR-GRF secretion.

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Is putrescine mediating neurotoxic effects of kainic acid?

Recent literature [Williams et al. (1991) Life Sci 48: 169–198] shows that the polyamines putrescine (PUT), spermine and spermidine are involved in the amino-acidergic neurotransmission through the NMDA receptor. Also, results from this group [Martinez et al. (1991) Life Sci 48: 77–84; de Vera et al. (1991) J Neurochem (in press)] show that the cell damage induced by kainic acid (KA) after convulsions, runs in parallel with increased PUT brain levels. This association is seen even 9 days after the KA treatment. In addition, the treatment with DFMO (an irreversible inhibitor of ornithine decarboxylase, the polyamine-synthesizing rate-limiting enzyme) does not prevent the cell damage although it partially decreases the PUT content in the brain areas examined. To study if PUT is a mediator of the brain damage caused by KA we have examined the actions of this polyamine on behavioural, biochemical and histological variables. PUT was administered systemically (200 mg/kg, i.p.) and the behaviour of animals observed. To facilitate the crossing of PUT throughout the blood-brain-barrier (BBB) rats were pretreated with glycerol. Treated rats exhibited motor incoordination, flat body posture and lack of sensitivity. Polyamines were analyzed in brain areas using HPCL with fluorimetric detection. Brain PUT increased up to 300% over saline- or glycerol-treated animals. In agreement with Genedani and coworkers [Pharmacol Toxicol (1987) 61: 224–227], the histological examination of the brains revealed the presence of oedema. These preliminary results suggest that PUT may mediate in part the histochemical damage observed after treatment with KA. Also, it supports a possible role for PUT in the regulation of the BBB [Koenig et al. (1989) Brain Res 483: 110–116].

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Mechanism of the antiepileptic action of the association GABA-phosphatidylserine in the rat

The i.p. administration of relatively high amounts of GABA to rats (740 mg/kg) does not result in any significant antiepileptic effect in two different models of experimental epilepsy.

On the contrary, when the amino acid was administered i.p., together with an equal amount of phosphatidylserine (PS), an antiepileptic effect was found within 30 minutes from the administration of the two substances.

Studies about the modality of GABA passage to the brain after i.p. injection have shown that the passage from the blood to the brain, at the blood concentrations involved in our model, is mainly a diffusion process, with

V passage = k · [GABA] blood.

The simultaneous administration of PS appears to favour a rapid passage to the blood of the amino acid from the peritoneum. Thus [GABA] blood raises more quickly with a consequent proportional increase of the rate of accumulation of the amino acid into the brain. In turn, the greater amount of exogenous neurotransmitter arriving to the brain results in an increased (+ 40%) in vivo uptake into the nerve endings.

This increase appears to be critical in determining the antiepileptic effect of GABA-PS treatment in comparison with the administration of plain GABA.

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Presence of $GABA_A$ receptors on the cytoplasmic side of a GABA-acceptive neurone

A micromethod allowing the study of the permeability characteristics of single membranes from the rabbit Deiters neurone has been applied to be evaluation of the effect of GABA on $^{36}Cl^-$ permeation.

The results show that, as expected, GABA on the extracellular side of the membrane can increase the passage of $^{36}Cl^-$ in the out → in direction, an effect which is blocked by the $GABA_A$ antagonists bicuculline and picrotoxin.

However, GABA can interfere with ^{36}Cl permeability even when it is only on the membrane cytoplasmic side. In particular, when the neurotransmitter was on that membrane

side, at the steady state intracellular concentration of 3.3 mM (Okada and Shimada, 1976), renders $^{36}Cl^-$ in → out permeability around the double of the one in the opposite direction. This effect could be reversed by the $GABA_A$ antagonists.

The dose-response curve for the effect of $GABA_{36}$ on the membrane cytoplasmic side on the permeation of $^{36}Cl^-$ in → out reveals a peak at 10^{-6} M with a marked phenomenon of desensitization at (GABA) $\geq 10^{-5}$ M.

Thus, it seems that the Deiters' membrane possesses $GABA_A$ receptors with their GABA recognition sites in its cytoplasmic side.

An additional property of such receptors is that they activate Cl^- channels exposing positive charges at their cytoplasmic mouth.

On the basis of their characteristics, we propose that these $GABA_A$ receptors are devices for pumping Cl^- ions outside the Deiters' cell, building up and maintaining an electrochemical gradient for the anion.

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Phenylalanine: neutral amino acid ratios in tardive dyskinesia

"Large neutral" amino acids (phenylalanine, tyrosine, tryptophan, valine, leucine, isoleucine) compete for a common transport system across the blood-brain-barrier. Therefore the plasma concentration of any of these amino acids in relation to the concentrations of the other determines its uptake into brain. It has been proposed that a relatively high phenylalanine concentration may decrease the availability of the dopamine precursor, tyrosine [1].

Tardive dyskinesia (TD) is a movement disorder typically appearing after the withdrawal of neuroleptic drugs. Its origin may be an acquired hypersensitivity of brain dopamine receptors. PKU and plasma phenylalanine to neutral amino acid ratios in non-PKU patients have been described as risk factors for TD [2]. We tested the latter hypothesis in schizophrenic patients 28 of whom exhibited TD and 22 age-matched controls who did not. Plasma phenylalanine concentrations and phenylalanine to neutral amino acid ratios were 59.5 μmol/L and 0.123 respectively in TD patients and 60.5 μmol/L and 0.118 in controls. Neither of these differences were significant and no significant differences were obtained when similar comparisons were made for tyrosine and tryptophan.

We conclude that the plasma phenylalanine to neutral amino acid ratio is not a risk factor for TD and that its implication in earlier studies may have been an age-related effect.

1. Fernstrom JD, Wurtman RJ (1972) Brain serotonin content physiological regulation by plasma neutral amino acids. Science 178: 414–416
2. Richardson MA et al. (1989) The plasma phenylalanine/large neutral amino acid ratio: a risk factor for tardive dyskinesia. Psychopharmacol Bull 25: 47–51

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Serum amino acid neurotransmitter precursor profiles and dopamine in schizophrenic patients and healthy subjects

Altered turnover of dopamine has been postulated as underlying cause for schizophrenia. This is partially inferred from pharmacological studies and from changes in serum dopamine and dopamine metabolite levels. The question arises whether serum amino acid precursor availability regulates the dopamine turnover in healthy subjects and schizophrenic patient.

We analyzed basal serum amino acids (including central monoamine precursors), and central monoamines in schizophrenic patients (after a drug holiday of 3 or more days, n = 87; neuroleptic-treated n = 23), and in healthy subjects (n = 90).

Asparagine, citrulline, phenylalanine, and cystine were higher and tyrosine, tryptophan, and the ratio of tryptophan to competing amino acids lower in drug-free schizophrenic patients than in healthy subjects (P 0.05). Dopamine was increased in drug-free schizophrenic patients compared to healthy subjects.

We speculate that these results sustain the notion for dopamine overactivity in schizophrenia, which might be caused by altered amino acid precursor availability.

Free Radicals

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Free radical scavenger activity of dehydroalanine derivatives

The concept of radicophily, as proposed by Viehe et al. [1, 2], claims that capto-dative substitution of the geminal C-atom of an olefin produces efficient radical traps. The adduct formed by the reaction of a free radical with such a capto-dative olefin is a stabilized free radical which dimerizes or reacts with an other free radical to terminate the radical reaction chain.

The present communication summarize the overall effects of N-substituted dehydroalanine derivatives (the so called AD compounds) on oxygen radicals mediated processes. Therefore, we will discuss both (a) the in vitro scavenging activity of AD compounds towards superoxide anion and hydroxyl radicals, and (b) the in vivo inhibitory effects of AD compounds in two experimental models where toxicity has been principally mediated by free radicals: redox cycling drugs and ionizing radiation.

1. Viehe HG, Janousek L, Mérenyi R, Stella L (1985) The capto-dative effect. Acc Chem Res 18: 148–154
2. Roberfroid M, Viehe HG, Remacle J (1987) Free radicals in drug research. In: Testa B (ed) Advances in drug research, vol 16. Academic Press, London, pp 1–84

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A model of oxidative stress across cell membrane

The binding of metals, their chemical reduction and the detoxification of free radicals derived species in cellular systems is routinely reported to involve cellular glutathione directly. However, this mechanism does not take into account the extreme reactivity, low concentration and non-permeability of many of these species. A model is presented which allows intracellular glutathione to act as a reducing agent in reactions with extacellular species with the overall redox chemistry being mediated by the transmembrane proteins of the cell. Examples from studies on certain diseases and therapy will be presented to indicate that this model best explains the changes observed in terms of a mechanism involving cross membrane electron transfer.

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Intact cells as strong oxidising agents: estimation of their redox potential

Peripheral white blood cells (monocytes and polymorphonuclear white cells) are found to be capable of oxidising a range of species including Disulphides and iron II complexes. Their redox potential has been defined within a range and compared to other well understood oxidants such as hydrogen peroxide and permanganate and periodated anions. This enables the likely oxidative activity of particular cell types to be predicted on the basis of their in vitro chemistry.

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Glutamate/GABA ratio v.s. lipid peroxidation in lumbar cerebrospinal fluid of patients with an acute ischaemic stroke

Contents of glutamate, aspartate, glycine, and GABA were determined in the lumbar cerebrospinal fluid (CSF) of 43 patients with an acute ischaemic stroke during the first 48 hours of the impact, using HPCL with an electrochemical detection. In the same patients, tiobarbituric acid-reactive material (TBARM) representing lipid peroxides was measured in the CSF and serum and subsequently correlated with the glutamate/GABA ratio. Both glutamate and GABA contents in the CSF were several times increased during the 48 hours of the ischaemic stroke compared to the levels obtained in the control group of patients subjected to the diagnostic lumbar radiculography. Nevertheless, relative increase in the glutamate content prevailed (rise in the glutamate/GABA ratio), meaning predominance of the excitatory effects of glutamate against inhibitory of GABA. Neuroexcitatory amino acids are thought to induce calcium accumulation within the neurons during ischaemia and subsequent activation of free radical reactions, leading to lipid peroxidation of neuronal membranes. Indeed, content of TBARM was increased both in the lumbar CSF (1.65 ± 0.30 v.s. 0.56 ± 0.10 nmol/ml in the control group) and serum (5.04 ± 0.32 v.s. 2.92 ± 0.57 nmol/ml in the control group) during the acute period of ischaemic stroke. Also, glutamate/GABA ratio correlated well with the TBARM content in the CSF ($r = 0.601$, $p < 0.05$). These results implicate excitatory amino acids as a significant inductor of free radical generation and lipid peroxidation during the first 48 hours of the cerebral ischaemic stroke.

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Free radical forms of amino acids in relation to the irradiation of foods

Amino acid free radicals have long been of interest in connection with radiotherapy and radiation protection. More recently, renewed interest has arisen in view of the possibility of using radiation to preserve foodstuffs on a commercial scale. Radicals are produced both by oxidation (attack by hydroxyl radicals, or direct one-electron ionisation) and reduction (action of electrons). Rate constants for the formation of amino acid free radicals are strongly dependent on pH. At near-neutral pH, hydroxyl radicals attack all amino acids, with rate constants ranging from $2 \times 10^7 - 10^{10}\,M^{-1}\,S^{-1}$. At similar pH values, hydrated electrons are more selective, but react rapidly with arginine, asparagine, cysteine, cystine, phenylalanine, tryptophan and tyrosine with rate constants of $1.5 \times 10^{-8}\,M^{-}\,S^{-1}$ or more. Many properties and reactions of amino acid radicals are known. They can themselves cause reduction (e.g. the radical formed by OH· attack on glycine) or oxidation (e.g.the radicals formed by loss of an electron from trypthophan). In simplified systems, the radical reactions provide a semi-quantitative explanation of the formation of radiolysis products. Reactions are strongly modified by the presence of a peptide group. In foodstuffs they are still further modified by the presence of other food components.

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Oxygen-derived free radicals: their effects on free amino acids proteins

The effect of oxygen derived free radicals (OFR) on aromatic and sulphur containing amino acids can be investigated, when amino acids exist both in their free form and within protein backbones. three discrete OFR generating systems; (1) gamma radiation in the presence or absence of formate (2) photolysis by UV light as 254 and 360 nm, and

(3) site specific modification by H_2O_2 in the presence of CuII ions can be used to initiate OFR damage to aerated amino acids and proteins in solution. A sensitive reverse-phase HPLC technique with dual detections systems (UV absorbance and fluorescence monitoring) has been developed by our group to analyse the products of amino acid oxidation. OFR denatured amino acids were chromatographed by this procedure, and all radical species generated, with the exception of the superoxide anion, resulted in the formation of identifiable fluorescent metabolites of tryptophan, kynurenines. After complete proteolytic hydrolysis, OFR treated proteins were also analysed by this technique; again the dose dependent production of kynurenines was detected in IgG, lens crystallins and albumin. Bityrosine was not detected in any of the proteins studied using this procedure, however, several novel unidentified fluorophores were detected in proteolytic hyrolysates, possibly the product of two different amino acid radicals. This work demonstrates that the measurement of a specific product of an oxidised amino acid can be applied to biological macromolecules, and that it may be an important technique to use in identifying the consequences of free radical reactions in certain disease processes.

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Oxygen radical damage to amino acids in vivo: relationship to autoimmune pathology

Free radicals are described classically as being produced by homolytic fission of a covalent bond between two atoms, A and B, which form the molecule AB. Gamma radiation, ultraviolet light and environmental pollutants are among the many exogenous initiators of free radical reactions; however, the most important sources of these radical species in vivo are univalent biochemical redox reaction involving oxygen. These reactions play an important role in many physiological processes. Although the products are highly toxic to biological material, the reactions are kept "in check" by a series of enzymic and non-enzymic protective agents. Profound tissue damage or disease may result from the excessive production of oxygen radicals during pathophysiological processes such as inflammation, ischaemia-reperfusion injury, normal metabolism or inadequate protection from the barrage of antioxidants available in vivo. This review will consider the generation of oxygen radicals in vivo; the involvement of these reactive substances in the denaturation of important biomolecules, particularly amino acids and proteins; and the importance of such reactions in the development of chronic autoimmune inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematosus.

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γ-Glutamylcysteinylethyl ester: transport into hepatocytes, conversion to glutathione, and preventive effect on oxidative damage

We have demonstrated that γ-glutamylcysteinylethyl ester (γ-GCE), which can be expected to function as a precursor of glutathione (GSH), prevents the damage of hepatocytes treated with lipid peroxidase (LPO). However, how γ-GCE shows a preventive effect on LPO-induced liver injury is still unclear. We, therefore, examined the transport of γ-GCE into hepatocytes and its intracellular conversion to GSH. In addition, the preventive effect of γ-GCE on oxidative damage in hepatocytes treated with t-butyl hydroperoxidase (t-BuOOH) was examined.

Hepatocytes (4×10^6 cells/ml) isolated from male Wistar rats aged six weeks were treated with 1 mM diethyl maleate (DM) for 10 min. This treatment resulted in an about 90% decrease in hepatic GSH content. When DM-treated hepatocytes were incubated with 5 mM γ-GCE for 30 min, GSH level was returned to about 80% of the level of DM-untreated ones. This effect of γ-GCE was intensified by the addition of glycine in the medium but weakened by the addition of bis(p-nitrophenyl)phosphate, an inhibitor of esterase. In contrast, the addition of GSH (a final conc. of 5 mM) in the medium enhanced GSH level in DM-treated hepatocytes, but this level was about half to that of γ-GCE-treated ones. When DM-treated hepatocytes were incubated with 1 mM t-BuOOH for 30 min after treatment with 5 mM γ-GCE for 30 min, an increase of hepatic LPO level and release of asparatate and alanine transaminases were reduced about half. Such effects were hardly found in the case of GSH.

The present results indicate that γ-GCE is transported into hepatocytes more easily than GSH, resulting in conversion to GSH, and that γ-GCE can prevent oxidative damage in hepatocytes, this effect being much stronger than that in GSH.

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Changes in plasma amino acids during conditioning therapy prior to bone marrow transplantation

Bone marrow transplant (BMT) recipients undergo a bimodal regimen of conditioning therapy, the precise prescription being dependent upon the primary disease of the individual patient. Generally, this treatment consists of chemotherapy and total body irradiation prior to transplantation, although the latter may or may not be included in the regimen. We have investigated amino acid metabolism in this situation.

Plasma amino acids were measured by HPLC on 10 BMT recipients prior to commencement of conditioning therapy, and again one week later before transplantation. There were many changes in the overall plasma amino acid pattern between these times, with highly significant reductions in methionine, cysteine, glutamine, glutamic acid, serine and taurine. Erythrocyte glutathione concentration was also significantly reduced post-conditioning ($p < 0.001$). These elements play an important role in the antioxidant processes.

Some of the observed changes in amino acids are attributable to general metabolic upset caused by the radiation and chemotherapy, but there is also a significant reduction of antioxidant capability, which may influence post-transplant recovery and graft function. There may also be an influence upon the transfusion requirement in terms of erythrocyte and platelet support post-grafting.

Such data adds to the evidence for the conditional essentiality of some amino acids such as taurine and glutamine, and may support the case for specific antioxidant intervention treatment prior to, and/or after conditioning therapy along with a review of antioxidant status during the post-grafting period.

Arginine

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Interaction of arginine and substituted guanidino-compounds with the vascular endothelium

Most of the peptides which release the endothelium dependent relaxating factor (EDRF) possess the amino acid L-arginine in their sequence. However, in some preparations arginine is a poor stereospecific vasodilator which does not require endothelium. In contrast to this several N-substituted arginine compounds such as Na-benzoyl L-arginine ethyl ester (BAEE) are potent vasodilators in the rat aorta, mesentery, isolated kidney, porcine coronary artery and internal mammary artery. Further several substituted guanidino compounds other than L-arginine of BAEE are also potent vasodilators. These guanidino compounds also prevent platelet aggregation. The effects of the guanidino compounds are inhibited by the EDRF inhibitors, methylene blue, haemoglobin, superoxide anion and potentiated by superoxide dismutase. Metabolism studies indicate that these guanidino compounds are converted to the corresponding citrulline derivative via the intermediate formation of a hydroxylamine (R-NHOH). In addition to this, several dipeptides containing arginine are also potent vasodilators when compared to L-arginine. These results suggest that properly substituted arginines may be more authentic substrates for EDRF synthesis than free arginine.

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Studies on the central role of arginase in the amino acid metabolism of macrophages

Currie (1978) explored that macrophages produce and secrete arginase. Hibbs et al. (1987) described that NO, a vasodilatator is synthesized in macrophages using arginine as the source of NO. Arginine being an amino acid found in proteins is also incorporated into proteins.

We showed earlier that murine macrophages produced a higher amount of arginase than rat macrophages and the produced arginase is not synthesized de novo after harvesting the cells during the first 6 hours (1990). The arginase and NO synthesis during long term cultures is now studied. The de novo synthesis of murine arginase was proved and the effect of various agents on the enzyme production was also investigated. The arginase production in rat cells could not be increased by the long term culturing.

Nitric oxide and its derivative, NO_2^- production can be measured only in long term cultures both in murine and rat macrophages. Rat macrophages produced a higher amount of NO_2^- than murine cells. The effect of various agents on nitrite production was also studied.

We studied various inflammatory agents both in mice and rats on the arginase and nitrite production. In vitro utilization of arginine was investigated on mice macrophage (de novo protein synthesis urea formation and NO to production).

The fate of arginine and several other amino acids and the occuring of the enzymes of urea cycle in murine and rat macrophages was investigated by ion exchange thin layer chromatography.

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The role of arginine and arginine peptides in modifying the pH of dental plaque

It has been known for some years that arginine and arginine peptides such as the salivary tetrapeptide sialin (glycine-glycine-arginine-lysine) reduce the sugar-associated drop in the pH of dental plaque and suspensions of oral bacteria. Previous work has shown that both with suspensions of salivary bacteria and with the cariogenic organism *Streptococcus mutants NCTC 10449* arginine is able to reduce the rate of glycolysis. Very little work has been done to investigate the effect of arginine on acid production by other common species of oral bacteria.

In the experiments reported here the effect of arginine on acid production by *Actinomyces viscosus NCTC 10951* is described. The bacteria were grown in supplemented proteose peptone yeast, centrifuged as resuspended in PBS in a range of glucose concentrationis. No evidence for any effect of 10 mM arginine on the rate of production of fermentation products could be detected. This is different from the reduction in lactate production seen when *Streptococcus mutants NCTC 10449* is incubated with glucose solutions plus 10 mM arginine.

It is concluded that the effect of arginine and (very probably) arginine peptides of glycolysis is species specific. It will be necessary to test both arginine and arginine peptides on a wide range of the more than 300 different species of dental plaque bacteria in order to understand the complete role of arginine in moderating the pH of dental plaque.

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Metabolism of guanidino-*N* methylated L-arginines in rats

The enzyme systems involved in the metabolism of guanidino-*N* methylated L-arginines, which are known to occur

in a variety of proteins methylated post-translationaly and to be released in body fluids after in vivo breakdown of these proteins, have been characterized in rats. N^G, N^G- and N^G, N'^G-dimethyl-L-arginine and N^G-monomethyl-L-arginine were catabolized to the corresponding α-keto acids by dimethyl-arginine: pyruvate aminotransferase, which was shown to be identical with the known aminotransferase, alanine: glyoxylate aminotransferse isozyme 2 (EC 2.6.1.44). This pathway is operated exclusively within mitochondria of kidney and liver. Furthermore, a part of N^G,N^G-dimethyl-L-arginine and N^G-monomethyl-L-arginine was degraded via another pathway leading to the formation of L-citrulline and dimethylamine or monomethylamine, in which a unique enzyme, N^G,N^G-dimethylarginine dimethylaminohydrolase widely distributed in rat tissues, was shown to participate. The latter pathway has been discussed in connection with the degradation of N^G-monomethyl-L-arginine, a blocker of the formation of endothelium-derived relaxing factor (EDRF, nitric oxide) by endothelial cells or activated phagocytic cells and also with the possible formation of a precursor of the potent carcinogen, nitrosodimethylamine, in rats in vivo.

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Inhibition of EDRF-synthesis by N^G-nitro-L-arginine (L-NNA) in conscious dogs

EDRF is derived from L-arginine. Blockade of EDRF synthesis by the arginine analogue L-NNA in vivo was shown to increase blood pressure in conscious rodents. The present study evaluates the hypertensive and counterregulatory effects (plasma renin activity (PRA), aldosteron (ADLO)) of a single oral dose of L-NNA 830 mg/kg) over an observation period of 24 h in 5 conscious dogs. Plasma concentration of L-NNA was measured with HPLC.

24 h after the oral administration of L-NNA the plasma concentration still amounts to approximately 80% of its maximum which was reached after 3–6 h. However, the increase in blood pressure returned to 25% of its maximum effect at 24 h. PRA and ALDO were still decreased at this time.

It is concluded, that the L-NNA-induced increase in blood pressure is attenuated by pronounced counterregulatory endocrin effects.

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Free arginine alterations in skeletal musculature and plasma following hindlimb ischaemia in rats

Increased protein breakdown in directly injured muscles has been documented and supposed to occur in the whole musculature during the late posttraumatic period. Thus, in the absence of increased utilization, increased content of free amino acid should be present. On the other hand traumatized organism has increased demands for arginine (arg). In the present work we investigated posttraumatic Arg alteration in musculature (injured and uninjured) and circulation. Examinations have been done during the early (0.5; 1; 3 hours) and late (48; 72 hours) period following 128-minute bilateral.hindlimb ischaemia in 50 male Wistar albino rats.

In directly injured muscle (m. femoralis) content of free Arg was transiently increased only during the early period, while phenylalanine (Phe) was few times greater than in controls throughout the examined period and followed by increased liberation of amino acids from the injured region. In spite of elevated Phe in uninjured muscles (m. biceps brachi) of traumatized organism, Arg contents decreased, reaching half of control values after 3 hours and its low level persisted even during the late period. Both in the injured and uninjured muscles of traumatized organis Arg/Phe molar ratios were decreased comparing to the controls. These results indicate elevated utilization of Arg associated with greater (directly injured) or smaller (uninjured muscles) increase in net protein degradation. At the site of injury processes are balanced keeping free Arg in the control limits, while in the uninjured musculature relative lack of this amino acid exist.

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New role for arginine: immunological activation and suppressive effect on tumor growth

To evaluate the role of arginine on cellular immune responses and tumor growth, Donryu rats were subcutaneously inoculated with Yoshida sarcoma cells and infused with solutions containing various levels of arginine for 8 days. There was no significant difference in the body weight gains between groups during infusion. The suppressed effect of arginine-supplemented solution on tumor growth reached a maximum

Results

	Control	1	3	6	12	24 h
HR (bpm)	126 ± 5.7	108 ± 9.2[a]	106 ± 5.4[a]	109 ± 6.9[a]	111 ± 5.9	108 ± 7.9
$PART_{syst}$ (kPa)	17.3 ± 0.6	20.4 ± 1.3	20.9 ± 0.9[a]	19.4 ± 0.4[a]	18.1 ± 0.7	18.2 ± 0.9
$PART_{DIAST}$ (kPa)	9.2 ± 0.4	12.9 ± 1.2[a]	13.9 ± 0.9[b]	12.8 ± 0.7[b]	11.9 ± 0.7[b]	10.6 ± 0.8[a]
CO/ (l/min)	3.1 ± 0.4	2.1 ± 0.2[a]	2.4 ± 0.3	2.2 ± 0.2[a]	2.1 ± 0.4[a]	2.1 ± 0.3[a]
PRA (ng/ml/h)	2.0 ± 0.8	1.2 ± 0.6[a]	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	1.4 ± 0.5
ALDO (pg/ml)	109 ± 49	80 ± 40.4	17 ± 5.8	7 ± 2.8	12 ± 5.1	50 ± 26.2
L-NNA (μg/ml)	0	34.4 ± 6.8	38.2 ± 8.4	37.1 ± 2.1	35.2 ± 2.0	29.9 ± 1.6

(mean ± SEM; [a] $P < 0.05$; [b] $P < 0.01$)

at 5.5% arginine at an early stage after inoculation in rats, and further increase or decrease in amount of arginine did not result in further inhibition of tumor growth. This arginine-enriched solution enhanced the phagocytic activity of alveolar macrophages. In subsequent in vitro studies, we established that natural killer cell (NK) activity was increased 3 fold after incubation with arginine and arginine induced 1.5 fold increase of macrophage-mediated cytotoxicity. Production of tumor cytotoxic factor from macrophages significantly increased after incubation with arginine for 24 hr. At high concentration of arginine, in vitro growth of tumor cells was evidently suppressed.

