Free Amino Acids in Cytosol of Rat Brain after Intraventricular Administration of 5,6-Dihydroxytryptamine and 6-Hydroxydopamine

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Levels of 24 free amino acids were estimated in the brain after administration of 5,6-dihydroxytryptamine and 6-hydroxydopamine into the lateral brain ventricles of male Wistar rats. These neurotransmitters caused serotoninectomy and sympathectomy in the diencephalon, striatum, brain stem and medulla, thalamus and hypothalamus, cerebral cortex and cerebellum. The most abundant amino acids in these brain structures were: glutamic acid, serine, aspartic acid, cystine, gamma-aminobutyric acid, glycine, tryptophan and alanine. We detected and quantified changes in the levels of these and other amino acids in the investigated regions of the rat central nervous system, under the influence of these two neurotransmitters.

Introduction

Free amino acids (AA) found in hyaloplasm of nerve cells play an essential role in these cells' metabolism: they are precursors for proteins and comprise their reserve energetic pool [1]. The role of some amino acids as neurotransmitters has been suggested when receptors for GABA, glycine, taurine, glutamine and asparagine were identified in the Central Nervous System (CNS) [2, 3]. Amino acids are also the main precursors for the neurotransmitters noradrenaline, dopamine, 5-hydroxytryptamine and acetylcholine [4, 5]. While studies regarding the amino acids' content in the CNS are numerous and concern mostly leucine, methionine, glycine, tryptophan and GABA, studies on the amino acid content in specific parts of the CNS, especially the three basic components of the nerve tissue (neurons, glial cells and neurophiles) are rare [6]. In addition, the amino acid content in the nerve tissue depends on the metabolic activity of each given part of the CNS, and on external factors, *e.g.* diet, drugs, excitation and toxins [7].

Some synthetic derivatives of natural neurotransmitters administered exogenously are taken up by the nerve terminals and consequently may cause degeneration of these terminals. Such substances include 6-OHDA and 6-ADA, which are capable of destroying terminals of both adrenergic and dopaminergic neurons. Other substances are 5,6-DHT, and 5,7-DHT, which damage serotoninergic neurons [8]. Intensive search is being carried out for substances which specifically damage pathways of other neurotransmitter, to be used as "pharmacological tools" in psychopharmacology, since they facilitate investigations of CNS functions and mechanisms of action of psychotropic drugs. Unfortunately, such effects are not always selective [9].

In view of the limitations mentioned above we undertook to investigate the effect of one compound producing chemical sympathectomy of the CNS (6-OHDA) and one compound yielding chemical serotoninectomy (5,6-DHT), upon the content of free amino acids in specific structures of rat's brain.

Materials and Methods

Seventy-five white male rats of the Wistar strain weighing 200–220 g each were used for this study. The animals were divided into three groups: the first was administered 250 μg of 6-OHDA · HBr (calculated as a base) into the right brain ventricle, dissolved in 10 μl saline containing 0.1% ascorbic acid, according to Herman *et al.* [10]. Two days later an identical dose was administered in the same way, and

Abbreviations: GLU, glutamic acid; SER, serine; ASP, aspartic acid; CYS, cystine; GABA, gamma-aminobutyric acid; GLY, glycine; TRY, tryptophan; ALA, alanine; OHDA, hydroxydopamine; ADA, aminodopamine; DHT, dihydroxytryptamine.

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seven days later the rats were decapitated and their brains divided into the following parts: diencephalon, striatum, thalamus plus hypothalamus, cerebral cortex, stem plus medulla oblongata and cerebellum. These brain fragments were immediately frozen in liquid nitrogen, weighed and kept at −20 °C. Free amino acids' contents were assayed by gas chromatography (GC) within ten days after killing the animals.

In rats of the second group chemical serotoninectomy of the CNS was induced by 75 μg 5,6-DHT · HBr (calculated as a base). This compound was also injected in a volume of 10 μl saline. After 10 days the rats were killed by decapitation and the same brain fragments collected. The third group consisted of control animals which were administered 10 μl saline containing 0.1% of ascorbic acid into both lateral brain ventricles on the 9th and 7th day before killing. No determinations of noradrenaline, dopamine or 5-hydroxytryptamine were made in this study, as they have been estimated elsewhere [9].

