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SHORT COMMUNICATIONS

Clorgyline and deprenyl insensitive monoamine oxidase in rat brain soluble fraction

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Monoamine oxidase, MAO (monoamine:O_2 oxidoreductase, EC 1.4.3.4) has been widely studied and characterized in different tissues from several species and the results obtained have been interpreted as indices for the existence of multiple forms of the enzyme [1, 2]. The original report by Johnston [3], demonstrated that the two forms of MAO could be differentiated on the basis of their sensitivity to inhibition by clorgyline.

In the present study, another form of MAO in the soluble fractions is reported, which is distinctly different from the well-established MAO forms associated with the outer mitochondrial membrane with regards to its inhibitor sensitivity. To study the MAO associated with the soluble fraction as distinct from the two mitochondrial forms, MAO-A and MAO-B, a partially purified mitochondrial preparation was used for comparison.

Materials and methods

The whole brain homogenates were prepared as described earlier [4]. The subcellular fractionation was performed according to the method of Student and Edwards [5]. A crude nuclear pellet fraction (P_1) was obtained by centrifugation at 1000 *g* for 10 min. The resulting supernatant (S_1) was centrifuged at 14,000 *g* for 15 min to yield the crude mitochondrial pellet (P_2) and the supernatant fraction (S_2). Centrifugation of S_2 at 70,000 *g* for 1 hr gave a microsomal fraction (P_3) and a cell soluble fraction (S_3). Pure mitochondrial pellet (M) was obtained from the fraction (P_2) using Ficoll sucrose discontinuous density gradient centrifugation as described by Student and Edwards [5]. The mitochondrial sonicate was prepared according to the method of Fowler *et al.* [6] and the 'high speed supernatant' thus obtained was further purified by gel filtration on Sephadex G-25, to yield an enzyme preparation of activity 2.30 units/mg protein. The supernatant fraction S_2 (1.08 units/mg protein), microsomal fraction P_3 (0.75 units/mg protein), partially purified cell soluble fraction S_3 (0.88 units/mg protein), the mitochondrial membrane vesicles M (1.17 units/mg protein) and the 'high speed supernatant' (2.30 units/mg protein) were used for MAO assay according to the method of Catravas *et al.* [7].

For *in vitro* studies, clorgyline was added to the assay mixture at the final concns of 10^{-1}–10^{-10} M, and was preincubated with the enzyme preparation for 30 min, before the addition of substrate kynuramine to allow for the irreversible inhibition of MAO-A [8]. For *in vivo* studies, rats were given an intramuscular injection of both clorgyline and deprenyl (12 mg/kg body wt) 2 hr before sacrifice to achieve maximum inhibition of MAO-A and MAO-B. The control rats were injected with 0.9% saline. Subcellular fractionation of the brains was then carried out as described above and assayed for MAO activity.

One unit of enzyme activity is defined as 1 μmole 4-hydroxyquinoline formed/90 min/g of tissue. Protein was estimated by the method of Lowry *et al.* [9].

Clorgyline, [*N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy)propylamine hydrochloride, M and B 9302] was kindly provided by Dr. R. A. Robinson of May & Baker, Dagenham, U.K. (−)-Deprenyl (phenyl-isopropylmethyl propionlyamine hydrochloride, E-250) was a gift from Dr.

