The cholinergic system thus is involved in the resistance of animals to acute anoxia in the recovery period.

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EFFECT OF BENZAMIDE DERIVATIVES ON TOXIC OXYGEN SEIZURES IN RATS

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Traditional anticonvulsants are known not to prevent or delay the onset of "oxygen epilepsy" [8]. In recent years monoaminergic neurotransmitter systems have been shown to play an important role in the mechanism of onset and development of this form of oxygen poisoning [1]. It has been shown, for instance, that during oxygen seizures the brain concentration of monoamine transmitters falls sharply [6, 9]. If serotonin is injected into animals before they are placed in the pressure chamber, oxygen convulsions may be considerably delayed [7]. In the light of these data the protective effect of typical antidepressants, which are monoamine oxidase (MAO) inhibitors, such as iproniazid, nialamide, and tranylcypromine, is evidently by delaying oxidative deamination of monoamines, with their resulting accumulation in the CNS [8, 10]. The ability of chlorgiline, an irreversible selective type A MAO inhibitor, to exert a protective action on rats exposed to hyperbaric oxygen, was demonstrated in [5].

The aim of this investigation was to study the effect of the reversible selective type A MAO inhibitor moclobemide, which has antidepressant properties, and also of original benzamide derivatives closely resembling moclobemide in structure, on the appearance of oxygen seizures in rats.

EXPERIMENTAL METHOD

The convulsive form of oxygen poisoning was simulated by exposing male albino rats weighing 180-250 g in a pressure chamber containing oxygen under a pressure of 6 atm for 27 and 60 min (the periods of compression and decompression were each of 20 min). The drugs were injected intraperitoneally in a dose of 1 or 5 mg/kg in the form of aqueous solutions 15 min before the animals were placed in the pressure chamber. The time of onset of seizures was recorded as the time when the rats lay in the side position. At the end of decompression the

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TABLE 1. Effect of Chlorgiline, Moclobemide, and Benzamide Derivatives on Development of the Convulsive Form of Oxygen Poisoning (6 atm) in Rats Exposed for 60 min (M ± m)

Drug	Dose, mg/kg	No. of animals	Percentage of animals observed to have seizures	Mortality, %	Time of onset of seizures, min
Control	—	40	90±10	80±10	28±6
Chlorgiline	1	12	83±10*	25±8*	44±3***
	5	5	0	0	—
Moclobemide	1	12	100	50±10***	31±2*
Compound No. 1: p-Chloro-N-(4-morpholinobutyl)-benzamide hydrochloride	5	5	0	0	—
	5	4	100	0	55±2*
Compound No. 4: p-Bromo-N-(3-morpholinopropyl)-benzamide hydrochloride	5	5	100	20	40±3**
Compound No. 6: N-(4-benzylpiperazinoethyl)-amide of p-chlorobenzoic acid dihydrochloride	5	4	100	50	49±6***

Legend. For experiments with several series of animals differences between experimental and control parameters are given: *$p \leq 0.01$, **$p \leq 0.02$, ***$p \leq 0.05$. Resynthesis of moclobemide and synthesis of compounds Nos. 1, 4, and 6 were carried out in the Department of Chemistry, Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, under the direction of Professor V. A. Zagorevskii.

animals were decapitated and their brains and hearts removed in the cold and kept in liquid nitrogen. MAO activity was determined in 25% homogenates of the organs by isothermic diffusion of ammonia followed by nesslerization [2]. MAO substrates were used in previously selected saturating concentrations. The results were subjected to statistical analysis with calculation of mean values and their confidence intervals at p = 0.01, 0.02, and 0.05.

