THE INHIBITION OF MITOCHONDRIAL AMINE OXIDASES IN VITRO BY PROFLAVINE

V.Z.Gorkin, N.V.Komisarova, M.I.Lerman and I.V.Veryovkina
Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR, Moscow (USSR)

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Although the oxidative deamination of various monoamines in animal tissues is usually ascribed to the action of a single enzyme - monoamine oxidase /MO; monoamine:O_2 oxidoreductase (deaminating), EC 1.4.3.4/ which occurs mainly in mitochondria, numerous data may be cited in favour of the "multiplicity" of mitochondrial amine oxidases (cf. Gorkin, 1963). It seems likely that "monoamine oxidase" may represent a complex system of amine oxidases with relatively narrow substrate specificities.

In the course of experiments planned for another purpose we found that certain features of the inhibitory effect of proflavine on the activity of mitochondrial amine oxidases may be of interest for investigations on the metabolism of biogenic amines and possibly for the design of new monoamine oxidase inhibitors with selective action on the oxidative deamination of different biogenic amines.

MATERIALS AND METHODS. Rat liver mitochondria isolated from 10 per cent homogenate in isotonic sucrose (Schneider, 1948) were treated with hypotonic phosphate solution (Gorkin and Veryovkina, 1963), and the partially purified MO preparation was lyophilized. Experimental conditions and methods for the assay of MO activity by the determination of ammonia liberated in the course of oxidation were as described earlier (Gorkin and Romanova, 1959).

Soluble beef serum amine oxidase (spermine oxidase) was partially purified by ammonium sulphate fractionation and column chromatography on DEAE-cellulose (Gorkin, 1962; Yamada and Yasunobu, 1962); its activity was measured by spectrophotometric observation of m-nitro-p-hydroxybenz-

aldehyde formation (λ_{max} 315 mμ;Gorkin et al.,1964).Protein content was determined and the specific activity calculated as described earlier (Gorkin, 1961).

Chemically pure preparations of tyramine, tryptamine, benzylamine, m-nitro-p-hydroxybenzylamine (all hydrochlorides), serotonin creatinine sulphate, isoamylamine and 3,6-diaminoacridine sulphate (proflavine) were used,

RESULTS. The effects of proflavine on deamination of various amines by rat liver mitochondrial MO are shown in fig.1. Appreciable inhibition of deamination of serotonin was noted already in samples containing 10^{-6}M/L of proflavine. Concentrations of proflavine inhibitory for the deamination of tyramine were significantly higher than those for serotonin:pI_{50} values 3.09±0.04 and 4.97±0.12, respectively ($P < 0.001$).

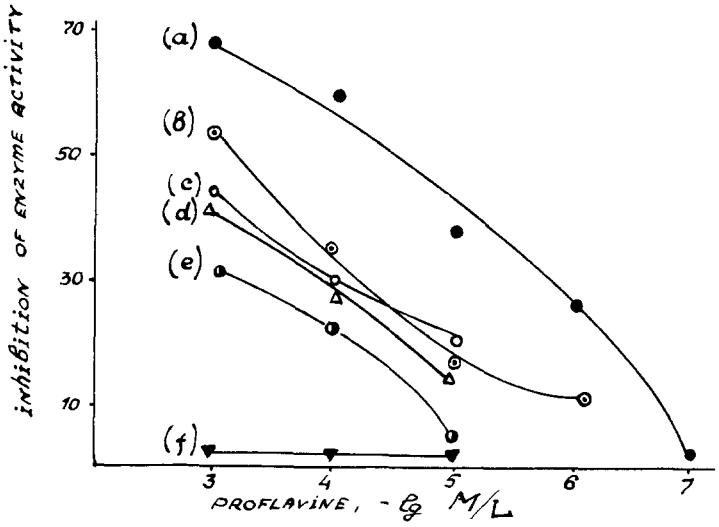

Fig.1 Effect of proflavine on the oxidative deamination of various amines by rat liver mitochondrial MO. The samples contained, in a total volume of 1.8 ml (0.1 M K-phosphate buffer pH 7.4), - 5 mg of MO preparation, proflavine and the following substrates in saturating concentrations (in μM per sample): (a) serotonin - 10, (b) tyramine - 6, (c) dopamine - 6, (d) isoamylamine - 5.5, (e) tryptamine - 6, (f) benzylamine - 8. Incubation in oxygen at 37.5°C for 50 minutes. Fixation, determination of ammonia liberated - see Gorkin and Romanova (1959).

