Effect of brain monoamines on the secretion of adrenocorticotrophic hormone

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Abstract. The role of brain monoamines (5-HT, NA and DA) in the secretion of adrenocorticotrophic hormone (ACTH) was studied in view of contradictory reports. Plasma corticosterone levels and the rate of synthesis of corticosterone in vitro by the adrenal gland were estimated in albino rats and have been taken as the index of ACTH activity. These estimations were done in unstressed and stressed, and in untreated and treated rats. Drugs were administered intracerebroventricularly to the rats to cause selective degeneration of tryptaminergic, noradrenergic or dopaminergic neurons.

The results show that plasma corticosterone levels and the rate of synthesis of corticosterone were significantly decreased after selective degeneration of tryptaminergic neurons in unstressed rats. After selective degeneration of either tryptaminergic or noradrenergic neurons, the acute increase in the plasma corticosterone levels and rate of synthesis of corticosterone in vitro by adrenal glands in stressed rats were significantly inhibited. These results have been interpreted to suggest that the central tonic control on adrenal glands may be 5-HT mediated and that during stress ACTH secretion may be both 5-HT and NA mediated. DA does not seem to have significant role in the regulation of ACTH secretion.

The literature on the role of brain biogenic amines in the secretion of adrenocorticotropic hormone (ACTH) is contradictory. Brain noradrenaline (NA) has been reported either to stimulate (Kawa et al. 1978), inhibit Scapagnini et al. 1972; Scapagnini & Preziosi 1973; Jones et al. 1976; Oishi 1979) or to have no effect (Schaepdrijver et al. 1969; Kumeda et al. 1974; Bhattacharya & Marks 1970; Abe & Hiroshige 1974) on ACTH secretion. Catecholamines have also been reported to stimulate (Kaplanski et al. 1974) or inhibit (Scapagnini et al. 1970) ACTH secretion. Similarly brain 5-hydroxytryptamine (5-HT) has been reported either to stimulate (Jones et al. 1976), inhibit (Ulrich et al. 1975; Telegdy & Vermes 1976) or to have no effect (Bhattacharya & Marks 1970; Dixit 1971) on ACTH secretion. 5-HT has also been reported to play an important role in the maintenance of the circadian rhythm of corticoids (Scapagnini et al. 1971; Van Delft et al. 1973). Dopamine (DA) has been reported either to inhibit (Van Loon et al. 1971; Ulrich & Yuwiler 1973) or to have no effect (Kawa et al. 1978) on ACTH secretion.

In view of these contradictions this study was planned to investigate the role of the three brain monoamines (5-HT, NA and DA) on ACTH secretion in albino rats. ACTH activity has been assessed by estimating plasma corticosterone levels and its rate of synthesis by the adrenal gland in vitro.

Materials and Methods

Male Wistar albino rats weighing between 120–180 g were used. The animals were fed Hind Level pellet diet for albino rats and were used for experiments a week after procurement. Five to six rats were housed together in a cage. Experiments were conducted at a controlled room temperature of 25 ± 2°C.

To study the effect of brain monoamines on ACTH secretion, plasma corticosterone levels and corticosterone synthesis rate by the adrenal gland in vitro were estimated and taken as the index of ACTH secretion. The role of tryptaminergic, noradrenergic and dopaminergic neurons has been studied by administering drugs known...
to cause selective degeneration of the respective neurons. Brain monoamines, plasma corticosterone and corticosteroid synthesis rate have been estimated in separated groups of rats. Rats were immobilised in order to induce ACTH secretion.

**Brain monoamines**

To affect tryptaminergic, noradrenergic and dopaminergic neurons selectively, all the drugs, i.e. methyl ester of parachlorophenylalanine (PCPA), 5,6-dihydroxytryptamine (DHT), 6-hydroxydopamine (6-OH-DA) except benztpine (Benz) and desmethylimipramine (DMI) were administered through a chronically implanted intracerebroventricular (icv) cannula (Sanyal et al. 1979). For icv administration of drugs a constant volume of 10 μl was maintained. Control (untreated) animals received an equal volume of artificial CSF. Benz and DMI were administered intraperitoneally (ip).

