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The significance of tryptophan in human nutrition

Minireview Article

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Summary. Aside from its role as one of the limiting essential amino acids in protein metabolism, tryptophan (TRP) serves as precursor for the synthesis of the neurotransmitters serotonin and tryptamine as well as for the synthesis of the antipellagra vitamin nicotinic acid and the epiphyseal hormone melatonin.

By involvement in so manifold pathways, TRP and its metabolites regulate neurobehavioral effects such as appetite, sleeping-waking-rhythm and pain perception. TRP is the only amino acid which binds to serum albumin to a high degree. Its transport through cell membranes is competetively inhibited by large neutral amino acids (NAA). The TRP/NAA ratio in plasma is essential for the TRP availability and thus for the serotonin synthesis in the brain.

Due to its high TRP-concentration, human milk protein provides optimal conditions for the availability of the neurotransmitter serotonin. Low protein cow's milk-based infant formulas supplemented with α-lactalbumin – a whey protein fraction containing 5.8% TRP – present themselves as a new generation of formulas, with an amino acid pattern different from the currently used protein mixtures of adapted formulas, resembling that of human milk to a much higher degree.

Keywords: Amino acids – Tryptophan (TRP) metabolism – Human nutrition – Serotonin – Nicotinic acid

Occurrence of tryptophan in foods

Tryptophan (TRP) is in many aspects one of the most interesting amino acids. It serves as a precursor of the neurotransmitter serotonin and the majority of the requirement for the pellagra preventive vitamin nicotinic acid is met by TRP. It is the limiting amino acid in almost all protein sources which are of importance for human nutrition. TRP is completely lacking in gelatine and its content in yeast and corn is remarkably low. Nutrients of relatively high TRP

content (expressed in g/16 g nitrogen) are eggs, milk, meat, soybean, potatoes and cereals such as rice, barley, wheat, rye and oats (Wissenschaftliche Tabellen Geigy, 1977). The distribution of TRP in food proteins differs considerably among the different fractions. In cow's milk protein, α-lactalbumin contains around 5.8% TRP, whereas bovine serum albumin and β-casein are extremely poor in TRP (Heine et al., 1991). The amino acid composition of the α-lactalbumin fraction and the amino acid sequences of human milk α-lactalbumin and cow's milk-lactalbumin are similar.

However, human milk protein consists of 28% lactalbumin, whereas lactalbumin in cow's milk contributes to only 3% of total protein. TRP supply from human milk is, consequently, much higher than from equinitrogenous amounts of cow's milk.

TRP analysis

Due to the lack of specific and sensitive methods quantitative determinations of TRP in proteins and biological fluids were not accessible in former times.

Therefore data on TRP concentration in proteins and biological fluids were often missing in older studies (Dickinson et al., 1965; Pohlandt, 1975; Widdowson et al., 1979). Even present data on the TRP concentrations of human milk for example as reported in the literature vary broadly (Heine, 1994), which is at least in part due to analytical difficulties. Acidic hydrolysis of proteins was found to be correlated with almost complete destruction of TRP. Quantitative determination of TRP in protein requires tryptic digestion or careful alkaline hydrolysis. Detection of free TRP was formerly based on colour reactions and microbiologic determinations. Although numerous colorimetric-, ion-exchange chromatographic-, fluorimetric or electrochemical detection methods have been proposed (Friedman and Finley, 1975; Williams et al., 1982), the determination of TRP is currently a problem (Nielsen and Hurrell, 1985). The determination of TRP is dependent on whether

i) total protein TRP (with acid- or alkaline-hydrolysis), or
ii) free TRP (without hydrolysis but protein precipitation), or
iii) albumin-bound TRP is to be analysed.

Normally, free TRP amounts to only 5–12% of total TRP in human plasma (Eccleston, 1973). Between 80 and 90% of circulating TRP is bound to serum albumin which is unique among the amino acids (Foller and Roush, 1973; Tricklebank et al., 1979). High concentrations of free fatty acids may compete with the TRP binding and displace TRP from the transport proteins (Greiling and Gressner, 1989).

In order to estimate total TRP (free and albumin bound), one of the most promising techniques appears to be human plasma treatment with 10% trichloracetic acid (20:80 vol-%), (Körbel, 1984). It is assumed that mild semi-hydrolytic conditions at pH-levels lower than 2 help to release the TRP from the serum albumin. Therefore, the solution is allowed to stand for 1 hr at 4°C

prior to centrifugation. The supernatant is than used for HPLC-analysis. The HPLC apparatus consists of a Merck-Hitachi controller, a Rheodyne injector fitted with a 20 μL loop, a gradient pump L-6200, and a fluorescence spectrophotometer F-1050. Plasma supernatant constituents are isocratically separated at a flow rate of 1 mL/min using a reversed phase column (RP-18, 5 μm, 4.0 × 250 mm). The mobile phase consists of 20 mM sodiumphosphate-buffer (pH 3.25) and acetonitrile 70:30 vol-%. The eluants are monitored fluorimetrically (excitation at 278 nm, emission at 363 nm), and peak areas are determined using an integrator.

Another conventional method is also used very often; (Maire, 1994): Plasma (20 μL) is crudely deproteinized by mixing with 980 μL methanol. After centrifugation, 600 μL of the supernatant solution is diluted with 600 μL water. The solution is then ultrafiltered (Ultrafree-MC 10,000 MW cut-off, Milipore UFC3 TGC) to remove all large molecular weight compounds. The HPLC-system consists of a refrigerated automatic injector, using a reverse phase water C_{18} resolve column (5 μm, 3.9 × 150 mm). The liquid phase consists of 75 mM ammonium acetate buffer (pH 4.5) containing 10% methanol. The eluants are monitored by electrochemical detection on a glassy carbon working electrode with a KCl-reference electrode.

