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# SPECIFIC CHANGES IN TYPE A AND B MONOAMINE OXIDASE ACTIVITY IN DIFFERENT TISSUES OF HYPOPHYSECTOMIZED RATS

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**Abstract**—MAO activity was tested in four organs of young male rats 16 days after hypophysectomy. Five monoamines were used as substrates. In the adrenal glands and the thyroid gland MAO activity towards the substrates preferably deaminated by MAO-A was severely decreased. In the adrenal glands, but not in the thyroid gland, the effect of hypophysectomy could be simulated with dexamethasone, a steroid which inhibits ACTH secretion. A significant rise in MAO activity towards substrates for MAO-A and MAO-B occurred in the heart of hypophysectomized rats. There was little change in the MAO activity of whole brain homogenates after hypophysectomy.

It is generally accepted that hormones affect the activity of monoamine oxidase [amine:oxygen oxidoreductase (deaminating) (flavin-containing), EC 1.4.3.4] (MAO) (for review see, for example, Ref. 1). The best studied effects are those of progesterone and oestrogens which cause significant changes in the enzyme activity of uterus, ovaries, adrenal glands and hypothalamus during the oestrous cycle of the rat [2]. A positive correlation between the progesterone concentrations in the ovaries and adrenal glands during the oestrous cycle and the MAO activity of these tissues was established [2]. In the canine adrenal gland, the highest progesterone concentrations per mg protein were found in a purified mitochondrial fraction which also showed the highest enzyme activity. Both of these parameters were increased by pretreatment of the animal with ACTH [3]. An inhibitory effect of cortisol on the increased cardiac MAO after adrenalectomy was reported by Avakian and Callingham [4]. Thyroid hormones were also found to influence MAO activity [5]. A decrease in adrenal MAO activity after hypophysectomy was observed by Wurtman and Axelrod [6] and by Bhagat *et al.* [7]. Both of these latter groups of workers only used tryptamine as substrate.

In the present paper, results of a more detailed study on the effect of hypophysectomy on tissue MAO activity are given. Observations were made on two organs whose major function is directly controlled by trophic hormones from the anterior pituitary, namely the adrenal gland and the thyroid gland and two organs on which the effect of pituitary hormones is either indirect or, as yet, less well defined, the heart and the brain. Five substrates were used in order to see whether the multiple forms of MAO are affected in different ways by the absence of pituitary hormone. Preliminary reports of these observations were presented in context with other considerations on the physiological control of MAO [8, 9].

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## METHODS

Male Wistar rats (200–260 g) were housed at 28°. Food and water was provided *ad lib*. Hypophysectomy was carried out by the parapharyngeal approach, and the operated rats were given a 5% glucose solution instead of drinking water. For sham-operations, the same surgical procedures were followed without removing the gland. ACTH (Sigma, porcine adrenocorticotrophic hormone) was injected intraperitoneally (20 mU./rat/day) for 8 days. In hypophysectomized rats, injections were started 1 week after the operation. Dexamethasone (2 mg/rat/day) was injected i.p. for 8 days.

The thyroid and adrenal glands and the whole brain or the apex of the heart were removed immediately after decapitation, frozen and kept at −18° for not longer than 14 days. For the enzyme assay one pair of adrenal glands or one thyroid gland were homogenized in 2 ml, the apex of the heart usually in 4 ml, 0.1 M sodium phosphate buffer (pH 7.4). The final protein concentrations lay between 0.5 and 2.5 mg/ml. MAO activity towards 5-OH tryptamine (5-HT), dopamine (DA), phenylethylamine (PEA) or tyramine (TY) was assayed by a radiochemical method similar to that of Robinson *et al.* [10]. Each assay sample consisted of 0.4 ml buffer (as earlier) to which 0.1 ml tissue homogenate was added. After a preincubation period of 5 min at 37°, 0.1 ml substrate solution containing 100 nmoles unlabelled 5-HT, DA or TY or 6 nmoles PEA plus approximately 50,000 dpm of the $^{14}$C-labelled amine were added. The samples were incubated for 30 min at 37°, chilled and passed through a column (0.5 × 2.5 cm) of Amberlite CG-50 (mesh 200–400) to separate the amines, which are adsorbed on the column, from their metabolites. The radioactivity in the metabolite fraction was counted in a liquid scintillation spectrometer. For blanks boiled homogenates were used. When kynuramine (KY) was used as substrate the method of Kraml [11] was followed (for details see Ref. 12). Proteins were estimated by the method of Lowry *et al.* [13].

