ANTIHYPOXIC AND ANTIOXIDATIVE PROPERTIES OF BEMITIL

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Antihypoxic agents have a protective action on brain tissue in ischemia: they reduce the oxygen consumption, lower the free fatty acid level, prevent degradation of phospholipids, preserve ATP reserves, and exhibit an antioxidant and membrane-stabilizing action [3, 7]. In their metabolic effects, drugs with what have been called actoprotector properties closely resemble antihypoxic agents.

The aim of this investigation was to study the antihypoxic activity of a member of this group (bemitil) on models of asphyctic and circulatory hypoxia and to study the effect of the drug on the blood supply and oxygen supply to the brain, and the concentration of lipid peroxidation products in the brain during circulatory hypoxia, and also on the respiratory and phosphorylating functions of the brain mitochondria, in order to evaluate the antioxidative activity of the drug.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats and on inbred Wistar rats of both sexes, weighing 180-200 g. Asphyctic hypoxia was produced in noninbred rats after preliminary implantation of electrodes for recording the ECoG. The animals were immobilized with succinylcholine (10 mg/kg) and artificially ventilated. Hypoxia was was induced by disconnecting the artificial respiration apparatus consecutively for 90, 120, 150, and 180 sec at intervals of 10 min. The following parameters were taken into account: the time from the beginning of asphyxia until disappearance of the ECoG, the time from the beginning of reventilation to restoration of the ECoG, the total duration of electrical silence, the time from the beginning of asphyxia until the appearance of bradycardia, and the number of animals with restored ECoG. Resistance to circulatory hypoxia was assessed on Wistar rats after ligation of both carotid arteries under ether anesthesia. Bemitil was injected intraperitoneally in a dose of 50 mg/kg 1 h before the experiment. Activity of the drug was assessed as the number of animals surviving 24 h after occlusion of the carotid arteries. In the experiments to study the effect of bemitil on the blood and oxygen supply to the brain and on its content of lipid peroxidation (LPO) products in circulatory hypoxia, noninbred rats anesthetized with urethane (1 g/kg, intraperitoneally) were used. Both carotid arteries were ligated 30 min after intraperitoneal injection of bemitil (50 mg/kg). The total cerebral blood flow was estimated from the venous outflow by the hydrogen clearance method [8]; pO_2 in the parietal cortex was recorded polarographically [4]. The action of bemitil on LPO processes during hypoxia in the lipid fraction of brain tissue was assessed by determining concentrations of diene conjugates (DC) and Schiff bases (SB) [13, 14]. The antioxidative properties of bemitil in vitro were assessed by determining inhibition of malonic dialdehyde (MDA) accumulation: in brain homogenates during Fe^{++}-ascorbate-stimulated LPO [12]; in a solution of Tween-80 [1]; and in a suspension of brain mitochondria (MCH). MCH were isolated under conditions helping to maintain their native state [7]. Respiratory activity of the brain MCH was recorded by a polarographic method in several different metabolic states (after Chance), during oxidation of a mixture of malate and pyruvate (3×10^{-3} M of each) and MDA accumulation was recorded after a cycle of phosphorylation at the 15th and 30th minutes of "aging" of the organelles at 25°C.

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TABLE 1. Effect of Bemitil on Resistance of Rat Brain to Asphyctic Hypoxia

Duration of asphyxia, sec	90				120			
	I	II	III	IV	I	II	III	IV
Control	45,4±3,8	22,4±2,5	66,6±6,0	100	48,6±3,3	45,3±3,5	110,5±7,0	86
Experiment	75,1±7,8*	4,5±0,5*	18,1±3,4*	100	82,7±9,1*	12,5±1,8*	49,1±6,3*	100

TABLE 1 (cont.)

Duration of asphyxia, sec	150				180			
	I	II	III	IV	I	II	III	IV
Control	60,1±3,6	57,3±12,6	147,9±15,5	54	76,4±7,6	—	—	17
Experiment	85,1±10,1	51,1±7,6	105,8±22,4	100	89,3±10,1	35,3±4,2	103,1±14,4	50

Legend. I) Time from beginning of asphyxia until disappearance of ECoG (in sec); II) time from beginning of ventilation to restoration of respiration (in sec); III) total duration of cerebral cortical electrical silence (in sec); IV) number of animals with restored ECoG (in %): *p < 0.05.

