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EFFECT OF CERTAIN DRUGS ON THE γ-AMINOBUTYRIC ACID SYSTEM OF THE CENTRAL NERVOUS SYSTEM

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Abstract—The central nervous system stimulants caffeine and centedrin increase the γ-aminobutyric acid content of the brain by 25–30 per cent. Administration of andaxin, which caused a general depression, results in a 23 per cent decrease in the brain γ-aminobutyric acid level. Intraperitoneal injection in rats of picrotoxin, metrazol, caffeine, centedrin, hept-amyl, 20–28 M.D., barbamil and andaxin does not produce changes in the enzymatic activities of L-glutamic acid decarboxylase and γ-aminobutyric acid-α-ketoglutaric acid transaminase. Addition of these pharmacological preparations to the incubation mixture of brain homogenates of rats also does not alter the activity of the enzyme systems connected with γ-aminobutyric acid in the brain. Administration *in vitro* of caffeine (600 mg/100 g brain tissue) suppressed L-glutamic acid decarboxylase by about 10 per cent.

THE DISCOVERY that γ-aminobutyric acid (γ-ABA) has an inhibitory effect on the nervous system of animals has led to the suggestion that it participates in regulating the physiological activity of the brain.[1–7] The appearance of certain convulsive states is connected with a decrease in the γ-ABA content of the brain by decreasing the activity of L-glutamic acid decarboxylase (GAD).[8–11] The increase of γ-ABA in the brain after administration of hydroxylamine or amino-oxyacetic acid may be due to an inhibition of γ-aminobutyric acid-α-ketoglutaric acid transaminase (γ-ABA-T). This enzyme catalyzes the reversible transamination of γ-ABA with α-ketoglutarate to give glutamate and succinic semialdehyde, which can be oxidized further to succinic acid and so enter the tricarboxylic acid cycle.[12–14] Many extremely contradictory papers concerning the quantitative determination of γ-ABA in the brain during different functional states of the central nervous system have been published.[15–22] A number of investigators assume that γABA is a specific 'inhibitory agent' affecting the activity of neurones and synaptic transmission and participating in the regulation of physiological activity in the brain, the excitability of which is an inverse relation to the concentration of γ-ABA.[4, 5, 22–25]

The purpose of the present investigation was to study the effect of a number of pharmacological agents on the γ-ABA content of the brain and the activities of the enzymes involved in the synthesis and destruction of this amino acid (GAD and γ-ABA-T) in order to determine whether the γ-ABA system is an index of the functional activity of the central nervous system.

METHODS

Albino rats weighting approximately 150 g were used and the brain tissue was prepared according to our modification[26] of the method of Roberts.[23] The rats were dipped into liquid nitrogen, the frozen cortex was quickly removed and stirred with liquid nitrogen, the proteins were then precipitated by 75 per cent ethanol (1:7) and centrifuged. The supernatant fluid was collected and the residue re-extracted with ice-cold 75 per cent ethanol and evaporated to dryness. The residue was dissolved in water and centrifuged, and the supernatant was again evaporated to dryness. The amount of γABA- was determined by one-dimensional chromatography on Whatman No. 1 paper using butanol-acetic acid- water [4:1:5 (v/v)]. The chromatograms were dipped into a 0·5% ninhydrin solution in acetone. Then they were heated at 80° in a drying cabinet for 20–25 min. The portions carrying the γ-ABA spots were cut out and eluted with 0·005% $CuSO_4$ in 75% ethanol. All spectrophotometic readings were made with spectrophotometer model SF-4A at 512 mμ.

Determination of brain glutamic decarboxylase activity[28]

The activity of GAD in brain homogenates was measured by the determination of the increase in the amount of γ-ABA during incubation of brain homogenates with glutamic acid. The animals were killed by decapitation and the brains dissected as rapidly as possible. The tissue samples were homogenized immediately in ice-cold 0·05M phosphate buffer pH 6·3 (1:2) with glass homogenizer at 4°. The incubation mixture was comprised of 1·0 ml of the brain homogenate and 1·0 ml of 0·05 M glutamic acid (neutralized to pH 6·3–6·7). Samples were incubated in closed tubes for 30 min at 37° in anaerobic conditions (N_2). At the end of the incubation period the reaction was stopped by heating for 10 min at 100°. Five ml of water was then added and after shaking the samples were centrifuged. The control samples were heated immediately till incubation. The samples were assayed for γ-ABA in the usual manner which is based on one-dimensional chromatograms.[26] Activity is expressed as micromoles of γ-ABA formed per g of fresh tissue per hour.

