THE REGULATION OF MONOAMINE OXIDASE ACTIVITY BY ADRENAL CORTICAL STEROIDS

By

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ABSTRACT

The activity of the enzyme monoamine oxidase (MAO) in the brain, heart, liver and spleen of rats 10 weeks after adrenalectomy was determined. Another group of adrenalectomized rats received 5 mg of hydrocortisone daily for the period of 10 days and their MAO activity was compared with that of control adrenalectomized rats. There was a significant rise in MAO activity following adrenalectomy in the tissue homogenate, mitochondrial and supernatant fractions of all the organs studied. Hydrocortisone administration decreased MAO activity to the level of normal rats. These results suggest that corticoids serve as a rate limiting factor for the activity of the enzyme MAO. This hypothesis was further tested in normal rats by blocking the biosynthesis of glucocorticoids with Metopirone®. Four hours after a single injection of 75 mg Metopirone the cerebral, hepatic and cardiac MAO showed marked increases from control levels. These results suggest that adrenal cortical steroids present in the circulation of normal animals regulate the activity of the enzyme MAO in most organs.
Early studies suggest that hormones of the adrenal cortex and the catecholamines act as a single physiological unit (Ramey & Goldstein 1958). Many effects of catecholamines cannot be induced in the absence of corticosteroids (Britton 1930). Inhibition of adrenal corticosteroidogenesis by drugs or by adrenalectomy produces marked differences in the release, biosynthesis and metabolic degradation of adrenaline and noradrenaline (Parvez et al. 1972; Wurtman & Axelrod 1965, 1966; Margolis et al. 1966; Parvez & Parvez 1972a). The enzyme phenylethanolamine-N-methyl transferase (PNMT) which catalyzes the conversion of noradrenaline to adrenaline (Axelrod 1962) requires high concentrations of glucocorticoid hormones for normal maintenance (Wurtman & Axelrod 1965; Margolis et al. 1966). The high concentrations of these hormones apparently induce the synthesis of the enzyme PNMT. The literature in the past decade provides sufficient evidence that corticoids regulate many other enzymes of metabolic significance (Lang 1971). Administration of glucocorticoids to animals is followed by rises in the activities of a number of enzymes mostly of the glycolytic cycle, urea cycle and amino acid metabolizing enzymes of the liver by increasing the synthesis of new enzyme protein. But our previous observations (Parvez & Parvez 1972b) provide evidence that the enzymes of catecholamine degradation respond in a contrary manner to the glucocorticoid hormones. The inhibition of glucocorticoid action in normal rats results in marked increases in the activities of monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT). Brief observations of Avakian & Callingham (1968) also support our findings since they observed a 2-5 fold rise in myocardial MAO activity a few days after adrenalectomy.

The present study was undertaken to determine the prolonged effects of adrenalectomy on the MAO activity in the mitochondria and other fractions of the brain, liver heart and spleen. The adrenalectomized rats were also injected with hydrocortisone for 10 days and their MAO activity was compared with that of control adrenalectomized rats. The biosynthesis of glucocorticoids in normal rats was inhibited by Metopirone® (Chart & Sheppard 1959; Kaplan 1963) and the MAO activity in different organs was investigated to confirm the results in adrenalectomized rats.

**MATERIALS AND METHODS**

Male albino rats bred in our own laboratory were used throughout the experiments. Their weight ranged from 200-250 g while their age varied from 10 to 11 weeks. The rats were kept in an animal house at a constant temperature of 23°C with exposures to natural light and darkness during the months of September and December. Bilateral adrenalectomy was performed under ether anaesthesia. The adrenalectomized rats were maintained on 0.9% saline for drinking and regular commercial laboratory food for the period of 10 weeks. The tissues from 14 normal and 9 control adrenalecto-
mized rats were excised after killing by a blow on the head. The tissues were kept in 0.25M sucrose for a few hours. Eight adrenalectomized rats were administered daily with 5 mg of hydrocortisone (Ciba) per rat for a period of 10 days. The tissues were excised at the termination of the treatment.

**Metopirone administration.** – Ten normal rats in each group were administered 75 mg of Metopirone®, Ciba (1,2-bis beta pyridyl, 2-methyl propanone Lot A) in sterilized olive oil. The tissues were rapidly dissected out at specified intervals.

**Enzyme preparation.** – Freshly excised tissues were homogenized in ice cold 0.25 M sucrose in glass homogenizers. 50% of the homogenate was kept at short time for the assay of MAO. The other 50% of homogenate was used to isolate mitochondria and supernatant fractions according to the methods of Oswald & Strittmatter (1963).