TABLE 2. Effect of Bemitil on Cerebral Blood Flow (CBF, in ml/100 g·min), pO_2 of Cerebral Cortex (in % of initial background). Systemic Blood Pressure (SBP, in mm Hg), and Content of DC (in OD_{232}/mg lipids) and SB in Brain Tissue (in optical units/mg lipids) in Circulatory Cerebral Hypoxia

Parameter	Control			Experiment				
	initial background	duration of hypoxia, min		initial background	time before hypoxia, min		duration of hypoxia, min	
		15	30		15	30	15	30
CBF	193±28	127±15*	90±12*	172±15	205±15*	222±16*	104±20*	101±9*
pO_2	100	35±4*	35±3*	100	118±6*	120±6*	45±5*, **	46±5*, **
SBP	109±11	118±14	106±14	103±3	104±5	104±7	114±6	112±6
DC	0,21±0,04	—	0,51±0,05*	0,21±0,04	—	—	—	0,28±0,10**
SB	2,03±0,60	—	4,18±0,80*	2,03±0,60	—	—	—	2,13±0,80**

Legend. *p < 0.05 compared with initial background , **p < 0.05 compared with control.

The antiradical activity of the drug was estimated on the basis of binding with diphenylhydrazine [9]. The results were subjected to statistical analysis by Student's t test and Wilcoxon's nonparametric test [5].

EXPERIMENTAL RESULTS

Bemitil clearly increased the resistance of the brain to asphyctic hypoxia, especially after the first episodes (90 and 120 sec): it increased the time from the beginning of asphyxia until disappearance of the ECoG and accelerated restoration of this parameter. Under the influence of bemitil the number of animals with restored ECoG after the end of exposure to hypoxia also was increased (Table 1). The antihypoxic properties of bemitil in circulatory hypoxia were manifested as a marked increase in the number of surviving animals (40%) compared with the control (10%). Thus bemitil has a distinct protective action on the brain in asphyctic and circulatory hypoxia. It was therefore interesting to study the possible mechanisms of the antihypoxic effect of bemitil.

The dynamics of the cerebral blood flow and of pO_2 of the brain tissue in rats receiving bemitil revealed certain particular features of the action of the drug before and during the period of circulatory hypoxia. Injection of bemitil clearly increased the cerebral blood flow (by 19-29%) and the value of pO_2 (by 18-20%) of the cerebral cortex of the rats in the period before exposure to hypoxia (Table 2). The response developed against the background of a stable systemic blood pressure, evidence of the selective dilator action of the drug on the cerebral vessels. Occlusion of the carotic arteries in rats after prophylactic administration of bemitil was accompanied by a smaller reduction of pO_2 than in the control (Table

TABLE 3. Effect of Bemitil on Respiration Rate of Rat Brain MCH at Rest (V_4) and after Addition of ADP (V_3, nanoatoms O_2/min·mg protein), Phosphorylation Time (T_p, in sec), ADP/O, and MDA Concentration in Medium (10^{-6} M/mg protein) after Phosphorylation Cycle and during "Aging" of MCH

Experimental conditions	Parameter				
	V_4	V_3	T_p	ADP/O	MDA
Initial background:					
control	14,4	47,5	86	3,1	0,275
experiment	14,5	46,9	90	3,1	0,270
15-30 min of "aging"					
control	15,4	32,3	166	2,5	0,372
experiment	16,4	36,2*	145*	2,7*	0,304*

Legend. *$p < 0.05$ compared with control.

2). Meanwhile, in circulatory hypoxia, bemitil had no significant effect on the blood supply of the brain.

