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# THE OXIDATION OF DOPAMINE BY THE SEMICARBAZIDE-SENSITIVE AMINE OXIDASE (SSAO) FROM RAT VAS DEFERENS

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**Abstract**—The activities of monoamine oxidase A and B and the semicarbazide-sensitive amine oxidase from rat vas deferens were compared towards benzylamine and dopamine. The selective inhibitors (−)-deprenyl and clorgyline were used to allow the contributions of the A and B forms of monoamine oxidase to be determined separately. Comparison of the kinetic constants of the three enzymes towards dopamine indicated that, although each of them had activity towards this substrate, their relative contributions to the total oxidative deamination would depend on the substrate concentration. At all concentrations in the range 1 μM to 10 mM monoamine oxidase-B would contribute about 50% of the total activity. In the range 1 to 10 μM the contributions made by activities of monoamine oxidase-A and the semicarbazide-sensitive enzyme were similar but at higher concentrations the activity of the latter enzyme became more important, its contribution to the total activity rising to some 35% of the total at 500 μM dopamine. The activity of the semicarbazide-sensitive enzyme towards dopamine might thus be important under conditions where either or both the monoamine oxidases were inhibited in pharmacological studies. Its possible relevance to Norrie disease, in which both forms of the human enzyme are deficient, requires further examination.

Although the recognized neurotransmitter amines are normally regarded as being oxidized by the enzyme monoamine oxidase [monoamine: oxygen oxidoreductase (deaminating) (flavin-containing) EC 1.4.3.4] (MAO) in the tissues (see Ref. 1 for review) another amine oxidase which differs from MAO is also present in many mammalian tissues. This enzyme differs from MAO in being insensitive to the specific acetylenic inhibitors clorgyline and (−)-deprenyl but sensitive towards carbonyl-group reagents such as semicarbazide. It has been variously termed clorgyline-resistant amine oxidase or semicarbazide-sensitive amine oxidase (SSAO) (see Refs 2 and 3 for reviews). Although the enzyme from several species is active towards benzylamine, it is not clear what the physiological substrates are. The enzyme is classified as EC 1.4.3.6 [amine: oxygen oxidoreductase (deaminating) (copper-containing)] but that class appears to be composed of a family of enzymes which are all carbonyl-reagent sensitive but may differ in a number of other respects. For example the semicarbazide-sensitive enzyme present in the plasma seems to differ in properties from the enzyme present in other tissues and there also appear to be considerable species differences (see Refs 2–4).

The recent observation that in some cases of Norrie disease an inherited condition involving a lack of both forms of MAO leads to impairment of 5-hydroxytryptamine (5-HT) metabolism but apparently not that of dopamine [5], suggests that some other activity must be capable of metabolizing the latter amine. There have not been detailed studies on the relative abilities of SSAO and MAO to metabolize this amine, although it has been shown to be a substrate for the SSAO enzymes from human plasma and rat aorta [5, 6]. In the present study we report the oxidation of dopamine by SSAO from rat vas deferens, a particularly rich source of that enzyme, in comparison with the activities of the two forms of MAO.

## MATERIALS AND METHODS

*Preparation of rat vas deferens homogenates.* Rat vasa deferentia were obtained from 250–300 g Sprague–Dawley male rats which had been fasted overnight. Vas deferentia were chopped finely with scissors and homogenized (1:10, w/v) in 50 mM potassium phosphate buffer, pH 7.2, using a mechanical homogenizer (Polytron). The resultant homogenate was filtered through gauze and stored in aliquots at −80°. Before each assay, the preparations were disaggregated by sonication at low frequency for 10 sec.

*Amine oxidase assay.* Amine oxidase activities were assayed radiochemically at 37° by a modification [7] of the method of Otsuka and Kobayashi [8], using the following substrates [$^{14}$C]benzylamine (sp. act. 10 mCi/mmol) and [$^{14}$C]dopamine (2 mCi/mmol). Unless otherwise stated the reaction mixture contained, in a final volume of 225 μL, 50 mM potassium phosphate buffer, pH 7.2, 200 μg of protein and 25 μL of radioactively labeled substrate. The reaction was stopped by adding 100 μL of 2 M

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citric acid, and the products were extracted into 1:1 (v/v) toluene/ethyl acetate containing 0.6% (w/v) 2,5-diphenyloxazole (PPO). The radioactivity of the organic extract was determined by liquid scintillation counting. In all cases time-courses were used to ensure that activity determinations were made in the region where product formation was occurring linearily with time.

