Br. J. Pharmac. (1970), **40**, 732–739.

Recovery of hypothalamo-pituitary-adrenal function in the rat after prolonged treatment with betamethasone

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Summary

1. Betamethasone caused growth retardation, adrenal atrophy and impaired hypothalamo-pituitary-adrenal (HPA) activity in the rat.

2. In spite of the profound impairment, recovery of normal HPA function was rapid, but the growth retardation persisted.

3. The ability of the pituitary gland to secrete basal corticotrophin (ACTH), recovered more rapidly and the adrenocorticotrophic response to stress less rapidly than the ability of the adrenal cortex to respond to ACTH.

4. The degree of HPA suppression was not determined by the total dose of steroid.

5. The possible significance of the results is discussed.

Introduction

Hypothalamo-pituitary-adrenal (HPA) activity may be impaired in patients on corticosteroids (Landon, Wynn, James & Wood, 1965 ; Shuster & Williams, 1961 ; Treadwell, Savage, Sever & Copeman, 1963). The impairment may persist and result in serious adrenocortical insufficiency in a stressful situation such as surgery (Carreon, Canary, Meyer & Kyle, 1960 ; Salassa, Bennett, Keating & Sprague, 1953 ; Sampson, Winstone & Brooke, 1962). It is not yet known whether the corticosteroid induced suppression of HPA function is due mainly to a failure of the mobilization of endogenous corticotrophin (ACTH) or to inability of the adrenal cortex to respond to the hormone. It is also not clear to what extent the degree of HPA suppression is related to steroid dose or the duration of therapy.

Most of the observations on this subject have been made in patients. There is a need for controlled animal experiments and the work described here was done in an attempt to solve some of the problems which accompany cessation of steroid therapy. Rats were given betamethasone for long periods and the functional activity of the HPA system was studied after the steroid had been withdrawn.

Methods

Approximately 300 male albino Sprague-Dawley rats (Fisons Pharmaceuticals Ltd.) were used. The animals were housed two/cage during the experiments and for at least 10 days before the beginning of any treatment. Food (Diet 41B, Lane-

Petter & Dyer, 1952) and water were allowed *ad lib* before and throughout the experimental period. The laboratory was lit by daylight and kept at a constant temperature of 23° C.

Betamethasone (Betnesol-Glaxo) was dissolved in tap water in concentrations of 0·5 and 2·0 μg/ml. These solutions were given to the rats instead of drinking water for periods of 7 or 2 weeks. The two dose schemes were selected on the basis of a series of preliminary experiments to ensure that all the animals ingested a total dose of approximately 450 μg betamethasone/100 g. The steroid solution was abruptly withdrawn and replaced by tap water. The rats were weighed throughout the experimental period. Adrenal weights were measured at various times after the end of steroid administration.

At intervals after withdrawal of betamethasone, basal plasma corticosterone concentrations and the increment in these concentrations 30 min after either the

