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Distribution of L-tryptophan in normal and glucose – loaded mice

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Summary. L-tryptophan is an essential amino acid in food, but is also widely used as a drug on the basis of several physiological actions. Lately, tryptophan's uses as a drug and as a food supplement have been discontinued in several countries due to its severe side-effects.

In the present study, the distribution of tryptophan in mice was studied with special attention on the target organs, where the drug has been shown to have pathological or physiological effects.

The results showed that several organs took up tryptophan and that glucose loading increased the accumulation. An interesting finding was that the highest concentration of tryptophan was found in the pancreas. The hypophysis and adrenal glands were also sites of accumulation. Within the brain the highest accumulation was found in the cerebrum. High concentrations were also seen in the gastrointestinal tract and bone marrow.

The connection between the accumulation of tryptophan and its normal and pathophysiological effects is discussed.

Keywords: Amino acid – Whole-body autoradiography – L-Tryptophan – Densitometric image analysis – Mouse

Introduction

Tryptophan is nutritionally an essential aromatic amino acid and one of the most critical amino acids in micro-organisms and the mammalian body. Having the lowest tissue concentration of all the amino acids usually renders it the rate-limiting component of protein synthesis (Munro, 1969). In addition, active neurotransmitters such as 5-hydroxytryptamine (serotonin) are formed from tryptophan in the brain. Quantitatively the most important pathway of tryptophan is its degradation via the kynurenine in the liver.

Tryptophan has been widely marketed as a non-prescription drug in the USA since 1974. Individuals have been reported to take it for sleeping

difficulties, depression or anxiety in rather large daily doses of up to 15 grams. Several recent reports refer to the serious disease called the eosinophilia-myalgia syndrome (EMS) which has appeared after using tryptophan for some weeks or up to several years. In some cases, the symptoms have begun several weeks after discontinuing the medication. EMS has been recognized since 1989 as a malady characterized by peripheral eosinophilia with scleroderma-like features (CDC, 1989, a,b; CDC, 1990; Silver et al., 1990). EMS is now reported in the United States to affect more than 1500 persons, about thirty of whom have succumbed. The latest reports, however, suggest that it was the bacterial contamination (Yamaoka et al., 1991) or chemical contaminant (Driskell et al., 1992; Ito et al., 1992) during manufacture of the product followed by autoimmune disease in the patients which caused the EMS (Criswell and Sack, 1991).

Tryptophan has been shown in many studies to cause hypoglycemia (Smith and Pogson, 1977; McDaniel et al., 1973). It also affects fatty acid synthesis (Fears and Murrell, 1980). Tryptophan is a powerful amino acid; it has been suspected of causing liver injuries in large doses (Sidransky, 1986), and even cancer (Trulson and Sampson, 1987). However, the evidence of its role in liver tumorgenesis is conflicting.

Tryptophan has been shown significantly to affect the hormones of the hypophyseal-adrenal axis (Modlinger et al., 1979, 1980; Träskman-Bendz et al., 1986).

In view of the variety of actions of tryptophan in different organs, the purpose of the present investigation was to study its tissue distribution in the body. Because glucose is known to have an effect on amino acid uptake, and proteins are usually taken with carbohydrates, we also studied tryptophan distribution when glucose was given simultaneously with tryptophan. The accumulation of tryptophan was measured in various organs using computer-assisted image analysis.

Materials and methods

Animals

The experimental animals were six male NMRI mice (30 g), maintained under constant light periods (light 14 h, dark 10 h) and with free access to food (standard diet from Hankkija Ltd. Turku, Finland) and water. L-[5-^{3}H] Tryptophan (specific activity 30.0 Ci/mmol, code TRK 460) was purchased from Amersham, U.K. and carboxymethylcellulose (CMC) (Ph. Nord grade). Hexan (n-hexane), ethylether and glucose were p.a. grade from E. Merck, Darmstadt, Germany.

Autoradiographic methods

The whole-body autoradiographic method used has been described earlier (Sainio and Sainio, 1991). The autoradiograms were scanned on a light box with a CCD video camera (WV-CD130L/G), Panasonic, Japan). Focus and exposure were kept standardized. Images were analyzed by a MicroScale TC program with a resolution of 720 × 512 with 256 grey values. The micro-computer used in analysis was the IBM compatible EKTACO EW-286PC-02 (Estonia). Point measurements were made and standards used for each exposure.

Experimental procedures

L-[5-^{3}H] Tryptophan 100 μCi in 0.9% NaCl (100 μl) was injected into the mice via a tail vein. Glucose (1 g/kg) in saline was injected intraperitoneally 15 min before tryptophan. The animals were killed with ether 45 mins after tryptophan administration and then embedded right side down in a shallow container constructed of a floor and a frame and containing 1.5% CMC (w/v). The temperature was −74°C and freezing was complete in about 30 min. The frozen specimens were allowed to stabilize overnight at −20°C before sections were cut.

