SELECTIVE INHIBITION OF TYPE A MONOAMINE OXIDASE BY PYRAZIDOL

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At least two functionally different types of monoamine oxidase (MAO) are found in animal tissues. MAO of type A, which deaminates serotonin and noradrenalin, is sensitive to the action of low concentrations of chlorgyline, whereas MAO of type B, which deaminates 2-phenylethylamine, is sensitive to the action of low concentrations of deprenil. Both types of MAO deaminate tyramine, dopamine, and tryptamine and are equally inhibited by high concentrations of chlorgyline and deprenil [7, 9, 13]. However, the properties of the MAO do not fit into this binary classification for all tissues and species of animals [5, 6].

Derivatives of pyrazino-indole and, in particular, pyrazidol (1,10-trimethylene-8-methyl-1,2,3,4-tetrahydropyrazino[1,2-a]-indole), which has the properties of an antidepressant, inhibit MAO activity in rat liver and brain [2, 3].

The investigation described below was devoted to a comparative study of the action of pyrazidol on deamination of various monoamines.

EXPERIMENTAL METHOD

The effect of pyrazidol on MAO activity was studied *in vivo* and *in vitro* on noninbred male rats weighing 180-200 g. Pyrazidol in doses of 5-50 mg/kg, or isotonic NaCl solution (control rats), was injected intraperitoneally. The animals were killed 30 min after injection of the preparation. MAO activity was determined in 50% homogenates of rat liver and brain prepared in 10M phosphate buffer, pH 7.4, containing 2% of the nonpolar detergent Triton X-100. Fragments of mitochondrial membranes of liver [1] and brain [12] were used as the source of MAO in experiments *in vitro*. In the experiments with brain MAO the following substrates were used (in μmoles/1.8 ml of sample): noradrenalin bitartrate (from Sigma) 4, serotonin creatinine-sulfate (from Reanal) 12, dopamine hydrochloride (from Ferak) 6, tyramine hydrochloride (from Merck) 8, 2-phenylethylamine hydrochloride (USSR origin) 2. To determine liver MAO activity the substrates were used in the following optimal concentrations (in μmoles/1.8 ml of sample): serotonin and dopamine 10, tyramine 6, 2-phenylethylamine 0.8. The optimal concentrations of substrates were chosen in preliminary experiments. MAO activity was judged from liberation of ammonia during incubation of the samples for 50 min at 37°C in an atmosphere of oxygen. Purified MAO preparations from hog liver were obtained by Oreland's method [10]. Protein in suspensions of fragments of mitochondrial membranes was determined by Lowry's method and in tissue homogenates by Peterson's method [11].

EXPERIMENTAL RESULTS

Pyrazidol in concentrations of $1 \cdot 10^{-5}$-$1 \cdot 10^{-6}$ M inhibited by 50% the deamination of serotonin and dopamine by MAO from rat liver mitochondria and also of serotonin, dopamine, and tyramine by MAO from rat brain mitochondria. In these concentrations pyrazidol had virtually no effect on deamination of 2-phenylethylamine by liver mitochondrial MAO. Only in a concentration of $1 \cdot 10^{-3}$ M was the deamination of this amine observed to be inhibited by 40%. These experiments thus demonstrated the selective action of pyrazidol on MAO of type A. In experiments with liver mitochondria pyrazidol inhibited deamination of serotonin and dopamine more strongly and that of tyramine less strongly. For instance, in a concentration of $1 \cdot 10^{-5}$ M py-

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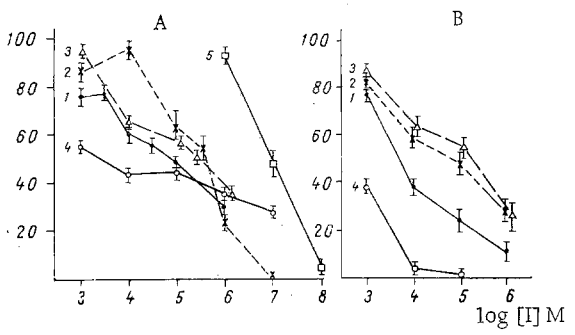

Fig. 1

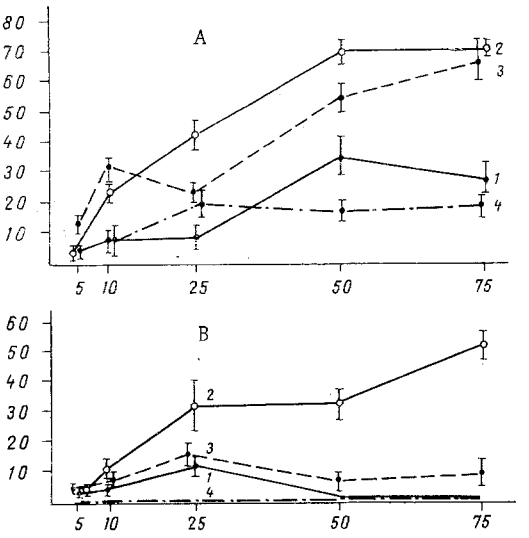

Fig. 2

Fig. 1. Effect of different concentrations of pyrazidol on deamination of biogenic amines by mitochondrial membranes of rat brain (A) and liver (B). Samples 1.8 ml in volume contained 1.5 mg or 4 mg protein of mitochondrial membrane fragments from rat liver or brain, respectively, 50 mM phosphate buffer, pH 7.4, optimal concentrations of substrates, and pyrazidol in the concentrations indicated. Results of 4-6 experiments shown. Inhibition of deamination of tyramine (1), serotonin (2), dopamine (3), 2-phenylethylamine (4), and noradrenalin (5) by rat brain (A) and liver (B) mitochondria. Abscissa, final concentrations of pyrazidol in samples; ordinate, inhibition of deamination (in %).