In studies of the effects of inhibitors the enzyme preparation was incubated with the inhibitor, at the stated concentration for 30 min at 37° before the reaction was started by the addition of substrate. For kinetic studies MAO-A and MAO-B activities were inhibited by preincubation under the above conditions with 0.5 μM clorgyline or 0.5 μM (−)-deprenyl, respectively, and SSAO activity was inhibited by preincubation with 1 mM semicarbazide. Control samples were incubated under the same conditions in the absence of inhibitor to ensure that no significant loss of activity occurred during this period. To ensure that semicarbazide did not significantly affect the extraction of the aldehyde products of amine oxidation [9] assays were carried out in the absence of semicarbazide and that compound was added after termination of the reaction by acidification with 2 M citric acid [7]. The results indicated that this addition had no effect on the measured extends of product formation from the amine substrates under the conditions used.

Kinetic constants were determined from the variation of initial velocity with respect to substrate concentration by use of non linear regression analysis [10]. Double-reciprocal plots are used for illustration purposes only.

*Protein assay.* Protein concentration was determined by the Hartree method [11] with bovine serum albumin as standard.

*Materials.* [7-$^{14}$C]Benzylamine hydrochloride and [2s-$^{14}$C]dopamine hydrochloride, were purchased from Amersham International (Amersham, U.K.). 2,5-Diphenyloxazole (PPO) was purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). All other compounds were standard, analytical-grade laboratory reagents.

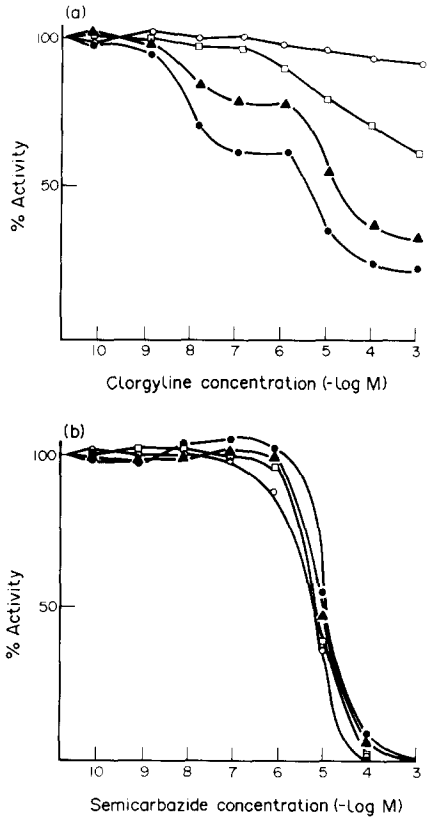


Fig. 1. Inhibition of amine oxidase activities from rat vas deferens by different concentration of clorgyline (a) and semicarbazide (b) enzyme samples were incubated with the inhibitor at the concentration indicated for 30 min at 37° before assay of the activity towards benzylamine at 1 μM (○) or 64 μM (□) and towards dopamine at 100 μM (●) or 500 μM (▲). For the studies on the inhibition by semicarbazide (b) the monoamine oxidase activities were inhibited by preincubation with 1 mM clorgyline for 30 min at 37°. Each point is the mean value of the percentage activity remaining, determined from three experiments.

## RESULTS AND DISCUSSION

Figure 1a shows the inhibition of the oxidation of dopamine and benzylamine (BZ) at two different concentrations by clorgyline. It can be observed that preincubation with 1 mM clorgyline inhibited the oxidation of 1 μM benzylamine by only about 10%. Thus BZ at this concentration is mainly (90%) metabolized by SSAO activity. Similar results have been described in other rat tissues (see Ref. 12). The inhibition curves in Fig. 1a also show that MAO-B contributes the remaining 10% of activity towards this substrate. When the BZ concentration was increased to 64 μM the contribution of MAO-B was about 38% and the contribution of SSAO activity was about 62%. Studies on the inhibition by semicarbazide (Fig. 1b) demonstrated that the clorgyline-resistant activity was completely inhibited by preincubation for 30 min at 37° with 1 mM semicarbazide.