The methyl ester of PCPA, a synthesis blocker of 5-HT (Koe & Weissmann 1966) was given in a dose of 100 μg/rat once daily for 3 days and experiments were conducted 48 h after the last injection. DHT, a tryptaminergic neuron degenerator (Baumgarten et al. 1971), was administered in a dose of 75 μg/rat and experiments were conducted 72 h after injection.

6-OH-DA (200 μg/rat), a known adrenergic neuron degenerator, was administered to rats 30 min after pre-treatment with either Benz (25 mg/kg, ip) to cause more selective degeneration of noradrenergic neurons, or DMI (25 mg/kg, ip) to cause selective degeneration of dopaminergic neurons (Neff et al. 1977). Experiments were conducted 72 h after 6-OH-DA injection.

Brain monoamines (5-HT, NA and DA) were estimated in unstressed rats by a modified method based on the methods of Ansell & Beeson (1968) and Haubrich & Denzer (1973) in untreated and treated (as above) animals. Animals were sacrificed by decapitation at appropriate times after pre-treatment; the sacrifice was always between 12.00–13.00 h. All operations were carried out at 4°C. Brain tissue was homogenised in 10 ml of acidified butanol and centrifuged. Four ml of homogenate was taken and 10 ml of n-heptane and 5 ml of 0.003 N HCl were added to it. The mixture was shaken for 5 min and centrifuged for 10 min. Four and a half ml of acid layer was taken in a tube containing 200 mg alumina and 1 ml of 2 m sodium acetate. It was shaken for 5 min and centrifuged. Supernatant was taken for the estimation of 5-HT (Ansell & Beeson 1968), and catecholamine adsorbed on alumina was estimated following the method of Chang (1964).

**Plasma corticosterone and rate of synthesis of corticosterone**

Plasma corticosterone was estimated by the method of Mattingly (1962). The in vitro rate of synthesis of corticosterone by the adrenal gland was studied by incubating the adrenals in 1 ml of Hanks solution at 37°C for 30 min; the incubating fluid was thereafter discarded. The

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**Table 1.**

Effect of 5,6-dihydroxytryptamine (DHT, 75 μg/rat icv), methyl ester of parachlorophenylalanine (PCPA, 100 μg/rat icv for 3 days), and 6-hydroxydopamine (6-OH-DA, 200 μg/rat icv) in benztpine (Benz, 25 mg/kg ip) and desmethylimipramine (DMI, 25 mg/kg ip) treated rats on whole brain monoamine (5-HT, NA and DA) levels.

Estimations were done at time intervals after pre-treatment as indicated in the text.

<table>
<thead>
<tr>
<th>Group</th>
<th>Brain monoamine concentration (μg/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-HT Mean ± SEM</td>
</tr>
<tr>
<td>Vehicle</td>
<td>8 0.5809 ± 0.0197</td>
</tr>
<tr>
<td>DHT</td>
<td>5 0.3102* ± 0.0154</td>
</tr>
<tr>
<td>PCPA</td>
<td>5 0.2640* ± 0.0152</td>
</tr>
<tr>
<td>Benz-6-OH-DA</td>
<td>3 0.05701 ± 0.0364</td>
</tr>
<tr>
<td>DMI-6-OH-DA</td>
<td>4 0.5975 ± 0.0500</td>
</tr>
</tbody>
</table>

* Indicates statistical significance at *P* < 0.001 in relation to vehicle treated controls.

**n** = Number of animals in each group. - or + indicate decrease or increase compared to vehicle treated controls.
adrenal gland was divided into quadrants and transferred to fresh tubes containing 2 ml of Hank's solution for incubation in an incubator shaker (SICO) for 2 h. Corticosterone was estimated in Hank's solution by the method of Mattingly (1962). Venous blood for plasma corticosterone estimation was collected from pentobarbital anaesthetized rats. The animals were always sacrificed between 12.00 and 13.00 h.

