COMPARATIVE STUDY OF SOME DRUGS ON MODELS OF CEREBRAL HYPOXIA

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UDC.613.2 4.3:547.745+615.
547.473.2].036.8:616.831

KEY WORDS: cerebral hypoxia; gutimin; pyracetam; sodium hydroxybutyrate; lithium hydroxybutyrate.

The treatment of cerebral hypoxia is one of the most important problems in modern medicine. There are various approaches to the correction of hypoxic states of the brain. However, the prevalence of diseases accompanied by cerebral hypoxia compels a search for new and pathogenetically based methods of treatment of such states.

Drugs capable of considerably alleviating the harmful action of hypoxia on brain tissue and participating in the most intimate mechanisms of cell metabolism are particularly interesting. Representatives of this group of compounds include gutimin (guanylthiourea), pyracetam [a Soviet GABA analog], and sodium and lithium hydroxybutyrate which, in various tests and with different technical approaches, possess marked antihypoxic properties [1-4]. Of these four substances lithium hydroxybutyrate has been studied the least.

This paper describes a comparative study of these drugs on resistance of the brain in rats with various forms of cerebral hypoxia.

EXPERIMENTAL METHOD

The antihypoxic effect of the drugs was studied on models of asphyctic, ischemic, and anemic cerebral hypoxia.

The asphyctic form of hypoxia was produced by the method in [5]. Under ether anesthesia nichrome electrodes were inserted into the sensomotor cortex of noninbred male rats weighing 150-180 g. The following day the animals were immobilized by intravenous injection of succinylcholine in a dose of 10 mg/kg and, after tracheotomy, were connected to an artificial respiration apparatus ("Ugo Basile," Italy). Asphyctic hypoxia (anoxia) was induced by disconnecting the artificial respiration apparatus repeatedly for 90, 120, 150, and 180 sec at intervals of 10 min. The electrocorticogram (ECoG) and the ECG were recorded throughout this experiment. The time after disconnection of the apparatus until disappearance of the ECoG, the time to resumption of the ECoG after the beginning of reventilation, the total time of electrical silence of the cortex and the time until appearance of bradycardia, evidence of myocardial hypoxia, were measured. Ischemic hypoxia was induced by bilateral ligation of the carotid arteries under ether anesthesia. The antihypoxic effect of the drugs was assessed from the number of animals surviving 24 h after the operation. Anemic hypoxia was induced by intraperitoneal injection of sodium nitrite in a dose of 200 mg/kg.

All the drugs were injected intraperitoneally 1 h before the beginning of the experiment in the following doses: gutimin 100 mg/kg, pyracetam 1000 mg/kg, and sodium and lithium hydroxybutyrates 50, 100, and 250 mg/kg in asphycitic hypoxia, 50, 100, 250, and 500 mg/kg in ischemic hypoxia, and 250 and 500 mg/kg in anemic hypoxia.

EXPERIMENTAL RESULTS

In the overwhelming majority of animals the first 90-sec period of anoxia led to disappearance of the ECoG after 45.4 ± 3.8 sec on average. Only in 8.3% of rats of the control group did the first exposure to anoxia not cause the appearance of an isoelectric ECoG.

Department of Pharmacology of Adaptation, Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 11, pp. 567-570, November, 1984. Original article submitted February 7, 1984.

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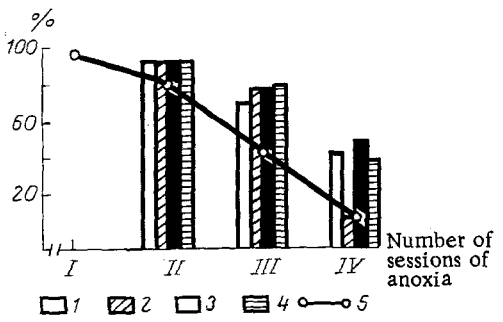

Fig. 1

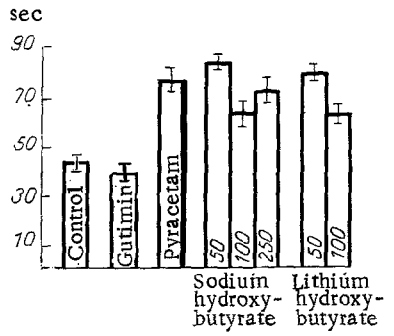

Fig. 2

Fig. 1. Effect of drugs on time of disappearance of ECoG after anoxia for 90 sec. I-IV) Number of sessions of anoxia. 1) Gutimin; 2) pyracetam; 3) lithium hydroxybutyrate; 4) control.

Fig. 2. Effect of drugs on number of animals in which cortical function was restored after exposure to anoxia (in %). Legend as in Fig. 1.

