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ABSTRACTS

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Selective determination of all D-amino acids in mammalian brain

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Along with the advances on sensitive and enantioselective analytical methods, D-amino acids, the enantiomers of L-amino acids, are gradually recognized as the candidates of novel physiologically active substances and/or biomarkers. Especially, the enzymes synthesizing D-Ser and D-Asp have been found in mammals, and these two D-amino acids are now considered as endogenous physiologically active biomolecules. However, besides D-Ser in frontal brain areas and D-Asp in endocrine tissues, the amounts of D-amino acids in mammalian tissues and physiological fluids are extremely small in most cases. Therefore, for the accurate determination of D-amino acids, a sensitive and selective analytical method is needed to avoid severe interferences with large amounts of L-amino acids, uncountable numbers of peptides and other compounds present in biological matrices. In the present study, we have established a selective two-dimensional high-performance liquid chromatographic (2D-HPLC) system consisting of a microbore-monolithic ODS column as a first dimension and a narrowbore-enantioselective column for the second dimension, for the determination of all proteinogenic amino acid enantiomers. For the sensitive determination, amino acids were derivatized with a fluorescence labeling reagent, 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F). By using the 2D-HPLC system, D- and L-amino acids were determined in the brain and physiological fluids of various strains of mice. In the brain of D-amino acid oxidase deficient mouse, high amounts of D-Ser, D-Ala and D-Leu were observed widely in most of the brain areas, although high amount of D-Ser was present only in the frontal brain areas in the control mice. On the other hand, the amounts of D-Ser drastically decreased in the brain of serine racemase knockout mice. In the brain of D-aspartic acid oxidase knockout mice, high amounts of D-Asp not D-Glu were observed in all brain areas. Further studies on their origins, physiological functions and diagnostic values are now being investigated.

Study of the possibility of using genetically engineered enzymes—nucleoside phosphorylases in the synthesis of modified nucleosides

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This work is dedicated to investigation of synthesis of structural analogues of natural nucleosides and testing of it's properties. The investigation was carried out using immobilize Glycosyl Transfering Enzymes, such as Purine nucleoside phosphorylase and Thymidine phosphorylase obtained from Laboratory of Biotechnology of Institute of Bioorganic Chemistry, Russian Academy of Sciences. We studied the effect of 19 nitrogen base derivatives on nucleoside phosphorylase activity in phosphate buffer reaction mixture. Also, we studied substrate-specific properties of nucleoside phosphorylase enzymes.

Conclusion: The conditions of 5-methyl substituted derivatives (1) and (2) (analogs of Ribavirin) fermentative synthesis have been developed:
R = OH (1), R = H (2)

It was shown the principle possibility of synthesis of the novel modified nucleosides using bases modified at 6-th position of Purine.

Keywords: Natural nucleosides, Glycosyl Transfering Enzymes, Purine nucleoside phosphorylase, Thymidine phosphorylase, Nucleoside phosphorylase

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Molecular recombinant transporters for the selective delivery of photosensitizers into cancer cells

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This work is dedicated to investigate the efficacy of the polypeptide modular recombinant transporter for the targeted delivery of local-acting drugs to the nuclei of cancer cells. The in vitro and in vivo activity of free photosensitizer bacteriochlorin was compared with the effects of its conjugate with transporter. Photocytotoxicity of bacteriochlorin against B16-F1 melanoma cells was enhanced 50-folds when this compound was applied as conjugate. Also, we detected 18-days delay in tumor growth after the five-cycles of photodynamic therapy of mice C57 Black with the bacteriochlorin-transporter conjugate. In contrast, the effect of free bacteriochlorin was non-significant. Results demonstrate the potency of modular transporters to be used as local-acting drug carriers in cancer therapy.

Keywords: Recombinant transporter, Photodynamic therapy, Photosensitizer, Bacteriochlorin, Photocytotoxicity, Melanoma B16-F1

This work was supported by Ministry of Education and Science of Russian Federation/Federal Education Agency of Russian Federation (grant 2.1.1/5939).

Development of method of search and activation of functional fragments in 5′-regulatory region, which provide transcription activity of genes

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The study of regulation of expression and in particular of transcription as an important stage of expression is a key problem of contemporary genetics. *D. melanogaster* gene *Trithorax-like* is known to encode the regulatory protein GAGA—a transcription factor that is in answer for the transcription of at least 30 genes. The regulation of transcription of this gene is not studied yet, though its exon–intron structure is already known. The aim of the work was to find out gene fragments that are most probable to influence the transcription. Four mutations of 5′-regulatory region of *Trl* gene were studied and the obtained mutants were examined from the point of view of their level of *Trl* expression and possible disturbances in ovary cells. It was concluded

in the result of the work that two fragments of 5′-regulation regions 479 and 197 bp are more important for the maintenance of level of *Trl* expression and mutants' viability. It was established that the deletion of fragment 479 bs leads to the male sterility and the deletion of fragment 99 bs that contains two first transcription initiation sites—to the female sterility. The deletion of fragment 306 bp results in the decrease of viability and fertility and of Trl expression that indicates the localization of functionally important sites in it.

Keywords: Regulation of expression, Transcription factor, Exon–intron structure, Mutations, Deletion, Initiation sites

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Analysis of skin secretions from the *Lithobates pustulosus* frog using high-resolution mass spectrometry

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The lack of efficacy of conventional antibiotics against an increasing number of pathogenic microorganisms constitutes a serious public health problem. One of the strategies applied in order to overcome microorganism resistance resides in the search for novel antimicrobial agents. Antimicrobial peptides (AMPs) are widespread in living organisms and constitute an important component of innate immunity against microbial infections. Amphibians are known to produce a large number of amphipathic peptides which manifest a broad range of pharmacological activities. They have been shown to inhibit the growth of numerous species of bacteria, fungi, protozoa, and also some types of tumor cells without evidence of significant hemolytic activity. *Lithobates pustulosus* is an endemic Mexican frog which inhabits the deciduous forest of Morelos State and to the best of our knowledge, no data is available in the current literature regarding the composition of its skin secretions. At present, liquid chromatography coupled with high-resolution mass spectrometers constitute the most powerful analytical procedure for studying complex mixtures of peptides and proteins. Here we used a LC-MS system composed of a nanoflow liquid chromatography coupled with an Orbitrap Velos mass spectrometer to determine the molecular mass of all components and the primary structure of most of these. Primary structure comparison was searched against a public data bank showing that *L. pustulosus* skin secretion is mainly composed of ranatuerin, brevinin, esculetin and bradykinin related-peptides. Analysis of large proteins by gel electrophoresis was also performed and many tryptic peptides were de novo sequenced using CID and HCD dissociation methods.