Tryptophan requirement

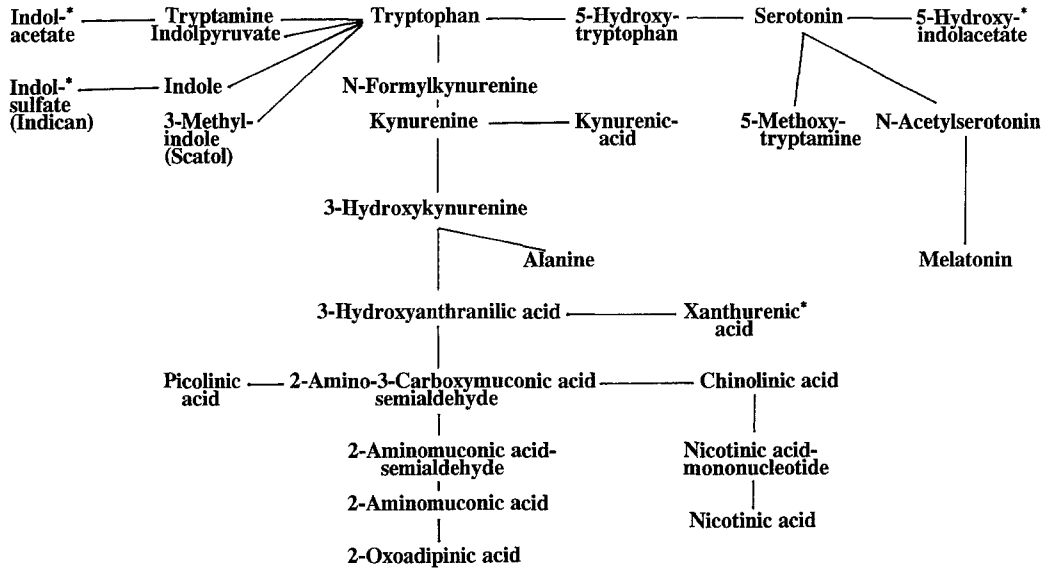
TRP belongs to the group of essential amino acids. In contrast to lysine and threonine, TRP can theoretically be synthesized by the human organism by transamination if the carbohydrate skeleton is provided. However, this is of no consequence in amino acid metabolism.
The bulk of dietary TRP flows into the synthesis of body proteins.

Minimal requirement of TRP was estimated to be 250 mg/day for males and 157 mg/day for females (Food and Nutrition Board, 1959, 1974). The average intake of TRP on a normal diet in adults is around 250–500 mg/day.

In relation to the body mass, the TRP requirement of the fast growing infant is relatively high. The daily need for TRP in infants is 19 mg/kg, whereas children aged 10–12 years require at least 4 mg/kg/day and adults 3 mg/kg/day. Snyderman and co-workers estimated the minimal requirement for TRP in infants to be 22 mg/kg/day (Snyderman et al., 1961; Snyderman, 1974). On human milk feeding, TRP intake of the infant amounts to approximately 145 mg/day. To meet the nutritional needs of infants the proportion of TRP to the total of all essential amino acids was determined by Snyderman (1986), Jürgens (1985, 1986) and Bürger to be 2.2–2.8 mmol%.

Tryptophan degradation products (Fig. 1)

With regard to the formation of bioactive metabolites arising as degradation products, TRP holds an exceptional position among the group of amino acids. Hydroxylation in position 5 of the indolring by TRP-5-monooxygenase results in the formation of 5-hydroxytryptophan. Dioxygen and tetrahydrobiopterin



* Eliminated with the urine.

Fig. 1. Metabolic pathways of tryptophan

serve as cofactors in this metabolic step. 5-hydroxytryptophan is then converted by decarboxylation to 5-hydroxytryptamin (serotonin). This conversion is catalyzed by an aromatic amino acid decarboxylase. Serotonin serves as an inhibitory neurotransmitter. Its synthesis takes place in serotonergic nerves, enterochromaffinic cells, thrombocytes and mast cells. Serotonin is widely distributed in the hypothalamus. Its concentration is particulary high in the suprachiasmatic and arcuatic nuclei (Saavedra et al., 1974). Like other neurotransmitters such as acetylcholine, suprarenin, noradrenaline, dopamine and gamma-aminobutyrinic acid serotonin is stored in the presynaptic vesicles at the nerve endings. The release of serotonin from the vesicles is specific for serotonergic neurons and is triggered by nerve stimulation-related changes of the action potential. Serotonin released from the vesicles diffuses through the synaptic cleft giving rise to changes in the permeability and of the potential of the postsynaptic membrane. In contrast to its precursor 5-hydroxytryptophan, serotonin is unable to pass the blood-brain-barrier. Cerebral serotonin-deficiency states, therefore, react exclusively positive to treatment with this precursor.

Serotonin is further metabolized in the pineal body by acrylaminoacetyltransferase to N-acetylserotonin and by acetylserotonin methyltransferase to melatonin. The latter enzyme also converts serotonin to 5-methoxytryptamine. Melatonin is of importance for the control of the day- and night-rhythm and serves as an intracellular scavenger of hydroxyl- and peroxide-radicals. In this connection it is supposed to inhibit formation and growth of malignomas, the development of cataracts and to protect organs from bacterial endotoxin affections. It is also claimed to be active in the decay of age-dependent nerve degeneration and in preventing tissue damages in the

course of cardial infarctions (Hardeland et al., 1993; Reiter, 1993; Reiter et al., 1994).