These results suggest that arginine action against tumor cells is due to, in part, not only via the enhancement of host immune functions (NK activity and macrophage-mediated cytotoxicity), but also direct effect to tumor cells.

Radiation and Isotopes

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The use of amino acids in Positron Emission Tomography

Positron Emission Tomography allows a regional quantitative measurement of biochemical functions with radioactive tracers in vivo. Although different biochemical fates of a special tracer can often not be distinguished unambiguously, careful selection of the labelling position in a molecule and tracer-kinetic modelling approaches usually allow quite exact determinations of the main biochemical pathways.

So far amino acids have been used mostly as tracers for the determination of amino acid influx special organs and tumors. Despite the fact that ist is incorrect to attribute the unindirectional influx to protein synthesis, this has often been done as a first approximation, at least when using natural amino acids. Currently two approaches have been reported to quantify the metabolic turnover rate. The first method employs a graphical evaluation of dynamic accumulation data, usually referred to as the Gjedde-Patlak plot. The second method uses a kinetic modelling approach. While the former is less accurate in terms of identifying the turn-over rate as protein synthesis, the latter can be used only with few simple amino acids as leucine, labelled with 11 C in the carboxyl position.

Whereas early investigations concentrated on diagnostic procedures for the pancreas, recent investigations have shown that there is a great potential for amino acids to help in various aspects of the diagnosis and treatment of tumors, especially in the brain and the chest. It has been shown that the accumulation in tumors correlates well with the degree of malignancy of tumors. The regional accumulation pattern can serve therapy planning with regard to radiation- and surgical treatment. Furthermore post treatment measurements can identify the degree of metabolic perturbation and/or differentiate recurrencies from radiation necrosis and surgical scars.

In basic research projects it has been shown that special synthetic amino acids which are not incorporated into protein, may serve as tools for identification of individual transport channels.

Besides the field of tumor diagnosis and therapy planning it seems very likely that regional protein synthesis rates may be a useful indicator for a variety of mental disorders. Although special diagnostic questions and applications have not yet been identified, there is evidence that frontal brain activity is related to protein synthesis.

Special amino acid tracers have already reached a dominant importance for the investigation of movement disorders. While the therapeutic value of DOPA for the treatment of Parkinson's disease had become clear since several years, analysis of its way of action and the optimization of treatments as well as a deeper insight in the development became possible only after the introduction of Positron Emission Tomography with 6-[18 F]Fluoro-DOPA.

Recent investigations have demonstrated the role of special amino acids and their derivatives as precursors for regioselective biomolecules. E.g. the use of 6-F, m-(hydroxy) tyrosine as a tracer for the visualization of the presynaptic dopamine pool has overcome problems with the metabolites which perturbate the measurements of the dopamine pool when 6-F-DOPA is used as a tracer itself.

Other interesting research and application areas will be opened when small polypeptides and neuroregulatory peptides labelled with short-lived positron emitting nuclides will become available. First attempts for the synthesis of such compounds have been made already.

It seems to be clear that this kind of application remains an exciting field of research.

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Carbon isotope fractionation during radiation-induced decarboxylation of solid phenylalanine-1-^{13}C

The radiation-induced decarboxylation of solid phenylalanine-1-^{14}C enantiomers was measured for the first time in 1976 [1]. The results exhibited different $^{14}CO_2$ cleavage rates for D- and L-phenylalanine. Similar investigations with D- and L-leucine-1-^{14}C showed comparable results [2]. The reason for these findings has not yet been clarified. To tacke this problem further, the question was raised whether or not a fractionation effect is operating during the radiation-induced decarboxylation. Beyond this question it was of interest to know to what extent this effect depends on the radiation dose. Therefore two mixtures of differently labelled L-phenylalanine-1-^{13}C were produced by thoroughly mixing L-phenylalanine-1-^{13}C (99% enriched) with the corresponding unlabelled compound. The mixtures produced were designed to contain about 1200 and 3000‰ 13 C respectively in the carboxyl group. This resulted in compounds with δ^{13}C values of 101 ± 0.2‰ and 304 ± 0.8‰ respectively. Roughly 3 g of

each mixture was irradiated with a ^{60}Co-source. The dose applied covered the range from 30 to $2.7 * 10^3$ Gy. Since irradiation of amino acids also induces deamination, the CO_2 produced during radiation-induced decarboxylation had to be especially purified in order to measure it with an isotope ratio mass spectrometer. These measurements proved that trapping of NH_3 on a molecular sieve was the best way for obtaining pure CO_2. The results indeed show that radiation induced decarboxylation leads to a fractionation of the carbon isotopes. The higher the dose applied, the smaller the effect found. These results are discussed with respect to the role of isotope effects in chiral selection mechanisms.

1. Merwitz O (1976) Radiat Environ Biophys 13: 63–69
2. Norden B, Liljenzin JO, Tokay RK (1985) J Mol Evol 21: 364–370

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The origin of differences in ^{13}C-content of amino acid enantiomers

In order to investigate the reason for the enantiospecifity found in the radiation-induced decarboxylation of 1-^{14}C labelled solid amino acids (Merwitz 1976, Nordén et al., 1985), similar experiments were planned for 1-^{13}C-labelled analogues. A first step in starting this stable isotope work was to examine the ^{13}C-content of non-labelled amino acid enantiomers. Surprising at first glance, these studies revealed significant differences in the $^{13}C/^{12}C$ ratios of the enantiomers.

Commercially available L-enantiomers of amino acids (serine, alanine, valine, proline, leucine, tyrosine, and tryptophan) usually showed a higher ^{13}C-content than the corresponding D-antipodies; the differences $\Delta_{D\text{-}L} = \delta^{13}C_D - \delta^{13}C_L$ varied from -1.5‰ for tyrosine to -7.6‰ for tryptophan.

In order to elucidate these findings, the non-proteinogenic amino acid DL-diethylalanine (2-amino-3-ethylpentanoic acid) was synthesized via the azlactone route, N-acetylated and submitted to the L-specific cleavage with acylase I from pig's kidney. The residual N-acetyl-D-amino acid was hydrolysed with hydrobromic acid. Small but distinct differences in the δ^{13}C-values found for the crystalline enantiomers vanished after careful purification. The δ^{13}C-values became identical within $\pm$ 0.02‰, the overall error in determination being $\pm$ 0.05‰. Hence, the fairly large $\Delta_{D\text{-}L}$ values quoted for L-enantiomers of natural origin versus the D-antipodes of synthetic origin may be due to the different ^{13}C-contents of the respective sources; the relatively low ^{13}C-content present int he D-amino acids accordingly reflects the apparently low ^{13}C-content of the petrochemicals used in the corresponding chemical synthesis.

For some amino acids, small but positive $\Delta_{D\text{-}L}$ values were found. In this case, the unused D-enantiomer had been recycled by racemization. Thereby, a $^{13}C/^{12}C$ primary isotope effect should have occurred in the rate determining step, i.e., the breakage of the C-H bond at the stereogenic center; the preferential inversion of the 2-^{12}C-isotopomer eventually led to an enrichment of ^{13}C in the residual D-enantiomer. *Vice versa*, a small but negative $\Delta_{D\text{-}L}$ value indicates that the D-enantiomer had been found by inversion of the L-antipode.

Hence, accurate analysis of the $^{13}C/^{12}C$-ratio is a promising method for establishing the chemical purity and possibly the origin of amino acid enantiomers.

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Amino acids and their derivatives as radioprotective agents

Over 40 years ago, the first studies were conducted to investigate the protective abilities of cysteine and cysteamine against a lethal dose of radiation. During the 1950s, the United States Army initiated an extensive program to develop agents to protect soldiers on the battlefield in the event of a nuclear attack. This massive effort succeeded in producing several thiophosphate derivatives of cysteamine with the ability to reduce radiation injury. Subsequently, the interest in these compounds shifted to a study of their use as protective agents during the radiation therapy of cancer. Other thiol-containing amino acids and derivatives have been investigated as radioprotectants; examples are taurine, *N*-acetylcysteine, homocysteine thiolactone, 2-mercaptoproprionyl glycine, ergothioneine (2-thiol-L-histidine betaine), and D-penicillamine (β, β-dimethyl-D-cysteine). Some thiol-containing dipeptide derivatives, such as S-acetyl-*N*-glycyl cysteamine and glutaurine (*gamma*-L-glutamyl taurine), have also been studied as radioprotectants. The endogenous tripeptide, glutathione, has long been considered crucial in detoxifying the reactive species produced from radiation. However, it's role in radioprotection remains ambiguous. These thiol compounds are generally believed to exert their protective effects through a free radical scavenging mechanism, although additional mechanisms have proposed as well.

Some very interesting non-thiol-containing amino acids are also being investigated for their potential use as radioprotective agents. *N*-Acyl dehydroalanines have shown free radical scavenging activity and appear to protect against X-ray-induced toxicity by this mechanism. Glutamine has been shown to possess protective qualities and apparently exerts its effects by accelerating the healing process. Recent work with organic zinc salts, especially zinc aspartate, has demonstrated their ability to spare the bone marrow from radiation-induced damage. Histidine and two histidine-containing dipeptides, carnosine (β-alanyl-L-histidine) and anserine (methyl carnosine), showed excellent radioprotective capacity in a bacteriophage inactivation system.

A historical review of the development of these amino acids and amino acid derivatives as protective agents against radiation-induced toxicity will be presented.

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^{3}H and ^{1}H high resolution NMR study of multicomponent isotopic mixtures of tritium-labelled glycine, alanine, tryptophan, 4-hydroxyproline and its derivates

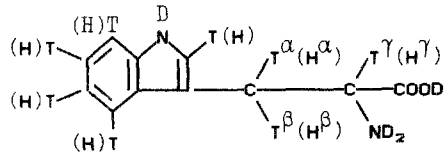
The NMR spectra of the samples obtained by hydrogenolysis and high-temperature solid-phase isotopic exchange (HSCIE) was carried out in ^2H_2O and $C^2H_3O^2H$ solutions on Bruker spectrometers AG 250 and WM 500 at frequencies 266.8 MHz (for tritium) and 250.13 and 500.13 MHz (for

protons). For simulate calculations was used standart "Panic" Bruker's software. Have been determined the compositions of mixtures, geminal (^{2}J) and vicinal (^{3}J) spin coupling constants (SCC), geminal ($^2\triangle$) and vicinal ($^3\triangle$) isotope chemical shifts (ICS). The more important results:

I. For Glycine three-component mixture $^2HNCH_n{}^3H_{2-n}COO^2H$ (n = 0, 1, 2) $^2J_{TH} = -16.4 \pm 0.2$ Hz; $^2\triangle_T = 0.026 \pm 0.001$ ppm; $^2\triangle_H = -0.021 \pm 0.001$ ppm.

II. For Alanine eight-component mixture $CH_n{}^3H_{3-n}C^3HN^2H_2COO^2H + CH_n{}^3H^{3-n}CHN^2H_2COO^2H$ (n = 0, 1, 2, 3, 4) $^2J_{TH} = -14.2 \pm 0.3$ Hz, $^3J_{TT} = 8.1 \pm 0.2$ Hz, $^3J_{TH} = 7.6 \pm 0.2$ Hz; ICS ($\pm$ 0.001 ppm) for α,β tritons $^2\triangle^\beta_T = 0.026$, $^2\triangle^\beta_H = -0.022$, $^3\triangle^\beta_T = 0.010$, $^3\triangle^\beta_H = -0.010$, $^3\triangle^\alpha_T = 0.007$, $^3\triangle^\alpha_H = -0.007$.

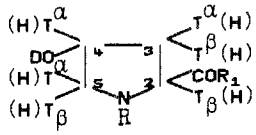
III. The parameters of highly labelled tryptophan with general formula



contained SCC ($\pm$ 0.25 Hz): $^2J_T\alpha_H\backsim = 16.64$, $^2J_T\alpha_T\beta = -17{,}20$, $^3J_T\alpha_H\gamma = 5.20$, $^3J_T\alpha_T\gamma = 5.40$, $^3J_T\beta_H\gamma = 8.40$, $^3J_T\beta_T\gamma = 8.90$; ICS ($\pm$ 0.001 ppm): $^2\triangle^{\alpha,\beta}_T = 0.021$, $^2\triangle^{\beta,\alpha}_T = 0.032$, $^3\triangle^{\alpha,\gamma}_T = 0.011$, $^3\triangle^{\beta,\gamma}_T = 0.008$, $^3\triangle^{\gamma,\alpha}_T = 0.009$, $^3\triangle^{\gamma,\beta}_T = 0.004$.

IV. The table shown the distribution of tritium (mol %) in 4-Hydroxyproline and derivates.

R	R_1	2-T_β	3-T_α	3-T_β	4-T_α	5-T_α	5-T_β
D	OD	6	6	1	5	19	64
$COCF_3$	OD	0	31	19	36	7	7
D	ND_2	16	13	9	4	29	29
$COCF_3$	ND_2	0	4	4	27	53	12



Conclusion: A method has been developed for the qualitative and quantitative analysis of complex isotopic mixtures of labelled aminoacids by using high resolution NMR-spectroscopy. The information of the distribution of tritium in labelled aminoacids has been used for study of solid-phase catalitic reactions mechanisms.

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Measuring internuclear distances in a membrane protein with isotopically labelled amino acid residues

Bacteriorhodopsin (bR) is a membrane protein, that carries out a light-driven proton translocation from the inside to the outside of the *Halobacterium halobium* cell. The proton gradient generated is used in the cell for the synthesis of ATP and, as a consequence, in the production of metabolic energy. Since the linkage between the aldehyde group of the chromophore retinal and the ε-amino group of lysine residue 216 of bR (a C = N Schiff base linkage) is directly involved in the pumping mechanism, it is important to consider the C = N configuration of the different intermediates which occur in the deprotonation and subsequent reprotonation of the Schiff base.

The investigation of isotopically labelled proteins with non-invasive and non-destructive techniques, such as solid-state NMR spectroscopy is an efficient approach to the study of proteins in intact membranes. Selective information can be obtained with ^{13}C (or ^{15}N) at various atoms in the active site [1].

It is the ultimate goal of the present study to determine the configuration about the C = N bond in the photointermediates of bR. This can be achieved by measuring samples of bR which are isotopically enriched at well chosen atomic positions.

Highly enriched (99% ^{13}C) [ε-^{13}C]-L-lysine [2] and [14-^{13}C]-retinal were synthesized. The preparation of isotopically labelled bR was acieved by growing *Halobacterium halobium* in a synthetic medium in which the labelled lysine was substituted for the lysine normally present. From the bacteria, bR was isolated with lysine residues labelled at the ε-^{13}C position. After bleaching bR the labelled chromophore was incorporated.

In the presentation it will be shown that C-C distances can be determined accurately ($\pm$ 0.3Å), even in a large (36 kDa) membrane protein. From the results it is clear that two different conformations of the chromophore-lysine linkage are present in dark adapted bR.

1. Creuzet F, McDermott A, Gebhard R, van der Hoef K, Spijker-Assink MB, Herzfeld J, Lugtenburg J, Levitt MH, Griffin RG (1991) Science 251: 783
2. Raap J, van der Wielen CM, Lugtenburg J (1990) Recl Trav Chim Pays-Bas 109: 207

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L-cysteine prodrug protects against cyclophosphamide urotoxicity without compromising therapeutic activity

2(R,S)-D-*ribo*-(1',2',3',4'-Tetrahydroxybutyl)thiazolidine-4(R)-carboxylic acid (RibCys) is a prodrug of L-cysteine which releases the sulfhydryl amino acid after nonenzymatic ring opening and hydrolysis. The L-cysteine then elevates glutathione (GSH) levels by stimulating its biosynthesis. RibCys was investigated for its ability to protect against the potent urotoxicity of cyclophosphamide (CTX) in CDF_1 mice without compromising the therapeutic utility of the drug. RibCys showed significant reduction in weight loss of the animals and in bladder inflammation 48 hours after CTX administration; however, bladder tissue remained inflammed

compared to controls. Bladder histology also showed some pathological changes in the presence of RibCys. In contrast, all parameters of toxicity (body weight loss, bladder inflammation and pathological abnormalities) were virtually reversed by 21 days after administration. In tests against L1210 leukemia, RibCys did not interfere with CTX anticancer activity. In fact, a slight therapeutic advantage was seen after coadministration of the two agents. RibCys appeared, from these preliminary studies, to be a likely candidate for protecting against long-term CTX toxicity, perhaps reversing the original damage caused by a very high dose, without compromising the therapeutic utility of the drug.

Biology

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Nucleotide sequence of the *Saccharomyces cerevisiae CYS3 (CYI1)* gene

A DNA clone containing the *S. cerevisiae CYS3* gene, which was originally identified as a cycloheximide inducible gene (*CYI1*) and was later found to be identical with *CYS3* and to be the structural gene for cystathionine γ-lyase, was sequenced. It has a single ORF of 1182 bp (394 amino acids), which was thus concluded to the the *CYS3* (*CYI1*) gene. *CYS3 (CYI1)* was a moderately expressed gene as judged by codon usage. The amino acid sequence deduced from the nucleotide sequence of *CYS3 (CYI1)* had substantial homology with the following enzymes; rat cystathionine γ-lyase (47%), and *Escherichia coli* cystathionine γ-synthase (36%) and cystathionine γ-lyase (25%) coded for by *METB* and *METC*, respectively. In addition, the N-terminal half of it was highly homologous with the N-terminal half of *S. cerevisiae* O-acetylserine and O-acetylhomoserine sulfhydrylase (39%) coded for by *MET17 (MET25)*. It was further found that the *CYS3 (CYI1)* gene complemented the *E. coli metB* mutation and conferred cystathionine γ-synthase activity as well as cystathionine γ-lyase activity to *E. coli*. In contrast, the *S. cerevisiae cys3* mutation caused deficiency of cystathionine γ-lyase and increase (about 6 fold) of cystationine γ-synthase. Based on these findings, we discuss the role and evolution of the *S. cerevisiae CYS3* gene.

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Co-purification of cystathionine γ-lyase and OAS/OAH sulfhydrylase of *Saccharomyces cerevisiae*

Cystathionine γ-lyase (CTLase) was purified about 200 fold from *S. cerevisiae*. The final preparation revealed two bands by PAGE; one had the CTLase activity and the other not. According to 2D separation (PAGE and SDS-PAGE), the band with the CTLase activity gave rise to a major (41.2 kD) and a minor (39.2 kD) spots while the other gave rise to a single (46.8 kD) spot. The three SDS-PAGE spots were separately subjected to N-terminal amino acid sequencing. The 41.2 kD and 39.2 kD spots had the same sequence, which was identical with that deduced from the nucleotide sequence of the *CYS3(CYI1)* gene except that terminal methionine is missing. On the other hand, the 46.8 kD spot had the sequence identical with the *MET17(MET25)* gene except the absence of terminal methionine. The corresponding PAGE band was then shown to have OAS/OAH sulfhydrylase (SHLase). From the results, we conclude that *CYS3(CYI1)* and *MET17(MET25)* are the structural genes for CTLase and SHLase, respectively, and that these enzymes are co-purified in our procedure, indicating their similarity in physical properties.

J. P. Daub, R. F. Dietrich, R. W. Reiser, L. M. Shalaby, D. V. Mesaros

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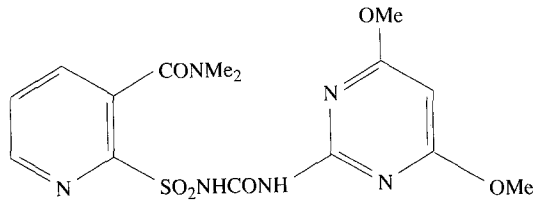
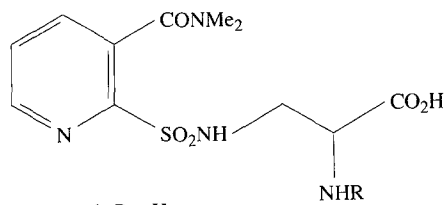
The structure determination and synthesis of two serine-conjugated soybean metabolites from a crop rotation study of accent® herbicide

Two serine-conjugated soybean metabolites were isolated from a crop rotation study of the corn herbicide Accent®.



Accent®



1: R = H
2: R = $COCH_2CO_2H$

Accent® was metabolized in soil primarily to a pyridine sulfonamide through hydrolysis of the urea bridge. Soybean plants grown in the soil absorbed this pyridine sulfonamide and converted into two major metabolites by conjugation. Structure determination of these metabolites was achieved by using various mass spectral techniques and high-resolution NMR. These data indicated one of the metabolites to be formed by conjugation of the pyridine sulfonamide with serine through loss of water. Two possible structures were synthesized and the amino-acid 1 was found to be identical to the serine-conjugate. The second metabolite appeared to be this serine-conjugate further conjugated with malonic acid. Total synthesis of the malonyl-conjugate 2 confirmed the structure of this second metabolite.

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Variations in the free amino acid pool of cassava leaves during the pathogenesis caused by *xanthomonas campestris* pv. *manihotis*

Xanthomonas campestris pv. *manihotis* induces a blight symptom in the latter stage of the disease on Cassava leaves, which partly results from the accumulation of various low molecular weight carboxylic acids in the leaf tissue. In vitro, these acids are produced directly by transamination and subsequent decarboxylation of amino acids such as methionine, phenylalanine and branched chain amino acids. No significant variation was found in the total amino acid content of the leaf during the pathogenesis suggesting that the proteolysis was not taking a great part in this pathological process. The importance, in the pathogenesis, of the amino acid catabolism was therefore evaluated by analyzing variations on the free amino acid pool of the leaf tissue. Amino acid variations were discussed with regard to the bacterial growth in the intercellular leaf spaces and the appearance of the symptoms.

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The effect of alpha aminoisobutyric acid (2 methylalanine) on the physiology and development of Basidiomycete fungi

The alanine analogue alpha aminoisobutyric acid is taken up actively by mycelium of the fungus *Serpula lacrymans* and accumulated in the intracellular free amino acid pool. It competitively inhibits uptake of glutamic acid, and utilisation of glutamic acid and alanine in several Basidiomycete fungi. It replaces glutamic acid in the free amino acid pool, and can depress its concentration in the pool by 50%. AIB is not metabolised to CO_2 nor is it incorporated into the water insoluble fraction of cellular material. It can thus be used as a marker for the tanslocation of amino acid (the normal form in which nitrogen is moved in the fungal colony). Mycelium of *S. lacrymans* fed with AIB shows an altered pattern of colony differentiation, in which leading hyphae are inhibited and the frequency of branching is increased. Growth measured as dry weight production is little changed. There is some evidence that the intrahyphal presence of AIB may subvert signalling systems that control the pattern of branching under normal growth conditions in vivo. AIB may have practical application in limiting the spread of "dry rot" (Hausschwamm) caused by *S. lacrymans*.

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From amino-acids to synthetic vaccines: two examples with the diphteria and the cholera toxins

A-Diphteria toxin. The m-nitro benzhydrylamine resin is a polymer leading to the peptidyl-resine complex after the synthesis and HF treatment. Using this polymer we synthesized the octa, deca, dodeca and tetradeca and hexadecapeptidyl-resin corresponding respectively to the sequences 194-,192-,190-188-and 186-201 which compose the loop A in the Diphteria toxin, an area of that protein recognized to be an hinge in its pathogenic process. Iodine labelled antibodies raised against the entire toxin recognized preferentially the 188-201-resin-complex. The free synthesized tetradeca-peptide 188-201 coupled to natural carriers like bovine serum albumin, ovalbumin or synthetic carrier lika a poly-alanine-lysine chain then conjugated to muramyl dipeptide elicited in guinea-pigs antibodies which not only bound specifically with the toxin but neutralize its dermonecrotic and lethal effects.

B-Cholera toxin. To avoid the synthesis of several peptides overspaning a protein, then determine their immunogenic potency, several criteria were used in order to select a sequence possessing the maximum of changes to raise antibodies. So the two peptides P-30-50 and P-50-75 were choosen in the B subunit of the Cholera toxin (CT) according to: 1-their hydrophilicity, 2-the specificity of the amino-acids chain, 3-their configuration and 4-the importance of the Arginines 35, 67 and 73 in the pathogenic process. The principal results obtained are:

- The antibodies cross-link the peptides and the toxin.
- The anti-toxin antibodies do not recognize the peptides
- Only the anti-50-75 antibodies neutralize the toxin
- Animals orally immunized with the 50-75 peptide are protected against the toxin by production of secretory IgAs at the intestinal level.

To avoid the risky coupling of an hapten to a carrier protein but to increase the degree of protection induced by the P 50-75 two carriers werer synthesized, one in a globular form (I) the other one in a linear form (II). Each polymer carries 8 anchoring points formed by the ε-NH 2 of the Lysine and so 8 identical peptides can be simultaneously and univocally synthesized. P 50-75 was then synthesized on each polymer. Peptide obtained on I was nicknamed "Star" this one prepared on (II) "Comb". After oral or intraperitoneal immunizations without carrier or adjuvant anti CT antibodies titers were not significantly incresed comparatively to these elicited by P 50-75. However as determined with the illeal loop test the protection against the cholera toxin is much more efficient with the "Comb" than with the "Star".

Details of investigation and principal results will be presented.

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The effect of amino acids on protein turnover in Bacillus megaterium under heat-stress conditions

Kinetics of intracellular protein catabolism indicated the presence of two metabolically different protein fractions: the short-lived proteins of the half life of about 1 h and the long-lived proteins. The half-life of the latter fraction was more than 10 h and decreased substantially during sporulation which represents cytodifferentiation in bacteria. Degradation of proteins in both-growing and sporulating populations os controlled by two main external factors: by the composition of the medium and by the temperature. The presence of aminoacids suppressed protein degradation decreasing mainly the portion of the short-lived proteins without affecting substantially their half-life. On the contrary, the increasing temperature brought about an increase of the short-lived protein fraction. The addition of aminoacids reduced partially the stimulation of protein catabolism caused by an increased temperature. However, when the temperature exceeded the temperature optimum, the aminoacids failed to suppress protein degradation. The growth in the presence of an aminoacid analogue (ethionine) brought about stimulation of protein catabolism. The size of a short-lived protein fraction as well as the rate of degradation of long-lived proteins were both increased. The optimum growth temperature was reduced at the same time

Protein turnover in a sporulating population was substantially higher as compared to asporogenic mutants or under inhibition of sporulation by specific inhibitors, e.g. netropsin. The increased protein catabolism was characteristic for the irreversible sporulation phase. Addition of amino acids slowed down protein catabolism during the reversible sporulation phase much more than degradation of proteins during later sporulation phases. Sporulation was much more sensitive against temperature than was the growth. The permissive temperature for sporulation was by 3–5 °C lower than that for the growth. An increased temperature stimulated the degradation of short-lived proteins but suppressed the degradation of long-lived proteins. Amino acids could not relieve the effect of supraoptimal temperature either on sporulation or on protein catabolism.

