THE GABA SYSTEM AS A FACTOR COMPENSATING THE DISTURBED CEREBRAL HEMODYNAMICS

S. A. Mirzoyan and V. P. Akopyan

UDC 616.831-005-085.31:547.466.3

Changes in the system of conversion of γ-aminobutyric acid (GABA) in ischemia and during specific inhibition of GABA-transaminase under conditions of quantitative measurement of the blood flow by the hydrogen clearance method revealed definite correlation between elevation of the GABA level in the brain and tissues of the cerebral arterial walls and the onset of compensation of the disturbed cerebral hemodynamics. Consequently, one of the principal manifestations of the increased quantity of endogenous GABA during insufficiency of the cerebral blood supply is its ability to improve the cerebral hemodynamics.

KEY WORDS: GABA system; cerebral circulation.

γ-Aminobutyric acid (GABA), the mediator of inhibition in the CNS [1, 10, 13], can increase the blood supply to the brain [3, 4]. The presence of GABA and of enzymes participating in its metabolism in the tissues of the cerebral arterial walls [5, 6] and the existence of GABA receptors in them [11] served as the basis for a study of the content of GABA and the enzymes of its metabolism in the tissues of the brain and its arteries during disorders of the cerebral circulation and the comparison of these indices with the onset and development of compensation of the disturbed cerebral hemodynamics.

EXPERIMENTAL METHOD

Experiments were carried out on 60 adult albino rats anesthetized with ether, 36 cats anesthetized with urethane and chloralose, and 20 dogs anesthetized intraperitoneally with pentobarbital sodium. To study changes in the system of GABA conversions in the tissues of the brain and walls of its arteries the common carotid (in rats) or the vertebral and carotid arteries simultaneously (in cats and dogs) were ligated unilaterally.

The GABA content and the activity of glutamate decarboxylase (GD) and GABA-transaminase (GABA-T) were determined by the method of Roberts et al. [12, 14] 5, 10, 20, and 30 min after ligation of the arteries. Weighed samples of tissues from the contralateral region served as the control. The volume velocity of the local blood flow in the cerebral cortex of the cats was recorded quantitatively by the hydrogen clearance method [9].

EXPERIMENTAL RESULTS

The results obtained in albino rats after ligation of the common carotid artery showed a marked increase in the GABA concentration (Table 1).

Slight changes in the GABA concentration and GD activity were observed as early as 3 and 5 min after ligation. The GABA-T activity was depressed but the changes compared with the control were not statistically significant. Consequently, changes in the GABA level were due mainly to an increase in GD activity.

After 10 min the GABA level in the cortex had risen from 11.2 ± 0.36 to 15.4 ± 0.42 mg%, i.e., by 37.5%, whereas in the hypothalamus it had risen from 15.8 ± 0.52 to 18.7 ± 0.29 mg%, i.e., by 18.3%. The GABA content in the cortex 20 min after ligation was increased by 67.8% and in the hypothalamus by 56.3%.

The quantitative changes in GABA are inseparately bound up with changes in the activity of the enzymes responsible for its biosynthesis and breakdown. Table 1 shows that 10 min after ligation of the common carotid

Department of Pharmacology, Erevan Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 1, pp. 45-48, January, 1978. Original article submitted May 13, 1977.

 0007-4888/78/8501-0050$07.50

TABLE 1. Effect of Unilateral Ligation of Common Carotid Artery in Rats on Content of GABA and Activity of Enzymes of Its Metabolism in the Brain (M ± m)

Experimental conditions and times of taking samples	Number of experiments	Cortex			Hypothalamus		
		GABA	GD	GABA-T	GABA	GD	GABA-T
Fixed control	10	10,8±0,25	168±12,6	1183±17,4	14,9±0,47	186±11,3	1213±14,6
Incubated control	10	11,2±0,36	204±14,6	1337±19,4	15,8±0,52	218±10,6	1344±19,7
10 min after ligation	15	15,4±0,42*	290±13,7*	1280±16,8	18,7±0,29*	267±11,8*	1318±16,8
20 min after ligation	12	18,8±0,69*	316±14,4*	1254±17,9	24,7±1,2*	297±10,4*	1280±17,4

Legend. Here and in Tables 2 and 3: GABA content expressed in mg%; GD activity in μg GABA formed per gram brain tissue during incubation for 60 min; GABA-T activity in μg glutamic acid formed per gram tissue in 60 min. Fixed control – tissues boiled without incubation; incubated control – tissues incubated for 60 min with addition of essential components in accordance with method described. Statistically significant change compared with incubated control marked by asterisk.

