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Selective influences of age and thyroid hormones on Type A monoamine oxidase of the rat heart

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The specific activity of rat heart MAO, towards both tyramine and benzylamine as substrates, was found to increase with the age of the animal, and also after administration of (−)-thyroxine to young male rats. Conversely, enzyme activity was decreased in animals made hypothyroid by including 2-thiouracil in their diet. However, with both age and altered thyroid status, relatively greater changes in the deamination of tyramine rather than in that of benzylamine, were obtained. Clorgyline and deprenyl, used as inhibitors of rat heart MAO, indicated that tyramine is metabolized solely by MAO-A, whereas benzylamine is a substrate for both MAO-A and -B, and also a clorgyline- and deprenyl-resistant enzymic activity. The proportional contribution of MAO-A, -B and the clorgyline-resistant enzyme towards the total benzylamine deamination in the rat heart was found to vary with the age and with altered thyroid status of the animal in such a way that selective changes in the activity of MAO-A appear to be largely responsible for the overall changes in the specific activity of rat heart MAO which occur in response to these developmental factors.

The enzyme monoamine oxidase (MAO, EC 1.4.3.4) is located predominantly in the outer mitochondrial membrane and is responsible for the oxidative deamination of a variety of biogenic amines. The activity of this enzyme may be influenced, in some animal tissues, by both the development and the hormonal status of the animal (see Youdim & Holzbauer 1976 for review).

It is well documented that the specific activity of this enzyme in rat heart shows an increase with the age of the animal at least up to about 25 weeks or 600g (Novick 1961; Prange et al 1967; Horita 1967; de Champlain et al 1968; Vaccari et al 1972; Inagaki & Tanaka 1974; Lowe et al 1975). Similarly, an increase in cardiac MAO activity has been found to occur after administration of thyroid hormones to the rat, particularly the young animal (Novick 1961; Utley 1964; Ho-van-Hap et al 1967; Lyles & Callingham 1974; Moonat et al 1975; Tong & D'Iorio 1976). Conversely induction of the hypothyroid state produces a decrease in MAO activity (Dubnick et al 1960; Skillen et al 1962; Lyles & Callingham 1974).

In many of the earlier studies, the changes in cardiac MAO activity brought about by age and thyroid state were assessed by the use of a single amine as substrate. However, MAO is known to exist in at least two catalytically separable forms, called MAO-A and MAO-B, which differ in their relative sensitivities towards the inhibitors clorgyline and deprenyl (Johnston 1968; Knoll & Magyar 1972), and which also show differences in their substrate specificities, depending upon the animal tissue studied (see Fowler et al 1978 for review). In the rat heart, we have previously shown that tyramine is a substrate for MAO-A, whereas benzylamine is metabolized by both MAO-A and MAO-B. Also, the increase in activity of rat cardiac MAO in response to administration of thyroid hormone appeared to be greater in magnitude when tyramine was used as substrate than with benzylamine, which would suggest that there was a selective increase in the MAO-A component (Lyles & Callingham 1974).

We have used both tyramine and benzylamine as substrates in a more detailed investigation of the changes produced in the activities of MAO-A and -B in the rat heart in response to changes in the age and thyroid status of the animal. Some of these results have previously been communicated in a preliminary form (Callingham & Lyles 1975).

MATERIALS AND METHODS

Materials

Radioactive substrates for MAO, [^{3}H]tyramine and [^{14}C]benzylamine were obtained from the Radiochemical Centre, Amersham, U.K., and ICN Pharmaceuticals, Hersham, U.K., respectively.

Clorgyline hydrochloride (M & B 9302) was a gift from May & Baker Ltd, Dagenham, U.K. and (±)-deprenyl hydrochloride was a gift from Professor J. Knoll, Institute of Pharmacology, University Medical School, Budapest, Hungary. Pargyline hydrochloride

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was a gift from Abbott Laboratories, Queenborough, U.K. (−)-thyroxine and 2-thiouracil were from Sigma London, U.K. Other reagents were analytical grade where possible. Male Wistar rats were from A. J. Tuck & Son, Rayleigh, U.K.