The lyophilizer was manufactured by VEB MLW Labortechnik, GDR, the evaporator by Buchi, Switzerland, and the gas chromatograph by Varian: model 3700 with data analyzer CDS 111C and A25 recorder. All chemicals were of analytical grade. The Dowex 50 X-W 8/H+/100−200 mesh was produced by Fluka, Buchs, Switzerland. Standard amino acids were obtained from Pierce, Rockford, IL, USA or BDH, poole, Great Britain. 6-OHDA was obtained from AB Hassle, Göteborg, Sweden and 5,6-DHT creatinine sulphate from Sigma.

The tissues were homogenized in saturated picric acid: ethanol 70% (1:5), centrifuged at $600 \times g$ for 15 min and the supernatant decanted and centrifuged at $105{,}000 \times g$ for 60 min. The sedimented proteins were rejected, while the deproteinized supernatant was introduced onto the ion-exchange column. The separation procedure was carried out according to Kaiser *et al.* [11]. Thirteen ml of the eluate were frozen in liquid nitrogen and lyophilized. The residue was transferred to an esterification vessel, and derivatization performed according to Zumwalt *et al.* [12].

Results and Discussion

These results are based on quantification of 24 amino acids, whose identification was practiced in our laboratory. The levels of eight amino acids are presented as ng per g brain tissue in Table I. The weights of each AA varied with the tissue location and reflected the different metabolic and physiological functions of each brain segment. They also represent the significance of the various elements of the nervous tissue *e.g.* neurons, glial cells, neurophiles and blood vessels. Irrespective of the brain structure assayed, GLU, SER, ASP, CYS, GABA, GLY, TRY and ALA predominated the brain tissues studied and therefore they were tabulated in Table I. Similar results were obtained by others [13]. GLU has the highest abundance in all investigated CNS structures, amounting to over 40% of all recovered amino acids. The abundance of the remaining acids vary, and is characteristic of each given part of the CNS. Analysis of the other most abundant amino acids show that ASP, GLY and TRY are most frequent, while GABA show marked variation in the individual brain areas: it could not be detected in the brain stem + medulla and thalamus + hypothalamus, but mounted to 14% in the cerebellum and diencephalon. Similarly, SER was hardly recovered in the diencephalon and the cerebellum, but amounted to 17% of the amino acids pool in the stem + medulla. Amino acids that play a role in the biosynthesis of neurotransmitters, *e.g.* GLU, ASP, GABA and GLY were found in markedly higher percentages. In view of the fact that the eight most abundant amino acids comprise over 90% of the amino acid pool in the untreated brains, only their weights are given in Table I.

The following changes in the content of the AAs were observed under the influence of 6-OHDA: marked changes in the content of the investigated amino acids were observed in the cerebellum: The contents of GLU, GABA and TRY were decreased, whereas that of SER was increased. GABA levels dropped from 14% of the total amino acids in the control group to 0.85%, while SER, which could hardly be detected in the control group increased to over 16% under the influence of 6-OHDA. In the thalamus + hypothalamus, increased amounts of GLU and GABA and decreases in ASP, GLY and TRY levels were observed. In conclusion, it seems that 6-OHDA decreases GLU and ASP levels, but increases SER and GABA percentages in most of the brain structures studied. The changes in the patterns of the remaining amino acids were inconsistent.

Under the influence of 5,6-DHT an increase in GLU and a decrease in ASP were noticed in most of

Table I. Weight (mean ± SEM) of the eight amino acids dominating the CNS, in rats injected 6-OHDA, 5,6-DHT or controls. All data are ng/g tissue (n = 7–10).