Table 1. Effect of hypophysectomy and ACTH on MAO activity in adrenal gland, thyroid and whole brain of the rat

| Treatment | MAO activity (nmoles product formed/mg protein/30 min) Substrate | | | |
|---|---|---|---|---|
| | 5-HT | PEA | DA | KY |
| | | Adrenal gland | | |
| Co | 35.5 ± 6.2 | 10.9 ± 0.5 | 17.1 ± 2.5 | 2.5 ± 0.36 |
| Co + ACTH | 25.2 ± 1.4 | 15.0 ± 0.6 | 16.7 ± 1.5 | 3.4 ± 0.35 |
| Sham-op. | 38.9 ± 2.3 | 13.3 ± 0.8 | 19.6 ± 1.5 | 3.0 ± 0.51 |
| Hypox | 9.9 ± 0.9 | 10.3 ± 1.6 | 8.0 ± 1.1 | 1.4 ± 0.21 |
| Hypox + ACTH | 7.8 ± 1.5 | 10.0 ± 2.0 | 5.3 ± 1.0 | 3.4 ± 0.35 |
| | | Differences between groups | | |
| | % P | % P | % P | % P |
| Co + ACTH vs Co | −29 n.s. | +38 <0.001 | +2 n.s. | +36 n.s. |
| Sham-op. vs Co | +10 n.s. | +22 <0.05 | +15 n.s. | +20 n.s. |
| Hypox vs Sham-op. | −75 <0.001 | −23 n.s. | −59 <0.001 | −53 <0.02 |
| Hypox + ACTH vs Sham-op. | −80 <0.001 | −25 n.s. | −73 <0.001 | +13 n.s. |
| | | Thyroid | | |
| Co | 18.5 ± 4.4 | 2.6 ± 0.2 | 9.3 ± 0.6 | — |
| Co + ACTH | 16.1 ± 0.8 | 2.9 ± 0.3 | 11.1 ± 1.3 | — |
| Sham-op. | 14.3 ± 2.1 | 4.4 ± 0.6 | 10.1 ± 1.4 | — |
| Hypox | 9.1 ± 0.7 | 3.9 ± 0.5 | 5.0 ± 0.6 | — |
| Hypox + ACTH | 9.1 ± 0.9 | 3.7 ± 0.7 | 6.4 ± 0.9 | — |
| | | Differences between groups | | |
| | % P | % P | % P | |
| Co + ACTH vs Co | −13 n.s. | +12 n.s. | −19 n.s. | |
| Sham-op. vs Co | −23 n.s. | +52 <0.05 | −9 n.s. | |
| Hypox vs Sham-op. | −46 <0.05 | −13 n.s. | −50 <0.01 | |
| Hypox + ACTH vs Sham-op. | −46 <0.05 | −16 n.s. | −47 <0.05 | |
| | | Whole brain | | |
| Sham-op. | — | 45.9 ± 2.4 | 34.7 ± 3.0 | 1.41 ± 0.07 |
| Hypox | — | 46.9 ± 1.2 | 33.8 ± 1.3 | 1.35 ± 0.06 |

Result of experiment 1. Male rats, mean body weight at start of experiment: 200 g. Co: unoperated controls; Co + ACTH: 20 mU. i.p./rat/day for 8 days. Sham-op.: Sham-operated. Hypox: hypophysectomized 16 days before decapitation. Hypox + ACTH: ACTH injected on days 8–16 after Hypox. MAO substrates: 5-HT: 5-OH tryptamine; PEA: phenylethylamine; DA: dopamine; KY: kynuramine. n.s.: not significant. P: probability of difference (Student's *t*-test). N = 6 for each group. Whole brain: the results for 'Co', 'Co + ACTH' and 'Hypox + ACTH' were equal to those obtained for Sham-op. and Hypox for all substrates.

