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EVIDENCE FOR THE ROLE OF ADRENOCORTICAL HORMONES IN THE REGULATION OF NORADRENALINE AND DOPAMINE METABOLISM IN CERTAIN BRAIN AREAS

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1 Bilateral adrenalectomy suppressed body growth and increased the activity of tyrosine hydroxylase in rat striatum in a time-dependent manner. Fifteen days after adrenalectomy, the concentrations of noradrenaline were decreased significantly in hypothalamus and striatum, as were those of dopamine in brain stem and striatum.

2 Catechol-*O*-methyltransferase failed to change in response to adrenalectomy, but the activity of monoamine oxidase in cortex was significantly increased 7 days after surgery. These changes in various neurochemical parameters were even more pronounced 15 days after adrenal ablation.

3 Administration of corticosterone (10 mg/kg i.p.) to adrenalectomized rats effectively reversed the observed effects on brain amine metabolism. Corticosterone treatment for 7 days beginning from the 8th day of adrenalectomy virtually restored the concentrations of noradrenaline and dopamine as well as the activities of striatal tyrosine hydroxylase and cerebrocortical monoamine oxidase to the values seen for sham-operated controls.

4 Our data suggest that changes seen in brain noradrenaline and dopamine of adrenalectomized rats are specific to adrenocortical steroids and that these hormones play a role in the regulation of catecholamine formation.

Introduction

In recent years, considerable evidence has accumulated to suggest that the metabolism of brain catecholamines is impaired during affective disorder (Schildkraut, 1965). Studies also indicate that severe depression is accompanied by adrenocortical hyperfunction. The plasma cortisol (hydrocortisone) levels have been found to be elevated in depressed patients and it was suggested that the rate of cortisol secretion might be increased during this psychiatric illness (Gibbons, 1964; McClure, 1966). Furthermore, evidence indicates that modifications in central monoamine metabolism induced by clinically used psychoactive agents (e.g. monoamine oxidase inhibitors, Ganong, 1964; reserpine or chlorpromazine Maickel, Westermann & Brodie, 1961; Ganong, 1964) are associated with changes in the function of the pituitary-adrenal axis. A consensus seems to have emerged from these findings that there is some relationship between adrenal corticoids and brain catecholamines.

Alterations in urinary output of adrenaline and noradrenaline following bilateral adrenalectomy or medullo-adrenalectomy have been reported in a variety of animal species (von Euler, Franksson & Hellstrom, 1954; Carpi & Oliverio, 1964). It has been proposed that bilateral adrenalectomy results in a compensatory increase in sympathetic nerve activity secondary to the decrease in blood pressure (Imms & Jones, 1968). These findings suggested that bilateral adrenalectomy influences the turnover of cardiac noradrenaline (NA). However, uncertainty still exists as to whether adrenalectomy exerts any effect on the synthesis of NA and dopamine in rat brain. The present study was therefore undertaken to examine the influence of bilateral adrenalectomy on the biosynthetic capacity for catecholamines in brain tissue.

Methods

Male Sprague-Dawley rats weighing 200–225 g were used in all experiments. Bilateral adrenalectomy was carried out under pentobarbitone sodium anaesthesia

through a mid-line incision from the back. Adrenalectomized animals were then placed in groups of 3 to a cage under constant environmental conditions (24°C, 60% relative humidity and regular alternate cycles of 12 h light and darkness) with free access to 0.9% w/v NaCl solution (saline) and Master Laboratory Chow until killed. Control rats which underwent a sham-adrenalectomy were kept under similar conditions except that they were given tap water. All biochemical parameters were measured either 7 or 15 days after surgery. In another experiment, groups of adrenalectomized rats were injected with corticosterone intraperitoneally (10 mg/kg), freshly dissolved in a 5:1 mixture of ethanol (95%) and saline for a period of either 3 or 7 days. The corresponding controls received an equal volume of the vehicle.