**Immobilisation**

Immobilisation was done by adopting the method of Fregly (1953) of strapping. The fully stretched albino rat was strapped to a wooden plank with adhesive tape after securing each limb to the plank individually as reported earlier (Amar & Sanyal 1981).

The preliminary results showed a highly significant ($P < 0.001$) increase in plasma corticosterone levels in rats sacrificed immediately after immobilisation (‘O’h) of the animals compared to the unstressed group. The corticosterone level thereafter showed no significant change up to 2 h of immobilisation. For further studies in the present series, animals were therefore administered 35 mg/kg, ip, of pentobarbital immediately after immobilisation and blood was collected in a heparinised tube and adrenal glands were removed for analysis. Control animals were not subjected to immobilisation but they also received the same dose of pentobarbital. Plasma corticosterone and its rate of synthesis by the adrenal gland in vitro were estimated in both untreated and treated groups of rats and also in unstressed and stressed animals in similar untreated and treated groups.

### Results

**Brain monoamines**

Pre-treatment with DHT and PCPA decreased the whole brain 5-HT level by 46.6% and 54.55%, respectively, compared to the vehicle treated chronically cannulated control group. In Benz-6-OH-DA treated rats, whole brain NA levels were found to be significantly decreased (42.5%) compared to similarly administered vehicle treated group with no significant change in DA and 5-HT levels. In DMI-6-OH-DA treated rats, whole brain DA levels were found to be significantly decreased (46.25%) compared to the vehicle treated group with no significant change in NA and 5-HT level. The results are summarized in Table 1.

**Plasma corticosterone**

In unstressed rats, plasma corticosterone levels were significantly decreased after pre-treatment with either DHT or PCPA, and not significantly affected in either Benz-6-OH-DA or DMI-6-OH-DA groups. Stress caused a significant ($P < 0.001$) increase in plasma corticosterone levels in the untreated group of rats. This increase in plasma corticosterone levels in stressed rats were significantly inhibited in animals pre-treated with DHT, PCPA and Benz-6-OH-DA, but not in the group pre-treated with DMI-6-OH-DA. The results are summarized in Table 2.

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**Table 2.**

Effect of 5,6-dihydroxytryptamine (DHT, 75 μg/rat icv), methyl ester of parachlorophenylalanine (PCPA, 100 μg/rat icv for 3 days), and 6-hydroxydopamine (6-OH-DA, 200 μg/rat icv) after benztpine (Benz, 25 mg/kg ip) and desmethyl-imipramine (DMI, 25 mg/kg ip) treatment, on plasma corticosterone (μg/100 ml) levels in unstressed and stressed rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Unstressed</th>
<th>Stressed (‘O’ h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Vehicle</td>
<td>22</td>
<td>32.90 ± 1.89</td>
</tr>
<tr>
<td>DHT</td>
<td>13</td>
<td>24.34* ± 2.86</td>
</tr>
<tr>
<td>PCPA</td>
<td>5</td>
<td>26.40* ± 1.93</td>
</tr>
<tr>
<td>Benz-6-OH-DA</td>
<td>8</td>
<td>31.75 ± 3.49</td>
</tr>
<tr>
<td>DMI-6-OH-DA</td>
<td>11</td>
<td>35.59 ± 3.14</td>
</tr>
</tbody>
</table>