Still higher proflavine concentrations were required to inhibit deamination of tryptamine, isoamylamine or dopamine,

whereas the deamination of benzylamine was practically insensitive to proflavine. The inhibitory effect of proflavine on deamination of serotonin or tyramine was not increased by preincubation (up to 60 minutes at room temperature) of the inhibitor with the MO preparation.

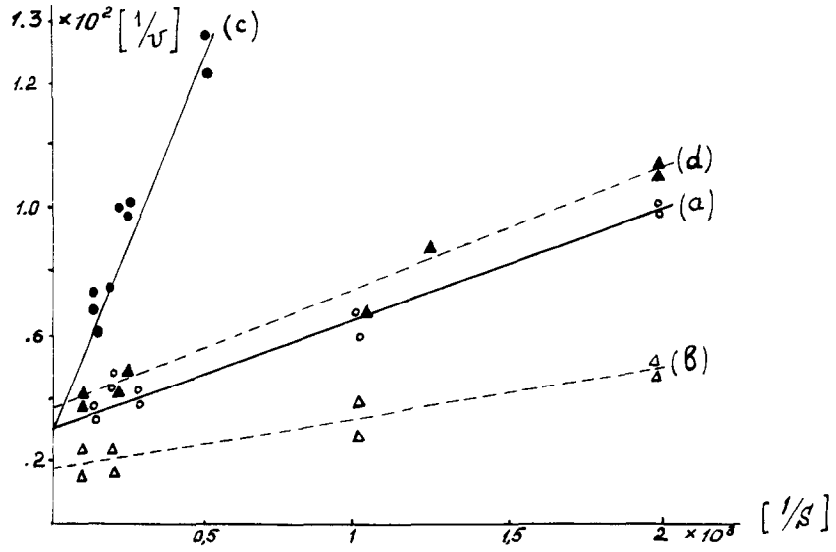

Fig.2 Competitive inhibition of serotonin and non-competitive inhibition of tyramine oxidative deamination by proflavine. Experimental conditions - see fig.1. Results plotted according to Lineweaver and Burk (1934).
Curve (a) - serotonin (no inhibitor), (b) - same for tyramine, (c) - serotonin + proflavine (10^{-5} M/L; final concentration), (d) - same as (c) for tyramine.

Proflavine acts as a competitive inhibitor in the case serotonin deamination catalysed by mitochondrial rat liver amine oxidase. In similar experiments with tyramine as the substrate proflavine causes non-competitive inhibition (fig.2).

Inhibition of the deamination of tyramine by proflavine was not reversed by repeated washing (Udenfriend et al., 1958) of the MO preparation. With serotonin as the substrate we could demonstrate partial reversibility of the inhibitory effect of proflavine (Table I).

TABLE I

Partial reversibility in the case of serotonin and irreversibility in the case of tyramine of the inhibitory effect of proflavine on oxidative deamination

Proflavine concentration (M/L)	Per cent inhibition serotonin		tyramine	
	before washing	after washing	before washing	after washing
8.10^{-4}	83	58.5	30	30
$3.1.10^{-5}$	62	17	-	-

Samples containing in a total volume of 2.7 ml (0.1 M K-phosphate buffer, pH 7.4), 10.5 mg of MO and proflavine were prepared. The MO-containing particles were than washed four times with 2.3 ml of the phosphate buffer (Centrifugations for 10 minutes at 10000 rpm). Control samples of MO without the inhibitor were washed in the same conditions. The suspensions of washed MO particles were made up to initial volume with the phosphate buffer, and aliquots of 1.6 ml were used for incubation with serotonin, resp. tyramine (experimental conditions - see fig.1)

As shown in fig.3, the activity of beef serum amine oxidase (spermine oxidase) is also inhibited by proflavine (I_{50} 6.10^{-5}M). Preincubation of spermine oxidase with proflavine (up to 90 minutes at room temperature) does not increase the inhibitory effect. Proflavine caused competitive inhibition of the oxidation of m-nitro-p-hydroxybenzylamine by this enzyme (fig.4).

DISCUSSION. Selective inhibition by proflavine of the oxidative deamination of various amines usually considered as typical substrates for a single enzyme ("monoamine oxidase") can readily be understood if we assume the existence in mitochondrial membranes of a system of multiple amine oxidases differing in substrate specificities. Competitivity and partial reversibility of the inhibition by proflavine of serotonin deamination (in contrast to the non-competitive and irreversible inhibition of tyramine deamination) is in accordance with this assumption. Partial separation of rat liver mitochondrial amine oxidases solubilized by a non-ionic detergent ("OP-10") was achieved by means of column chromatography on brushite (Gorkin, 1963). Synthetic amines suitable for spectrophotometric determination of MO

activity, namely p-nitrophenylethylamine (Zeller et all., 1962) and m-nitro-p-hydroxybenzylamine (Brusova et all., 1964), were used as substrates in these experiments.