EXPERIMENTAL RESULTS

The results of a study of the effect of moclobemide and of benzamide derivatives No. 1, 4, and 6, compared with that of chlorgiline, on the development of the convulsive form of oxygen poisoning in rats after an exposure of 60 min are shown in Table 1. It will be clear from Table 1 that the control animals lay in the side position on average after an exposure of 27-29 min to oxygen under a pressure of 6 atm. After injection of chlorgiline and moclobemide into the rats in a dose of 1 mg/kg, the onset of seizures was delayed a little. After injection of chlorgiline, fewer animals developed seizures. The mortality among the rats after injection of chlorgiline and moclobemide (1 mg/kg) was reduced by 33-50% compared with the control. It will be clear from Table 1 that after administration of chlorgiline and moclobemide in a dose of 5 mg/kg rats did not develop seizures in the course of a 60-min exposure in the pressure chamber (6 atm). Compound No. 1 (5 mg/kg) doubled the latent period of onset of convulsions but did not reduce the number of animals which developed them. Under these circumstances all the animals remained alive after decompression. Compounds Nos. 4 and 6 (5 mg/kg) also delayed the

TABLE 2. Effect of Chlorgiline and Moclobemide in a Dose of 5 mg/kg on MAO Activity (in %) in Rat Brain and Heart in Vivo (M ± m)

Drug	Brain				Heart			
	5 HT	2- PEA	DA	Tyr	5-HT	2-PEA	DA	Tyr
Control	100±27	100±10	100±26	100±3	100±5	100±8	100±6	100±10
Chlorgiline	25±3**	73±40	0	77±3*	0	12±4	0	0
Moclobemide	13±4**	14±11	89±30	63±7*	40±2*	74±11	62±23	38±9**

Legend. 5-HT) serotonin, 2-PEA) 2-phenylethylamine, DA) dopamine, Tyr) tyramine. 100% MAO activity in rat brain: 2.59 ± 0.7 nmoles 5-HT/mg protein/min; 0.82 ± 1.08 nmoles 2-PEA/mg protein/min; 2.57 ± 0.68 nmoles DA/mg protein/min; 4.05 ± 0.14 nmoles Tyr/mg protein/min. 100% MAO activity in rat heart: 3.93 ± 0.2 nmoles 5-HT/mg protein/min; 2.11 ± 0.16 nmoles 2-PEA/mg protein/min; 3.3 ± 0.2 nmoles DA/mg protein/min; 3.74 ± 0.36 nmoles Tyr/mg protein/min. *$p \leq 0.01$, **$p \leq 0.05$ compared with control. Here and in Table 3 MAO activity was measured in the presence of the following saturating concentrations of substrates: 4.0 mM 5-HT, 1.25 mM 2-PEA, 2.5 mM DA, 3.5 mM Tyr; number of determinations — 4.

TABLE 3. Effect of Chlorgiline and Moclobemide in a Dose of 5 mg/kg on MAO Activity (in %, M ± m) in Rat Brain and Heart after Exposure of 27 min to Toxic Hyperoxia (6 atm)

Drug	Brain				Heart			
	5-HT	2- PEA	DA	Tyr	5-HT	2-PEA	DA	Tyr
Control	100±16	100±9	100±20	100±7	100±1	100±25	100±5	100±2
Chlorgiline	47±4**	40±4*	45±1***	26±2*	57±4*	51±15	29±11*	60±9**
Moclobemide	6±1*	48±4**	80±10	53±13***	51±1*	65±11	46±11**	33±4*

Legend. 100% MAO activity in rat brain: 3.33 ± 0.54 nmoles 5-HT/mg protein/min; 2.52 ± 0.23 nmoles 2-PEA/mg protein/min; 3.07 ± 0.60 nmoles DA/mg protein/min; 5.10 ± 0.36 nmoles Tyr/mg protein/min. 100% MAO activity in rat heart: 7.89 ± 0.05 nmoles 5-HT/mg protein/min; 1.61 ± 0.4 nmoles 2-PEA/mg protein/min; 4.68 ± 0.08 nmoles DA/mg protein/min; 7.46 ± 0.40 nmoles Tyr/mg protein/min. *$p \leq 0.01$, **$p \leq 0.02$; ***$0.02 \leq p \leq 0.05$ compared with control.

onset of convulsions in all the animals and reduced their mortality. Thus the protective effect of the benzamide derivatives in "oxygen epilepsy" in rats differs: moclobemide (5 mg/kg), like chlorgiline in the same dose, completely prevented the onset of seizures, whereas compounds Nos. 1, 4, and 6 (5 mg/kg) delayed the onset of seizures, although as in the control group all the animals developed seizures, and reduced the mortality rate.

