ASYMMETRY OF THE FREE AMINO ACID POOL IN SOME PARTS OF THE RAT BRAIN

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Information on the neurochemical basis of functional asymmetry of the brain suggests that an important contribution to the biochemical mechanisms of lateralization of parts of the CNS is made by the amino acid pool, which is characterized by relative constancy [1]. However, no data are available on the asymmetry of its distribution.

In the investigation described below the content of free amino acids was studied in symmetrical areas of the frontal cortex, hypothalamus, and midbrain of rats and the asymmetry thus revealed was compared with the animals' learning ability.

EXPERIMENTAL METHOD

Experiments were carried out in March and April on 18 male Wistar rats (from the Rappolovo Nursery), weighing 180-200 g. The animals were kept on a standard diet with unrestricted access to water and food. In the course of 14 days a motor conditioned reflex was formed in all the animals to the ringing of a bell, reinforced with food after a delay of 10 sec. On the 15th day (depending on the ability of the animals to respond correctly to the conditioned stimulus) the rats were divided into two groups: trained and untrained. Immediately after the test the animals were decapitated and the brain removed and frozen in carbon dioxide. Symmetrical areas of the frontal lobes of the cortex, hypothalamus, and midbrain were taken for quantitative determination of free amino acids. The structures were isolated in accordance with [2]. The tissue homogenates were deproteinized and free amino acids isolated as in [3]. Free plasma amino acids were isolated by addition of 10% sulfosalicyclic acid solution. The levels of free amino acids in the blood plasma and brain tissues were determined on an LC 7000 amino-acid analyzer ("Biotronik"). The coefficient of asymmetry for each amino acid was calculated as the ratio L:R, where L denotes the concentration of the amino acid in the left, and R the same in the right half of the corresponding part of the brain. Statistical analysis (by Student's t test and calculation of the coefficient of correlation) was carried out on the Iskra-226 computer.

EXPERIMENTAL RESULTS

Concentrations of five of the 18 free amino acids determined by the analyzer were found to be significantly ($p < 0.05$) higher in blood of the untrained animals (Fig. 1). Negative correlation was found between levels of serine, alanine, valine, phenylalanine, and lysine and the ability of the animals to respond correctly to the conditioned stimulus (r between -0.320 and -0.430).

Under the same conditions, concentrations of free amino acids in different parts of the brain were not so uniform (Table 1). It was found, for instance, that the concentrations of virtually all free amino acids except cysteine were significantly ($p < 0.05$) higher in the trained than in the untrained rats, only in the right half of the midbrain. Changes in the amino acid spectrum were not so uniform in the other parts of the brain. Nevertheless, concentrations of glutamate, glycine, and lysine were higher in the trained animals ($r = 0.450$,

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TABLE 1. Free Amino Acid Concentrations in Separate Parts of Brain (in μmoles/g wet weight) of Wistar Rats, Trained (I) and Untrained (II) in Performing Motor-Food Reflex with Delayed Reinforcement

Part of brain	Group of rats	Asp	Thr	Ser	Glu	Gly	Cys	Tyr	Phe	His	Hys
Left cortex	I	1,217±0,006*	0,519±0,020	0,229±0,017	5,84±0,15	0,415±0,019	0,389±0,007	0,079±0,005	1,058±0,060	0,253±0,050	0,114±0,004
	II	1,183±0,005*	0,581±0,021*	0,257±0,018	5,59±0,05*	0,411±0,014	0,412±0,005*	0,101±0,002*	1,500±0,070*	0,105±0,011*	0,116±0,007
Right cortex	I	1,164±0,004	0,548±0,015	0,289±0,014	5,52±0,20	0,369±0,007	0,334±0,012	0,047±0,005	0,842±0,009	0,078±0,002	0,101±0,003
	II	0,978±0,006*	0,590±0,014	0,289±0,015	4,91±0,15*	0,357±0,012	0,401±0,012*	0,107±0,007*	0,705±0,008*	0,102±0,009*	0,108±0,007
Left midbrain	I	0,543±0,041	0,532±0,015	0,149±0,008	2,98±0,10	0,737±0,025	0,234±0,017	0,098±0,005	0,680±0,100	0,077±0,003	0,126±0,002
	II	1,027±0,038*	0,599±0,025	0,155±0,007	2,84±0,09	0,714±0,032*	0,560±0,062*	0,181±0,016*	1,394±0,100*	1,077±0,005	0,109±0,004*
Right midbrain	I	0,585±0,012	1,154±0,004	0,458±0,036	3,35±0,05	0,980±0,027	0,394±0,040	0,079±0,003	0,788±0,120	0,101±0,004	0,188±0,009
	II	0,447±0,012*	0,912±0,005*	0,306±0,025*	3,08±0,02*	0,777±0,025*	0,692±0,09*	0,075±0,003	0,446±0,055*	0,088±0,006	0,130±0,007*

Legend. Groups I and II each consisted of nine animals. Data for groups I and II differing significantly ($p < 0.05$) indicated by an asterisk.

