FORMATION OF THE AMINO ACID POOL OF THE BRAIN IN RATS DIFFERING IN PREFERENCE FOR ETHANOL

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The phenomenon of consumption of ethanol solutions in preference to water by animals kept under ordinary conditions have attracted attention for a long time as a possible analog of the alcohol motivation which is one of the principal biological components in the pathogenesis of alcoholism [5, 8]. One problem which still remains unsolved is that of the mechanisms of this preference. There are two main opposite points of view: animals consume ethanol solution voluntarily as a readily metabolized substrate, or they do so because of the pharmacological effect of alcohol. Correspondingly, in biochemical investigations, these animals have been divided into two groups. The state of energy metabolism, of the turnover of carbohydrates, lipids, and ethanol itself, has been characterized, whereas investigators of the other group have concentrated their attention on the study of the state of the brain neurotransmitter systems [2]. At the Third International Congress on the biomedical study of the alcoholism, this second point of view received much support [11], for the blood alcohol level of animals preferring ethanol, tested under experimental conditions, reached pharmacologically active concentrations. It was shown previously [3] on noninbred rats preferring ethanol or water that the free amino acid pool differed with respect to some parameters in the animals compared. In those preferring ethanol, concentrations of phosphoethanolamine, and alanine in the blood plasma and glycine in the brain were raised. Thus the differences related only to nonessential amino acids, which are used for plastic and energy-producing purposes, and also in the neurotransmitter pool [7].

The aim of the present investigation was to develop the research mentioned above. To reduce the heterogeneity of the test population, experiments were carried out on inbred lines

TABLE 1. Parts of the Brain of AA and ANA Rats Differing in Their Content of Free Amino Acids and Their Derivatives (in nmoles/g)

Amino acids	Hemisphere		Cerebellum
	right	left	
AA rats			
Taurine	4340±730	5860±200	4900±800
Cysteine	69±18	90±24	129±46
Leucine	52±9	85±34	43±10
α-Aminobutyric acid	81±9	73±8	58±20
Glutamine	1170±200	1300±180	1050±170
Urea	560±160	480±140	340±50
ANA-rats			
Taurine	7610±1430*	7730±1340	4420±1360
Cysteine	120±8*	90±24	130±47
Leucine	101±17*	75±16	68±22
α-Aminobutyric acid	92±11	167±42*	67±15
Glutamine	1090±130	1300±230	590±100*
Urea	1050±310	630±280	190±30*

Legend. *$p < 0.05$.

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TABLE 2. List of Within-Group Differences ($p < 0.05$) in Amino Acid Concentrations between Parts of the Brain of AA and ANA Rats

Amino acids	AA	ANA
	differences in pairs	
Cysteine	RS, CS	None
Glutamate	None	SC, RC
Glutamine	None	LC, RC
Glycine	None	LC, RC, SC
Alanine	None	LC
α-Aminobutyrate	None	LC
Valine	LS	None
Methionine	LR, RS	None
Cystathionine	LS, RS	CS, LS
Tyrosine	LS	None
GABA	LC	LC, RC
Histidine	RC, RS	None

Legend. R) Right hemisphere, L) left hemisphere, S) brain stem, C) cerebellum.

of mice preferring (AA) or rejecting (ANA) ethanol solutions [8]. The free amino acid pool also was studied in several parts of the brain.

EXPERIMENTAL METHOD

Rats (7 in a group) weighing 280-320 g were kept on a standard diet. After decapitation the brain was quickly removed and divided into right and left hemispheres, cerebellum, and brain stem. All these parts were quickly frozen in liquid nitrogen, and then homogenized in 3% sulfosalicyclic acid. Amino acids in the supernatant were determined [1] on a T-339 automatic analyzer (Czechoslovakia). The results were processed (Mera Camac CM4 computer) by a program testing the significance of differences and determining the coefficient of correlation [6].

EXPERIMENTAL RESULTS

The results are noteworthy from several standpoints. First, there were no differences in the amino acid level of the brain stem of the groups of animals compared. Second, there were minor differences (Table 1) in the concentrations only of cysteine, leucine, and taurine in the right hemisphere and α-aminobutyric acid in the left. Finally, concentrations only of glutamine and urea were lower in the cerebellum of the ANA animals. The differences noted above are evidence that the functional state of the CNS is different in AA and ANA animals, and this conclusion is confirmed by a more detailed examination (Table 2) of correlations between individual amino acids in the groups of animals and between different parts of the brain.