The main metabolic pathway of TRP degradation leads to nicotinic acid ribonucleotide. Sixty mg TRP are equivalent to 1 mg nicotinic acid. This pathway depends on thiamine, riboflavin and pyridoxine; Greiling and Gressner (1989). Intermediary metabolites formed in the course of TRP degradation are N-formylkynurenine, kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, 2-amino-3-carboxymuconic acid semialdehyde and chinolenic acid, Nicotinic acid and nicotinic acid amine represent the pellagra-preventive vitamin. Nicotinic acid amide and its phosphate derivate serve as coenzymes of dehydrogenases, which play an important role in energy synthesis within the respiratory chain.

Further metabolic products derived from the metabolite 2-amino-3-carboxymuconic acid semialdehyde are picolinic acid and 2-oxoadipinic acid, which flows into further degradation to acetacetyl CoA.

Decarboxylation of TRP by aromatic L-amino acid decarboxylase results in formation of tryptamine, which has a stimulating effect on smooth muscles and on the central nervous system.

TRP originating from food proteins and from endogenous sources when subjected to microbial degradation in the large bowel, is degraded to indole and β-methyl-indole (skatol), indolacetate, indolpyruvate and indoxylsulfate (indican).

The significance of nutritional tryptophan supply in infants, children and adults

It was already mentioned that human milk is comparatively rich in TRP. This is mainly due to the high proportion of α-lactalbumin, immunoglobulin A and lactoferrin (Harzer, 1989). A protein high in TRP as supplied to the breast-fed infant has apparent beneficial effects on conscious behavior and sleep patterns (Pollet and Leathwood, 1983). This is due to the TRP-dependent serotonin synthesis in the brain.

There is evidence that low TRP-intakes are correlated with low serotonin levels. Though cow's milk protein as compared with human milk is relatively poor in TRP, TRP-deficient conditions do not occur on formula feeding if the protein concentration of the formula – as it is currently the case – is 1.5 to 2 times higher than in human milk. The supplementation of cow's milk with whey proteins rich in TRP – which were introduced in the 1950s – met the requirements for reducing the protein concentration in infant formulas. At present, protein concentrations in infant formulas are still 2 times higher than in human milk. Protein administered in abundance results in the formation of urea and ammonia and other end-products which are usually eliminated with the urine. Thus, excessive protein intake represents a useless metabolic load. For this reason, a further reduction of the protein concentration in infant formulas is desirable. However, a further adaption of formula protein concentrations towards the standard value of human milk causes a reduction of the

TRP and taurine concentrations of the serum in infants fed with these formulas even when they contain whey protein in excess (Janas et al., 1985, 1987; Jarvenpää et al., 1982a,b), and the surplus of neutral amino acids in relation to the TRP content of the protein component does not guarantee a sufficient TRP supply of the brain (Anderson and Johnson, 1983; Wurtman, 1982, 1988).

Low TRP serum concentrations when compared with similar values as observed in human milk feeding may reflect TRP depletion states, which are probably of importance for whole body protein synthesis and serotonin-related disturbances of the sleeping-waking-rhythm, appetite regulation and other serotonin-dependent abnormalities in behavior. There are two options to attain protein-reduced formulas with enriched TRP concentration.

As shown by Fazziolari-Nesci and co-workers (1992) supplementation of protein-reduced infant formulas with free TRP succeeds in TRP serum levels in infants fed these preparations which do not differ from those of breast-fed infants.

However, in view of absorption kinetics of free and protein-bound TRP as well as in toxicological and economic aspects, protein fractions rich in TRP seem to be a better option, especially since such fractions offer the opportunity to adapt the whole amino acid pattern including cystine and other amino acids more closely to that to human milk. For this purpose, α-lactalbumin presents itself as the most suitable fraction of cow's milk (Forsum, 1974; Heine et al., 1991).

Modern ultrafiltration techniques currently available allow a selective enrichment of the low molecular α-lactalbumin in whey protein. Our own studies with an α-lactalbumin enriched formula (1.3% protein, TRP content 2.21% of the total protein) revealed TRP and taurine serum concentrations in 10 infants fed on this formula which did not differ significantly from those of a control-group fed on human milk, whereas a formula containing 1.3 g% of protein and 1.88% TRP in its protein component presented significantly lower TRP serum levels (Heine et al., 1995).

Correlations between tryptophan supply and serotonin synthesis

TRP intake in adults is around 0.25 to 0.5 g/day. A small proportion of this amino acid serves as a precursor for serotonin synthesis. The proportion of serotonin synthetized in the brain amounts to only 1–2% of the total body serotonin synthesis (Cooper et al., 1986). Diurnal fluctuation of plasma- and brain-TRP was shown in rats by Maher (1984).

Plasma and brain TRP levels peaked between 10 pm and 2 am (8 hours later than in humans since rats eat at the onset of the dark cycle). Serotonin levels in the brain lagged behind by a few hours but showed a clear relationship.