Determination of brain γ-aminobutyric acid-α-ketoglutaric acid transaminase activity

The brains of rats, killed by decapitation, were homogenized with ice-cold 0·05 M phosphate buffer pH 7·8 (1:2) at 4°. The incubation mixture was comprised of 1 ml of the brain homogenate, 0·5 ml of α-ketoglutaric acid (0·05M in pH 7·8–8·2 phosphate buffer) and 0·5 ml of γ-ABA (0·05M in pH 7·8-8·2 phosphate buffer). Then the procedure used was carried out as described for determination of brain GAD activity. Activity is expressed as micromoles of glutamic acid formed per g of fresh tissue per hour.

The pharmacological substances were injected intraperitoneally (experiments *in vivo*) or they were tested *in vitro* by addition to the incubation mixture. The following pharmacological substances were injected intraperitoneally in doses presented in Table 1. As a result of its action different changes in the central nervous system were detected. Picrotoxin and metrazol caused seizures, caffeine and hept-amyl* (heptaminol, chlorhydrate amino-2-methyl-6-heptanol-6 of Delalande manufacture—Paris) caused noticeable excitation of the central nervous system. Centedrin [Richter—of

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Hungarian manufacture, methyl ether L-phenyl-L-piperidyl (2) hydrochloride] increased spontaneous motor capacity along with a sharply increased orienting reaction. 20–28 M.D. [4′-fluoro-4(1-4-2 methoxy) phenyl-piperazine—butyrophenone of Delalande manufacture—Paris] and andaxin (of Hungarian manufacture)—2 methyl-2n-propyl-1,3-propanediol dicarbamate) produced a state of depression. After the injection of barbamil (sodium amytol, sodium salt of ethyl-isoamylbarbiturate acid) a state of deep sleep of the animals was detected. The animals were killed 5–10 min after the injection of picrotoxin, metrazol, caffeine, centedrin, hept-amyl; 15 min after 20–28 MD and 40 min after the administration of andaxin and barbamil.

The doses of these drugs in experiments *in vitro* (expressed as mg/100 g of brain tissue) were picrotoxin-6, metrazol-60, caffeine-600, centedrin-30, hept-amyl-15, 20–28 M.D.-6·6, andaxin-300 and barbamil-60.

The data obtained were examined statistically by the t-test.[29]

RESULTS

In the normal state of relative physiological quiescence the individual variations in the γ-ABA level in the brain were not large (14· 4–17·8 mg%). The γ-ABA content of the hemispheres of rat brains in a state of centedrin or caffeine excitation was 25–30% higher (see Table 1). No alteration in γABA- concentration of the mammalian brain was observed during 5 min seizures induced by picrotoxin and metrazol. The effect of andaxin, seen as muscular weakness and a state of depression in the animals, decreased the amount of γ-ABA present in the brain by 23 per cent. These changes in γ-ABA content as compared with the normal are statistically significant: $P < 0{\cdot}01$. Barbamil depresses cortical activity but its effect is not accompanied by the change of γ-ABA level in the brain.

The activity GAD of brain rats showed normal activity *in vivo* and *in vitro* (Table 2). Only caffeine *in vitro* (600 mg/100 g) suppressed GAD, but there was only about a 10 per cent inhibition. No significant inhibition was noted of γ-ABA-T either *in vitro* or *in vivo* by administering of some of the pharmacological agents (Table 3).

DISCUSSION

Our results indicate that γ-ABA must not be considered an 'inhibitory agent' and that neither increase nor decrease in its content in the brain serve as an index of the

TABLE 1. γ-ABA CONTENT OF RAT BRAIN (IN mg/100g WET WT. OF TISSUE; MEAN VALUES ± S.E.M.)

Experimental conditions	No. of experiments	Dose (mg/100 g)	γ-ABA
control	15	—	16·2 ± 0·08
picrotoxin*	12	1·0	17·6 ± 0·46
metrazol*	10	10·0	16·8 ± 0·9
caffeine	11	80·0	20·4 ± 0·56
centedrin	12	10·0	21·7 ± 0·95
hept-amyl	13	10·0	17·2 ± 0·53
20–28 M.D.	9	1·0	15·8 ± 0·39
andaxin	11	100·0	12·5 ± 0·08
barbamil	9	10·0	16·3 ± 0·57

* Musaelyan experiments ([30]).

TABLE 2. EFFECT OF PHARMACOLOGICAL SUBSTANCES UPON GLUTAMIC ACID DECARBOXYLASE IN RAT BRAIN *in vivo* AND ADDITION OF IT *in vitro* (ACTIVITY—μMOLES γ-ABA PER g TISSUE PER HR; MEAN VALUES $\pm$ S.E.M.)