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Mechanism of O-Acetylserine sulfhydrylase from *Salmonella typhimurium* LT-2

O-Acetylserine sulfhydrylase is a pyridoxal 5′-phosphate (PLP) dependent enzyme that catalyzes the final step of L-cysteine biosynthesis in *Salmonella*, viz. O-acetyl-L-serine + sulfide → L-cysteine + acetate. A spectrophotometric assay is available using 5-thio(2-nitrobenzoate) (TNB) as an analog of sulfide and monitoring the disappearance of absorbance at 412 nm. The enzyme catalyzes a ping pong mechanism with α-aminoacrylate in Schiff base with the active site PLP. Using a combination of the pH dependence of UV-Vis and ^{31}P NMR spectral studies and initial velocity kinetic parameters, the acid-base chemical mechanism and the optimum protonation state of enzyme and substrate functional groups necessary for binding has been determined. The Schiff base is protonated and the α-amine of the substrate O-acetyl-L-serine (OAS) is unprotonated for binding. There also appears to be a requirement for one active site general base to accept the α-proton and a Lewis acid to polarize the leaving group carbonyl.The enzyme als catalyzes an OAS hydrolase activity, and the pH dependence of this reaction suggests that the active site lysine that participated in the Schiff base linkage is protonated to start the second half reaction. The stereochemistry of ^{3}H-borohydride reduction of the Schiff base in free enzyme and the α-aminoacrylate intermediate has been determined by degradation of the resulting pyridoxyllysine and pyridoxylalanine to pyridoxamine 5′-phosphate measuring ^{3}H-release with apo-aspartate aminotransferase. The sequence around the active site lysine is AsnProSerPheSerValLysCysArg.

This work was supported by grants from the National Science Foundation (DMB 8912053) and the Robert A. Welch Foundation (B-1031) to PFC and a grant from Deutsche Forschungsgemeinschaft (Schn 139/9-2) to KDS).

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An increase in levels of free amino acids and glutathione in *E. coli* B as a result of osmotic stress

In all living cells, maintenance of turgor pressure is a prerequisite for normal growth and division, and closely connected with the adaptive mechanism that protects cells from the dehydration caused by salt or freezing.

When *Escherichia coli* B growing logarithmically in minimum medium was stressed suddenly by 0.5 M NaCl or sucrose, intracellular levels of amino acids (peptides), in addition to K^+, increased temporarily. Among the peptides, levels of glutathione increased to about 2.5 (the addition of sucrose) and 4 fold (the addition of NaCl) that in the control cells in the first 15 and 30 min, respectively.

Proliferation of cells resumed after 15 (the addition of sucrose) and 30 min (the addition of NaCl). The increased levels of amino acids and glutathione decreased with the resumption of growth. Levels of betaine and proline were very low and scarcely increased during these periods.

In complex medium, intracellular levels of betaine, proline, and other amino acids (peptides), in addition to K^+, increased in that order; when betaine and proline were present in the medium, the cells accumulated these solutes during stress. The increased levels of proline, and other amino acids (peptides) also decreased with the resumption of growth.

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Binding of kynurenine to catecholamine

Pale yellow pigment (Papiliochrome II) of the wing-scales of *Papilio* butterflies consists of one molecule each of L-

kynurenine and N-β-alanyldopamine (NBAD). The structure has been reported to be $N^{\alpha\gamma}$-[α-(3-aminopropionylaminomethyl)-3,4-dihydroxybenzyl]-L-kynurenine, in which aromatic amino nitrogen of kynurenine is bonded to the β-carbon of dopamine.

There are two isomers of the pigment which have been named Papiliochrome II a and II b. The II a/II b ratio is about 1.17. The II a and II b show opposite ORD and CD curves. And the II a and II b are convertible to each other in 0.1 N HCl solution.

Papiliochrome II a and II b readily decompose to L-kynurenine and N-β-alanylnorepinephrine (NBANE) by being heated in water at 80 °C for 30 min. The NBANE gives a positive CD curve at 280 nm in the cases of both II a and II b. On hydrolysis in 1 N HCl at 100 °C for 2 hr, the NBANE gives (±) norepinephrine and β-alanine. By the hydrolysis, NE is readily racemized.

As Yago (1989) reported, the II a and II b can be enzymatically synthesized by incubating L-kynurenine, NBAD with phenoloxidase. The reaction mechanism may be explained by Sugumaran's quinone-methide theory (Review, 1988) as follows: (1) BNAD is oxidized to NBAD *o*-quinone by phenoloxidase. (2) The NBAD *o*-quinone become its tautomeric isomer, NBAD quinone-methide. (3) Aromatic amino nitrogen of L-kynurenine attacks the β-carbon of the quinone-methide.

Now, the stereochemistry and biosynthesis of II a and II b are being investigated in more detail.

Gastroenterology

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Effect of injecting excess lysine on rat pancreas

In view of the current interest in transfusion of large dose of amino acids following the popularization if parenteral nutrition, author examined the effect of excess lysine, which is a basic amino acid like arginine, on various tissues.

I.P. injection of excess lysine into Wistar rats (400 mg/100 g body weight) caused remarkable and selective damage of pancreatic acinar cells, such as pancreatic necrosis. The first changes in the acinar cells were marked swelling of mitochondria with increase in their calcium content and decrease in their ATP content. Cytochemically early calcium deposits occurred inthe matrices of swollen mitochondria and later various pattern occurred. Subsequently focal areas of the cytoplasm were degraded. Autophagic vacuoles appeared in these areas, and then acid phosphate activity in their periphery as a result of fusion with lysosomes. In addition, the effects of excess lysine on in vitro morphological and functional changes of isolated acinar cells and mitochondria of rat pancreas were examined. In isolated acinar cells also, excess lysine caused marked swelling of mitochondria as an early change, followed by dilatation of endoplasmic reticulum and then appearance of autophagic vacuoles. Addition of excess lysine of suspensions of mitochondria induced their swelling and inhibited oxidative phosphorylation. These results suggest that the first intracellular target of excess lysine in pancreatic acinar cell damage is mitochondria due to the direct effect of depolarizing mitochondrial membrane, resulting their swelling and inhibition of oxidative phosphorylation.

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Basal amino acid profiles are altered in epileptic patients: influence of drug-induced alterations of liver function?

Amino acids which serve as excitatory or inhibitory neurotransmitters or as neurotransmitter precursors have been speculated to participate in the etiology of epilepsy. Most studies focus on data obtained from a limited number of patients; and most patients are treated with anticonvulsants that are known to induce enzymes in the liver, the main organ involved in amino acid metabolism. The question arises, whether changes in amino acid metabolism can be observed in anticonvulsant-free epileptic patients and if the change in liver metabolism is related to alterations in the amino acid profile.

We investigated serum amino acid profiles and serum liver parameters in 73 epileptic patients and in 90 healthy subjects and evaluated the data by an analysis of variance. Seventy-two per cent of the anticonvulsant-treated patients had increased serum transaminases, and none of the drug-free patients showed these changes compared with healthy subjects. In anticonvulsant-treated epileptic patients we observed increased serum concentrations of glutamine and glycine and decreased levels of taurine, threonine, serine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, tryptophan and arginine. These deviations were not observed in untreated patients.

These results suggest that altered liver function may be involved in the changes in amio acid profiles that are observed in anticonvulsant-treated epileptic patients.

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Amino acid neurotransmitter receptors in the cerebral cortex of chronic alcoholic cases with and without cirrhosis of the liver

Following up our previous findings on chronic alcoholics with Wernicke's Encephalopathy (WE), we studied glutamate and GABA receptors in pathologically abnormal (superior frontal gyrus, SFG) *cf* control (motor cortex) brain regions in non-WE chronic alcoholics with an without liver cirrhosis. We have processed samples from four cases in each group; major differences *cf* alcoholic WE cases were found, while cirrhosis had a lesser effect. Diazepam (Dz) and flunitrazepam (Fz) did not behave as identical ligands at the "central-

type" benzodiazepine (Bz) receptor in human brain, in that Dz sites were markedly *reduced* in the SFG from alcoholics with WE, but *increased* in non-WE alcoholic, whereas Fz binding was little altered in either group. There was also a closer corresondence between changes in Dz and $GABA_A$ sites in non-WE *cf* WE alcoholic cases. Thogether with studies of Dz : Fz cross-inhibition, the results suggest that there may be variations in the expression of specific sub-types of the $GABA_A$-Bz receptor complex in these diseases. We also found changes in the glutamate activation of MK 801 binding on the NMDA sub-type of glutamate receptors. Using a goat model, we showed that the changes seen in WE alcoholics are unlikely to be due to the associated thiamine deficiency. To gain a better characterization of the cases, we studied desialyltransferrin in human brain as a potential quantitative marker of alcohol abuse; however, we were unable to detect a clear correlation.

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Amino acid metabolism in acute pancreatitis, measured with L-(1-^{13}C)-leucine

A common part in therapy of the acute pancreatitis is the total parenteral nutrition (TPN). In the literature are recommended 70–100 g of amino acid solutions with a branched chain amino acid content of 14–20%. Until now there are no studies done if (and in which quantities) these amino acids can be metabolised.

With the ^{13}C-leucine-tracer-technique it is possible to get quantitative results in the metabolism of the branched chain amino acids, especially concerning protein-oxydation, -turnover, -synthesis, -breakdown and -retention.

In the Surgical University Clinic Mannheim a clinical prospective study was done to investigate the branched chain amino acid metabolism in patients with acute pancreatitis. The patients had been divided in 3 groups: 1) patients with mild pancreatitis; 2) patients with severe (necrotizing) pancreatitis and 3) patients with no disease of the pancreas. In group 1 and 3 the ^{13}C-leucine tracer-test was performed on the 3. and 5. day after hospitalisation, in group 2 on the 3. and 5. day after the operation. Also the common parameters of protein metabolism had been determined. For the blood samples are in work, the results can be expected in june 1991.

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Plasma amino acid profile in patients undergoing liver transplantation; an HPLC study in evaluating the acceptance and rejection of transplanted graft

It is well known that the patients with advanced hepatic failure have abnormalities in their plasma acid profile due to impaired metabolism by the diseased liver. In an attempt to gain this information, we have conducted a study to investigate whether amino acid profiles obtained in plasma samples after liver transplantation could reflect liver function which may have a predictive role for the acceptance and rejection of transplanted graft. These patients are followed for the period of 2–3 weeks. 20 patients, 9 having a successful outcome with rapid normalization of liver function whereas 11 having an unsuccessful course due to failure of liver graft. Blood samples were collected in the morning hours after an overnight fast and plasma separated were subjected to HPLC quantitation of amino acids. An automatic online HPLC method based on pre-derivatization with OPA was developed to quantitate 24 amino acids in plasma samples. The method of analysis is sensitive and reproducible providing an excellent technique to monitor plasma amino acids in these patients. The results accumulated suggest that the function of the liver graft as well as the nutritional status is reflected in plasma aminogram and among all amino acids, the level of methionine may be an important parameter in assessing the graft function after successful and unsuccessful liver transplantation.

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High-performance liquid chromatography for the quantitation of dopamine and its metabolites in cerebrospinal fluid of male rats. Application of the method in defining the role of dopamine in food intake mechanism

In this study, an HPLC method based on ion-pair elution is used to monitor catecholamines, tryptophan and their metabolites with electrochemical detection. The whole separation is achieved within 20 minutes and the concentrations of all substances analysed are linearly related to the integrated areas between 0–100 pmol/l. The system of analysis is sensitive, stable and reproducible.

The method of analysis is applied to study the effect of food intake and deprivation mechanism in male rats on the levels of these substances in CSF. The results accumulated show that the level of dopamine is clearly related with the food intake conditions decreasing under the deprivation condition. These observations further show that on injection of cholecystokinin restored the level of dopamine as under free food intake conditions in rats. Injection of dopamine receptor agonist i.e. apomorphine inhibited food intake and this effects were reversed by pre-treatment with dopamine receptor antagonist i.e. cis-flupentixol.

It is suggested that cholecystokinin interacts with dopamine in the control of food intake.

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Long term use of aminoacids is required to improve the hepatic regenerating potential

In order to stimulate the proliferation of the tissues, it is essential to supply the adequate amounts of energy sources and nitrogenous precursors, for the purpose of which varying formulae of aminoacids have been developed. Especially in case of hepatic failure treatment, a specific formula enriched in branched chain aminoacids has been successfully recom-

mended. However, there are many disputes about the effects of aminoacid formulae on the improvement in regenerating capacity. Most of the aminoacid studies, related with liver regeneration has been confined to their use only for limited period immediately prior or during the induction of liver regeneration by partial hepatectomy or chemical hepatic damage. Therefore in the present experiment, we have designed to compare the effect of length of the administration period with varying formulae of amino acids prior to liver regeneration. The results showed that the formula including branched chain aminoacids, arginine and glutamine was most effective in increase of H^3-thymidine uptake in the regenerating hepatic DNA only when the administration period was longer than two weeks. These data indicated that to make best use of aminoacids for the tissue regeneration, it would be required to induce the varying enzymes related with aminoacid metabolism prior to stimulation of liver regeneration.

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Significant role of central gamma-aminobutyric acid (GABA) in maintenance of gastric mucosal integrity and in regulatory mechanisms of acid secretion in the rat

Gastric acid secretory response is regulated by cephalic, gastric and intestinal mechanisms. Substantial significance of its central mechanisms with respect to gastric function has been recently proposed because of abundant studies on dopamine, ACh, norepinephrine, GABA and neuropeptides. GABA is a unique substance to stimulate gastric function while this has been identified as an inhibitory transmitter. GABA-mimetics; muscimol, baclofen ($GABA_A$- and $GABA_B$-receptor agonists, respectively) and regular GABA, augmented basal acid secretion by central activation. This led us to examine the implications of GABA mechanisms in the pathogenesis of experimental peptic ulceration. Exposure of the rat to cold stress resulted in stimulation of gastric acid output and this response was completely abolished by surgical vagotomy. Increment or decline in brain GABA contents evoked by AOAA or SC, respectively, remarkedly potentiated or reduced acid secretory and ulcerogenic responses to the cold exposure. Significant correlation were found between the brain GABA levels and the acid secretory activity or the ulcer index of gastric lesions induced by the cold stress ($r = 0.978$ or $r = 0.996$, respectively). These evidence indicate that hypothermia evoked by the cold exposure triggers to stimulate gastric acid secretion and to induce gastric lesions through central GABA mechanisms in the rat.

(*AOAA* Amino-oxy acetic acid, *SC* Semicarbazide.)

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Long-term effectiveness of high-dosed ornithin-aspartate on urea synthesis rate and portal hypertension in human liver cirrhosis

The effectiveness of ammonia reducing amino acids on hyperammonemia and hepatic encephalopathy is well known in patients suffering from liver cirrhosis. Data concerning long-term therapy on hepatic function and urea synthesis rate (*UNSR*) ist still lacking. According to Vilstrup/Poulsen it is a good standard for functioning liver mass. Therefore, 25 patients with histologically proven liver cirrhosis and distinct portal hypertension were treated daily with 9 gr. ornithinasparte over 13 years (8–20 years). Shunt operations, esophageal varicosis sclerosis, or portal pressure reducing medication were not applied. Rigorous alcohol abstinence and 80 gr protein/day were prescribed. During the investigation, 3 laparoscopies and 4 liver biopsies were performed, on the average, on each individual. Significant improvements of clinical and biochemical results (Child-Pugh-Index; Composite Clinical and Laboratory Index) were obtained during the long-term therapy with ornithinaspartate. Esophageal varicosis II-III was either reduced to 0-I or totally eliminated. Also significant was an increased urea synthesis rate a decreased hyperammonemia.

A plausible explanation for the long-term therapy effectiveness with ornithinaspartate is the possible recovery of the functioning mass without hepatic size increase. Also important is the rigorous alcohol abstinence. It leads to a significant reduction of portal hypertension in patients suffering from alcohol induced liver cirhosis (Reynolds, own observations).

Additional favorable factors are intensive muscle training and absence of gastrointestinal bleeds.

Nutrition

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Indicator amino acid oxidation as a new method for estimating amino acid requirement in humans

Lysine (LYS) requirement was measured by the oxidation (OX) of L-[1-^{13}C]phenylalanine (PHE) in 7 adult males consuming an energy sufficient diet with graded levels of dietary LYS, adequate PHE (14 mg/kg/d) and excess tyrosine (TYR) (40 mg/kg/d). Each subject was studied at 6 of 7 LYS intakes. Primed, 4 h continuous IV infusions of [^{13}C]PHE (1.2 mg/kg/h) and [ring-$^2H_5$]PHE (0.5 mg/kg/h) resulted in isotopic steady state in plasma and breath CO_2 by 2 h.

PHE flux was not affected by LYS intake nor by the isotope infused. Mean PHE conversion to TYR was low (3.4%) and unaffected by LYS. PHE OX decreased at LYS intakes between 5 and 30 mg/kg/d, but was not different between 30 and 60 mg/kg/d. Treatment means and individual

	LYS Intake (mg/kg/d; n = 6 per intake)							
	5	10	20	30	40	50	60	(SD)
FLUX (μmol/kg/h)								
[^{13}C]PHE	41.9	44.8	40.6	40.7	43.2	40.9	40.6	(5.9)
[$^{2}H_5$]PHE	42.1	44.6	40.6	40.1	42.5	41.1	41.7	(7.0)
OX (μmol/kg/h)	3.1	2.7	2.5	1.8	1.6	1.5	1.6	(0.7)

subject data suggest that a breakpoint in OX representing requirement occurs near 30 mg/kg/d. These studies suggest that the dietary LYS requirement of adult males is greater then the FAO/WHO/UNO (1985) recommendation of 12 mg/kg/d.

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Experimental evaluation of various cysteine analogues as cysteine source in amino acid solutions for parenteral nutrition in the rat

Utilization of intravenously administered N-acetylcysteine, N,N-bis-acetylcysteine, bis acetylglycylcystine, glutathiondisulfide and L-thiazolidine-4-carboxylic acid was evaluated by means of weight gain, nitrogen balance and amino acid concentrations in plasma and urine. Young rats were maintained entirely by TPN for 15 days. The average weight at the beginning of the TPN was 177.5 ± 4.0 g. All groups (n = 4–8) were given the same isocaloric regimen supplying 350 kcal and 1 g $N*kg^{-1}*24\,h^{-1}$. Group I received a solution containing the required amount of methionine for rats, while to group II was given a solution, in which 2/3 of the required methionine was substituted by glycine. The solutions given to groups 3 to 7 also had only one third of the required methionine and the rest was substituted by the cysteine derivatives listet above, respectively. Statistical comparison of the groups receiving glycine, N,N-bis-acetylcysteine and bis-acetylglycylcysteine with the group receiving the adequate amount of methionine yielded significant lower N-balances ($p < 0.001$, $p < 0.0025$, $p < 0.001$), while the groups receiving the N-acetylcysteine, glutathiondisulfide, and L-thiazolidine-4-carboxylic acid had significantly higher N-balances ($p < 0.001$, $p < 0.001$, $p < 0.0005$) when compared to the group receiving 2/3 of the methionine as glycine. Release of cysteine as evaluated by Vmax and Km from N,N-bis-acetylcystine was found in the cytosol of liver and to be very slow when compared to the release of cysteine from acetylcysteine, which was more prominent in the kidney than in the liver. Release of cysteine from L-thiatzolidine-4-carboxylic acid occurred rapidly in liver and kidney. Amino acid concentrations of methionine, cystine and taurine in plasma and urine support the conclusion that cysteine is readily available from acetylcysteine, glutathiondisulfide and L-thiazolidine-4-caboxylic acid-(4) but not from N,N-bisacetylcysteine or bis-acetylglycylcysteine when supplied intravenously.

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Applications of chemically-defined diets to the solution of nutrition problems

Chemically-defined amino acid diets have been developed for most laboratory and meat-producing animals species. In many cases, growth performance of animals fed these diets equals that obtained with standard intact-protein diets. The pattern of both essential and nonessential amino acids is critical to obtaining excellent voluntary food intake. Other factors such as carbohydrate and fat type and level, acid-base balance (i.e., cation-anion ratio), and texture are important of the success of purified diets. Chemically-defined diets provide amino acids, mineral elements and vitamins in forms that are maximally bioavailable. Also, virtually any nutrient can be manipulated at will for studies of 1) requirements, b) bioavailability, c) factors affecting requirement and bioavailability, d) nutrient-nutrient interrelationships, 3) nutrient-drug or nutrient-toxin interrelationships, f) absorption phenomena and g) efficiency and priority aspects of nutrient utilization. Requirements for essential nutrients are generally lower with purified diets than for practical diets because the nutrients in the former are more bioavailable, but also because purified diets generally lack antagonizing factors such as phytate and soluble fiber. That chemically-defined diets for pigs, rats and dogs yield such excellent rates of growth suggest that a specific peptide requirement may not exist for these species. Also, this suggests that all known nutrients necessary for maximal growth must be present in the diet. Whether additional nutrients, or different levels, may be necessary for optimal health and immunocompetency, or for maximal life span, needs further study.

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Effect of tryptophan administration on food intake in piglets and broilers

The effect of intraperitoneal administration of l-tryptophan (Trp) on food intake (FI) was compared in piglets and broilers, both selected for intensive body mass growth.

Single 100 mg · kg^{-1} doses of Trp were adminstered i.p. to ten piglets aged 3, 9, 12, 14 and 16 d. The animals were reared individually in cages from day 1 after birth and bottle-fed a liquid diet 9 times a day at 2 h intervals between 6 and 22 h. Five piglets served as intact controls. Trp began to suppress the meal sizes in 3-d-old animals and did so signif-

icantly ($P < 0.05$) in those aged 9, 14 and 16 d. This decrease in FI made itself also felt in whole-day diet consumption.

In another experiment, 100 Hybro broilers divided into 8 groups were used. They were reared from day 1 post hatching in heated batteries with food and water available ad libitum. Four groups were injected i.p. with single 100 mg · kg^{-1} doses of Trp. Four control groups were treated with saline. FI was measured 30 min post injection and then at 1 h intervals until 14 h. The FI of broilers aged 2 d was unaffected by Trp adminsitration. On day 3, their FI was suppressed ($P < 0.02$) 30 min post injection. The birds treated at 6, 10, 20 and 24 d of age had invariably lower FI 30 min post injection.

It is concluded that i.p. administration of Trp exerts a suppressive effect on FI during the early postnatal period in piglets and broilers despite the different patterns of their food intake (meal eaters versus nibblers). Being functional during the intensive protein synthetic phase in both piglets and broilers the aminostatic component of food intake regulation seems to be of paramount importance in early postnatal period of these two animal categories.

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Increase in glutamine pools after ornithine alpha-ketoglutarate treatment

Glutamine pools are replenished during parenteral ornithine alpha-ketoglutarate (OKG) treatment of catabolic patients. Whether or not this effect is obtained when OKG is administered enterally was assessed in an experimental model of acute denutrition. Male Wistar rats (5 in each group) were starved for 3 days with lab · chow (290 Kcal · $Kg^{-1} \cdot d^{-1}$ and 2.7 g N $kg^{-1} \cdot d^{-1}$) plus either 0, 2, 4 or 8 g of OKG $Kg^{-1} \cdot d^{-1}$ or isonitrogenous amounts of glycine (control groups). Glutamine and glutamate contents were measured in small bowel (10 cm proximal), muscle (anterior tibialis) and serum before and after food deprivation and after the 3 days of refeeding.

Food deprivation induced a fall in smal bowel glutamate (−31%, $p < 0.001$) and muscle glutamine (−59%, $p < 0.001$). After refeeding, OKG treatment counteracted the fall in small bowel glutamate, event at the lowest OKG dose. Muscle glutamine content was increased by OKG in a dose-dependent fashion. Similar variations of glutamine occurred in serum:

In isonitrogenous glycine control groups, glutamate and glutamine pools were the same as or poorer than in the unsupplemented goup (OKG free).

Oral OKG at high doses can thus be as efficient as parenteral OKG in restoring the glutamine pool; possibly through a sparing effect of OKG on small bowel uptake of endogenous glutamine.

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Plasma free amino-acids and nutrition markers of vietnamese malnourished children during proteino energetic renutrition

We evaluated the outcome of plasma free amino-acids and biochemical markers of nutritional status (NM): visceral proteins, apolipoproteins in a population of malnourished children (kwashiorkor) from Vietnam followed during three weeks of protein-energy renutrition.

The children ($n = 25$) were aged 7 to 30 months and selected according to clinical and anthropometric parameters. The samples were collected at the admission of patients (D0) and at 1 to 3 weeks (D 7, D 14, D 21) of controlled dietary therapy (OMS).

Highly variable levels of NM have been found at admission. Only in 7 severely malnourished children werer the levels of NM recuded much below normal values. In this group ($n = 7$) the levels of total essential amino-acids (EAA) among which the branched-chain amino-acids (BCAA) were greatly reduced as compared with the normal pattern for subjects of comparable age. The level of non-EAA (NEAA) remained within the lower normal range.

A significant increase of EAA ($P = 0.05$) particularly BCAA ($P = 0{,}05$) was found after two weeks of dietary therapy, whereas the NEAA were not significantly increased. The normal range of EAA and BCAA where only reached after three weeks of renutrition. In fact a highly significant of all amino-acids levels were observed between DO and D21 ($P < 0.05$). The normal range of NM were reached earlier. No correlation was found between both amino-acids and protein levels.

The difference between amino-acid and NM profiles may reflect various degrees of denutrition in agreement with clin-

	Starved	Refed		
	OKG free	OKG 2 g	OKG 4 g	OKG 8 g
Small bowel Glu nmol/cm	224 ± 19	246 ± 8	266 ± 8**[a]	254 ± 8*[a]	271 ± 8*[a, b]
Muscle Gln nmol/tibialis	579 ± 26	1 226 ± 93	1 372 ± 56	1 422 ± 98	1 634 ± 85*[b]
Serum Gln µmol/l	839 ± 31	696 ± 22	733 ± 34	941 ± 12*[a, b]	1 002 ± 20**[a, b]

Mean ± SEM: * $p < 0.05$, ** $p < 0.001$, [a] vs starved, [b] vs refed OKG free.

ical findings in these infants and amino-acids levels give a better evaluation of their denutrition.

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Comparison of amino acid requirements of the human and animal models using chemically defined diets

The study of amino acid requirements, metabolism and functions of individual amino acids in humans has been greatly enhanced by the use of animal models. Chemically defined diets are imperative to quantitate the metabolic effects of altering individual dietary amino acid levels. The knowledge of dietary essentiality and the levels at which specific amino acids are required are important factors to consider in the selection of animal models for the study of the role of amino acids in human metabolism. This presentation will focus on the comparison of amino acid requirements of man and several laboratory animals.