Methods

Rats were weighed individually and killed by cervical dislocation. Hearts were dissected, washed in 0·9% sodium chloride solution (saline), blotted and larger blood vessels removed. After having been weighed, each heart was stored deep-frozen in saline until used. Before assay, each heart was homogenized in 1 mM potassium phosphate buffer, pH 7·8 (1 : 10 w/v) in a conical glass homogenizer. The homogenate was centrifuged at 600*g* for 10 min to remove nuclei and cell debris. The supernatant was decanted and used as the source of MAO for immediate assay. Subsequent inhibition studies were performed upon thawed samples of heart homogenates that had been deep-frozen immediately after preparation.

MAO activity was assayed radiochemically by the method of McCaman et al (1965), as modified by Callingham & Laverty (1973), with tyramine and benzylamine as substrates at 1 mM concentrations. This concentration saturated MAO activity with respect to these substrates, without producing high substrate inhibition. Protein contents of homogenates were estimated by the microbiuret method (Goa 1953). Statistical significance was tested by the Wilcoxon rank sum test (Goldstein 1967).

RESULTS

Developmental changes in the specific activity of rat heart MAO

Rats of approximately 40, 60, 110, 160, 210, 300 and 500 g (3–30 weeks old), separately grouped, were used to investigate the possibility of changes in cardiac MAO activity occurring during growth. Rats weighing 40 g were chosen as the lower limit of the weight range, since these were the youngest rats after weaning which could be reliably shipped by our animal supplier. Mean heart weights and corresponding body weights are shown in Table 1.

MAO activity was assayed in each rat heart homogenate with tyramine and benzylamine as substrates. When the relationship between enzyme activity and body weight was plotted as mean values for each group (Fig. 1), the specific activity of MAO towards both substrates showed an increase with growth, but the relative size of the increases was dependent upon the substrate used. Rats from the 500 g group had mean MAO activities greater than those of the 40 g group, by factors of 21·6 and 6·1 for tyramine and benzylamine respectively (Table 1).

Table 1. Comparison of the relative changes in specific activity of rat heart MAO towards tyramine and benzylamine with increasing body weight. Mean cardiac MAO activities from the various groups of rats used to obtain the data of Fig. 1, are expressed relative to the activity for the corresponding substrate in the smallest group studied (42 g). * corresponds to 45·6 nmol (mg protein)$^{-1}$ h^{-1}; ** corresponds to 5·7 nmol (mg protein)$^{-1}$ h^{-1}.

Body wt (g ± s.e.m.)	Heart wt (mg ± s.e.m.)	Relative MAO specific activity: Tyramine	Benzylamine
42 ± 1	208 ± 5	1·0*	1·0**
62 ± 2	289 ± 22	1·2	1·1
109 ± 2	440 ± 9	1·7	1·2
160 ± 2	585 ± 27	3·5	1·7
208 ± 2	716 ± 14	5·8	2·1
302 ± 2	855 ± 14	16·9	4·2
489 ± 11	1287 ± 43	21·6	6·1

These substrate-dependent differences appear to arise largely during the early stages of growth. For example, between the 40 g rats and 210 g rats, MAO activity increased by a factor of 5·8 for tyramine and 2·1 for benzylamine. In contrast, between the 210 g and the 500 g rats the corresponding increases were by factors of 3·7 and 2·9 respectively, suggesting that the increases in activity towards the two substrates occurred at a similar rate.

Effects of age upon irreversible inhibition of rat cardiac MAO

MAO activity was assayed in rat heart homogenates in the presence of various concentrations (5×10^{-11}–5×10^{-4} M) of the MAO inhibitors clorgyline and deprenyl. Samples of the homogenates were pre-incubated with the inhibitor for 20 min at 37 °C be-

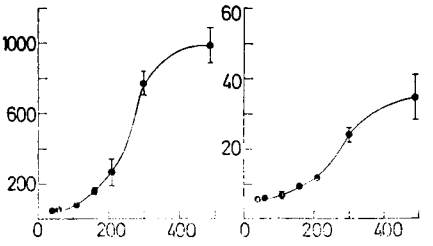

FIG. 1. Developmental changes in specific activity, of rat heart MAO. Left panel, tyramine; right panel, benzylamine as substrate. MAO activities (ordinate: nmol mg protein^{-1} h^{-1}) were assayed in quadruplicate for each heart and are expressed as mean ± s.e., n = 5–9 animals in each group. Abscissa: body weight (g).

fore the addition of substrate for assay of remaining MAO activity. Enzyme activities of inhibited samples were expressed as percentages of untreated controls, and plotted against the negative log of inhibitor concentration.