The neurons synthesizing the neurotransmitter serotonin are involved in the regulation of appetite, in the sleeping- and waking-rhythm, in affective reaction control as well as in aggression and sexual behavior (Faust et al.,

Table 1. Neurobehavioral effects of tryptophan (TRP) as a precursor of serotonin

TRP-free diets	TRP-loaded diets
– Depressed mood	– Elevated mood
– Insomnia	– Calmness, drowsiness
– Increased carbohydrate intake	– Decreased appetite for carbohydrates
– Disturbances in affective reaction control and sexual behavior	– Decreased pain threshold

1990), (Table 1). Furthermore, the TRP precursor 5-hydroxytryptophan was shown to increase secretion of growth hormone (Collu et al., 1972; Lancranjan et al., 1977). There is evidence that growth hormone may be stimulated by excessive serotonin secretion in patients with Carcinoid syndrome (Feldman and Lebowitz, 1972). Serotonin increases the motility of the gastrointestinal tract and may result in protein deficiency due to increased TRP demand of the serotonin-producing tumor (Siegenthaler, 1987). There is also evidence that serotonin may stimulate prolactin secretion via vasoactive intestinal peptide and prolactin stimulating factor (Shimatzu et al., 1985).

The synthesis of the neurotransmitter serotonin is probably correlated with physiological fluctuations of the intake of foods even if eaten at a single meal (Wurtman, 1988). Dietary TRP depletion may reduce the levels of free and total plasma TRP by more than 80% from baseline levels (Barr et al., 1994; Zimmermann et al., 1993).

Serotonin synthesis is directly dependent on the availability of the specific precursor tryptophan and on the nutritional status of individuals (Goodwin et al., 1987), and on the local concentration of TRP (Eccleston et al., 1965; Fernstrom and Wurtman, 1971; Moir and Eccleston, 1968; Schaechter and Wurtman, 1990).

As shown in numerous studies elevation of TRP concentration in the brain results in an increased release of serotonin (Auerbach and Lipton, 1982; Broderick and Jacoby, 1988; Carboni et al., 1989; DeSimoni et al., 1987; Eccleston et al., 1965; Fernstrom and Wurtman, 1971; Moir and Eccleston, 1968 Schaechter and Wurtman, 1990; Ternaux et al., 1976; Yokogoshi et al., 1987). By contrast, Elks and co-workers (1979) could not confirm a dependence between tryptophan and serotonin synthesis using brain slice techniques. Analogous results were obtained by Marsden et al. (1979) and Trulson (1985).

In rat experiments, amino acid mixtures deficient of TRP have proves to decrease the serotonin tissue concentration of the brain and the serotonin neurotransmission (Benedetti and Moja, 1993). In monkeys, significant correlations between serotonin and its precursor 5-hydroxy-TRP and TRP levels in blood and cerebral fluid have been described (Yan et al., 1993).

Healthy human subjects reacted to dietary restriction of TRP intake to 50% of the minimal requirement with a decreased urinary excretion of 5-

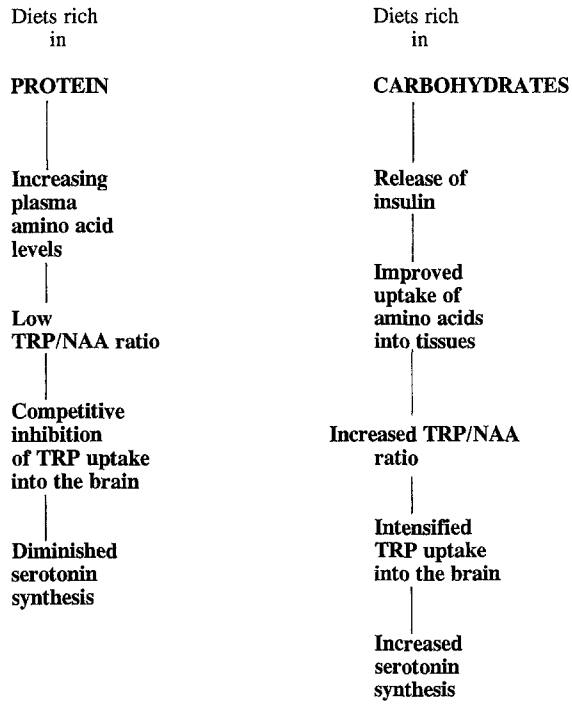


Fig. 2. Consequences of dietary protein and carbohydrate intake on tryptophan availability to serotonin synthesis in the brain

hydroxyindolacetic acid, which rapidly normalized after restoration of the regular TRP supply (Alfieri and Cubeddu, 1994).

Dietary TRP supply is considered the most effective and easiest method to alter gastrointestinal serotonin (Anderson and Johnson, 1983; Wurtman, 1983, 1988).

Intracellular transport of tryptophan is mediated by a common carrier mechanism for the large neutral amino acids (NAA) leucine, isoleucine, valine, phenylalanine, tyrosine and methionine. TRP-passage through the blood-brain-barrier is competetively influenced by the concentration of these amino acids, which increase in plasma following protein ingestion. Meals rich in protein may, therefore, paradoxically lower the tryptophan availability of the brain. Controversely, ingestion of carbohydrates increases plasma TRP and consecutively TRP availability to the brain (Anderson and Johnson, 1983; Wurtman, 1983); (Fig. 2).