The most variable part of the brain in this respect was the cerebellum of ANA rats: differences in concentrations of glutamate, glutamine, glycine, alanine, α-aminobutyrate, cystathionine, and GABA in it relative to other parts of the brain were observed in 12 cases. In AA rats, the cerebellum behaved in this way in only 3 cases, in 2 of which (histidine, cysteate) these amino acids were uniformly distributed in the parts of the brain of ANA rats. There was another important finding: differences in the distribution of amino acids present in the distribution of amino acids present in the animals of one line were absent in the other. The results thus confirm that functional equilibrium (optimal interaction between different part of the brain) in AA and ANA lines of rats in achieved by means of different mechanisms of utilization of individual amino acids, to match the very great diversity of their functions.

The same conclusion can be drawn by correlation analysis of the data. For instance, changes in taurine and phosphoethanolamine concentrations, reported to be in the same direction in [10], were recorded significantly only in the right hemisphere ($r = 0.76$) of AA rats and were not found in the left hemisphere of the animals of either group. Significant correlations between the levels of taurine and glycine, which are inhibitory mediators [7], were discovered only in the right (r = 0.71) and left (r = 0.87) cerebral hemispheres of AA rats. Conversely, a uniform direction of changes in tyrosine and phenylalanine levels in the left hemisphere of AA and ANA rats (r = 0.91 and r = 0.85 respectively) was found with a lower significance (r = 0.62, $p < 0.1$) in the right hemisphere of AA rats, and not at all (r = 0.02) in rats preferring water. Significant correlations were found in the GABA pool for the neurotransmitter only with aspartate (r = 0.83) and glycine (r = 0.76), and only for the left hemisphere of ANA rats.

The cerebellum contains more glycine than the other parts of the brain. From both metabolic and neurophysiological aspects, glycine is closely linked with other amino acids and neurotransmitters [7, 12]. Appropriate analysis of our own data revealed striking differences for the cerebellum of AA and ANA rats. For instance, coefficients of correlation for glycine relative to GABA, glutamine, taurine, serine, and the ammonia level were highly significant for ANA animals (p = 0.05-0.000) (r = 0.69-1.0), whereas in AA rats, with a lower level or absence of significance (p = 0.5-0.05), all correlations can be described by a negative coefficient of correlation of between −0.72 and −0.35.

Amino-acid transport [9] also differed in the left hemisphere of the AA and ANA rats. Isoleucine did not compete with tyrosine in the AA rats and they accumulated harmoniously (r = 0.77) in the ANA rats. Valine competed with tyrosine (r = −0.86) and with phenylalanine (r = −0.74) only in ANA and not in AA rats (r = 0.06 and 0.12 respectively).

Differences also were found in metabolism of sulfur-containing amino acids in the animals compared. Levels of cysteic acid and serine showed very similar changes in the right (r = 0.90) and left (r = 0.96) hemispheres and in the brain stem (r = 0.89) of ANA rats only, and not of AA rats, in which the corresponding coefficients of correlation were very low and not significant. Correlations between cysteic acid and methionine were opposite: in AA rats in the right hemisphere r = 0.73, but in ANA animals r = −0.47, in the left hemisphere the values were 0.82 and 0.08 respectively, and in the brain stem 0.05 and 0.87. Since both serine and methionine are converted into cysteic acid only through cysteine [12], the differences between the two lines of rats must be looked for in the metabolic behavior of this amino acid.

It can be concluded from these results that inbred lines of rats (AA, ANA), differing in their voluntary preference for ethanol solutions, differ also in the character of distribution, functional use, and metabolism of individual amino acids in the right and left hemispheres, cerebellum, and brain stem.

LITERATURE CITED

1. J. D. Benson, in: New Methods of Analysis of Amino Acids, Peptides and Proteins [Russian translation], Moscow (1974), p. 9.
2. N. P. Bobrova, Ethanol and Metabolism [in Russian], Minsk (1982), p. 104.
3. S. Yu. Ostrovskii and M. N. Sadovnik, Vopr. Med. Khimii, No. 4, 502 (1980).
4. Yu. M. Ostrovskii, Ethanol and Metabolism [in Russian], Minsk (1982), p. 6.
5. Yu. M. Ostrovskii, V. I. Satanovskaya, and M. N. Sadovnik, The Biological Component in the Genesis of Alcoholism [in Russian], Minsk (1986).
6. P. F. Rokitskii, Biological Statistics [in Russian], Minsk (1973).
7. F. E. Bloom, Am. J. Physiol., 246, No. 3, C184 (1984).
8. K. Eriksson, Animal Models in Alcohol Research, ed. by K. Eriksson, et al., London (1980), p. 3.
9. R. A. Hawkins, Fed. Proc., 45, No. 7, 2055 (1986).
10. A. Lechman and A. Hamberger, J. Neurochem., 42, No. 5, 1286 (1984).
11. K.-H. Li, L. Lumeng, W. F. McBride, and J. M. Murphy, Alcohol and Alcoholism, 21, No. 2, 37 (1986).
12. J. Musil, O. Novakova, and K. Kunz, Biochemistry in Hematic Perspective, Prague (1977).