EFFECT OF BENZAMIDE DERIVATIVES ON RAT BRAIN MONOAMINE OXIDASE ACTIVITY *IN VITRO*

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A new effective antidepressant with low toxicity, moclobamide [compound Ro 11-1163, p-chloro-N-(2-morpholinoethyl) benzamide], a derivative of benzamide, has been obtained in the West [3, 8]. It is claimed that the antidepressant effect of this compound is based on its ability to exert a selective inhibitory action on the type A monoamine oxidase — MAO (amine: oxygen-oxidoreductase, deaminating, flavine-containing; EC 1.4.3.4) — relative to its substrate serotonin [6]. However, it is not yet clear what components of the chemical structure of moclobamide are directly connected with its inhibitory action on serotonin deamination.

Accordingly, in the investigation described below, an attempt was made to discover the functional groups in the molecule of benzamide derivatives essential for inhibition of serotonin-deaminating and other monoamine-oxidase reactions.

EXPERIMENTAL METHOD

A 25% homogenate and the coarse mitochondrial fraction (CMF) of the brain of noninbred male albino rats weighing 180-200 g were used as the source of the enzyme [1]. The CMF was obtained by the standard method [1].

The 25% homogenate or CMF was added to the samples in a dose of 3-5 mg protein, together with one of the substrates in the following final concentrations [2]: serotonin (5-HT); 6.6 mM; noradrenalin (NA), 2.2 mM (substrates of type A MAO [5]); dopamine (DA), 3.3 mM; tyramine (TYR), 4.4 mM (mixed substrates of MAO of types A and B [7]); 2-phenylethylamine (2; PEA), 1 mM (a specific substrate of type B MAO [4]); K,Na-phosphate buffer, 0.05 M (pH 7.4); and MAO activity was determined after incubation of the samples for 30 min in an atmosphere of oxygen at 37°C, with shaking. The reaction was stopped by the addition of 50% TCA. In the experiments to determine antimonoamine-oxidase activity of original benzamide derivatives synthesized in the Scientific-Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, they were added to the sample as an aqueous solution. Compounds 1-6 are benzamide derivatives: Compound 1 is a homolog of moclobamide, in four compounds the chlorine atom is shifted from the para position to the ortho position (compound 4), or replaced by a methyl group in the ortho position of the benzene ring (compound 5), and in compound 6 the morpholine heterocyclic ring is replaced by a benzylpiperazine moiety. Besides the compounds listed above, substance 7, in which the benzamide moiety is replaced by isonicotinamide, also was studied for comparison. Chemically speaking, all the compunds are water-soluble salts.

The velocity of the enzyme reaction was estimated from the quantity of ammonia removed from the substrate, determined by the isothermic diffusion method followed by nesslerization, and calculated per milligram protein per minute. The results were subjected to statistical analysis with calculation of mean values and their confidence intervals at $P = 0.02$ and $P = 0.05$ relative to the control (results of 4-8 determinations).

EXPERIMENTAL RESULTS

The preparation moclobamide had maximal antiserotonin-deaminase activity and in a concentration of 100 μM it completely inhibited 5-HT deamination (Table 1). Compounds 3 and 5, in the same concentration, inhibited 5-HT deamination less actively, and in a concentration of 10 μM they were ineffective. Substances 1, 2, 4, 6, and 7 had no antiserotonin-deaminase properties. Moclobamide, and also compounds 1, 3, and 7 (100 μM) completely inhibited MAO

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TABLE 1. Effect of Benzamide Derivatives on Deamination of Biogenic Amines in Rat Brain *in Vitro* (M ± m)

Substance	Concentration, μM	MAO activity, %					
		5-HT	NA		DA	TYR	2-PEA
		homogenate	homogenate	CMF	homogenate	homogenate	CMF
Control	—	100±30	100±36	100±10	100±25	100±12	100±30
Moclobamide	10	88±13	—	—	—	—	—
	100	0	0	—	92±16	84±13	—
Compound:							
1	100	139±11	0	0	130±11	73±12	—
2	100	119±38	100±25	—	90±30	123±22	—
3	10	98±10	—	—	—	—	71±15
	100	48±13*	0	0	67±19**	63±8	59±7*
4	10	—	—	—	—	—	122±38
	100	82±11	—	72±6*	68±17	76±23**	58±18*
5	100	40±5**	84±19	—	82±20	82±20	48±18**
6	10	—	—	—	—	78±8	80±10
	100	78±13	—	—	69±2	47±5*	44±16*
7	100	121±15	—	10±2*	115±40	98±11	—