The inhibition by clorgyline of MAO activity towards tyramine was studied in two groups of rats with mean body weights of 41 and 385 g (Fig. 2). For each group a single-sigmoid curve for the inhibition of tyramine deamination was obtained, and on the basis of the original classification of MAO into two enzyme types with differing relative sensitivities towards clorgyline (Johnston 1968), it would appear

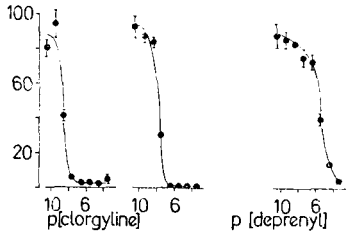

FIG. 2. In vitro inhibition of tyramine deamination by clorgyline and deprenyl in homogenates of hearts from ageing rats. The inhibition curves (from left to right) correspond to homogenates of hearts from rats of mean body weights 41, 385 and 385 g, respectively. Each curve represents the mean ± s.e. of the ratio, derived from duplicate estimations of individual homogenates from 3–4 animals. Inhibitor concentrations (expressed as negative logarithms) range from 5×10^{-11} M to 5×10^{-4} M. Activities are expressed as percentages of uninhibited activity of their appropriate controls. Ordinate: MAO activity (%).

that MAO-A is responsible for the metabolism of tyramine in the hearts of these young (41 g) and older (385 g) rats. This finding is in agreement with our earlier conclusion that MAO-A is also responsible for cardiac deamination of tyramine in rats of about 130g (Lyles & Callingham 1974).

The sensitivity towards inhibition by deprenyl was also studied with the heart homogenates from the 385 g rats. While a single-sigmoid inhibition curve was again obtained, higher concentrations of deprenyl than of clorgyline were required to inhibit the tyramine deamination. As MAO-A in animal tissues shows a low sensitivity towards inhibition by deprenyl (Knoll & Magyar 1972), the present results provide additional evidence that tyramine is deaminated by MAO-A in these hearts.

In contrast to the relatively simple nature of the inhibition curves for MAO activity towards tyramine, the shapes of the corresponding plots for the inhibition of benzylamine metabolism by clorgyline and deprenyl were more complex, and depended upon the age of the animals studied. With clorgyline (Fig. 3), in agreement with earlier findings (Lyles & Callingham 1974), a double-sigmoid inhibition curve for cardiac MAO was obtained in 151 g rats, indicating that benzylamine can be deaminated by both MAO-A and -B in this tissue. However, the relative proportions of these activities appeared to alter with age. The proportion of MAO-A activity seen in younger rats (63 g), was smaller, and in the youngest group studied (36 g) the inhibition curve was essentially a single-sigmoid, indicating inactivation of MAO-B alone. As the rats grew older, an increasing proportion of MAO-A was seen, until in the oldest group (546 g), the inhibition curve produced indicated that MAO-A was by far the predominant activity.

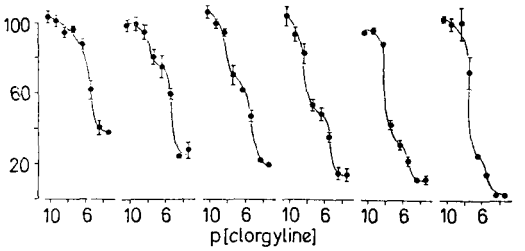

FIG. 3. In vitro inhibition of benzylamine deamination by clorgyline in homogenates of hearts from ageing rats. Legend as in Fig. 2 except that the inhibition curves (from left to right) correspond to homogenates of hearts from rats of mean body weights 36, 63, 151, 317, 414 and 546 g, respectively.