The aim of the next stage of the investigation was to determine possible differences in the effect of selective type A MAO inhibitors (chlorgiline and moclobemide) on brain and heart MAO levels of normal rats and rats with toxic hyperoxia. As Table 2 shows, the velocity of the serotonin-deaminase reaction was considerably reduced in the rat brain and heart following injection of chlorgiline and moclobemide (5 mg/kg); the inhibitory effect of chlorgiline was more marked in the heart than in the brain. The results are in agreement with those obtained by other workers who observed marked antiserotonin-deaminase activity of moclobemide in vivo, and with our own data showing inhibition of serotonin deaminase by moclobemide in vitro in the rat brain [3, 11]. The anti-2-phenylethylamine-deaminase action of moclobemide in the brain was stronger than that of chlorgiline which, as in the case of serotonin deamination, was much more active in the rat heart than in the brain. Deamination of dopamine in the brain was almost unchanged by moclobemide, whereas in the heart it was reduced more considerably. Chlorgiline was found to be a strong inhibitor of this process in both organs studied. All the preparations tested inhibited tyramine deamination in the rat brain moderately but significantly, and inhibited it very considerably in the heart. Thus the antimonoamine-oxidase spectra of chlorgiline and moclobemide differed in the rat brain and heart: as a rule chlorgiline had a stronger action in the heart and moclobemide a stronger action in the rat brain. Data showing the powerful inhibitory action of all the substances tested on tyramine deamination in the heart, but not in the brain, are interesting.

Table 3 shows that after a 27-min exposure to toxic hyperoxia the inhibitory effect of chlorgiline and moclobemide on serotonin deamination in the rat brain was virtually indistinguishable from that in control animals (Table 2), whereas in the heart, the effect of chlorgiline was much weaker than in intact animals. More or less the same pattern was observed in the case of 2-phenylethylamine deamination. Just as in intact animals, moclobemide did not affect dopamine deamination in the brain of rats breathing hyperbaric oxygen, but inhibited it in the heart. The inhibitory effect of chlorgiline, as in the cases examined above, during hyperoxia was weaker, especially in the animals' heart. Conversely, oxygen poisoning potentiated the antityramine-deaminase action of chlorgiline in the rat brain and weakened it in the heart. The results are thus evidence that in rats exposed in a pressure chamber to a raised oxygen pressure (6 atm), type A MAO inhibitors, just as in intact animals, exhibit antimonoamine oxidase activity, and this may be the reason for their anticonvulsant protective action. However, in some cases the spectrum of this activity differs from that normally observed, reflecting the presence of neurochemical disturbances, uncompensated by administration of the drugs tested, due to a raised partial pressure of oxygen. Differences in the action of the compounds on MAO under normal and extremal conditions, revealed by this investigation, confirm once again the importance of the study of the neurochemical mechanisms of the action of drugs on models of this pathological state. The conclusion can be drawn from these investigations that not only irreversible, but also reversible type A MAO inhibitors can exert a protective action in oxygen poisoning, and that this action is evidently largely due to inhibition of deamination of serotonin and other monoamines in the CNS and peripheral organs. However, as exemplified by compounds Nos. 1, 4, and 6, whose antimonoamine-oxidase action is absent or weak [4], but which reduce the percentage of animals which die, it can be postulated that the protective action of these benzamide derivatives may be unconnected with their effect on oxidative deamination of monoamines in the tissues.

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