Sample preparation and biochemical assays

Animals were killed between 09 h 00 min and 11 h 00 min by the near-freezing technique of Takahashi & Aprison (1964). Following decapitation, the brains were rapidly excised and stripped of adherent meningeal tissue and grossly visible blood vessels on a glass plate resting on crushed ice. In order to study the regional changes in NA and dopamine, specific brain areas, were dissected out, according to the procedure of Glowinski & Iversen (1966). The 'brain stem' consisted of whole brain minus olfactory lobes, cerebral cortex, striatum, hypothalamus and cerebellum. The striatum was used for measuring the activity of tyrosine hydroxylase (TH) and parts of the cerebral cortex for assessing monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT) activity. The tissues were homogenized in 20 volumes of 0.28 M ice cold sucrose. The activity of TH was determined under linear kinetic conditions according to the procedure of McGeer, Gibson & McGeer (1967) with the modifications described by Rastogi & Singhal (1974). In order to calculate the V_{max} of tyrosine hydroxylase, the endogenous concentrations of tyrosine were determined in the striatum as described by McGeer *et al.* (1967) modified from Waalkes & Udenfriend (1957). The activity of MAO was assayed by measurement of the [^{14}C]-indoleacetic acid (IAA) metabolite of [^{14}C]-tryptamine bisuccinate according to the method of Wurtman & Axelrod (1963) with a slight modification (Rastogi, Lapierre & Singhal, 1976). The activity of COMT was measured by the method of McCaman (1965) adapted from D'Iorio (1961). S-adenosyl-L-methionine iodide [methyl-^{14}C] was used as the methyl donor and 3,4-dihydroxybenzoic acid as the substrate. NA was estimated essentially according to the method of Maickel, Cox, Saillant & Miller (1968) and dopamine according to Spano & Neff (1971) with the modification described previously (Hrdina, Ghosh, Rastogi & Singhal, 1975).

Chemicals

All reagents were of the purest grade available. (±)-Arterenol and L-tyrosine were purchased from

Table 1 Effect of adrenalectomy (Adx) and replacement therapy with corticosterone on body weight and striatal tyrosine hydroxylase (TH) activity

Treatment	*Body weight (g)*	*Striatal TH (nmol DOPA g^{-1} h^{-1})*
Sham-operated	257 ± 15 (100)	63.3 ± 4.2 (100)
Adx (7 days)	209 ± 19 (81)	76.6 ± 3.1 (121)*
Adx (15 days)	180 ± 14 (70*; 100)	82.9 ± 3.8 (131*; 100)
Adx + Costicosterone (3 days)	203 ± 16 (79*; 113)	84.6 ± 4.8 (134*; 102)
Adx + Corticosterone (7 days)	221 ± 31 (86; 123)	65.5 ± 3.6 (103; 79†)

Each value represents the mean ± s.e. mean for 6 rats. Rats were killed 7 or 15 days after adrenalectomy. Two groups of adrenalectomized rats were injected with corticosterone (10 mg/kg daily, i.p.) for either 3 or 7 days (beginning on day 12 or day 8 after adrenalectomy, respectively) and killed 15 days after adrenalectomy. The corresponding controls (adrenalectomized rats in this case) received an equal volume of the vehicle (5:1 mixture of 95% ethanol and saline). Data in parentheses express results in percentages taking the values of sham-operated or 15 day adrenalectomized rats as 100%.

* Statistically significant difference when compared with the values for sham-operated rats: $P < 0.05$.

† Statistically significant difference when compared with the values of adrenalectomized (15 days) rats: $P < 0.05$.

Calbiochem (LaJolla, Calif.), L-[^{14}C]-tyrosine and *S*-adenosyl-L-methionine iodide-[methyl-^{14}C] from Amersham Searle Corp. (Arlington Heights, Ill.), tryptamine bisuccinate-[2-^{14}C] from New England Nuclear (Boston, Mass.) and N-Methyl-*N*-3-hydroxybenzylhydrazine (NSD-1034) from Smith and Nephew Ltd. (Lachine, Que.). Corticosterone (Sigma Chemical Co., St. Louis, Mo.) was freshly dissolved in a 5:1 mixture of ethanol (95%) and saline.

Results

Influence of bilateral adrenalectomy on noradrenaline and dopamine metabolism

The data in Table 1 demonstrate that adrenalectomy produced a time-dependent suppression of body growth. The activity of TH in the striatum was increased by 21%, 7 days after adrenalectomy and by 31%, 15 days after adrenalectomy. Despite increased activity of TH, the endogenous concentrations of NA in the brain stem, hypothalamus and striatum 7 days after adrenalectomy were the same as in control rats (Table 2). Noradrenaline was lowered significantly in the hypothalamus and striatum, 15 days after adrenalectomy. The data in Table 3 show that adrenalectomy caused a significant decrease in the dopamine concentrations in the brain stem and striatum. The maximal change (−23%) was seen in striatum 15 days after adrenalectomy.

Effect of replacement treatment with corticosterone on noradrenaline and dopamine concentrations in brains of adrenalectomized rats

In order to investigate whether the observed changes in brain catecholamine metabolism were due to the absence of adrenocortical hormones, adrenalectomized rats were treated with corticosterone. Administration of corticosterone (10 mg/kg daily, i.p.) for 3 days produced no appreciable change in body weight or TH activity of the striatum. However, increasing the duration of treatment to 7 days did result in a significant decrease (by 21%) in TH activity to values similar to those obtained in sham-operated controls (Table 1). Administration of corticosterone also produced a dose-dependent rise in NA, the significant change being observed in the hypothalamus of adrenalectomized rats receiving corticosterone for 7 days beginning on day 8 after adrenalectomy (Table 2). The concentrations of dopamine were also increased in adrenalectomized animals treated with corticosterone. Treatment with corticosterone for 7 days restored dopamine levels to almost the same values as seen in sham-operated animals (Table 3).

