FUNCTIONAL ACTIVITY OF THE HYPOTHALAMO-PITUITARY COMPLEX IN THE RAT AFTER BETAMETHASONE TREATMENT

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SUMMARY

The precise nature of the impairment of hypothalamo-pituitary-adrenocortical (HPA) activity which follows prolonged corticosteroid treatment is not yet understood. To study this problem, hypothalamic corticotrophin releasing hormone (CRH) content, pituitary ACTH content and the functional capacity of adenohypophysial tissue in vitro were measured in rats after treatment with betamethasone. The content of CRH and ACTH in the hypothalamus and pituitary gland respectively were markedly reduced. After stopping the treatment the hormone concentrations in both structures returned to normal with the rise in the hypothalamus preceding that in the pituitary gland. Adenohypophysial tissue from betamethasone-treated rats incubated with hypothalamic extracts from control animals showed a considerable reduction in its ability to synthesize and release ACTH. However, corticotrophin release was impaired in adenohypophyses removed from untreated rats and incubated with betamethasone but synthesis was not affected. The physiological significance and the possible clinical relevance of the results are discussed.

INTRODUCTION

The impairment of hypothalamo-pituitary-adrenocortical (HPA) activity which follows prolonged treatment of rats with corticosteroids is associated with both a failure of the pituitary gland to mobilize endogenous corticotrophin (ACTH) and the inability of the adrenal cortex to respond to it (Hodges & Mitchley, 1970a, b). However, it is not clear whether these defects are due to a direct action of the steroid on the pituitary gland or to suppression of production of corticotrophin releasing hormone (CRH) or both. Recently methods for the assessment of both the corticotrophic activity of adenohypophysial tissue and the corticotrophin releasing activity of hypothalamic tissue were developed in this laboratory (Buckingham & Hodges, 1977). This paper describes the results of some experiments in which these methods were used to study the effect of betamethasone treatment on the functional activity of the hypothalamo-pituitary complex in the rat.

METHODS

Animals

Male albino Sprague-Dawley rats (Charles River; SPF) were kept at a constant temperature of 22 °C in stock cages in a room where the experiments were performed. Food and water were available ad libitum. Animals, weighing 100–125 g, were housed two per cage for at least 7 days before the beginning of the experiments and handled three times a week (Hodges & Mitchley, 1970c).
Betamethasone

Betamethasone disodium phosphate (Glaxo Laboratories Ltd) was administered to rats in the drinking water in concentrations of 20-0, 2-0 and 0-5 μg/ml for periods of 24 h, 13 days and 7 weeks respectively. These dose régimes were such that every animal ingested a total dose of approximately 450 μg betamethasone/100 g body weight (Hodges & Mitchley, 1970a, b). At the end of the treatment the steroid solution was withdrawn and replaced with tap water. Pituitary ACTH content, hypothalamic CRH content and pituitary adrenocorticotropic activity in vitro were measured immediately, and 24, 48 and 96 h afterwards on tissue removed at 08.00 h to assess the functional activity of the HPA system.

Pituitary ACTH content

Anterior pituitary glands were removed from the rats immediately after decapitation. Each gland was ground in 1:0 ml 0-1 m-HCl and stored in a refrigerator for 24 h before freezing and storing at −30 °C (Hodges & Vernikos, 1960). ACTH was estimated by the cytochemical bioassay method (Chayen, Loveridge & Daly, 1972; Alaghband-Zadeh, Daly, Bitensky & Chayen, 1974).

Functional activity of adenohypophysial tissue in vitro

The capacity of the anterior pituitary tissue to synthesize and release ACTH in vitro was investigated both in the presence and absence of betamethasone (Buckingham & Hodges, 1977).

