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HYPOTHALAMO-PITUITARY ADRENOCORTICAL FUNCTION IN THE RAT AFTER TREATMENT WITH BETAMETHASONE

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1 Hypothalamo-pituitary-adrenocortical (HPA) activity was suppressed in rats treated with betamethasone.

2 Recovery of normal HPA function occurred after corticosteroid withdrawal.

3 Although corticotrophin release was rapidly restored to its basal rate there was a delay in the return of the normal adrenocorticotrophic response to stress and normal adrenocortical function was evident only after the plasma adrenocorticotrophic hormone had reached 'supra-normal' levels.

4 The physiological significance and possible clinical relevance of the results are discussed.

Introduction

Corticosteroid treatment results in impairment of hypothalamo-pituitary adrenocortical (HPA) activity which appears to be due both to failure of the pituitary gland to mobilize adrenocorticotrophic hormone (ACTH) and to inability of the adrenal cortex to respond to the hormone. The HPA system recovers from corticosteroid-induced suppression in a way which is still not understood despite the existence of data on this aspect of HPA function both in man (Daly, Myles, Bacon, Beardwell & Savage, 1967) and in the rat (Hodges & Mitchley, 1970a). Such studies are often limited by the fact that only indirect indices of pituitary adrenocorticotrophic activity have been used. This paper describes the results of experiments in the rat in which direct estimates of circulating ACTH were made after betamethasone treatment.

Methods

Animals

Male albino Sprague-Dawley rats, obtained from a closed colony (Fisons Pharmaceuticals Ltd.), 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 lib.* Animals, weighing 100–125 g, were housed two per cage 3 days before the beginning of the experiments and handled three times a week from then onwards (Hodges & Mitchley, 1970b).

Betamethasone

Betamethasone (Betnelan, Glaxo) 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 schemes were such that all animals ingested a total dose of approximately 450 μg betamethasone/100 g body weight (Hodges & Mitchley, 1970a, c). At the end of the treatment period the steroid solution was withdrawn abruptly and replaced with tap water. At various times after the cessation of treatment adrenal weights, plasma corticosterone concentrations, and plasma ACTH concentrations before and after stress were measured to assess the functional activity of the HPA system.

Collection of blood

Animals were killed by rapid decapitation. Blood was collected from the trunks into chilled heparinized tubes. The plasma was divided into two portions and stored at −30°C for ACTH estimation (Chayen, Loveridge & Daly, 1972, modified by Alaghband-Zadeh, Daly, Bitensky & Chayen, 1974) and at −4°C for corticosterone estimation (Zenker & Bernstein, 1958).

Stress

Rats were exposed for 1 min to ether vapour in a concentration sufficient to induce anaesthesia. Blood samples for ACTH estimation, were collected 2.5 min after the onset of anaesthesia (Hodges & Vernikos, 1960).

Removal of adrenal glands

Adrenal glands were removed from treated and untreated control animals immediately after the

