Inhibition of Enzymatic Synthesis of Epinephrine by Low Doses of Glucocorticoids

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ABSTRACT. Low doses of dexamethasone depress the activity of the epinephrine-forming enzyme, phenylethanolamine-N-methyl transferase, in the rat adrenal medulla; they also decrease its content of epinephrine. This inhibition can be blocked by the concurrent administration of small amounts of ACTH. Very high doses of the steroid do not lower the enzyme activity, nor do they depress the catecholamine content in the adrenal. (Endocrinology 80: 825, 1967)

The conversion of norepinephrine to epinephrine in mammals is catalyzed by phenylethanolamine-N-methyl transferase (PNMT), an enzyme which is highly localized to the adrenal medulla (1). It has recently been shown that the activity of this enzyme is controlled by hormones secreted from the pituitary and adrenal cortex (2). The rate of epinephrine synthesis and the content of this amine in the adrenal medulla decline following hypophysectomy; both can be restored by the administration of ACTH or glucocorticoids. The increase in PNMT activity produced by treating hypophysectomized rats with dexamethasone can be blocked by the concurrent administration of actinomycin D or puromycin, compounds that inhibit protein synthesis (3).

The amounts of glucocorticoid required to restore PNMT activity in the hypophysectomized rat are several orders of magnitude higher than those needed to restore most other steroid-dependent enzymes (e.g., more than 30 mg of hydrocortisone/day) (3, 4). These large doses are consistent with the high levels of hydrocortisone or corticosterone normally present in the adrenal venous effluent. A major portion of the rat adrenal medulla is perfused by an intra-adrenal portal system, which carries steroid-rich blood that has already passed through the adrenal cortex (5). It has been suggested that the location of the medulla within the cortex is, therefore, an important factor in maintaining the normal rate of epinephrine synthesis in this animal (4).

When intact rats are treated with low, physiologic doses of glucocorticoids, the release of endogenous ACTH from the pituitary is suppressed, and the adrenal cortex subsequently atrophies (6). Such treatment produces no signs of adrenocortical insufficiency in peripheral target organs of the adrenal gland, because circulating glucocorticoid activity is maintained at normal levels by the exogenous steroid. However, the contents of glucocorticoid within the adrenal gland and the adrenal venous effluent decline. It will be shown that the repeated administration of low doses of glucocorticoids depresses epinephrine synthesis in the intact rat. Larger doses, on the

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Table 1. Effect of various doses of dexamethasone on PNMT activity and corticosterone levels

<table>
<thead>
<tr>
<th>Dexamethasone administered (µg)</th>
<th>Adrenal weight (mg/pair)</th>
<th>PNMT activity (U/adrenal)</th>
<th>Corticosterone (µg/adrenal)</th>
<th>Corticosterone (µg/100 ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>52.4 ± 1.8</td>
<td>4.05 ± 0.14***</td>
<td>1.17 ± 0.14</td>
<td>58 ± 10</td>
</tr>
<tr>
<td>1</td>
<td>49.6 ± 3.6</td>
<td>3.37 ± 0.48*</td>
<td>1.77 ± 0.32*</td>
<td>54 ± 8</td>
</tr>
<tr>
<td>10</td>
<td>47.1 ± 1.7</td>
<td>2.20 ± 0.11</td>
<td>1.02 ± 0.16</td>
<td>50 ± 6</td>
</tr>
<tr>
<td>100</td>
<td>39.3 ± 2.5</td>
<td>2.82 ± 0.25*</td>
<td>0.32 ± 0.08***</td>
<td>9 ± 3***</td>
</tr>
<tr>
<td>1000</td>
<td>34.9 ± 4.2</td>
<td>3.41 ± 0.35*</td>
<td>0.21 ± 0.07***</td>
<td>6 ± 2*</td>
</tr>
</tbody>
</table>

Groups of 6 rats were treated for 9 days.
* Differs from group treated with 10 µg, p < 0.05.
** Differs from group treated with 100 µg, p < 0.01.
*** Differs from group treated with 1000 µg, p < 0.001.

The other hand, do not depress PNMT activity, even though they cause an even greater atrophy of the adrenal.