In several of the inhibition curves with benzylamine as substrate, at the highest concentration of clorgyline used (5×10^{-4} M), a significant proportion of the total enzyme activity remained uninhibited, and represented about 40% of the total activity in the youngest rats (36 g), decreasing as the rats grew older. The properties of this residual activity for benzylamine metabolism bear a strong resemblance to those of connective tissue amine oxidases and may well be the source of this activity in the rat heart (Lyles & Callingham 1975).

The variation with age in the relative proportions of MAO-A and -B in the rat heart was also shown by the use of deprenyl (Fig. 4). Here, the increasing proportion of MAO-A with age was indicated in the curves by the gradual increase of the enzyme component showing the lower sensitivity towards deprenyl. In these experiments, there were some quantitative differences in the respective proportions of MAO-A and -B detected by clorgyline and deprenyl. For example, the inhibition curves for heart MAO in the 36 g rats showed a small MAO-A

component with deprenyl, but not with clorgyline. Similarly, in the oldest group of rats (546 g), a small component of MAO-B was indicated by the inhibition curve obtained with clorgyline, but not with deprenyl. Also, as with clorgyline, the inhibition curves obtained with deprenyl showed the presence of a residual component of benzylamine deamination which was resistant to the highest inhibitor concentration used (5×10^{-4} M), and which again decreased in proportion with the growth of the rat.

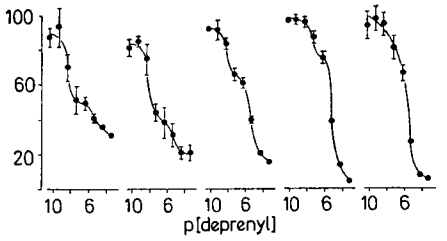

FIG. 4. In vitro inhibition of benzylamine deamination by deprenyl in homogenates of hearts from ageing rats. Legend as in Fig. 2 except that the inhibition curves (from left to right) correspond to homogenates of hearts from rats of mean body weights 36, 63, 227, 385 and 546 g, respectively.

Effects of thyroid hormones on rat cardiac MAO activity

We have shown previously that the specific activity of rat cardiac MAO is increased in hyperthyroidism, and decreased in the hypothyroid state due mainly to changes in the activity of MAO-A (Lyles & Callingham 1974). This was therefore considered in some further experiments with hyperthyroid and hypothyroid rats.

Rats, 30–50 g at the start of the experiment were made hyperthyroid as previously described (Lyles & Callingham 1974) by daily injection of (−)-thyroxine (1 mg kg^{-1} s.c.) for 16 days, while control animals received corresponding daily injections of saline. Alternatively, rats were made hypothyroid by feeding them, without restriction, on a ground meal diet containing 2-thiouracil (0·2% w/w) for about 16 weeks. Controls received ground meal diet alone.

MAO activity was assayed in all groups using tyramine and benzylamine as substrates. These results are shown in Table 2. In agreement with previous studies, the specific activity of cardiac MAO was increased in hyperthyroid animals and decreased in hypothyroid animals, compared with their age-matched controls. Furthermore, the percentage changes in activity were greater in magnitude when tyramine was used as substrate.

Table 2. Effect of hyperthyroid and hypothyroid state upon rat cardiac MAO activity. Quadruplicate assays for MAO activity in each heart were performed with both tyramine and benzylamine as substrates. All values are expressed as mean ± s.e., n = 8 in each group.

	Mean body weight (g)	Mean heart weight (mg)	MAO specific activity [nmol (mg prot.)$^{-1}$ h^{-1}] Tyramine	Benzylamine
Hyperthyroid	99±3	542±19	289±36*	11·4±0·9
Control	104±4	385±10	167±35	9·4±1·0
Hypothyroid	175±6	478±24	294±28*	9·0±0·4
Control	306±8	835±22	496±59	11·9±1·0

* $P < 0.05$.