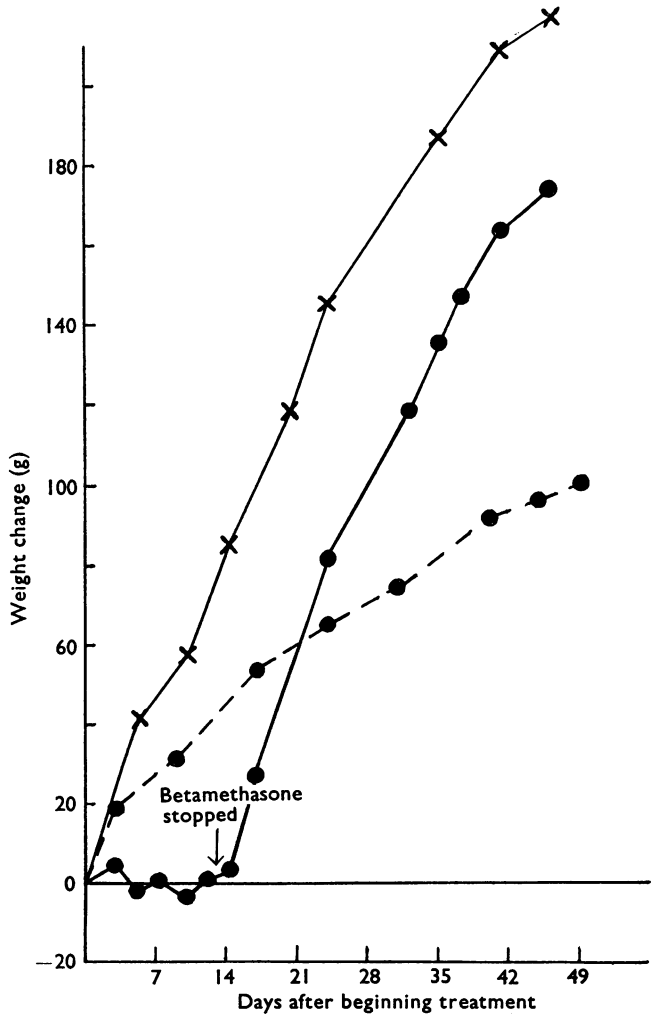

FIG. 1. Growth rate in rats given betamethasone in the drinking water. (×——×) controls; (●——●) betamethasone 2 μg/ml for 2 weeks; (●- - -●) betamethasone 0·5 μg/ml for 2 weeks.

stress of exposure to ether vapour for 1 min or the injection of tetracosactrin (Cortrosyn-Organon) were determined. The circadian rhythm in plasma corticosterone concentration was also studied as described by Hodges & Mitchley (1970a).

Corticosterone production by the adrenal glands *in vitro* was measured using the method of van der Vies, Bakker & de Wied (1960), modified by Flack (1970). Adrenal glands were removed rapidly under ether anaesthesia, cleaned and weighed. Each adrenal was quartered and placed in a flask containing 3 ml Krebs-Ringer-bicarbonate-glucose medium and incubated with shaking for 1 h at 36·5° C under oxygen + 5% Co_2. The glands were then transferred to fresh medium and incubated for a further hour.

Corticosterone concentrations in the *in vitro* medium and in plasma were estimated as described by Zenker & Bernstein (1958). Adrenal glands and plasma samples (Hodges & Sadow, 1967) were all obtained between 09.00 and 12.30 h to minimize circadian rhythmical variations.

Results

The growth rates of the betamethasone treated rats are shown in Fig. 1. Body growth was almost completely prevented by the higher concentration of the steroid and considerably reduced by the lower concentration. Some rats (about 5%) died, usually with an infection. The death rate was higher when younger rats or greater steroid concentrations were used. More of the treated rats died during the first 1 or 2 days of steroid withdrawal and the survivors remained growth retarded.

Table 1 shows adrenal weights after betamethasone treatment. The adrenal atrophy induced by the higher dose of the steroid was significant ($P<0·01$) but the adrenal weights returned to normal within 3 or 4 days of stopping the treatment. The lower dose of betamethasone had no effect.

Figs. 2 and 3 show the plasma corticosterone changes in response to stress and tetracosactrin, respectively, at various times after withdrawal of the steroid. Stress was used to assess hypothalamo-pituitary-adrenocorticotrophic activity and tetracosactrin (0·25 μg/100 g s.c. which produces a submaximal elevation in plasma corticosterone concentration) to test adrenal sensitivity. Betamethasone, 2 μg/ml for 2 weeks, obliterated the rise in plasma corticosterone concentration which normally occurs in response to ether or ACTH, but both responses returned to normal 4 days after the end of steroid treatment. Treatment with 0·5 μg/ml beta-

TABLE 1. *Adrenal tissue (mg/100 g) at various times after treatment with betamethasone*

Treatment	Days after end of treatment	Controls	Treated
Betamethasone 2 μg/ml for 2 weeks	0	18·0±1·6 (18)*	11·0±1·5 (16)†
,,	3	17·7±1·2 (12)	15·0±2·3 (24)
,,	4	17·3±1·3 (10)	16·9±1·7 (18)
Betamethasone 0·5 μg/ml for 7 weeks	1	11·0±2·4 (9)*	10·4±1·8 (10)
,,	4	11·0±1·8 (9)	11·5±2·5 (18)

Number of animals in parentheses.
* Difference between the controls occurred because older animals were used for the 7 weeks experiment.
† Difference from controls significant ($P<0·01$).

methasone for 7 weeks only reduced the responses and the return to normal was rapid.

