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Regional brain studies on indoles and tyrosine in Mongolian gerbils during nutrition with artificial mixtures high in branched chain amino acids compared to a protein rich diet

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With 2 tables

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Introduction

Mongolian gerbils (Meriones unguiculatus) are uniquely susceptible to experimental cerebral infarction because of the frequent inadequacy of the circle of Willis, which permits induction of cerebral ischemia by unilateral common carotid artery ligation (13).

Physiological changes of tryptophan concentration are known to influence brain indole metabolism (4, 25, 19). No data are available regarding the behaviour of indoles and tyrosine in various brain areas of the Mongolian gerbil after alteration of food intake. Therefore comparative studies of the regional distribution of tryptophan (TRP), serotonin (5-HT), 5-hydroxy-indole-3-acetic acid (5-HIAA), and tyrosine (TYR), as well as on the effects of altered brain indole and tyrosine concentrations are of interest in studies of cerebral infarction and in animal models of metabolic comata.

Experimental data have shown profound alterations of neurotransmitter metabolism in these experiments (15, 16, 17). However, evidence is lacking on possible differential responses of various brain regions after ischemia or cerebral infarction. Therefore, to elucidate the role of biogenic amines in infarcted and non-infarcted brain regions, a detailed stereomicroscopical dissection technique is needed.

Besides the importance to rule out the basal level of the indoles and tyrosine, in comparison with what is known from other animals and man (2, 8, 9) the effect of an artificial amino acid mixture high in L-valine and a protein rich diet on such values should be determined. This is of special interest as a competition between the large neutral amino acids regarding an uptake across the blood brain barrier has been shown by *Wurtman* and *Fernstrom* (26).

Moreover, an imbalance of plasma amino acids is able to alter synthesis and metabolism in neurotransmitter systems (4, 8, 12, 25, 26). In the last years, special amino acid compositions have been used for therapeutical reasons to influence brain amino acids concentrations in experimental

animals (6, 9) and in patients with hepatic encephalopathy (9, 11, 22, 25). The aim of the present study is to describe the regional distribution of indoles and tyrosine in the brain of the Mongolian gerbil, and to examine the influence of an artificial and a protein rich diet on the levels of these substances in peripheral organs and various regions of the brain in this species.

Material and methods

a) Groups of 20–25 Mongolian gerbils weighing 20–40 g were deprived of food for 22 hours, while getting water *ad libitum*. Animals were decapitated and brains were taken for further regional dissection in the early afternoon (2–4 pm). These animals were used as a fastened control group.

b) Another group of 25 animals was fed *ad libitum* for 22 hours with a parenteral nutrition, including high concentrations of L-valine. Every three hours each animal was fed ad libitum with the diet using a pipette. Artificial nutrition I contained L-leucine (0.061 M), l-isoleucine (0.054 M), l-valine (0.06 M), l-arginine (0.034 M), l-ornithine (0.030 M; as chloride and aspartate), sorbit (0.27 M), Na^+ (100 mVal), K^+ (10 mVal), Cl^- (70.3 mVal), acetate (47.4 mVal), aspartate (7 mVal), malate (22.5 mVal) in water (1000 ml).

The composition of this solution has been proposed for treatment of metabolic coma, as a favourable effect of similar amino acid mixtures has been shown in experimental animals as well as in human patients with hepatic coma (6, 11, 22).

c) The same experiment as designed in part b) has been performed, except that the concentration of L-valine has been increased to 0.49 moles/l (artificial nutrition II).

d) 25 Mongolian gerbils were fed ad libitum with a special diet containing 18% protein (RK 88, Kerber Futter).

After decapitation, the brain was quickly removed, placed on a chilled plate and dissected with the aid of a stereomicroscope (magnification 15 to 40 times). Samples of the following regions were cut or punched out using a microknife or a 1 mm needle: cortex (frontal plus parietal), hemispheral white matter plus corpus callosum; lenticular nuclei (nucleus caudatus, putamen and globus pallidus), thalamus; hippocampus plus amygdaloid nucleus; cerebellum (cortex, white matter and deep nuclei), nucl. dorsalis raphe of the pons including some adjacent parts of the formatio reticularis pontis; and the remaining parts of the pons and medulla. For recognizing the nuclei the help of various atlasses was obtained including those of *Tigges* and *Shanta* (23) and *Zeman* and *Innes* (28). The dissected samples were rapidly frozen at −30 °C. For the determination of indoles and tyrosine in brain areas it was necessary to pool samples of several animals (cerebellum 2, lenticular nuclei 2–3, raphe + R.F. 2–3, hippocampus 3–4, rest of the brainstem 2, frontal cortex 4, white matter 8).