The effect of clorgyline on benzylamine metabolism in rat heart homogenates from each of these groups was also studied. In hyperthyroid rat hearts, the plateau region of the resulting double-sigmoid inhibition curve was significantly lower than in the control hearts, indicating an increased proportion of MAO-A in the hyperthyroid state (Fig. 5). Conversely, the inhibition curves for MAO from hypothyroid rat hearts indicated a significantly smaller proportion of MAO-A (Fig. 6).

DISCUSSION

Two types (A and B) of MAO activity have been described in a variety of animal tissues, on the basis of the differences in sensitivity of these enzyme activities towards inhibition by clorgyline and deprenyl (Johnston 1968; Knoll & Magyar 1972; Fowler et al 1978). Although both forms of MAO also exist in the rat heart, their overall substrate specificities differ somewhat from those reported for

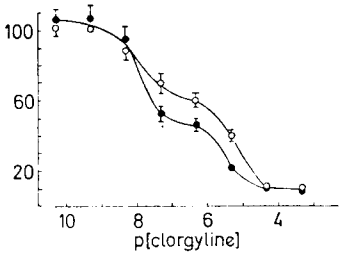

FIG. 5. In vitro inhibition of benzylamine deamination by clorgyline in heart homogenates from control and hyperthyroid rats. ○——○ control; ●——● hyperthyroid. Each curve represents the mean ± s.e. of the ratio, derived from duplicate estimations of individual homogenates from 4 animals in each group. Activities (ordinate: MAO % activity) are expressed as percentages of uninhibited activity of their appropriate controls. Significant differences between the curves for the two groups are seen at clorgyline concentrations of 5×10^{-8} M ($P < 0.05$), 5×10^{-7} M ($P < 0.05$) and 5×10^{-6} M ($P < 0.01$), calculated from the absolute values of the specific activity.

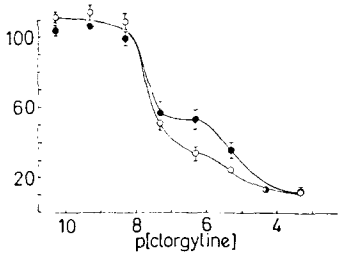

FIG. 6. In vitro inhibition of benzylamine deamination by clorgyline in heart homogenates from control and hypothyroid rats. Legend as in Fig. 5 except ○——○ control; ●——● hypothyroid rats. Significant differences between the two curves are seen at a clorgyline concentration of 5×10^{-7} M ($P < 0.05$).

other tissues. For example, tyramine (Lyles & Callingham 1974), noradrenaline (Fuentes & Neff 1977), and other substrates including dopamine, 5-hydroxytryptamine, kynuramine, 2-phenethylamine and tryptamine when assayed at 1 mM substrate concentration (Lyles & Callingham 1975) all appear to be deaminated predominantly, if not exclusively, by MAO-A in this tissue. In contrast, benzylamine is a substrate for both forms of the enzyme (Lyles & Callingham 1974). In the light of these previous findings, tyramine and benzylamine were considered to be a suitable combination of substrates to investigate whether or not selective age-related changes might occur in the activities of these different forms of rat heart MAO.

In agreement with previous reports (see introduction for references), the specific activity of rat heart MAO was found to increase with the growth of the animal. However, there was a strong indication that the relative changes in activity were not identical for each substrate. Tyramine deamination was found to increase by several-fold more than that of benzylamine, particularly during the early growth of the animal when the gain in body weight was most rapid.

An investigation into the inhibition of MAO activity by clorgyline and deprenyl in heart homogenates from animals of different ages, provided a possible explanation for these age-related changes in the specific activity of MAO towards the different substrates. These results were interpreted as indicating that these changes were due predominantly, if not exclusively, to a selective increase in the specific activity of MAO-A. From the appropriate inhibition curves, MAO-A alone, appears to be responsible for the cardiac deamination of tyramine in rats of all ages, and thus changes in MAO activity towards this substrate should reflect fully the changes in the activity of the MAO-A component. In contrast, the metabolism of benzylamine appears to be brought about by three enzymic activities, whose relative proportions were found to vary with the age of the rat. In the very young rat, almost all the benzylamine oxidation proceeds by way of MAO-B together with a clorgyline- and deprenyl-resistant component which is believed to resemble the connective tissue amine oxidases (Lyles & Callingham 1975). With growth, an increasing proportion of the total deamination of benzylamine occurs by means of MAO-A, with a concomitant decrease in the proportions of MAO-B and the inhibitor-resistant activity.