The circadian rhythm in plasma corticosterone concentration was abolished by treatment for 2 weeks with betamethasone (Fig. 4). There was some afternoon rise on the third, and a normal increment on the fourth day after withdrawal of the steroid.

Fig. 5 shows the corticosterone produced *in vitro* by adrenal glands removed from rats under ether anaesthesia at various intervals after treatment with betamethasone (2 μg/ml) had been stopped. Corticosterone produced during the first

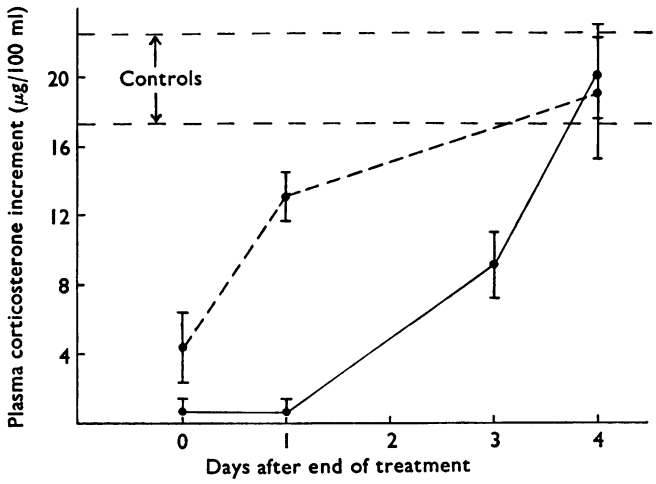

FIG. 2. Plasma corticosterone increments 30 min after stress (exposure to ether vapour for 1 min) in rats at various times after withdrawal of betamethasone (●——●, 2 μg/ml for 2 weeks; ●– –●, 0·5 μg/ml for 7 weeks). Each point is the mean of at least six determinations (twelve rats) and the vertical bars indicate the standard errors.

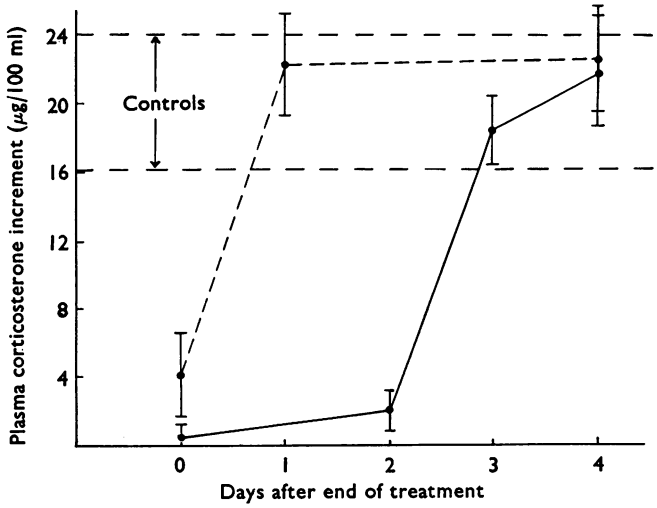

FIG. 3. Plasma corticosterone increments 30 min after tetracosactrin (0·25 μg/100 g s.c.) in rats at various times after withdrawal of betamethasone. Results are expressed as in Fig. 2.