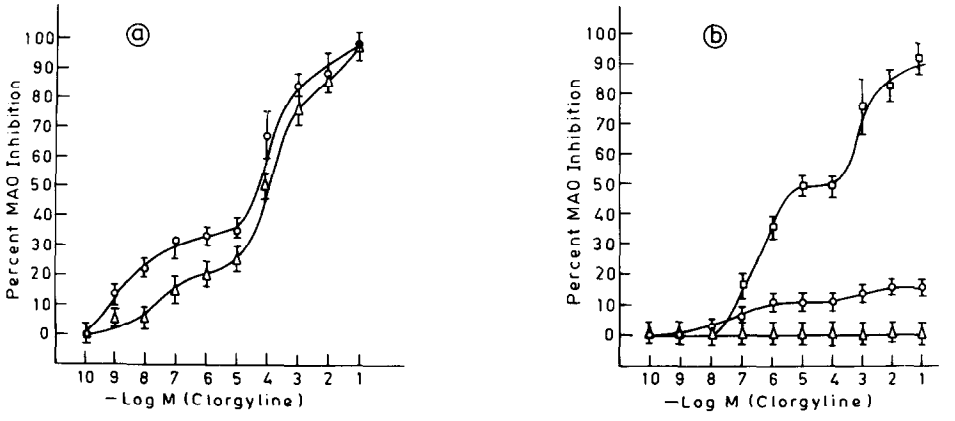

Fig. 1. (a) Percentage inhibition of MAO activity towards kynuramine by various concns of clorgyline. (○) The pure mitochondrial and (△) 'high speed supernatant' fractions obtained from the sonicated mitochondrial fraction were preincubated at 37° for 30 min in the presence of different concns of clorgyline in a total vol. of 3 ml prior to addition of kynuramine. MAO activity is expressed as μmoles/g/90 min. Each point is the mean ± S.D. of three determinations. (b) Percentage inhibition of MAO activity towards kynuramine by various concns of clorgyline. (○) The supernatant fraction, S_2, (△) the soluble fraction, S_3, and (□) the microsomal fraction, P_3, were preincubated at 37° for 30 min in the presence of different concns of clorgyline in a total vol. of 3 ml prior to addition of kynuramine. MAO activity is expressed as μmoles/g/90 min. Each point is the mean ± S.D. of three determinations.

Table 1. *In vivo* effect of inhibitors on the activity of monoamine oxidase in different subcellular fractions of rat brain

Subcellular fraction	Control rats (μmoles/g/90 min)	Clorgyline + deprenyl treated rats (μmoles/g/90 min)
Whole homogenate	25.05 ± 1.46	7.52 ± 1.08 (70%)
Nuclear pellet (P_1)	18.14 ± 2.14	1.90 ± 0.90 (89%)
Supernatant fraction (S_1)	16.89 ± 0.90	4.80 ± 0.20 (72%)
Crude mitochondrial fraction (P_2)	18.43 ± 0.80	1.82 ± 0.20 (90%)
Mitochondrial fraction (M)	15.84 ± 0.86	1.44 ± 0.44 (91%)
Synaptosomal fraction	11.08 ± 0.12	2.42 ± 0.40 (78%)
Myelin	10.45 ± 0.66	2.00 ± 0.60 (81%)
Supernatant fraction (S_2)	12.96 ± 0.44	10.05 ± 0.72 (22%)
Microsomal fraction (P_3)	5.76 ± 0.16	0.36 ± 0.10 (94%)
Soluble fraction (S_3)	9.72 ± 1.12	9.36 ± 0.82 (4%)
'High speed supernatant' fraction	15.12 ± 0.90	0.36 ± 0.10 (98%)

Each value is a mean ± S.E.M. of three determinants. Values in parentheses represent the percentage inhibitions of MAO in clorgyline + deprenyl treated rats.

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Results and discussion

The inhibition of MAO by clorgyline in the mitochondrial and supernatant fraction, S_2, was biphasic when kynuramine was used as a substrate (Figs. 1a and 1b). The MAO activity from the mitochondrial fraction, M, was inhibited by 98%. If the supernatant fraction, S_2, were to have both A and B forms of MAO then inhibition similar to that in the mitochondrial preparation would be expected, but the inhibition was only 22%. This observation leads us to believe that the S_2 fraction, apart from having the clorgyline-sensitive MAO activity, which shows the usual biphasic dose-response to increasing concn of the inhibitor, may also contain a clorgyline-insensitive form of MAO.