Neonatal Intrahepatic Cholestasis caused by Citrin Deficiency (NICCD): clinical analysis of 50 cases

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Aims: Citrin deficiency is resulted from dysfunction of citrin, a mitochondrial aspartate/glutamate carrier encoded by SLC25A13 gene. This study aims to investigate the clinical and laboratory features of Neonatal Intrahepatic Cholestasis caused by Citrin Deficiency (NICCD).
Methods: Fifty NICCD cases confirmed by SLC25A13 analysis were enrolled as research subjects. Major clinical manifestations and the features of blood biochemistry, hepatopathology, medical imaging and metabolome were analyzed by means of a cross-sectional study.
Results: Clinical presentations included jaundice, abnormal coagulation tests, chubby face, failure to thrive, steatorrhea, motor retardation, hepato/hepatosplenomegaly, anemia, echinocytosis, and light stool. Besides the elevated biochemical indices of cholestasis and dyslipidemia, reduced fibronectin, retinol binding protein and ceruloplasmin along with zinc deficiency were also revealed. Diffused fat deposition, cholestasis in hepatocytes and canaliculi, and varying degrees of inflammation and fibrosis were observed, suggestive of hepatopathological features of non-alcoholic fatty liver disease (NAFLD). Ultrasound, CT and MRI revealed fatty livers in some cases, respectively. Metabolome features included coexistence of indices of tyrosinemia type I and markers for galactosemia at urine samples, and abnormal amino acid spectrum and acylcarnitine profile in blood specimens, with dramatically elevated free, myristyl and palmityl carnitine.
Conclusions: This study revealed motor retardation, reduced serum fibronectin, retinol binding protein and ceruloplasmin and zinc deficiency as novel clinical and biochemical features for NICCD, and proposed, for the first time, that NICCD might be a specific etiology for non-alcoholic fatty liver disease (NAFLD). Moreover, the changes of acylcarnitine profile in this study further expanded the metabolome feature of NICCD.

Hub molecule p75^{NTR} regulates homeostatic function linked with recognition memory in the basal forebrain–prefrontal cholinergic system

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Recognition memory operates through coordinated activities between perirhinal cortex, hippocampus and medial prefrontal cortex (mPFC). However, molecular and cellular mechanisms underlying recognition memory remain elusive. Using p75$^{NTR-/-}$ mice crossed with knock-in mice expressing enhanced green fluorescence protein (EGFP) in cholinergic neurons, we explored the roles of p75^{NTR} in recognition memory and its related molecular, cellular and neural network mechanisms. Disruption of p75^{NTR} gene expression resulted in both defective temporal order memory and aberrant neuronal network oscillations in the mPFC. In vitro studies revealed a robust decrease in

cholinergic neuron excitability in the nucleus basalis, but not in the medial septum in $p75^{NTR-/-}$ mice. Using whole-cell patch clamp recording, unchanged excitatory and inhibitory synaptic inputs onto the pyramidal neurons were observed in the layer V of the mPFC. However, combined electrophysiological and biochemical experiments revealed an unexpected marked depolarizing shift in the reversal potential for $GABA_A$-mediated inhibitory postsynaptic currents (E_{IPSCS}), which reduced synaptic inhibition, in the mPFC from $p75^{NTR-/-}$ mice. This shift in reversal potential resulted from a down-regulation of the potassium-chloride cotransportor 2 (KCC2) expression. Pharmacological cholinergic enhancement rescued the KCC2 expression deficit both in vitro and in vivo and restored synaptic inhibition by reestablishing chloride homeostasis. Taken together, these findings suggest that $p75^{NTR}$ serves as a hub molecule within the basal forebrain–prefrontal system in controlling recognition memory, and reveals an unexpected physiological role for $p75^{NTR}$ in the modulation of cholinergic excitability in the nucleus basalis.

Keywords: $p75^{NTR}$; Basal forebrain cholinergic neurons; Prefrontal cortex (PFC); Reversal potential of E_{IPSCs}; Potassium chloride cotransporter 2 (KCC2); Recognition memory

Taurine and analogues: components of Nano Drugs

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Nano recognized as one billionth of measurement, Nano scale has properties which can solve biomedical challenges. At this stage functional properties show uniqueness to the engineered technology. Effectiveness of medicines is totally depends upon the effectiveness of the ingredients and mode of preparation. Drugs are basically chemicals and follow; rule of chemistry. Chemistry improves the manipulation of matter at nano scale regulating chemical products with specific intrinsic properties that effect how they interact with their environments. Food and medicine can not be separated; food contains bio-molecules, minerals and amino acids, importance of amino acids are increasing, they are involved in protecting activities. Sulfur amino acids play a vital role in life process providing elemental sulfur and involved in host defense. Taurine is involved in almost every cellular process. Beneficial action of taurine is regarded to effective; between 1 and 3 g. Taurine seems to well effective at nano level. Its compound Taurox 6×: "COBAT", is carbobenzoxy beta alanyl-taurine, modified efficiently to absorbed via the oral mucosa, is a engineered product of Nano technology. This nano compound when compared to known anti-fatigue, is more than one thousand to one million times potent. Other examples may include; taurine is of chicken embryos, Hypertension pills, Hyperexol, Nano coffee. In nature, birds used spiders to make their young one bold. Spiders contain taurine, amount of taurine utilized by chick is in nano scale, taurine in their diet makes them bolder and better learner in adulthood. Nano nutrition can be vital tool to supply nutrients. This technology is yet not time tested hence "wait watch & move" "must follow" and is essential for growth of nano Drugs.