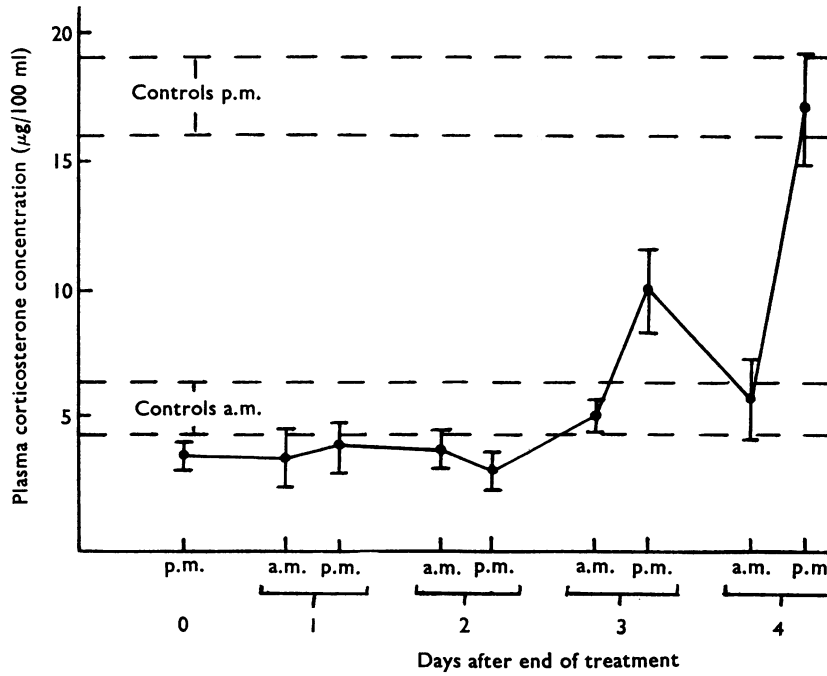

FIG. 4. Circadian rhythm in plasma corticosterone concentration in rats at various times after withdrawal of betamethasone (2 μg/ml for 2 weeks). Each point is the mean of at least six determinations and the vertical bars indicate the standard errors (a.m. 10.00–11.00 h and p.m. 16.00–17.00 hours).

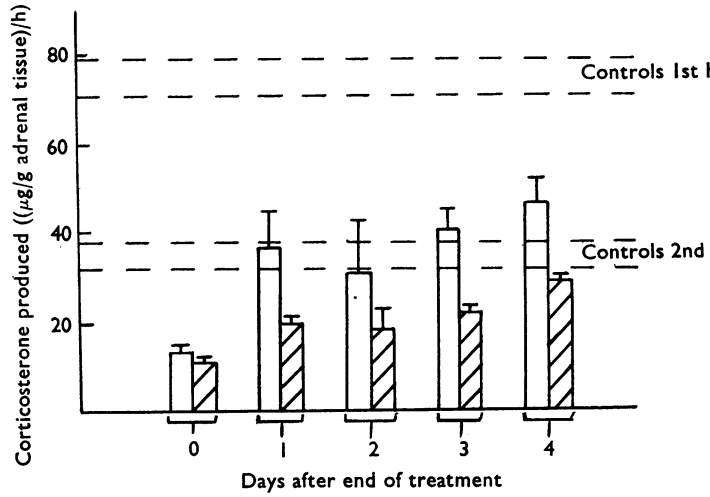

FIG. 5. Corticosterone production *in vitro* by quartered adrenal glands removed from rats anaesthetized with ether at various times after withdrawal of betamethasone (2 μg/ml for 2 weeks). (□) First hour of incubation; (▨) second hour of incubation. Each result is the mean of four determinations and the vertical bars indicate the standard errors.

and second hour incubation periods is shown. Production of corticosterone during the first hour of incubation was considerably reduced even 4 days after cessation of treatment. During the second hour, however, corticoidogenesis *in vitro* was normal in adrenal glands removed 4 days after the end of the betamethasone treatment.

Discussion

A previous study (Hodges & Sadow, 1969) of the effect of prolonged treatment with cortisol was unsatisfactory both because many animals succumbed to infection and because of the stress associated with injections (Hodges & Mitchley, 1970b). In the present work these disadvantages were minimized by administering betamethasone in the drinking water as first described by Purves & Sirett (1965) for dexamethasone. Nevertheless, some animals in the steroid treated groups died. Like cortisol (Hodges & Sadow, 1969), betamethasone profoundly inhibited HPA activity. However, recovery of HPA function was rapid whereas after cortisol it was slow. Such differences in the properties of corticosteroids may help to explain the fact that, in man, corticosteroid induced suppression of HPA function has been variously reported to take hours, weeks or months to recover (Roe, Mitchell & Pennington, 1966; Ferriman & Page, 1960; Graber, Ney, Nicholson, Island & Liddle, 1965).