This is due to the carbohydrate-induced release of insulin which results in an increased uptake of free plasma amino acids into the muscle tissue with exception of the serumalbumin-bound proportion of TRP. It is speculated that the protein-bound proportion of TRP is then released during the perfusion of the brain capillaries, leading to an increased ratio between TRP and the bulk of other neutral amino acids which meets the requirements for the enhanced transport of TRP into the brain tissue. This thesis is supported by rat experiments, in which increased 5-hydroxyindol concentrations were observed after feeding low-protein diets enriched with TRP (Yokogoshi et al., 1987). These correlations were confirmed by animal experiments conducted

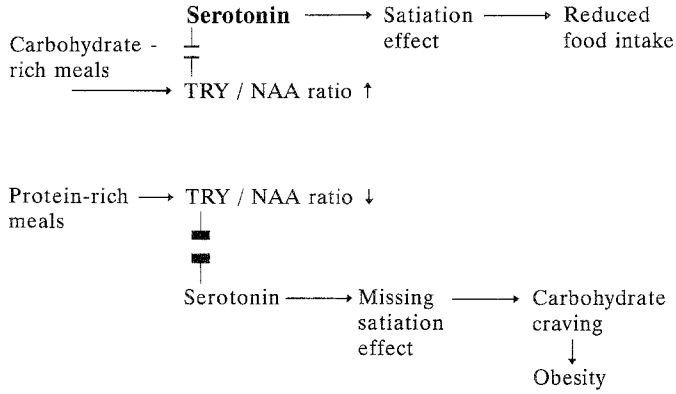


Fig. 3. Serotoin-dependent regulation of food intake

by Ng and Anderson (1992) who observed a significant suppression of food intake following intraperitoneal TRP injections. However, no preferential effect was shown for either the high carbohydrate or high protein diet choice. This pathway is supposed to play an important role in the serotonin-dependent regulation of food intake (Fig. 3).

The amino acid pattern of human milk protein provides a high TRP/NAA ratio, which brings about high plasma TRP levels in relation to the bulk of other neutral amino acids, a high TRP transport across the blood-brain-barrier and thus a sufficient serotonin synthesis. This may explain neurobehavioral differences between breast-fed and formula-fed infants.

Serotonin deficiency is regarded as an active principle in the pathogenesis of neurological damages in biopterin deficiency-related phenylketonuria. In the synthesis of serotonin, tetrahydrobiopterin serves as a cofactor of tryptophanhydroxylase, which is essential for the synthesis of the serotonin precursor 5-hydroxytryptophan (Boehles, 1991). Clinical application of a TRP-reduced diet has been reported to be effective in Huntington's disease (Pascoe, 1993). In combination with lysine restriction and riboflavin/carnitine supplementation TRP-restricted diet was shown to arrest the neurologic deterioration in children with glutaric acidemia type I (Yannicelli et al., 1994).

The pathogenesis of migraine is also linked with an increased release of serotonin, causing vasoconstriction and consecutive vasodilatation. Concomitant release of mediator substances such as substance P contributes to the formation of local edemas and a decreased pain threshold.

Whether dietary tryptophan intake is involved in triggering migraine attacks has not been proven. However, avoidance of excessive food intake in the evening together with reduced fluid intake is recommended as a prophylactic measure in the intermediate treatment of migraine (Mutschler, 1991).

Inborn errors of tryptophan metabolism

Normal TRP degradation is dependent on a variety of different enzymes. By means of detection of accumulated breakdown products, numerous inherited defects of TRP metabolism have mostly been described as case reports. This

refers to disorders such as Tada-syndrome (Tada et al., 1963), Price-syndrome (Price et al., 1967), Vitamin B_6-dependent xanthuremic aciduria (Knapp, 1962) and Komrower's syndrome (Komrower et al., 1964). The underlying genetic defect in Hartnup's syndrome is a disturbed transport of neutral amino acids by the intestinal mucosa and renal tubules. For absorption of TRP, at least two transport mechanisms are present in the gut. One of these transport mechanisms is specific for TRP, the other is shared by other neutral amino acids. In Hartnup's syndrome, plasma concentrations of tryptophan and the other neutral amino acids are usually within normal limits. Malabsorbed TRP is subjected to bacterial conversion to indole which after absorption is oxidized, sulfated and renally excreted as indican.

In the Blue Diaper syndrome, a familial genetic TRP malabsorption disorder, characterized by nephrocalcinosis, hypercalcemia and indicanuria, the blue change of the colour of the urine is due to the oxidation of indican to indigo on exposure to air.

Hartnup's disorder responds to treatment with nicotinic acid or nicotinamide at a dosage between 50 and 300 mg/day and to a high-protein diet. In phenylketonuria, due to biopterin deficiency, supplementation of tetrahydrobiopterin at a dosage of 1.5–3 mg/kg/day in combination with L-dihydroxyphenylalanine (dopa) (1–1.5 mg/kg/day), carbidopa (1.5 mg/kg/day) and 5-hydroxytryptophan is recommended in order to prevent neurological damages which occur in these disorders in spite of dietary phenylalanine restriction (Boehles, 1991). Diets selectively low or high in TRP do not play a role in the treatment of inborn errors of TRP metabolism.

Tryptophan-loading tests

Oral TRP loadings with 5 g TRP in vitamin B_6 deficiency states result in increased renal excretion of xanthurenic acid, kynurenine, hydroxykynurenine and kynurenic acid, whereas in riboflavin deficiency kynurenic and anthranilic acid are the main metabolites, which are excreted in excess.

A xanthurenic acid excretion of <25 mg in 6 hours following an oral load with 100 mg TRP reflects a normal vitamin B_6 supply. Excessive xanthurenic aciduria and kynurenineuria have been described as an inherited, vitamin B_6-dependent disturbance by Knapp in 1962.

Therapeutic effects of excessive tryptophan intake

The sleep-inducing effect of TRP was first described in 1962 (Siegenthaler, 1987). The substance was then widely used as a therapeutic agent in patients with difficulties in falling asleep and due to its stimulating effect also in depressive syndromes, the single dose being in the range of several grams. Side effects of this treatment such as bloating, nausea and vomiting were relatively rare.

