PHARMACOLOGIC ANALYSIS OF THE ROLE OF GABA- AND OPIOIDERGIC SYSTEMS IN THE DEVELOPMENT OF CEREBRAL EDEMA

V. E. Novikov, V. V. Yasnetsov, and A. N. Sharov UDC 616.831-005.98-02:615.31:547.466.3]-07

KEY WORDS: cerebral edema; naloxone; GABA-negative drugs

The writers showed previously [8] that the use of GABA-positive drugs prevents the development of cerebral edema (CE) in animals. This fact is evidence in support of a role of the GABA-ergic system in the formation of CE. We know that the GABA and opioid systems are quite closely interconnected both morphologically and functionally [2]. Both systems also perform a stress-limiting function in vivo [4], and this may be of definite importance in the pathogenesis of CE. However, no investigations that would shed light on the character of interaction of the GABA system with opioidergic processes in the formation of CE have yet been undertaken.

The aim of this investigation was to study the effect of GABA-negative substances and of the universal opioid receptor blocker naloxone on the development of CE.

EXPERIMENTAL METHOD

Experiments were carried out on 95 noninbred albino rats of both sexes weighing 150-220 g. The animals were anesthetized with ether and the skull trephined in the region of the left parietal bone. This manipulation is an invariable part of the creation of several models of CE [6]. The following pharmacologic analyzers were injected intraperitoneally into the rats 24 h after the trephining operation: GABA-negative agents (bicuculline, picrotoxin, thiosemicarbazide) and naloxone. The animals were decapitated 1 h after injection of the drugs. The total water content and the tissue density were determined in brain tissue from the left parietal lobe [5].

Mitochondria were isolated from the brain by high-speed centrifugation and were used for polarographic study of oxidative phosphorylation [7]. The oxidation substrate was glutamic acid (3 mM). The following parameters were calculated from the polarographic results: the rate of mitochondrial respiration in different metabolic states (V_0, V_3, V_4), the rate of uncoupled respiration (V_{DNP}) in ng-atoms oxygen/min·mg protein; the respiratory controls according to Lardy and Wellman (RC_L) and after Chance and Williams (RC_C); the ratio ADP/O; stimulation of respiration by the uncoupler 2,4-dinitrophenol (DNP); the rate of phosphorylation of the ADP additive (ADP/t), in nanomoles ADP/min·mg protein. Protein was determined by Lowry's method [10]. The significance of the results was assessed by Student's t test [3].

EXPERIMENTAL RESULTS

The brain tissue of intact rats contained 77.75 ± 0.14% of water and the density of the brain was 1.0412 ± 0.0002 g/cm^3.

Medical Institute, Smolensk. (Presented by Academician of the Russian Academy of Medical Sciences P. V. Sergeev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 8, pp. 163-165, August, 1992. Original article submitted January 16, 1992.

 0007-4888/92/0008-1132$12.50

TABLE 1. Effect of GABA-Negative Substances and Naloxone on Water Content and Density of Brain Tissue (M ± m)

Groups of animals	Total water, %	Density of brain, g/cm ^{3}
Control (n = 13)	77,75±0,14	1,0412±0,0002
Bicuculline, 0.8 mg/kg (n = 8)	77,46±0,48	1,0413±0,0003
Bicuculline, 1 mg/kg (n = 8)	77,43±0,15	1,0414±0,0002
Bicuculline, 2 mg/kg (n = 6)	77,38±0,34	1,0417±0,0002
Bicuculline, 3 mg/kg (n = 6)	78,65±0,42*	1,0403±0,0002*
Bicuculline, 4 mg/kg (n = 7)	78,74±0,59*	1,0403±0,0003*
Thiosemicarbazide, 15 mg/kg (n = 14)	78,44±0,18*	1,0400±0,0002*
Picrotoxin, 1 mg/kg (n = 7)	77,93±0,55	1,0410±0,0002
Naloxone, 2 mg/kg (n = 6)	78,34±0,46	1,0408±0,0003
Naloxone, 4 mg/kg (n = 6)	78,42±0,40	1,0407±0,0003
Bicuculline, 2 mg/kg + naloxone, 2 mg/kg (n = 10)	78,88±0,32*	1,0384±0,0002*
Picrotoxin, 1 mg/kg + naloxone 2 mg/kg (n = 8)	78,57±0,28*	1,0406±0,0003

Legend. Here and in Table 2, asterisk indicates values for which $p < 0.05$.

TABLE 2. Effect of GABA-Negative Substances and Naloxone on Oxidative Phosphorylation in Brain Mitochondria (M ± m)

Series of experiments	V_o	V_3	V_4	V_{DNP}	RC_L	RC_C	ADP/O	DNP	ADP/t
Control (n=10)	26,88 ±2,36	76,51 ±5,28	38,73 ±3,16	97,40 ±9,34	3,02 ±0,31	2,05 ±0,17	2,48 ±0,13	2,58 ±0,22	193,71 ±20,59
Thiosemicarbazide, 15 mg/kg (n=8)	20,93 ±1,10*	74,70 ±3,07	28,05 ±1,52*	94,22 ±4,41	3,60 ±0,12	2,69 ±0,09*	3,16 ±0,12*	3,37 ±0,08*	240,72 ±11,64
Bicuculline, 2 mg/kg (n=5)	32,23 ±3,64	122,72 ±10,03*	54,30 ±7,37*	132,11 ±10,28*	3,89 ±0,20	2,34 ±0,16	2,13 ±0,28	2,54 ±0,22	253,82 ±15,01*
Bicuculline, 2 mg/kg + naloxone, 2 mg/kg (n=9)	24,25 ±0,50	78,21 ±2,68	33,05 ±3,49	101,52 ±3,18	3,23 ±0,11	2,58 ±0,29	2,23 ±0,12	3,33 ±0,34	173,50 ±10,97
Picrotoxin, 1 mg/kg + naloxone 2 mg/kg (n=8)	34,35 +4,85	88,50 +4,53	37,07 ±4,56	115,24 ±7,08	2,92 ±0,42	2,67 ±0,39	2,37 ±0,10	3,54 ±0,57	212,58 ±15,93

