EFFECT OF γ-AMINOBUTYRIC ACID AND ITS DERIVATIVES ON RESISTANCE OF ANIMALS TO ANOXIA

S. V. Osipova, N. V. Uskova, and R. A. Khaunina

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γ-Hydroxybutyric acid and β-phenyl-γ-aminobutyric acid, derivatives of γ-aminobutyric acid, increase the resistance of mice and rats to acute anoxia caused by a fall of atmospheric pressure. Neither γ-aminobutyric acid itself nor its derivative β-hydroxy-γ-aminobutyric acid possess this effect.

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When the organism is exposed to certain harmful agents (hypoxia [1], irradiation and overloading [8]), the concentration of γ-aminobutyric acid (GABA) in the brain of laboratory animals is increased. γ-Hydroxybutyric acid (GHBA), possessing tranquilizing activity [7, 9, 12, 14, 15], closely related in its chemical structure to GABA, is used in surgery and obstetrics for premedication and narcosis [4, 14, 16, 17]. It has been observed that GHBA does not lower the oxygen demand during narcosis, a feature distinguishing it advantageously from other narcotics [14].

Since under the conditions listed above, both clinical and experimental, anoxia may be one of the most injurious factors encountered, in the present investigation the effect of several different derivatives of GABA on the resistance of animals to anoxia caused by a fall of atmospheric pressure was compared. Besides GABA, the action of its hydroxy-derivative β-hydroxy-GABA (BHGABA) was investigated. This later compound, like GABA, is a natural product of brain metabolism, although more active than GABA [13]. The synthetic derivatives GHBA and β-phenyl-GABA (BPGABA) were also studied. The latter compound was synthesized at the Department of Organic Chemistry, A. I. Gertsen Pedagogic Institute. Pharmacological investigation of BPGABA [5, 10, 11] has shown that it belongs to the group of minor tranquilizers. The action of these substances was compared with the influence of other substances with depressant properties on resistance to anoxia: chlorpromazine, Nembutal, chloral hydrate, and hemithiamine – a thiamine derivative possessing a sedative action [2].

EXPERIMENTAL METHOD

Experiments were carried out on adult mice of both sexes, weighing 20-25 g, and on rats of both sexes, weighing 110-120 g. The substances for testing were injected intraperitoneally in aqueous solutions at various times before the animals were placed under a bell jar connected to a vacuum pump. Rarefaction continued until 200 mm (10,000 m above sea level) in the experiments on mice and to 130-150 mm (12,000-13,000 m above sea level) in the experiments on rats, over a period of 40 sec; the duration of exposure was 10 min.

The efficacy of the action on resistance to anoxia was assessed by: 1) the time of onset of convulsions, 2) the number of animals developing convulsions, 3) the period of survival under the bell jar, and 4) the number of animals dying in the course of 10 min in the chamber.

Before the animals were placed under the jar their motor activity was recorded. The effect of most of the tested compounds on body temperature was studied in parallel experiments on intact animals. The data were analyzed by statistical methods.

EXPERIMENTAL RESULTS

According to all indices, neither GABA nor BHGABA in a dose of 1000 mg/kg increased the resistance of the mice to anoxia (Table 1).

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TABLE 1. Effect of GABA, BHGABA, BPGABA, and GHBA on Resistance on Mice to Anoxia Caused by Lowering Atmospheric Pressure (Compounds Injected 30 min before Beginning of Rarefaction)

Compound tested	Dose in mg/kg	Dose in % of LD_{50}	No. of mice	Effect: Time of onset of convulsions (in min)	No. of mice with convulsions abs.	No. of mice with convulsions %	Length of survival (in min)	No. of mice dying abs.	No. of mice dying %
Control	—	—	22	2,5±0,43	19	86,4	3,6±0,42	17	77,3
GABA	1000	20	12	1,8±0,47	11	91,6	2,8	9	75
BHGABA	1000		12	2,4	8	66,6	3,6	8	66,6
BPGABA	100	11	10	5,7±0,94	9	90	5,9±0,32	8	80
»	200	25	12	4,7±0,9	7	58	7,7±0,54	8	66,6
»	300	33	12	6,2±0,11	5	41,6	9	2	16,7
GHBA	50	1,3	10	2,1	9	90	3,7	10	100
»	100	2,6	10	2,0±0,03	6	60	3,4	9	90
»	250	6,6	12	3,4	5	41,6	6	1	8,3
»	500	13	12	1,25	4	33,3	9	2	16,7

Note. Statistically significant differences ($P \leq 0.05$) are underlined.

TABLE 2. Comparison of Effect of Chlorpromazine and Narcotics with that of GHBA and BPGABA on Resistance of Mice to Anoxia Caused by Lowering of Atmospheric Pressure and on other Indices

Compound	Dose in μg/kg	Dose in % of LD_{50}	Time bet. inj. of comp. and beg. of rarefaction, min	No. of animals: Total	No. of animals: No. dying	Motor activity (before placing mice in chamber)	Body temperature (before placing mice in chamber)
Control	—	—	—	14	14	Normal	Normal
GHBA	100	2,6	60	10	9	Normal	Lowered by 2°
GHBA	250	6,6	60	12	1	Limited	Lowered by 5.5°
BPGABA	100	11	60	10	8	Normal	Lowered by 3°
BPGABA	300	33	60	12	2	Limited	Lowered by 6.6°
Chlorpromazine	5	39	60	10	1	Limited	Lowered by 5°
Chlorpromazine	10	77	60	10	9	Limited	Lowered by 8°
Nembutal	30	25	30	10	7	Normal	Lowered by 2°
Nembutal	50	42	15	10	2	Limited	Lowered by 5°
Chloralhydrate	200	33,3	15	10	3	Limited	Lowered by 1.5°
Hemithiamine	50	13	15	10	7	Normal	Lowered by 2.5°
Hemithiamine	100	26	15	10	3	Limited	Lowered by 6°

Note. Statistically significant differences ($P \leq 0.05$) are underlined.