Occlusion of the carotid arteries was accompanied by activation of LPO — by considerable accumulation of primary and secondary LPO products in the brain (Table 2), in agreement with observations made by other workers [10]. The conditions for activation of LPO in circulatory hypoxia were created through the predominance of free-radical oxidation over neutralization of its products on exhaustion of reserves of biological antioxidants [15]. Bemitil distinctly inhibited the accumulation of LPO products in the brain (Table 2). Toward the end of ischemia, in animals receiving bemitil the increase in DC and SB in the brain tissue was less than in the control, by 4.3 and 20 times respectively.

Inhibition of LPO processes in brain tissue by bemitil may be due not only to weakening of the intensity of cerebral hypoxia, but also the direct antioxidative activity of the drug. This last property of bemitil was demonstrated during incubation of the drug with Tween-80 (containing a derivative of monounsaturated oleic acid), brain homogenate, and a suspension of brain MCH.

In a solution of Tween-80 with bemitil (10^{-4} M) and during incubation for 48 h under conditions of LPO activation, accumulation of MDA was less than in the control ($0.028 \times 10^{-6} \pm 0.02 \times 10^{-6}$ and $0.078 \times 10^{-6} \pm 0.02 \times 10^{-6}$ M respectively). In a concentration of 10^{-5} M, bemitil limited MDA formation in brain homogenate by the 60th minute of incubation to $0.93 \times 10^{-6} \pm 0.13 \times 10^{-6}$ M/kg protein). Addition of bemitil (5×10^{-5} M) to the isolation medium of brain MCH preserved the respiratory and phosphorylating function of the organelles during "aging" at close to the initial level and prevented the MDA level in the incubation medium from rising (Table 3). However, addition of bemitil to a solution of diphenylpicrylhydrazyl (2.5×10^{-4} M) in equimolar concentration did not change the optical density of the solution, evidence that the preparation has no antiradical activity. Consequently, a decrease in the concentration of LPO products in the brain of the bemitil-treated rats in hypoxia takes place on account of the effect of the drug on LPO processes. The antioxidative properties of bemitil were demonstrated by the writers on model systems with activation of free-radical oxidation: in medium containing a monounsaturated fatty acid and in brain homogenate. The favorable action of bemitil was confirmed under conditions of "aging" of MCH, on a model of swelling of the organelles in ischemic hypoxia [11]. It can be tentatively suggested that bemitil, by weakening the severity of the hypoxic state of the brain tissue when affected by ischemia and by limiting activity of LPO processes, inhibits degradation of the energy-forming functions of the brain MCH and has a protective action on the brain when affected by hypoxia.

Thus the sunscreen bemitil possesses antihypoxic properties in asphyctic and circulatory hypoxia. The drug limits the rise in the level of LPO products in the brain, increases the oxygen supply to the brain tissue in circulatory hypoxia, and exhibits definite antioxidative activity.

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EFFECT OF AMINO ACID DERIVATIVES OF β-CARBOLINE-3-CARBOXYLATE ON BEHAVIOR IN RATS

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When the possible existence of endogenous anxiogenic compounds is examined, particular attention is drawn to β-carboline derivatives, for in experiments in vivo and in vitro the possibility of their formation in the body has been demonstrated [4, 10, 11], and certain derivatives of β-carboline-3-carboxylate have been shown to possess marked anxiogenic activity [3, 5, 6].

One possible source of β-carbolines in the body may be fragments of peptides with a N-terminal tryptophan residue. Previously the writers synthesized the methyl ester of N-(β-carboline-3-carbonyl) glycine (GA) and the methyl ester of N-(β-carboline-3-carbonyl) leucine (LA) and showed that the action of GA on evoked electrical activity of hippocampal neurons is similar to the action of the known anxiogenic compound β-carboline-3-carboxylate methylamide (FG 7142) [2].

The aim of this investigation was to study the effect of amino-acid derivatives of β-carboline-3-carboxylate on rat behavior.

EXPERIMENTAL METHOD

Experiments were carried out in the fall and winter on male Wistar rats weighing 200-250 g. GA and LA were injected intraperitoneally in the form of a 0.4% solution of a mixture of physiological saline with dimethylformamide (2:1). Control animals received the corresponding volume of a mixture of physiological saline with dimethylformamide (2:1).

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