The presence of the form of MAO in the supernatant fraction, S_2, which is sensitive to clorgyline inhibition was earlier reported by Student and Edwards [5], who in their *in vivo* studies showed that MAO-A and MAO-B activities were 3% and 3.5%, respectively, of the total MAO activity in S_2. The recovery of the total MAO activity in our experiments from the mitochondrial fraction, M, is found to be approx. 35%, which is almost consistent with the earlier reports of approx. 30.5% [5]. The figure for the supernatant fraction, S_2, reported by Student and Edwards, including forms A and B was 6.5%, compared with the recovery of supernatant MAO activity of 24–30% in our studies (including forms A and B and an inhibitor-insensitive enzyme).

To find out the nature of this MAO activity present in the supernatant fraction, S_2, which is insensitive to clorgyline inhibition, the *in vitro* inhibition by clorgyline studied in the 'high speed supernatant' obtained from the sonicated mitochondrial fraction gave the same biphasic dose-response kinetics as the non-sonicated mitochondrial fraction. However, the soluble fraction, S_3, did not show the same biphasic curve as that of the parent supernatant fraction, S_2. The activity was found to be localized in the microsomal pellet fraction, P_3, which showed the usual biphasic patterns (Figs. 1a and 1b). The inhibition was nearly 100% in the microsomal fraction and no inhibition was observed in the soluble fraction, S_3. These results, therefore show the presence of a new form of MAO localized in the soluble fraction, which is insensitive to clorgyline inhibition.

The results from the *in vivo* studies showed that the MAO activity in the mitochondrial preparation, M (which is believed to have A and B forms of MAO), was inhibited by 91% while the inhibition was about 30% in the supernatant fraction, S_2, by clorgyline. This confirms our *in vitro* finding, where the usual biphasic dose-response curve was obtained, but the inhibition was not 100% (Table 1). The MAO activity was inhibited 98% in the 'high speed supernatant' obtained from the mitochondrial fraction, while it remained unaffected in the soluble fraction, S_3. The enzyme activity in the microsomal fraction, however, showed an inhibition of 94% as observed during our *in vitro* experiments.

It may be speculated that the ineffectiveness of some of the antidepressant drugs, which act by inhibiting the monoamine oxidase, may be attributed to the fact that these inhibitors of MAO-A and MAO-B may not be inhibiting the enzyme totally, i.e. both the mitochondrial and the soluble forms, thereby causing a partial effect by inhibiting only the mitochondrial forms; or, that the process of inhibition may be mediated through specific drug receptors present on the particulate fractions of the cell, the drug being unable to inhibit the enzyme in the soluble fraction.

Evidence is presented to show that the soluble fraction of the rat brain contains a MAO form which is different from MAO-A and MAO-B in its sensitivity to MAO inhibitors, namely clorgyline and deprenyl. This MAO can be termed as (1) soluble MAO (MAO-S) or (2) unsedimentable MAO (?). Further studies to ascertain the above are in progress.

In summary, a substantial percentage of monoamine oxidase activity was found in the soluble fraction of rat brain homogenates. The enzyme was not inhibited *in vivo* by high doses of clorgyline and deprenyl (12 mg/kg for 2 hr). Monoamine oxidase activity associated with the soluble fraction did not show the usual biphasic dose-response kinetics with clorgyline when kynuramine was used as the substrate. The present data show that there may be a new form of monoamine oxidase associated with the soluble fraction of the cell which has properties different from the two well-known monoamine oxidases A and B.

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REFERENCES

1. M. B. H. Youdim, *Br. Med. Bull.* **29**, 120 (1973).
2. M. D. Houslay, K. F. Tipton and M. B. H. Youdim, *Life Sci.* **19**, 467 (1976).
3. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
4. C. S. K. Mayanil, S. M. I. Kazmi and N. Z. Baquer, *J. Neurochem.* **38**, 179 (1982).
5. A. K. Student and D. J. Edwards, *Biochem. Pharmac.* **26**, 2337 (1977).
6. C. J. Fowler, B. A. Callingham, M. D. L. O'Connor and E. K. Matthews, *Biochem. Pharmac.* **29**, 1185 (1980).
7. G. N. Catravas, J. Takenaga and C. G. Mchale, *Biochem. Pharmac.* **26**, 211 (1977).
8. C. J. Fowler, B. A. Callingham, T. J. Mantle and K. F. Tipton, *Biochem. Pharmac.* **27**, 97 (1978).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).