Sagittal sections of 30 μm in thickness from the whole animal were cut at − −20°C with a microtome (PMV Cryo Microtome 450 MP, Stockholm) on adhesive tape (Scotch Brand Magic Transparent Tape, No 810, 3 M). The sections were allowed to dry for 24 h at −20°C before being placed on X-ray film (Hyperfilm- ^{3}H code RPN. 12, Amersham made by CEA Ltd. Sweden) for a five-week exposure. The films were developed by normal photographic processing. Commercial standards (RPA 510 Amersham) with 0.1–100 nCi/mg activity were pre-cut strips of 30 μm in thickness and were exposed in the same way as the samples. The higher activity range was 1.4–32.0 nCi/mg and the lower activity range 0.012–6.2 nCi/mg. The standard strips were expanded before use by floating them on a water bath at 50°C for 15 minutes. Flat and easily mountable strips were then obtained.

Results

The pancreas had the highest tryptophan accumulation, 24 Ci/mg in normal and 33 nCi/mg in glucose-loaded mice (Fig. 1 and Table 1). The other gastrointestinal organs also contained more tryptophan than many others. In all cases glucose, increased the tryptophan uptake. The wall of the large and small intestine contained 4.0 nCi/mg in normal and 5.1 nCi/mg, respectively, in glucose loaded mice, and 4.8 in normal and 5.6 nCi/mg in glucose loaded mice. The wall of the stomach contained 3.3 nCi/mg in normal and 4.0 nCi/mg in glucose loaded mice.

Uptakes of tryptophan by the hypophysis, salivary glands and adrenal glands were also high. The pineal glands had the lowest detected tryptophan concentration.

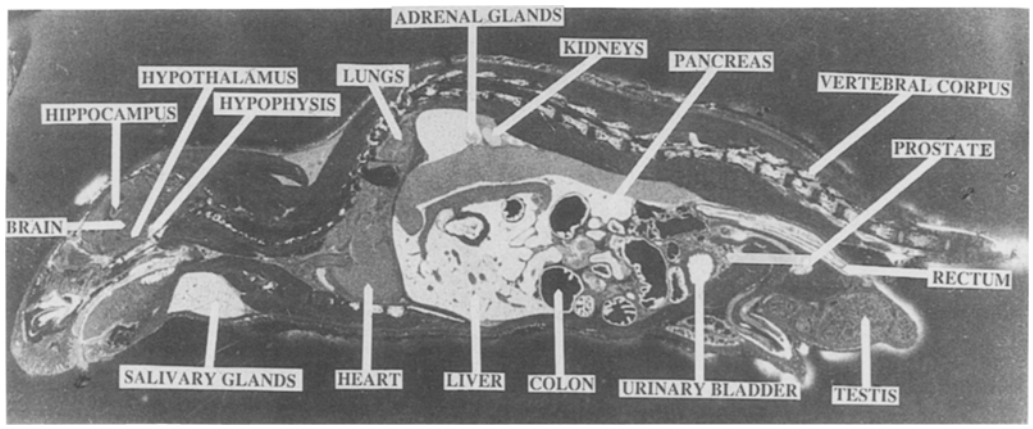

Fig. 1. Autoradiographic distribution of L-[^{3}H] Tryptophan in the glucose- loaded mouse. The mouse was handled as described in Materials and methods

Table 1. The concentration of L- [^{3}H] tryptophan in some organs

Organ	Tissue content[a] (n Ci/mg)	
	normal	glucose-loaded
Pancreas	24.0	33.0
Hypophysis	5.4	4.9
Intestinal wall (small)	4.8	5.6
Adrenal gland (cortex)	4.2	6.9
Intestinal wall (large)	4.0	5.1
Stomack wall	3.3	4.0
Vertebral corpus	3.2	11.0
Liver	3.1	12.5
Kidney	3.1	10.2
Submandibular salivary gland	3.0	11.5
Testis	2.8	3.9
Adrenal gland (medulla)	2.6	5.5
Lung	1.8	3.0
Cerebrum	1.7	3.1
Muscle	1.5	2.2
Hypothalamus	1.4	4.2
Hippocampus	1.4	3.7
Heart	1.4	3.7
Cerebellum	1.4	3.1
Pineal gland	1.3	4.4

[a] The mouse selected for analysis was handled as described in Materials and methods. L-[5-^{3}H] tryptophan (100 μCi) was injected into the mice via a tail vein. Glucose (1 g/kg) in saline was injected intraperitoneally 15 min before tryptophan. The animals were killed 45 min after tryptophan administation. The radioactivity was measured from an autoradiogram as described in Materials and methods. The numerical values are means of two measurements

In the central nervous system, the concentration of tryptophan was low. The highest accumulation of tryptophan appeared in the cerebrum, hippocampus, and hypothalamus.