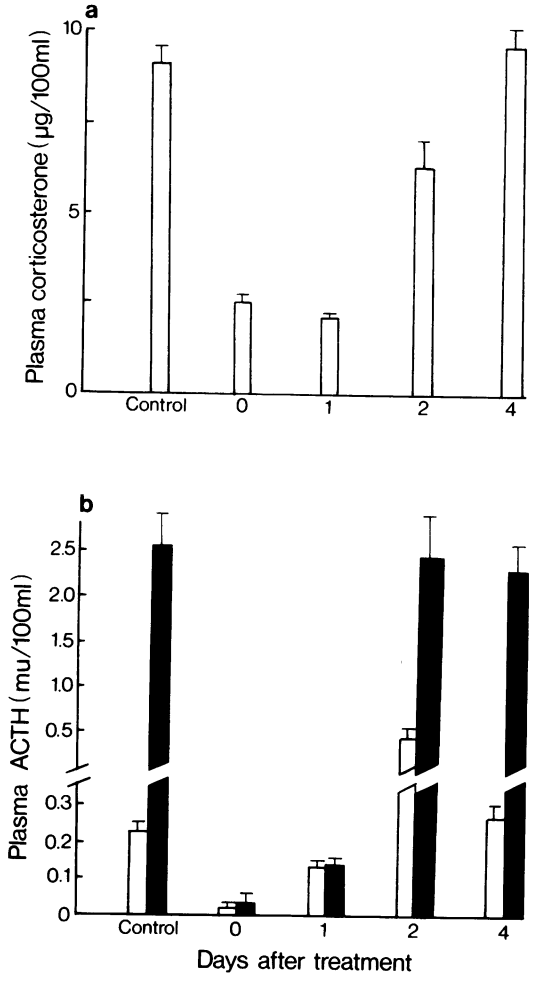

Figure 1 (a) Plasma corticosterone and (b) plasma ACTH concentrations in rats after 24 h treatment with betamethasone (20 μg/ml) in drinking water. Open columns, before ether stress; closed columns, 2.5 min after ether stress. Each column shows mean of six observations. Vertical lines indicate s.e. mean.

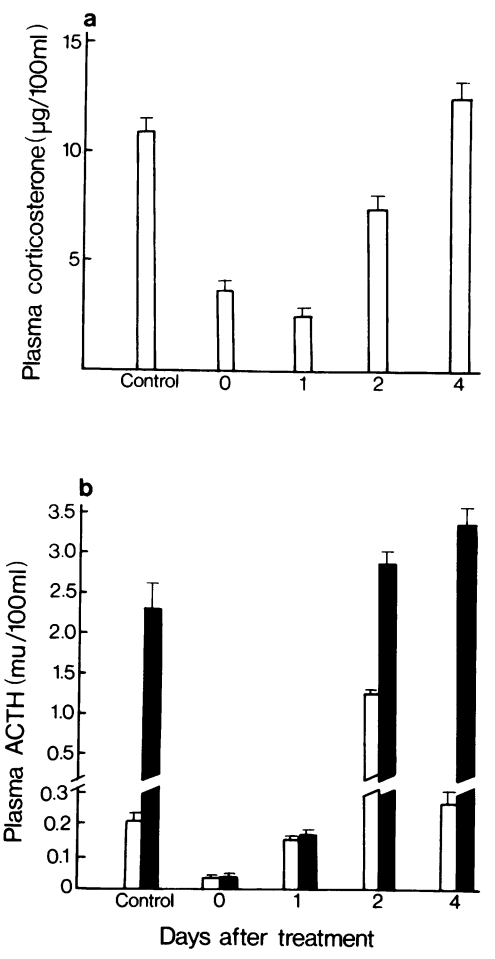

Figure 2 (a) Plasma corticosterone and (b) plasma ACTH concentrations in rats after 13 days treatment with betamethasone (2 μg/ml) in drinking water. The results are expressed as in Figure 1.

collection of blood. Each gland was dissected free from periadrenal tissue and weighed on a torsion balance.

Results

Overnight treatment

The results obtained in rats which received betamethasone in their drinking water (20 μg/ml) for 24 h are shown in Figure 1. Overnight treatment with this high concentration of betamethasone resulted in suppression of HPA activity although there was no reduction in adrenal size (18.6 ± 1.2 mg/100 g body weight compared with untreated controls, 19.2 ± 1.4 mg/100 g body weight). The plasma corticosterone concentration was reduced but two days after steroid withdrawal it had risen significantly ($P < 0.001$) to 6.2 ± 0.8 μg/100 ml, reaching the control value (9.0 ± 0.54 μg/100 ml) within 4 days. The plasma ACTH concentration in the same animals was also reduced considerably by the treatment to 0.029 ± 0.001 mu/100 ml. Twenty-four hours after withdrawal of the steroid it had risen significantly (0.137 ± 0.015 mu/100 ml) but was still lower than

the control value (0.23 ± 0.03 mu/100 ml). It continued to rise and within 2 days reached a maximum value of 0.50 ± 0.07 mu/100 ml which was significantly ($P < 0.01$) higher than normal. It then declined. Stress caused a rise in plasma ACTH concentration in untreated controls from 0.23 to 2.55 mu/100 ml. The stress-induced release of the hormone was completely inhibited immediately and 24 h after the treatment. However within 2 days the response to stress had returned.