In a dose of 100 mg/kg (Table 1), BPGABA increased resistance to anoxia, prolonging the latent period of onset of convulsions and increasing the survival period, while in a dose of 300 mg/kg it significantly reduced the number of animals which developed convulsions and the number which died.

Compound GHBA, in doses of 50 and 100 mg/kg, had no protective effect on resistance to anoxia, but in doses of 250 and 500 mg/kg its effect was positive according to all the indices.

A clear increase in resistance to anoxia (shown by the number of dying mice) due to GHBA and BPGABA was observed with doses causing definite limitation of motor activity.

Increased resistance to anoxia under the influence of BPGABA (200 mg/kg) and GHBA (250 mg/kg) was also observed in the experiments on rats. Whereas all ten of the control rats died, only 1 of the 10 animals receiving BPGABA (or GHBA) died.

Hence GHBA, like BPGABA, has a definite protective action during anoxia.

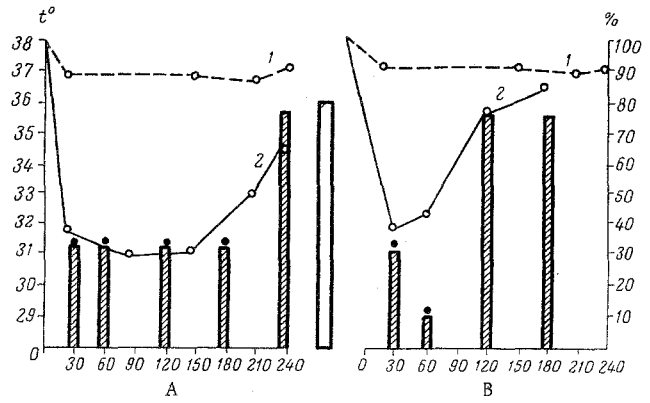

Fig. 1. Comparison of protective (in anoxia) action of BPGABA and GHBA with their hypothermic effect. Vertical axes: rectal temperature of mice (A) and mortality among mice in % (B) during anoxia caused by lowering atmospheric pressure. Horizontal axes: time after injection of compound (in min). 1) Body temperature of control mice; 2) body temperature after injection of 210 mg/kg BPGABA (A) and 250 mg/kg GHBA (B). The columns denote mortality among mice (in %) during anoxia: unshaded—control shaded: A) BPGABA (200 mg/kg); B) GHBA (250 mg/kg). Statistically significant differences are indicated by black circles.

To investigate the duration of the protective action of GHBA and BPGABA, the animals were placed in the chamber 15, 30, 60, 120, 180, and 240 min after injection of the compounds. These experiments (see Fig. 1) showed that the protective action of BPGABA (200 mg/kg) began 30 min after administration and continued for 3 h. The protective effect of GHBA (250 mg/kg) also began after 30 min, became stronger after 1 h, but could no longer be detected after 2 h. The data for the protective action of BPGABA and GHBA during anoxia coincided with their hypothermic action and with their depression of the motor activity of the mice.

As Table 2 shows, the coincidence between the protective action (preventing death of the mice) during anoxia and the hypothermic and sedative effects was also observed in experiments with chloropromazine, Nembutal, chloral hydrate, and hemithiamine.

Hence, of the four GABA derivatives, only two(GHBA and BPGABA) exhibited a protective action in acute anoxia caused by lowering the atmospheric pressure. This may be because GHBA and BPGABA penetrate more readily than GABA and BHGABA into the brain tissue.

In our experiments increased resistance to anoxia coincided with two other effects characteristic of GHBA and BPGABA: limitation of the animals' mobility and hypothermia. Lowering of the body temperature in anoxias of different origin is regarded as a protective reaction [3, 6]. However, if our results obtained in experiments with chloral hydrate, Nembutal (30 mg/kg), and hemithiamine (50 mg/kg), causing the same degree of hypothermia (Table 2), are compared it will be seen that only chloral hydrate had a protective action during anoxia. This may be because it limits the animals' mobility. It may therefore be postulated that in our experiments the increased resistance of the animals to anoxia was dependent both on hypothermia and on limitation of motor activity under the influence of the tested substances. Undoubtedly other factors may also be concerned in their protective effect. In particular, the antianoxic effect of chlorpromazine is known to be connected with its ability to inhibit oxidative processes in the tissues.

The protective effect of BPGABA lasts about twice as long as that of GHBA. Nevertheless, comparison of the protective action of GHBA and BPGABA with their toxicity (the most effective doses expressed in % of LD_{50} for GHBA are about one-fifth of those for BPGABA; see Table 2) shows that GHBA is to be preferred for clinical use in anoxic states.

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