Protective effects of taurine on ischemic myocardial injury

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Objective: To study the effects of taurine (Tau) on ischemic myocardial injury and the relation between Tau and inositol 1,4,5-triphosphate receptor 1 (IP_3R1) in ischemic injury.
Methods: Ischemic myocardial injury was established by hypodermic injection of isoprenaline (ISO) (10 mg/kg day). Fifty rats with normal electrocardiogram were randomly divided into five groups: control (saline 20 mg/kg day), model (ISO 10 mg/kg day), and taurine groups administered with 100, 200 and 300 mg/kg day Tau, respectively by intraperitoneal injection 30 min before injection of ISO. Seven days later, the effects of Tau on heart index, tectology and ultrastructure of cardiac muscle, the levels of CK-MB, cAMP, cGMP and antioxidant ability were investigated. In vitro, the effects of silencing CSD mRNA by siRNA on the expression of CSD and IP3R1 mRNA were analyzed by realtime fluorescent quantitative PCR.
Results: In vivo, Tau could significantly prevent the increases of heart index induced by ISO, protect the tectology and ultrastructure integrity of cardiac muscle. Compared with the model group, intraperitoneal administration of 100 and 200 mg/kg Tau could significantly decrease the levels of cAMP and cGMP in myocardium, but there was no significant effect on serum CK-MB. Meanwhile the levels of GSH, T-AOC in myocardium and GSH-Px, SOD, T-AOC in serum were significantly increased but the serum MDA was significantly decreased by Tau administration. And the results showed 200 mg/kg day Tau had better protective effects. In vitro results showed that the expression levels of cysteine sulfinate decarboxylase (CSD) and IP_3R_1mRNA in H9c2 cells were significantly up-regulated after being cultured under 95% N_2 + 5% CO_2 for 1 h. And the expression level of IR_3R_1 mRNA was significantly down-regulated after the inhibition of CSD mRNA expression in cultured H9c2 cells both under the ischemic and normal conditions.
Conclusions: The results indicated 200 mg/kg day Tau had better protective effects on ISO induced ischemic myocardial injury and the inhibition of CSD mRNA expression could down-regulate the expression level of IR_3R_1 mRNA in cultured H9c2 cells.
Keywords: Taurine, Ischemic myocardial injury, CSD, IR_3R1, siRNA

Alterations in energy metabolism mediated by taurine deficiency

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Rats treated with the taurine transport inhibitors, guanidinoethylsulfonate or β-alanine, lose about 50% of their myocardial taurine content. Associated with the decline in taurine levels is a modest change in myocardial relaxation ($-dP/dt$) but no apparent change in other measures of myocardial function. However, the taurine depleted hearts experienced significant changes in energy metabolism, which are characterized by a shift away from aerobic metabolism in favor of anaerobic metabolism. The taurine deficient hearts experienced a twofold increase in glucose utilization, a twofold increase in pyruvate production and a three- to fourfold increase in lactate production. These effects were exaggerated in hearts perfused with buffer containing insulin. Crossover plots revealed that the stimulation in glycolysis by the taurine depleted heart was largely caused by the activation of phosphofructokinase, in part because of a decrease in citrate levels. The size of the creatine phosphate and adenine nucleotide pools of the taurine deficient heart fell by 26 and 6%, respectively. These data are consistent with evidence in isolated

cardiomyocytes that β-alanine-mediated taurine depletion slows flux through the electron transport chain secondary to impaired assembly of electron transport chain complexes.

Taurine biosynthesis in pancreatic islet β cells and its effects on glucagon secretion in rats

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Taurine is a semi-essential amino acid and has many important physiological functions including the regulation of diabetes. As a first step toward understanding how taurine is involved in the development of diabetes, we first analyzed the expression of cysteine sulfinate decarboxylase (CSD), the key rate-limiting enzyme in taurine biosynthesis, in pancreatic islet cells of normal and streptozotocin (STZ)-induced diabetic rats, and the effects of taurine on insulin and glucagon in vivo and in vitro, and further examined the effects of taurine on glucagon gene transcription, regulation of transcription and post-translational processing of gene expression. Our results showed that, firstly, CSD was expressed at both mRNA and protein levels in pancreatic islet β cells, suggesting that taurine could be synthesized in islet β cells by the CSD pathway; moreover, the transcript abundance was decreased upon STZ treatment, suggesting a low level of taurine synthesis in diabetic pancreatic cells. Secondly, the levels of blood glucose and glucagon (GN) in STZ-induced diabetic rats were significantly decreased, while insulin levels increased not significantly upon taurine administration. In vitro results showed that taurine can significantly reduce the level of glucagon secretion, while the effect was not obvious for insulin in STZ-treated islet cells. Thirdly, taurine can significantly reduce the gene transcription level of proglucagon, key regulators of proglucagon pax6, HNF-3 and PC2 which is key regulator from proglucagon into glucagon. Finally, we concluded that the pancreatic islet cells can synthesize taurine by CSD pathway, which is beneficial to reducing the synthesis of glucagon by way of paracrine in the development of diabetes.

Keywords: Taurine, Cysteine sulfinate decarboxylase (CSD), Pancreatic islet cells, Glucagon, Diabetes, Rat

A unique approach for enantioselective extraction of amino acid: reversal resolution with a single chiral receptor

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The enantioselective liquid–liquid extraction (ELLE) of amino acids is undergoing a fast development. However, this field still suffered from low selectivity and narrow application scope. Herein, we report a new and highly enantioselective extraction agent for the resolution of amino acids. This reagent serves the highest levels of selectivity for 11 amino acids in a new ELLE system and easy recovery. Moreover, a unique temperature-induced reversal enantioselective extraction of racemic amino acids was observed, which can be rationalized on the basis of a kinetic-thermodynamic control. Thus, high purity L- or D-form amino acids can be achieved by using a single chiral receptor. We believe that these findings will serve as a fresh path for the design of new enantioselective extraction agents for the field of ELLE.

Chirality converting reagent based on binol aldehyde pendant with linear-chained guanidinium

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Optically pure D-amino acids are useful intermediates as synthesis of pharmaceuticals and food ingredients. In the past years, we designed and synthesized novel binol based chiral receptors for amino acids and amino alcohols called as ARCA (Alanine Racemase Chiral Analogue) systems.

These receptors have good selectivity for amino alcohols and can convert L-amino acids to D-amino acids. These receptors make reversible imine bond with amino alcohols which have the advantage of being much stronger and structurally well defined compared to the noncovalent interactions. Besides the imine bond, a resonance assisted hydrogen bond (RAHB) and other hydrogen bonds between the acid/alcohol group and the receptor play important roles in determining the stereoselectivity.

We constantly report here a novel ARCA receptor which has many advantages such as solubility in organic solvents and high enantioselectivity towards the chiral amino acids and amino alcohols, compared to the other developed receptors. Guanidinium based chiral receptor has been designed, the synthetic details of receptors and the recognition properties will be presented.

Conversion of L-amino acid to D-form by naphthol-based aldehyde

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Amino acid is the most representative chiral material. In particular, D-amino acids are used in food technology and as drug intermediates in Pharmaceuticals. Nevertheless, compared to L-amino acids, these D-amino acids are not abundant in nature. Therefore, the synthesis of D-amino acids in an easy and cost-effective way is very important. Naphthol-based aldehyde can convert the L-amino acids to D-amino acids.

Receptor is designed to enhance the stereoselectivity of existing Naphthol-Based Aldehyde as the movement between the naphthalene rings of the receptor is restricted by steric hindrance. Receptor reacts with L-amino acids in $CDCl_3$-d to form imines. After the addition of DBU, and the time dependant of ^{1}H-NMR spectrum of the imines proves the conversion of the L-amino acids to D-amino acids, through decreasing the L-form imine peak and increasing the D-form imine peak.