Bicuculline in doses of 0.8, 1, and 2 mg/kg did not change these parameters, but if the dose was increased to 3 and 4 mg/kg an increase in the total water content and a decrease in the density of the brain tissue were observed (Table 1). After injection of bicuculline in a dose of 4 mg/kg the animals developed seizures, their activity was sharply inhibited, and external respiration disturbed; autopsy showed congestion of the cerebral vessels and edema of the brain tissue.

Picrotoxin in a subconvulsant dose of 1 mg/kg did not change the water content or brain density. In a dose of 2 mg/kg it led to the development of seizures and to death of the animals. At autopsy the brain showed signs of edema and the total water content was increased. Thiosemicarbazide, in a dose of 15 mg/kg, increased the total water content and reduced the brain density (Table 1). In this case the animals were inhibited and their motor activity reduced. Clonicotonic convulsions developed 65-70 min after injection of the drug. At autopsy the brain was wet and edematous.

Naloxone in doses of 2 and 4 mg/kg caused a tendency for the total water content to rise (the changes were not significant).

A combination of a subconvulsant dose of bicuculline (2 mg/kg) with naloxone in a dose of 2 mg/kg caused inhibition of the animals, and when touched they displayed aggressiveness (each substance separately, in the above-mentioned dose, did not cause any such changes). Marked CE was observed in the rats.

A combination of picrotoxin in a dose of 1 mg/kg + naloxone in a dose of 2 mg/kg led to the appearance of tremor of the skeletal muscles and inertia of the animals. The water content in the brain tissue increased and a tendency was noted for density to fall (Table 1).

The results show that GABA-negative substances in convulsant doses cause CE; naloxone, moreover, can potentiate the edema-generating action of bicuculline and picrotoxin, in subconvulsant doses, on brain tissue. This effect of naloxone may perhaps be partly connected with its GABA-negative properties [9].

Considering the important role of changes in carbohydrate and energy metabolism in the development of CE, the state of oxidative phosphorylation was studied in the brain mitochondria against a background of the action of the drugs mentioned above.

It will be clear from Table 2 that under the influence of thiosemicarbazide both the initial rate of substrate oxidation (V_0) and the rate of its oxidation after phosphorylation (V_4), i.e., the rate of nonphosphorylating oxidation, fell. The rates of phosphorylating (V_3) and uncoupled (V_{DNP}) oxidation showed no significant change.

Bicuculline in a dose of 2 mg/kg stimulated the respiration velocity (attention is particularly directed to the increase in the rate of phosphorylating oxidation V_3). The coupling parameters remained at the control level. As a result the rate of phosphorylation ADP/t increased, i.e., in unit time the mitochondria were able to synthesize more ATP. This increase in oxidative activity in the brain mitochondria under the influence of bicuculline can probably be explained by the attempt of the mitochondria to compensate the increased energy demands of the brain tissue as a result of predominance of excitation over inhibition (due to the action of GABA-receptor antagonists).

Naloxone abolished the stimulating effect of bicuculline on oxidative processes in the brain mitochondria (Table 2). Similar results were obtained with a combination of another GABA antagonist, picrotoxin, with naloxone.

The antagonism described above of naloxone, which by itself does not significantly change the level of oxidative processes [1], relative to the effects of bicuculline and picrotoxin is difficult to explain at the present time.

The results of this investigation, together with those published previously [8], are thus pharmacologic proof of the participation of the GABA-ergic system in the pathogenesis of CE. In all probability, endogenous opioids, which play a modulating role in relation to GABA, are definitely involved in this process, for naloxone potentiates the edema-generating action of GABA antagonists.

REFERENCES

1. N. N. Ardentova, K. I. Bender, E. V. Panchenko, et al., Farmakol. Toksikol., No. 5, 554 (1981).
2. I. P. Ashmarin and M. A. Kamenskaya, Neuropeptides in Synaptic Transmission [in Russian], Moscow (1988).
3. V. A. Kokunin, Ukr. Biokhim. Zh., No. 6, 776 (1975).
4. F. Z. Meerson and M. G. Pshennikova, Adaptation to Stress Situations and to Physical Exercise [in Russian], Moscow (1988).
5. V. E. Novikov, Abstract No. 2662-V90 lodged with the All-Union Institute of Scientific and Technical Information (1990).
6. I. A. Platonov and V. S. Yasnetsov, Farmakol. Toksikol., No. 5, 565 (1981).
7. A. N. Sharov and N. B. Kozlov, Neirokhimiya, No. 3, 269 (1982).
8. V. S. Yasnetsov and V. E. Novikov, Byull. Éksp. Biol. Med., No. 1, 67 (1985).
9. R. Dingledine, L. L. Iversen, and E. Breuker, Eur. J. Pharmacol., **47**, 19 (1978).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., **193**, No. 1 265 (1951).