*, ** = indicate statistical significance at $P < 0.025$ and $P < 0.001$ in relation to vehicle treated unstressed rats. a, b = indicate statistical significance at $P < 0.01$ and $P < 0.001$ in relation to vehicle treated stressed rats (Student's t-test). n = indicates number of rats in each group.
Table 3.
Effect of 5,6-dihydroxytryptamine (DHT, 75 µg/rat icv) and 6-hydroxydopamine (6-OH-DA, 200 µg/rat icv) after benztropine (Benz, 25 mg/kg ip) and desmethyl-imipramine (DMI, 25 mg/kg ip) treatment, on corticosteroid synthesis rate (µg/100 mg/h) in vitro in unstressed and stressed rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Unstressed</th>
<th>Stressed (0' h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Vehicle</td>
<td>12</td>
<td>2.04 ± 0.27</td>
</tr>
<tr>
<td>DHT</td>
<td>10</td>
<td>1.17* ± 0.09</td>
</tr>
<tr>
<td>Benz-6-OH-DA</td>
<td>5</td>
<td>2.32 ± 0.46</td>
</tr>
<tr>
<td>DMI-6-OH-DA</td>
<td>7</td>
<td>1.45 ± 0.28</td>
</tr>
</tbody>
</table>

*, ** indicate statistical significance at P < 0.01 and P < 0.001 in relation to vehicle treated unstressed rats. a = indicates statistical significance at P < 0.001 in relation to vehicle treated stressed rats (Student’s t-test). n = indicates number of rats in each group.

Corticosteroidogenesis

The rate of synthesis of corticosterone by adrenal glands was not significantly altered in unstressed rats after pre-treatment with either Benz-6-OH-DA or DMI-6-OH-DA. There was, however, a significant decrease in the rate of synthesis in the group pre-treated with DHT and PCPA. There was a significant (P < 0.001) increase in the in vitro rate of synthesis of corticosterone by the adrenal gland in stressed untreated animals. This increase in rate of synthesis by stress was significantly inhibited in animals pre-treated with DHT and Benz-6-OH-DA, but not in the group pre-treated with DMI-6-OH-DA (Tables 3 and 4). ACTH (Sigma) significantly increased the rate of synthesis when added to Hank’s solution in a concentration of 2 × 10⁻⁴ IU/ml for 30 min. Hank’s solution was discarded and quadrants were incubated in 2 ml of fresh Hank’s solution as described. ACTH was found to cause an increase in the rate of synthesis of corticostrone by adrenal glands of both control (untreated and unstressed) and PCPA treated unstressed animals (Table 4).

Table 4.
Effect of ACTH (2 × 10⁻⁴ IU/ml, 30 min) on in vitro corticosteroid synthesis rate (µg/100 mg/h) in vehicle and methyl ester of PCPA (100 µg/rat × 3 days, icv) treated rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Untreated</th>
<th>ACTH-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Vehicle</td>
<td>8</td>
<td>2.28 ± 0.24</td>
</tr>
<tr>
<td>PCPA</td>
<td>4</td>
<td>0.80* ± 0.18</td>
</tr>
</tbody>
</table>

* = indicates statistical significance at P < 0.001 in relation to vehicle treated group. a = indicates statistical significance at P < 0.01 in relation to untreated PCPA group (Student’s t-test). n = indicates number of rats in each group.
Discussion

The plasma corticosterone level and in vitro rate of synthesis of corticosterone by the adrenal gland have been taken as the index of ACTH activity. The possible role of brain monoamines on ACTH activity was studied by selectively inhibiting the tryptaminergic, noradrenergic or dopaminergic neuronal activity by administering drugs known to affect the desired neuron selectively. DHT and PCPA selectively decreased the 5-HT levels of the whole brain while DMI-6-OH-DA and Benz-6-OH-DA treated groups showed significant inhibition in dopamine and noradrenaline levels in the whole brain, respectively. Plasma corticosterone levels and rate of synthesis of corticosterone were estimated in unstressed and stressed animals and also in untreated and treated groups in order to study the possible role, if any, of 5-HT, NA or DA on the resting adrenal cortical activity and on the activity of adrenal cortex under stressful condition.