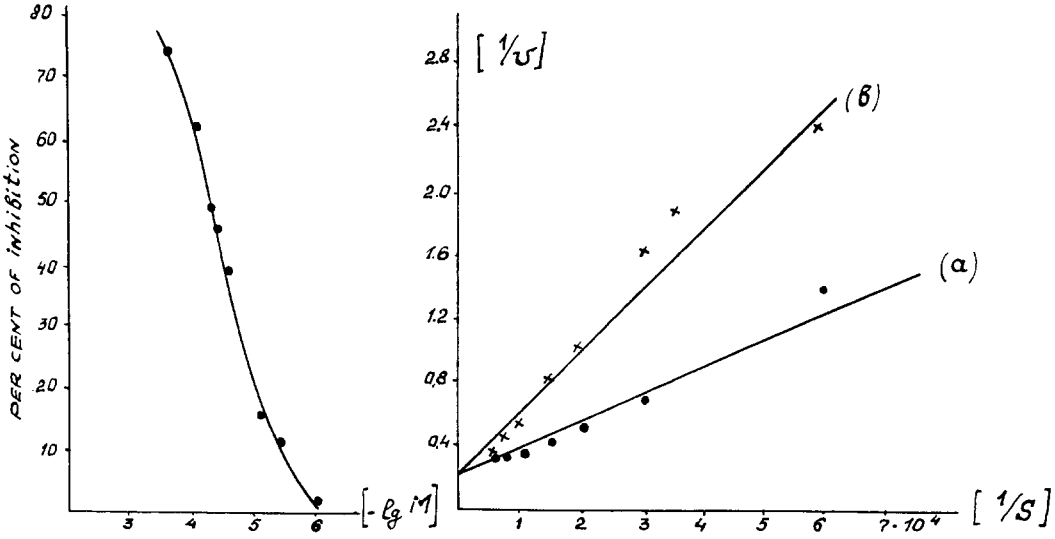

Fig.3 Effect of proflavine on the activity of serum amine oxidase (spermine oxidase). The sample contained in a total volume of 3.0 ml,0.39 mg of partially purified spermine oxidase (sp.act. 74 U/mg),phosphate buffer,pH 7.4 (200 µM) and m-nitro-p-hydroxybenzylamine as substrate (final concentration 7.10^{-5}M/L). Measurements of optical density at room temperature in 10 mm silica cells (SF-4 spectrophotometer) at 315 mµ (Gorkin et all., 1964).

Fig.4 Competitive inhibition of serum amine oxidase activity by proflavine. Experimental conditions - see fig.3. Curve (a) - without inhibitor, (b) - in the presence of proflavine (5.10^{-5}M)

We have recently found that besides proflavine other relatively simple tricyclic compounds, including xanthene, oxazine, azine, phenothiazine and acridine derivatives, also strongly inhibit MO activity (Veryovkina et al.,1964). According to our data (Chodera et al.,1964) harmine inhibits serotonin deamination in much lower concentrations (pI_{50} 7.3) than the deamination of tyramine (pI_{50} 4.62) or of tryptamine (pI_{50} 5.22). Similar results were obtained by L.A.Romanova with nialamid (1-isonicotinyl-2-(benzyl-carboxamidoethyl)-hydrazine). As opposed to these data, no difference in iproniazid (1-isonicotinyl-2-isopropyl hydra-

zine) concentrations inhibitory to the deamination of tyramine, serotonin or benzylamine could be noted. These observations are of interest in connection with reported data on differences in the effects of various MO inhibitors on the concentrations of dopamine,noradrenaline and normetanephrine in rat and mouse brain (Carlsson et al.,1959).

Data on the participation of a flavine component in MO action were published (Sourkes, 1958; Wiseman-Distler and Sourkes, 1963). The inhibitory effect of harmine upon MO has been ascribed (Belleau and Moran,1963) to its interaction with the flavine component (possibly involving charge-transfer complex formation).It seems possible to explain the inhibitory effect of proflavine (as well as of other tricyclic compounds) on the enzymatic activity of mitochondrial amine oxidases in a similar way. However, this interpretation is not necessarily correct, since the deamination of amines by spermine oxidase (beef serum amine oxidase), an enzyme that does not appear to contain a flavine component (Gorkin,1961; Yamada and Yasunobu,1963), is also inhibited by proflavine in rather low concentrations, as demonstrated in experiments reported above, and likewise by the related compound, quinacrine (atebrine; Tabor et al., 1954).

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