Materials and Methods

Female Sprague-Dawley rats weighing 180-200 g were treated for 9-14 days with daily intraperitoneal injections of 1-1000 µg of dexamethasone. The animals were then killed at 9 AM, and the adrenals were removed. One gland and serum obtained from carotid artery blood were assayed for their content of corticosterone; the other gland was assayed for its PNMT activity, and, in some experiments, for its epinephrine content. In one experiment, animals received ACTH (0.1–1.0 U subcutaneously daily) in addition to the dexamethasone.

Corticosterone was assayed by the method of Glick et al. (7). Adrenal catecholamines (6) and PNMT activity (3) were estimated by methods described previously, except that the PNMT assay was modified as follows: Phenylethanolamine was used in place of normetanephrine as the methyl acceptor, and the 14C-N-methyl phenylethanolamine formed enzymatically was extracted into a mixture of toluene:isooctyl alcohol (30:1). This modification decreases the blank values and increases the sensitivity of the assay. One unit of enzyme represents 1 mumole of 14C-methyl N-methyl phenylethanolamine formed per hour.

Each experimental group contained 6 animals. Data are expressed as mean ± standard error.

Results and Discussion

Groups of animals were treated for nine days with 1, 10, 100 or 1000 µg of dex-

1 9α-Fluoro-16α-methyl-Δ1-dehydrocortisol, obtained as dexamethasone-21-phosphate.
2 Partially purified porcine ACTH, obtained as ACTHar gel.

amethasone/day; control animals received an equal volume (0.25 ml) of isotonic saline. Ten µg of the steroid constituted the minimum dose needed to produce a significant decline in the weight of the adrenals (Table 1). This dose also lowered the content and concentration of corticosterone in the adrenal, when the glands were compared with those obtained from rats treated with 1 µg of the glucocorticoid. In addition, 10 µg of dexamethasone produced a highly significant fall in PNMT activity (46%). Larger doses had progressively less inhibitory activity, so that rats treated with 1 mg of the steroid showed epinephrine-forming activity which did not differ significantly from that observed in control animals.

Since the turnover of epinephrine in the adrenal proceeds very slowly (9), hypophysectomy is not followed by a rapid decline in the epinephrine content of this gland. However, previous studies had shown such a decrease, when animals were examined two to four weeks after the removal of the pituitary (4). To determine whether the prolonged administration of dexamethasone in doses that inhibited PNMT activity could also depress adrenal epinephrine levels, rats were treated for 14 days with 10 or 100 µg of the steroid. Both doses produced an atrophy of the adrenal gland (Table 2). The lower dose caused a 61% fall in PNMT activity, and a significant decline (21%) in the epinephrine content of the adrenal. The higher dose produced less inhibition of PNMT activity.
(37%), and no change in the adrenal epinephrine content.

The observation that very high doses of dexamethasone were less effective in inhibiting PNMT activity than lower doses (which produced less adrenal atrophy) suggested that enzyme activity depended upon the high level of glucocorticoid within the adrenal venous effluent. Low doses of the steroid would be expected to suppress the release of endogenous ACTH; this would cause a selective decline in the amount of glucocorticoid delivered to the adrenal medulla. Higher doses, while shutting off the synthesis of corticosterone in the adrenals, might restore the physiologic hyperadrenocorticism, or "Cushing’s disease," within the medulla, and thus not depress PNMT activity. If this explanation were correct, treatment with ACTH might be expected to antagonize the inhibitory effects of low doses of dexamethasone on PNMT activity. Rats were treated for nine days with both dexamethasone and small amounts of ACTH. Animals receiving the steroid alone showed a 30% decline in PNMT activity (Table 3). This fall was effectively blocked by administering the exogenous ACTH.