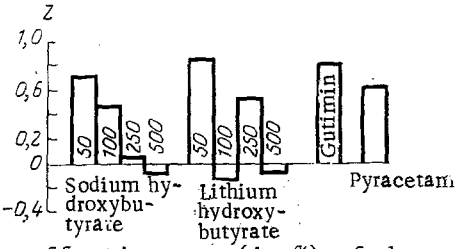

Fig. 3. Relative effectiveness (in %) of drugs in acute cerebral ischemia caused by bilateral ligation of the carotid arteries, calculated by the equation: $E = D - C/1 - C$, where D denotes the fraction of surviving animals receiving the drug, C the fraction of surviving animals in the control group 24 h after the operation. Legend as in Fig. 1.

All the drugs tested (except gutimin) delayed disappearance of the ECoG. Sodium hydroxybutyrate was most active in this respect: In a dose of 50 mg/kg it increased the number of animals whose ECoG did not disappear after the first session of anoxia to 60% (Fig. 1). With an increase in the number of sessions of anoxia, the number of animals of the control group in which cortical function was restored after resumption of respiration decreased progressively, to 51.4% after the third session, but only 17.1% after the fourth session of anoxia. The mean total duration of cortical electrical silence in the control animals after the fourth session of anoxia, lasting 180 sec, was 163.9 ± 40.6 sec.

Preliminary injection of the drugs increased the ability of the brain to resume its functional activity after hypoxia in virtually all cases (Fig. 2). After the fourth session of anoxia, the ECoG was restored in 50% of the animals under the influence of gutimin and lithium hydroxybutyrate, but in 60% of animals under the influence of sodium hydroxybutyrate.

The principal parameters used to assess the antihypoxic effect of the drugs are listed in Table 1. Virtually all the drugs tested increased the resistance of the rats' brain to hypoxia to some degree or other, delayed the time of disappearance of the ECoG after the beginning of anoxia, and considerably reduced the time before resumption of electrical activity and the total duration of cortical electrical silence. The least active drug in this respect was gutimin, the most active sodium hydroxybutyrate, especially in a small dose (50 mg/kg), which had a considerable antihypoxic effect, until the very last 180-sec session of anoxia. Lithium hydroxybutyrate (100 mg/kg) also had a long-lasting antihypoxic action. Pyracetam had a beneficial effect mainly during the first two sessions of anoxia.

Under the influence of all the drugs studied the total duration of absence of the ECoG was considerably reduced, especially during the first and second sessions of anoxia, suggest-

TABLE 1. Basic Parameters (in sec) for Estimation of Antihypoxic Activity of Drugs (M ± m)

Experimental conditions	Duration of anoxia, sec							
	90				120			
	I	II	III	IV	I	II	III	IV
Control (n=35)	45,4±3,8	22,4±2,5	66,6±6,0	19,9±2,3	48,6±3,3	45,3±3,5	110,5±7,0	20,1±2,4
Gutimin (n=10)	32,3±1,8	20,9±1,4	75,8±2,3	14,2±2,6	43,2±5,1	30,9±4,4	92,9±9,9	20,2±3,9
Pyracetam (n=9)	76,6±4,8***	5,3±0,2***	17,8±4,9***	15,1±3,2	85,8±7,3***	21,8±6,9**	53,3± ±12,0***	15,1±1,6
Sodium hydroxybutyrate:								
50 mg/kg (n=10)	84,9±3,0***	3,3±1,9***	8,1±0,8***	32,5±4,4*	94,7± ±10,1***	9,1±3,7***	19,2± ±10,3***	25,0±4,8
100 mg/kg (n=9)	64,0±4,6***	19,1±6,3	25,9±3,9*	17,3±4,5	59,8±3,5***	49,4±13,1	106,1±15,5	15,4±4,6
250 mg/kg (n=6)	73,7±5,8***	5,7±2,5***	21,6±4,3***	17,6±4,8	78,5± ±14,4*	73,7±33,4	118,6±49,9	20,7±7,0
Lithium hydroxybutyrate								
100 mg/kg (n=6)	63,6±3,3***	18,5±3,6*	44,5±7,0**	13,6±1,8	60,8±4,1**	28,3±3,8**	73,3±4,0***	19,1±4,0
50 mg/kg (n=8)	79,4±4,4***	5,3±1,9***	15,9±6,1***	10,8±2,2*	73,6±4,5***	15,6±1,7***	61,3±5,9**	11,3±2,1**