These findings would appear to account for the different relative rates of the increase in specific activity of MAO towards tyramine and benzylamine. We have no evidence from these results to suggest that these changes in MAO-A arise from a transformation from MAO-B or the clorgyline-resistant component. Rather, it seems more likely that a selective accumulation of MAO-A occurs in the growing rat heart, which would account for the proportional decrease in benzylamine deamination by the other enzyme activities. Whether or not MAO-B and the clorgyline-resistant enzyme do, themselves, show any age-related increase at all in their specific activity cannot be determined directly at present, since we know of no substrate which is specific *solely* for either of these activities in the rat heart. In this respect, it is relevant to wonder what might be the physiological substrate for MAO-B and the clorgyline-resistant enzyme in the rat heart, although some evidence for the in vitro deamination of 2-phenethylamine by the latter enzyme has recently been reported (Fuentes & Neff 1977).

The experiments described in the present paper also indicated that changes in cardiac MAO activity, arising from the alteration of circulating levels of thyroid hormones in the rat, also appear to be due to selective changes in the specific activity of MAO-A. Thus, the effects of thyroid hormones seem, qualitatively, to be similar to those of increasing age. However, there are important differences in the way in which age on the one hand and thyroid hormones and also adrenalectomy on the other, give rise to changes in MAO activity. It does not appear that changes in either heart or body weights of the rats are directly responsible since while adrenalectomy leads to a decrease in both heart and body weights when compared with age-matched controls, thyroid hormones cause a relatively greater increase in heart weight. When tyramine was used as a substrate for MAO activity thyroid hormones and adrenalectomy affected the apparent rate of synthesis, whereas

ageing affected the rate constant for apparent degradation of rat heart MAO (Lyles & Callingham 1974; Della Corte & Callingham 1977). This suggests that the changes in the half-life of MAO-A in the rat heart are related in a fundamental way to the age rather than to the weight of the animal or its heart. Although the MAO-A component of some animal tissues such as rat pineal gland (Goridis & Neff 1971) and vas deferens (Jarrott 1971) may be partly, or wholly, neuronal in origin, it seems likely that the changes in MAO-A described here occur predominantly at extra-neuronal locations, probably within the cardiac muscle cells (Lowe et al 1975).

The molecular basis for the biochemical differences between MAO-A and -B in animal tissues has not yet been fully characterized. There is now good evidence, in the rat liver at least, that these enzyme forms may represent antigenically-identical protein molecules (Dennick & Mayer 1977), which possess different characteristics with respect to inhibitor and substrate specificities, by virtue of being surrounded by different types or amounts of lipid material in the outer mitochondrial membrane (Tipton et al 1976). Thus the selective effects of both ageing and thyroid hormones upon the activity of MAO-A in the rat heart may well depend also upon accompanying influences of these factors upon particular mitochondrial membrane lipid components. Although we are not aware of any studies into the effects of ageing upon mitochondrial phospholipid content in the rat heart, thyroid hormones have been reported to control the synthesis, composition and resulting membrane concentrations of some mitochondrial phospholipids in several rat tissues (Nelson & Cornatzer 1965; Hulbert et al 1976). At the present time, the exact molecular alterations responsible for the selective influences of thyroid hormones and ageing upon MAO-A remain unknown, although recent experiments would seem to indicate that changes in the total number of enzyme active sites, are responsible for the observed changes in specific enzyme activity (Lyles & Callingham 1974; Fowler & Callingham 1979). Whatever the ultimate biochemical differences between multiple forms of MAO may turn out to be, the present study shows clearly that differences in the physiological control of their individual activities may occur, at least, in the rat heart.

Acknowledgements

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