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Gamma-glutamyl-tyrosine and -glutamine as potential sources of tyrosine and glutamine in total parenteral nutrition

Amino acid mixtures currently used in pediatric total parenteral nutrition (TPN) contain little tyrosine (Tyr) and no glutamine (Gln), due, respectively, to limited solubility and to instability in solution. When 29 μmol of the gamma-glutamyl peptide of Tyr (Glu/Tyr) or of glutamine (Glu(Gln)) are injected intravenously into mice, the peptides are hydrolyzed , producing significant elevations of plasma Tyr or of Gln 10 min after injection, compared to levels in saline-treated controls or to levels 60 min post-injection. Studies using acivicin, an inhibitor of gamma-glutamyl transpeptidase (GGTase), indicate that GGTase catalyzes this hydrolysis. Neither Glu(Tyr) nor Glu(Gln) appears in the urine, except when mice are pretreated with acivicin. These peptides, which are normal metabolites, contain an unusual peptide linkage which renders them stable for month at room temperature in a commercial solution of amino acid used for TPN.

Amino acid mixtures for pediatric TPN contain high molar ratios of phenylalanine (Phe) to Tyr, producing elevated plasma Phe/Tyr ratios in treated infants, compared with those in breastfed babies. Rats on TPN containing a pediatric amino acid mixture also show elevations of plasma Phe/Tyr, compared to this ratio in chow-fed rats infused with saline. Because of the importance of Tyr as a precursor of catecholamines which function in brain development and of Gln in maintaining the normal architecture of the gut, addition of these amino acids to TPN mixtures, in the form of Glu(Tyr) and Glu(Gln), may contribute to an improved outcome in treated preterm infants.

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Aspartame and sucrose produce a similar increase in the plasma phenylalanine to large neutral amino acid ratio in healthy subjects

Aspartame (L-aspartyl-L-phenylalanine methyl ester) consumption has been postulated to increase brain phenylalanine levels by increasing the molar ratio of the plasma phenylalanine concentration to the sum of the plasma concentrations of the other large neutral amino acids (Phe/LNAA). Dietary manipulations with carbohydrate or protein can also produce changes in the Phe/LNAA value. To compare the effects of aspartame and carbohydrate on Phe/LNAA, beverages sweetened with aspartame, sucrose, and aspartame plus sucrose, and unsweetened beverage were ingested by eight healthy, fasted subjects in a randomized, four-way crossover design. The beverages were sweetened with an amount of aspartame (500 mg) and/or sucrose (100 g) approximately equivalent to that used to sweeten a liter of soft drink. The baseline-corrected plasma Phe/LNAA values did not differ significantly following ingestion of aspartame or sucrose. Following aspartame alone, the high mean ratio increased 26% over baseline 1 h after ingestion. Following sucrose alone, the high mean ratio increased 19% at 2.5 h. Sucrose increased the Phe/LNAA value due to an insulin-mediated decrease in the plasma LNAA, while aspartame increased the ratio by increaing the plasma Phe concentration. These findings indicate that similar increases in plasma Phe/LNAA occur when healthy, fasting subjects ingest amounts of equivalent sweetness of sucrose or aspartame.

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An experimental model for studies on the utilization of amino acid analogues during long-term parenteral nutrition in the growing rat

Clinical studies do have the advantage to reveal information on the metabolic response of man; standardized conditions, however, are difficult to achieve in patients and also there are problems in testing compounds of which the metabolic action is not known. An experimental animal model for studying the utilization of amino acid analogues in vivo as well as of the quality of various nutritional regimen with special regard to amino acid composition is presented. Young male Sprague Dawley rats (weighing approx. 170 gs. when entering a study) are entirely maintained by parenteral nutrition via a central vein catheter for up to several weeks. The animals are kept in metabolic cages and are freely movable during the entire infusion period. The rats are given sufficient energy ($350\,kcal * kg^{-1} * 24\,h^{-1}$), electrolytes, trace elements and vitamins according to their requirement but are given only a minimum of nitrogen ($0.6–1.0\,g\,N * kg^{-1} * 24\,h^{-1}$) resulting in submaximal nitrogen balances and growth rates. Under these conditions nitrogen balance reflects very accurately and rapidly qualitative and quantitative alterations in the amino acid supply as is demonstrated by several studies. Differences in the concentration

of free amino acids or their respective analogues in plasma, tissues and urine give additional information on the metabolic fate of the substance under investigation. The model is extremely sensitive and the contribution of single substances to the biological value of a nutrient solution can be detected. It thus can be used as a secreening method to trace the most efficient substances but also for other nutritional, pharmacological or toxicological studies.

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Transferibility of in vivo animal infusion experiments to man as evaluated by in vitro and in vivo studies on the utilization of glutamine analogues in rats and in humans

Utilization of intravenously administered acetyl-L-glutamine (AC-GLN), glycyl-L-glutamine (GLY-GLN) and alanyl-L-glutamine (ALA-GLN) was studied in growing rats during TPN for 15 days. Six groups of rats (n = 5–8) all received besides adequate calories $0.6\,g\,N * kg^{-1} * 24\,h^{-1}$ as essential amino acids (EAA) and $0.4\,g\,N * kg^{-1} * 24^{-1}$ as either AC-GLN, GLY-GLN, ALA-GLN or equimolar amounts of the respective free AAs. Compared to the free AAs, cumulative nitrogen balance was 82% for AC-GLN ($p < 0.05$), 86% for GLY-GLN (n.s.) and 87% for ALA-GLN (n.s.). Daily fractional excretion rates for AC-GLN, GLY-GLN and ALA-GLN werre 20.8, 4.3 and 3.7% of the infused amount, respectively.

In vitro, hydrolysis of AC-GLN, GLY-GLN and ALA-GLN in various tissues of rats and in man was compared by estimating Vmax and Km. Both in man and in the rat, AC-GLN is hydrolyzed by liver and kidney, but not by muscle, lung or blood. The peptides are efficiently hydrolyzed in kidney, liver, muscle and lung and very low or no activity was found in blood. Activities in man are either similar or higher when compared to rats.

It is concluded that all 3 substances are potential glutamine sources for PN although the best results are obtained with the peptides, especially with ALA-GLN. Comparison of the enzymatic data of animal and human tissues could serve to estimate the transferibility of in vivo animal infusion experiments to man.

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Plasma amino acids in septic patients: parenteral versus enteral nutrition

We have measured the plasma amino acid concentrations in two groups of septic patients received the first one total parenteral nutrition (PN) (G-1, n = 10) and the second group continuos enteral nutrition (EN) (G-2, n = 9) during two weeks. Blood samples were taken: one imediately before the administration of nutritional solutions and another at the end of the study period. Amino acid analyses were performed by HPLC of phenylthiohidantoin-amino acid derivates. The energy supply was calculated according to the Harris-Benedict equation modified by consideration of the patient ideal weight. The nitrogen intake was 90 Kcal/g N for the G-1 and 135 Kcal/g N for the G-2. The nitrogen balance and the Bristian index improved in both groups, although they were more favourable in G-2. Plasma amino acid imbalances were corrected with both types of nutrition. However, in G-1 the levels of glutamate (Glu) and aspartate (Asp) were increased and that of glutamine (Gln) decreased as compared to G-2. PN not contain those amino acids while EN was specially rich in Glu, Gln and Asp. The relative higher supply of Glu. Gln and Asp do not produce increased levels of these amino acids in plasma. Those differences could be attributed to a higher utilization of Gln, Glu and Asp by peripheral tissues, specially the small intestine as an energetic fuel. Phe Phe/Tyr ratio was more decreased in G-2 at the end of study which apparently would lead to a less risk of encephalopathy in septic patients.

Glycosylation

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Structure of advanced glycation endproducts

In food chemistry the very complex reactions of reducing sugars with the free amino groups of amino acids or proteins are known as non-enzymatic browning or Maillard reaction. In biological systems like the human body the reaction of glucose with proteins is termed as glycosylation, glucosylation, fructosylation or glycation. It is generally accepted that the Maillard reaction in vivo contributes to aging processes which can be observed in diabetics ten or twenty years earlier. There are great similarities between the Maillard reaction in foods and in vivo, several degradation pathways are similar. It is supposed that aging process in biological systems are often produced by crosslinking of proteins thus changing their functions and properties. Recently substances with cross-linking abilities formed from sugars and amines have been found. An imidazo pyridinium compound was isolated from biological materal indicating that pentoses may also contribute to the Maillard reaction in vivo.

In model systems pyrrols were detected which react with functional groups in the side chains of amino acids forming more or less stable products. Experiments with glucose-6-phosphate reveal that there are differences in the degradation pathways compared to that of glucose. That means that the Maillard reaction in cells to some extent differs from that in the extracellular tissue. It is known that aminoguanidine and similar compounds have inhibitory effects on the sugar amine interactions in biological systems. Further informations are necessary to learn more about the inhibitory mechanism and the compounds formed with amino-guandine.

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Maillard reaction mediated protein crosslinking in diabetes and aging

Work from this laboratory has focussed on structure elucidation of short and long-wave fluorescent molecules from human extracellular matrix and lens crystallins. Pentosidine is a 335/385 nm fluorescent crosslink involving lysine and arginine residues linked together by a pentose in an imidazo (4,5 b) pyridinium ring. Pentosidine levels increase with age in a variety of human and animal collagen-rich tissues. Presence of diabetes and especially uremia greatly catalyze pentosidine synthesis in skin. Plasma levels of pentosidine are mildly elevated in diabetes but dramatically so in uremia. Skin pentosidine levels correlate with the cumulative severity of diabetic complications and may thus become a metabolic marker for long-term damage to proteins by the Maillard reaction in diabetes. Ribose is the most reactive pentosidine precursor sugar, but other sugars such as glucose, fructose and ascorbate were also found to form pentosidine. The reaction is catalyzed at alkaline pH and is 12 × faster with Amadori products than with free ribose. Very high pentosidine levels were found in highly pigmented human lenses but not in diabetic lenses, suggesting that ascorbate is the precursor sugar. Pentosidine formed in lens crystallins incubated with ascorbate and dehydroascorbate only with O_2. On the contrary, a 370/440 nm fluorophore (LM-1) formed also from DHA under N_2. Thus pentosidine in cells may reflect an imbalance of the redox state.

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Biological and clinical studies with aminoguanidine

In tissues that develop diabetic complications, excessive amounts of irreversible advanced glycosylation products (AGPs) accumulate on long-lived extracellular matrix proteins and intracellular nuclear constituents, due to chronically elevated plasma glucose. Abnormalities in extracellular protein cross-linking, cell/matrix interactions, and DNA structure have been demonstrated in vitro, and inhibitors of AGP formation have been developed. The prototype compound, aminoguanidine HCl, effectively inhibits AGP formation. Aminoguanidine's mechanism of action has been shown to involve the formation of an aminoguanidine-substituted Amadori product by fast atom bombardment mass spectrometry. In vitro, aminoguanidine effectively inhibits AGP-induced matrix abnormalities involving collagen, fibronectin, and laminin. AGP-matrix induced defects in endothelial cell adhesion and mesangial cell proliferation are also prevented. In vivo aminoguanidine treatment prevents diabetes-induced increases in retinal vascular permeability, AGP formation, and the subsequent intercapillary deposition of extravasated proteins in long-term diabetic rats. Pathologic development of retinal microaneurysms and an 18-fold increase in acellular capillaries are also dramatically reduced. In diabetic kidney, aminoguanidine treatment decreases glomerular basement membrane AGP content, reduces basement membrane thickening and mesangial expansion, and reduces urinary albumin excretion to near-normal values. In diabetic peripheral nerve, axonal atrophy, moto and sensory nerve conduction velocities, and vasa nervorum blood flow are also normalized in animals treated with aminoguanidine. Aminoguanidine and its analogues thus have a potential therapeutic role in the prevention and treatment of chronic diabetic complications.

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Site specificity of protein glycation

The rate of nonenzymatic glycation of a protein amino group is enhanced by acid-base catalysis of the Amadori rearrangement, which is the rate-limiting step of the reaction [Watkins NG, Thorpe SR, Baynes JW (1985) *J Biol Chem* 260: 10629–10636]. Examination of crystal structures on the computer showed that each of the glycation "hot-spots" of liver alcohol dehydrogenase (LADH), ribonuclease A, and haemoglobin is close to a histidine imidazole group, which presumably acts as an acid-base catalyst. Therefore, distances between amino and imidazole groups may be an important factor in dictating the site specificity of glycation of proteins. Glycation of LADH, conducted in a HEPES buffer containing sodium sulphate, occurred almost exclusively at Lys-231, which is only 9 Å distant from the imidazole of His-348. When the sulphate in the buffer was replaced by phosphate, additional glycation occured at Lys-228, which is not close to an imidazole group. In this case the catalyst may have been inorganic phosphate, immobilised by binding to a cluster of basic residues. NAD^+ blocked glycation at Lys-228, probably by binding to the same basic cluster. LADH therefore provides a good example of a protein in which the site specificity of glycation can be interpreted in terms of the influence of (a) local acid-base catalysis, and (b) ligand binding.

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Prevention and reversible solubilization of advanced glycation end products (AGE) by derivatives of amino acids containing germanium as the substitute of carbone

The Maillard reaction of bovine lens crystallin, serum albumin, or skin collagen with glucose was investigated so as to find effective means to prevent the formation of Advanced Glycation End Products (AGE), and to induce reversible solubilization of polymerized proteins as one of the models of cataract treatment. Derivatives of amino acids consisting of germanium as a carbone-substitute, 2-Carboxyethylgermanium sesquioxide (Ge-132), 2-Carboxy-2-amino-1-phenylethylgermanium sesquioxide (Ge-373), and 2-Carboxy-2-aminoethylgermanium sesquioxide (Ge-385), were combined by the box titration method to determine what dose would be most effective, comparing with Aminoguan-

idine-HCl (AMG), Vitamin E (VE), and pirenoxine (Catalin-K, CK). Though AMG suppressed the formation of AGE, the effective concentrations were higher than 20 mM. However, Ge-385, when administered by itself at a low dose, induced the reversible solubilization of AGE made from crystallin, and albumin. When either two of such reagents as AMG, VE, CK and Ge-132 or 385 were added together to proteins, the effective range became lower, and the peaks of smaller molecules in the profiles of HPLC and polyacrylamide gel electrophoresis could be prominently observed. The effect of Ge-132 on the eyes of SAM mice, which manifest senescence accerelating cataracts at a relatively young age, were examined. Prevention of cataract-genesis and induction of reversible transparency of turbid lenses could be observed by administering Ge-132 in the eyes 4 times a day. Organic germanium compounds appeared uniquelly capable of dissecting the sugar-parts from AGE by the specific reaction of decarbonylation, and of causing AGE to be smaller sizes.

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Enzymatic glycosylation and deglycosylation of hydroxylysine in normal and pathological kidney

Glycosylation and Hydroxylysine (Hyl) by UDP-Gal and UDP-Glc is catalysed successively by a Galactosyltransferase (EC 2.4.1.50) and a Glucosyltransferase (EC 2.4.1.66) resulting in Hyl-linked Glc-Gal disaccharide units (DU) characteristic of collagens. Deglycosylation of the DU is catalysed by a specific alpha-glucosidase (EC 3.2.1.107), but the galactosidase activity on Gal-Hyl-peptides is so weak in rat kidney that its existence is discussed.

In the Glomerular Basement Membranes (GBM) of *diabetic patients*, Garlick and Spiro have shown increased enzymatically glycosylated Hyl (Glc-Gal-Hyl and Gal-Hyl) concentration: 20.8 residues per 1 000 aminoacids vs 15.9 in normoglycemic controls, whereas glycated Lys (Glc-Lys) and Hyl (Glc-Hyl) levels were respectively 0.3 and .19 per 1 000 (vs. 0.18 and 0.08 in controls). The quantitative importance of enzymatic glycosylation is much greater than that of glycation. In *experimental diabetes* increased specific activity of Glc-transferase and Gal-transferase have been reported; the activity of Glc-Gal-Hyl glucosidase is inhibited by elevated glucose levels but the enzyme concentration is increased; all these abnormalities are reversed by insulin treatment. Preventive treatment by an aldose-reductase inhibitor, sorbinil, prevents cataract, hypoalbuminemia, GBM thickening, 3-Hyp accumulation and increase in Glc-Gal-Hyl glucosidase concentration, without modifying hyperglycemia.

In *spontaneously hypertensive rats*, which develop microangiopathy with GBM thickening as diabetic animals, the Glc-Gal-Hyl glucosidase specific activity is decreased. This is not corrected by a preventive treatment by hydralazin which normalises arterial blood pressure. Therefore this abnormality appears to be under genetic control.

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Glycation and oxidation (glycoxidation) of skin collagen in aging and diabetes

The glycation and free radical theories of aging hypothesize that the chemical aging of long-lived tissue proteins is caused by reactions with reducing sugars and oxygen radicals, respectively. We have measured the concentration of fructoselysine (FL), the initial product of glycation of lysine residues in protein, and the concentration of two products of sequential glycation and oxidation (glycoxidation) of protein, carboxymethyllysine (CML) and pentosidine (P), in insoluble skin collagen from persons, aged 0–80 years, using GC/MS assays for FL and CML and an HPCL assay for P. FL was present at ~ 5 mmol FL/mol Lys in adult collagen, but increased only ~ 33% between ages 20 and 80. In contrast, CML and P increased 5-fold between ages 20 and 80 and correlated strongly with one another and with age, reaching maxima of 2 mmol CML/Lys and 50 µmol P/mol Lys at age 80. The concentration of FL was 3-fold higher in diabetic, compared to age-matched non-diabetic subjects, while CML and P increased up to 2-fold. The age-dependent increase in CML and P in skin collagen indicates that glycoxidation reactions are involved in the natural aging of tissue protein and the acceleration of protein aging in diabetes. Two other markers of oxidative damage to protein,, *o*-tyrosine and dityrosine, were not significantly increased with age, suggesting that glycoxidation products are sensitive biomarkers of oxidative damage to tissue protein during aging and in diabetes. The sensitivity of glycation products to oxidation may result from their chelation of transition metal ions and the relative ease of oxidation of reducing sugars and their adducts to proteins.

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Protein oxidation during glycation (non-enzymatic glycosylation)

Exposure of protein to glucose in vitro is associated with protein fragmentation, conformational change, the generation of new fluorophores and oxidation of amino acid side chains. These changes appear to be caused by powerful hydroxylating agents generated by the transition metal-catalysed oxidation of glucose and glycation adducts. Inhibition of oxidative reactions during the glycation process (by the inclusion of metal chelating agents) prevents many of these effects. In addition, we find that that the binding of transition metal to selective sites on the protein surface (such as copper ions to histidine) affects the specificity of the glycation process as well as the parts of the protein which are oxidatively damaged. These effects will be reviewed. In addition, we provide evidence that protein fluorophore formation in vivo is unlikely to the dependent upon glucose but rather upon the presence of "decompartmentalised" transition metals which catalyse the oxidative formation of protein-reactive aldehydes from compounds such as vitamin C and polyunsaturated fatty acids. These latter compounds appear to be far more potent fluorogens than glucose, at least in vitro.

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Pentosine-mediated skin collagen crosslinking correlates with the severity of diabetic complications

Pentosidine is an advanced glycosylation end-product and protein crosslink that results from the reaction of pentoses with proteins. Recent data indiacte that long-term glycation of proteins with hexoses also leads to pentosidine formation through sugar fragmentation. In this study,we investigated the hypothesis of whether the severity of diabetic complications is related to cumulative tissue damage by advanced glycosylation as reflected by pentosidine formation in skin collagen obtained by biopsy. Pentosidine was quantitated in acid hydrolyzed insoluble collagen from skin-punch biopsies obtained from 25 nondiabetic and 41 type I diabetic subjects with diabetes duration greater than 20 yrs. These specimens were used previously to describe the association between collagen-linked fluorescence and diabetic complications [N Engl J Med (1986) 314: 403]. Skin collagen pentosidine was significantly elevated in all diabetic vs. control subjects ($P < 0.0001$). It correlated strongly with age ($P < 0.0001$) and weakly with duration ($P < 0.082$). Age-adjusted pentosidine levels were highest in grade 2 (= severe) vs. grade 1 and 0 complication in all four parameter tested (retinopathy, nephropathy, arterial stiffness and joint stiffness). Although overlap was present within groups for all parameters, significant differences were found for retinopathy ($P < 0.014$) and joint stiffness ($P < 0.041$). The highest degree od association of pentoside levels was with the cumulative grade of complication ($P < 0.005$) determined by summing indexes of all four parameters. Pentosidine was also significantly elevated in the serum of diabetics vs. controls ($P < 0.0001$); however, there was no recognizable association with age, duration, complication, or skin collagen pentosidine ($P < 0.05$). Pentosidine reflects a form of sugarmediated molecular damage to protein which is enhanced in type I diabetic individuals with severe complications. Determination of skin pentosidine level may be a useful marker for the risk of developing complications and a biochemical endpoint for the assessment of therapeutic interventions aimed at preventing reversing their progression.

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Formation of pentosidine crosslinks by the reaction of ascorbate with lens crystallins: a mechanistic role for oxidation

Pentosidine is a fluorescent Maillard product and crosslink originally isolated from aging human collagen. It could be synthesized from the reaction of L-arginine and L-lysine with pentoses; however, recent data indicate that glucose is also a precursor. Levels are elevated in diabetic collagen and very high levels are found in plasma and collagen from uremic individuals. Because both of these conditions are associated with cataracts, pentosidine was also quantitated in human lens crystallins. It highly correlated with the grade of cataract with up to an 8-fold elevation in the brunescent-type. In the present study, lens crystallins were incubated for 8 days with ascorbic acid (ASA), dehydroascorbic acid (DHA), 2,3 diketogulonic acid (DKG), xylosone, reductic acid and erythrose. All compounds except reductic acid reacted to form pentosidine, with erythrose six-times more reactive than all others. In a longer study, lens crystallins were incubated up to 3 weeks with either ASA, DHA and DKG in the presence or absence of oxygen. Pentosidine formed when crystallins were incubated with all three of these compounds in the presence of oxygen, however, very litte was detected in the absence of oxygen with ASA and DHA, and partial inhibition occurred with DKG. The results suggest that pentosidine may be derived from ascorbic acid through oxidative degradation reactions. In the presence of oxygen, ASA is oxidized to DHA which rapidly breaks down to DKG. In turn, DKG may undergo a further decarboxylation reaction to form pentose precursors of pentosidine. The importance of this reaction in vivo is not understood, but may play a role in understanding pentosidine formation in complications of diabetes, uremia and aging.

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Formation of cross-linking between N^{α}-acetyltrypthophan molecules through the reaction with hexanal

Proteins undergo various deteriorative changes such as polymerization and browning on the reaction with secondary oxidized lipids. These changes accompany damages of tryptophan and other amino acid residues. We examined the products from the reaction of N^{α}-acetyltryptophan (Ac-Trp) and hexanal which is a major secondary product of lipid peroxidation.

Four Ac-Trp derivatives were isolated with HPLC after exposure of solid Ac-Trp to hexanal vapor. They were identified as 1-[N^{1}-(N^{α}-acetyltryptophano)]-1-(N^{α}-acetyltryptophan-2-yl)hexane (Trp-HL-1 a, Trp-HL-1 b), 1,1-bis[N^{1}-(N^{α}-acetyltryptophano)]hexane (Trp-HL-2) and 1,1-bis(N^{α}-acetyltryptophan-2-yl)hexane (Trp-HL-3). Hexanal became a cross-linker between two Ac-Trp molecules. Trp-HL-1 a and -1 b were diastereomers. When the reaction was done in the presence of cation such as N^{α}-acetyllysine, two other Ac-Trp derivatives (Trp-HL-4 a, Trp-HL-4 b) were separated in adition to cross-linking compounds. They were identified as diastereomers of N^{α}-acetyl-1-(1-hydroxyhexyl)tryptophan. Trp-HL-4 a and -4 b were assumed to be intermediates of cross-link formation. Trp-HL-1 and Trp-HL-3 readily became brown when they were spotted on a silica gel thin layer plate. The characteristics of Ac-Trp derivatives found in this study indicate that tryptophan residues are responsible for the polymerization and browning of proteins caused by oxidized lipids.

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Enzymatic metabolism of Maillard intermediates

The Amadori rearrangement compound, the product in the early stage of the Maillard reaction of proteins with glucose, is known to be degraded into 3-deoxyglucosone (3-DG). 2-Oxoaldehydes such as methylglyoxal (MG) and 3-DG were reported to be highly reactive and cytotoxic compounds. We have obtained the evidence that 3 DG inhibited the DNA replication of cells. Glucose-derived pyrrole formed during Maillard reaction have been reported to be detected in vivo. The pyrrole is considered to be formed from 3-DG and free amino groups in proteins. The experiments of intravenous and oral administration of ^{14}C-3-DG to rats were shown that the absorbed 3-DG was not biologically utilized by the rats, but was rapidly excreted in the urine as 3-deoxyfructose (3-DF). Metabolizing activities of the crude extracts prepared from porcine organs were examined using MG and 3-DG as substrates. NAD- and NADP-dependent 2-oxoaldehyde dehydrogenase activity was detected in liver, kidney, small intestine and lung. On the other hand, NADH- or NADPH-dependent 2-oxoaldehyde reductase activity was detected in all porcine organs in which liver and kidney contained higher activity of NADPH-dependent ones than the other organs. The reductases which catalyze the reduction of 3-DG to 3-DF and MG to acetol, were purified and characterized from porcine liver and kidney. In addition, the NADPH-dependent 2-oxoaldehyde reductase was extensively distributed in chicken, rat and plant tissues. The reductase is speculated to prevent the advanced stage of the Maillard reaction as a self-defence enzyme.

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Antimutagenic effects of amino acids-sugars browning reaction products

The antimutagenic effect of Maillard browning products (MRPs) prepared by heating three sugars (fructose, glucose and xylose) and four amino acids (arginine, glycine, lysine and tryptophan) at 100 °C for 10 hr was evaluated by *Salmonella*-microsome assay against some mutagens. The highest extent of browning was found in the MRPs of sugars-lysine and xylose-amino acids. The MRPs of xylose-amino acids and sugars-tryptophan showed stronger antioxidative activity and reducing power than other combinations. No mutagenicity was found in all MRPs to *Salmonella typhimurium* TA 98 either with or withour S 9 mixture. Most MRPs strongly inhibited the mutagenicities of 2-amino-2-methylimidazo (4,5-f) quinoline (IQ), 3-amino-1,4-dimethyl-5 H-pyridol(4,3-b)indol(Trp-P-1) and 2-amino-6-methyldiprido(1,2-a : 3′,2′-d)imidazole(Glu-P-1) toward TA 98 in the presence of S 9 mixture, especially the MRPs of sugars-tryptophan and xylose-amino acids. However, the MRPs of fructose-glycine and fructose-arginine enhanced the mutagenicity of Trp-P-1. The antimutagenic effect of MRPs to IQ, Glu-P-1- and Trp-P-1 as well correlated to their antioxidative activity and reducing power. The mutagenicity of benzo(a)pyrenen also moderately inhibited by most MRPs, while its mutagenicity was increased by some MRPs such as glucose-arginine, fructose-arginine and fructose-glycine. In addition, the mutagenicity of aflatoxin B 1 was remarkedly enhanced my MRPs, except xylose-tryptophan. MRPs might have bifunctional properties of mutagenicity and antimutagenicity in certain cases. The antimutagenic mechanism of MRPs was also discussed.