Effect of adrenalectomy and corticosterone treatment on monoamine oxidase and catechol-O-methyltransferase activity

The results in Table 4 demonstrate that whereas adrenalectomy exerted no appreciable change in

Table 2 Influence of adrenalectomy (Adx) and replacement therapy with corticosterone on noradrenaline (NA) concentrations in 3 brain regions

Treatment	*NA (μg/g)* *Brain stem*	*Hypothalamus*	*Striatum*
Sham-operated	0.61 ± 0.02 (100)	1.92 ± 0.10 (100)	0.26 ± 0.01 (100)
Adx (7 days)	0.65 ± 0.02 (107)	2.00 ± 0.07 (104)	0.26 ± 0.01 (100)
Adx (15 days)	0.54 ± 0.04 (89; 100)	1.40 ± 0.08 (73**; 100)	0.20 ± 0.01 (76*; 100)
Adx + corticosterone (3 days)	0.55 ± 0.03 (90; 102)	1.54 ± 0.07 (80*; 110)	0.22 ± 0.01 (85; 111)
Adx + corticosterone (7 days)	0.62 ± 0.04 (102; 114)	1.74 ± 9.06 (91; 124†)	0.23 ± 0.02 (88; 115)

Each value represents the mean ± s.e. mean of 6 rats. For experimental details see legend to Table 1. Data in parentheses express results in percentages taking the values of sham-operated or 15 day-adrenalectomized rats as 100%.

Statistically significant difference when compared with the values for sham-operated rats: $^{*}P < 0.05$; $^{**}P < 0.01$.

† Statistically significant difference when compared with the values for adrenalectomized (15 days) rats: $P < 0.05$.

COMT activity, the activity of MAO was elevated by 19%. Replacement therapy with corticosterone for 3 days produced no significant change in either of the catabolizing enzymes when compared with the appropriate controls. However, when the corticosterone treatment was extended to 7 days, the activity of MAO decreased to 74% of that of the controls (Table 4). The data in this table also show that COMT activity failed to change in adrenalectomized rats treated with corticosterone.

Discussion

The data presented show an apparent increase in brain catecholamine synthesis following adrenalectomy. This agrees with observations of Javoy, Glowinski & Kordon (1968) who found NA turnover to be significantly enhanced 6 days after adrenalectomy. Despite increased TH activity, the endogenous concentrations of NA and dopamine remained low, indicating that the utilization of catecholamines was enhanced and could not be compensated for by an enhanced amine formation. This observation is corroborated by the findings of Caesar, Collins & Sandler (1970) who reported high urinary excretion in adrenalectomized animals of 4-hydroxy-3-methoxyphenylglycol (MOPEG) and homovanillic acid (HVA), the major metabolites of NA and dopamine. Since brain MAO activity was significantly increased following adrenal corticoid deficiency, as has also been reported by Caesar *et al.* (1970), the possibility remains that the low concentrations of NA and dopamine could, in part, be due to enhanced deamination of these monoamines. Using histochemieal techniques, Fuxe, Hokfelt & Ungerstedt, (1970) and Olson & Fuxe (1971) found that adrenalectomy increased monoamine disappearance in most parts of the brain, particularly in the hypothalamus and cerebral cortex, suggesting that there is a general increase in cerebral NA turnover following adrenalectomy. More recently, Shen & Ganong (1976), by determining the endogenous [^{3}H]-NA levels in rats pretreated with labelled NA by the intraventricular route, demonstrated that adrenalectomy did indeed increase NA turnover in rat hypothalamus.