Thirteen day treatment

The results obtained in rats which received betamethasone in their drinking water (2 μg/ml) for thirteen days are shown in Figure 2. This treatment resulted in severe suppression of HPA activity with marked adrenal atrophy (11.3 ± 1.6 mg/100 g compared with 16.6 ± 1.4 mg/100 g in control rats). The adrenal weight returned to normal within 4 days of cessation of treatment (Table 1). The plasma corticosterone concentration was significantly ($P < 0.001$) reduced from 10.92 ± 0.69 μg/100 ml to 3.85 ± 0.49 μg/100 ml by the treatment. It rose to 7.58 ± 0.62 μg/100 ml within 2 days of withdrawing the steroid and on the 4th day it was not significantly ($P > 0.05$) different from the control value. The plasma ACTH concentration was markedly reduced (0.039 ± 0.001 mu/100 ml compared with 0.21 ± 0.031 mu/100 ml in the untreated group). However, two days after stopping the treatment it rose to 1.26 ± 0.12 mu/100 ml which was significantly ($P < 0.001$) higher than in the controls. The plasma ACTH concentration then declined returning, within 4 days, to the control value. The rise in plasma ACTH concentration which normally occurs in response to stress was absent immediately and 24 h after withdrawing the betamethasone. However, some response was evident on the second day and it was normal on the fourth day after withdrawal of the steroid.

Table 1 Adrenal size in rats after 13 days treatment with betamethasone in drinking water (2 μg/ml)

Days after withdrawal of betamethasone	*Adrenal wt (mg/100 g body wt ± s.e. mean)*
0	11.3 ± 1.6*
1	10.2 ± 1.5*
2	14.3 ± 1.7
4	15.6 ± 1.9
Controls	16.6 ± 1.4

Values are mean of six determinations ± s.e. mean.
* Significantly different from the control value ($P < 0.01$).

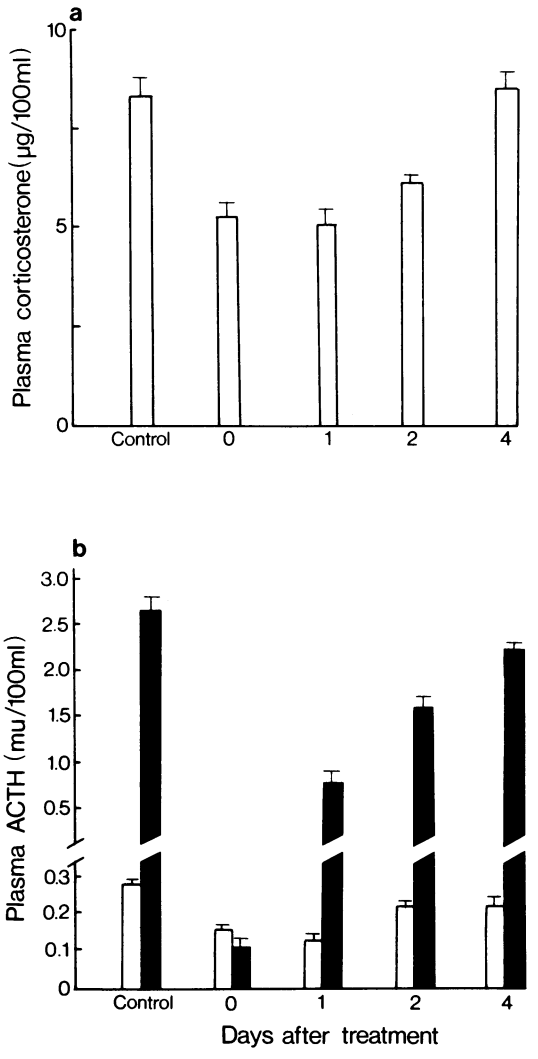

Figure 3 (a) Plasma corticosterone and (b) plasma ACTH concentrations in rats after 7 weeks treatment with betamethasone (0.5 μg/ml) in drinking water. The results are expressed as in Figure 1.

Seven weeks treatment

The results obtained in rats which received betamethasone in their drinking water (0.5 μg/ml) for seven weeks are shown in Figure 3. This treatment with the lowest concentration of betamethasone resulted in only mild suppression of HPA activity. There was no adrenal atrophy when the treatment was withdrawn (10.62 ± 0.5 mg/100 g compared with 11.2 ± 0.6 mg/100 g in the untreated group). The

plasma corticosterone concentration was significantly ($P < 0.01$) reduced but only to about 60% of the control value (5.27 ± 0.38 µg/100 ml compared with 8.33 ± 0.43 µg/100 ml). After 2 days it had risen to 6.10 ± 0.20 µg/100 ml and was normal within 4 days. The plasma ACTH concentration was also found to be reduced by about 60% immediately and 24 h after the end of the treatment. However it rapidly returned to normal and was not significantly ($P < 0.01$) different from the control value (0.29 ± 0.01 mu/100 ml) within 2 days. No rise in plasma ACTH occurred in response to stress immediately after the treatment. Twenty-four hours later there was a small but significant ($P < 0.01$) stress-induced rise from 0.14 to 0.76 mu ACTH/100 ml. Within 4 days the response was normal.