In 1989 the registration of TRP was worldwide cancelled due to suspected correlations with the eosinophilia-myalgia syndrome (EMS). The features of

this syndrome are pain in muscles and joints, often accompanied with fever, swelling of the limbs, skin reactions and occasionally with dyspnea. The blood picture is generally characterized by an excessive eosinophilia. Carr and co-workers (1994), reviewing the epidemiologic features and the incidence of EMS in Germany, registered 105 patients who fulfilled the criteria for EMS. The study supported the pathophysiologic role of a contaminant in the L-TRP preparations as the causal connection with EMS. The contaminant implicated in the manifestation of EMS was suspected to be 1,1-ethylidenebis tryptophan. However, binding studies with rat liver nuclear envelope protein did not confirm this suspicion (Sidransky et al., 1992). There is evidence that other impurities of the product which are unknown at present may be envolved in the development of EMS. The only TRP-derivate which is still licensed for the treatment of serotonin-deficiency states is 5-hydroxytryptophan. However, its indication is strictly limited to biopterinsynthase- and dihydrobiopterin-reductase-defects in which substitution of the serotonin precursor 5-hydroxytryptophan is life-saving.

Observations made before biotechnologically-produced TRP was withdrawn from the market point at the efficacy of TRP in patients with depressions. While TRP depletion did not show mood changes, return to normal TRP intake resulted in an improvement in mood in 1/3 of the patients (Miller et al., 1992).

The beneficial effects of therapeutic doses of TRP in patients with sleeping disorders has been substanciated in many cases in the past. Presently it is just a matter of speculation, whether comparable effects on neuropsychological behavior can be achieved with food proteins rich in TRP. Corresponding studies ought to be performed with α-lactalbumin-enriched proteins.

References

Alfieri AB, Cubeddu LX (1994) Effects of inhibition of serotonin synthesis on 5-hydroxyindoleacetic acid excretion in healthy subjects. J Clin Pharmacol 34: 153–157

Anderson GH, Johnson J (1983) Nutrient control of brain neurotransmitter synthesis and function. Can J Physiol Pharmacol 61: 271–281

Auerbach S, Lipton P (1982) Vasopressin augments depolarization-induced release and synthesis of serotonin in hippocampal slices. J Neurosci 2: 477–482

Barr LC, Goodman WK, McDougle CJ, Delgado PL, Heninger GR, Charney DS, Price LH (1994) Tryptophan depletion in patients with obsessive-compulsive disorder who respond to serotonin reuptake inhibitors. Arch Gen Psychiatry 51: 309–317

Benedetti F, Moja EA (1993) Failure of a tryptophan-free amino acid mixture to modify sexual behavior in the female rat. Physiol Behav 54: 1235–1237

Boehles HJ (1991) Ernährungsstörungen im Kindesalter. Wiss. Verlagsgesellschaft, Stuttgart, S 352

Broderick PA, Jacoby JH (1988) Diabetes-related changes in L-tryptophan-induced release of striatal biogenic amines. Diabetes 37: 956–960

Carboni E, Cadoni C, Tanda GL, DiChiara G (1989) Calcium-dependent, tetradotoxin-sensitive stimulation of cortical serotonin release after a tryptophan load. J Neurochem 53: 976–978

Carr L, Ruther E, Berg PA, Lehnert H (1994) Eosinophilia-myalgia syndrome in Germany: an epidemiologic review. Mayo Clin Proc 69: 620–625

Collu R, Fraschni F, Bisconti P, Martini L (1972) Adrenergic and serotoninergic control of growth hormone secretion in adult male rats. Endocrinology 90: 1231–1237

Cooper JR, Bloom FE, Roth RH (1986) Catecholamines. II. CNS aspects. In: Cooper JR, Bloom FE, Roth RH (eds) The biochemical basis of neuropharmacology. Oxford University Press, New York

DeSimoni MG, Sokola A, Fodritto F, DalToso G, Algeri S (1987) Functional meaning of tryptophan-induced increase of 5-HT metabolism as clarified by in vivo voltametry. Brain Res 411: 89–94

Dickinson JC, Rosenblum H, Hamilton PB (1965) Ion exchange chromatography of the free amino acids in the plasma of the newborn infant. Pediatrics 36: 2–13

Eccleston EG, Ashcroft GW, Crawford TBB (1965) 5-Hydroxyindole metabolism in rat brain: a study of intermediate metabolism using the technique of tryptophan loading, II. J Neurochem 12: 493–503

Eccleston EG (1973) A method for the estimation of free and total acid-soluble plasma tryptophan using an ultrafiltration technique. Clin Chim Acta 48: 269–272

Elks ML, Youngblood WW, Kizer JS (1979) Serotonin synthesis and release in brain slices: independence of tryptophan. Brain Res 172: 461–469

Fazzolari-Nesci A, Domianello D, Sotera V, Räihä NCR (1992) Tryptophan fortification of adapted formula increases plasma tryptophan concentrations to levels not different from those found in breast-fed infants. J Pediatr Gastroenterol Nutr 14: 456–459

Faust V, Baumhauer H, Dietmaier D (1990) Psychopharmaka. Kurzgefaßter Leitfaden für Klinik und Praxis. Ecomed, Landsberg

Feldman J, Lebowitz HE (1972) Control of insulin and growth hormone secretion by serotonin and dopamine. Proceedings of the 4th Congress of Endocrinology, Excerpta Medica Foundation Series, 256, p 32

Fernstrom JD, Wurtman RJ (1971) Brain serotonin content: physiological dependence on plasma tryptophan levels. Science 173: 149–152

Foller RW, Roush BW (1973) Binding of tryptophan to plasma proteins in several species. Comp Biochem Physiol (B) 46: 273–276

Food and Nutrition Board. Committee on Amino Acids (1959) Evaluation of protein nutrition. National Academy of Sciences – Nat Res Council Publ 711