The concentration of tryptophan in bone was 3.2 nCi/mg in normal and 11.0 nCi/mg in glucose-loaded mouse which was very close to the liver accumulation (3.1 in normal and 12.5 nCi/mg in glucose loaded). The kidneys also contained abundant tryptophan (3.1 in normal and 10.2 nCi/mg in glucose-loaded).

Figure 2 shows the photographic magnifications of some organs taken from the autoradiogram (Fig. 1).

Discussion

Quantitative measurements of the distribution of soluble compounds can be made in autoradiograms of cryosections and small differences detected in concentrations in different organs. We measured the tissue tryptophan concentrations in about twenty organs; the levels on other tissue were lower than

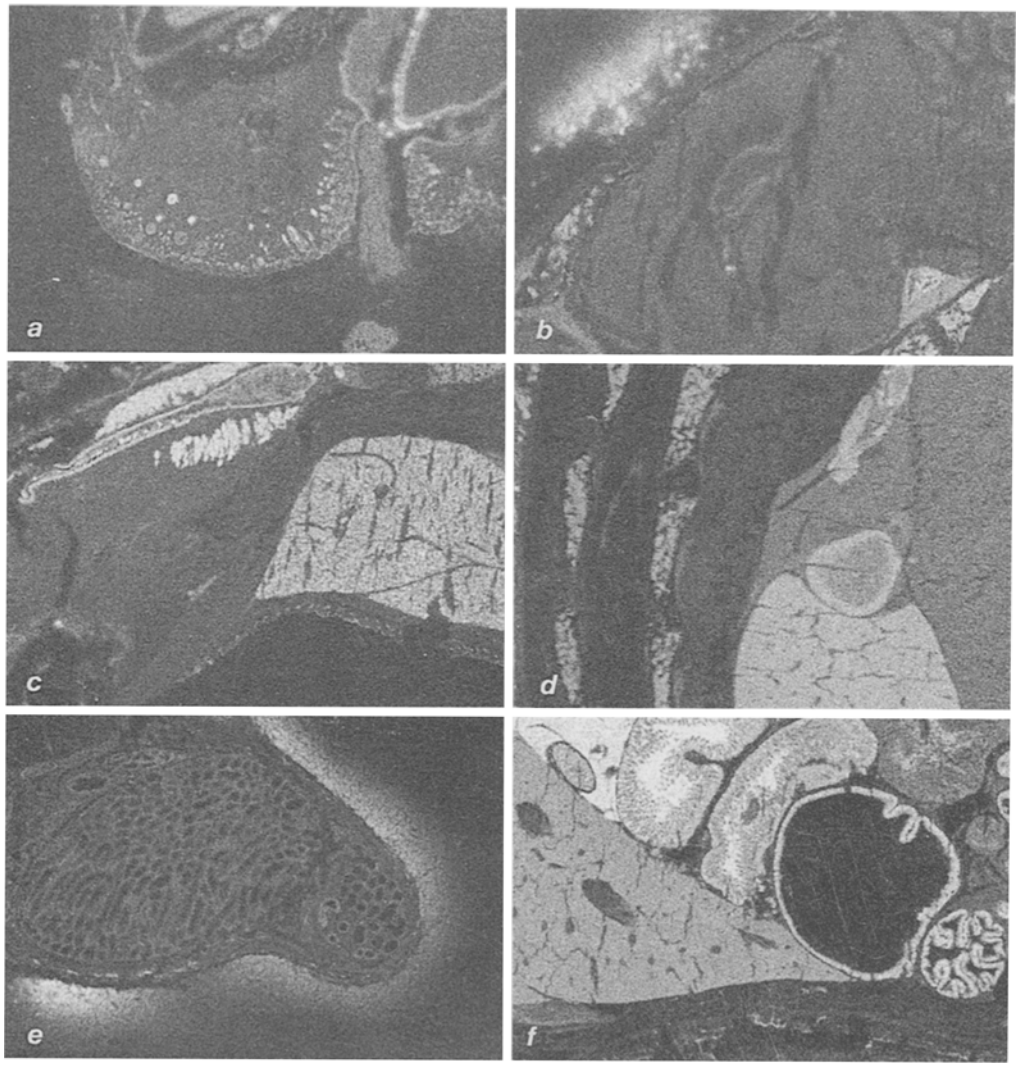

Fig. 2. The concentration of L-[^{3}H] tryptophan in some organs. **a** Hair follicle, **b** hippocampus, **c** salivary gland, **d** adrenal gland, **e** testis, **f** large intestine (magnified from Fig. 1)