**Assay of MAO.** – MAO was determined using 14C-tryptamine bisuccinate (specific activity 8.9 mCi/mm) as a substrate according to the method of Wurtman & Axelrod (1963). All the features of the original technique were used except for the lower concentrations of 14C-tryptamine (0.55 nmole per incubation tube) while 0.2 M phosphate buffer instead of 0.5 M was used. The enzyme activity is expressed in disintegrations per minute (dpm) extracted after 20 min of incubation. The dpm extracted represent a linear proportionality between moles of indole acetic acid transformed during the incubation time.

**In vitro studies.** – The livers from 10 male adult rats were excised in chilled 0.9% KCl at 2°C. The tissues were immediately homogenized in 4 vol. of KCl 0.9% for the assay of enzyme MAO. The incubation mixture for the assay of MAO was prepared as described previously. The required concentration of Metopirone bitartrate solution or Hydrocortisone in water was added by means of an Eppendorf micro pipette in the incubation tube just before the start of the start of the incubation. The remaining procedure was as described above.

**Statistical analyses** were performed according to the method of Fisher's t-test. The mean values are expressed with ± SEM.

**RESULTS**

Fig. 1 shows the activity of the enzyme MAO in the brain of the control rats, adrenalectomized rats and adrenalectomized rats administered with hydrocortisone. The enzyme activity was determined in total tissue homogenate, mitochondrial and supernatant fractions of the brain. Ten weeks following adrenalectomy significant rises in all the three fractions were observed ($P < 0.001$). The administration of hydrocortisone to adrenalectomized rats for 10 days decreased the MAO activity in all the three fractions. The MAO in adrenalectomized rats treated with hydrocortisone was statistically insignificant compared with the control rats.

Fig. 2 shows the activity of MAO in control, adrenalectomized and adrenalectomized rats given hydrocortisone in total homogenate, mitochondria and supernatant fractions of the heart. Adrenalectomy produced uniformly and
The activity of enzyme MAO in tissue homogenate ■, mitochondria ▼ supernatant fraction □ of brain of control rats (C), adrenalectomized rats (A) and adrenalectomized rats injected with 5 mg of hydrocortisone daily for 10 days (A+H). The enzyme activity is expressed in DPM with SEM of the mean values (C, 14 rats, A, 9 rats, A+H, 8 rats).

Fig. 1.

The activity of enzyme MAO in hearts of control rats (C) adrenalectomized rats (A) and adrenalectomized rats given 5 mg of hydrocortisone daily for 10 days (A+H). The activity of MAO was determined in three different fractions of heart: Tissue homogenate ■, mitochondria ▼, and supernatant fraction □.

The C, A, A+H contained 14, 9, and 8 rats respectively.

Fig. 2.
Hepatic MAO activity in 14 control rats (C), 9 adrenalectomized rats (A) and 8 adrenalectomized rats receiving 5 mg of hydrocortisone daily for 10 days (A + H). The MAO was measured in tissue homogenate  and mitochondrial and supernatant fraction of the liver.

Fig. 3.

highly significant rises ($P < 0.001$) in all the fractions. Hydrocortisone administration to adrenalectomized rats was followed by a decrease and the MAO activity returned to control values. The statistical significance due to adrenalectomy disappeared after 10 days of hydrocortisone administration in all three fractions.

As appears from Figs. 3 and 4 the activity of the enzymes MAO is demonstrated in the liver and spleen of the control rats, adrenalectomized rats and adrenalectomized rats given 5 mg of hydrocortisone daily for 10 days. The activity of MAO in both organs was determined in tissue homogenate, mitochondrial fraction and supernatant fraction. There was a significant rise ($P < 0.05$) in MAO activity in all the three fractions following adrenalectomy, in the liver as well as in the spleen. Hydrocortisone administration lowered the activity in homogenate and supernatant fractions of the spleen but the mitochondrial MAO activity remained slightly higher than in the control rats. Prolonged administration of hydrocortisone to adrenalectomized rats brought about a return of the activity of MAO to control values in all the three fractions of the liver.

Fig. 5 shows the effects of blocking glucocorticoid synthesis with Metopirone on the MAO activity in heart, liver and brain in the total tissue homogenate.
The activity of enzyme MAO in spleen of control rats (C), adrenalectomized rats (A) and adrenalectomized rats injected with 5 mg of hydrocortisone daily for 10 days (A + H). The individual mean values for each group consisted of 14 rats, 9 rats, and 8 rats respectively. ■ Tissue homogenate. □ Mitochondrial fraction. □ Supernatant fraction.

The MAO activity was measured in all the tissues 4 hours after a single injection of 75 mg of Metopirone. The hepatic MAO increased by 48% as compared with its control value. This rise was significantly higher ($P < 0.001$). The cardiac and cerebral MAO were respectively 28 and 37% higher compared with control levels. These increases proved to be highly significant ($P < 0.001$).

The activity of MAO in rats 4 hours after the injection of 75 mg Metopirone. All the groups consisted of 10 rats. The tinted columns for each tissue show the control values. The enzyme activity was assayed in total tissue homogenate.