Multiple SNP analysis in genome-wide association studies

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In recent years, genome-wide association (GWA) studies have successfully led to many discoveries of genetic variants affecting common complex traits, including height, blood pressure, and diabetes. Although GWA studies have made much progress in finding single nucleotide polymorphisms (SNPs) associated with many complex traits, such SNPs have been shown to explain only a very small proportion of the underlying genetic variance of complex traits. This is partly due to that fact that most current GWA studies have relied on single-marker approaches that identify single genetic factors individually and have limitations in considering the joint effects of multiple genetic factors on complex traits. Joint identification of multiple genetic factors would be more powerful and provide a better prediction of complex traits, since it utilizes combined information across variants. Recently, a new statistical method for joint identification of genetic variants for common complex traits via the elastic-net regularization method was proposed. In this study, we applied this joint identification approach to a large-scale GWA dataset (i.e., 8,842 samples and 327,872 SNPs) in order to identify genetic variants of several phenotypes of interest for the Korean population. In addition, in order to test for the biological significance of the jointly identified SNPs, gene ontology and pathway enrichment analyses were further conducted.

Prediction of biological properties of some groups of heterocyclic derivatives by their effects on the polyamine oxidative deamination

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The investigations of the aberrant regulation of polyamine metabolism in tumors made it possible to suppose that inhibitors of the polyamines' oxidative deamination could have the carcinogenic properties. In contrast, compounds, which activate the polyamine catabolism, may be potential antineoplastic agents.

The aim of our study was the prediction of carcinogenic and antiproliferative properties of the novel compounds. The objective was to evaluate the influence of benzimidazole, azofluorene, dioxaboreninopyridine and aniline derivatives on the rate of putrescine, spermidine and spermine oxidative deamination in the acellular testing system of the high mitotic index tissue.

We studied 32 test compounds of 3 groups.

Conclusions: (1) Among the 12 compounds from group A 7-aminopyrido[1,2-α]benzimidazole and 7-nitropyrido[1,2-α]benzimidazole demonstrated 1.5- to 1.7-fold inhibition of amino-oxidase activity in regenerating liver and can be potential cancerogenic. 1-amino-9-phenylamino-4-azofluorene and 1,4-diazoacetonaphtyleno[1.2-f]-fluorinanten were activators of polyamine catabolism, thus can probably have antiproliferative effects. (2) Aniline derivatives, especially 3-(4-iodinanilino)-1-phenylpropanone-1, activated the process of oxidative deamination and may be considered as potential cancerostatics. (3) The repression of amino-oxidase activity by all dioxaborenynopyridine derivatives enables to consider them as potential tumorogenic due to the presence of common 6-methyl-4,8a-diphenylperhydro[1,3,2]-dioxaborynino[5,4-c]pyridine core structure.

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Lipid glycation in food and biological systems

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An amino group of phosphatidylethanolamine (PE) is a target for nonenzymatic glycation and its involvement in lipid glycation reaction has been confirmed in the food processing, in the aged animals and in the pathogenesis of human disease such as diabetic complications. Amadori-glycated PE (gPE, deoxy-D-fructosyl PE) is a Primary and early glycation product of PE, while carboxymethyl-PE (CM-PE) and carboxyethyl-PE (CE-PE) are the advanced glycation end products (AGE-PE). These glycation products have been analyzed by UV-labeled HPLC and quadrupole/linear ion-trap mass spectrometer, QTRAP. Among several food samples, infant formula and chocolate contained 1.5–112 mg/kg of glucose-PE (Glc-PE) and lactose-PE. Human milk did not. Rat plasma showed 0.8 mol% Glc-PE/PE in 50 weeks-old, but below the detection limit (4.5 ng) in 6 weeks-old rats. Human plasma gPE concentration was 0.08 mol% of PE in normal subjects and 0.15–0.29 mol% in diabetic patients. For AGE-PE concentrations, no significant difference was observed between normal and diabetic patients. The gPE induces angiogenic differentiations in human umbilical vein endothelial cells and causes membrane lipid oxidation via superoxide-mediated free radical reactions in vitro and in vivo. Pyridoxal 5′-phosphate and pyridoxal (vitamin B_6) were the effective inhibitor of lipid glycation. Pyridoxal 5′-phosphate-PE adduct was detectable in human red blood cells. The increased plasma gPE concentration in streptozotocin-induced diabetic rats was decreased by dietary supplementation of pyridoxal 5′-phosphate. The lipid glycation reaction is proncipary involved in the food deterioration, in the ageing of animals and in the diabetic complications.

Monosodium glutamate intake increases haemoglobin level over 5 years among Chinese adults

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Objective: The aim of this analysis was determine the relationship between monosodium glutamate (MSG) intake and change in haemoglobin (Hb) levels over 5 years.

Methods: Data from 1197 Chinese men and women who participated in the Jiangsu Nutrition Study (JIN) were analyzed. In this study, MSG intake and Hb were quantitatively assessed in 2002, and followed-up in 2007.

Results: MSG intake was associated with a significant increase in Hb among men but not women. Among anemic participants at baseline, there was a significant inverse association between MSG intake and the risk of anemia at follow up. The association was independent of dietary patterns and lifestyle factors. A dose response relationship

between MSG intake and increase in Hb levels among anemic participants was seen. Comparing extreme quartiles of MSG intake among those on anemic at baseline, the OR for incident anemia was 0.29 (95% CI 0.10–0.83).
Conclusion: MSG intake may have independent Hb-increasing effects, especially among men and those anemic at baseline.

Therapeutic approaches for patients with adult onset type II citrullinemia (CTLN2)—low carbohydrate diet and oral administration of sodium pyruvate

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Adult-onset type II citrullinemia (CTLN2) is an autosomal recessive disease characterized by highly elevated plasma levels of citrulline and ammonia due to the urea cycle dysfunction associated with citrin deficiency. Patients with CTLN2 present various neurological symptoms with hyperammonemia that closely resemble those of hepatic encephalopathy. Since 1990, 29 CTLN2 patients have been admitted and treated in Shinshu University Hospital. Of 29 patients, fourteen patients received liver transplantation (LT). After LT, neurological symptoms soon disappeared and all had returned to their previous social lives. Among the 15 patients who have not undergone LT, six died of intractable encephalopathy or development of hepatic cancer. Recently, eleven patients have been treated with oral intake of sodium pyruvate and low carbohydrate diet. One patient stopped taking the sodium pyruvate in a few days because of nausea. Of 10 patients, seven patients have had relatively good clinical courses (ranged from 0.5 to 4 years) with decrease in frequency of encephalopathy. However, three patients underwent LT during 1 month to 1 year after starting of sodium pyruvate therapy because frequency of attacks of encephalopathy did not decrease. Hepatic steatosis markedly ameliorated after the treatment with sodium pyruvate in 4 patients. Our observation indicates that liver transplantation is a very promising therapy but other therapeutic approaches are being established since all patients do not always undergo LT because of shortage of donor. The therapy with low carbohydrate diet and sodium pyruvate can cure many patients of CTLN2.