Fig. 2. Effect of different doses of pyrazidol on deamination of biogenic amines in rat brain (A) and liver (B) tissues. Results of six experiments shown. Abscissa, dose of pyrazidol (in mg/kg); ordinate, inhibition of deamination (in %). Remainder of legend as in Fig. 1.

razidol inhibited the deamination of serotonin and dopamine by 50% and of tyramine by 25%. In experiments with brain mitochondria the concentration of pyrazidol inhibiting deamination of 2-phenylethylamine by 50% differed from the concentrations for serotonin and dopamine by almost two orders of magnitude (Fig. 1A); nevertheless, the selectivity of action of pyrazidol was less clearly demonstrated than in the experiments with liver mitochondria. In fact, in concentrations of $1 \cdot 10^{-6}$ to $1 \cdot 10^{-7}$ M pyrazidol continued to inhibit deamination of 2-phenylethylamine by 35-25%. The character of the curve of the dependence of the degree of inhibition of deamination of 2-phenylethylamine on the pyrazidol concentration indicates the possibility that this amine may be oxidized by both types of MAO from rat brain mitochondria. The high selectivity of action of pyrazidol relative to noradrenalin must be emphasized. In a concentration of $1 \cdot 10^{-6}$ M pyrazidol completely blocked the deamination of this amine. Concentrations of pyrazidol inducing 50% inhibition of oxidative deamination of 2-phenylethylamine and noradrenalin differed by more than three orders of magnitude ($4 \cdot 10^{-4}$ and $1.2 \cdot 10^{-7}$ M, respectively). The selectivity of action of pyrazidol relative to the inhibition of deamination of different monoamines was manifested more clearly still in experiments *in vivo*. For instance, in a dose of 50 mg/kg pyrazidol inhibited deamination of serotonin in brain tissue by 70% (Fig. 2A) but inhibited deamination of 2-phenylethylamine by only 18%. In all doses tested pyrazidol had no effect on deamination of 2-phenylethylamine in liver tissue (Fig. 2B). These experiments also revealed the tissue selectivity of action of pyrazidol. In liver tissue pyrazidol, in a dose of 50 mg/kg, blocked serotonin deamination by 35% but had virtually no effect on deamination of tyramine and dopamine (Fig. 2B). These data agree with those of experiments *in vitro* and evidently reflect different ratios between the types of MAO in liver and brain tissues. The higher degree of inhibition of tyramine and dopamine in brain tissues may perhaps be attributable to the fact that the ratio between types A and B MAO in this tissue in rats is close to 1, and in addition, dopamine is predominantly deaminated by MAO of type A [8]. It must be pointed out that pyrazidol in a dose of 50 mg/kg, which produces

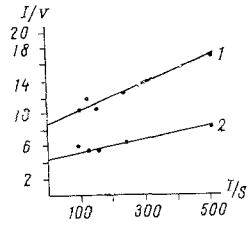

Fig. 3. Inhibition of tyramine deamination by purified preparations of hog liver MAO as a function of substrate concentration. Samples contained 63 μg protein of purified MAO preparation (specific activity 7142, substrate benzylamine hydrochloride, unit of activity defined as change in optical density at 250 nm by 0.001 unit/min); tyramine in concentrations of $1 \cdot 10^{-2}$ to $2 \cdot 10^{-3}$ M and 50 mM phosphate buffer, pH 7.4. Results of four determinations given. 1) In presence of pyrazidol, 2) control.

maximal inhibition of deamination of serotonin in liver tissue, had virtually no effect on tyramine deamination in liver tissues (Fig. 2A and B). During the therapeutic use of nonspecific MAO inhibitors or of those selectively blocking type A MAO, circulatory disturbances may arise if foods containing tyramine are taken at the same time (the so-called cheese syndrome), Allowing for these particular features of the action of pyrazidol it can be tentatively suggested that the use of this drug should not be accompanied by this complication.

It was shown previously that the character of MAO inhibition induced by pyrazidol depends on the nature of the substrate used: Inhibition of deamination of serotonin is "mixed" but that of dopamine is noncompetitive [2]. These data were confirmed in experiments using purified MAO preparations from hog liver mitochondria which, as has now been shown, contain both types of MAO [4]. Inhibition of tyramine deamination by pyrazidol in these experiments also was noncompetitive (Fig. 3). These results evidently indicate that pyrazidol does not interact with the catalytic center of MAO, but mainly blocks substrate combining sites.

Pyrazidol, a member of a new group of polycyclic antidepressants which are derivatives of pyrazino-indole, thus has a characteristic effect on deamination of monoamines in rat tissues. The character of pyrazidol-induced inhibition of oxidative deamination of serotonin and 2-phenylethylamine in experiments *in vivo* and *in vitro* thus suggests that it belongs to the group of selective inhibitors of type A MAO. The property of pyrazidol to block type A MAO selectively in rat liver and brain tissues is important to an understanding of the particular features of antidepressant activity of this preparation.

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