TRICYCLIC DYES AS MONOAMINE OXIDASE INHIBITORS

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Highly purified specimens of monoamine oxidase were obtained from bovine liver mitochondria. Pyronine G and acridine orange NO in low concentrations were shown to selectively inhibit oxidative deamination of tyramine or serotonin by the preparations of purified monoamine oxidase. These tricyclic dyes, in experiments in vitro, inhibit monoamine oxidase activity of rat or human liver mitochondria to a much greater degree than that of bovine liver mitochondria. The ability of pyronine G and acridine orange NO to inhibit monoamine oxidase activity under the experimental conditions in vivo was confirmed by a radiometric method.

Previous work in the writers' laboratory showed [1, 3, 4, 6, 9] that some tricyclic compounds in low concentrations inhibit monoamine oxidase activity of rat liver mitochondria and can selectively block the deamination of certain biogenic amines [4, 6, 9]. These effects are also exhibited in experiments in vivo [8].

The object of the present investigation was to discover whether the enzyme activity of highly purified specimens of mitochondrial monoamine oxidase can be inhibited by tricyclic dyes. Since a modified method of isolating and purifying this enzyme, developed in the writers' laboratory by Zh. I. Akopyan, was used on bovine liver mitochondria, it was also decided to study the sensitivity of the mitochondrial monoamine oxidase activity in the liver of different species of animals to the inhibitory action of tricyclic dyes. The final purpose of the investigation was to use a radiometric method of determining monoamine oxidase activity [11] to study the action of tricyclic dyes in vivo as monoamine oxidase inhibitors.

EXPERIMENTAL METHOD

The liver of a person dying accidentally was obtained 12 h after death from the mortuary of the Department of Pathological Anatomy, First Moscow Medical Institute.

The sources of the other materials and methods used to isolate the mitochondria were described previously [6].

In the course of purification of the enzyme, monoamine oxidase activity was determined colorimetrically with p-nitrophenylethylamine · HCl as substrate [7]. The rate of deamination of tyramine · HCl or serotonin creatinine-sulfate was investigated as described previously [6]. In the experiments in vivo, the test compounds were injected subcutaneously into rats 60 min before decapitation. Monoamine oxidase activity was determined in 10% homogenates radiometrically [11] with tyramine-C^{14} (New England Nuclear Corp., specific activity 5.3 μCi/mmole) as substrate. The control samples contained the boiled homogenate.

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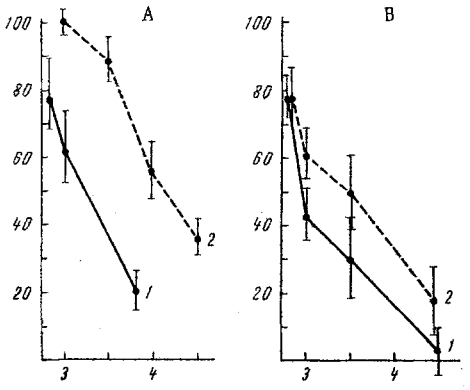

Fig. 1

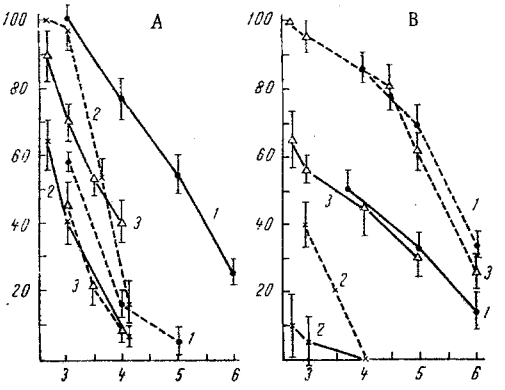

Fig. 2

Fig. 1. Inhibition of deamination of tyramine (1) and serotonin (2) by purified specimens of mitochondrial monoamine oxidase from bovine liver by pyronine G (A) and acridine orange NO (B). Abscissa, negative logarithm of final concentrations (M) of inhibitors in samples; ordinate, arithmetic means values (results of 4-6 experiments) with standard deviation, expressing degree of inhibition of deamination of tyramine serotonin. All samples contain 0.025 (experiments with tyramine) or 0.06 mg (experiments with serotonin and substrate) protein of purified enzyme preparations (specific activity 580-600). Other components of samples and experimental conditions described previously (1, 6).