Impairment of HPA function following prolonged corticosteroid therapy may be due to failure of the pituitary gland to secrete ACTH as well as to insensitivity of the adrenal cortex to the hormone. Some workers, using direct estimates of plasma corticotrophin (Meakin, Tantongco, Crabbé, Bayles & Nelson, 1960; Graber *et al.* 1965), found that pituitary adrenocorticotrophic activity recovers before the ability of the adrenal cortex to respond to ACTH, whereas others (Treadwell *et al.*, 1963, Hodges & Sadow, 1969) reported that the sensitivity of the adrenal cortex returned to normal before the capacity of the pituitary gland to release corticotrophin. From our work, without ACTH estimates, it was difficult to determine whether the impaired HPA activity was due to an inability to secrete ACTH, because any failure of its release mechanism was masked by insensitivity of the adrenal cortical cells to corticotrophin. However, ACTH secretion probably occurs very soon after the end of steroid administration, because both adrenal size and the circadian rhythm in plasma corticosterone, which reflects small changes in ACTH release, returned rapidly to normal. Probably, the adrenal cortex cannot respond normally to ACTH until it has been 'primed' by the trophic hormone. Although there may be some adrenocorticotrophic activity associated with the resumption of normal basal steroid concentrations and some circadian variation, normal HPA function may still be impaired (Livanou, Ferriman & James, 1967). Certainly the mechanisms controlling ACTH release differ in their sensitivity to the inhibitory effects of corticosteroids (Hodges & Mitchley, 1970a).

In our experiments there was a delay between the return of the HPA response to stress and the adrenocortic response to ACTH. The existence of a residual defect of stress induced ACTH release is also suggested by the reduced capacity for adrenal corticoidogenesis *in vitro*. According to Bakker & de Wied (1961) the initial corticosteroid output by the adrenal glands *in vitro* is a measure of the circulating corticotrophin at the time of adrenalectomy. Since the adrenal glands were removed

from our animals under ether anaesthesia it appears that the release of ACTH in response to the anaesthetic in the steroid treated animals was reduced, even though HPA activity appeared to be normal in the other tests we used. The possibility that the initial reduced corticosterone production was due to a failure of synthesis is unlikely because steroid production was normal during the second incubation period.

The degree of suppression of HPA function does not appear to be related to the total amount of steroid administered. Our rats were given the same total dose of betamethasone over different periods and the effects on the endocrine system were different. The total dose alone appeared not to be of prime importance in this respect, in contrast to the findings of Treadwell *et al.* (1963) and Landon *et al.* (1965). Since the steroid was administered in the drinking water it attained high blood levels only infrequently, if at all. Steroids may have to reach a critical level before inhibition of HPA function is produced (Livanou *et al.* 1967; Shuster & Williams, 1961; Landon *et al.*, 1965; Knol, 1969) and hence the short-lived effect of betamethasone in our experiments may be explained. Thus, minimal HPA disturbance would probably follow multiple small doses of steroid spaced so that there is no cumulative effect. Such treatment might be preferable to alternate day therapy which does not always give good symptom relief, although effects on the endocrine system are reduced (Ackerman & Nolan, 1968; Harter, Reddy & Thorn, 1963; Jasani, Boyle, Carson-Dick, Williamson, Taylor & Watson-Buchanan, 1968).

Although the smaller dose of betamethasone given over a long period had little inhibitory effect on HPA activity, it caused marked inhibition of body growth. The higher dose of steroid affected growth even more profoundly. Retardation of growth is a well recognized effect of corticosteroids both in children and animals (Friedman & Strang, 1966; 1969; Norman & Sanders, 1969; Bellamy, 1964; Hodges & Sadow, 1969). The effect is probably due to negative nitrogen balance (Bellamy & Leonard, 1964) although some workers have implicated inhibition of growth hormone release (Hartog, Gaafar & Fraser, 1964; Frantz & Rabkin, 1964).

Failure of the adrenal cortex to respond to stimulation by ACTH and inability of the hypothalamo-pituitary complex to release ACTH follow corticosteroid treatment. We have attempted to distinguish between the two, and also to investigate the relationship between the dose regime and the effects on the HPA system. It appears that the nature, degree and duration of inhibition of HPA function is not dependent so much on the total dose of steroid as on its chemical identity and the dose regime. Further work on laboratory animals is in progress in an attempt to solve some of the important clinical problems associated with the withdrawal of steroids.

Our thanks are due to the Wellcome Trust for a generous grant which made this work possible, to Dr. W. J. Tindall of Organon Laboratories Ltd. for gifts of corticosterone and tetracosactrin, and to Dr. L. M. Flacks of Glaxo Laboratories Ltd. for betamethasone. We are grateful to Miss Elizabeth Roberts for invaluable technical assistance.

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(*Received September* 12, 1970)