Tryptophan was measured according to *Denckla*, et al. (5). 5-HT and 5-HIAA were determined as described by *Ashcroft* (1) and tyrosine by the method of *Waalkes* et al. (24). All values are given as mean ± S. D.

Results

TRP, 5-HT, 5-HIAA and TYR were measured in various peripheral organs of Mongolian gerbils after short (4 hours) and prolonged (22 hours) food deprivation. The liver, lung, heart and kidney showed essentially uniform concentrations of TRP and TYR, while the 5-HT and 5-HIAA levels were higher in the lung.

Table 1. Indoles and tyrosine in various organs of Mongolian gerbils during artificial and protein rich nutrition.

Organ	Diets	TRP µg/g	5-HT ng/g	5-HIAA ng/g	TYR µg/g
Liver	4 hrs. fasted controls (5)	24.2 ± 2.9	50 ± 6.1	23 ± 2.2	8.5 ± 0.9
	22 hrs. fasted controls (10)	47.0 ± 3.1*)	111 ± 37**)	20 ± 1.8	9.6 ± 2.3
	Artificial nutrition I (10)	39.5 ± 6.5*)	32 ± 3.1*)	20 ± 2.0	8.6 ± 2.6
	Artificial nutrition II (10)	40.5 ± 4.7*)++)	36 ± 4.7**)	17 ± 1.9	5.8 ± 2.2++)
	Protein rich diet (10)	70.5 ± 9.0*)	72 ± 8.3*)	23 ± 3.0	22.8 ± 3.6*)
Lung	4 hrs. fasted controls (5)	21.4 ± 2.2	632 ± 75	169 ± 32	9.8 ± 1.9
	22 hrs. fasted controls (10)	39.9 ± 3.6*)	822 ± 106**)	270 ± 15*)	6.8 ± 1.7
	Artificial nutrition I (10)	29.0 ± 4.6**)++)	467 ± 73**)	115 ± 10**)+)	7.2 ± 1.8
	Artificial nutrition II (10)	26.5 ± 5.0++)	436 ± 68	121 ± 18**)+)	7.5 ± 1.5
	Protein rich diet (10)	58.0 ± 9.1*)	765 ± 104	259 ± 33**)	16.6 ± 2.0*)
Heart	4 hrs. fasted controls (5)	29.0 ± 3.1	51 ± 3.7	39 ± 4.1	8.2 ± 1.6
	22 hrs. fasted controls (10)	46.6 ± 4.4*)	137 ± 55*)	55 ± 3.9**)	5.6 ± 1.5
	Artificial nutrition I (10)	36.5 ± 4.9*)++)	38 ± 3.4*)	33 ± 2.9	4.8 ± 1.6*)
	Artificial nutrition II (10)	39.8 ± 3.4*)	40 ± 4.1**)	31 ± 2.8	4.3 ± 1.0*)
	Protein rich diet (10)	58.3 ± 3.1*)	48 ± 2.0	39 ± 7.2	11.1 ± 1.6**)
Kidney	4 hrs. fasted controls (5)	25.2 ± 3.0	14 ± 1.2	44 ± 12	10.1 ± 2.3
	22 hrs. fasted controls (10)	36.0 ± 2.4*)	19 ± 0.9*)	35 ± 9	13.5 ± 1.6
	Artificial nutrition I (10)	29.8 ± 3.2++)	15 ± 1.7	37 ± 1.9	10.2 ± 0.9
	Artificial nutrition II (10)	36.6 ± 2.6++)	17 ± 1.3	35 ± 2.2	7.7 ± 1.4**)+)
	Protein rich diet (10)	67.6 ± 6.8*)	17 ± 2.3	44 ± 6.3	13.8 ± 2.1