Legend. Number of determinations for each point was 4-8. 100% MAO activity: 2.67 ± 0.24 nmole 5-HT/mg protein/min; 1.50 ± 0.57 nmole NA/mg protein/min (homogenate); 1.4 ± 0.11 nmole NA/mg protein/min (CMF); 3.53 ± 0.21 nmole DA/mg protein/min; 5.4 ± 0.12 nmole TYR/mg protein/min; 3.6 ± 1.1 nmole 2-PEA/mg protein/min. —) Not determined. *P = 0.02, **P = 0.05.

activity with respect to NA in 25% rat brain homogenate. These results agree with those of experiments on CMF. Compounds 2, 4, and 5 had no antinoradrenalin-deaminase activity.

Moclobamide did not inhibit deamination of DA and TYR in rat brain homogenate. All the other compounds either inhibited MAO activity with respect to DA weakly in a concentration of 100 μM, or did not affect deamination of DA. Compound 6 had the strongest antityramine-deaminase activity of all the benzamide derivatives. Substances 1, 3, and 4 weakly inhibited TYR deamination, whereas compounds 2 and 5-7 did not inhibit it.

The effect of those benzamide derivatives which inhibited deamination of 2-PEA in rat brain homogenate was studied on 2-PEA deamination in the CMF. The whole series of benzamide derivatives tested either had a very weak anti-2-phenylethylaminedeaminase effect or had no effect on 2-PEA deamination.

All the results obtained thus indicate that introduction of a chlorine atom (compound 2) and a bromine atom in the para position (compound 4) of the benzene ring of the moclobamide molecule causes loss of antiserotonin-deaminase activity. The antiserotonin-deaminase effect of compound 6, in which the morpholine ring is replaced by a benzylpiperazine moiety, was no stronger than that of moclobamide. When the results were analyzed, differences were found between inhibition of the deamination reaction of 5-HT and NA (substrates of type A MAO) by the compounds studied: For instance, introduction of a methyl group in the para position of the benzene ring (compound 5) caused loss of antinoradrenalin-deaminase activity, but under these circumstances the antiserotonin-deaminase effect was preserved; lengthening the side chain by two methyl groups (compound 1), however, made this compound an evidently selective inhibitor of NA deamination, whereas moclobamide inhibits deamination of both 5-HT and NA.

Replacement of the benzamide moiety of the moclobamide molecule by isonicotinamide (compound 7) preserved the antinoradrenalin-deaminase effect and abolished the antiserotonin-deaminase effect. It can be tentatively suggested that besides the benzamide, the isonicotinamide moiety in the chemical structure of compounds of this series is responsible for the fact that this compound has only antinoradrenalin-deaminase activity.

The absence of substituents in the benzene ring (compound 3) led to the appearance of about equal antimonoamine-oxidase activity relative to all substrates tested, suggesting a possible connection between the presence of a chlorine atom in the para position of the benzene ring in moclobamide with its selectivity relative to 5-HT and NA.

Another fact which deserves attention is that changes in the morpholine ring of compound 6 (replacement by a benzylpiperazine moiety) led to the appearance of very weak but steady antitryamine-deaminase activity. It is evidently the morpholine ring in the moclobamide

molecule that is responsible for the absence of the antityramine-deaminase effect of this compound.

Since the majority of compounds tested were found to be active type A MAO inhibitors with virtually no effect on the dopamine- and 2-phenylethylamine-deaminase reactions, it can be postulated that DA in rat brain is deaminated mainly by type B MAO.

Thus modifications of different components of the chemical structure of moclobamide enabled the functional groups of this compound determining the limits of its antiserotonin-deaminase action, to be determined to some extent.

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