## RESULTS

Experiments on several groups of treated and control rats were carried out. In Table 1 results of the effect of hypophysectomy and of replacement therapy with ACTH on adrenal and thyroid MAO activity are listed. A significant decrease in the MAO activity per mg protein occurred in both endocrine tissues 16 days after hypophysectomy when either 5-HT (MAO type A) or DA was used as substrate, but not when PEA (MAO type B) was used. Because of the severe atrophy of the adrenal cortex after hypophysectomy there was a decrease in the total protein content of the adrenal glands. The mean value for the control animals was 6.7 ± 0.52 mg protein/pair of glands (for the hypophysectomized animals 3.5 ± 0.2) (mean ± S.E., N = 6 in each case). Post-operative treatment with ACTH (days 8–16) did not restore the MAO activity towards 5-HT or DA in adrenal glands or the thyroid glands, nor did the adrenal glands regain their normal size (protein content per pair 4.8 ± 0.87 mg). Adrenal MAO activity towards KY, which was also significantly decreased after hypophysectomy, had returned to normal in the ACTH-treated rats. Table 1 includes values for the MAO activity in homogenates of the whole brain of these rats measured against PEA, DA and KY. No changes in the brain MAO were detected after hypophysectomy.

In a second group of rats, hypophysectomy decreased MAO activity in the thyroid gland towards 5-HT and DA by one half whereas MAO activity towards PEA remained again unaltered.

In Fig. 1A the effect of hypophysectomy on adrenal MAO activity in a third group of rats is illustrated. As in the first experiment the enzyme activity was decreased by more than one half when 5-HT or DA were used as substrate. The much smaller fall seen with PEA was in this instance statistically significant. Again, ACTH treatment did not restore the enzyme activity. In the same rats, MAO activity in the thyroid gland towards 5-HT was decreased by 64% after hypophysectomy.

Fig. 1B shows results obtained on the same occasion with rats which were treated with dexamethasone, a steroid which is often used to suppress specifically ACTH secretion from the pituitary gland (e.g. Ref. 14). When 5-HT or DA was used as

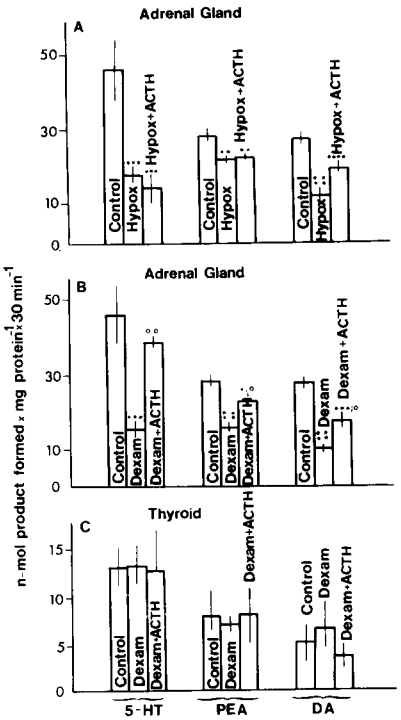


Fig. 1. Effect of hypophysectomy, ACTH and dexamethasone on the MAO activity of the adrenal gland (A and B) and the thyroid (C) of the male rat. Control: untreated rats; Hypox: hypophysectomized, 16 days before decapitation; Hypox + ACTH: ACTH (20 mU. i.p./rat/day) injected on days 8–16 after hypophysectomy; Dexam: dexamethasone (2 mg i.p./rat/day) for 8 days. Dexam + ACTH: dexamethasone and ACTH injected combined for 8 days. Substrates: 5-HT: 5-OH tryptamine; PEA: phenylethylamine; DA: dopamine (N = 6 for each group). Significance of difference from untreated controls: $^{*}P < 0.05$; $^{**}P < 0.02$; $^{***}P < 0.01$; $^{****}P < 0.001$. Significance of difference from Dexam alone: $^{\circ}P < 0.01$; $^{\circ\circ}P < 0.001$ (Student's *t*-test).

substrate the adrenal glands of the dexamethasone treated rats showed a large fall in the MAO activity. There was also a fall by 45% in the MAO activity towards PEA. From Fig. 1B it can be seen that the effects of dexamethasone on adrenal MAO were antagonized by simultaneous injections of ACTH. In Fig. 1C the MAO activity in the thyroid glands from the dexamethasone-treated rats is shown. In contrast to the adrenal glands, the MAO activity in the thyroid glands was not affected by this steroid. There was also no change in the MAO activity of the whole brain after dexamethasone treatment (not shown).