TABLE 2. Effect of Unilateral Ligation of Common Carotid and Vertebral Arteries in Dogs on Content of GABA and Activity of Enzymes of Its Metabolism in the Brain and Tissues of the Walls of Its Arteries (M ± m)

Experimental conditions and times of taking samples	Number of expts.	Cortex			Hypothalamus			Cerebral vessels		
		GABA	GD	GABA-T	GABA	GD	GABA-T	GABA	GD	GABA-T
Fixed control	8	17,8±0,21	263±18	1156±48	26,4±0,27	332±29	1250±46	2,5±0,1	26,5±2,7	914±30
Incubated control	8	18,0±0,23	337±31	1408±38	28,7±0,25	528±43	1558±52	2,6±0,12	48,2±2,7	1102±25
10 min after ligation	6	22,6±0,3*	378±34	1280±36	32,1±0,43*	558±39	1315±58*	3,2±0,13*	60,5±2,8	1030±23
30 min after ligation	6	29,4±0,43*	480±39*	1090±44*	42,2±0,53*	668±44*	1195±46*	5,1±0,27*	80,5±3,7*	848±24*

artery in rats GD activity was significantly increased in the cortex and hypothalamus, and it was increased still more after 20 min.

To determine GABA and the enzymes of its synthesis not only in brain tissues, but also in the walls of the cerebral vessels, experiments were carried out on dogs since the necessary quantity of tissues of the vessel walls required by the method used could be obtained only from large animals (Table 2).

The GABA content in the cortex 10 min after ligation of the carotid and vertebral arteries was increased significantly by 25.5% and in the hypothalamus by 11.8%. This increase was more marked still after 30 min. GD activity in the cortex and hypothalamus 30 min after ligation of the main vessels reached exceptionally high values, whereas GABA-T activity was depressed. It will be noted that as a result of ligation the content of GABA and the enzymes of its metabolism in the tissues of the cerebral arterial walls also was appreciably changed. After 10 min of interruption of the blood flow the GABA level was significantly increased by 23%, GD activity was increased by 25.5%, and GABA-T activity reduced by 6.5%. The considerable rise in GD activity evidently depended directly on the accumulation of acid intermediate products of metabolism, caused by the hypoxia, in the tissues of the brain [2, 8] and of its arteries, leading to a marked increase in the GABA concentration in those tissues. These essentially opposite changes in GABA biosynthesis and metabolism in the animals with disturbances of the circulation in the brain nevertheless gave a similar result: a marked increase in the level of endogenous GABA in the brain and in the tissues of the walls of its arteries. It seemed reasonable to suppose that GABA participates in the stimulation of the collateral circulation and so facilitates compensation of the disturbed cerebral hemodynamics. This hypothesis is also supported by the results of experiments [7] in which GABA was found to increase the number of functioning capillaries after ligation of the main vessels.

TABLE 3. Quantitative Measurements of Local Cerebral Blood Flow in Cats after Unilateral Ligation of Common Carotid and Vertebral Arteries and after Preliminary Injection of 5 mg/kg Aminohydroxyacetic Acid (M ± m)

Experimental conditions	Mean blood pressure, mm Hg	Cerebral blood flow, ml/100 g/min			
		1st minute	5th minute	10th minute	20th minute
Control	110±9,8	34,2±4,1	34,2±3,7	34,2±4,2	34,2±3,9
Ligation	115±11,5	13,3±2,7	14,6±5,2	16,3±4,9	18,2±5,4
Control	112±8,6	37,5±4,6	37,2±3,6	37,5±5,8	37,5±6,4
AHAA + ligation	116±7,9	2,21±5,7	22,7±5,4	23,4±6,2	23,7±5,3

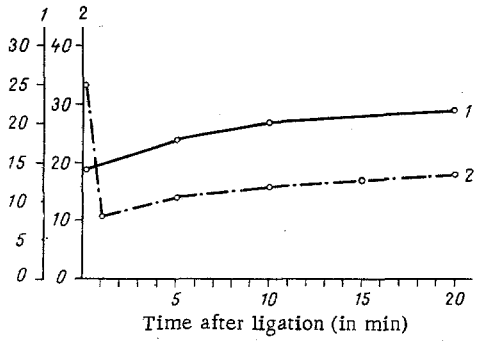

Fig. 1. Quantitative local blood flow and GABA content in cerebral cortex of cats on side of ligation: 1) GABA content (in mg%); 2) velocity of local blood flow (in ml/100 g/min).

Immediately after ligation of the artery the blood supply to the brain was reduced by 61.2%, and by the 5th minute the decrease was 57.3% (Table 3). Later, the deficiency of the blood supply was appreciably reduced. The volume velocity of the cortical blood flow 10 min after ligation of the artery was reduced by 52.4%, and 20 min after ligation by 46.8%. This means that the appearance of a deficiency of the cerebral blood supply was accompanied by an increase in the GABA contentration in the brain, and the increase in the GABA level led to an improvement of the circulation in the brain (Fig. 1). The increase in the content of endogenous GABA during hypoxia evidently facilitated the activation of arterial anastomoses which were not previously functioning, and in this way the collateral inflow of blood into the ischemic region of the brain was stimulated. The mechanism examined above may be an example of a self-regulating system, operating by the feedback principle and ensuring adaptation of the cerebral hemodynamics to constantly changing conditions.