Discussion

The low plasma ACTH and corticosterone concentrations reflect the marked suppression of HPA activity which betamethasone treatment produces. This HPA inhibition has been shown to be due to failure of the pituitary to mobilize endogenous ACTH and impairment of adrenocortical sensitivity to the hormone (Graber, Ney, Nicholson, Island & Liddle, 1965; Hodges & Sadow, 1969; Hodges & Mitchley, 1970a). However most of the workers in this field have used only either indirect or insensitive methods for the assessment of pituitary adrenocorticotrophic activity which do not show clearly how the HPA system recovers from such inhibition. The results described in this paper indicate that soon after the steroid has been withdrawn the pituitary gland begins to secrete ACTH and the adrenal size is rapidly restored to normal. The 'basal' plasma ACTH concentration rises quickly to a 'supra-normal' level but there is no concomitant rise in plasma corticosterone concentration indicating that the adrenal sensitivity must still be impaired. This suggests that an elevated 'basal' level of blood ACTH is necessary to 'prime' the adrenal cortex and thus restore adrenal sensitivity to normal (Graber *et al.*, 1965). The mechanism leading to the hypersecretion of ACTH is not known but it may well be associated with negative feedback regulation of basal ACTH secretion (Buckingham & Hodges, 1974, 1975).

Acute oral administration of high doses of corticosteroids to guinea-pigs and rats has been reported to leave adrenal sensitivity to ACTH unaffected immediately after the treatment (Purves & Sirett, 1965; Hodges & Mitchley, 1970c; Hodges & Hotston, 1971). However, the present work suggests that there is a reduction in adrenal activity since 48 h after overnight treatment with betamethasone, despite the hypersecretion of ACTH which then occurs, there is no concomitant rise in plasma corticosterone concentration. Adrenocortical insensitivity to ACTH is believed to be the direct result of abnormally low circulating concentrations of the hormone (Holub, Jailer, Kitay & Frantz, 1959; Daly *et al.*, 1967) and hence the delay in the development of adrenal insensitivity may be explained by the persistence for at least 24 h of an abnormally low blood ACTH concentration.

Although the capacity of the pituitary gland to maintain 'non-stress' plasma levels of ACTH returned rapidly after the withdrawal of betamethasone, there was a marked delay before it exhibited the normal adrenocorticotrophic response to a stressful stimulus. This dissociation confirms earlier studies which suggest that the mechanisms controlling the release of ACTH under 'basal' and 'stressful' conditions are different (Zimmerman & Critchlow, 1965, 1969; Hodges & Mitchley, 1970a, c; Buckingham & Hodges, 1974).

The present work indicates that the degree of HPA suppression is not related to the total amount of steroid administered. Our rats were given the same total dose of steroid over different periods and different effects on the HPA axis were evident. This confirms earlier studies (Hicklin & Wills, 1968; Hodges & Mitchley, 1970a) but is contrary to the findings of Treadwell, Savage, Sever & Copeman (1963). Prolonged treatment with the lower concentration of the steroid caused only slight suppression of HPA activity. It has been suggested, on the basis of observations in man, that steroids may have to reach a critical level in the blood and tissues before inhibition of HPA function develops (Shuster & Williams, 1961; Daly *et al.*, 1967; Knol, 1969) and that minimal HPA disturbance may follow prolonged treatment with very small doses of the steroid when there is no cumulative effect. Our results are in accord with such a hypothesis and add further support to the importance of properly designed and controlled studies on laboratory animals to assist in the solution of clinical problems associated with corticosteroid therapy.

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References

ALAGHBAND-ZADEH, J., DALY, J.R., BITENSKY, L. & CHAYEN, J. (1974). The cytochemical section assay for corticotrophin. *Clinical Endocrinology*, **3**, 319–327.