Some restoration in brain catecholamine metabolism after adrenalectomy could be obtained with corticosterone treatment. Corticosterone given for 3 days starting on day 12 after adrenalectomy, did not produce any significant effect on TH activity and NA concentrations. However, when corticosterone was given for 7 days beginning on day 8 after adrenalectomy, cerebral TH activity and NA and dopamine concentrations returned to values similar to those seen in sham-operated controls. The decrease in MAO activity following corticosterone treatment, may be responsible for the increases in NA and dopamine concentrations. Mass & Mednieks (1971) found that hydrocortisone *in vitro* increased the uptake of NA by cerebrocortical slices; thus an increase in amine uptake may have also occurred in the corticosterone-treated adrenalectomized rats and contributed to the observed increase of brain NA and dopa-

Table 3 Effect of adrenalectomy (Adx) and replacement therapy with corticosterone on dopamine concentrations in 3 brain regions

Treatment	*Dopamine (μg/g)* *Brain stem*	*Hypothalamus*	*Striatum*
Sham-operated	1.23 ± 0.08 (100)	0.55 ± 0.02 (100)	7.87 ± 0.51 (100)
Adx (7 days)	1.15 ± 0.07 (94)	0.53 ± 0.02 (97)	7.24 ± 0.62 (92)
Adx (15 days)	1.02 ± 0.05 (83*; 100)	0.51 ± 0.03 (93; 100)	6.06 ± 0.28 (77**; 100)
Adx + corticosterone (3 days)	1.09 ± 0.05 (89; 107)	0.60 ± 0.02 (109; 117†)	7.03 ± 0.31 (99; 116†)
Adx + corticosterone (7 days)	1.19 ± 0.04 (97; 117†)	0.61 ± 0.02 (110; 119†)	7.33 ± 0.39 (104; 121†)

Each value represents the mean ± s.e. mean for 6 rats. For experimental details see legend to Table 1. Data in parentheses express results in percentages taking the values of sham-operated or 15 day adrenalectomized rats as 100%.

Statistically significant difference when compared with the values of sham-operated rats: $^*P < 0.05$, $^{**}P < 0.02$.

† Statistically significant difference when compared with the values of adrenalectomized (15 days) rats: $P < 0.05$.

mine. It is of interest that the rise in cerebral dopamine following corticosterone treatment was more pronounced than that seen for NA. Dopamine is readily accessible for deamination by MAO within sympathetic neurones and may even exhibit a greater susceptibility to deamination than NA (Roth & Stone, 1968) thus explaining, at least in part, why suppression of MAO by corticosterone elevated dopamine more than NA.

Table 4 Effect of adrenalectomy (Adx) and replacement therapy with corticosterone on cerebrocortical monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT) activity.

Treatment	*MAO*[a]	*COMT*[b]
Sham-operated	138.4 ± 6.1 (100)	335.3 ± 7.75 (100)
Adx (7 days)	148.1 ± 3.2 (107)	342.1 ± 7.03 (102)
Adx (15 days)	164.7 ± 6.8 (119**; 100)	358.8 ± 7.63 (107; 100)
Adx + corticosterone (3 days)	159.8 ± 4.1 (116*; 97)	359.3 ± 8.18 (107; 100)
Adx + corticosterone (7 days)	121.9 ± 3.8 (88; 74†)	369.6 ± 10.5 (110; 103)

[a]MAO activity is expressed as nmol [^{14}C]-IAA $g^{-1}h^{-1}$; [b]COMT activity is expressed as nmol [^{14}C]-*O*-methylated metabolite $g^{-1}h^{-1}$.
Each value represents the mean ± s.e. mean for 6 rats. For experimental details see the legend for Table 1. Data in parentheses express results in percentages taking the values of sham-operated or 15 day-adrenalectomized rats as 100%.
* Statistically significant difference when compared with the values of sham-operated rats: *$P < 0.05$; **$P < 0.02$.
† Statistically significant difference when compared with the values of adrenalectomized (15 days) rats: $P < 0.05$.

The reason for an increased catecholamine turnover in brains (Shen & Ganong, 1976) of adrenalectomized rats is not clear. Previous studies have shown that adrenalectomy results in cardiovascular depression (i.e. decrease in blood pressure and cardiac output; Ingle, 1952; Imms & Jones, 1968). Total peripheral resistance (Imms & Neame, 1974) and NA turnover in the heart were increased following adrenalectomy (Landsberg & Axelrod, 1968; Westfall & Osada, 1969; Dailey & Westfall, 1970). However, it is not known whether there is a parallel rise in central noradrenergic activity in response to increases in peripheral sympathetic activity. In fact, van Zwieten (1973) has shown that central mechanisms involving noradrenaline inhibit peripheral sympathetic discharge. The effect of corticosterone on NA metabolism in brains of normal rats was not examined in the present study. However, if we were to assume that excess corticosterone affects the metabolism of this catecholamine in a manner opposite to that found after adrenalectomy, a reduction in the turnover of brain NA would be expected. It is interesting that *in vitro* studies by Mass & Mednieks (1971) have shown that hydrocortisone enhanced the uptake of NA by slices of cerebral cortex and lowered the turnover of this monoamine. The increased adrenocortical activity seen during depressive illness (Coppen, 1970) may be responsible for diminished turnover of NA and suppressed psychomotor drive in such patients.

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