The effects of hypophysectomy on cardiac MAO are demonstrated in Fig. 2. In contrast to the adrenal and the thyroid glands, hypophysectomy caused an increase in MAO activity towards all five substrates used. No preference for MAO-A or MAO-B was indicated. Treatment with ACTH did not alleviate the effect of the operation on MAO activity.

Fig. 3 represents a summary of the results on hypophysectomy. The mean values obtained with the tissues from hypophysectomized rats of different groups of rats are expressed as percentages of the

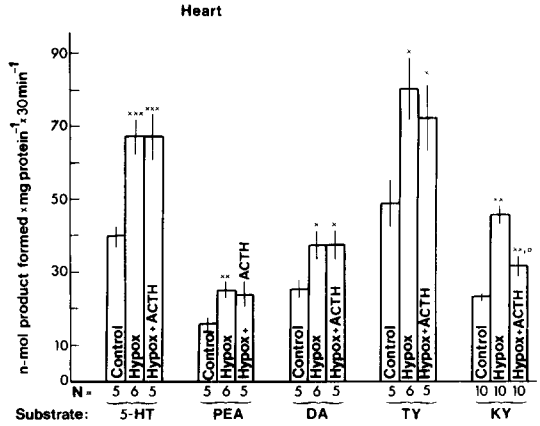


Fig. 2. Effect of hypophysectomy on the MAO activity in the heart of the rat (male rats, 200–260 g). Treatments and abbreviations as in Fig. 1. TY: tyramine; KY: kynuramine. N = number of observations. Signficance of difference from untreated controls: $^{*}P < 0.05$; $^{**}P < 0.02$; $^{***}P < 0.01$. Significance of difference from Hypox: $^{\circ}P < 0.001$.

values obtained with the control tissues. Fig. 3 demonstrates the substrate and organ specificity in the response of MAO to hypophysectomy.

## DISCUSSION

In a hypophysectomized animal the 'trophic' hormones which control the function of the adrenal glands, the thyroid gland or the gonads are missing. This causes structural and functional changes in these endocrine glands and also in tissues which are influenced by their secretion products. In addition, the lack of the neurohormones from the posterior lobe as well as the lack of growth hormone, prolactin, endorphins and other pituitary hormones will affect the biochemistry of the body in general. After this drastic interference with biological processes changes in the activity of enzymes are to be expected.

In the present experiments a large fall in the activity of MAO towavds substrates known to be preferentially deaminated by MAO-A in adrenal tissues 16 days after hypophysectomy was observed. The lack of ACTH was probably not the only reason for this fall in enzyme activity as treatment with ACTH from day 8 to 16 after hypophysectomy did

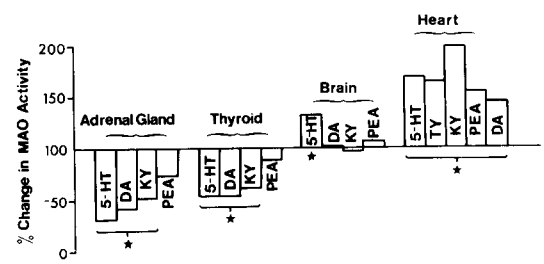


Fig. 3. Summary of observations on the substrate and organ specificity of the effect of hypophysectomy on MAO activity in the male rat. Results combined from various groups of rats. Abbreviations for substrates as in Figs 1 and 2. Activity of untreated control rats = 100%. Statistical significance of difference from controls: $^{*}P < 0.05$ (or less).

not increase significantly MAO activity towards 5-HT or DA. The absence of ACTH alone can however cause a significant decrease in adrenal MAO activity as indicated by the rats treated with dexamethasone, a steroid which specifically inhibits the release of ACTH. Simultaneous treatment with ACTH antagonized the dexamethasone effect on adrenal MAO activity.