Number of analysis in parentheses;
control animals had free access to water;
the composition of the artificial nutritions I and II are given in the methods section;
protein rich diet contained 18% protein;

TRP = tryptophan
5-HT = 5-hydroxytryptamine
5-HIAA = 5-hydroxyindole-3-acetic acid
TYR = tyrosine
*) $p < 0.01$ when compared to 4 hrs. fasted controls
**) $p < 0.05$ when compared to 4 hrs. fasted controls
++) $p < 0.05$ when compared to 22 hrs. fasted controls
+) $p < 0.01$ when compared to 22 hrs. fasted controls

Food deprivation for 22 hours led to a significant increase of TRP in all organs when compared to the short term deprived animals (table 1). An artificial nutrition including the branched chain amino acids with or without high concentrations of L-valine also increased control levels (4 hours deprivation) to a significant degree. Nutrition of the animals with food high in proteins (18%) led to an even further significant increase in TRP.

5-HT was increased significantly in the liver after ingestion of protein rich food, whereas there were no significant alterations in the lung, heart and kidney. A 22 hour fast increased 5-HT in all organs, indicating an increased synthesis of this biogenic amine. Artificial nutrition showed a drop of 5-HT in liver, lung and heart when compared to the 4 hour fast. This decrease of 5-HT and the concomitant decrease of TRP indicate a dependence of 5-HT synthesis from TRP concentration.

5-HIAA increased significantly in lung and heart after a 22 hour fast, but did not show any alterations in liver and kidney. Artificial nutrition showed a significant decrease of 5-HIAA only in the lung, which is one of the peripheral organs with highest concentrations of 5-HT. After a protein rich food intake 5-HIAA increased only in the lung.

In contrast to the increase of TRP after a long term fast TYR did not show any change. Artificial nutrition, specially that with high concentrations of L-valine, reduced TYR levels particularly in the liver and the heart. The protein rich diet led to a significant increase of TYR in liver, lung and heart.

Regional distributions found for TRP, 5-HT, 5-HIAA and TYR demonstrated higher values of TRP, 5-HT and 5-HIAA in the raphe/reticular formation system, when compared to the other brain areas. The lowest concentrations of 5-HT and 5-HIAA were measured in the frontal cortex and white matter (table 2). It is also shown that long lasting food deprivation increases TRP in most of the brain areas. On the contrary, artificial nutrition with branched chain amino acids leads to a significant decrease in comparison to short and long term fast in most of the brain areas. Especially, those with serotoninergic innervation were involved.

However, 5-HT and 5-HIAA only show little changes in comparison to the short term fast. After a 22 hour food deprivation an artificial application or nutrition with or without high concentrations of L-valine results in slightly reduced values in comparison to those of the short term deprived animals, indicating a competition of branched chain and aromatic amino acids on uptake mechanisms across the blood brain barrier. However, comparison with the 22 hour fast resulted in a marked reduction of TRP, 5-HT, 5-HIAA and TYR in most of the brain areas.

Administration of protein rich food resulted in changes of TRP, 5-HT, 5-HIAA and TYR, which are not different from those of controls.

Discussion

The increases in regional brain concentrations of TRP, 5-HT and 5-HIAA after fastening are in good agreement with previous findings in the rat (4, 12, 19). It is suggested that food deprivation results in higher concentrations of plasma non esterified fatty acids (NEFA). NEFA seem to increase by a release of tissue lipase into plasma which hydrolyses more

Table 2. Brain indoles and tyrosine in Mongolian gerbils during artificial and protein-rich nutrition.