Tryptophan

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Comparative hypotensive effects of D & L-tryptophan, 5-hydroxyl-L-tryptophan and L-kynurenine in spontaneously hypertensive rats

D and L-tryptophan and L-kynurenine (100 mg/kg, intraperitoneal/2 hours) and 5-hydroxy-L-tryptophan (50 mg/kg, 25 mg/kg and 12.5 mg/kg, intraperitoneal/2 hours) were tested for their effects on arterial (systolic) blood pressure in male ~ 3 months old spontaneously hypertensive rats (SHRs). Reductions in blood pressure were pronounced with all three doses of 5-hydroxy-L-tryptophan; also, both D and L-tryptophan showed significant antihypertensive (-△BP) effects in SHRs, but these were less pronounced as compared to 5-hydroxy-L-tryptophan. The effect of L-kynurenine was much less, i.e., 1/3rd of D and L-tryptophan.

The brainstem amine metabolites [5-hydroxy-tryptamine (5-HT), 5-hydroxyindole-3-acetic acid (5-HIAA), norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were analyzed employing HPLC/electrochemical detection. These neurochemical data revealed that in 5-hydroxy-L-tryptophan treatments, the higher the concentrations of brainstem 5-HT and 5-HIAA, the more pronounced were the blood pressure reductions; the concentrations of NE, DA, DOPAC and HVA were almost identical in these three groups of rats as well as in the controls, and as such, these neurochemicals did not exert any effect on blood pressure reduction. Also, the brainsteim concentrations of NE, DA, DOPAC and HVA did not show any significant difference, compared to controls, in rats treated with 100 mg/kg of D or L-tryptophan, although there were significant increases in brainstem 5-HT and 5-HIAA. Thus, the brainstem conversions, L-tryptophan → 5-hydroxy-L-tryptophan → 5-hydroxytryptamine (5-HT) → 5-hydroxyindole-3-acetic acid (5-HIAA) appear to play a key role in blood pressure reduction in SHRs.

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Distribution of tryptophan in normal and glucose loaded mice

Tryptophan is an essential aromatic amino acid in the mammalian body. It renders the rate-limiting component of protein synthesis in tissues. In addition, active neurotransmitters are formed from tryptophan in the brain. Glucose is generally known to increase the cell permeability to amino acids.

The present study was performed to clarify the distribution of tryptophan by quantitative whole-body autoradiography. Distribution was studied in fed and starved normal and glucose loaded mice. The study also bore a toxicological intention, because latest reports have shown that administration of tryptophan has caused a severe illness, eosinophilia myalgia syndrome (EMS) to which at least 1 500 persons have succumbed.

The results showed that tryptophan was widely distributed in different organs in the body and that glucose loading increased the accumulation. An interesting finding was that there was much more tryptophan in the pancreas than liver (up to seven times). Hypophysis, adrenals (cortex and medulla) werer also places for accumulation. In brain the major cumulation was found in the hippocampus and pineal gland. High concentrations were seen in the gastrointestinal tract and bone marrow.

It is concluded that glucose enhance the penetration of tryptophan to certain tissues. The possible active role of pancreas in accumulation of tryptophan and the high cumulation of tryptophan to endocrinological glands need further research. High concentrations of tryptophan in the bone would likely explain eosinphilia and myaglia in the EMS.

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L-tryptophan uptake by luminal membrane vesicles from proximal tubule of rabbit kidney

The characteristics of L-tryptophan transport in luminal membrane vesicles isolated either from pars convoluta or pars recta of rabbit proximal tubule were studied by a rapid filtration technique and by a spectrophotometric method using a potential sensitive carbocyanine dye. In vesicles from pars convoluta, the uptake of L-tryptophan was mediated by a single, Na^+-dependent and electrogenic process (K_m = 1.93 mM). By contrast in pars recta, the influx of L-tryptophan occurred via a dual transport component (K_{m1} = 0.49 mM, K_{m2} = 9.18 mM). Furthermore, it was found that an inwardly directed H^+ gradient could drive the transport of L-tryptophan at low concentrations in vesicles from pars recta both in the presence and absence of Na^+ (K_m = 0.81 mM). Investigation of the coupling ratio by using the "activation method" suggested that 1 Na^+ : 1 L-tryptophan is cotransported in vesicles from pars convoluta. In pars recta, approximately 1 H^+, 2 Na^+ (high-affinity) and 1 Na^+ (low affinity) are involved in the transport of the amino acid. Competition experiments indicated the existence of a common Na^+-dependent transport component for L-tryptophan, L-phenylalanine, L-serine, L-alanine and L-valine in vesicles from pars convoluta. No effect was observed with L-proline and glycine. By contrast, the high affinity, Na^+-dependent transport system localized in pars recta was only inhibited by L-phenylalanine. The test compounds had no effect on the H^+-dependent uptake of L-tryptophan.

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Clinical features of the L-tryptophan (LT) associated eosinophilia myalgia syndrome (EMS)

In November 1989, the Centers for Disease Control (CDC) criteria for EMS were: 1) total eosinophil count > 1 × 10^9/

Symptoms	Number	Signs	Number
Myalgias	82	Fasciitis	51
Oedema	60	Oedema	40
Rash	53	Rash	32
Pruritus	46	Muscle tenderness	32
Muscle cramps	45	Peripheral neuropathy	26
Uyspnea	40	Joint contractures	25
Paraesthesias	36		

Laboratory studies	Number*
Eosinophilia > 1 × 10^9/L	79/88
C-reactive protein	17/25
Aldolase level	35/65
Antinuclear antibody	31/66
Rheumatoid factor	3/38
Electromyogram	37/50

* Positive or abnormal/total tested

L; 2) disabling, myalgias; 3) exclusion of known causes of 1 and 2. 96% of cases consumed LT. In our first 88 LT associated EMS cases, 74 met CDC criteria, 14 did not. Some lacked eosinophilia; other lacked myalgias. Analysis of clinical and laboratory data from these groups revealed both eosinophilia and myalgias are not essential to define a case. We report the clinical spectrum in our 88 patients. 75 were female. LT exposure varied from 2 weeks to 15 years; dose ranged from 200–4 000 mg/day. Patients exhibited these features (see Table on page 160).

Cytopenias (2) and a myelodysplastic disorder evolving into acute leukemia (1) occurred. Skin (35), muscle (49), fascia (40), peripheral nerve (10), lung (3), and heart (2) biopsies revealed mononuclear infiltrates, often surrounding and invading vessel walls, few eosinophils, and an occlusive microangiopathy in most specimens. Fibroplasia was striking in some.

Treatment included withdrawal of LT in all; corticosteroids in most; and, methotrexate, hydroxychloroquine, cyclophosphamide and/or plasma exchange in some. Most responded, the extent being related to severity and tissues affected. The long-term prognosis is uncertain.

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Effect of L-tryptophan and L-5-hydroxytryptophan on the blood pressure of patients with mild to moderate hypertension

Nine patients (6 male and 3 female), ranging in age from 34 to 64 years, with mild to moderate essential hypertension were studied. Blood pressures (supine) were measured weekly for 4 weeks (control period) while the patients were off all medication. Medical histories, physical examination, chest X-ray, liver function test, complete hemogram, urinalysis, plasma creatinine concentration, creatinine clearance, and a Zung Depression Test were carried out on each patient at the end of the control and treatment periods. Following completion of the control period, treatment with L-tryptophan (4 g/day, 1 g every 6 hr) began and continued for 6 to 16 weeks. The initial systolic, diastolic, and mean blood pressures prior to treatment were 154 ± 4 (S.E.), 101 ± 2, and 119 ± 2 mm Hg, respectively. The final systolic, diastolic, and mean blood pressures at the end of the treatment period were 142 ± 2, 92 ± 2 and 109 ± 1 mm Hg, respectively. The mean decrease ($P < 0.01$) in systolic, diastolic, and mean blood pressure were 12 ± 3, 9 ± 2, and 9 ± 1 mm Hg, respectively. Linear regression analysis of blood pressures versus time revealed significant ($P < 0.05$-0.01) negative correlation coefficients for systolic, diastolic or both blood pressures of 8 of the 9 patients. There were no significant adverse effects of therapy. Body weight was not affected significantly by treatment. An additional 8 patients (6 male and 2 female), ranging in age from 40 to 71 years, with mild to moderate essential hypertension were treated with L-5-hydroxytryptophan (800 mg/day, 200 mg every 6 hr) as described above. The initial systolic, diastolic, and mean blood pressures were 158 ± 6, 101 ± 2, and 121 ± 3 mm Hg, respectively. The final systolic, diastolic, and mean blood pressures were 145 ± 3, 91 ± 3 and 109 ± 3 mm Hg, respectively. The mean decrease ($P < 0.01$) in systolic, diastolic, and mean blood pressures were 13 ± 5, 11 ± 3 and 12 ± 4 mm Hg, respectively. Linear regression analysis of blood pressures versus time revealed significant ($P < 0.05$-0.01) negative correlation coefficients for systolic, diastolic, or both blood pressures in 6 of 8 patients. There were no significant adverse effect of therapy. Body weight was not affected significantly by treatment. The results of these sutdies, while of relatively short duration and a limited number of patients, confirm a previous study and suggest that mild to moderate essential hypertension can be treated effectively with either L-tryptophan or L-5-hydroxytryptophan. The similarity of the results with both compounds suggests further that the common mechanism mediating the effect is increased production of serotonin.

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Is xanthommatin formation related with the coloration of human cataractous lenses?

Xanthommatin (XM) is produced from two moles of 3-hydroxykynurenine (3-OH-Kyn), a metabolite and L-tryptophan, through oxidative condensation. The color of XM is similar to that of human brown cataractous lenses. However, the relationship between XM formation and the coloration of human cataractous lenses has not been clarified well. In order to clarify whether or not XM formation is related with the coloration of human cataractous lenses, therefore, we measured visual spectra of both XM bound with bovine serum albumin (BSA) and pigments extracted from human cataractous lenses.

3-OH-Kyn was incubated with BSA under ultraviolet irradiation for 48 h, resulting in the formation of XM-bound BSA. This protein was purified by gel filtration and ion-exchange column chromatography. Lenses were enucleated from eyes of patients with various types of cataracts and solubilized by ultrasonic treatment and trypsin digestion. XM was detected by measuring the difference spectrum of the oxidzed type and reduced one of each sample. When free XM was reduced by ascorbic acid, an absorption maximum was found at 500 nm. When XM bound with BSA was reduced under the same conditions, an absorption maximum was present at 515 nmn. However, no human cataractous lenses showed an absorption maximum at 500 to 515 nm under the same measurement conditions. We further determined both fructosamine, an index of glycation, and absorbance at 450 nm, an index of coloration, in these human lenses since glycation has been proposed to be involved in lens coloration. The level of fructosamine was correlated with that of absorbance at 450 nm ($r = 0.68$).

The present results suggest that glycation plays a more important role in the coloration of human cataractous lenses than XM formation.

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Preventive effect of L-tryptophan on the development of acute, carbon tetrachloride-induced liver injury in rats

L-tryptophan (Trp) is known to stimulate liver protein synthesis in rats and mice. This amino acid is expected to

prevent the development of liver injury with damaged protein synthesis. Although this effect has been examined in experimental liver injury models, it is still controversial. We, therefore, examined whether or not Trp can prevent the development of liver injury in rats treated with carbon tetrachloride (CCl_4).

In male Wistar rats (6 weeks old) with a single i.p. injection of CCl_4 (1 ml/kg body weight), the development of liver injury over 48 h was prevented by a single i.p. injection of Trp (100 mg/kg body weight) 30 min before or 0, 6, 12, or 36 h after CCl_4 treatment. Trp injection at 6 h after CCl_4 treatment caused the strongest preventive effect. But, this effect could not be observed immediately after Trp injection. The activity of tryptophan 2,3-dioxygenase, an index of liver protein synthesis inhibition in CCl_4-induced liver injury, had been reduced since 6 h after CCl_4 treatment. This reduction of activity was prevented by Trp injection at 6, 12, and 36 h after CCl_4 treatment. In addition, this prevention had been observed before the recovery of liver injury could not be detected. Liver calcium ion, triglyceride, and lipid peroxidase levels were increased by CCl_4 treatment, but these increases were prevented by Trp injection.

The present results indicate that Trp can prevent the development of acute, CCl_4-induced liver injury in rats, and suggest that this preventive effect is due to not only the recovery of protein synthesis inhibition, but also other actions such as an anti-oxidative one.

Synthesis

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Asymmetric synthesis of α-amino phosphonic acids

The study of phosphorus analogs of the natural α-aminoacids has accelerated in the past ten years due to the finding of molecules with useful biological activity. In several cases their activity has been shown to depend upon their absolute configuration. These aminophosphonic acids are found as free compounds or in complex structures such as lipids, peptides, proteins or polysachharides.

We have developed a general and efficient method via reaction with electrophiles of Schiff bases prepared from phosphonic analog of glycine and R,R,R or S,S,S 2-hydroxypinan-3-one easily accessible from S,S or R,R α-pinene. The best results were obtained using LDA as base.

In order to prepare functionalized products (phosphonic analogs of chloralanine, homoserine) we have applied the same methodology but other bases (MgDA) or adjuvants (HMPA, crown ether) were necessary to obtain excellent results. Enantiomerically pure phosphonic analog of homoserine was a good precursor for the synthesis of several new aminophosphonic acids which will be described.

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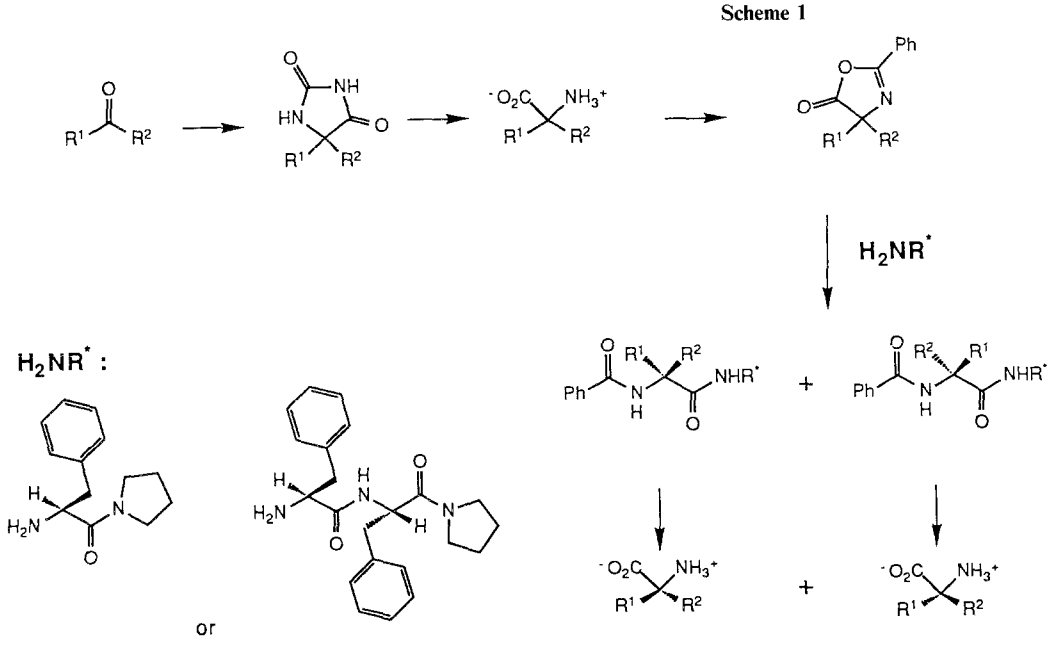
A new efficient method for the preparation of enantiomerically pure alpha,alpha-disubstituted amino acids

Peptides containing alpha,alpha-disubstituted amino acids have been the focus of a large number of studies over

Scheme 1



the last ten to fifteen years due to their interesting stereochemical properties [1, 2]. The majority of these studies have been on Aib (aminoisobutyric acid), the prototype achiral member of this family of non-proteinogenic amino acids. Recently however, more attention has been turned to the conformational effects of chiral alpha,alpha-disubstituted amino acids on peptide secondary structure [3].

To be able to perform a comprehensive structural study on such amino acids, it is necessary to have both enantiomeric forms available and in sufficient quantities. With these two criteria principally in mind, we have developed an efficient and general preparation of alpha,alpha-disubstituted amino acids providing gram quantities of optically pure material [4, 5].

The racemic amino acids are synthesized using the Bucherer reaction starting from the appropriate ketones and proceeding via the hydantoins. The amino acids are then N-acylated, transformed into oxazolines and coupled to the chiral amine. Separation of the resulting diastereomers is performed by flash chromatography on silica gel. Selective cleavage followed by aqueous acid hydrolysis gives the enantiomerically pure amino acid hydrochlorides in high yields (Scheme 1, page 162).

The chiral amines used in this separation are readily synthesized from L-phenylalanine and can be recovered after celavage in good yield.

1. Venkataram Prasad BV, Balaram P (1984) CRC Crit Rev Biochem 16: 307–348
2. Toniolo C, Benedetti E (1988) Biochemistry, 225–230
3. Wipf P, Heimgartner H (1988) Helvetica Chimica Acta 71: 258–267
4. Stierli F, Obrecht D, Heimgartner H (1984) Chimia 38: 432
5. Spiegler C, Obrecht L, to be published

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Thia-analogs of amino acids – the N,S-acetal type analogs

Thia-analogs of amino acids are isosters of naturally occurring amino acids. Due to their chemical nature they can be classified in three groups:

1. *Thioether compounds* (e.g. 4-thialysine = S-aminoethyl-L-cysteine
2. *Carbonic acid derivatives* (e.g. thiaglutamine = S-carbamoyl-L-cysteine),
3. *Aldehyde derivatives* (N,S-acetals: e-thialysine, 3-thiaphenylalanine, 3-thialeucine and the thiaprolines: thiazolidine-4-caboxylic acid an thiazolidine-2-carboxylic acid.

While the chemistry and biochemistry of the thioether analogs is the topic of a previous review (P. Hermann, in: Organic Sulfur Chemistry, ((Eds. R. Kh. Freidlina, A. E. Skorova)), Pergamon Press, Oxford 1981, pp 51–67), the N,S-acetal type analogs got more attention only recently.

This report deals with the synthesis of these amino acid analogs (including stereoselective enzymatic reaction steps), their utilization as substrates for the estimation and hydrolases, their behaviour in biochemical systems and the results of conformational calculations.

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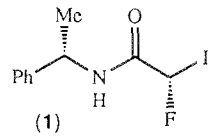
The asymmetric synthesis of α-fluoroglycine derivatives

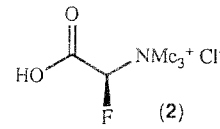
A series of α-fluoroglycine derivatives have been synthesized, via a simple asymmetric route from (S,S)-N-(1-phenylethyl)-1-fluoro-1-iodoethanamide (**1**). The crystal structure of this key intermediate has enabled us to deduce the absolute configuration of the fluorinated carbon in all our chiral products.

Further elaboration of one of these α-fluoroglycyl derivatives has led to the formation of a free α-fluoro amino acid [α-fluorobetaine (**2**)] and work is currently underway on the coupling of this to other amino acids.

Free α-fluoroglycine itself is inherently unstable, and so N-acylation using standard peptide chemistry is not viable. To address this problem, we are developing a new strategy using the piperazine-2,3,6-triones (**3**); for example the (*L*)-phenylalaninamide derivative (**3a**) can be alkylated on the imide nitrogen, and initial results with compound (**1**) suggest that protected dipeptides such as (**4**) are accessible using this methodology. Eventually this could provide a route to many different dipeptides containing the fluoroglycyl residue.



(**1**)



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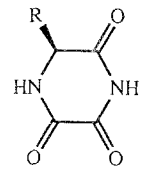
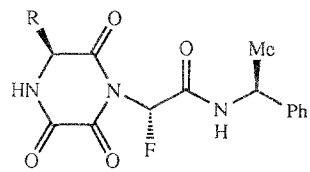


(**3**)
(**3a**) ; R = CH_2Ph



(**4**)

Other nitrogen-containing groups have been introduced α to the fluorine (e.g. azide, cyanide, phthalimide and succinimide) and attempted modification of some of these polyfunctional compounds has led to unexpected and interesting chemistry.

Our results demonstrate for the first time that α-fluorinated α-amino acids and peptides are viable synthetic targets, and such molecules should be of considerable biological interest.

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Stereoselective synthesis of chain extended analogues of quisqualic acid – potential agonists and antagonists for excitatory amino acid receptors

Of the two L-glutamic acid receptor subtypes, namely NMDA and non-NMDA (AMPA/kainate), the NMDA receptor has been the subject of the most detailed pharmacological and biophysical studies. This is, in part, due to the availability of potent and selective antagonists and to the important roles in neurotransmission and neurodegeneration played by NMDA receptors.

Acyclic phosphona-α-amino acids D-AP 5 (1, n = 2), D-AP 7 (1, n = 4) and the cyclic piperazine analogue of AP 7 (2, CPP), developed by Watkins, are amongst the most potent and selective NMDA antagonists; the cyclic analogue is 4–10 times more potent than the acyclic derivative. The prominent structural feature in these derivatives is the separation of the second acidic function from the α-carbon by a chain of 3–5 carbon atoms. We reasoned therefore that the chain extended analogues (3, n = 2 and n = 3) of D-QUIS (3, n = 1) will be novel, target molecules as NMDA antagonists. These analogues have the two acidic functions separated by a chain length comparable to that of AP 5 and AP 7. We report the synthesis of both D-homo-(3, n = 2) and D-hishomoQUIS (3, n = 3). The corresponding L-enantiomers were also synthesized as potential, novel agonists for the L-QUIS receptor [1].

The general synthetic methods followed our published synthesis of QUIS and its analogues [2]. L-Aspartic acid was the starting material for L-homoQUIS and D-glutamic acid the starting material for D-bishomo-QUIS. Strict attention was paid to the retention of chiral integrity throughout the synthetic procedures. L- and D-QUIS analogues were tested on the well-characterized QUIS-sensitive glutamate receptor found on locust (*Schistocerca gregaria*) muscle. They were less active than L-QUIS. The D-enantiomers which are potential antagonists of NMDA receptors will also be tested in rat brain. The L- and D-isomers were prepared and tested as part of our on-going structure-activity profile studies into the mapping of the agonist binding site. These compounds will provide new leads for insecticides and for pharmaceutical agents.

[We thank the UK SERC for a studentship (to N.H.).]

1. Boden P, Bycroft BW, Chhabra SR. Chiplin J, Crowley PJ. Grout RJ, King TJ, McDonald E, Rafferty P, Usherwood PNR (1986) Brain Res 385: 205–211
2. Bycroft BW, Chhabra SR, Grout RJ, Crowley PJ (1984) JCS Chem commun, 1156–1157

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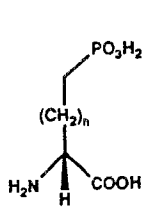
Asymmetric synthesis of Tryp-Leu dipeptide lactam isosteres: conformational constrained analogs of cholecystokinin tetrapeptide (CCK_4)

The introduction of conformational constraints into peptides has been an important approach toward understanding the bioactive conformation of these molecules. The information gained could lead to the discovery or design of peptide analogs with omproved activity, selectivity and bioavailability. A dipeptide lactam with a carbon bridge connecting the α-center of the first residue to nitrogen of the second moiety will fix the amide bond in the *trans* configuration as well as introduce further constraints into the peptide in which this fragment is incorporated. During the course of our work to design mimics of the peptide neurotransmitter/neuromodulator cholecystokinin (CCK), we prepared a constrained derivative of CCK_4 with the N-terminal dipeptide Trp-Met replaced by a Trp-Leu dipeptide γ-lactam isostere. As it was desirable to test both diastereomers, we initially pursued the synthesis in racemic form at the quaternary lactam center via a known procedure, followed by separation of diastereomers prior to testing. One of the diastereomers exhibited higher affinity for the CCK-B receptor than the parent peptide while the other was inactive. Therefore, an asymmetric synthesis of the Trp-Leu dipeptide lactam was developed. The absolute configuration of quaternary lactam center was determined unambiguously by single X-ray crystal of a key intermediate. The chiral syntheses of Trp-Leu dipeptide lactam constrained analogs and their biological acitivty will be presented.



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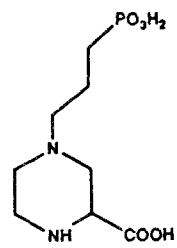

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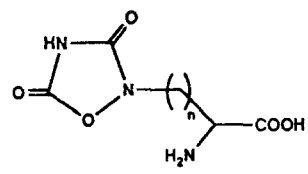

(3)

C. K. Acosta[1], M. L. Bahr[1], J. E. Burdett Jr[1], J. W. Cessac[1]. R. A. Martinez[1], D. M. Peterson[1], P. Narasimha Rao[1], H. K. Kim

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Synthesis of unnatural amino acids for incorporation into GnRH antagonists

Recent structural modifications of the potent GnRH antagonist [N-Ac-D-2-Nal[1],D-*p*-ClPhe[2], D-3-Pal[3], D-Arg[6], Trp[7], D-Ala[10]]GnRH have led to the development of the new GnRH antagonist, Antide[N-Ac-D-2-Nal[1],D-*p*-ClPhe[2],D-3-Pal[3], Niclys[5], D-Niclys[6], I-Lys[8], D-Ala[10]]GnRH.. Antide causes negligible histamine release while maintaining almost equipotent anti.ovulatory activity (AOA) in the rat.

Our laboratory has synthesized all of the unnatural amino acids incorporated into the structure of Antide. Presented are the details of the efficient preparative synthesis of these unnatural amino acids, in particular, the D and L isomers of N^{α}-*t*-BOC-N^{ε}CBZ-N^{ε}-isopropyllysine dicyclohexylamine salt (N^{α}-BOC-N^{ε}-ZIlys DCHA salt), the D and L isomers of N^{α}-*t*-BOC-N^{ε}-nicotinollyssine (N^{α}-BOC-Niclys), β-(3-quinolyl)-DL-alanine (3-Quinal) and its resolution into its D an L isomers and the *cis* and *trans* isomers of 4-Aminocyclohexyl-D-alanine (D-4-Achal).