Amino acid	6-OHDA	5,6-DHT	Control	6-OHDA	5,6-DHT	Control
	Diencephalon			Striatum		
GLU	390 ± 22*	392 ± 15*	609 ± 68	183 ± 27	521 ± 41*	171 ± 37
SER	61 ± 7.6*	3 ± 0.2	1 ± 0.1	52 ± 13*	26 ± 4.8	33 ± 9.2
ASP	71 ± 12*	71 ± 12*	110 ± 21	32 ± 0.9*	81 ± 27	22 ± 6.1
CYS	1 ± 0.4	0 ± 0.0	20 ± 0.2	0 ± 0.0	0 ± 0.0	1 ± 0.2
GABA	82 ± 6.6*	130 ± 25*	189 ± 31	22 ± 1.0*	131 ± 10*	15 ± 8.2
GLY	54 ± 11	41 ± 12	40 ± 9.2	13 ± 1.6	1 ± 0.3*	14 ± 6.3
TRY	30 ± 6.2*	237 ± 51	30 ± 6.1	22 ± 1.0*	21 ± 6.4*	6 ± 0.5
ALA	29 ± 8.4	39 ± 13	21 ± 0.1	14 ± 0.9	20 ± 6.1	11 ± 1.5
	Cerebellum			Thalamus + Hypothalamus		
GLU	320 ± 50*	520 ± 41	421 ± 30	264 ± 22	310 ± 50*	181 ± 35
SER	91 ± 12*	110 ± 5.3*	3 ± 0.1	71 ± 13	100 ± 5.6*	53 ± 10
ASP	52 ± 11	60 ± 5.4	62 ± 4.6	41 ± 9.9*	62 ± 5.2	62 ± 5.2
CYS	–	5 ± 0.3	–	0 ± 0.0	10 ± 2.2	43 ± 5.8
GABA	1 ± 0.2*	1 ± 0.1*	107 ± 12	1 ± 0.3*	0 ± 0.0	0 ± 0.0
GLY	11 ± 4.2	31 ± 9.6	11 ± 0.7	11 ± 3.2*	23 ± 6.2	31 ± 6.9
TRY	11 ± 6.6*	10 ± 0.8*	35 ± 7.1	10 ± 2.1*	31 ± 10	32 ± 5.4
ALA	21 ± 8.1	26 ± 2.4	25 ± 0.0	11 ± 0.9	11 ± 2.2	6 ± 1.1
	Cerebral Cortex			Stem + Medulla		
GLU	330 ± 42	650 ± 86*	260 ± 61	121 ± 21*	490 ± 25	198 ± 10
SER	96 ± 11*	1 ± 0.2*	61 ± 5.6	34 ± 9.8*	36 ± 4.9*	63 ± 5.6
ASP	52 ± 18*	121 ± 11*	80 ± 5.1	36 ± 9.2	9 ± 1.5*	47 ± 5.2
CYS	111 ± 21*	–	31 ± 0.6	5 ± 1.9	5 ± 1.2	1 ± 0.0
GABA	1 ± 0.2	140 ± 16*	1 ± 0.0	–	11 ± 2.0	1 ± 0.0
GLY	1 ± 0.2*	21 ± 0.7*	59 ± 9.6	22 ± 3.8	3 ± 0.8	17 ± 0.1
TRY	166 ± 25*	27 ± 0.2	27 ± 6.6	32 ± 3.0*	3 ± 1.8*	21 ± 2.0
ALA	13 ± 0.7*	29 ± 5.7	22 ± 0.5	6 ± 1.2	1 ± 0.3	16 ± 1.2

* = Significantly different from the control.

the investigated CNS structures, while the changes in the remaining amino acids assayed in the individual CNS structures were variable. In the majority of the CNS structures investigated, 6-OHDA and 5,6-DHT acted in an antagonistic pattern with reference to GLU, *i.e.* 5,6-DHT increases GLU content while 6-OHDA decreases it in the same structures. Such reciprocity was noticed with GABA, whose levels in the various CNS structures varied between 6-OHDA and 5,6-DHT. Similar changes were found with regard to TRY, GLY, ASP and SER. From analysis of all investigated amino acids (including 16 acids whose levels were assayed but were not reported here) it seems that the greatest changes produced by 6-OHDA were observed in the diencephalon, thalamus + hypothalamus and stem + medulla, whereas 5,6-DHT caused changes mainly in the cortex, cerebellum and striatum. 6-OHDA and 5,6-DHT produced the greatest changes in GABA, SER and GLU levels of the CNS.