Participation of the GABA system in the mechanisms of the onset and development of compensation of the disturbed cerebral hemodynamics was confirmed by direct experiments in which the endogenous GABA level in the brain was initially raised by means of aminohydroxyacetic acid (AHAA, Table 3). After injection of AHAA (5 mg/kg) the blood supply of the cerebral cortex 1 min after ligation of the arteries was reduced by 41.1%, whereas in cats not receiving the GABA-T inhibitor, as stated above, it was reduced by 61.2%. The fact will be obvious that AHAA was particularly effective 1 min after ligation of the arteries. It must be remembered that the inhibitor was injected into the animals 1.5 h before ligation of the arteries (i.e., at the time most favorable for endogenous accumulation of GABA in the brain and for the manifestation of its action on the cerebral vessels) and that the animal was more prepared to withstand the marked deficiency of the blood supply to the brain caused by ligation of the main vessels.

To sum up the results of these experiments it can thus be concluded that the GABA system is one of the specific chemical components of the mechanism for compensation of the disturbed cerebral hemodynamics by stimulation of the collateral circulation.

LITERATURE CITED

1. V. V. Zakusov, The Pharmacology of Central Synapses [in Russian], Moscow (1973), p. 16.
2. M. N. Maslova and V. I. Rozengart, in: Collected Proceedings of the 3rd All-Union Conference on Biochemistry of the Nervous System [in Russian], Erevan (1963), p. 153.

3. S. A. Mirzoyan and V. P. Akopyan, in: The Role of γ-Aminobutyric Acid in the Activity of the Nervous System [in Russian], Leningrad (1964), p. 44.
4. S. A. Mirzoyan and V. P. Akopyan, Farmakol. Toksikol., No. 5, 572 (1967).
5. S. A. Mirzoyan, V. P. Akopyan, and B. A. Kazaryan, Dokl. Akad. Nauk SSSR, 186, 231 (1969).
6. S. A. Mirzoyan, V. P. Akopyan, and B. A. Kazaryan, Dokl. Akad. Nauk SSSR, 190, 1241 (1970).
7. S. A. Mirzoyan and V. P. Akopyan, in: The Physiology and Biochemistry of Mediator Processes [in Russian], Moscow (1976), p. 92.
8. S. S. Musaelyan, in: The Nervous System [in Russian], Vol. 3, Leningrad (1962), p. 17.
9. K. Aukland, B. F. Bower, and R. W. Berliner, Circulat. Res., 14, 164 (1964).
10. G. G. L. Collins, Brain Res., 66, 127 (1974).
11. M. Fujiwara and I. Muramatsu, Br. J. Pharmacol., 55, 561 (1975).
12. E. Roberts and S. Frankel, J. Biol. Chem., 188, 789 (1951).
13. F. Schon and G. S. Kelly, Brain Res., 66, 275 (1974).
14. A. Waksman, M. K. Rubinstein, K. Kuriyama, et al., J. Neurochem., 15, 351 (1968).

EFFECT OF CHOLINOLYTIC AND ADRENOBLOCKING AGENTS ON RESISTANCE OF RAT ERYTHROCYTES TO HYPOOSMOTIC HEMOLYSIS

A. N. Petrov

UDC 612.111.32:612.118.221.3].014.46:615.217.24

The effect of central cholinolytics and adrenoblockers on hemolysis of rat erythrocytes in hypoosmotic buffer was studied in vitro. At pH 7.4 and in a concentration of 10^{-4} M, hemolysis was prevented to the greatest degree by the central nicotinic (n) cholinolytics ethyldiphacil, diphacil[1], pediphen[2], tropacin[3], and the β-adrenoblocker propranolol. Erythrocytes were protected against hemolysis to a lesser degree by the central muscarinic (m) cholinolytics amizil[4] and glypin[5] and the α-adrenoblockers pyrroxan[6], sympatholytin[7], and phentolamine. The antihemolytic effect of the drugs reached a maximum in the course of 30 min and continued for several hours. A lower level of ionization of the drugs containing a tertiary nitrogen atom in their molecule was shown to correspond to greater protection of the erythrocytes against hemolysis. The prevention of hypoosmotic hemolysis is evidence of stabilization of the erythrocyte membrane by the substances studied. The possibility of stabilization of membrane formations not containing synaptic contacts must be taken into account when considering the mechanism of action of central n-cholinolytics and β-adrenoblockers.

KEY WORDS: central cholinolytics; adrenoblockers; erythrocytes; membranes; stabilization.

Evidence has now been obtained to show that not all effects of cholinolytics and adrenoblockers can be explained entirely by blockage of the postsynaptic receptors of the corresponding mediator system [1, 3, 6]. It is accordingly interesting to study interaction between such compounds and biological membranes not containing synaptic contacts. Erythrocytes are a widely used model for the study of the action of various compounds on membranes [5].

[1]Adiphenine; [2]1,1-diphenyl-5-diethylaminopentane; [3]2,3-dihydro-3-hydroxy-8-methylnortropidine diphenylacetate hydrochloride; [4]Benactyzine; [5]Unidentified; [6]Central α-adrenoblocker of USSR origin; [7]Dibenamine.

(Presented by Academician of the Academy of Medical Sciences of the USSR S. N. Golikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 1, pp. 48-51, January, 1978. Original article submitted April 27, 1977.