BUCKINGHAM, J.C. & HODGES, J.R. (1974). Interrelationships of pituitary and plasma corticotrophin with plasma corticosterone in adrenalectomized and stressed

adrenalectomized rats. *J. Endocr.*, **63**, 213–222.

BUCKINGHAM, J.C. & HODGES, J.R. (1975). Interrelationships of pituitary and plasma corticotrophin and plasma corticosterone during adrenocortical regeneration in the rat. *J. Endocr.*, **67**, 411–417.

CHAYEN, J., LOVERIDGE, N. & DALY, J.R. (1972). A sensitive bioassay for adrenocorticotrophic hormone in human plasma. *Clinical Endocrinology*, **1**, 219–233.

DALY, J.R., MYLES, A.B., BACON, P.A., BEARDWELL, C.G. & SAVAGE, O. (1967). Pituitary and adrenal function during corticosteroid withdrawal in rheumatoid arthritis. *Ann. rheum. Dis.*, **26**, 18–25.

GRABER, A.L., NEY, R.L., NICHOLSON, W.E., ISLAND, D.P. & LIDDLE, G.W. (1965). Natural history of pituitary-adrenal recovery following long-term suppression with corticosteroids. *J. clin. Endocr. Metab.*, **25**, 11–16.

HICKLIN, J.A. & WILLS, M.R. (1968). Plasma 'cortisol' response to synacthen in patients on long-term small dose prednisolone therapy. *Ann. rheum. Dis.*, **27**, 33–37.

HODGES, J.R. & HOTSTON, R.T. (1971). Suppression of adrenocorticotrophic activity in the ascorbic acid deficient guinea-pig. *Br. J. Pharmac.*, **42**, 595–602.

HODGES, J.R. & MITCHLEY, S. (1970a). Recovery of hypothalamo-pituitary-adrenal function after prolonged treatment with betamethasone. *Br. J. Pharmac.*, **40**, 732–739.

HODGES, J.R. & MITCHLEY, S. (1970b). The effect of 'training' on the release of corticotrophin in response to minor stressful procedures in the rat. *J. Endocr.*, **47**, 253–254.

HODGES, J.R. & MITCHLEY, S. (1970c). The effect of betamethasone on circadian and stress-induced pituitary-adrenocortical function in the rat. *Br. J. Pharmac.*, **38**, 719–724.

HODGES, J.R. & SADOW, J. (1969). Hypothalamo-pituitary-adrenal function in the rat after prolonged treatment with cortisol. *Br. J. Pharmac.*, **36**, 489–495.

HODGES, J.R. & VERNIKOS, J. (1960). The effect of hydrocortisone on the level of corticotrophin in the blood and pituitary glands of adrenalectomized and stressed adrenalectomized rats. *J. Physiol., Lond.*, **150**, 683–693.

HOLUB, D.A., JAILER, J.W., KITAY, J.I. & FRANTZ, A.G. (1959). Direct and indirect estimation of pituitary adrenocorticotrophin reserves in man following adrenal steroid therapy. *J. clin. Endocr. Metab.*, **19**, 1540–1522.

KNOL, K. (1969). The function of the adrenal glands during prolonged corticosteroid therapy. *Scand. J. Resp. Dis. Suppl.*, **68**, 41–48.

PURVES, H.D. & SIRETT, N.E. (1965). Assay of corticotrophin in dexamethasone treated rats. *Endocrinology*, **77**, 366–374.

SHUSTER, S. & WILLIAMS, I.A. (1961). Pituitary-adrenal function during administration of small doses of corticosteroids. *Lancet*, **ii**, 674–678.

TREADWELL, B.L.J., SAVAGE, O., SEVER, E.D. & COPEMAN, W.S.C. (1963). Pituitary-adrenal function during corticosteroid therapy. *Lancet*, **i**, 355–358.

ZENKER, N. & BERNSTEIN, D.E. (1958). The estimation of small amounts of corticosterone in rat plasma. *J. biol. Chem.*, **231**, 695–701.

ZIMMERMAN, E. & CRITCHLOW, V. (1965). Dissociation of feedback control of ACTH release. Compound 'B' in corticoid treated rats before and after stress. *Fed. Proc.*, **24**, 191.

ZIMMERMAN, E. & CRITCHLOW, V. (1969). Effects of intra-cerebral dexamethasone on pituitary-adrenal function in female rats. *Amer. J. Physiol.*, **217**, 392–396.

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