Hypothalamic CRH content

Hypothalami removed from rats immediately after decapitation, exactly as described by Jones, Hillhouse & Burden (1976), were extracted separately in 10 µl 0-1 m-acetic acid/0-9 % saline for 1 h at 2 °C (Hiroshige, Sakakura & Itoh, 1969). After addition of 1-0 ml of an artificial medium similar to cerebrospinal fluid (Bradbury, Burden, Hillhouse & Jones, 1974) the extract was centrifuged at 1×10^3 g for 5 min. The supernatant fluid was stored on ice for not longer than 1 h before CRH estimation which was done by the method of Buckingham & Hodges (1977) using the rise in pituitary ACTH content, which is not affected by the presence of betamethasone, as the index of hormone activity.

RESULTS

The results shown in Figs 1, 2 and 4 are all expressed as percentages of control values. This was necessary because, although every treatment was properly controlled, the control values varied from experiment to experiment. Thus, in control animals, the pituitary ACTH content (Fig. 1) varied from 107±9 to 170±9 (s.e.m.) μu./pituitary gland, the increment in ACTH released into the medium (Fig. 2a) from 80±2 to 167±10 μu./ml/mg pituitary tissue, the rise in pituitary ACTH content (Fig. 2b) from 103±8 to 160±30 μu./mg pituitary tissue and the hypothalamic CRH content (Fig. 4) from the equivalent of 168±2 to 278±40 μu. ACTH/mg pituitary tissue.

Pituitary ACTH contents at various times after stopping the betamethasone treatment are shown in Fig. 1. The pituitary ACTH was significantly (P < 0-001) reduced to approximately 38 % of the control value by the 24-h and 13-day treatments, and to 55 % by the 7-week treatment. Increases in pituitary ACTH were evident in all three groups within 24 h of steroid withdrawal. The pituitary ACTH content returned to normal within 2 days in the 24-h- and 7-week-treated animals and within 4 days in the 13-day-treated group.

The release of ACTH from pituitary glands removed from betamethasone-treated rats in response to a submaximal dose (0-5 ml) of hypothalamic extract in vitro was significantly
Fig. 1. Pituitary ACTH content in rats at various times after betamethasone treatment. Hatched bars, 24-h treatment (20 μg/ml); white bars, 13-day treatment (2 μg/ml); stippled bars, 7-week treatment (0.5 μg/ml). Every column shows the mean ± S.E.M. of the results obtained from five animals.

Fig. 2. Effect of betamethasone treatment on the pituitary adrenocorticotrophic responses to hypothalamic extract in vitro. (a) Increment in ACTH release; (b) increment in ACTH content. Hatched bars, 24-h treatment; white bars, 13-day treatment; stippled bars, 7-week treatment. Dosages as in Fig. 1. Every column shows the mean ± S.E.M. of the results obtained from five animals.

(P < 0.001) reduced. At the end of the steroid treatment ACTH release in response to a single dose of hypothalamic extract was 57.9 ± 14.5, 19.8 ± 1.6 and 42.0 ± 5.9 % of the control values in rats which received 24-h, 13-day and 7-week treatments respectively. Twenty-four hours later there was a significant (P < 0.001) increase in the responsiveness to hypothalamic extract in all groups and, within a further 24 h, pituitary ACTH release in vitro was normal (Fig. 2a).

The capacity of pituitary tissue to synthesize ACTH in vitro was also reduced by the
treatment (Fig. 2b). However, adrenocorticotrophic responses were normal within 2 days of steroid withdrawal in rats which received the overnight treatment and within 4 days in the 13-day- and 7-week-treated groups.

The effect of betamethasone on the adrenocorticotrophic activity in vitro of adeno-hypophysial tissue from untreated rats is shown in Fig. 3. Betamethasone, the presence of which does not affect the cytochemical assay, added to the incubation medium in concentrations of 1 and 10 ng/ml significantly \( (P < 0.001) \) reduced the amount of ACTH released.

Fig. 3. Effect of betamethasone in vitro on the pituitary adrenocorticotrophic response to hypothalamic extract. ●, Control; ○, 1 ng betamethasone/ml; ×, 10 ng betamethasone/ml. Every point is the mean of five determinations and is shown with its standard error.