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Regulation of brain and hepatic glutathione-*S*-transferase by sex hormones in rats

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The presence of glutathione-*S*-transferase (GST)* activity in mammalian and avian brains and its characterization in rat brain has been reported by us recently [1, 2]. Brain and hepatic GSTs were found to catalyze the conjugation of acrylamide with GSH [3] and to bind this potent neurotoxin [4]. The enzyme may therefore play a biological role in removal and expression of the toxicity of certain neurotoxic xenobiotics.

The brain regulates the secretion of hormones in pre- and post-pubertal animals. Recent studies by Lamartiniere [5] have shown that the hypothalamic–hypophyseal–gonadal axis plays an important role in the sexual differentiation and regulates the hepatic GST activity in the rat. Evidence has been presented to show that the hypothalamic nerve endings of female rats exposed to androgen during the critical period of early development result in the programming of a male type of metabolism that is expressed post-pubertally via the hypothalamic–hypophyseal–gonadal axis [6–10]. Therefore it would be of potential interest to investigate whether brain GST is regulated by these sex hormones. The present study deals with some of our observations on the regulation of brain and hepatic GSTs by sex hormones.

Material and methods

The experiments were performed on young and post-pubertal Wistar albino rats derived from the ITRC animal breeding colony. The animals were raised on a commercial pellet diet (Hindustan Lever, Bombay, India) and allowed free access to water. The litters were kept with their mothers until weaning, i.e. until 3 weeks of age (five pups with one mother per cage). After weaning five animals were housed in a cage.

Post-pubertal male rats (12 weeks old) were castrated by removing both the testicles after anaesthetising the animals with ether. Anaesthetic ether was found to produce no significant change in GST activity of the brain and liver. Castration or sham operations in neonatal (1-day-old) male rats were performed by placing them in an ice-water bath prior to surgery.

TP and DES [each from Steriod Inc. (Wilton, NH)] were dissolved in peanut oil and injected at a dose of 2.5 mg/kg body weight subcutaneously in the adult rats daily for 7 days beginning 1 week after castration. The control rats received the same volume of vehicle. All the animals were killed 7 days after the last treatment. In the case of neonatal rats the pups were given TP and/or DES from day 7 to 13 of their life and killed by decapitation at 3 and 9 weeks of age.

The brain and liver homogenates were centrifuged at 14,000 and 9000 *g* for 15 min respectively to obtain post-mitochondrial fractions which were used for the measurement of GST activity by the method of Habig *et al.* [11].

GSH and protein contents were estimated according to the methods of Ellman [12] and Lowry *et al.* [13] respectively.

Results

Effect of neonatal castration and influence of sex hormones on brain and hepatic GST activity in pre- and post-pubertal rats.

At prepubertal (3 weeks) age, brain and hepatic GST activity towards CDNB did not show any significant difference between male and female rats (Fig. 1 and Table 1). The GST activity in both tissues remained unaffected on neonatal castration at prepubertal age but administration of TP and DES to castrated pups induced enzyme activity significant in comparison to uncastrated male or female animals (Fig. 1 and Table 1). No change in brain or hepatic GSH content was observed on castration or treatment of castrated rats with TP or DES (data not shown).

Fig. 2 demonstrates that brain GST activity towards CDNB of male rats at post-pubertal age (9 weeks) was significantly lower than that of females. Neonatal castration

* Abbreviations: GST, glutathione-*S*-transferase; GSH, glutathione; TP, testosterone propionate; DES, diethystilbestrol; CDNB, 1-chloro-2,4-dinitrobenzene.