Amino acids are a source for neurotransmitters in the CNS, and some of them play neurotransmitters' role in the brain. For this reason, amino acids raised growing interest within the past few years in understanding CNS physiological and physiopathological changes. So far there is very little information on the interaction between biogenic amines, amino acids and the CNS. Previously it was shown that chemical sympathectomy and serotoninectomy of the CNS change GABA content in some brain areas of a rat [14]. On the other hand, it has been shown that taurine administered into the lateral brain ventricle of a rat changes the content and degree of acetylcholine synthesis in individual brain areas. Our

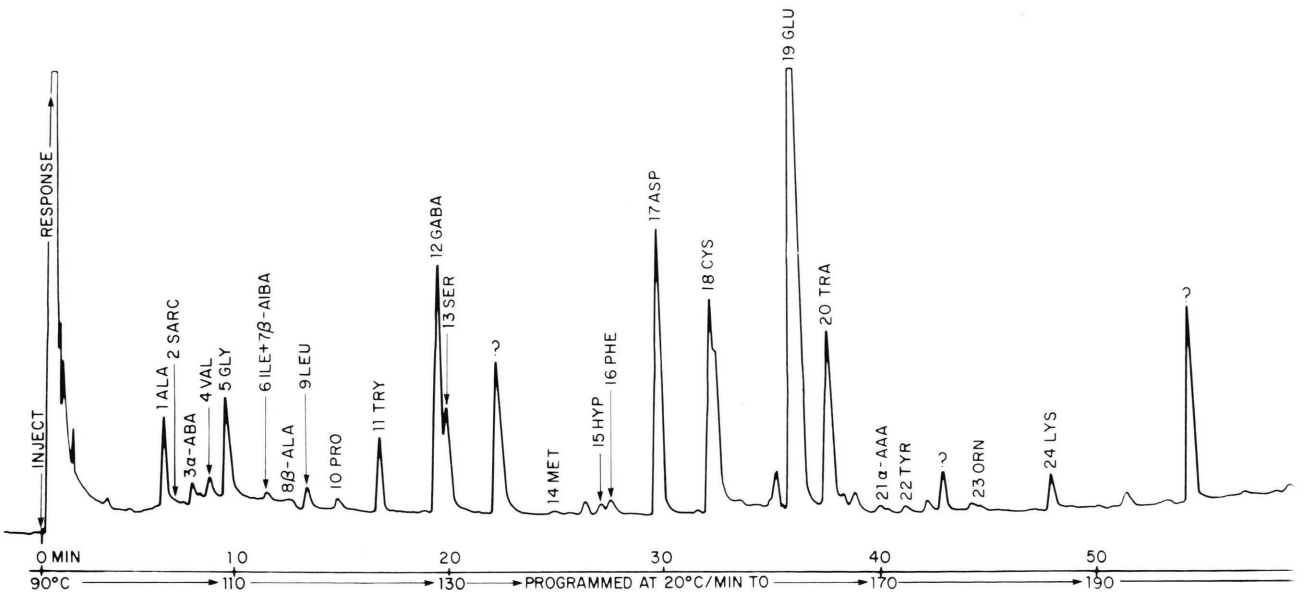

Fig. 1. Simultaneous GLC separation of N-TFA n-butyl esters of amino acids in cytosol of rat cerebellum. Sample injected: 1 µl; Column filled with 0.65% EGA-PS on 80–100 mesh Chromosorb WAW. Attenuation: 2×10^{-10} a.u.f.s.; Temperature: 90 °C–220 °C; programming rate: 2 °C/min; flow-rate of the helium gas: 10 ml/min; of hydrogen: 30 ml/min and of air: 300 ml/min. Internal standard: tranexamic acid.

results indicate that damage of central serotoninergic and adrenergic neurons significantly affects the content of free amino acids in the rat brain. This may indicate an interaction between adrenergic and serotoninergic systems on one hand and potential neurotransmitters, *e.g.* amino acids, on the other, on controlling cerebral activity.

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