Food and Nutrition Board (1974) Recommended Dietary Allowances. Washington DC, 8th edn. National Academy of Sciences, pp 37–48

Forsum E (1974) Nutritional evaluation of whey protein concentration and their fractions. J Dairy Sci 57: 665–670

Friedman M, Finley JW (1975) Evaluation of methods for tryptophan analysis in proteins. Part 1. In: Friedman M (ed) Protein nutritional quality of food and feeds. Marcel Decker Inc, New York, pp 423–452

Goodwin GM, Fairburn CG, Cowen PJ (1987) The effects of dieting and weight loss on neuroendocrine responses to tryptophan, clonidine and apomorphine in volunteers. Important implications for neuroendocrine investigations in depression. Arch Gen Psychiatr 44: 952–957

Greiling H, Gressner AM (1989) Lehrbuch der Klinischen Chemie und Pathobio – chemie, 2. Aufl. Schattauer, Stuttgart New York, S 501–503

Hardeland R, Reiter RJ, Poeggeler B, Tan DX (1993) The significance of the metabolism of the neurohormone melatonin: antioxidative protection and formation of bioactive substances. Neurosci Biobehav Rev 17: 347–357

Harzer G (1989) Über die Zusammensetzung von Muttermilch zur Adaptation von Säuglingsnahrungen In: Renner E (Hrsg) Milchwissenschaft, vol 7. Verlag B. Renner, Gießen, S 37

Heine W (1994) Qualitative aspects of protein in human milk and formula: amino acid pattern. In: Räihä NCR (ed) Protein metabolism during infancy. Nestlé-Nutrition Workshop Series vol 33, Nestec Ltd. Vevey, Raven Press Ltd. New York, pp 121–132

Heine WE, Klein PD, Reeds PJ (1991) The importance of α-lactalbumin in infant nutrition. J Nutr 121: 277–283
Heine W, Radke M, Wutzke KD, Peters E (1995) Clinical and biochemical studies with low-protein infant formulas enriched with α-lactalbumin (Submitted for publication)
Holt LE jr, György P, Pratt EL, Snyderman SE, Wallace WM (1960) Protein and amino acid requirements in early life. University Press, New York
Janas LM, Picciano MF, Hatch TF (1985) Indices of protein metabolism in term infants fed human milk, whey predominant formula, or cow's milk formula. Pediatrics 75: 775–784
Janas LM, Picciano MF, Hatch TF (1987) Indices of protein metabolism in term infants fed either human milk or formulas with reduced protein concentration and various whey/casein ratios. J Pediatr 110: 838–848
Jarvenpää AL, Räihä NCR, Rassin DK, Gaull GE (1982a) Milk protein quantity and quality in the term infant: I. Metabolic responses and effects on growth. Pediatrics 70: 214–220
Jarvenpää AL, Rassin DK, Räihä NCR, Gaull GE (1982b) Milk protein quantity and quality in the term infant. II. Effects on acidic and neutral amino acids. Pediatrics 70: 221–230
Jürgens P (1985) Zur Korrelation zwischen extrazellulärer Aminosäurenhomöostase und Deckung des Aminosäurenbedarfs. In: Kleinberger G, Bürger U (Hrsg) Aminosäuren-Transferlösungen. Klinische Ernährung, vol 15. Zuckschwerdt, München Bern Wien, S 186–200
Jürgens P (1986) Zum Aminosäurenbedarf Früh- und Neugeborener sowie junger Säuglinge bei enteraler und parenteraler Ernährung. In: Dölp R, Löhlein D (Hrsg) Aktuelle Entwicklung und Standard der künstlichen Ernährung. Karger, Basel München Paris, S 14–53
Knapp A (1962) Über eine erbliche Störung im Tryptophanstoffwechsel in Abhängigkeit von der Vitamin B_6-Zufuhr. Z Menschl Vererb Konstit Lehre 36: 258–267
Komrower GM, Wilson V, Clamp R, Westall RG (1964) Hydroxykynuremia. Arch Dis Child 39: 250–256
Körbel IM (1984) Untersuchungen über die Tryptophankonzentration im Plasma von Kindern und Erwachsenen. Promotion, Universität Düsseldorf
Lancranjan I, Wirz-Justice A, Puhringer W, PelDozo E (1977) Effect of 1-5-hydroxytryptophan infusion on growth hormone and prolactin secretion in man. J Clin Endocrinol Metabol 45: 588–593
Maher TJ (1984) Plasma branched chain amino acids as regulator of brain neurotransmitters. In: Adibi SA, Fekl W, Langenbeck U, Schauder P (eds) Branched chain amino and keto acids in health and disease. Karger, Basel, pp 242–259
Maire JC (1994) Personal communication
Marsden CA, Conti J, Strope E, Curzon G, Adams RN (1979) Monitoring 5-hydroxytryptamine release in the brain of the freely moving unanaesthesized rat using *in vivo* voltametry. Brain Res 171: 85–99
Miller HL, Delgado PL, Salomon RM, Licinio J, Barr LC. Charney DS (1992) Acute tryptophan depletion: a method of studying antidepressant action. J Clin Psychiatry 53 [Suppl]: 28–35
Moir ATB, Eccleston D (1968) The effects of precursor loading in the cerebral metabolism of 5-hydroxyindoles. J Neurochem 15: 1093–1108
Mutschler E (1991) Arzneimittelwirkungen. Lehrbuch der Pharmakologie und Toxikologie, 6. Aufl. Wiss. Verlagsgesellschaft, Stuttgart
Nielsen HK, Hurrell RF (1985) Tryptophan determination of food proteins by HPLC after alkaline hydrolysis. J Sci Food Agr 36: 893–907
Ng LT, Anderson GH (1992) Route of administration of tryptophan and tyrosine affects short-term food intake and plasma and brain amino acid concentrations in rats. J Nutr 122: 283–293