Fig. 4.
Fig. 5.
The results of the present investigation are in agreement with our previous findings of higher urinary excretion of vanil-mandelic acid in hypophysectomized and adrenalectomized rats (Parvez & Parvez 1972a,c). The ablation of the adrenal gland is followed by highly significant rises in MAO activity in all the organs of the body. These rises in enzyme activity are not only limited to specific form of MAO in the mitochondrial or supernatant fractions but also in all the different forms of MAO present in total tissue homogenates. This suggests that hormones of the adrenal cortex interfere with the synthesis of new molecules of the enzyme protein of MAO. The activity of the enzyme PNMT is highly dependent on the concentration of glucocorticoid hormones present in the intra-adrenal portal circulation (Pohorecky & Wurtman 1971; Harrison & Hoey 1960). This supply of glucocorticoids induces the PNMT activity in the adrenal medulla (Wurtman & Axelrod 1965; Wurtman 1966). The observations of Wurtman & Axelrod (1966) and Roffi (1965, 1968) in hypophysectomized adult rats or in decapitated rat and rabbit foetuses, show that the administration of hydrocortisone, dexamethasone or ACTH to these animals result in marked increases in the adrenaline content of the adrenals as well as in the activity of the enzyme PNMT. The pre-administration of inhibitors of protein synthesis (puromycin or actinomycin) to hypophysectomized rats prevents the increase in PNMT activity after the administration of ACTH, natural or synthetic glucocorticoids (Wurtman & Axelrod 1966; Wurtman et al. 1972). These extensive studies provide good evidences that the activity of enzymes of adrenaline synthesis is augmented due to a rise in the synthesis of new enzyme protein. Contrary to PNMT, the enzyme MAO is inhibited by the high concentrations of glucocorticoids in the portal circulation of normal animals (our present results). The increase in MAO activity following adrenalectomy and the decreases due to hydrocortisone administration suggest that hormones of the adrenal cortex act as an inhibitory factor.

Our experiments on the inhibition of glucocorticoid synthesis by means of Metopirone (Chart et al. 1958; Liddle et al. 1958; Waxman et al. 1961) show that the block of biosynthesis of these hormones affects the activity of MAO already after 4 hours. The administration of Metopirone is immediately followed by a fall in glucocorticoid concentration and is reduced to zero in 4 to 6 hours (Waxman et al. 1961). The results presented in Fig. 5 show that MAO in the heart, brain and liver rises significantly 4 hours after Metopirone administration.

Two possible mechanisms by which MAO activity is inhibited by glucocorticoids are suggested: 1. Influence on the synthesis of noradrenaline and 2. Interference of glucocorticoids with the enzyme protein of MAO at the molecular level.

515
Our previous study (Parvez et al. 1972) indicated that the inhibition of synthesis of glucocorticoids augments the adrenal stores of noradrenaline. Adrenalectomy in rats is also followed by a significant increase in the urinary noradrenaline (Parvez & Parvez 1972a). Westfall & Osada (1969) observed a marked increase in the synthesis of noradrenaline in the heart after adrenalectomy. They found that hydrocortisone and deoxycorticosterone prevent the accelerated synthesis. Adrenalectomy increases the activity of the central noradrenaline containing neurones (Javoy et al. 1968). The turnover of noradrenaline is increased in the heart (Landsberg & Axelrod 1968) and in the adrenals (Roffi 1965; Wurtman & Axelrod 1965; Wurtman 1966) of hypophysectomized rats. Our present study showing a higher MAO activity after adrenalectomy or inhibition of corticoid synthesis raises the question as to whether this is secondary to the increase in the synthesis rate of noradrenaline. If MAO activity is blocked by suitable inhibitors with a resultant increase in noradrenaline levels, there is an end product inhibition of noradrenaline synthesis.

Recent literature suggests that hormonal control of protein synthesis occurs at the translational level (Pitot 1964; Tomkins & Thompson 1967) as well as directly on the genetic material (Sekeres 1967). The existence of regulator genes has been claimed by Jacob & Monod (1961). The sensitive phase which is frequently defined as the start of potential enzyme regulation (Hadron 1958; Hermann & Tootle 1964) might be identified with a depression of a regulator gene, while the biosynthesis of active enzyme protein might eventually be dependent on a sufficient amount of hormonal stimulated effectors or other factors. The comprehensive review by Litwack & Singer (1972) and previous studies of Feigelson & Feigelson (1964) clearly indicate that glucocorticoids interfere with protein synthesis at RNA transcription from DNA.

The exact molecular basis of increased MAO activity after blocking of glucocorticoid synthesis needs to be more thoroughly investigated which is beyond the limit of present study. It is suggested that MAO activity is probably controlled by the glucocorticoids because of their effect on protein synthesis.

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