Fig. 4. Hypothalamic corticotrophin releasing hormone content in rats at various times after betamethasone treatment. Hatched bars, 24-h treatment; white bars, 13-day treatment; stippled bars 7-week treatment. Dosages as in Fig. 1. Every column shows the mean ± s.e.m. of results obtained from five animals.
from the tissue in response to hypothalamic extract, when it was added simultaneously. Its effects were dose related. The dose–response lines in the presence and absence of betamethasone deviated significantly ($P < 0.01$) from parallelism. The rise in pituitary ACTH content induced by the hypothalamic extract was unaffected by the steroid.

The CRH content of the hypothalamus was significantly ($P < 0.001$) reduced to $20.2 \pm 3.2$, $4.5 \pm 3.1$ and $30.6 \pm 7.0$ % of the control value by the 24-h, 13-day and 7-week steroid treatments respectively. However, it rose rapidly and returned to normal within 24 h in rats which received the lowest concentration of betamethasone and within 48 h in the other groups. These results are shown in Fig. 4.

**DISCUSSION**

Until comparatively recently, studies in laboratory animals and in man on HPA function following corticosteroid treatment have been limited by the lack of sensitive methods for the direct assessment of the functional integrity of the system. The development of the cytochemical bioassay method for ACTH (Chayen et al. 1972; Alaghband-Zadeh et al. 1974) enabled us to measure the changes in circulating ACTH which occurred in the rat after betamethasone treatment (Buckingham & Hodges, 1976). This assay technique has been adapted for the determination of CRH and exploited to study the effect of betamethasone on the hypothalamo-pituitary complex.

Corticosteroid treatment caused a reduction in hypothalamic CRH content associated with a reduced ability of the pituitary gland to synthesize and release ACTH. The data support the findings of Hillhouse & Jones (1976) and explain our previous observations of a reduction in the basal secretion of ACTH and its release in response to stressful stimuli in corticosteroid-treated rats. They suggest that the impairment of HPA function is due to the action of corticosteroids on the hypothalamus or higher centres of the brain, although a reduction in hypothalamic CRH content does not necessarily reflect a reduction in the functional capacity of the gland. The rapid rise in plasma ACTH to a supranormal concentration after corticosteroid withdrawal suggests that changes in ACTH secretion can occur without simultaneous fluctuations in hypothalamic corticotrophin releasing activity (Buckingham & Hodges, 1976). A direct action of the corticosteroids on the adrenocorticotrophic activity of the pituitary gland is evident from the observation that incubation of adenohypophysial tissue with betamethasone reduces the release of corticotrophin without affecting its synthesis in response to hypothalamic extract. The mechanism of this inhibitory action is not clear but the deviation from parallelism of the dose–response lines in the presence and absence of betamethasone indicates that it is not of a competitive nature.

During recovery of normal HPA function after withdrawal of corticosteroid treatment, the ability of the pituitary gland to secrete basal amounts of ACTH returns more rapidly than the ability of the adrenal cortex to respond to it (Hodges & Mitchley, 1970a). The present work suggests that restoration of the normal functional activity of the hypothalamus precedes that of the pituitary in a similar way. Suppression of pituitary activity by corticosteroids is probably the result of both a direct action on the pituitary gland and of effects mediated via the hypothalamus while suppression of the hypothalamus may be due to a direct action on the hypothalamus (Jones et al. 1976) and to actions on higher centres in the brain.

The degree of impairment of HPA function is not related to the total amount of corticosteroid administered. All our rats received the same total dose of steroid but, when the treatment was spread over the longest period, the least profound changes in hypothalamic CRH and pituitary ACTH occurred. The findings add further support to the hypothesis
(Shuster & Williams, 1961; Daly, Myles, Bacon, Beardwell & Savage, 1967; Knol, 1969) that prolonged treatment with very small doses of corticosteroid causes only very low HPA disturbance if there is no cumulative effect.

The relevance of experimental findings in laboratory animals to clinical situations is impossible to define. However, the type of data described here cannot be obtained from studies in man and emphasizes the need for such experiments to explain the nature of steroid-induced suppression of HPA function to solve problems associated with steroid withdrawal and to lead to a clearer understanding of other types of hypothalamo-pituitary dysfunction.

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