Plasma corticosterone levels and the in vitro rate of synthesis of corticosterone by adrenal gland were significantly inhibited in DHT and PCPA treated groups but not significantly affected in DMI-6-OH-DA and Benz-6-OH-DA treated groups in unstressed rats. Because of the known circadian rhythm of adrenal corticoid activity, animals were always sacrificed between 12.00 and 13.00 h. These results are in agreement with those of Van Delft et al. (1973) who reported similar inhibition of plasma corticosterone levels and in vitro rate of synthesis of corticosterone in ip administered PCPA treated animals compared to their peak levels (at 20.00 h) in the untreated group. The ability of the adrenal gland to synthesize corticosterone in response to ACTH was not affected when tryptaminergic neurons were selectively inhibited by PCPA pre-treatment, which indicates that even when PCPA treatment had caused a significant inhibition in the rate of synthesis of corticosterone by the adrenal gland in vitro, the gland did not lose its responsiveness to ACTH. These results have been interpreted as suggesting a central tonic control mechanism for adrenal cortex activity and this central control mechanism is probably 5-HT mediated.

To study the effect of stress, the animals were sacrificed immediately after immobilisation and has been called '0' h of immobilisation. This was done because of the observation that a significant increase in plasma corticosterone levels is observed at '0' h of immobilisation and thereafter up to 2 h of immobilisation there is no further significant increase (Amar & Sanyal, unpublished data). The significant increase in the plasma corticosterone level at '0' h of immobilisation is interpreted to mean that due to the stress of handling, the hypothalamo-pituitary-adrenal axis is stimulated through the release of ACTH by the pituitary and its action on adrenal steroidogenesis and corticosterone release in the plasma. This is substantiated by the observations of the present study that not only are plasma corticosterone levels raised at '0' h but that the mean rate of synthesis of corticosterone by the adrenal gland per hour is also increased. The increase in plasma corticosterone levels and rate of synthesis of corticosterone are, however, significantly inhibited in stressed rats treated with DHT, PCPA, and Benz-6-OH-DA but not in the group treated with DMI-6-OH-DA. These results indicate that ACTH release under acute stress is both 5-HT and NA mediated. DA does not seem to play any significant role in the release of ACTH during stress.

The observation that the DHT and PCPA treatments decrease the significant increase in plasma corticosterone level and rate of synthesis of corticosterone during acute restraint stress is again in conformity with the observations of Van Delft et al. (1973) but in contrast to those of Telegdy & Vermes (1976). The latter investigators reported significant decrease in hypothalamic 5-HT levels after restraint with a simultaneous increase in plasma corticosterone levels. They concluded that the tryptaminergic system has an inhibitory action on the pituitary-adrenal axis. We, however, did not observe any significant decrease in the hypothalamic 5-HT levels from 0 to 2 h of immobilisation (Sanyal & Amar 1981). There are very few reported studies which indicate the role of more than one monoamine on ACTH secretion. Jones et al. (1976) concluded that while 5-HT was stimulatory, NA was inhibitory to ACTH release. The results of the present study, however, indicate that both 5-HT and NA stimulate ACTH secretion during stress. The stimulatory role of NA on ACTH secretion is in conformity with the reported conclusion of Kawa et al. (1978), but in disagreement with others who have reported that NA either inhibits ACTH secretion (Scapagnini et al. 1972; Jones et al. 1976; Oishi 1979) or has no effect on it (Kumeda et al. 1974; Abe & Hiroshige 1974).

Our data indicate that DA does not have any
significant role on ACTH secretion or adrenal steroidogenesis. This observation again is in conformity with those of Kawa et al. (1978) but contradictory to those of Van Loon et al. (1971) and Ulrich & Yuwiler (1973). The observation that cyproheptadine is of value in the treatment of Cushing’s syndrome (Weiner & Ganong 1978) and that l-dopa has no significant effect on the symptoms of Cushing’s syndrome (Liddle & Melman 1974) may be considered as supportive evidence that the central tonic control of adrenal steroidogenesis may be 5-HT mediated alone and that DA has no significant role.

It is, therefore, concluded that there is a central tonic control mechanism of adrenal steroidogenesis and that this is 5-HT mediated. During acute stress ACTH secretion may be both 5-HT and NA mediated.

References


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