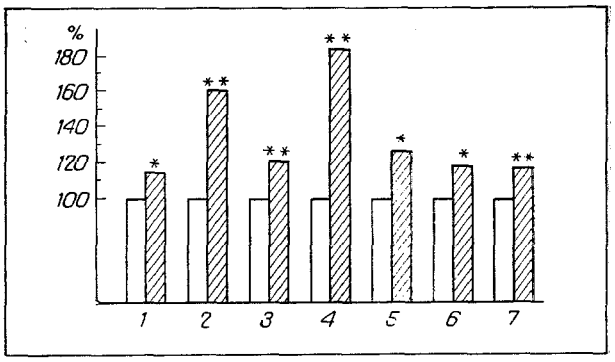

Fig. 1. Plasma levels of free amino acids in trained and untrained rats. Abscissa: 1) Ser; 2) Ala; 3) Nal; 4) Phe; 5) Lys; 6)Amm; 7) Totals; ordinate, amino acid concentrations (in %). Unshaded columns – trained rats, shaded – untrained. Following concentrations taken as 100% (in μmoles/ml): Ser 0.087, Ala 0.086, Val 0.036, Phe 0.031, Lys 0.111, Amm 0.388, total 1.285. *p < 0.05, **p < 0.01.

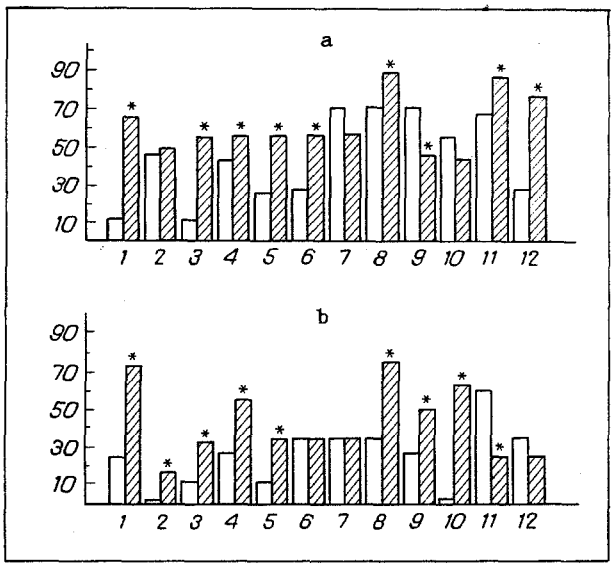

Fig. 2. Number (in %) of trained and untrained rats with higher free amino acid levels in left half of brain. Abscissa, free amino acids: 1) Asp; 2) Thr; 3) Ser; 4) Glu; 5) Gly; 6) Ala; 7) Cys; 8) Phe; 9) His; 10) Lys; 11) Amm; 12) totals; ordinate, number of animals (in %). a) Frontal cortex, b) midbrain. Unshaded columns – trained, shaded – untrained animals. Significant differences indicated by an asterisk.

0.459, and 0.412, respectively), whereas in untrained rats, especially on the left side of the brain, concentrations of cysteine, tyrosine, and phenylalanine were higher (r = −0.631, −0.384, and −0.425, respectively). A unique feature of the amino acid distribution in the hypothalamus was that phenylalanine and histidine levels were 1.5-2 times higher only in the left half of the brain of the untrained rats, whereas the cysteine level, on the contrary, was definitely higher in other parts of the brain of the trained animals. Levels of alanine, valine, methionine, ammonia, arginine, and of total amino acids were virtually identical in all symmetrically opposite parts of the brain in all animals.

Attention must be drawn not only to quantitative differences in free amino acid levels in trained and untrained animals, but also to the asymmetry of their distribution, as is shown conclusively by the value of the L:R ratio. Negative correlation (r between −0.340

and −0.492) was found between the ability of the animals to respond correctly to the conditioned stimulus and the shift of the L:R ratio toward an increase. It was also found that animals with left-sided asymmetry of most of the free amino acids determined predominated in the group of untrained rats (Fig. 2).

Asymmetry of distribution of the free amino acid pool was thus demonstrated in the brain of these animals, and it probably makes a definite contribution to their learning ability.

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EFFECT OF THE CALCIUM IONOPHORE A23187 ON PLASMA AND MITOCHONDRIAL POTENTIALS OF RAT BRAIN SYNAPTOSOMES: FLUORESCENCE STUDY

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The use of isotopic methods has shown that A23187, in the presence of Ca^{++}, lowers the potential of intrasynaptosomal mitochondria [5] and induces slow depolarization of the synaptosomal plasma membrane [5, 10]. Meanwhile Bartschat and Blaustein [6], using the potential-sensitive fluorescent probe diS-C_3-(5), obtained hyperpolarization of the synaptosomal plasma membrane by the addition of Ca^{++} in the presence of A23187. These workers linked hyperpolarization with activation of Ca-dependent K channels. Several investigations [1, 3, 4, 9] on different objects, including synaptosomes [4, 9], have now shown that positively charged voltage-sensitive probes respond to changes in both plasma potential and intracellular mitochondrial potential. In this connection the problem of whether hyperpolarization of the plasma membrane during the action of pharmacologic agents (A23187, valinomycin), simultaneously inducing mitochondrial depolarization also, can be recorded with the aid of diS-C_3-(5), is not yet clear. The aim of this investigation was to study, with the aid of diS-C_3-(5), the effect of A23187 and of Ca^{++} on the synaptosomal plasma and mitochondrial potentials and to evaluate the possibility of using this method to study the Ca-activated K channel in synaptosomes.

EXPERIMENTAL METHOD

Synaptosomes were isolated from the rat cerebral cortex [8] and suspended in modified Krebs–Ringer medium of the following composition (in mM): NaCl 132, KCl 5, NaH_2PO_4 5, glucose 10, $MgSO_4$ 1.3, HEPES 20, pH 7.4 (at the temperature of the measurements). The fluorometric measurements were made on a "Hitachi MPF-4" spectrofluorometer. Fluorescence of the probe was excited by light with wavelength 650 nm and was recorded at 670 nm. Except where

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