CORTICOSTERONE AND METHYLATION OF NORADRENALINE BY EXTRA-ADRENAL CHROMAFFIN TISSUE

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SUMMARY

The effects of corticosterone and deoxycorticosterone in concentrations of 10 and 30 µg./ml. on organ cultures of extra-adrenal chromaffin cells of neonatal rabbits were investigated.

The presence of corticosterone resulted in synthesis and storage of about 35% of adrenaline. Deoxycorticosterone did not induce methylation so that the ratio of catecholamines remained at about 90% noradrenaline.

The results indicate the importance of the 11-oxy group in steroid-induced methylation of catecholamines.

INTRODUCTION

The close proximity of adrenaline (A)-storing chromaffin cells to adrenocortical tissue and the more distant association of noradrenaline (N)-storing cells with these elements has suggested the possibility that high concentrations of corticosteroids may be involved in some way in the methylation process (Coupland, 1953, 1965).

Wurtman & Axelrod (1966) demonstrated in rats that the activity of phenylethanolamine N-methyltransferase, which catalyses the N-methylation of noradrenaline to form adrenaline, falls after hypophysectomy but that this change can be restored by injections of corticotrophin (ACTH) or dexamethasone. These restorative effects were blocked by the concurrent administration of puromycin or actinomycin D and hence it is likely that the activity of the corticosteroids is exerted through their effects on RNA transcription from DNA and involves the synthesis of new enzyme protein.

In the rabbit (Coupland, 1953, 1956, 1965) chromaffin tissue forms a well-defined body or bodies in the pre-aortic region, which may or may not be continuous with the adrenal medullae. Assay or histochemical examination of the pre-aortic tissue shows a catechol content of more than 90% noradrenaline, whereas the catecholamine of the adrenal medulla after birth is more than 90% adrenaline (Coupland & MacDougall, 1966). Since the N-storing extra-adrenal tissue may be readily identified in the living specimen up to some 2 weeks after birth this makes a convenient test object for studies in vitro on amine synthesis and storage.

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Previous work involving assay and electron histochemistry (Coupland & MacDougall, 1966) has demonstrated that the addition of the natural corticosteroid, corticosterone, at a concentration of 10 \( \mu g./ml. \) to organ cultures of extra-adrenal chromaffin cells, effects synthesis and storage of adrenaline in cells which normally store noradrenaline. This work has now been extended in order to determine the effects of different concentrations of corticosterone and also whether the phenomenon is dependent on the chemical structure of the natural steroid employed.

METHODS

Organ cultures of pre-aortic chromaffin bodies of 2-day-old New Zealand white rabbits (14) were prepared as described previously (Coupland & MacDougall, 1966; MacDougall & Coupland, 1967). In order to minimize variation due to inadvertent stimulation by stress, all animals were obtained from two litters born on the same day and animals from both groups were included in the control and each experimental group. The medium consisted of 80 % Parker’s tissue culture medium 199 and 20 % horse serum. The dishes were divided into groups. One group served as controls, and either corticosterone or deoxycorticosterone in concentrations of 10 or 30 \( \mu g./ml. \) in 20 \( \mu l. \) ethanol was added to the others. The tissues were incubated at 37° for 6 days under oxygen at 1 atmosphere absolute pressure, sufficient CO\(_2\) being added to maintain the pH at 7.2–7.4.

Tissues were subsequently homogenized at 0–4° in 2 ml. 0·1 N-HCl, and to this 0·5 ml. 1 % aqueous ascorbic acid + 0·5 ml. 1 % aqueous ethylenediamine tetra-acetic acid, disodium salt, was added. After centrifugation, catechol amines were adsorbed from the supernatant by alumina (Woelm neutral, activity grade 1) using the method of Weil-Malherbe & Bone (1952), eluted with 0·2 M-acetic acid and assayed fluorimetrically (Udenfriend, 1962). Fluorescence was recorded by a Locarte fluorimeter using an LF 3 primary and LF 7 + Kodak Wratten 61 as secondary filters. Other specimens were prepared for electron microscopy by the method previously described (Coupland & MacDougall, 1966).

