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Presence of Urea in Rat Brain

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This paper reports the presence of urea in rat brain and the evidence that it does not come from blood contamination. The amount of urea is higher during the first 4 days after birth, and it decreases thereon as the brain develops. Urea concentration in brain remains constant from the age of 9 days to 1 year. A possible mechanism of brain urea formation is discussed.

Early studies indicated that the amount of urea which could be synthesized in brain is quantitatively insignificant when compared to the amount produced by liver. Although ammonia is constantly produced during neural activity (15) most of it is believed to be disposed of as glutamine (2). The meaning of urea formation and the presence of the urea cycle in brain is not yet understood, because the complete enzyme systems participating in urea synthesis via Krebs mechanism (12) have not been described thus far in nervous tissues. It has been observed that brain is unable to synthesize citrulline (11) but citrulline was, however, found in brain (13), although it seems to be derived from blood. Carbamylphosphate synthetase (N-acetylglutamate-dependent) has not been clearly detected in brain and carbamylphosphate synthetase II (glutamine-dependent) was, only recently, found to be present mainly in the soluble fraction of brain (20) in a low amount, but this enzyme seems to be only implicated in the production of pyridine nucleotides. GRISOLIA *et al.* (8) reported the presence of a very high carbamylphosphatase activity but the significance of this enzyme in brain is not known. RATNER *et al.* (14) observed that brain can synthesize arginine from citrulline by means of two enzymes, arginosuccinate synthetase and arginosuccinase (3) and SPORN *et al.* (19) have shown that the brain has an appreciable amount of arginase and, recently, SADASIVUDU and HANUMANTHARAO (17) have identified arginosuccinate synthetase, arginosuccinase and arginase in differents regions of the rat brain. In this paper we give date about urea presence in rat brain and a possible mechanism of its formation is discussed.

Materials and Methods

White albino rats were used throughout. They were killed by previous anesthesia

and brains were quickly removed, washed with cold 9 $^{0}/_{00}$ NaCl and immediatly homogenized with 4 volumes of 0.2 M sodium phosphate buffer pH 7.4. Then, they were centrifuged at 10.000 × *g* for 15 minutes. The supernatant was considered as the homogenate.

Urease was prepared with 4 mg of urease disolved in 2 ml of 0.1 M sodium phosphate buffer pH 7.4.

Hemoglobin was determined by cyanmethemoglobin method (5). Ureides were determined by HUNNINGHAKE and GRISOLIA method (10).

Results and discussion

Table I shows the presence of ureides in rat brain homogenates. The possibility that these ureides might be a compound with group R-NH-CO-NH_2 other than urea was controlled by the addition of urease. It can be seen that in presence of urease the ureides disappear. This fact confirmes urea presence in brain. It is well know that urea is found in blood. In order to check whether the urea present in brain arises by blood contamination, we assayed hemoglobin and urea in both, brain homogenates and blood. Table II shows that there is not a detectable amount of hemoglobin in brain and urea in blood is not as high as to assume that urea presence in brain could be due to a blood contamination because if 4.5 μmoles of urea in blood cerrespond to 16.5 % of hemoglobin; 2.65 μmoles of urea from brain homogenates would correspond to 10.4 % of hemoglobin; this amount of hemoglobine could be detected in brain homogenates in spite of the low efficiency of cyanmethemoglobine method (5). On the other hand we can notice in table III that urea concentration in brain homogenates decreases with the developing rat brain. This change, together with brain weight, increase, agrees with the fact that during brain maturation there is a higher metabolism. The whole mechanism which forms this urea is unknown. SADASIVUDU *et al.* (17) have recently reported the distribution of some enzymes from urea cycle but carbamylphosphate synthetase I (N-acetylglutamate-dependent) and ornithine transcarbamylase have not been yet found in brain. SPORN *et al.* (19) reported that brain can form urea from arginine. Urea is normally synthesized by Krebs-Henseleit urea cycle, however, the excretion of homocitrulline and homoarginine in hyperlysinemia (21) or following an oral lysine load (16) and the secretion of homocitrulline in a patient with hiperamonemia (18) raised

Table I. *Demonstration of the presence of urea in rat brain.*
0.5 ml of brain homogenate were incubated for 15 minutes at 37° C with 1 ml of 0.2 M sodium phosphate buffer pH 7.4 or 1 ml of urease solution. Incubation was stopped by addition of 2 ml of 1 M $HClO_4$. The mixture, was centrifuged in a clinical centrifuge. Ureides were determined in 1 ml of supernatant, as indicated in the methods.

