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Abstract

The present paper describes each pattern of the free amino acids in different parts of the dog brain determined by ion-exchange chromatography. The parts examined have been the cerebral cortex, cerebral white matter, cerebellar hemisphere, cerebellar vermis, caudate nucleus, thalamus, hypothalamus, and medulla oblongata. Gamma-aminobutyric acid concentration was the highest in the hypothalamus. Glutamic acid showed lower values in the white matter, hypothalamus, and medulla oblongata. Aspartic acid showed lower values in the white matter and caudate nucleus and higher values in the medulla oblongata. Glutathione and cystathionine showed higher values in the thalamus. N-Acetylaspartic acid showed lower values in the white matter and medulla oblongata. Glycine and alanine showed higher values in the medulla oblongata.

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AMINO ACID CONCENTRATION IN DIFFERENT PARTS OF THE DOG BRAIN

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By application of MOORE and STEIN's ion-exchange chromatographic procedure[1], the pattern of the amino acids in the brain of various animal species of the vertebrate and its changes in the rat brain due to the therapies effective to mental diseases and the drugs producing mental disorders have been reported previously by us[2,3]. These and many other author's data[4,5], however, were obtained from the whole brain *en masse*, and only scanty data[6,7,8] exist on the distribution of amino acids in different parts of the brain. The knowledge of each pattern of amino acids in anatomically and functionally differentiated parts of the brain aids to obtain a better understanding of the specific role of each free amino acid in the nervous system.

The present paper describes each pattern of the free amino acids in different parts of the dog brain determined by ion-exchange chromatography.

EXPERIMENTALS

Materials and Preparation of Tissue Extracts

Healthy female dog, weighing about 8 kg., 2 dogs were employed in the experiments. The dogs were slightly anaesthesized and killed by decapitation, and the brains were removed. The parts examined were the cerebral cortex, cerebral white matter, cerebellar hemisphere, cerebellar vermis, caudate nucleus, thalamus, hypothalamus, and medulla oblongata. Protein-free extracts of the tissues were prepared with 90 per cent ethanol as described previously[2,3].

Chromatographic Analysis

The samples were analyzed on 150×0.9 cm. columns of Dowex 50-X4 by MOORE and STEIN's procedure[1]. A fraction collector was used to obtain 2 ml. fractions. One ml. of each fraction was analyzed by the color reaction with modified ninhydrin reagent, and the concentration of amino acid

was calculated by comparison with a calibration curve obtained with L-leucine[9].

RESULTS

Table I gives the distribution of amino acids and related compounds

Table 1. Free Amino Acid Concentration in Various Parts of Dog Brain
(mg per 100 g wet weight)

	Cerebral cortex	Cerebral white matter	Cerebellar hemisphere	Cerebellar vermis	Caudate nucleus	Thalamus	Hypothalamus	Medulla oblongata
Amino Acid N	35.7	22.8	30.7	47.0	21.7	28.0	—	—
Glycerophosphoethanolamine	17.2	14.1	22.4	23.7	12.3	23.2	20.2	26.4
Phosphoethanolamine	24.2	7.5	21.7	8.5	39.1	20.8	26.8	10.8
Taurine	15.1	19.9	17.6	21.2	14.5	15.1	16.1	10.4
Urea	18	15	14	15	15	16	12	27
Aspartic acid	29.2	16.7	26.1	31.1	18.5	29.8	31.4	32.8
Threonine	2.9	4.4	2.8	2.6	4.0	3.1	4.6	4.2
Serine	4.4	3.7	3.8	3.6	4.5	4.0	7.7	5.2
Glutamic acid	127.0	85.5	127.8	176.0	125.3	137.3	89.2	77.4
Glycine	4.2	3.4	3.3	)13.5	4.6	9.2	)18.8	)32.1
Alanine	5.9	5.7	5.8		7.1	6.6		
Glutathione	16.4	11.2	26.6	36.2	23.3	62.1	28.2	16.7
Cystathionine	9.1	10.4	3.5	2.6	5.0	19.7	7.0	3.9
Isoleucine	) 2.6	) 0.8	1.7	1.7	1.2	) 2.4	) 3.5	2.1
Leucine			2.8	3.4	1.1			1.1
γ-Aminobutyric acid	18.7	5.8	18.4	28.8	22.6	27.5	54.4	17.2
Lysine	0.9	1.7	—	2.8	—	1.2	—	1.2
Histidine	1.2	2.2	—	2.7	—	—	—	1.0
Arginine	—	—	—	—	—	—	4.4	2.1
N-Acetylaspartic acid	106.5	73.5	106.5	92.0	91.1	99.6	102.6	58.9