The syntheses of the lysine derivatives were carried out by selectively forming the N^{ε}-CBZ derivatives from the lysine copper complexes followed by N^{α}-*tert*-butyloxycarbonlylation. In the case of the N^{α}-BOC-N^{ε}-ZIlys isomers, catalytic hydrogenolysis in basic acetone gave the corresponding N^{ε}-isopropyl derivatives which were subsequently converted to the N^{ε}-CBZ DCHA salts by standard methods. Catalytic hydrolysis of N^{α}-BOC-ZIlys in methanol followed by reaction with *p*-nitrophenyl nicotinate gave the D and L isomers of N^{α}-BOC-N^{ε}-Niclys. The synthesis of 3-Quinal was accomplished by base condensation of diethyl acetamidomalonate with 3-chloromethylquinoline hydrochloride. A mixture of the *cis* and *trans* isomers of D-4-Achal was prepared by nitration of D-phenylalanine followed by catalytic reduction. Separation of the isomeric mixture was carried out by preparative HPCL and characterization of their stereochemistry was established *via* proton NMR.

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Enantioselective syntheses of ^{13}C-, ^{15}N- and ^{2}H-isotopomers of L-lysine and their incorporation into the membrane protein bacteriorhodopsin

Bacteriorhodopsin (bR) is a membrane protein, that carries out a light-driven proton translocation from the inside to the outside of the *Halobacterium halobium* cell. The proton gradient generated is used in the cell for the synthesis of ATP and, as a consequence, in the production of metabolic energy. BR contains one molecule retinal bound as "Schiff's base" to the ε-amino group of Lys-216. Assuming that the retinylidene Schiff-base NH^+ is directly involved in the proton pumping mechanism, it is important to consider i) the C = N configuration of the different retinylidene-intermediates which occur in the photochemical reaction and ii) the conformation of the lysine side chain which is connected to each of the retinylidene-intermediates. To test a hypothetical model (based on a computer modelling study) concerning the orientation or the retinal within the folded protein, the present study was undertaken. An approach involving different spectroscopic techniques (solid-state NMR, laser-Raman and FT-IR spectroscopy) has been used [1]. Selective information can be obtained by using bP containing isotopically labelled Lys-216.

Syntheses of isotopically labelled lysines: In the presentation an asymmetric synthesis of various isotopomers of L-lysine is described. The synthesis is based on a general method starting from the bislactim-ether of cyclo(d-Val-Gly)[2] and simple labelled reagents like $K^{13}CN$, $K^{13}C^{15}N$, $^{13}CH_3CN$, $Br^{13}CH_2COOH$, $BrCH_2{}^{13}COOH$, and $NH_2{}^{13}CH_2COOH$. Using this route isotopically labelled lysines were prepared in ca. 45% yield based on the labelled starting compounds. It is now possible to specifically substitute each carbon and nitrogen by a heavier isotope on a gram scale. Moreover, via this route many multiply ^{13}C- and ^{15}N-labelled lysines are accessible. Finally, the preparation of [ε-$^{2}H_2$]-L-lysine and [R ε-$^{2}H_1$]-L-lysine is described. The isotopomers of L-Lys were characterized using various spectroscopic techniques (e.g. NMR and mass spectrometry). The configuration of the reaction product was assigned by optical rotation and by means of enzymatic hydrolysis experiments with LysOMe (ca. 99% e.e.).

Preparation of isotopically labelled R: Halobacterium halobium was grown in a synthetic medium in which the isotopically labelled lysine (0.25 g/l) was substituted for the lysine normally present. After 6 days 35 mg/l protein was isolated. A method was developed for checking the level of incorporated labelled lysine in bR. In this method the following steps can be distinguished: i)acid (or alkaline) hydrolysis of the protein, ii) derivation of the liberated amino acids (TFA-Bu), iii) separation of the derivatized amino acids based on gas-chromatography and iv) determination of the ratio labelled/unlabelled $(TFA)_2$-Lys-O*n*Bu by means of mass spectrometry.

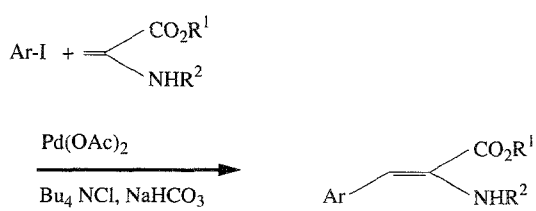
1. Lugtenburg J, Mathies HA, Griffin RG, Herzfeld J (1988) Trends Biochem Sci 13: 388
2. Schöllkopf U (1983) Top Curr Chem 109: 65

A.-S. Carlström, T. Frejd

Chemical Center, Organic Chemistry, Lund Institute of Technology, Lund, Sweden

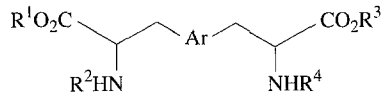
Synthesis of bis(amino acids) via a palladium-catalyzed coupling reaction

(Z)-Didehydroamino acid derivatives can be prepared by a palladium-catalyzed coupling between aryl iodides and 2-amidoacrylates [1].



The didehydroamino acid derivatives are useful synthetic intermediates; they can e.g. be hydrogenated asymmetrically to produce unusual aromatic amino acids in optically active from [2].

The coupling reaction has now been used to synthesize some aromatic bis(didehydroamino acid) derivatives, starting from diiodo or bromo-iodo aromatic compounds [3]. When two different olefins with orthogonal protecting groups are used, bis(amino acid) derivatives 1, suitable for use in peptide synthesis, are obtained after hydrogenation. Using this approach, we have synthesized an analogue of the peptide antibiotic Biphenomycin.



1

Curiously, when 1,2-diidobenzene is subjected to the reaction, neither mono- nor bis-coupling occurs. This is due to the formation of an organopalladium intermediate which prevents palladium from taking further part in the reaction.

1. Carlström AS, Frejd T (1989) Synthesis, 414
2. For a recent review see: Williams RM (1989) Synthesis of Optically Active α-Amino Acids. Pergamon Press
3. Carlström AS, Frejd T (1991) J Org Chem 56: 1289

Z. Zhang, S. Lam, G. Malikin, A. Karmen

Albert Einstein College of Medicine, Bronx, NY, USA

A novel polymeric reagent for synthesizing FMOC-L-proline amino acids for chiral HPLC in CU(II)-L-histidine methylester eluants

The development of a novel FMOC-L-proline polymeric reagent for derivatizing amino acids is described. The new polymeric reagent is highly reactive. Significant improvements in the ease of use and speed of derivatization of strong nucleophiles, such as amines and amino acids, and soft nucleophiles, such as alcohols and thiols, were realized. This study demonstrates the utility of the solid phase reagent for chiral HPLC of amino acids. In the off-line mode, the derivatization is assomplished by mixing the solid phase reagent with a sample of the amino acid in acetonitrile at room temperature for 10 minutes. Since the derivatization is facilitated under mild conditions, reacemization of amino acid is practically eliminated. Despite FMOC-L-proline-D,L-amino acids are diastereoisomers, the separation of the optical isomers is not trivial. Several procedures, including reversed phase chromatography, for resolving the optical isomers of FMOC-L-proline amino acids were explored. However, excellent resolution of all the D and L enantiomers of natural amino acids was achieved by using CU(II)-L-histidine methylester eluants. The separated derivatives were detected in low manograms by fluorescence at 315 nm with excitation at 275 nm.

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Amino acid synthesis from D,L-5-monosubstituted hydantoin derivatives

The great advantages of producing optically pure amino acids from the corresponding D,L-5-monosubstituted hydantoin derivatives are (i) the fact, that racemic substrates can be directly and totally converted to the optically pure products, and (ii) the wide substrate specificity of the enzymes involved in this process.

In our previous investigations a process was developed for the production of L-tryptophan (L-Trp) from the corresponding D,L-5-indolymethylhydantoin (D,L-5-IMH) [1, 2]. It was shown that 3 inducible enzymes are involved in this reaction: a L-hydantoinase, a hydantoin-racemase, and a N-carbamoyl-L-amino acid amidohydrolase. Using resting cells of *Arthrobacter* sp. DSM 3 747, which were grown before in presence of the not degradable inducer D,L-5-indolylmethyl-N-3-methylhydantoin, 10 g l^{-1}, D,L-5-IMH could be converted totally to L-Trp within 1–2 h after optimization of the reaction parameters.

Starting from these investigations, we describe the production of various unnatural L-amino acids by this method in this work.

1. Syldatk,Mackowiak, Höke, Gross, Dombach, Wagner (1990) J Biotechnol 14: 285–302
2. Gross, Syldatk, Mackowiak, Wagner (1990) J Biotechnol 14: 363–376

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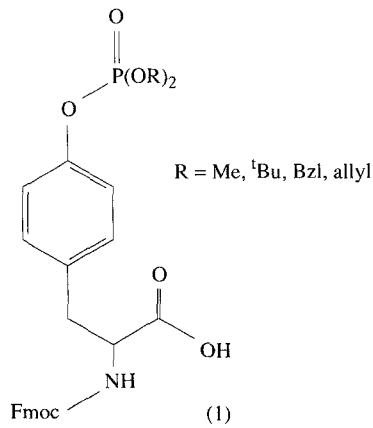
F. Hoffmann-La Roche Ltd., Basel, Switzerland

The synthesis of phosphotyrosine building blocks suitable for solid phase peptide synthesis

Building blocks for the phosphotyrosine amino acid have been prepared. These are of the type (1) and can be directly incorporated in Fmoc-solid phase peptide synthesis using 1,1,3,3-tetramethyl-2-(2-oxo-1(2H)-pyridyl) uronium tetrafluoroborate (TPTU) or related coupling reagents. Such derivatives were prepared by phosphite-triester phosphorylation of suitably protected Fmoc-Tyr-OH using a variety of



(1)

phosphoramidite reagents following by appropriate oxidation procedures. The choice of R protecting groups allows for felxibility in selecting the final deprotection reagent in peptide syntehsis.

J. Pospíšek, T. Barth

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague, Czechoslovakia

Tertiary leucine and neopentylglycine preparation of diastereoisomers

Investigation of the relationship between the peptide structure and its biological activity resulted in the preparation of non-proteogenic amino acids exhibiting some properties different from those of natural amino acids. We synthesized racemates of tertiary leucine with a bulky tert. butyl group and highly hydrophobic neopentylglycine. For the preparation of peptides it was essential to obtain both amino acids as optically active isomers. The resolution was carried out both chemically by means of crytallization of the brucine salt and enzymatically. Resolution with brucine if relatively simple as formyl derivatives of both amino acids form easily salt and the D-form of that crystallized preferentially. Phenylacetyl derivatives of both amino acids can be cleaved enzymatically by penicilinamidohydrolase taking advantage of the stereospecifity of the enzyme which cleaves the protective group only from the L-form. This enzyme can be used either in solution or in an immobilized form. We employed new carriers, chemically treated glass with a high density of pores and binding ligands for the immobilization of penicilinamidohydrolase. Resolution under these conditions yielded gramm amounts of the L.forms and highly purified protected D-form of both amino acids. Purity of the both stereoisomers was checked by chromatography on chiral plates (Meckerey - Nagel) and by optical rotation.

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Further insights into the synthesis of high specific activity tritium and C-14 labelled peptides by asymmetric reduction of dehydroamino acids

The assembly of peptide hormone analogs containing structurally and/or optically unnatural amino acid residues has become an important area of research in the pharmaceutical industry. At Syntex one such compound, Synarel® (Nafarelin acetate), has recently received FDA approval and is currently marketed for the treatment of endometriosis. The problems we encountered in the synthesis of Synarel-^{3}H led to the development of a strategy for the synthesis of high specific activity, specifically labelled peptides which is completely general, affords products with predictable specific activities, and can be used to prepare both tritium and C-14 labelled amino acids and peptides.

Our approach originally involved the synthesis of a dehydroamino acid, 2,3-dehydro-3-(2-naphthyl)-alanine, followed by reduction with tritium gas in the presence of Wilkinson's catalyst and enzymatic resolution (H. Parnes and E. J. Shelton, J. Labelled Compds. Radiopharm. XXI, 263, 1984). This methodology was refined with the use of the chiral homogenous catalyst (S,S)BPPMRh^{+} which gave D-3-(2-naphthyl)-alanine-(2,3-^{3}H) in 97% optical purity directly and at a specific activity of 44 Ci/mmole. The corresponding C-14 amino acid was prepared in a similar fashion from the requisite C-14 labelled dehydroamino acid. The 50% loss in yield associated with enzymatic resolutions was thus avoided.

(S,S)BPPMRh^{+} as well as other chiral homogenous catalyst were subsequently used to prepare D and L high specific activity tritiated and C-14 amino acids from their respective dehydroamino acid precursors. Optical purities in excess of 95% were obtained for amino acids in both the aromatic and aliphatic series. The labelled amino acids thus obtained were used in solution phase syntheses to prepare a variety of peptide analogs with predictably high specific activities. In addition to the synthesis and purification of these compounds, the merits and flexibility of our strategy will be discussed.

Lecture

R. A. Nichols

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Protein phosphorylation

Protein phosphorylation is an essential means by which biochemical processes are regulated within cells. It involves the reversible enzymatic transfer of the terminal phosphate of ATP to distinct amino acid acceptor sites in certain proteins, usually to serine or threonine residues, but also to tyrosine residues. The enzymes which accomplish this phosphate transfer are termed protein kinases. The presence of phosphate on a protein often results in a change in the activity of said protein; in other words, phosphorylation is a kind of "molecular switch". As a result, attention has been focused on the roles for protein phosphorylation events in signal transduction. Many protein kinases that phosphorylate proteins on serine and threonine residues have been found to be activated in response to physiological stimulation. In addition, signal transduction often utilizes tyrosine phosphorylation, which was originally discovered in oncogenic elements and later, found to be component to certain growth factor receptors and even to growth factors themselves. Substantial levels of tyrosine phosphorylation activities have also been observed in adult brain, strongly implicating it in complex intercellular interactions.

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Central Institute of Nutrition, Rehbrücke,
Federal Republic of Germany

Effects of ketoanalogues of essential amino acids

Ketoanalogues of essential amino acids are used in the conservative therapy of chronic renal failure to form essential amino acids by transamination and to spare nitrogen. But we doubt on the basis of metabolic changes and the stoichiometry weather this is the mean mechanism of action.

Therefore we studied metabolic parameters in 5/6-nephrectomized rats 6 months after operation when a chronic uremia was developed and feeding of different protein- and with ketoanalogues supplemented diets.

Fractional rates of protein synthesis were determined in rats of each feeding group using the 6-hour infusion method of ^{14}C-labelled amino acids and defining the tissue free amino acids as precursor pool and using directly measured values for the exponential rate constant for the rise to plateau levels of specific radioactivities in tissues. Specific radioactivities were determined by semipreparative HPLC (ion-exchange chromatography with postcolumn ninhydrin derivatization).

The main results (see table) demonstrated the advantageous role of ketoanalogues e.g. on N-balance. The mechanism remains to be elucidated but the results of e.g. the fractional rates of protein synthesis and excretion rates of 3-methylhistidine confirmed an influence on the regulation of protein degradation.

Diet	8% casein	1% casein	1% casein * KA
N-balance (mgN/d)	0.2	−78.1 ($P < 0.01$)	2.3
Protein synthesis rates (%/d)	4.22	5.02 (n.s.)	4.98
3-methylhistidine excretion (μmol/d)	2.82	4.22 ($P < 0.01$)	2.76

F. Ciardella[1], F. Romano[1], M. Massitelli[1], A. Sagripanti[1] A. Carpi[2]
[1] Clinica Medica I, [2] Centro Regionale di Medicina Nucleare, Università di Pisa, Italy

Effects of a low protein diet and a mixture of essential amino-acids and keto analogues on serum total cortisol and acidosis in patients with chronic renal failure (CRF)

The low-protein diet (LPD), and a mixture of essential amino-acids (EAAs) and keto-acids (KAs) reduces high cortisol (C) in CRF (Walser, 1989; Ciardella, 1989). Since acidosis induces hypercorticism (Mitch, 1986) and enhances protein catabolism in CRF, we studied the effects of LPD, EAAs, and KAs in 17 pts (9 M, (f, 18–49 yrs aged) with CRF (sCr: 7.4 ± 2.5 mg/dl; CRcl: 8.9 ± 7.0 ml/min).

We measured sCR, CRcl, serum urean (sU), capillary pH, HCO_3-, BE, C and body weight (BW) beore and after 2–12 months of diet. LPD supplied 0.3 g/Kg/day protein from vegetables, 35 KCal/Kg/day and 5–6 mg/Kg/day inorganic phosphorus; EAAs and KAs were given according to Zimmermann's formula (1979).

After treatment sCr (7.4 ± 2.5 to 7.8 ± 3.2 mg/dl 9 and CRcl (8.9 ± 7.0 to 9.1 ± 7.3 ml/min) were not significantly modified. Metabolic acidosis improved: pH rose from 7.32 to 7.38 ($p < 0.001$), HCO_3- from 17.6 ± 2.6 to 21.9 ± 2.2 mEq/1 ($p < 0.001$) and BE from −6.2 ± 2.3 to −2.1 ± 2.0 ($p < 0.001$). C fell from high-normal values (275.9 ± 125.9 to 184.2 ± 86.1 ng/ml; $p < 0.005$). At the 2nd month of LPD we observed a fall of C into the normal range in several cases with very high basal levels. sU fell from 99.3 ± 44.1 to 59.5 ± 37.1 mg/dl ($p < 0.001$). BW did not change (69.2 ± 17.0 to 68.6 ± 9.8 Kg).

Metabolic acidosis was then corrected together with hypercorticism and protein catabolism. LPD corrects uremic acidosis thanks to the abundant alkali rich foods (it is a vegan diet) (Giovannetti, 1989): this fact lowers C. Furthermore, EAAs/KAs mixture decreases C directly, by lowering B-EP secretion in CNS, and then, ACTH in the pituitary (Ciardella, 1989). Protein catabolism can be reversed thanks to the fall of C, but also to the effect of branched-chain-keto-acids (BCKA) and amino-acids. These stimulate powerfully insulin and testosterone secretion (Strauch, 1989).

Keto-leucine exerts an "insulin like" effect on peripheral target cells (Adibi, 1984).

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[1] Clinica Medica I, [2] Centro Regionale di Medicina Nucleare, Università di Pisa, Italy

Effects of single amino acids and their analogues on metabolic and hormonal derangements in chronic renal failure (CRF): the role of the diet of the supplementation

Low protein diet (LPD) plus essential acids (EAAs) and keto analogues (KAs) improve the polyendocrinopathy in CRF. LPD, due to the low phosphorus intake, lowers PTH (Ciardella, 1989). Other endocrine-metabolic effects may be consequence of PTH fall, or direct effect of EAAs/KA mixture.

20 male CRF pts (sCr: 8.1 ± 1 mg/dl, CRcl: 9.9 ± 0.6 ml/min, 20–36 yrs) were recruited and followed LPD, receiving also the EAAs/KAs tablets, for 2–12 months. Every 2nd mont we measured: sCR, CRcl, endocrine-metabolic features. sCr

(8.1 ± 1.0 to 8.4 ± 0.2 mg/dl), and CRcl (9.9 ± 0.6 to 10.0 ± 0.7 ml/min) did not change significantly. Serum lipids improved. Triglycerides (190.1 ± 82 to 143.4 ± 5 mg/dl; $p < 0.001$), cholesterol (210.0 ± 11.2 to 168.1 ± 10.1 mg/dl, $p < 0.001$), Apo-B (157.3 ± 11.2 to 60.2 ± 12.3 mg/dl, $p < 0.005$) fell. HDL-cholesterol (32.2 ± 4.5 to 51.1 ± 3.3 mg/dl, $p < 0.05$), Apo-A I (108.3 ± 190.6 ± 11.3 mg/dl, $p < 0.001$) increased. Clucose tolerance test improved. Hormones improved. PTH (265.8 ± 45.2 to 51.7 ± 25.2 pg/ml, $p < 0.001$), beta-endorphin (B-EP) (62.2 ± 23.6 to 42.1 ± 11.1 pg/ml, $p < 0.005$), TSH (4.1 ± 0.8 to 1.9 ± 1.0 uU/ml, $p < 0.001$), cortisol (237.0 ± 21 to 110.0 ± 11.2 ng/ml, $p < 0.005$), GH (7.2 ± 1.2 to 1.8 ± 1.0 ng(ml, $p < 0.001$), TT4 (5.4 ± 1.2 to 12.7 ± 2.1 µg/dl, $p < 0.001$), FT4 (8.1 ± 0.6 to 12.5 ± 1.1 pg/ml, $p < 0.05$), TT3 (60.4 ± 12.2 to 181 ± 21.3 ng/dl, $p < 0.005$), FT3 (2.7 ± 0.6 to 5.1 ± 1.1 pg/ml, $p < 0.05$) increased.

These endocrine-metabolic effects are independent from changes in renal function. LPD lowers PTH, and this favours gonadal, thyroid functions and triglycerides levels. The EAAs/KAs mixture acts independently on glucose tolerance (lysine, k-phenyl-alanine and methionine stimulate insulin secretion (Mackowiak, 1989). k-leucine exerts and "insulin-like" action (Strauch, 1989). Most hormones are directly influenced by threonine, methionine, tryptophan (Yogokoshi, 1989), Tyrosine favours thyroid function (Ciardella, 1988). Phenylalanine influences neuroamines, active on hormones. Also B-EP fall due to the enhancement of monoaminergic CNS pathways. Branched-chain-keto-acids exert a potent protein anabolic stimulus.

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Amino acids and the kidney

The kidney plays a major role in amino acid metabolism and nutrition. Amino acid reabsorption by the renal tubules recovers about 70 g/day whilst a proportion of total kidney proteins and plasma proteins are degraded in the proximal cells and resynthesized. Measurement of arterio venous differences for free amino acids across rat kidney show that glycine and citrulline are removed and serine and arginine are added to the circulation, whilst glutamine may be taken up in large quantities to maintain acid-base homeostasis. The total amino acids reaching the kidney is also dependent on the dietary supply, and this also affects the haemodynamics. High intakes increase both plasma flow and glomerular filtration rate either by direct effect of specific amino acids (e.g. arginine) or by mediators such as glucagon and prostaglandins. This effect may contribute to the accelerated progression of renal failure associated with high protein intakes.

In chronic renal failure, some abnormalities of amino acid metabolism, can be attributed directly to loss of functional kidney with impaired ability to synthesise or catabolise certain amino acids, proteins or hormones. Secondary effects such as uraemia, acidosis or hormonal imbalance may adversely influence amino acid metabolism both in the kidney and elsewhere. Factors contributing to nutritional depletion such as loss of appetite, or losses of amino acids or proteins during dialysis therapy, will exacerbate existing abnormalities. However, whilst we can rationalise aberrations of the amino acid profiles in muscle and plasma, reduced protein synthesis and increased catabolism, the regulation of protein and amino acid metabolism remains a problem.

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Plasma free aminoacid profiles and nutrition proteins in chronic renal failure (CRF); effect of dialysis treatment

A well preserved nutritional status is beneficial in chronically uremic patients for slowing the pace of deterioration of renal function and delaying the need for dialysis therapy. The purpose of this study was to assess the nutritional profile of 10 patients in a steady state of advanced CRF and of 15 patients with terminal renal failure immediately prior to their first hemodialysis session (JO) and 7, 14, 45, 60 days post start of dialysis. Patients were 18 to 65 with total plasma proteins > 60 g/l. Plasma concentrations of amino acids (AA), nutrition proteins, apolipoproteins A 1 and B and somatomedin were evaluated. Non inflammatory reaction was evaluated by determination of alpha-acid glycoprotein and C reactive protein. The data (mean ± 1 SD) were compared with mean values of 15 healthy individuals. In steady CRF patients the initial essential AA (EAA) plasma concentrations were found decreased (valine: 206 ± 59/280 ± 95, leucine: 128 ± 21/162 ± 64 µmol/l, $p < 0.01$) and those of ornithine, citrulline, proline, 3-methyllhistidine, cystine were increased. Visceral protein concentrations are in the lower range of normal values except for retinol binding protein (RBP) whose concentration is very high. In predialysis patients (JO) almost all EAA are decreased particularly methionine ($p < 0.04$) and branched chain AA (BAA $p < 0.01$). Concentrations of nutrition proteins and non EAA are similar to that found in stable advanced CRF, but inflammatory proteins are increased and RBP is higher. At J 7 all AA are increased, at J 14 alanine, glycine, proline and at J 30 methionine and BAA are decreased. At J 60 methionine is back to normal and almost all AA are in the lower range of normal. BAA concentrations stay low. These results are in agreement with literature (specially BAA and 3-methylhistidine). Hemodialysis does not restore the plasma AA concentrations to normal and possibly enhances protein catabolism. The increase of the inflammatory marker CRP may reflect the adverse effect of dialysis, possibly in relation with membrane. The AA profiles reported in this study appear less modified than that found in a previous one carried out in our laboratory.

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Glutathione and its associated enzymes in peripheral blood cells in chronic renal failure

Many products which accumulate in the blood of patients suffering from chronic renal failure might be expected to affect the level of glutathione (GSH) and its metabolizing

enzymes. Recently, we have shown a significant negative correlation between the degree of renal insufficiency and increase in intracellular reduced glutathione level. To elucidate why such high GSH level is present in the cells in uremia, the activities of glutathione reductase (E.C.1.6.4.2) and glutathione S-transferase (E.C.2.5.1.18) were estimated in isolated erythrocytes and lymphocytes from patients with varius degree of chronic renal insufficiency.

Patients were divided according to their creatinine clearance into four groups. Blood samples from healthy adults were used as controls. The erythrocytes and lymphocytes were separated by the density gradient procedure of Boyum (1968). The supernatant solution, obtained by centrifugation of cells lysate for 15 min at 10.000 g, was used for the biochemical assays. Reduced glutathione concentration was determined by the fluorometric method of Hissin and Hilf (1976); glutathione reductase (GR) activity was measured by the method of Carlberg und Mannervik (1985) and glutathione S-transferase activities by spectrophotometric procedure of Habig (1974).

Reduced glutathione levels and glutathione reductase and glutathione S-transferase activities in erythrocytes and lymphocytes from uremic patients were higher compared to the corresponding controls. There is also significant negative correlation between the degree of renal insufficiency and GSH levels as well as GR and GST activities. Hemodialysis did not significantly alter erythrocyte and lymphocyte GSH content and activities of its associated enzymes. The increase in glutathione reductase and glutathione S-transferase activities of blood cells in uremia may be a protective mechanism for the cells due to the accumulation of toxic, oxidizing wastes in the blood as a result of the uremic state. This view is supported by the results of in vitro experiments, which have shown that GR and GST activities of normal lymphocytes are increased when incubated with serum from uremic patients.