In vitro ACE inhibitory activity and the functional factors of Chinese traditional fermented soy product—Douchi

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Douchi is one the frequently consumed fermented soy products in China as a condiment from ancient time. The angiotension I converting enzyme (ACE) inhibitory activities of both commercial Douchi samples and the ones produced in the labs with different conditions were investigated. After investigating more than 50 commercial Douchi samples, one Douchi sample (ZSX) produced in the South area of China was found to have very high ACE inhibitory activities and this product was fermented by mixed cultures, and the ripening process played an important role in this activity of the final product. The contents of hydrophobic amino acids, Val or Ile, were obviously high in two of the purified fractions, which would be considerably contributing to the potent ACE inhibitory activities of these fractions. The ACE inhibitory activities of douchi (koji) pure-cultured by *Aspergillus Egyptiacus* for 48, and 72 h were compared with douchi secondary-fermented for 15 days. The results also suggested the inhibitory substances were pro-drug-type or a mixture of pro-drug-type and inhibitor-type inhibitors. The ACE inhibitors in 48 h-fermented Douchi were fractionated into four major peaks by gel filtration chromatography on Sephadex G-25. Peak 2, which had the highest activity, had only one peptide, composed of phenylalanine, isoleucine and glycine with a ratio of 1:2:5.

A $p75^{NTR}$ mutation implicated in cognitive deficits decreases prefrontal synaptic inhibition by down-regulation of potassium-chloride co-transporter KCC2

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The p75 neurotrophin receptor ($p75^{NTR}$) is a pan-neurotrophic receptor. In the adult, $p75^{NTR}$ is mainly expressed in the cholinergic neurons of basal forebrain including nucleus basalis and medial septum. However, little is known about physiological function for $p75^{NTR}$ in adulthood. Using $p75^{NTR-/-}$ mice crossed with knock-in mice expressing enhanced green fluorescence protein in cholinergic neurons, we explored the role of $p75^{NTR}$ in cholinergic intrinsic properties, neuronal circuitry functions of medial prefrontal cortex (mPFC) and cognitive functions. Disruption of $p75^{NTR}$ gene expression resulted in a robust decrease in neuronal excitability of cholinergic neurons in nucleus basalis, but not in medial septum. Further studies showed the impaired neuronal network oscillations in the mPFC. Using whole-cell patch recording, however, unchanged excitatory and inhibitory inputs onto the pyramidal neurons were observed in the mPFC. Combined with electrophysiological and biochemical experiments demonstrated an unexpected marked depolarizing shift in the reversal potential for $GABA_A$-mediated inhibitory postsynaptic currents, which it reduced GABAergic strength in mPFC. This shift in reversal potential resulted from a down-regulation expression of potassium-chloride co-transporter 2 (KCC2). The behavioral studies revealed an impaired temporal order recognition memory in $p75^{NTR-/-}$ mice. Pharmacological cholinergic enhancement rescued the KCC2 expression deficit both in vitro and in vivo and restored synaptic inhibition by reestablishing chloride homeostasis. Taken together, these findings suggest that $p75^{NTR}$ serves as a key molecule within the basal forebrain–prefrontal system in controlling recognition memory, and reveals an unexpected physiological role for $p75^{NTR}$ in the modulation of cholinergic excitability in the nucleus basalis.

Stable isotopic dilution analysis tandem mass spectrometry in quantitative studies of oxidative and nitrosative protein damage

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Studies of oxidative damage and nitrosative damage to proteins in samples collected from physiological systems by indirect methods and immunoassays has often led to qualitative estimates and overestimates of the levels oxidation and nitration adducts. Poor antigen specificity of antibodies to oxidation and nitration adducts may also have produced misleading detection of oxidised and nitrated proteins by immunocapture protocols in redox proteomics studies. Stable isotopic dilution analysis tandem mass spectrometry (LC–MS/MS) provides for robust quantitative analysis of oxidized and nitrated proteins. Appropriate pre-analytic processing and sample storage conditions are required to avoid overestimation of oxidation and nitration adducts. Generally for the most secure analysis pre-analytic processing is simplified as much as possible and lengthly sample storage is avoided. Security of estimates can also be validated in mathematical systems models knowing rates of reaction of physiological oxidants with proteins and rates of elimination of oxidised proteins. LC–MS/MS Studies over the last decade of oxidative and nitrosative damage to proteins in physiological system have found low extents of protein damage are ubiquitous throughout the microbial, plant and animal kingdom. Oxidised and nitrated proteins undergo cellular proteolysis with release of oxidised and nitrated amino acids—also called oxidation and nitration free adducts. In mammalian systems, oxidation and nitration free adducts such as methionine sulfoxide, *N*-formyl-kynurenine, *o*,*o*′-dityrosine (DT) and 3-nitrotyrsosine (3-NT) are cleared from the body by urinary excretion. For methionine sulfoxide there is enzymatic repair by methionine sulfoxide reductases, and for *N*-formyl-kynurenine there is further metabolism to kynurenine and other products. The use of LC–MS/MS in studies of formation and metabolism of oxidised and nitrated proteins in diabetes, renal failure, other disorders and ageing are described.

Proteome and free amino acid of *Polygonum minus* leaf

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The aromatic plant, *Polygonum minus*, produces essential oil of high economic value with high demands in the food, flavor and fragrance industry. The essential oil contains a variety of secondary metabolites, including aliphatic aldehydes and terpenes that have been associated with this plant unique smell. To understand the secondary metabolite biosynthesis, a proteomic approach was undertaken to identify the proteins and amino acids produced in this plant. *P. minus* leaf proteins were resolved into 1869 polypeptides with pI values ranged between 4 and 7 and relative molecular masses from 10 to 100 kDa. A master leaf polypeptide profile was generated based on the consistently expressed protein pattern. Proteins present in 97 high quality spots were identified using Mascot software and Viridiplantae database (NCBI) and by comparison with in-house EST database of this plant. Enzymes involved in carbohydrate metabolism and photosynthesis were among the main proteins identified. Subsequent amino acid analysis using LC–TOF–MS showed that from 19 amino acids detected, leucine was found to be the most abundant amino acid. The enzyme involved in the precursor biosynthesis of leucine was identified from the proteomic study. The proteome map obtained provides the basis for further study on *P. minus* physiology and will contribute to improve our understanding of plant metabolism in aromatic plant thus aiding in crop improvement effort.