RESULTS AND DISCUSSION

The results are summarized in Table 1. These findings using naturally occurring steroids indicate that the 11-OH grouping is essential for the induction or stimulation of methylation in extra-adrenal chromaffin cells of the rabbit. Since the appearance of adrenaline in the adrenal medulla of the foetal rabbit follows the development of a well-defined adrenal cortex (Roffi, 1964; Coupland & Weakley, 1968) the results suggest also that this may be the essential factor in the induction of methylation in the adrenal medulla. The degree of methylation obtained with a corticosteroid concentration of 10 \( \mu g./ml. \) is as great as 30 \( \mu g./ml. \) and this may represent a maximal response. A concentration of 10 \( \mu g./ml. \) is about five times the normal level present in the adrenal vein (Holzbauer & Vogt, 1961). There is a suggestion that the total concentration of amine may rise in response to higher concentrations (30 \( \mu g./ml. \)) of corticosterone, but not to higher concentrations of deoxycorticosterone which may even produce a diminution in the total. A larger number of cultures (currently
precluded by lack of facilities) is, however, required to follow this aspect of the work and also to produce a complete dose-response curve.

Electron micrographs presented an appearance in keeping with those published previously (Coupland & MacDougall, 1966). Adrenaline-type (A) storage granules were seen in large numbers in cultures exposed to corticosterone. Some contained only A granules, others mixed A and noradrenaline (N) storage granules, while others contained only N granules. In cultured controls and in those exposed to DOC more than 90% of the granules were of the N type; the few less electron dense elements may represent A storage or amine-depleted (probably during procurement and fixation) N granules.

Table 1. Catecholamine content of rabbit chromaffin tissue cultured with corticosterone and deoxycorticosterone

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Amine (μg./specimen)</th>
<th>Percentage adrenaline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>A</td>
</tr>
<tr>
<td>Control cultured PAB</td>
<td>3.20</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>1.98</td>
<td>0.19</td>
</tr>
<tr>
<td>Cultured PAB + CS, 10 μg./ml.</td>
<td>2.12</td>
<td>1.22</td>
</tr>
<tr>
<td>10 μg./ml.</td>
<td>2.32</td>
<td>1.31</td>
</tr>
<tr>
<td>30 μg./ml.</td>
<td>3.00</td>
<td>1.60</td>
</tr>
<tr>
<td>30 μg./ml.</td>
<td>3.12</td>
<td>2.10</td>
</tr>
<tr>
<td>Cultured PAB + DOC, 10 μg./ml.</td>
<td>3.02</td>
<td>0.34</td>
</tr>
<tr>
<td>10 μg./ml.</td>
<td>2.80</td>
<td>0.28</td>
</tr>
<tr>
<td>30 μg./ml.</td>
<td>2.71</td>
<td>0.33</td>
</tr>
<tr>
<td>30 μg./ml.</td>
<td>1.92</td>
<td>0.19</td>
</tr>
</tbody>
</table>

CS = corticosterone; DOC = deoxycorticosterone; PAB = pre-aortic chromaffin bodies from a 2-day-old rabbit; N = noradrenaline; A = Adrenaline.

It is of interest to note that the powerful synthetic corticoid dexamethasone (9α-fluoro-16α-methyl-11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione) used by Wurtman & Axelrod (1966) also contains an 11-OH group. Hence this grouping may again have accounted, at least in part, for the stimulation of methylating enzyme activity.

Organ cultures are particularly useful for investigations of this type, providing normal morphological features are preserved, since the general systemic effects of various corticosteroids and their specific effects on other endocrine organs do not mask or modify their effects on the target organ—in this case the chromaffin cell.

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REFERENCES