the minimum organ level measured. Concerning the method, excellent linearity between the thickness of the section and the radioactivity counted, and the precision of the measurements has been demonstrated (Sainio and Sainio, 1991). Several biochemical, physiological, and pathophysiological findings associated with tryptophan can be explained on the basis of the above results. The very marked accumulation of tryptophan in the *pancreas* could explain its ability to increase insulin secretion (Ekholm et al., 1971) followed by hypoglycemia (Mc Daniel et al., 1973; Smith and Pogson, 1977). Teff and Young (1988) have proposed that the increase in insulin secretion induced by tryptophan is under the seronergic control, because after oral or intraperitoneal tryptophan administration the concentration of 5-HT in the pancreas increases.

The powerful effect of tryptophan on insulin secretion has also been suggested to cause increased hepatic fatty acid synthesis, followed by elevated free tryptophan in the blood, because free fatty acids are bound to albumin molecules instead of tryptophan (Fears and Murrell, 1980). Insulin increases the level of tryptophan in plasma and, thus, also in the brain.

Obviously tryptophan's effect on the pancreas targets the Islets of Langerhans, because the low concentration of tryptophan in the small intestine contents indicates that it had not accumulated in the secretory cells of the pancreas. The essential role of tryptophan in normal pancreatic function is suggested by a study which described pancreatic atrophy in quinea pigs and rats fed a diet completely devoid of tryptophan (Samuels et al., 1951). Normal pancreatic function would thus seem to be dependent on this macronutrient. Nevertheless, high doses of tryptophan have been suspected of causing pancreatitis (Chiba et al., 1990), although a tumor-promoting effect of tryptophan on the pancreas has not been observed (Sidransky, 1985).

Our study showed that the concentration of tryptophan in the *liver* was not the highest, although tryptophan has been suspected of causing liver cancer; the issue remains controversial (Hartman, 1987; Pollack and Kravitz, 1987; Trulson and Sampson, 1987).

It is not surprising that tryptophan was concentrated on *gastrointestinal* tissue, since tryptophan 5-monooxygenase has a high affinity for tryptophan (Othsuka et al., 1991). Tryptophan's side-effects include gastrointestinal disturbances and vomiting, which could be caused by the high accumulation in the walls of the digestive tract. The stimulation of acid secretion by tryptophan (Cooke, 1978) could also be due to its accumulation. The high concentrations in the intestinal walls apparently built up via the systemic circulation, while there was very little tryptophan in the contents of the large intestine and stomach. These findings are in agreement with an earlier study (Teff and Young, 1988).

Tryptophan accumulated *in the endocrine glands* (adrenals, testes, thyroid and pineal). The effect of tryptophan on endocrine secretions has been revealed previously (Modlinger et al., 1979, 1980). Our study showed that tryptophan was highly concentrated in the hypophysis and the adrenals, more in the cortex than medulla, which could explain the above mentioned endocrinological effects. Tryptophan was located in the testes in the interstitial tissue, while none was detected in the tubuli, probably due to the blood-testis barrier.

The salivary glands seem to accumulate tryptophan and the mouth also had high concentration of it. It is possible that tryptophan is secreted via the salivary glands, transported via saliva to the stomach and thereafter is reabsorbed from the small intestine. Another possible explanation for the slight accumulation of tryptophan in the small intestine is that tryptophan has an enterohepatic circulation.

Various parts of the brain contained small, but clearly detectable accumulation of tryptophan. This is probably due to the small proportion (1%) of tryptophan which passes the blood-brain barrier.

Bone marrow and vertebral corpus showed some accumulation of tryptophan. Whether this is involved in the etiology of EMS calls for further studies.

Bladder carcinogenesis is also thought to be caused by tryptophan (Sidransky et al., 1985) The content of tryptophan in the urinary bladder was high due to the kidney excretion.

Glucose enhanced the accumulation of tryptophan in almost all organs studied. This is in agreement with previous findings (Teff and Young, 1988). According to our study, tryptophan accumulates more in tissues in fed than in fasted mice (Sainio et al., 1991). The advantage of glucose means that smaller doses of tryptophan as a drug are needed when given with glucose (Polack and Kravits, 1987). The enhanced accumulation of tryptophan into tissues may also explain the finding that glucose loading in humans decreased the concentration of tryptophan in plasma by half (our unpublished result). Another explanation for the enhancement could be that the insulin released due to glucose elevates the ratio of tryptophan to other large neutral amino acids, which is followed by increased penetration of tryptophan into the tissues (Yokogoshi et al., 1987).

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