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Amino acid as well as polyols and methylamines accumulated in rat kidney in dehydration

During antidiuresis cell in the renal inner medulla contain large amounts of the polyols sorbitol and myo-inositol and the methylamines glycerophosphorylcholine (GPC) and glycine-betaine to adjust the intracellular osmolality to the extracellular hyperosmolality. Although the accumulation of these four major organic osmolytes in the renal inner medulla of the dehydrated animal has been a consistent finding, the role of another organic osmolyte, amino acid, in osmoregulation in the kidney remains controversial. In the present study, renal responses of four major osmolytes and amino acids to 4 days dehydration were investigated. Using two HPLC systems, we determined the amino acid content as well as that of other osmolytes in the renal tissues along the corticopapillary axis from the control and dehydrated rats. Four major osmolytes and taurine were significantly higher in the inner medulla of the dehydrated rats as compared with the control rats. Phosphoethanolamine and taurine in the cortex of the dehydrated rats were significantly higher than that of the control rats. For the content gradients of osmolytes along the corticopapillary axis, not only polyols and methylamines but also taurine, glutamine, aspartic acid, alanine and proline increase monotonically from the cortex to the inner medulla in the dehydrated rats, on the other hand, phosphoethanolamine decrease from the cortex to the inner medulla. As for four major osmolytes, we can confirm previously reported patterns in antidiuresis in greater detail. In conclusion, not only four major osmolytes but also amino acid play a salient role in the osmoregulation on the kidney.

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Plasma amino acids are highly dependent on acid base balance in uremic rats

Plasma amino acid concentrations are frequently used in order to assess the nutritional status of uremics. The impact of changes in acid base balance have only rarely been considered as a relevant influencing factor. The aim of the study was to assess the impact of changes in acid base balance in uremic rats on plasma amino acids and keto acids. 42 SD rats were rendered uremic by 5/6 nephrectomy. 19 rats received a normal (18%) protein diet while 23 rats received 8%-protein diet. Following a random cross over design all rats received either $CaCo_3$ or CaC_{12} in the drinking water for a fortnight each. The application of $CaCl_2$ resulted in a significant drop in blood-pH: 18% protein: $CaCO_3 = 7.19$ $CaCl_2 = 7.11$; 8% protein $CaCO_3 = 7.26$ $CaCl_2 = 7.09$. Comparabe changes occurred with respect to the base excess: 18% protein: $CaCo_3 = -6$ $CaCl_2 = -10$; 8% protein $CaCO_3 = -3$ $CaCl_2 = -12$. During the $CaCl_2$-period the animals of the 18%-protein group exhibited a significant increase of the amino acid his, while ile, pro and val droped. In the 8% protein diet a significant increase of the following amino acids was observed: asp, his, ile, leu, phe, thr, trp, val, cys, lys and tyr. With respect to the keto acids of val, ile and leu no signficant changes were observed. Thus our data demonstrate that the plasma amino acid concentration is highly dependent on the acid base balance and the protein intake.

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Regulation of taurine transporter activity in LIC-PK I cells: role of protein synthesis, exocytosis and protein kinase C activation

Taurine transporter activity increases after culture renal epithelial cells are exposed to taurine-free medium for 24 hours and decreases after the cells are incubated in high (500 μM) taurine. To elucidate mechanisms potentially invovled in regulating β-amino acid transporter activity, the role of RNA transcription, protein synthesis, protein import (trafficking),and protein kinase C (PKC) activation on the control of taurine transport was examined in the continuous proximally derived LLC-PK_1 renal cell line. Inhibiting RNA

transcription with actinomycin D did not alter the up- and down-regulatory adaptive responses. Inhibiting protein synthesis with cycloheximide prevented the increased taurine transport in response to taurine-free medium and the decreased taurine transport following exposure to high taurine. Colchicine prevented the response to taurine-free medium but had no effect on the response to high medium taurine. Exposure of confluent cell monolayers to the active phorbol esters phorbol 12-myristate 13-acetate (PMA) and phorbol, 12,13 dibutyrate (PDBu) resulted in reduced taurine uptake. Seen within minutes of exposure, the effect was not observed in the presence of the inactive phorbol 4 alpha. This inhibitory action was blocked by staurosporin, an inhibitor of PKC. Treatment of cells with the diacylglycerol (DG) kinase inhibitor R 59022, which results in increased intracellular DG, a natural stimulant of PKC, also inhibited taurine uptake, further revealing a specific effect of PKC activation. Exposure of LLC-PK_1 cells to the active phorbol esters PMA and PDBu increased taurine efflux in LLC-PK_1 cells loaded with taurine whereas incubation with the inactive phorbol 4-alpha did not alter efflux. Reduced taurine uptake is possibly a result of enhanced efflux in response to PKC activation. PKC might exert its action on taurine transport and efflux by phosphorylation of a membrane-bound protein or a regulatory protein involved in the control of β-amino acid transport.

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Effect of extracellular volume expansion on renal hyperfiltration induced by amino acid (AA) infusion in healthy man

The effect of extracellular volume expansion on renal hyperfiltration induced by AA infusion was investigated in 5 healthy young volunteers (23–25 years of age) who underwent two different infusion tests:

A) Infusion of a mixture of AAs (Freamine 8.5%) at a rate of 145 mg/min/1.73 sqm for 2 hours. The total amount of the solution infused was 200 ml and 17.4 gr of AAs.

B) Infusion of the same AAs solution, as the previous test, together with a saline wiht NaCl 45 mmol/l. The infusion rate was 15 ml/min for a 2 hours period and a total of 2 000 ml infused.

The following data were collected before and during the infusions: clearance of polyfructosan S as a measure of GFR, PAH clearance for renal blood flow and Filtration Fraction (GFR/Renal plasma flow).

	AA		AA + NaCl	
	before	during	before	during
GFR (sd) ml/min/1.73 m^2	102(8)	120(17)*	103(13)	100(13)
RBF(sd) ml/min/1.73 m^2	847(91)	929(114)	787(116)	754(174)
FF(sd)	0.22(0.3)	0.23(0.3)	0.23(0.5)	0.24(0.5)

* $p < 0.05$ (ANOVA)

The infusion of saline, 45 mmol/l, inhibits the hyperfiltrative response induced by AA infusion. Such response seems, therefore, influenced by changes of the extracellular volume.

Enantiomers

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Succinimide-mediated deamidation, racemization and peptide bond cleavage in aging αA-crystalllin

Alpha-crystallin, the major structural protein of almost all vertebrate eye lenses, undergoes a multitude of posttranslational modifications, both enzymatic and nonenzymatic.

Spontaneous deamidation of asparagine residues is thought to be one of the most prevalent nonenzymatic modifications in aging proteins. Studies have shown that deamidation occurs via formation of a succinimide intermediate. The propensity to form imide rings in native proteins largely depends on the local conformation around the residue. Deamidation by hydrolysis of the imide ring also leads to isomerization and pronounced racemization. Recently, the specific sites of in vivo deamidation in αA-crystallin have been identified. In bovine αA-crystallin, Asn-101 is subject to deamidation, whereas the only other asparagine residue, Asn-123, is not. Suprisingly, it was found that also the in vivo peptide bond cleavage after Asn-101 occurs via succinimide formation.

Imide ring formation can similarly occur at aspartic acid residues, leading to isomerization and racemization. Also this process takes place in aging αA-crystallin. Among the 14 aspartyl residues in αA-crystallin, only Asp-151 exhibits pronounced age-dependent racemization. Both in vivo and in vitro racemization studies indicate that this extreme and site-specific racemization proceeds via succinimide formation. Asp-151 resides in a flexible carboxyl terminal extension of α-crystallin, which would facilitate imide ring formation. Interestingly, in vivo truncation of αA-crystallin also occurs at this specific residue, and no enzyme has been found to cause such cleavage. It is therefore tempting to speculate that this peptide bond cleavage likewise proceeds via a similar process of intramolecular ring formation.

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Occurrence of D-amino acid in foods

The presence of D-amino acids has been detected in many natural systems such as biological fluids, tissues or foods thanks to the availability of very efficient methods of enantiomeric recognition.

Thus, the biological, biochemical, nutritional and eventually toxicological role of these "unusual" enantiomers has became a more and more fascinating subject and it warrants further study.

In the last few years we have been involved in the study of the mechanism of chiral recognition by both gas-chromatography (G.C.) and high-performance liquid chromatography (H.P.L.C.) and we have developed several methods (G.C., H.P.L.C. and T.L.C.) for performing enantiomeric separations of D,L-amino acids.

The combined use of these methods allowed us to approach the analytical determination of very complex mixtures such as foods and to cross-check the results obtained wich accuracy and avoiding pytfalls due to a single method or to derivatization provedures.

Here, we wish to discuss the occurrence of D-amino acids in several foods, either of microbiolgical origin or eventually generated during heating treatment (short or long, mild or severe), alkali treatment or radiation (low or high power microware treatment).

The results will be presented in connection with technological treatments: *heat*, with particular reference to the correlation temperature-duration of the treatment (UHT-milk); *alkali/acidic* treatments; *cooking* procedures (normal and microwave ovens); *radiation* treatments, fermentation procedures (yogurt, cheese, wines), preservation conditions.

In dairy products D-ala, D-glu and D-asp comes from the laysis of the bacterial cell walls or are products of natural fermentations, whereas in other cases (fruit-milk) they can be considered as markers of spoilage or of contamination.

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The presence of free D-alanine, D-proline, D-serine and D-glutamine in mouse serum and kidney

The sera and kidneys of mutant (ddY/DAO$^-$) mice lacking D-amino acid oxidase, and the sera of normal control (ddY/DAO$^+$) mice were investigated for the presence of free D-amino acids by high performance liquid chromatography (HPLC). Free amino acids were prepared from the sera and kidneys, and separated by two-dimensional thin layer chromatography. HPLC analysis of ten neutral amino acids demonstrated the presence of D-isomers of Ala, Pro, Ser and Gln in all the samples examined. The mutant mouse sera exhibited high contents (D/L ratio) of D-isomers; 10.2% fora Ala, 5.2% for Ser and 4.6% for Pro. The contents were moderate in the mutant mouse kidneys, and were low in the normal sera.

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Racemization of neutral amino acids in mouse tissues

It is generally believed that mammals neither synthesize nor possess D-amino acids whereas microorganisms, some insects, marine invertebrates and higher plants contain D-amino acids. Only D-aspartic acid is known to exist in human metabolically stable proteins. However, we have doubted if D-amino acids had been unable to be detected because of the function of D-amino acid oxidase in mammalian tissues. D-amino acid oxidase (EC 1.4.3.3) if a flavoenzyme widespread in many animal tissues, and catalyzes oxidative deamination of neutral free D-amino acids to produce 2-oxo acids in vitro.

A D-amino acid oxidase-lacking mutant mouse strain was used to clarify this point. Twelve neutral free amino acids extracted from the mouse kidney and serum were derivatized with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent) to diastereomers, separated by two-dimensional thin layer chromatography, and analyzed by reverse-phase high performance liquid chromatography for the resolution of D- and L-isomers. As the result, D-isomers of alanine, proline and serine were found. The highest D/L ratio was 0.09. The value was obtained with alanine in the sera of the mutant mice. As far as we know, this may be the first communication on the presence of free neutral D-amino acids in mammals. The D-amino acids seem to be neutral products of mice. Investigations in racemization of tissue proteins are in progress.

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D-Aminoaciduria in mutant mice lacking D-amino-acid oxidase activity

D-Amino-acid oxidase (EC 1.4.3.3) is present in the kidney, liver and brain of all higher animals. However, since its substrates, D-amino acids (stereoisomers of naturally occurring L-amino acids), are rare in higher animals, its physiological role is not known.

Mutant animals are useful for the study of the functions of enzymes. Mutant ddY/DAO$^-$ mice lacking D-amino-acid oxidase activity manifest a specific renal aminoaciduria. Large amounts of D-methionine and D-alanine are present in their urine. The D-methionine was determined to be derived from the DL-methionine supplemented in their diet, since an elimination of DL-methionine from the diet decreased the urinary methionine to normal levels. D-Methionine sulfoxide and/or D-methionine sulfone present in the urine was considered to be the metabolite(s) of the dietary D-methionine. The D-alanine was concluded to come from cell walls of intestinal bacteria, since oral administration of antibiotics reduced the urinary alanine to normal levels. D-Serine and D-proline were also detected in the urine but they were of neither dietary nor intestinal bacterial origin.

These results indicate that the ddY/DAO$^-$ mice are unable to metabolize the D-amino acids due to a lack of D-amino-acid oxidase, so they excrete them in urine, causing D-aminoaciduria. At the same time, these results also indicate that, in normal mice, the D-amino acids are constantly metabolized by this enzyme. Metabolism of D-amino acids would be one of the physiological roles of D-amino-acid oxidase.

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Regulation of L-alanine-initiated germination of *Bacillus subtilis* spores by alanine racemase

Bacterial spores are metabolically dormant and highly resistant to various physical and chemical agents. Once spores germinate, they lose their resistance irreversibly and differentiate metabolically and morphologically to the vegetative state. Spore germination is initiated by exogenous amino acids, sugars, nucleosides and inorganic ions depending on species. *Bacillus subtilis* spores can be effectively germinated by L-alanine and the germination is inhibited competitively by D-alanine; an alanine receptor is present on the spore. The dormant spores also show alanine racemase activity. In this paper the relationship between spore germination and alanine racemase was examined.

Germination by L-alanine was inhibited by diphenylamine and not by D-penicillamine, whereas alanine racemase was *vice versa*. Apparent affinity of L-alanine to the germinant receptor was more than 1 000 times higher than that to the racemase. Germination could be observed at 20 to 55 °C, and its optimum was 43 °C, while the racemase activity was observed at 20 to 90 °C and its optimum was 65 °C. the germinability of the spores at 37 °C preincubated with L-alanine at 70 °C for 1 h decreased without denaturation of the germinant receptor. Germinability increased in the presence of D-penicillamine when the concentration of L-alanine was low and that of spore was high. Thus, germination may be affected indirectly by the racemase which reduces the concentration of L-alanine and increased that of D-alanine. The results indicate a possible role of alanine racemase in the regulation of germination in the environmental conditions that do not favor vegetative growth, such as high temperature and high population in limited nutrients.

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D-amino acids in marine invertebrates

Previously we have shown that D-amino acids occur in about 40% of 90 species of marine invertebrates form 9 phyla as detected by a D-amino acid oxidase (DAO) based assay. We also confirmed using chiral HPLC that D-amino acids occur in 15 species from 8 phyla in concentrations ranging from 0.6 mM D-aspartate (D-asp) in the gut of the sipunculid *Siphonosoma rotumanum* to 66 mM D-alanine (D-ala) in the adductor muscle of the mollusk, *Mya arenaria*. In other experiments, we have shown that ^{14}C-D-alanine is transported and metabolized in a variety of species. In an attempt to identify the enzymes involved, we assayed tissue from selected species for DAO, D-asp oxidase and D-ala and D-asp aminotransferase. No activity was detected except for DAO in the tunicate, *Halocynthia pyriformis*. Chiral HPLC and coupled enzyme assays have demonstrated racemase activity in *Mya* and selected other species. These data suggest that an important pathway for D-amino acid metabolism in marine invertebrates involves enzyme mediated racemization.

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D-amino acid and aging

Recently, biologically uncommon D-aspartic acid (D-Asp) has been found in proteins which have little turn over, such as tooth, brain, and eye lens of aged mammals. Aspartic acid (Asp) is the most easily racemizable amino aicd. Therefore, it has been explained that D-Asp might form by racemization in the metabolically inactive tissues during the natural aging process. In the lens protein, D-Asp has been found in only water insoluble (WI) fraction of aged mammal lens. We have isolated a D-Asp-containing protein from WI fraction of bovine lens and confirmed that the origin of this protein was α-crystallin of main component of water soluble (WS) fraction.

In the present study, we report that the racemization of Asp in WS proteins proceeds like in WI fraction of aged human lens. The WS proteins contain, α, β, γ-crystallins. We isolated these crystallins and analyzed D/L ratio of Asp of these proteins. It was found that α-crystallin (D/L of Asp = 0.20) was one of the easily racemizable component of WS protein. α-Crystallin is composed of 2 types of polypeptide chain αA and αB, which show approximately 55% amino acid sequence homology. We separated αA and αB-crystallin by reverse phase chromatography. The racemization of Asp proceeds in both αA and αB crystallin, however, the D/L ration of Asp was higher in αA than in αB-crystallin. To determine D/L ration of individual Asp residues in αA-subunit, αA-subunit were digested with trypsin. We found two Asp residues pronounced racemization in αA-crystallin.

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Porcine pancreatic lipase-catalized enantioselective hydrolysis of N-protected amino acid methyl-esters

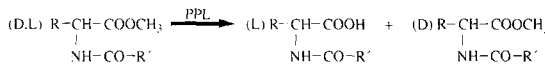
Enantioselective hydrolysis of racemic methyl esters of N-benzoyl phenylalanine and phenylglycine has been carried out by using crude porcine pancreatic lipase (Triacylglycerol lipase, EC 3.1.1.3) PPL, from Sigma (Tip II), as a hydrolytic enzyme.

The enzymatic hydrolysis of the corresponding N-protected racemic methyl esters were carried out in 0.1 M KH_2PO_4 aqueous solution at 30 °C and pH = 7.7 Reaction rates can be followed by the amount of NaOH 1 M necessary to neutralyze the liberated amino acid maintaining pH at the indicated constant value. In all cases studied, the reaction end point is obtained when pH is not changed during two or three hours. Then, 50% of racemic methyl ester was hydrolysed to the N-protected L-amino acid with very high stereoselectivity. Enantiomeric excess was determined from unhydrolysed esters by ^{1}H n.m.r spectroscopy (200 MHz) in the presence of Eu $(hfc)_3$ (20–30% mol/mol), was always 100%.

The preferential hydrolysis of the L-enantiomer was directly determined from the optical rotation values of both

enantiomers. They were obtained by successive extraction with chloroform form the reaction mixture. First, the unhydrolysed D-methyl esters were directly extracted. The corresponding N-protected L-amino acids were obtained from the acidified reaction mixture.

According to the results, these PPL-catalized enantioselective hydrolysis of N-protected amino acid methyl esters, can be considered as a part of a general process (claimed for a spanish patent, P 8903384m in 1989):



where R y R' can be not only aromatic substituents but also aliphatic ones. Thus, enzymatic resolution of racemic 3-cyclohexyl-N-carbonylcyclohexylalanine methyl esters was also obtained with 100% enantiomeric excess.

Peptides

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In vitro effect of Leu-OMe on polymorphonuclear and mono-nuclear cells

Polymorphonuclear (PMN from porcine) and macrophage (MO from mice) were treated in vitro with Leu-OMe or Leu-Leu OMe (1.5–5,0 mM). Investigation of the mechanism of uptake indicated that the entry of both derivatives occures either by simple diffusion or by a carrier system. These methylesters have a more lipophilic character than the non-ester forms of Leu or Leu-Leu. Our investigation on the fate of both derivatives after entry into PMN and MO showed a very rapid hydrolysis of ester and the peptide bounds of dipeptides. Within short period of time (1–5 min) the intracellular accumulation of Leu was found. An unexpected finding was that the saturation of Leu and Leu-OMe in PMN and in MO was proportional to the formation of Leu-Leu-OMe dipeptide.

The fact that cytoplasm was the site of hydrolysis for the derivatives was indicated by lack of ester-hydrolase activity in the incubation medium and in the plasma membrane. After longer time of incubation some in the intracellularly accumulated Leu gradually reentered the media. In the ultrastructural picture of the treated cells the formation of a large amount of vacuoles was observed. The viability of the treated cells did not show any significant alteration (based on the trypan blue exclusion test). The damage of the plasma membrane could be not excluded (based on the absence of lack of the LDH activity in the culture media). The damage of plasma membrane is based on the observation that of fMLPh and increasing exocytosis of various lysosomal enzymes (lysozyme and nonspecific neutral proteinases).

The present experiments provid evidence for the uptake of both methylesters (Leu-OMe, Leu-Leu-OMe) by the porcine blood granulocytes and by mice macrophages.

Incorporation of amino acids was blocked in treated MO. The Leu-OMe is used for depletation of the amino acid pools of MO (as glutamic acid and arginine).

(*Abbreviations: Leu* Leucine; *Leu-Ome* Leucine-0-methylester; *Leu* Leu; *Leucil* leucine; *PMN* polimorphonuclear; *MO* macrophage; *fMLPh* formil-methyonil-leucil-phenylalanine; *LDH* lactate dehydrogenase.)

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Amino acid residues in the quantitative structure-activity analysis of biologically active peptides: descriptors of biological properties and methods of their use

Biological activities of peptides are frequently linked to the structural features of side chains in individual positions of the peptide backbone. These "site-oriented" features can usually be assessed from physico-chemical properties of the corresponding free amino acids, or of short peptides containing this amino acid. Simple descriptors of structural features derived in this way are prerequisites for structure-activity studies. Four types of descriptors are used in peptides: 1) descriptors derived from the concept of linear free energy relationships (Hammett, Taft etc. constants); 2) descriptors derived from empirical parameters (hydrophobicity, rate and equilibrium constants, chemical shift, etc.); 3) descriptors obtained by transformation of experimental data (polarizability, electrical effect substituent constants, etc.); 4) descriptors derived from molecular models (accounting for molecular shape, bond properties, bulkiness, electrical charge, etc.). Some of them have recently been listed [Fauchère et al Int J Peptide Protein Res. 32: 269; Pliska, Charton, J Receptor Res 11 (*in print*)].

Two modes of their use in the studies of quantitative structure-activity relationships (QSAR) are conceivable. 1) When a single position of the peptide backbone is changed, multiple regression applied to several descriptors (and/or their functions) are applicable. 2) In cases of multiple-site substitutions, linear analysis can be used only when the effects of substituents in individual positions upon the biological activity in question are mutually independent (Free, Wilson, J Med Chem 7: 395). This participation rule may be quite frequently valid for peptides (Pliska, Heiniger, Int J Peptide Protein Res 32: 520). Then, the computed "fragmental (side-chain) contributions" can be treated as "fractional" biological activities; common correlation methods can be applied to them (Pliska, Experientia 34, 1190; Pliska, Charton, see above).

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Solid-phase C-terminal sequencing of peptides

Sequential C-terminal amino acid analysis seemed to be an established procedure, as a counter part of the Edman's N-terminal sequencing. However, still poor recovery of the C-terminal amino acids in the reaction in homogenous solution suggested further improvement of the method.

In the present study, N-terminal amino acid was fixed to the controlled pore glass (CPG) beads by covalent bonds, and then the C-terminal amino acid was activated (by treating with acetic anhydride), coupled with thiocyanate to form thiohydantoin (TH) ring at the C-terminus. Then the C-terminal amino acid was split as a TH derivative. Thus split TH was extracted with ethyl acetate 3 times, and the solvent was evaporated, and the residue was dissolved in the mobile phase solvent mixture for HPLC. Hydrolytic condition of TH derivative from the C-terminal amino acid was examined: the most satisfactory conditions were found to be that the mixture was heated in the presence of 2 N HCl at 60° for 2 h, or by microwave (2450 MHz) for 10 min.

So far obtained results in the heterogeneous mixture are not satisfactory in terms of the recovery of the C-terminal TH, and improvement of the recovery and further steps are under progress.

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Syntheses and structure-activity relationships of thymosin β_4 family

Four peptides related to thymosin β_4 family and its six fragments were synthesized by the solution method. Among them, the four peptides related to thymosin β_4 family and its two fragments were found to have restoring effect on the impaired blastogenic response of T-lymphocytes isolated from uremic patients, but the other four fragments had no effect. Studies on the structure-activity relationships suggest that the tricosapeptide moiety corresponding to amino acids 16–38 of thymosin β_4 is found to be an important moiety on impaired immunological deficiency.

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Studies on the anticoagulant and antithrombotic effects of D-MePHE-Pro-Arg-H (GYKI 14 766)

D-MePHE-Pro-Arg-H (I) synthesized in our Institute represents a special novel type of reversible highly specific inhibitor of thrombin having both anticoagulant and antithrombotic activity.

In vitro effect was examined on human fibrinogen, human plasma and whole blood. Coagulation parameters and platelet aggregation were recorded.

In vivo anticoagulant activity was studied in white New Zealand rabbits and beagle dogs by oral and parenteral administration. Changes in the coagulation parameters and platelet aggregation were dose dependent. There was no discrepancy between the in vitro and in vivo effects.

Antithrombotic activity was investigated by ateriovenous shunt model in rabbits, venous and arterial thrombosis models in rats after subcutanous and oral administration. The (I) significantly reduced the weight of the thrombus formed.

Bleeding time was measured by a standardized ear method in self controlled experiments. At the maximum of the anticoagulant action showed no significant prolongations in therapeutic doses.

Based in the results presented we may conclude that (I) can highly be recommended for clinical use.

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A method of sequencing glycated peptides

We are interested in the development of a method of identification of positions of N^{ε}-(1-deoxyfructosyl)lysine (FL) residues in sequences of glycated peptides. Borohydride reduction of an FL residue produces a relatively stale N^{ε}-(1-deoxyhexitolyl)lysyl residue which undergoes the Edman degradation, but does not give a detectable PTH derivative [Shilton, Walton, J Biol Chem. (in press)]. The goal was to produce a stable modification of FL that could be extracted and identified by HPLC. Under acidic conditions, 1,2-aminoalcohols are oxidized by periodate at smaller rates than 1,2-diols. This accounts for the observation that, on oxidation of N^{α}-acetyl-FL with 0.1 M periodate at pH 3, there is limited cleavage of the C-1-C-2 bond; more of the N^{α}-acetyl derivative of N^{ε}-carboxylmethyl-lysine (CML) is formed than N^{α}-acetyl-lysine. This has been exploited in the development of a sequencing method, in which an FL residue of a glycated peptide is similarly converted to a CML residue. The PTH derivative of CML, formed at the appropriate stage of Edman sequencing, is extracted from the sequencer disc and identified by HPLC.

(Supported by the Canadian Diabetes Association. This work is part of a project undertaken with Dr. R. Badoud., Nestlé Research Centre, Vevey, Switzerland.)