H_2S production and its neuroprotective effect

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Hydrogen sulfide (H_2S) is well known toxic gas. Its toxicity was initially described by Bernardino Ramazzini in 1713. Sulfurtransferases had been intensively studied in the 1950s to 1970s, and three enzymes, cystathionine β-synthetase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3MST) were found to be able to produce H_2S in vitro. Three papers published in 1989 and 1990 reported the levels of sulfide in the brains of humans, bovine and rats, suggesting that H_2S may have a physiological function. We demonstrated that H_2S may function as a signaling molecule; a neuromodulator in the brain and a smooth muscle relaxant in tissues including blood vessels in 1996 and 1997. Since H_2S has a strong impression as toxic gas, the cytoprotective effect of this molecule has not been predicted. In 2004 we demonstrated that H_2S protects neurons from oxidative stress by reinstating the levels of glutathione, which has been decreased by oxidative stress. H_2S enhances the activity of cysteine and cystine transporters and the activity of γ-glutamyl cysteine synthetase to increase the production of glutathione. In addition, H_2S produced in mitochondria by 3MST along with cysteine aminotransferase may directly decrease the levels of reactive oxygen species in this organelle. For example, Neuro2a cells overexpressing 3MST and CAT showed significant resistance to oxidative stress. H_2S protects neurons by these integrated activities. We will also show our recent findings of the neuroprotective effect of H_2S. This work is supported by a grant from Grant-in-Aid for Challenging Exploratory Research.

Co-administration of taurine and alcohol is lethal in mice

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Acute exposure of developing rodents to ethanol causes extensive apoptosis throughout the brain. Using activated caspase-3 and TUNEL staining as apoptotic markers we recently demonstrated that 2 g/kg taurine protects about 50% of granular neurons in the internal cerebellar layers of 7-day old mice against ethanol-induced apoptosis. In an attempt to rescue more neurons we then increased the taurine dose two- and threefold. Ethanol (20% w/v solution) was administered subcutaneously at a total dose of 5 g/kg (2.5 g/kg at 1 h and 2.5 g/kg at 3 h). Total doses of taurine were injected in two half-doses (at 0 and 4 h). Opposite to our expectations the increased taurine doses administered to ethanol-injected mice induced death. The dose of 4 g/kg co-administered with ethanol killed 50% of mice and the dose of 6 g/kg killed them all. All mice treated with ethanol or taurine alone survived. The results were similar on adult 10- to 12-month-old mice. Death was precipitated by a marked fall in the blood glucose content and at least a temporal reduction of the heart rate. No changes occurred in these parameters in the mice which received only taurine or ethanol.

Our present findings seem to be an important warning sign of the interactions of taurine and ethanol and their combined toxic effects.

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Site-directed spin labelling and EPR spectroscopy

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Site-directed spin labelling (SDSL) in combination with electron paramagnetic resonance (EPR) spectroscopy is a powerful tool for the analysis of structures and conformational dynamics of proteins. A major advantage of SDSL is the possibility to investigate large membrane proteins which do not crystallise and are not amenable for NMR analysis. SDSL is based on the attachment of a stable free radical, known as a nitroxide spin-label at different positions in a protein. Cysteine moieties are the standard targets—either natural occurring or engineered by mutagenesis. The sulfhydryl group of the cysteine is then modified by the attachment of the nitroxide spin-label, and the signal of this paramagnetic group is detected by EPR spectroscopy. Continuous wave (cw)-EPR provides information on the local structure, the solvent accessibility, the polarity of the environment, and dynamic conformational changes in response to a perturbation. Distances between the spin-label and another paramagnetic centre in the protein (e.g. a second spin-label or a metal ion) in the range of 0.8–2 nm can be additionally determined. Using pulsed EPR techniques, determination of distances between two spin labels up to 8 nm is possible.

Expanding the borders of the polyamine metabolism in mammalian cells helped by omics and biocomputational techniques

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Metabolism of biogenic amines (BA) derived from cationic (polyamines, histamine) and aromatic amino acids (serotonin, dopamine) metabolism has been considered a secondary metabolic module lately included in academic biochemistry texts. However, they are involved in modulation of the most important functions for mammals/human beings: cell cycle and death, differentiation, cell–cell intercommunication (including defense, and neurons and neuroendocrine systems). BAs are ligands of multiple macromolecules (for instance, receptors, transporters, other proteins, nucleic acids, proteoglycans). Their effects are elicited through these interactions that conform very complex networks. As explained by Barabasi's group, cellular networks are governed by specific laws, the understanding of which will be essential for a deeper comprehension of Biology. In a previous work, they built a pioneer "diseasome" (a bipartite network conformed by relationships between disease phenome- and disease genome-elements), from which it can be deduced that BA metabolism and signaling-related elements accomplish most of the requirements to be genes well represented in a future complete human diseasome. In this presentation, we will present evidence supporting this assessment, as well as some examples on on-going work made in our group to advance in deciphering the exact positions of BA-related elements in the origin and evolution of many different diseases by using combined biocomputational (text mining, graph theory, metabolic modeling) and experimental technologies (HTP technologies and others). These efforts are especially valuable for the advance of knowledge and for opening new intervention possibilities in the case of rare diseases (mainly neurological, neuroendocrine and inflammation-related diseases). Work funded by Grants SAF2008-02522 and CVI-6585. CIBERER is an initiative of "Instituto de Salud Carlos III".

L-Threonine plays an important role in regulation of self-renewal of mouse embryonic stem cells via PI3K/Akt, MAPKs, and mTOR pathways

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Amino acids are able to control many physiological functions and are involved in regulating early embryonic development. Therefore, it is now widely accepted that amino acids can stimulate signal transduction and function as signal molecules regulating many embryonic stem cell (ESC) functions. Nevertheless, amino acid-dependent regulation of ESC function and related signal pathways has not been described. Thus, we investigated the effect of L-threonine on regulation of mouse (m) ESC self-renewal and related signaling pathways. Depletion of L-threonine decreased the expression of undifferentiation marker genes (Oct4, nanog, FOXD3, and Rex1, except Sox2) and proliferation (cyclins D1/CDK4 and cyclin E/CDK2), but increased the mRNA of trophoectoderm and mesoderm marker genes of mESC, which were restored by L-threonine addition. Disruption of the lipid raft/caveolae using methyl-β-cyclodextrin or caveolin-1 specific small interfering RNA blocked L-threonine-induced proliferation. In addition, L-threonine induced phosphorylation of Akt, MAPKs, and mTOR in time-dependent manner, which were blocked by PI3K/Akt inhibitors (LY 294002, wortmannin, or Akt inhibitor). L-threonine-induced activation of mTOR signaling as well as cyclins and Oct4 were blocked by MAPKs inhibitors (PD 98059, SB 203580, or SP 600125). Furthermore, L-threonine induced phosphorylation of raptor and rictor was inhibited by rapamycin. L-threonine induced translocation of rictor from the membrane to the cytosol/nuclear, which blocked by pretreatment with rapamycin. In addition, rapamycin blocked L-threonine-induced increases in mRNA of trophoectoderm and mesoderm marker genes, and proliferation of mESC. In conclusion, L-threonine stimulated mESC G1/S transition as well as maintenance of undifferentiated states through lipid raft/caveolae-dependent PI3K/Akt, mTOR, and p70S6K signaling pathways.