Experimental conditions	Duration of anoxia, sec							
	150				180			
	I	II	III	IV	I	II	III	IV
Control (n=35)	60,1±3,6	57,3±12,6	147,9±15,6	20,3±3,1	76,4±76,4	—	—	26,7±9,2
Gutimin (n=10)	58,2±8,6	46,2±11,1	128,9±19,9	24,6±1,5	82,7±7,6	52,9±8,5	138,7±16,0	31,4±5,4
Pyracetam (n=9)	95,0±16,3*	67,6±42,1	131,2±53,8	23,2±4,2	79,4±23,8	—	—	26,2±5,8
Sodium hydroxybutyrate:								
50 mg/kg (n=10)	97,9±16,0*	11,1±4,1**	40,2±16,4*	34,8±10,6	146,0± ±24,6**	—	—	60,3±23,1
100 mg/kg (n=9)	62,1±8,7	207,3± ±41,3**	281,0± ±52,4**	21,1±3,3	60,6±13,9	—	—	40,0±6,7
250 mg/kg n=6)	53,9±18,1	75,5±42,4	158,9±50,6	20,5±6,7	80,3±6,7	—	—	28,7±7,0
Lithium hydroxybutyrate								
100 mg/kg (n=6)	98,5±8,9***	74,3±16,8	116,1±21,3	31,5±6,2	95,3±2,7***	—	—	43,2±
50 mg/kg (n=8)	60,2±4,6	60,5±1,9	140,1±4,4	12,0±2,5	68,5±2,6	94,0±8,3	203,4±11,9	16,3±3,1

Legend. I) Time from stopping artificial respiration apparatus until disappearance of ECoG, II) time from beginning of reventilation until restoration of ECoG, III) total duration of cortical electrical silence, IV) time from beginning of bradycardia. *P < 0.05, **P < 0.01, ***P < 0.001.

ing increased ability of the brain to resume electrical activity after exposure to hypoxia. However, this parameter can be used to judge the powers of recovery of the brain from another standpoint. We know that absence of an oxygen supply to brain tissue for 4-5 min leads to the appearance of irreversible changes in it. For that reason, restoration of brain function after a longer time interval than in the control can provide a good criterion of the antihypoxic activity of a drug. In the present experiments the longest individual time of cortical electrical silence under the influence of anoxia, after which recovery of the ECoG was observed, was 223.2 sec (3.7 min) in the control animals. Under the influence of sodium hydroxybutyrate, in some cases brain electrical activity was restored after longer intervals of

cortical silence. The longest period of electrical silence observed in animals receiving sodium hydroxybutyrate (250 mg/kg) was 504 sec (8.3 min). In another version of the experiment, in which the animals were subjected to a single interruption of oxygen supply for 4 min, the ECoG was not restored in the control animals during the 10 min of observation after the end of anoxia, whereas sodium hydroxybutyrate (250 mg/kg) restored brain function after anoxia for 4 min in all animals. The total duration of cortical electrical silence in this case averaged 228.7 ± 124.7 sec and its maximal duration was 470.7 sec.

None of the drugs tested had any significant effect on the time until the beginning of bradycardia after interruption of the oxygen supply.

In the control group of animals with ischemic hypoxia the survival rate after 24 h was 28%. Sodium hydroxybutyrate considerably increased the number of animals which survived this form of hypoxia; the smaller dose of this compound, moreover, was more effective. Sodium hydroxybutyrate and lithium hydroxybutyrate were a little more effective than gutimin and pyracetam (Fig. 3).

Sodium hydroxybutyrate in doses of 250 and 500 mg/kg increased the length of survival of rats exposed to anemic hypoxia by more than 1.5 times. Lithium hydroxybutyrate in the same doses was less effective, for it prolonged the animals' life by 18.8 and 21.3%, respectively.

Salts of gamma-hydroxybutyric acid (GHBA) were thus the most active of the antihypoxants tested on a model of asphyctic hypoxia. The antihypoxic action of gutimin and pyracetam was weaker and was exhibited mainly in the early stages of this form of hypoxia.

The two GHBA salts had a considerable antihypoxic action in the ischemic form of hypoxia: Lithium hydroxybutyrate in a dose of 50 mg/kg was a little more active than sodium hydroxybutyrate. Smaller doses of both drugs were more effective and had a significantly stronger antihypoxic action than pyracetam.

LITERATURE CITED

1. A. P. Arendarug, A. P. Skoldinov, V. V. Zakusov, et al., Author's Certificate No. 552094 (USSR).
2. V. M. Vinogradov, L. V. Pastushenkov, and É. N. Sumina, Patol. Fiziol., No. 4, 81 (1981).
3. M. D. Mashkovskii, L. F. Roshchina, A. I. Polzehaeva, et al., Khim.-farm. Zh., No. 8, 132 (1977).
4. R. U. Ostrovskaya, "Neuropharmacology of the gamma-aminobutyric acid shunt," Author's Abstract of Doctoral Dissertation, Moscow (1977).
5. J. van den Driesche, P. Lacroix, P. Linée, et al., Arch. Int. Pharmacodyn., 239, 62 (1979).