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Studies on the optimal immunization schedule of experimental animals using a peptide antibiotic as the antigen

Although a number of immunization schedules have been reported, it has been difficult to show that any one scheme is better than another. We have undertaken a series of studies of various immunizing conditions to establish the optimal immunization schedule using a peptide antibiotic viomycin (VM) as the common antigen and BALB/c mice as the animals. We developed two kinds of highly sensitive and accurate enzyme immunoassay methods for mouse IgG and mouse anti-VM antibody to evaluate the immune response of mice studied under various immunization conditions. The effecto of the immunogen dose on the immune responses of mice was first studied. It was found that the optimal dose depend on the kind of adjuvant used: 10 μg was the optimal immunogen dose using the adjuvant of either Freund's complete adjuvant (FCA) or aluminium gel. While 200 μg was the optimal dose with Freund's incomplete adjuvant (FICA). Concerning to the booster conditions, the combination of FCA and FICA as the primary and booster adjuvants, respectively, was found the most optimal for the immune response of mice. The effect of trial times and interval periods of boosters was greatly influenced by the immunogen dose. With the optimal antigen dose, the greater number of booster shots given the less was the response of total and specific IgGs. With low antigen dosage, the more boosters were given, the higher was the response level of specific IgG. It was also found that age was a more important factor than sex, and

the mice aged 8 weeks yielded the highest response of both total and specific IgGs. The choice of the injection rout was found to be a very important factor in immunization and intraperitoneal injection was the most optimal among the four rout studied. The effect of the controlled release of the antigen was also studied.

Analysis

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Determination of the optical purity of amino acid residues in synthetic peptides by means of gas chromatography/chemical ionization mass spectrometry (GC/CIMS). A preliminary report

It is well known that the acid hydrolysis of a peptide will cause partial racemization of the resultant free amino acids as well as the exchange of the α-hydrogen atoms with the hydrolysate. This paper describes the technique on how to take advantage of the ease of the α-hydrogen exchange for the determination of the real optical purity of amino acid resides in synthetic peptides. Here a deuterated acid is used for the hydrolysis and the "artifically" racemized amino acid(s) is tagged with deuterium. A mass spectrometer coupled to a gas chromatograph with a chiral column is utilized by means of selected ion monitoring (SIM) technique in the chemical ionization mode to quantify only non-deuterated amino acid molecules, thus avoiding the racemization contribution occurring during the hydrolysis. Experimental parameters are discussed in details and the analytical results are shown based on optical purity determination of amino acid residues of various types in synthetic peptides.

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A fast and simple in situ method for the determination of the absolute configuration of amino acids and amino acid esters using the chiroptical properties of their EU(FOD)$_3$ complexes

The NMR shift reagent, Europium (III)-tris-(1,1,1,2,2,3,3)-heptafluoro-7,7-dimethyl-4-6-octanedione [Eu(fod)3], complexes efficiently with α-amino acids and α-amino acid esters in chloroform. These 1 : 1 metal to ligand complexes exhibit characteristic circular dichroism (CD) spectral pattern in the 350-250 nm region. A fast and simple procedure (also in microscale) has been worked out which utilizes the signs of these CD bands for the determination of the absolute configuration of the α-carbon atom in situ. In L-series, a positive CD band is observed at around 310 nm and a negative one in the 290-280 nm region. The CD spectra of the Eu complexes of the D-isomers are mirror images of those of the L-configuration. An empirical rule is being proposed. The reaction parameters for the optimal complex formation are discussed in detail.

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A ninhydrin reagent for postcolumn derivatization of amino acids

Ion exchange chromatography with ninhydrin detection is still the method of choice for the analysis of amino acids in physiological samples.

Conventional procedures involve methylcellosolve which may be problematical because of its properties as an irritant and to form peroxides limiting of ninhydrin reagents.

Therefore we used ethanol as a solvent in the ninhydrin reagent.

The composition was optimized using Box-Hunter-models for statistic experiment planning. On this basis a new and reliable reagent for postcolumn ninhydrin detection systems for amino acid analysis was developed.

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Separation of amino-acids using ion-paired reversed-phase high-performance liquid chromatography with special reference to collagen hydrolysate

Collagens form about one third of all animal proteins. Its study includes the analysis of its characteristic amino-acids: proline, hydroxyproline, lysine, hydroxylysine. HPLC offers an interesting device to separate these amino-acids, mainly if associated with on-line radiometric detection for the determination of radiolabelled amino-acids in the case of metabolism studies.

To avoid pre or post-column derivatization which may be poorly quantitative in the case of the hydrolysate of unpurified samples, we developed an ion-paired reversedphase chromatography using a C 8 column (econosphere C 8 5 U, length: 250 mm, ID: 4.6 mm from Altech Ass.) and an elution carried out with an acetonitrile gradient in heptane-sulfonate solution. A direct detection at 210 nm was used. Nineteen amino-acids were separated within 40 min. The lag time was 7.3 min between hydroxyproline and proline, and 6.9 min between hydroxylysine and lysine. In the case of radiolabelled amino-acids, there was a linear correlation (r = 0.99) between HPLC and ion exchange chromatography.

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High resolution chromatography of *tert.*-butyldimethylsilyl amino acids

The biological, biochemical clinical and nutritional importance of amino acids, has stimulated efforts in the research for suitable analytical methods. In the past thirty years, a number of interesting procedures, both for gas chromatography and liquid chromatography have emerged, but only a few have made their way through the proof of everyday practive.

The high sensitivity, resolution power, speed of analysis, and hyphenation possibilities make capillary gas chromatography a technique of choice for analysis where those requirements must be met. The preparation of volatile derivatives of amino acids present some challenging questions, due to the variability of their functionality. The classical N,O(S)-acyl amino acid esters, althoug very popular, present difficulties in quantitation of histidine and arginine. Moreover, they do not allow simultaneous analysis of dicarboxylic amino acid amides such as glutamine and asparagine, which may have a special importance. The recently introduced N,O(S)-*tert.*-butyldimetylsilyl (TBDMS) derivatives present very interesting chromatographic and spectroscopic characteristics: they allow simultaneous analysis of amino acids and amino acid amides, have very typical mass spectra (CI and EI), and possess adequate FTIR responses in GC-FTIR experiments. A survey of these properties and some application examples are given.

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Quality of performance of amino acid analyses

Results from two years of an external quality assessment scheme for quantitative amino acid analyses have been presented [1]. The conclusions of that report were that the performance of amino acid analyses was suboptimal at normal and low concentrations and that it depended less upon method and choice of instrumentation than upon other postulated factors such as staff expertise.

Continuation of the scheme has allowed results to be analysed from 30 distributions to 25 laboratories. Mean coefficienty of variation for all amino acids at each distribution ranged from 13% to 60%, the differences relating to the nature of samples and the concentrations of amino acids in them; there was no clear trend in the overall results during five years of operation. Mean coefficients of variation for individual amino acids over all the distributions varied from 15% for glycine to 48% for glutamine. In the original report, chromatographic and pre-chromatographic factors were identified to explain these differences in performance and these are refined by the additional data.

1. Rattenbury JM, Townsend HC (1990) Establishment of an external quality assessment scheme for amino acid analyses: results from assays of samples distributed during two years. Clin Chem 36: 217–224

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Occurrence and detection of isoaspartate in peptides and proteins

Formation of isoaspartate (isoAsp) is a major pathway for spontaneous damage to covalent protein structure at physiological pH and temperature. IsoAsp forms *via* deamidation os asparagine or isomerization of Asp-X bonds. IsoAsp can be detected by enzymatic transfer of ^{3}H-methyl groups from S-adenosyl-L-methionine onto the α-carboxyl of the isoAsp. We estimated rates of isoAsp formation in proteins at pH 7.4, 37 °C and localized sites of isoAsp formation in calmodulin and human growth hormone (hGH). In hGH, isoAps froms at $\geqslant$ 1.8 residues per day per 100 molecules, with 60–70% occuring at Asn-149 and the remainder at Asp-130. In calmodulin, isoAsp is at three sites; Asn-97, Asp-131 and/or Asp-133, and Asn-44. All of the sites reported here are at Asx-Gly or Asx-Ser sequences. These data, plus data obtained from the literature, suggest that isoAsp formation is most likely to occur when (1) Asn or Asp is followed by Gly or Ser and (2) these sequences fall in a highly flexible domain of the protein. Enzymatic methylation provides a powerful analytical tool for evaluating the isoaspartate content of proteins.

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Glutaminase: "in situ" detection in polyacrylamide native gels and new HPLC method of assay

A new procedure for rapid purification of Ehrlich ascitic mitochondrial phosphate-dependent glutaminase (EC 3.5.1.2) has been developed. The method is based on the "in situ" detection of glutaminase activity in native polyacrylamide preparative gels containing 0.05% Triton X-100 as non-denaturant detergent. After electrophoretic separation, the enzyme is revealed by incubation of the gel in the dark with glutamine in the presence of the auxiliary enzyme glutamate dehydrogenase and NAD^+. The transfer of reducing power from NADH to nitro blue tetrazolium (NBT) resulted in the formation of an insoluble formazan. The protein band was excised from the negative gel and electroeluted at pH 8.3, in the presence of 0.1% Triton X-100. The soluble enzyme was recovered and its activity assayed with a new HPLC method. After incubation for 10 minutes at 37 °C with 20 mM glutamine and 200 mM phosphate, the glutamate formed was allowed to react with dansyl chloride. The Gln and Glu dansyl-derivatives were separated by HPLC on a C 18 reversed-phase column and quantified by fluorometric detection (γ_{exc} = 338 nm, Emission filter, 425 nm long pass). The reported method of glutaminase detection and isolation could be a valid alternative to the conventional procedures, taking into account that glutaminase is a very unstable and hard to purify enzyme. Moreover, the HPLC activity assay also present some advantages; particularly, it avoids the need for the auxiliary enzyme (GDH), a major drawback in some kinetic studies.

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Band shape analysis of electronic spectra of amino acid Schiff-bases

Pyridoxal 5'-Phosphate (PLP) and enzymes requiring it as a cofactor carry out a wide variety of chemical transformations of amino acids that rely on the ability of the cofactor to act as an electron sink. These enzymes have several common mechanistic and stereochemical features, many of which can be duplicated in enzyme-free chemical model as PLP-amino acid Schiff-bases.

A great deal of information about the enzyme mechanism and model systems have been obtained from the aplication of optical methods. In this work, we have studied the formation equilibria of Schiff-bases of PLP and different amino acids. The acid dissociation constant and absorption spectrum of every ionic species have been calculated. The latter has been resolved into components with log-normal curves, which allowed the estimation of tautomerization constants and microscopic acid dissociation constants.

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High performance liquid chromatographic determination of iodoamino acids in picomole range by pre-column dabsyl derivatization

A method for the sensitive determination and efficient resolution of iodinated amino acids is described. Iodinated amino acids used in the present study were monoiodohistidine (MIH), diiodohistidine (DIH), monoiodotyrosine (MIT) and diiodotyrosine (DIT). Iodoamino acids were dissolved in 0.1 M bicarbonate buffer, pH 9.0, and labelled with dimethylaminoazobenzenesulfonyl chloride (dabsyl-Cl) as 1.5. mM acetone solution at 70 °C. The dabsyl iodoamino acids were separated on a TSK gel ODS-80 TM CTR column (4.6 mm i.d. X 100 mm) by employing isocratic conditions of acetonitrile/25 mM Na acetate buffer, pH 6.5, used as the mobile phase, and the eluted derivatives were detected at 436 nm.

MIT and DIT, as well as tyrosine and histidine, formed bis-dabsyl derivatives, while MIH and DIH formed monodabsyl derivatives. Yields of dabsylated amino acids were influenced by the pH of bicarbonate buffer, concentration of dabsyl-Cl solution and the volumetric ratio of carbonate buffer: dabsyl-Cl. When the volumetric ratio was kept to 1 : 2, nearly maximum dabsylation yields were obtained for MIH, MIT and DIT. Dabsylation yield of MIH was lower than that of other iodoamino acids. The calibration curves for all dabsylated amino acids were linear in the range from 2 to 1 000 pmol.

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Identification of S-[2-carboxyl-1-(1 H-imidazol-4-yl)ethyl] cysteine in normal human urine

In the course of studies on the origin of 3-[(carboxymethyl)thio]-3-(1 H-imidazol-4-yl)propanoic acid (I) isolated from normal human urine (Kinuta M et al (1991) Biochem J 275 (in press)], and unidentified urinary compound was found on a high-voltage paper electrophoretogram sprayed with Pauly's reagent, a diazotized sulphanilic acid reagent for the detection of imidazole compounds. The concentration of the unidentified compound in human urine was too low for the detection of its reddish band on the paper electrophoretogram without a preceding concentration step. One hundred and twenty litres of the collected urine was treated successively with ion-exchanger columns SK-1, SA-100, Dowex 1, Dowex 50 and Amberlite CG-50, and applied to the paper electrophoresis and thin-layer chromatography (TLC). The compound had a relative mobility of 1.15, a mobility compared to that of imidazol-4-yllactiv acid in pyridine/acetic acid/water buffer (1 : 20 : 179, by vol.; pH 3.1), and its R_F value on TLC in butan-1-ol/acetic acid/water solvent (12 : 3 : 5, by vol.) was 0.20. The compound gave positive reactions with 1% (w/v) ninhydrin/2% (v/v) pyridine/acetone reagent and a chloroplatinate reagent for the detection of amino acid derivatives and sulphur-containing compounds, respectively. These characteristics are coincidental with those of authentic S-(2-carboxyl-1-(1 H-imidazol-4-yl)ethyl]cysteine (II). The method for the chemical synthesis of compound II described in the previous report has now been modified to produce the compound in greater yield by incubating a reaction mixture of 15 mmol of *trans*-urocanic acid, 45 mmol of L-cysteine and 45 mmol of Na_2CO_3 in 20 ml of deoxygenated water at 70 °C for one week. Judging from the chemical structure of compound II, it is suggested that compound II is one of the physiological precursors of compound I and that natural thiol compounds such as cysteine and glutathione may participate in the metabolism of urocanic acid, a key metabolite of histidine.

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Preparation and analyses of volatile derivatives of sulfuric acid, taurine and cysteic acid

In order to study cysteine metabolism in the animal body, we have prepared volatile derivatives of some metabolites of L-cysteine, and analyzed by gas chromatography [Masuoka N et al. (1989) Acta Med Okayama 42; 247–252, (1990) Acta Med Okayama 43: 253–259]. Sulfuric acid was converted to the silver salt by the reaction with silver oxide, then the silver salt reacted with methyl iodide to form dimethyl sulfate. Taurine was converted to the silver salt as above. The salt was reacted with isobutyl chloroformate to form N-isobutoxycarbonyl derivative and finally converted to N-isobutoxycarbonyltaurine methyl ester. These derivatives were analyzed by gas chromatography. Recoveries of the derivatives were quantitative, and the methods were applied for the determination of sulfuric acid and taurine in human urine.

Derivatization of cysteic acid to dimethyl N-isobutoxycarbonylcysteate was studied. Cysteic acid was reacted with isobutyl chloroformate after the addition of 0.5 M sodium

hydroxide. Then, the sodium ion in the reaction mixture was removed with a Dowex 50 W column. The N-isobutoxycarbonyl derivative was converted to the silver salt by the reaction with silver oxide, and the salt was converted to dimethyl N-isobutoxycarbonylcysteate. The derivative was analyzed by gas chromatography using a column of 3% OV-101 in Chromosorb W. When dimethyl N-isobutoxycarbonylhomocysteate was used as an internal standard, the calibration curve was linear between 0.5 and 5.0 μmol of cysteic acid and the recovery was more than 95%.

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The importance of enantiomeric separations of amino acids in medical science

High performance liquid chromatogaphy (HPLC) is a widely used method for amino acid analysis. Selective and sensitive determination of amino acids with high reproducibility is attained when using electrochemical detectors with high sensitivity analytical cells or with post column derivatization and fluorescence detection. It is possible to determine nanogram quantities of some aromatic acids, such as tryptophan and tyrosine, and sulfur-containing amino acids, cysteine and methionine.

It is possible to separate enantiomers which contain a protonated primary amine in close proximity of the chiral center using a choral crown ether stationary phase. Aqueous perchloric acid (a highly oxidizing solvent) is used as the mobile phase with 1–5% methanol (v/v). At low flow rates and low temperatures, these separations are greatly enhanced. Once the separation has been maximized, it is possible to determine trace quantities (0.1% to 0.0004%) of the D-amino acid present as an impurity in commercially available L-amino acids.

A comparison is made between the L-amino acid product of six prominent chemical companies. The molar concentration of the L-amino acid solutions was approximately 0.1 M. The chiral stationary phase was overloaded, however the enantiomers were still baseline resolved under maximum conditions. In some cases, it was necessary to use two chiral columns in sequence because the molar concentration of the amino acid solution had to be increased to determine the level of D-amino acid. It was determined that none of the L-amino acids were enantiomerically pure.

The human body is known to be composed of L-amino acids. Current research is focusing on the determination of the presence of D-amino acids in physiological fluids of humans using a column switching method. We have determined the existing "baseline" levels of D-amino acids in normal human beings. By monitoring the presence of trace levels of the D-enantiomers of various amino acids, it may be possible to determine the various stages of certain illnesses or of cell growth. Because of the low levels of the D-enantiomers great care must be taken to eliminate false positive due to racemization.

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Determination of amino acid enantiomers in food and beverages by capillary gas chromatography and high-performance liquid chromatography

Objective. Free amino acids (AA) were isolated from food and beverages and the presence of D-enantiomers was determined by gas chromatography (GC) employing capillary columns coated with Chirasil-L(or D)-Val and/or reversed-phase liquid chromatography (HPLC) after pre-column derivatization of AA-enantiomers with o-phthaldialdehyde (OPA) together with *N*-isobutyryl-L(or D)-cysteine (*i*-But-Gys).

Materials and Methods. Free AA were isolated from food and beverages (dairy products, Oriental fermented food, fruit and vegetable juices) by treatment with Dowex WX 8 cation exchanger and eluted therefrom with 2 N aq. ammonia. For GC, AA were converted into their *N*-trifluoroacetyl AA 1-propyl esters by treatment with acetyl chloride in 1-propanol and, subsequently, trifluoroacetic anhydride. Derivatives were investigated by capillary gas chromatography using fused silica columns coated with the chiral phase Chirasil-L(or D)-Val using a temperature program 70–190 °C in 40 min. For HPLC a Model 1090 liquid chromatography (HP) designed for the automated pre-column derivatization of AA was used. AA enantiomers were separated as diastereomeric isoindol derivatives obtained after pre-column derivatization with OPA together with *i*But-Cys [1]. Conditions: Shandon Hypersil ODS, column 250 mm × 4 mm i.d., eluent A, 23 mM NaOAc, pH 6.0; eluent B, MeOH/MeCN (600 ml + 50 ml); gradient 0% B to 53.5% B in 75 min; automated derivatization of AA with OPA/*i*But-Gys in 0.4 N sodium borate buffer pH 10.4; fluorescence detection at 230 nm (excitation) and 450 nm (emission).

Results. D-AA were found in all fermented dairy products and in soy sauces, in fermented and certain non-fermented (cabbage) vegetable juices, and in orange juices suspected of being adulterated by addition of DL-AA, or being contaminated by microorganisms [2]. GC as well as HPLC are shown to be suitable methods for the quantitative determination of DL-AA. HPLC as described above enables also the determination of Asn andGln.

1. Brückner H, Wittner R, Godel H (1989) J Chromatogr 476: 73
2. Brückner H, Lüpke M (1991) Chromatographia 31: 123

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Analysis of DABS-amino acids with sensitivity to femtomole levels using RP-narrow bore columns

In this paper we report the complete separation of amino acids as DABS-derivatives using a 3 μm Supelcosil LC-18 (25 cm × 2.1 mm I.D.) narrow bore column. The system described allows us to perform the analysis of DABS-amino acids, with a sensitibity to femtomole level. We have also studied the conditions necessary to use the narrow bore columns for routine analysis, paying particular attention to the problem of providing adeguate protection for the analytical column, otherwise it is possible that the column can become damaged after only a few runs. We found it very suitable to

use a (2 cm × 2.1 mm I.D.) guard column filled with a 40 µm Pelliguard LC-18, pellicular packung resin which did not affect the complete resolution of the DABS-amino acids. Furthermore we have shown that it is possible to use the narrow bore columns with a standard HPLC apparatus, such as the Gold system from Beckman, equipped with a 12 µl flow cell and using a 5 µl sample injection. Comparing the results obtained using standard HPLC columns (3–5 µm Supelcosil LC-18) of different lengths (15, 25 cm × 4.6 mm I.D.) with those obtainable with the narrow bore columns used in this work, it is possible to achieve, under the chromatographic conditions reported in the paper, a much greater sensitivitiy using the narrow bore columns. This significant increase in sensitivity allows us, in the case of DABS-amino acids separation, to perform their analysis up to femtomole level. However, we also applied this strategy to separate other compounds such as DABTC-peptides, DABTH-amino acids and nucleotides, and we were able to obtain the same benefits. Briefly, the conditions that must be followed to protect the analytical column, and the "standard" HPLC apparatus used, make the narrow bore columns very useful for rourtine analyses of DABS-amino acids with a sensitivity up to femtomole level.

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Isolation of indolic and kynurenine tryptophan metabolites using Sep-Pak C_{18} cartridges

Conventional extraction procedures for the isolation of tryptophan metabolites from physiological fluids are laborious and time consuming due to the complexity of the matrix. Sample extraction purification and concentration using Sep-pak is gaining wide acceptance. It was the purpose of this investigation to develop a simple and reproducible extraction procedure using Sep-pak C_{18} cartridges pretreated with sodium dodecyl sulphate (SDS).

Before use the methanol/water activated Sep-pak C_{18} cartridges were treated with 10 mM SDS at pH 2.10. Standards and samples were adjusted to pH 3.5 (± 0.01) and passed through the cartridge. This was sequentially washed with 10 mM SDS and methanol-SDS (5 : 95). The compounds were eluted using 1.0 M ammonia-methanol (80 : 20). Further extraction with 1.0 M ammonia-methanol (50 : 50) was necessary for the extraction of 5-hydroxy-tryptamine and indol-3-acetamide.

Recoveries obtained for the extraction procedure were studied by HPLC for six different indolic and six kynurenine compounds at four different concentrations (200; 50; 20 and 5 µM). The recovery for the compounds tested ranged between 80.4 and 97.9% probably due the decomposition during the extraction procedure. The recovery showed to be independent of the concentration since the standard deviation ranged between 2.37 and 9.92%.

The method is simple, reliable, reproducible, avoids lengthy and laborious operations and is applicable to routine use for the detection of tryptophan metabolism disorders. Moreover it has the advantage of being applicable to both kynurenine and indolic tryptophan metabolites.

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Homocystine assay in serum or urine by pre-column derivatization and reversed-phase liquid chromatography

The o-phthaldialdehyde (OPA)-2-mercaptoethanol(MCE) derivatization of primary amines is ideally suited to the detection of amino acids in physiological fluids using reverse-phase high performance liquid chromatography. One disadvantage of the OPA-MCE reaction is the very low fluorescence yield given by cysteine as well as by other sulfur amino acids such as homocysteine, cystathionine and s-sulfo-L-cysteine.

Since the determination of homocysteine is crucial in the differential diagnosis of the inborn errors of the sulfur amino acid pathway, the purpose of this study was to determine the optimal parameters for the quantitation of plasma and urine homocysteine. Optimized stepwise gradient elution was based on the following solvents: Solvent A, MetOH : THF : 0.05 M CH_3 COONa (9 : 9 : 982), pH 6.70 (± 0.01); Solvent B, MetOH : 0.05 M CH_3COONa (650 : 350), pH 5.00 (± 0.01). The pH of solvent B was critical in order to achieve a 70% base in line resolution for the pair homocysteine : methionine. The molar fluorescence intensity for different sulfur amino acids has been determined. Cystathionine, s-sulfo-L-cysteine and homocysteine exhibit a fluorescence intensity which is 12, 15 and 36 times higher than that of cysteine, respectively. Fluorescence enhancement was attempted using iodoacetic acid as a sulphydryl group blocker, prior to OPA/MCE reaction. However homocysteine derivative overlaps glycine under above chromatographic conditons. Therefore detection of homocysteine without iodoacetic reaction was used because, even though it quantum yield is 10 times lower than methionine, it is still enough for its quantitation under pathological conditions.

The method was successfully applied to the determination of sulfur amino acids in physiological fluids.

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Use of capillary zone electrophoresis in the determination of amino acids and their enantiomeric forms

Derivatisation with o-phthalaldehyde(OPA)/mercaptoethanol is a frequently used sample preparation technique for the amino acid analysis by HPLC. Derivatisation is done to improve retention behaviour and detection. A modified version of this reaction uses OPA together with a chiral mercaptan. The resulting diastereomers can be resolved by HPLC. In this way an amino acid analysis and an enantiomer determination can be done simultaneously.

Besides HPLC, also capillary zone electrophoresis (CZE) provides a good separation power for such kind of compounds. In this paper we present studies on amino acid separations and enantiomer determinations by CZE. Derivatisation is done with OPA together with a chiral mercaptan. Different chiral mercaptans are used to study their structural

influence on the diastereomer separation. Furthermore various buffer systems including micell forming additives are evaluated to improve resolution. By using a buffer system with hydrophobic organic cations as additives the selectivity strongly improves and a number of amino acids and corresponding diastereomers can be separated.

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Thiosugars – tools for chiral derivatization of amino compounds

Chiral amino compounds as α-amino alcohols or α-amino acids used in organic synthesis of pharmacological studies are frequently required in their enantiomerically pure forms, as the enantiomers can possess different activities.

For the resolution of these amino-compounds, their derivatization was carried out with o-phthaldialdehyde and various thiosugars. In the course of reaction, highly fluorescent, diastereomeric N-substitued 1-isoindolyl-(1-thio-β-D-glycosides) were formed, which were separated and analyzed by high performance liquid chromatography on conventional non-chiral analytical columns.

The chromatographic behaviour of a series of novel derivatives were described and the derivatization was optimized with respect to the pH, derivatization time and concentration of reagents. Application was evaluated for a number of amino alcohols, natural amino acids, halogenated- and dehydroamino acids.

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Chiral discrimination for the interaction between an helicoidal system and an amino acid molecule

The interaction between an helix characterized by its radius, its pitch and the atomic distribution inside its monomer and of an amino acid molecule is determined on the basis of semi-empirical potentials including Lennard-Jones, electrostatic, induction and hydrogen bond contributions. Calculations are performed for various values of the helix characteristics which schematize helicoidal polysaccharides (cellulose, cellulose derivatives...) or chiral cavities (cyclodextrin). The helix configuration acts as a chiral revealer as it frozes the amino acid inside its cavity. The chiral discrimination of D and L amino acid is about 2–5% of the total interaction for most of the helix geometries and it can reach 8% when the amino acid lies inside the helix.

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