Brain area	Treatment	TRP µg/g	5-HT ng/g	5-HIAA ng/g	TYR µg/g
Lenticular nuclei	4 hrs. fasted controls (5)	24.0 ± 2.1	228 ± 12.7	139 ± 16.9	5.2 ± 0.42
	22 hrs. fasted controls (9)	37.1 ± 2.8*)	200 ± 10.1	221 ± 24.0*)	6.9 ± 0.59**)
	Artificial nutrition I (10)	20.0 ± 1.7**)$^{+}$)	160 ± 9.3*)$^{+}$)	85 ± 9.1*)$^{+}$)	4.1 ± 0.37*)$^{+}$)
	Artificial nutrition II (10)	18.7 ± 1.6**)$^{+}$)	146 ± 10.1*)$^{+}$)	62 ± 7.2*)$^{+}$)	3.9 ± 0.32*)$^{+}$)
	Protein rich diet (–)	–	–	–	–
Raphe + reticular formation	4 hrs. fasted controls (5)	35.1 ± 1.7	507 ± 56	643 ± 58	5.8 ± 0.8
	22 hrs. fasted controls (10)	39.0 ± 2.8**)	494 ± 22	758 ± 63**)	9.1 ± 1.1*)
	Artificial nutrition I (9)	32.0 ± 3.1**)	386 ± 29*)$^{+}$)	375 ± 45*)$^{+}$)	4.6 ± 0.51**)$^{+}$)
	Artificial nutrition II (10)	32.5 ± 2.8**)	352 ± 30*)$^{+}$)	363 ± 38*)$^{+}$)	4.3 ± 0.38*)$^{+}$)
	Protein rich diet (7)	46.1 ± 3.9**)	515 ± 38	593 ± 78	8.0 ± 1.0*)
Brainstem without raphe + ret. form.	4 hrs. fasted controls (5)	22.4 ± 1.8	426 ± 31	530 ± 38	5.4 ± 0.40
	22 hrs. fasted controls (10)	25.2 ± 2.7	418 ± 22	576 ± 42	6.7 ± 0.51**)
	Artificial nutrition I (12)	18.1 ± 1.2**)$^{+}$)	325 ± 33**)$^{++}$)	444 ± 27**)$^{+}$)	4.5 ± 0.38**)$^{++}$)
	Artificial nutrition II (11)	16.1 ± 1.4**)$^{+}$)	350 ± 36	390 ± 33*)$^{+}$)	3.3 ± 0.29*)$^{+}$)
	Protein rich diet (11)	26.4 ± 2.5	385 ± 40	489 ± 29	4.5 ± 0.75
Hippocampus + amygdaloid n.	4 hrs. fasted controls (5)	18.4 ± 1.8	118 ± 11	132 ± 12.1	2.9 ± 0.15
	22 hrs. fasted controls (6)	24.2 ± 1.8**)	139 ± 12	179 ± 18.0**)	4.25 ± 0.38*)
	Artificial nutrition I (7)	14.2 ± 1.1**)$^{+}$)	107 ± 9^{++})	115 ± 8.2^{+})	1.1 ± 0.07*)$^{+}$)
	Artificial nutrition II (7)	13.8 ± 1.2**)$^{+}$)	90 ± 7*)$^{+}$)	109 ± 9.8**)$^{+}$)	0.95 ± 0.06*)$^{+}$)
	Protein rich diet (10)	28.2 ± 2.7*)	108 ± 7	148 ± 15.0	2.9 ± 0.35
Cerebellum	4 hrs. fasted controls (5)	19.2 ± 1.8	55 ± 5.1	41 ± 3.8	2.7 ± 0.17
	22 hrs. fasted controls (12)	20.9 ± 1.8	60 ± 5.3	48 ± 4.0**)	3.6 ± 0.29**)
	Artificial nutrition I (10)	18.2 ± 1.1	45 ± 4.0*)$^{+}$)	35 ± 3.1^{++})	1.5 ± 0.10*)$^{+}$)
	Artificial nutrition II (11)	17.6 ± 1.3^{++})	40 ± 3.9*)$^{+}$)	33 ± 3.2**)$^{+}$)	1.9 ± 0.12*)$^{+}$)
	Protein rich diet (12)	22.0 ± 2.1	62 ± 6.8	47 ± 4.3	3.5 ± 0.26**)