Experimental conditions	Experiments *in vitro*			Experiments *in vivo*		
	No. of experiments	Dose (mg/100 g)	Activity GAD	No. of experiments	Dose (mg/100 g)	activity GAD
control	44	—	18·1 ± 0·27	—	—	18·1 ± 0·26
picrotoxin	8	6·0	18·1 ± 0·31	7	10·0	18·8 ± 0·55
metrazol	9	60·0	18·8 ± 0·25	5	10·0	18·2 ± 0·71
caffeine	14	600·0	16·2 ± 0·37	6	80·0	18·7 ± 0·67
centedrin	6	30·0	17·7 ± 0·54	12	10·0	17·8 ± 0·31
hept-amyl	5	15·0	18·3 ± 0·62	5	10·0	18·0 ± 0·15
20–28 M.D.	8	6·6	17·9 ± 0·41	5	10·0	18·0 ± 0·19
andaxin	6	300·0	18·2 ± 0·49	11	100·0	18·7 ± 0·22
barbamil	10	60·0	17·6 ± 0·58	5	10·0	18·6 ± 0·23

TABLE 3. EFFECT OF PHARMACOLOGICAL SUBSTANCES UPON γ-AMINOBUTYRIC ACID TRANSAMINASE IN RAT BRAIN *in vivo* AND ADDITION OF IT *in vitro* (ACTIVITY—μMOLES GLUTAMIC ACID PER g TISSUE PER hr; MEAN VALUES $\pm$ S.E.M.)

Experimental conditions	Experiments *in vitro*			Experiments *in vivo*		
	No. of experiments	Dose (mg/100 g)	activity γ-ABA-T	No. of experiments	Dose (mg/100 g)	activity γ-ABA-T
control	40	—	29·4 ± 0·6	—	—	29·4 ± 0·6
picrotoxin	11	6·0	29·0 ± 1·6	8	1·0	29·6 ± 1·3
metrazol	6	60·0	31·0 ± 2·0	10	10·0	28·4 ± 0·9
caffeine	12	600·0	29·1 ± 1·1	10	80·0	29·6 ± 0·9
centedrin	5	30·0	31·0 ± 1·1	9	10·0	30·2 ± 0·5
hept-amyl	8	15·0	28·6 ± 1·3	10	10·0	30·0 ± 1·3
20–28 M.D.	10	6·6	29·2 ± 1·3	9	1·0	29·3 ± 0·8
andaxin	8	300·0	31·2 ± 2·0	10	100·0	29·2 ± 0·8
barbamil	10	60·0	28·1 ± 1·2	10	10·0	30·4 ± 0·9

functional state of the nervous system. The effect of andaxin, seen as a state of depression in the animals decreased the amount of γ-ABA present in the brain by 23 per cent. In the present experiments with barbamyl, which caused intracortical inhibition, no alteration was noted 40 min after injection. But as has been found earlier[31] a 30 per cent drop in the γ-ABA content of the brain was noted by 80 min after injection of the preparation instead of the conjectured rise. A similar contradiction was seen in the experiments with stimulants (caffeine and centedrin)—the γ-ABA content of the brain increased by 25–30 per cent, instead of decreasing as expected. Our results show that the changes of levels of γ-ABA in the brains of rats were not the results of the changes of GAD and γ-ABA-T activities while their activity in the experimental conditions practically did not change. Earlier we showed that it is necessary to immerse the animals in liquid nitrogen for obtaining values for γ-ABA

that represent true *in vivo* values more closely[35]. The results of our methodic investigations[36] indicated the necessity of keeping the shortest time intervals between decapitation of the rat and homogenization of the brain tissue. In the attempt to obtain the true values of enzymes activities special physiological precautions were taken in our experiments. The activities of the enzymes were determined only in brain homogenates without pyridoxal-phosphate and salts of metals. The extracts of brain acetone powder were not used in our experiments. However, the reaction conditions for the enzymes were optimal (pH, temp., N_2—anaerobic conditions).

According to Maslova[32] the γ-ABA level in brain is changed in conformity with the phases of neuronal excitability. Contradictory data about the γ-ABA content in the brain during different functional states of the central nervous system were explained by the absence of a true appropriate control of the state of whole body and especially of the states of the blood circulation and respiration. Chickvaidze[21] reported that γ-ABA content in brain and its alterations which were seen in the experiments with stimulants and depressants are not always connected with the degrees of activity of enzymes of γ-ABA metabolism. Results of Roberts[33] and Ersting[34] indicate that pharmacological substances do not greatly affect the activity of the enzyme systems connected with γ-ABA metabolism in the brain. At the present time we have no data about the biochemical mechanisms involved in phenomena such as seizure or sleep. The nature of drug effects on the enzymatic activities of γ-ABA metabolism remains to be determined. The results discussed in this paper have been limited to a consideration of absence of congruence between the ABA level in brain and its enzymes. At the same time it should be acknowledged that a stable γ-ABA level is an essential factor in normal brain activity, which indicates the great metabolic plasticity of the nervous system. γ-ABA is continuously formed in neuron cells and influences modulatory effect in the central nervous system. The decrease in its production in synapses would suggest an increase in neuronal excitability because γ-ABA plays a regulatory role influencing excitatory and inhibitory states by means of the formation of a general inhibitory background to the cortex.

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