Fig. 2. Species differences in inhibition of deamination of monoamines by pyronine G (A) and acridine orange NO (B): 1) rat liver mitochondria; 2) bovine liver mitochondria; 3) human liver mitochondria. Continuous lines represent tyramine, broken lines serotonin. Remainder of legend as in Fig. 1. All samples contain 2.5 mg (experiments with bovine or rat liver mitochondria) or 6 mg (experiments with human liver mitochondria) mitochondrial protein.

TABLE 1. Purification of Monoamine Oxidase from Bovine Liver Mitochondria

Stage of purification	Volume (in ml)	Activity (in units per ml)[1]	Total activity (in units)	Protein content (in mg/ml)	Specific activity (in units per mg protein)	Yield (in percent)	Degree of purity
Suspension of mitochondria	40	230	9 200	18.1	12.7	100	1
Solubilization.	36	190	6 840	14.7	12.9	74	1
Treatment with aluminum hydroxide .	74	35	2 590	0.28	126	28	10
Precipitation with ammonium sulfate.	15	110	1 683	0.79	139	18	11
Treatment with detergent OP-10 . . .	14.5	80	1 120	0.13	608	12	48

[1]The unit of enzyme activity was taken as the amount of enzyme catalyzing liberation of 1 mμ mole ammonia in 1 min under standard incubation conditions [7].

EXPERIMENTAL RESULTS

An example of a typical experiment to purify monoamine oxidase is shown in Table 1.

Mitochondria were isolated as described previously [2]. Solubilization of the mitochondrial suspensions in distilled water (20 mg protein/ml) was carried out by adding benzylamine base to the final concentration 14 mmoles and detergent OP-10 (final concentration 0.75%). The suspension (pH 8.6) was centrifuged (40000 g, 20 min) and the residue discarded.

The resulting supernatant was kept for 16-18 h at 4° (during which time the pH fell to 7.8) and was then treated with aluminum hydroxide C gel with a ratio of protein to gel (calculated as dry weight) of 1:6. After centrifugation (8000 g, 2 min) the residue was discarded.

TABLE 2. Inhibition of Monoamine Oxidase Activity by Tricyclic Dyes in Vivo (mean results of 6 experiments)

Compound	Dose (in mg/kg)	Inhibition of deamination (in percent) of tyramine-C^{14}	
		liver	brain
Pyronine G	10	0	0
	20	0	5.2
	50	15.5	11.2
Acridine orange NO	10	34.0	10.0
	20	34.0	10.0
	50	38	45.0

Proteins were precipitated from the supernatant with ammonium sulfate at pH 7.3. After a degree of saturation of 0.4 had been reached (by the addition of dry ammonium sulfate), the mixture was left for 2 h at 4° and then centrifuged (8000 g, 10 min). The floating residue was suspended in a small volume of 1 mmole phosphate buffer (pH 7.4) and dialyzed against this buffer until all ammonium ions were completely removed.

Treatment of the resulting dialyzate with OP-10 detergent (final concentration 2%) followed by centrifugation (8000 g, 10 min) yielded a transparent yellowish solution containing the enzyme purified on the average by 40 times compared with the original mitochondrial suspension.

In its degree of purification, the resulting specimen could not be distinguished from the highly purified monoamine oxidase obtained by other workers from bovine liver [10].

Pyronine G and acridine orange NO, in low concentrations, inhibited the deamination of tyramine and, in particular, of serotonin by purified preparation of mitochondrial monoamine oxidase from bovine liver (Fig. 1).

It is interesting to note that the mitochondrial monoamine oxidase activity of bovine liver was much less sensitive to the inhibitory action of acridine orange NO (but not of pyronine G) than the mitochondrial monoamine oxidase activity of rat or human liver measured for comparison (Fig. 2). These results are in full agreement with species differences found in the sensitivity of the mitochondrial monoamine oxidase to the inhibitory action of the natural tricyclic compound harmine [5, 6, 9]. In the course of its purification, the sensitivity of this enzyme to the inhibitory action of pyronine G and acridine orange NO increased (Figs. 1 and 2).

The activity of the purified monoamine oxidase from human or rat can be assumed to be blocked by particularly low concentrations of the tricyclic compound.

It follows from the results given in Table 2 that pyronine G and, in particular, acridine orange NO, under experimental conditions in vivo, inhibit the reaction of deamination of tyramine-C^{14}, recorded by a radiometric method [11]. This conclusion is supported by results obtained in the writers' laboratory previously by a different method.

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