Frontal cortex	4 hrs. fasted controls (5)	15.4 ± 1.2	20 ± 2.2	30 ± 2.7	3.5 ± 0.28
	22 hrs. fasted controls (6)	20.6 ± 1.8*)	25 ± 2.1	37 ± 2.0**)	5.3 ± 0.45*)
	Artificial nutrition I (5)	15.0 ± 1.1^{++})	16 ± 1.5*)$^{+}$)	24 ± 1.9**)$^{+}$)	2.5 ± 0.18*)$^{+}$)
	Artificial nutrition II (5)	10.8 ± 0.9*)$^{+}$)	15 ± 1.7*)$^{+}$)	22 ± 1.8**)$^{+}$)	2.0 ± 0.15*)$^{+}$)
	Protein rich diet (5)	20.8 ± 1.7**)	28 ± 3.1	37 ± 3.1	2.5 ± 0.47
White matter	4 hrs. fasted controls (3)	17.6 ± 1.61	20 ± 2.0	20 ± 1.8	3.2 ± 0.25
	22 hrs. fasted controls (3)	20.3 ± 0.90*)	22 ± 2.3	24 ± 1.7	3.8 ± 0.17
	Artificial nutrition I (3)	16.7 ± 0.85^{++})	18 ± 1.9	19 ± 1.5	2.4 ± 0.12*)$^{+}$)
	Artificial nutrition II (3)	16.3 ± 1.1^{++})	18 ± 2.0	19 ± 1.4	1.9 ± 0.12*)$^{+}$)
	Protein rich nutrition (3)	19.2 ± 1.7	22 ± 2.3	23 ± 2.1	2.9 ± 1.1

Number of analysis in parentheses; for analysis brain areas had to be pooled according methods section;
control animals had free access to water;
the composition of the artificial nutritions I and II are given in the methods section;
protein rich diet contained 18% protein;
– not estimated

TRP = tryptophan
5-HT = 5-hydroxytryptamine
5-HIAA = 5-hydroxyindole-3-acetic acid
TYR = tyrosine
*) $p < 0.01$ when compared to 4 hrs. fasted controls
**) $p < 0.05$ when compared to 4 hrs. fasted controls
$^{+}$) $p < 0.01$ when compared to 22 hrs. fasted controls
$^{++}$) $p < 0.05$ when compared to 22 hrs. fasted controls

plasma glycerides. These fatty acids are strongly bound to albumin. Therefore an increase in NEFA releases tryptophan from albumin sites thus increasing plasma free tryptophan and hence brain tryptophan. Moreover, an increase in brain 5-HIAA also could be observed in food deprived rats (4).

As a result of adaptive responses by the liver to meals the circulation is protected against excessive changes in the amount of free amino acids entering the body. Experimental data led to the suggestion that a catabolic response is finely adjusted to the amount of amino acids entering the body. Moreover, evidence exists for recycling of amino acids within organs like the liver (for review 18). These experimental findings could be an explanation for the data given in table 1, where rather small changes in indoles and tyrosine could be observed after artificial nutrition of Mongolian gerbils with amino acid mixtures highly concentrated with branched chain amino acids.

Artificial nutrition including high concentrations of L-valine resulted in small changes of indoles and tyrosine in peripheral organs, but TRP and TYR values were lowered in brain areas. Brain levels of tryptophan are suggested to be dependent on the plasma free-tryptophan concentration (4), as well as on the balance of competing amino acids across the blood brain barrier (26, 27). As artificial nutrition used in this study contained high concentrations of branched chain amino acids, particularly L-valine, a decrease of tryptophan and tyrosine in brain regions is suggested to be the result of competition for transport systems (7, 8, 14, 25). However, the strong antiserotoninergic action of this amino acid mixture cannot be explained only by competition of the large neutral amino acids across blood-brain barrier. 5-HT decreases significantly particularly in areas rich in serotoninergic neurons. This decrease is more pronounced than the drop of TRP. Therefore an inhibition of the large neutral amino acid uptake at neuronal sites is suggested.

Evidence for such a mechanism is also provided by experiments showing a strong inhibitory action of leucine on serotonin synthesis (10, 20, 26).