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Translational control and stress response in Parkinson's disease

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Parkinson's disease (PD) is a movement disorder that affects the maintenance of DA neurons. Most PD cases are sporadic, with oxidative stress being one prominent pathological feature. A small percentage of PD cases are inherited in a Mendelian fashion and several disease-causing genes have been identified.

Mutations in *leucine-rich repeat kinase 2* (*LRRK2*), a kinase gene that contains multiple domains, are known to cause autosomal-dominant late-onset PD. We found that both human LRRK2 and the *Drosophila* orthologue of LRRK2 (dLRRK) phosphorylate and inactivate eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP), a negative regulator of eIF4E-mediated protein translation and a key mediator of various stress responses. While modulation of the eIF4E/4E-BP pathway by LRRK2 stimulates eIF4E-mediated protein translation both in vivo and in vitro, it attenuates resistance to oxidative stress and survival of DA neuron in *Drosophila*. Moreover, we found that dLRRK as well as human LRRK2 negatively regulates microRNA-mediated translational repression through phosphorylation of 4E-BP. Searching for translationally upregulated proteins that are responsible for neurodegeneration by the impairment of microRNA pathway and the activation of eIF4E-dependent protein translation, we identified the E2F1-DP heterodimeric transcription factor complex. The expression of E2F1 and DP are regulated by miR-184* and let-7, respectively. Inhibition of E2F1 and DP expression by genetic deletion of these genes or introduction of miR-184*/let-7 attenuated pathogenic effects of LRRK2 in dopaminergic neurons. These results implicate chronic phosphorylation of 4E-BP by LRRK2 with pathogenic mutations induces overactivation of E2F-DP, which eventually results in age-dependent loss of DA neurons.

Translation regulation by leucyl-tRNA synthetase and methionyl-tRNA synthetase via amino acid signaling and catalytic activity control

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Deregulation or misregulation of protein synthesis is frequently observed in cancer and accurate control of translation is important for the maintenance of normal cell growth and proliferation. Assembly of eukaryotic initiation factor (eIF) 4F (eIF4E·eIF4G·eIF4A) and formation of ternary complex (eIF2·GTP·Met-tRNA$_i^{Met}$) are the rate limiting steps of translation initiation. Various signals are transduced to these steps via mTOR pathway and phosphorylation of eIF2 subunit α to cope with stresses by controlling translation. Here we demonstrated that aminoacyl-tRNA synthetases (ARSs), specifically leucyl-tRNA synthetase (LRS) and methionyl-tRNA synthetase (MRS), can control translation through signaling amino acid to mTOR and reducing Met-tRNA$_i^{Met}$ upon UV irradiation, respectively.

Leucyl-tRNA synthetase senses leucine concentration and mediates leucine-induced mTORC1 activation. LRS mutation of amino acid residues which are important for leucine binding renders the mTORC1 pathway insensitive to amino acid. LRS directly binds to Rag GTPase, a mediator of amino acid signaling to mTORC1, in an amino acid-dependent manner and functions as a GTPase-activating protein for Rag GTPase to activate mTORC1.

Methionyl-tRNA synthetase provides a cytosolic anchoring site for AIMP3/p18, a potent tumor suppressor that is translocated to the nucleus for DNA repair. Upon UV stress, MRS is phosphorylated by GCN2 at Ser662 residue. This phosphorylation induces AIMP3 release for nuclear translocation as well as catalytic inactivation of MRS resulting global translation inhibition.

This work reveals a novel mode of regulation of global translation as mediated by ARSs. In addition our research suggests that LRS and MRS can play as key mediators for translation and cancer control.

Leucine deprivation increases hepatic insulin sensitivity via GCN2/mTOR/S6K1 and AMPK pathways

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We have previously discovered that serum insulin levels decrease threefold and blood glucose levels remain normal in mice fed a leucine-deficient diet, suggesting increased insulin sensitivity. Recently, we confirmed this hypothesis by demonstrating that leucine deprivation improves insulin sensitivity in both normal and insulin-resistant conditions. Furthermore, we found that leucine-deprived mice exhibited increased insulin sensitivity in different tissues including liver. By using different molecular techniques in cell and animal models, we found that leucine deprivation improves hepatic insulin sensitivity by sequentially activating general control nonderepressible (GCN)2 and decreasing mammalian target of rapamycin/S6K1 signaling. In addition, we show that activation of AMP-activated protein kinase also contributes to leucine deprivation-increased hepatic insulin sensitivity. This study describes mechanisms underlying increased hepatic insulin sensitivity under leucine deprivation. Furthermore, we demonstrate a novel function for GCN2 in the regulation of insulin sensitivity.

Regional and cell-type-specific distribution of HDAC2 in the mouse brain

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Although the epigenetics was studied extensively in various fields of biomedicine, its critical role in brain functions has only recently been appreciated. As one part of epigenetics, it is now increasingly realizing that histone deacetylases (HDACs) get into the neural events including synaptic plasticity and neurodegenerative diseases through regulating acetylation status of target proteins, such as histones H3 and H4, to influence protein function and gene expression. Currently,

we restrict our focus on HDAC2, one member of HDACs family. We attempt to illuminate the regional distribution of HDAC2 in the C57BL/6J mouse brain using immunochemistry staining. Our data showed that HDAC2 is near ubiquitously expressed throughout the brain as a mainly nuclear localization protein. We also performed double immunofluorescence staining to identify the cell types at the level of subregions and cell layers in which the HDAC2 were expressed. We found that HDAC2 was mainly present in neuron-specific cells, such as cholinergic neurons, postsynaptic glutamatergic neurons, catecholaminergic neuron and GABAergic neurons. In glial cells, HDAC2 was also expressed in oligodendrocytes. However, no HDAC2 positive staining was found in astrocytes and microglial cells. This work provides information regarding the distribution of HDAC2 throughout the mouse brain, including cell type localization. We hope that this will offer a valuable resource for understanding the role of HDAC2 in brain functions and neurological diseases and disorders.