When considering the reasons for the observed fall in adrenal MAO after hypophysectomy one must take into account the fact that the adrenal gland is a compound organ in which MAO activity is present in the medulla as well as in the cortex and that it is only the cortex which becomes atrophic. If MAO activity per mg protein were the same in both tissues then the observed decrease indicates a true decrease per unit tissue (without being able to decide whether only one or both tissues were affected). If, however, in the normal gland MAO activity per mg protein were higher in the cortex than in the medulla (as found for the pig and the dog [15]) then the decrease after hypophysectomy may only be an expression of the fact that a higher percentage of the total adrenal mass consists of medullary tissue. For the rat, experiments on regenerated adrenal glands after demedullation have indicated that MAO activity per mg protein is probably similar in the cortex and medulla [15].

The atrophy of the adrenal cortex after hypophysectomy is mainly due to an atrophy of the zona fasciculata which goes hand in hand with a near cessation of steroid secretion. This is accompanied by a 40% decrease in the number of mitochondria per fasciculata cell [16], and also by a decrease in total mitochondrial protein [17]. As MAO is located in the outer mitochondrial membrane [18] this may be an important factor in the mechanisms which led to the observed loss in MAO activity. Hypophysectomy causes also a decrease in the adrenal activity of succinic dehydrogenase [19], an enzyme located in the inner mitochondrial membrane. Another factor which may be responsible for the preferential decrease in MAO type A activity in the adrenal gland after hypophysectomy may be the lipid loss from the adrenal gland which occurs under these conditions [20]. According to Tipton *et al.* [21] the lipid environment of the enzyme protein is particularly important for MAO-A activity whereas MAO-B activity appears to be less affected by this factor.

In the adrenal glands and in the thyroid MAO activity towards PEA was not affected by hypophysectomy. This substrate is regarded to be preferably metabolized by MAO-B.

There was also a fall in the MAO-A activity per mg protein in the thyroid gland after hypophysectomy. The absence of a dexamethasone effect on thyroid MAO activity confirmed the assumption that the observed effect of this steroid on the adrenal gland was probably an indirect one via the pituitary gland.

The observed rise in cardiac MAO after hypophysectomy may be linked with the fall in adrenal steroid secretion because adrenalectomy can cause an increase in cardiac MAO which is reversible by cortisol [4]. The role of thyroxine on cardiac MAO of the rat is not yet clear because of variations in rat strains, age and sex of the animals and the different substrates used by various investigations (for discussion see Ref. 9). An increase in MAO activity in the heart of young male rats after administration of (−)-thyroxine was reported by Callingham and Lyles [5]. This would concur with the observation that (−)-thyroxine is required for the synthesis of flavin, a cofactor of MAO [22]. From the present results it would appear that in the hypophysectomized rat the decreased glucocorticoid secretion from the adrenal gland is more decisive for the activity of MAO in the heart than the decreased thyroid function. There was apparently no difference in heart MAO activity between MAO-A or MAO-B as indicated by the similar results obtained with the five different substrates used. Our experiments do not allow conclusions to be drawn in respect of the clorgyline-resistant MAO [23]. Although a rise in adrenal steroid secretion after treatment of hypophysectomized rats with ACTH is to be expected, this rise was, in our experiments, clearly not sufficient to antagonize the effect on cardiac MAO. A rise in the cardiac turnover of noradrenaline was observed in chronically hypophysectomized rats [24, 25] which was associated with an increase in *de novo* synthesis of catecholamines [26]. The latter authors reported also a 37% increase in cardiac MAO in female rats hypophysectomized for at least 6 days using tryptamine as substrate.

Neither hypophysectomy nor dexamethasone affected MAO activity in whole brain homogenates appreciably. This does not exclude the possibility that enzyme activity in discrete brain regions was affected.

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