in different parts of the dog brain. The most remarkable difference was found in the concentration of γ-aminobutyric acid. It showed the highest value in the hypothalamus. In the neighboring parts of the hypothalamus, namely, in the thalamus, caudate nucleus, and cerebellar vermis, it was found in relatively higher concentrations. Glutamic acid showed lower value in the white matter, hypothalamus, and medulla oblongata. Aspartic acid showed lower values in the white matter and caudate nucleus and higher values in the medulla oblongata. Glutathione and cystathionine showed higher values in the thalamus, but the recovery of glutathione

was not satisfactory[2,3] and in the case of cystathionine, the identification of it was based solely on its position on the chromatogram and the results shown in Table I must therefore be considered as maximal figures. For the above mentioned reasons, the results of these two compounds were not fully reliable. N-Acetylaspartic acid showed lower values in the white matter and medulla oblongata. Glycine and alanine showed higher values in the medulla oblongata. In the neighboring parts of the medulla oblongata, namely, in the hypothalamus, thalamus, and cerebellar vermis the glycine and alanine were found in relatively higher concentrations.

DISCUSSION

In the white matter, having poor nuclear component, it has been reported that glutamic acid[10], γ-aminobutyric acid[6], and N-acetylaspartic acid[7] were contained in low concentrations. Aspartic acid was found also in a low concentration. The fact that the γ-aminobutyric acid was the most abundant in the hypothalamus arouses a great interest. γ-Aminobutyric acid may play a specific role on the function of hypothalamus. It was described proviously by AWAPARA[6] that the γ-aminobutyric acid is contained in the caudate nucleus in the largest amount, and he has not examined the concentration in the hypothalamus. PORCELLATI and THOMPSON[11] reported that the value, dividing the aspartic acid value by glutamic acid value was higher in the spinal cord than in the brain. We found that the concentration of the aspartic acid in the medulla oblongata was high in comparison with the low concentration of glutamic acid in the same part. Cystathionine has been found by TALLAN, MOORE and STEIN[12] in the human brain. Though our values of cystathionine may not be fully reliable, it appears to occur in the highest concentration in the thalamus, and in the same part, the concentration of the glutathione was also the highest. The free amino acid patterns of the cerebral cortex and cerebellar hemisphere were very similar with each other. It may be of some interest to note that both of these two parts are phylogenetically the parts of the more recent developments. Glycine and alanine concentrations of the human foetal brain was much higher than those of the adult brain. * In regard to this fact, it is interesting to note that the high concentration of the glycine and alanine are obtained in the phylogenetically older parts, e. g., the medulla oblongata, hypothalamus, thalamus, and cerebellar vermis rather than in the cerebral cortex or cerebellar hemisphere.

* unpublished data

SUMMARY

The present paper describes each pattern of the free amino acids in different parts of the dog brain determined by ion-exchange chromatography. The parts examined have been the cerebral cortex, cerebral white matter, cerebellar hemisphere, cerebellar vermis, caudate nucleus, thalamus, hypothalamus, and medulla oblongata.

Gamma-aminobutyric acid concentration was the highest in the hypothalamus. Glutamic acid showed lower values in the white matter, hypothalamus, and medulla oblongata. Aspartic acid showed lower values in the white matter and caudate nucleus and higher values in the medulla oblongata. Glutathione and cystathionine showed higher values in the thalamus. N-Acetylaspartic acid showed lower values in the white matter and medulla oblongata. Glycine and alanine showed higher values in the medulla oblongata.

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