The inhibition curves for the activities towards two different concentrations of dopamine (Fig. 1a) showed that MAO-A, MAO-B and the clorgyline-resistant, semicarbazide-sensitive, activities all contributed to the oxidation of this substrate. The SSAO activity was responsible for about 24% of the oxidative activity at a dopamine concentration of 100 μM and 35% at 500 μM dopamine. The values for the contribution of SSAO were confirmed by studies on the sensitivity by semicarbazide (Fig. 1b). Such values only give an approximate picture of the contributions of the different amines since they were determined at two fixed substrate concentrations and take no account of the kinetic parameters of the different enzymes (see Ref. 7).

The kinetic parameters of SSAO and MAO-B towards benzylamine were determined by the use of the selective inhibitors and the substrate concentration ranges were 1 to 64 μM for SSAO and 10 to 200 μM for MAO-B. The values obtained are shown in Table 1 and are consistent with the relative contributions of these two enzymes determined from

Table 1. Kinetic constants of MAO-A, MAO-B and SSAO for the metabolism of dopamine

| Enzyme | Substrate | $K_m$ ($\mu$M) | $V_{max}$ (pmol/min/mg protein) | $V_{max}/K_m$ |
|---|---|---|---|---|
| MAO-A | DA | 383 ± 42 | 647 ± 27 | 1.69 |
| | BZ | — | N.D. | — |
| MAO-B | DA | 960 ± 96 | 2470 ± 70 | 2.57 |
| | BZ | 54 ± 5 | 137 ± 9 | 2.53 |
| SSAO | DA | 1058 ± 122 | 1692 ± 185 | 1.59 |
| | BZ | 4.5 ± 0.7 | 183 ± 7 | 40.66 |

Values were determined from initial rate data obtained at 37° by the procedures described in the text.

Abbreviations: BZ, benzylamine; DA, dopamine; ND, not detectable.

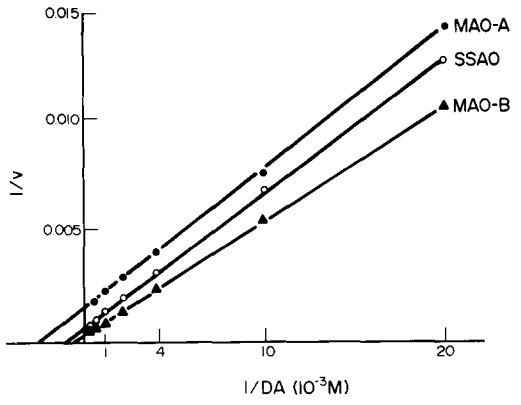


Fig. 2. Double-reciprocal plots for the deamination of dopamine (DA) by MAO-A, MAO-B and SSAO from rat vas deferens. The assay conditions are described in the text. Velocity is expressed in pmol/min/mg protein. Each point is the mean of three determinations.

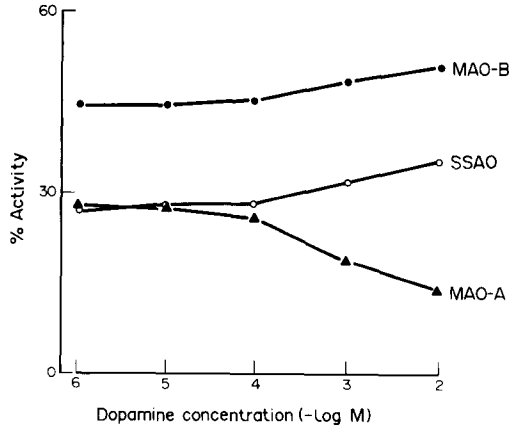


Fig. 3. Contributions of MAO-A, MAO-B and SSAO activities from rat vas deferens to dopamine oxidation calculated from the data shown in Table 1.

the data in Fig. 1. The kinetic parameters of MAO-A, MAO-B and SSAO towards dopamine were estimated in a similar way with various concentrations this substrate (0.05–4 mM for MAO-B and SSAO and 0.05–2 mM for MAO-A). Figure 2 shows representative double-reciprocal plots for these enzymes towards this substrate. The kinetic parameters determined for the activities of the individual enzymes are shown in Table 1. This result shows that MAO-A has the lowest $K_m$ value towards dopamine, with MAO-B and SSAO having similar $K_m$ values. However, MAO-B and SSAO have considerably higher $V_{max}$ values than MAO-A.