Keywords: Histone deacetylase; Epigenetics; Brain; Hippocampus

Preventions of taurine from damage of brain and kidney in mice exposed to arsenic subchronically

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To evaluate the preventions of taurine from damage of brain and kidney in mice exposed to arsenic (As) subchronically, adverse effects of As on nucleic acid, the critical protein expression related to mitochondrial respiratory chain and morphology were observed in brain or kidney tissues of mice exposed to As. Forty mice were randomly divided into 3 groups of 10 each. Group 1 received drinking water alone (control). Group 2 received 4 mg/L arsenic trioxide. Group 3 received both 4 mg/L arsenic trioxide and 150 mg/kg taurine (as protective group). Arsenic trioxide was given through drinking water for 60 days and taurine was affused to stomach twice a week. The group treated with As showed pathologic changes in the brain and kidney tissues and significant increase in the expression levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) and 8-nitroguanine (8-NO_2-G). The down-regulated protein expression of succinate dehydrogenase subunit A (Sdha) and the decreased activity of SDH were also observed in the mitochondria of brain tissue exposed to As. On the contrary, the protective group showed mild pathologic changes, very low level of 8-OHdG or 8-NO_2-G expression, light down-regulation of Sdha expression. These results suggested that the coadministered taurine in vivo protects against the adverse effects of As in brain and kidney tissues of mice. It was indicated that taurine antagonism to toxic effect of As on the SDH activity in the mitochondria may be another mechanism of its protections through controlling the ROS or RNS signaling pathway.

Neurological infections by Gram positive and Gram negative bacteria and their implication on neurotransmitters of central nervous system

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The mechanism of infection progression and the underlying neuro-circuits are yet to be explored. This study was done to determine the effect of bacterial metabolites on CNS by evaluating the neuro-transmitters of it along with the motor activity of the animal model. 37 cerebrospinal fluid samples were collected; causative bacteria were identified as *Neissaria* (N) and *L. monocytogenes* (L). *C. tetani* (C) and *B. cereus* (B) were also included in this study. Filter sterilized cell free supernatant (SCFS) were obtained from nutrient broth (NB) cultures of each bacteria. Six groups of Sprague–Dawley rats ($n = 7$) were injected intraperitoneally with SCFS. Control groups were injected with saline S and NB. Motor activity was visualized. The brains were analysed by HPLC-EC. L and B developed fever within 20 min. L exhibited curled body posture. C group's hind limb paralysed within 2 min. Hind paw of N swelled within 5 min. Drowsiness was the common trait for all test groups. The chromatograms of tests were compared with the controls. In N the quantity of DOPAC, DA, 5HIAA in brain was enhanced. DOPAC was raised in all test groups. 5HIAA in L, C, and B was comparatively enhanced. In L it raised significantly. HVA was elevated in C relative to S. Significant quantity of 5HT was found exclusively in B group. The intraperitoneal shot of SCFS in fact mimic the scenario of in vivo infection and implicates its effects on CNS. Paralysis of limb and inflammation of paw in C and N groups exhibited the blockade of nicotinic receptors of cholinergic neuron and activation of neuroendocrine, respectively. It is hypothesized that neurotransmission in response to infection is an epigenetical phenomena integrated to the immunogenetic of disease progression.

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The effects of leucine and Walker factor on cell signaling in Vero cells

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Branched-chain amino acids, especially leucine, exert regulatory influence on protein, inducing cellular signalling and inhibiting the activity of ubiquitin–proteasome pathway in muscle of cachectic rats and improves muscle protein synthesis in tumour-bearing rats. Therefore, the cancer cachexia state can be induced and enhanced by factors produced by tumours or host tissues. The Walker Factor (WF) is a proteolysis-inducing factor (PIF)-like protein, purified from the ascitic fluid of the Walker 256 tumour-bearing rat, and is immunologically identical to PIF. The WF acts on muscle proteolysis increasing the expression and activity of the ubiquitin–proteasome pathway. Since Vero cells have well defined specific cellular characteristics, this study investigated the effects of Walker Factor exposure, modulated by leucine treatment on key cell signalling proteins in Vero cells metabolism. We analysed the effects of leucine (50 μM) in Vero cells exposed to WF (5 and 25 μg/mL). The WF-treated Vero cell increased the phosphorylated and also the total STAT3, Akt and Erk on WF5-treated cell, but not at 25 μgWF. The leucine-treated cells showed increased on phosphorylation of STAT3, Akt and Erk, and the leucine treatment modulated the increased phosphorylation produced by WF exposure in Vero cells. The total protein degradation was increased as a potent effect of Walker factor in Vero cells, while the leucine treatment induced less protein degradation and improved the total net protein in WF-treated cells. These results strongly suggest an important modulatory effect of leucine

when Vero cells under WF effects were previously treated with this branched-chain amino acid.

Cancer prevention and incidence in rats submitted to nutritional supplementation of glutamine, Omega-3 and Omega-6 polyunsaturated fatty acids

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This work evaluated the nutritional supplementation with polyunsaturated fatty acids, Omega-3 and Omega-6, and glutamine on the prevention, incidence and evolution of Walker-256-tumour-bearing rats.

Methods: Rats were distributed into groups according to diet and tumour (T): C-control; CT-tumour-bearing; GT-tumour-bearing fed glutamine diet (4% L-glutamine); ω3T-tumour-bearing fed ω3-diet (24% omega-3); ω6T-tumour-bearing fed ω6-diet (24% omega-6); ω3/ω6T-tumour-bearing fed ω3/ω6-rich diet (12% omega-3 and 12% omega-6); Gω3/ω6T-tumour-bearing fed diet with glutamine, ω3 and ω6. The animals were killed after 20 days of tumor implant. Results showed that the body weight decreased in ω3T and ω3T/ω6T; and was maintained in GT, ω6T and G/ω3/ω6T in relation to C. All supplemented groups had higher relative weight tumor, spleen and liver than CT, except in GT. Fat tissue was increased in all groups analyzed; the hematocrit reduced in all tumour-bearing groups, but ω3T and G/ω3/ω6T groups recovery the glucose, total protein and albumin values. The tumour histopathology showed that there was a reduction in the number of cells but no change in mitoses index in ω3/ω6T group compared to CT group.

Conclusion: the polyunsaturated fatty acids-rich diet, especially rich in omega-3, can modulate tumor growth. On the other hand, diets rich in glutamine, ω-3 and ω-6 (G/ω3/ω6T) and omega 3 (ω3T) promoted recovering of blood glucose, total protein and albumin, parameters which evaluate the nutritional state in this cachexia model tumour-bearing host.