Conditions	Δ O.D./ml
— Urease	0.495
+ Urease	0.0

Table II. *Evidence that the urea found in brain is not a blood contamination.*
The incubation contained 0.5 ml of rat brain homogenate or 0.5 ml of heparinized blood, 1 mg of urease was added where indicated. Incubations were carried out for 30 minutes at 37° C. The reaction was stopped by adding 1 ml of 1 M $HClO_4$. Ureides were determined in 1 ml of supernatant, as indicated in the methods. Hemoglobin was determined in both, homogenate and blood. Ureides are expresed as μmoles of urea by ml of brain homogenate or blood.

Conditions	μMoles urea/ml	% Hemoglobine
Homogenate	2.86	0.0
Homog. + Urease	0.01	0.0
Blood	4.50	16.5
Blood + Urease	0.01	16.5

Table III. *Influence of rat age on brain urea concentration.*

Brain were homogenized with 2 volumes of 1 M $HClO_4$ and then centrifuged for 15 minutes in a clinical centrifuge. 0.1 ml of each supernatant was taken to check the ureides. Each experience is an average of, at least, six rats.

Age (Days)	Weight of brain (g)	μMoles urea/g
1	0.173±0.007	9.8±0.88
2	0.246±0.04	9.7±1.30
3	0.304±0.03	9.0±0.91
4	0.332±0.02	8.3±1.10
5	0.399±0.05	5.9±0.35
6	0.519±0.05	5.9±2.10
7	0.544±0.13	5.9±2.80
8	0.613±0.04	4.2±0.90
9	0.695±0.05	3.9±0.80
10	0.724±0.04	3.2±0.50
14	0.841±0.05	3.3±1.00
21	0.964±0.10	3.4±0.40
1 Year	1.830	3.2

the possibility of an other homologous of urea cycle consisting of lysine homocitrulline, homoarginosuccinic acid and homoarginine. Another possibility of urea formation could be, that, refered by COHEN *et al.* (6) from guanidosuccinic acid with the intervention of a new enzyme analogous to glycine-arginine-amidinotransferase using aspartate rather than glycine as the amide acceptor. Finally there is another possibility of the urea formation by means of carbamyl-γ-aminobutyric acid, GONZÁLEZ and GRISOLIA (7) reported that rat homogenates of brain, kidney and to a lesser extent, liver, formed urea from carbamyl-γ-aminobutyric acid. This finding indicated the existence of a pathway whereby carbamyl-γ-aminobutyric acid may be converted into γ-guanidobutyric acid and urea. This route could be important in brain where 4-aminobutyric acid is mainly found. This presupposed, first, a transcarbamylation between carbamylphosphate and 4-aminobutyric acid (ornithine in urea cycle) followed by a γ-guanidobutyrate synthetase which formed γ-guanidobutyrilsuccinate (equivalent to arginosuccinate); then, a γ-guanidobutylsuccinate hydrolase converted that into γ-guanidobutyric acid which by means of a γ-guanidobutyrate ureohydrolase transformed the γ-guanidobutyrate into 4-aminobutyrate and urea. In that way, we have a cycle very similar to urea cycle. It sould be noticed that the transformation of γ-guanidobutyrate into urea by a γ-guanidobutyrate ureohydrolase has been claimed to occur in pig liver and kidney (1).

During many years it was thought that carbamylphospate was formed with ammonia as donor but afterwards a carbamylphosphate synthetase II (glutamine-dependent) was reported (20); so, the possibility of another aminoacid ammonia donor can not be rejected. In this case, the synthesis of carbamylphosphate would be possible, and the presence of a high concentration of carbamylphosphatase in brain (8) could be understood because it might represent a protective influence against carbamylation by destroying the small amount of residual carbamylphosphate because it is known that carbamylphosphate can carbamylate several protein in brain (4, 9).

Resumen

Se demuestra la presencia de urea en cerebro de rata, observando que ésta no se debe a la contaminación por la sangre.

La concentración de urea en homogenados de cerebro disminuye con la edad del animal, manteniéndose constante a partir de los diez días.

Se discute un posible mecanismo de formación de la urea en cerebro.

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