These observations are consistent with the hypothesis that the maintenance of normal PNMT activity in the rat adrenal medulla depends on the perfusion of this organ with glucocorticoid-rich blood from the adrenal cortex. When PNMT activity was reduced by treating rats daily with 10 μg of dexamethasone, there was also a significant decrease in the weight of the whole adrenal gland (Tables 1, 2). This indicates that the release of ACTH from the pituitary had been partially suppressed, and suggests that the secretion of corticosterone had also declined. This dose of dexamethasone also lowered the content of corticosterone in the adrenal, but only if animals so treated were compared with rats that had received 1 μg of the synthetic steroid. It did not lower the concentration of corticosterone in the peripheral blood, nor did it appear to lower adrenal corticosterone levels if treated animals were compared with rats that had been given only isotonic saline (Table 1). Several explanations may be offered for this apparent paradox: 1) It is possible that, had the animals been killed later in the day (i.e., when adrenal corticosterone levels normally rise), the steroid levels in glands from control rats would also have been significantly higher than those from rats which received the 10-μg dose. This dose of dexamethasone might interfere with the 24-hour rhythm in corticosterone synthesis, and the daily peaks in the secretion of this hormone.

### Table 2. Effect of dexamethasone on PNMT activity and epinephrine content in the adrenal

<table>
<thead>
<tr>
<th>Dexamethasone administration (μg)</th>
<th>Adrenal weight (mg/pair)</th>
<th>PNMT activity (U/adrenal)</th>
<th>Epinephrine content (μg/adrenal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>58.2 ± 2.0*</td>
<td>6.56 ± 0.23*</td>
<td>30.6 ± 1.0*</td>
</tr>
<tr>
<td>10</td>
<td>45.6 ± 2.0</td>
<td>2.58 ± 0.16</td>
<td>24.2 ± 1.3</td>
</tr>
<tr>
<td>100</td>
<td>34.2 ± 1.8*</td>
<td>4.14 ± 0.22*</td>
<td>31.7 ± 1.6*</td>
</tr>
</tbody>
</table>

Groups of 6 rats were treated for 14 days.
* Differs from group treated with 10 μg, p < 0.01.

### Table 3. Effects of dexamethasone and ACTH administration on PNMT activity in the adrenal

<table>
<thead>
<tr>
<th>Dexamethasone administered (μg)</th>
<th>ACTH administered (U)</th>
<th>PNMT activity (U/adrenal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>4.85 ± 0.47*</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>3.27 ± 0.31</td>
</tr>
<tr>
<td>10</td>
<td>0.1</td>
<td>4.53 ± 0.24**</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
<td>4.61 ± 0.24**</td>
</tr>
</tbody>
</table>

Groups of 6 rats were treated for 9 days.
* Differs from group treated only with dexamethasone, p < 0.05.
** Differs from group treated only with dexamethasone, p < 0.01.
might be important in maintaining PNMT activity. The same argument could explain the apparent failure of 10 μg of dexamethasone to suppress circulating corticosterone levels. 2) It is also possible that the content of corticosterone in the adrenal is a poor index of small changes in the secretion of this steroid. Clearly the reduction in ACTH secretion was only partial; a much greater reduction in adrenal weight could be elicited by giving animals larger doses of dexamethasone (Tables 1, 2).

There are a variety of clinical situations in which patients are treated chronically with low, “replacement doses” of glucocorticoids. Since these doses produce little or no change in the mean blood corticoid level, they are generally assumed to be without metabolic side-effects. The studies described in this report suggest that such doses may interfere with normal rates of epinephrine synthesis, at least in the rat. It should be of interest to determine whether patients who have been maintained chronically on low doses of glucocorticoids show an abnormality in their ability to synthesize epinephrine.

An alternative interpretation of these data might be that, under the stimulus of ACTH, the adrenal cortex secretes a highly potent steroid, which differs from corticosterone, hydrocortisone, estradiol, testosterone and aldosterone (4), and which serves to maintain PNMT activity. Hypophysectomy or low doses of dexamethasone would depress the secretion of this substance; glucocorticoids might serve as a substitute for it, but only in very large doses. It does not seem necessary to invoke an unknown steroid to explain the above findings, inasmuch as the levels of glucocorticoid which are normally available to the medulla should be adequate to induce the epinephrine-forming enzyme. However, the identification of a natural (or synthetic) steroid which stimulates PNMT activity but lacks potency as a glucocorticoid might be of some clinical interest.

Acknowledgment
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References