Another mechanism of action, overlapping these competing effects, may involve inhibition of transmitter-synthesizing enzymes by large neutral amino acids. In fact, it could be shown by *Carlsson* et al. (3) that high concentrations of these amino acids inhibit the synthesis of 5-hydroxytryptophan. Thus the fast drop of 5-HT after application of high concentrations of branched chain amino acids (table 2) and previous results on a beneficial treatment of patients with metabolic encephalopathies by L-valine plus parenteral nutrition (9, 11, 22) are likely to be dependent both on the competition of amino acids across membrane structures (blood-brain barrier, neuronal) *and* on enzyme inhibition.

Protein rich food intake did not influence brain tryptophan or tyrosine and had no effect on 5-HT metabolism which is in agreement with findings in rats by *Wurtman* (26). It might be that the well-balanced composition of amino acids did not allow an imbalance in the uptake of single amino acids across blood-brain barrier. Further evidence for this derives from the results obtained from peripheral organs which show that TRP and TYR

increase in all organs after a protein rich diet. This effect was observable particularly in the liver and the lung and was less pronounced in the heart and the kidney.

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Summary

Up till now evidence is lacking regarding the regional distribution of indoles, like tryptophan (TRP), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) as well as tyrosine (TYR) in the brains of Mongolian gerbils. Therefore using a microdissection technique and pooling regional brain samples, it can be shown that there is a regional distribution of 5-HT and 5-HIAA in the brain of Mongolian gerbils which is highest in the raphe + reticular formation system followed by the rest of the brainstem and lenticular nuclei. A longterm fast (22 hours) increases TRP and 5-HIAA but not 5-HT, indicating an increase in the turnover rate of 5-HT. Brain TYR and TRP are only slightly increased after a protein rich diet, whereas 5-HT and 5-HIAA are not changed. Artificial nutrition with amino acid mixtures highly concentrated with branched chain amino acids lead to a decrease of TYR and TRP as well as 5-HT and 5-HIAA. Competing amino acid as well as inhibition of 5-HT synthesis is suggested to be responsible for these effects.

Mongolian gerbils show higher brain values of TRP and lower ones of TYR in comparison with other species of mice.

In peripheral organs, specially in the liver and lung, similar effects are observable. However, the changes are only mild in comparison to that observed in the brain. Moreover, TYR and TRP are significantly increased in peripheral organs after a protein rich diet.

Zusammenfassung

Die regionale Verteilung von Indolen wie Tryptophan (TRP), Serotonin (5-HT) und 5-Hydroxyindol-3-essigsäure (5-HIAA) sowie von Tyrosin (TYR) wurde in Gehirnen von mongolischen Springmäusen untersucht. Mit Hilfe einer Mikrosektionstechnik sowie durch „Poolen" von Gewebeproben war es möglich, regionale Unterschiede speziell für 5-HT und 5-HIAA festzustellen, wobei die höchsten Werte in Raphe + Formatio reticularis, restlichem Hirnstamm und Linsenkern meßbar waren. Ein 22stündiger Nahrungsentzug resultierte in einem Anstieg von TRP und 5-HIAA, nicht aber von 5-HT, was auf eine gesteigerte Umsatzrate von 5-HT hindeutet.

Die Verabreichung einer proteinreichen Diät führt nur zu einem leichten Anstieg von TYR und TRP, 5-HT und 5-HIAA zeigen keine signifikanten Unterschiede. Ernährung mit künstlichen Aminosäuregemischen, welche hohe Konzentrationen von verzweigtkettigen Aminosäuren enthielten, ergab einen signifikanten Abfall von TYR und TRP, 5-HT und 5-HIAA. Die kompetitive Wechselwirkung der aromatischen und verzweigtkettigen Aminosäuren um die Transport- und Aufnahmemechanismen in das Gehirn sowie die Hemmung der 5-Hydroxytryptophansynthese durch verzweigtkettige Aminosäuren dürften für die beobachteten Effekte verantwortlich sein.

Mongolische Springmäuse weisen im Gehirn, verglichen mit anderen Mäusearten, höhere Konzentrationen an TRP und geringere an TYR auf.

In peripheren Organen, speziell in Leber und Lunge, wurden ähnliche Ergebnisse erzielt. Die Unterschiede sind aber im Vergleich zum Gehirn geringer ausgeprägt. Proteinreiche Kost führt im Gegensatz zum Gehirn zu einem stärkeren Anstieg von TYR und TRP.

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