Pascoe M (1993) Huntington's disease and low tryptophan diet. Med Hypotheses 41: 325–326
Pohlandt F (1975) Zur Vermeidung von Aminosäurenimbalanzen bei Neugeborenen unter parenteraler Ernährung. Monatsschr Kinderheilkd 123: 448–450
Pollet P, Leathwood PD (1983) The influence of tryptophan on sleep in man. Int J Vitam Nutr Res 2: 53–58
Price JM, Yess N, Brown RR, Johnson SAM (1967) Tryptophan metabolism. A hitherto unreported abnormality occurring in a family. Arch Dermatol 95: 462–472
Reiter RJ (1993) The melatonin rhythm: both a clock and a calendar. Experientia 49: 654–664
Reiter RJ, Tan DX, Poeggeler B, Menendez-Pelaez A, Chen LD, Saarela S (1994) Melatonin as a free radical scavenger: implications for aging and age-related diseases. Ann NY Acad Sci 719: 1–12
Saavedra JM, Palkovits M, Brownstein M, Axelrod J (1974) Serotonin distribution in the nuclei of the rat hypothalamus and preoptic region. Brain Res 77: 157–165
Schaechter JD, Wurtman RJ (1990) Serotonin release varies with brain tryptophan levels. Brain Res 532: 203–210
Sidransky H, Verney E, Cosgrove JW (1992) Competitive studies relating to tryptophan binding to rat hepatic nuclear envelopes as a sensitive assay for unknown compounds. Toxicology 76: 89–100
Siegenthaler W (1987) Klinische Pathophysiologie, 6. Aufl. Thieme, Stuttgart New York, S 149, 1161
Shimatzu A, Kato Y, Ohta H, Tojo K, Kabayama Y, Inoue T, Imura H (1985) Involvement of vasoactive intestinal polypeptide in serotonergic stimulation of prolactin secretion in rats. In: McLeod RH, Thorner MD, Scapagnim U (eds) Prolactin. Basic and clinical correlates. Fidia Research Series, Liviana Press, Padova, p 73
Snyderman SE (1974) In: Nyhan WL (ed) Heritable disorders of amino acid metabolism. John Wiley and Son, New York, pp 641–651
Snyderman SE (1986) Cited in: Dölp R, Löhlein D (Hrsg) Aktuelle Entwicklung und Standard der künstlichen Ernährung. Karger, Basel München Paris, S 42
Snyderman SE, Boyer A, Phansalker SV, Holt LE (1961) The essential amino acid requirements of infants: tryptophan. Am J Dis Child 102: 163–167
Tada K, Ito H, Arakawa T, Tohoku J (1963) Congenital tryptophanuria with dwarfism. J Exp Med 80: 118–134
Ternaux JP, Boireau A, Bourgoin S, Hamon M, Hery F, Glowinski J (1976) In vivo release of 5-HT in the lateral ventricle of the rat: effects of 5-hydroxytryptophan and tryptophan. Brain Res 101: 533–548
Tricklebank MD, Pickard FJ, DeSouza SW (1979) Free and bound tryptophan in human plasma during the perinatal period. Acta Paediatr Scand 68: 199–204
Trulson ME (1985) Dietary tryptophan does not alter the function of brain serotonin neurons. Life Sci 37: 1067–1072
Widdowson EM, Southgate DAT, Hey EN (1979) Body composition of the fetus and infant. In: Visser HKA (ed) Nutriton and metabolism of the fetus and infant. Martinus Nijhoff, Boston, pp 167–177
Williams AP, Hewitt D, Buttery PJ (1982) A collaborative study on the determination in feeding stuffs. J Sci Food Agr 33: 860–865
Wissenschaftliche Tabellen Geigy (1977), 8. Aufl., S 259
Wurtman RJ (1982) Nutrients that modify brain function. Sci Am 246: 50–59
Wurtman RJ (1983) Behavioral effects of nutrients. Lancet I: 1145–1147
Wurtman RJ (1988) Effects of dietary amino acids, carbohydrates, and choline on neurotransmitter synthesis. Mt Sinai J Med 55: 75–86
Yamamoto H, Egawa B, Horiguchi K, Kaku A, Yamada K (1992) Changes in CSF tryptophan metabolite levels in infantile spasms. No To Hattatsu 24: 530–535

Yan D, Urano T, Pietraszek MH, Shimoyama I, Uemura K, Kojima Y, Sakakibara K, Serizawa K, Takada Y, Takada A (1993) Correlation between serotonergic measures in cerebrospinal fluid and blood of subhuman primate. Life Sci 52: 745–749

Yannicelli S, Rohr F, Warman ML (1994) Glutaric acidemia type I is a rare, autosomal recessive, inborn error of lysine and tryptophan metabolism. J Am Diet Assoc 94: 183–188

Yokogoshi H, Iwata T, Ishida K, Yoshida A (1987) Effects of amino acid supplementation to low protein diet on brain and plasma levels of tryptophan and brain 5-hydroxyindoles in rats. J Nutr 117: 42–47

Zimmermann RC, McDougle CJ, Schmumacher M, Olcese J, Heninger GR, Price LH (1993) Urinary 6-hydroxymelatonin sulfate as a measure of melatonin secretion during acute tryptophan depletion. Psychoneuroendocrinology 18: 567–578

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