When different enzymes compete for the same substrate their relative contributions to the overall metabolic flux will depend on the substrate concentration and the ratio $V_{max}/K_m$ for each enzyme [13]. Thus despite the relatively high $K_m$ value of SSAO towards dopamine its correspondingly high $V_{max}$ value means that it could make a significant contribution to dopamine oxidation in this tissue. Figure 3 shows curves, calculated from the data in Table 1, of the contributions of the three enzymes as a function of dopamine concentration. They show that, although the activity of MAO-B will make the major contribution to dopamine oxidation in rat vas deferens, the contributions of the semicarbazide-sensitive activity will be significant, over a wide range of dopamine concentration. Such studies take no account of possible effects of compartmentation on substrate accessibility. However, there is no indication that the localization of MAO (mitochondrial outer membrane, see Ref. 14) and SSAO (microsomal or plasma membrane, see Ref. 15) significantly affect substrate access.

In relation to the deficiencies of Norrie disease further work will be necessary to show whether the enzymes in human tissues show similar properties such that SSAO activity might be capable of compensating for the lack of MAO activities.

## REFERENCES

1. Youdim MBH, Finberg JPM and Tipton KF, Monoamine oxidase. In: *Handbook of Experimental Pharmacology* (Eds. Trendelenburg U and Weiner N), Vol. 90, pp. 119–192. Springer, Berlin, 1988.
2. Callingham BA, Resolution of amine oxidase activities by irreversible inhibitors. In: *Monoamine Oxidase:*

*Basic and Clinical Frontiers* (Eds. Kamijo K, Usdin E, and Nagatsu T), pp. 100–111. Excerpta Medica, Amsterdam, 1982.
3. Lewinsohn R, Mammalian monoamine-oxidizing enzymes, with special reference to benzylamine oxidase in human tissues. *Brazilian J Ed Biol Res* **17**: 223–256, 1984.
4. McEwen CM, Human plasma monoamine oxidase. I. Purification and identification. *J Biol Chem* **240**: 2003–2010, 1965.
5. Murphy DL, Sims KB, Karoum F, de la Chapelle A, Norio R, Sankila EH and Breakefield XO, Marked amine and amine metabolite changes in Norrie disease with an X-chromosomal deletion affecting monoamine oxidase. *J Neurochem* **54**: 242–247, 1990.
6. Elliot J, Callingham BA and Sharman DF, Semicarbazide-sensitive amine oxidase (SSAO) of the rat aorta. *Biochem Pharmacol* **38**: 1507–1515, 1989.
7. Fowler CJ and Tipton KF, Concentration-dependence of the oxidation of tyramine by the two forms of rat liver mitochondrial monoamine oxidase. *Biochem Pharmacol* **30**: 3329–3332, 1981.
8. Otsuka D and Kobayashi Y, A Radioisotopic assay for monoamine oxidase determinations in human plasma. *Biochem Pharmacol* **13**: 995–1006, 1964.
9. Lyles GA and Shaffer CJ, Substrate specificity and inhibitor sensitivity of monoamine oxidase in rat kidney mitochondria. *Biochem Pharmacol* **28**: 1099–1106, 1979.
10. Leatherbarrow RJ, *Enzfitter: A Non-linear Regression Data Analysis Program for the IMB PC*. Elsevier, Amsterdam, 1987.
11. Hartree EF, Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Anal Biochem* **48**: 422–427, 1972.
12. Callingham BA and Barrand MA, Some properties of semicarbazide-sensitive amine oxidase. *J Neural Trans* Suppl 23: 37–54, 1987.
13. Dixon M and Webb EC, *Enzymes* (3rd edn.), pp. 72–75. Longman, London, 1979.
14. Schnaitman C, Erwin VG and Greenawalt JW, The submitochondrial localization of monoamine oxidase. *J Cell Biol* **32**: 719–725, 1967.
15. Barrand MA and Callingham BA, Monoamine oxidase activities in brown adipose tissue of the rat: some properties and subcellular distribution. *Biochem Pharmacol* **31**: 2177–2184, 1982.