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The effect of betamethasone on circadian and stress-induced pituitary-adrenocortical function in the rat

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Summary

1. Both the circadian and stress-induced changes in plasma corticosterone concentration were abolished by the inclusion of betamethasone in the drinking water of rats.

2. Adrenal sensitivity to exogenous corticotrophin (ACTH) was unimpaired by the betamethasone treatment.

3. The normal circadian rhythm in plasma corticosterone returned within 1 day of withdrawal of the steroid, but the response to stress was normal only after 3 days.

4. The possible significance of these observations is discussed.

Introduction

Corticosteroids can prevent the release of corticotrophin (ACTH) which normally occurs in response to stress, but the various mechanisms controlling pituitary adrenocorticotrophic activity are not equally sensitive to inhibitory effects by steroids (Hodges, Jones & Stockham, 1968). According to Zimmerman & Critchlow (1965) small doses of dexamethasone obliterate the normal circadian ACTH rhythm without preventing the release of the hormone in response to stress and, hence, the mechanisms controlling circadian ACTH release appear to be more sensitive to the inhibitory effects of corticoids. In the experiments described here the acute effects of betamethasone on circadian and stress-induced pituitary adrenocortical activity were studied. The recovery of pituitary adrenocortical function after withdrawal of the steroid was also investigated.

Methods

Two hundred male Sprague-Dawley rats (Fisons Pharmaceuticals Ltd.) of weight 130–170 g were used. They were kept at an even temperature of 23° C in the room in which the experiments were carried out for at least 2 weeks. They were then transferred to opaque-sided cages, two rats per cage, and handled daily for a week. No attempt was made to exclude outside noise, and the room was illuminated by daylight only. Food (Diet 41B, Lane-Petter & Dyer, 1952) and tapwater were allowed *ad lib.*

Betamethasone (Betnesol, Glaxo), dissolved in tap water, was given to the test animals in place of drinking water for 24 h. A concentration of 20 μg beta-

methasone/ml was chosen after trial experiments on liquid intake, so that each rat ingested 450–500 μg/100 g body weight during the experimental period.

Blood samples were obtained as described by Hodges & Sadow (1967) and plasma corticosterone was estimated by the method of Zenker & Bernstein (1958). Changes in plasma corticosterone concentration were taken as the index of pituitary adrenocorticotrophic activity.

Results

The plasma corticosterone concentrations were determined in rats at intervals of approximately 2 h over a 24 h period. The concentration was lowest between 08.00 and 12.00 h, and rose sharply after midday to reach a peak value at 16.00 h. It remained high until approximately 04.00 h and then declined. The circadian rhythm of plasma corticosterone concentration is shown in Fig. 1. The pattern is similar to that described by Guillemin, Dear & Liebelt (1959), Critchlow, Liebelt, Bar Sela, Mountcastle & Lipscomb (1963) and Saba, Saba, Carnicelli & Marescotti (1963).

Subsequent experiments were done in the morning at 10.00–11.00 h and in the afternoon at 16.00–17.00 h—that is, when the plasma corticosterone concentrations were at their lowest and highest circadian values respectively.

Plasma corticosterone concentrations were determined in control and experimental rats, at the end of betamethasone treatment, and 1, 2 and 3 days later. The circadian rise in plasma corticosterone was absent immediately after treatment, but the rhythm returned rapidly and was normal within one day of steroid withdrawal. The results are shown in Fig. 2.

In another experiment, plasma corticosterone was estimated before and 0·5 h after stress (exposure to ether vapour for 1 min) in control and betamethasone-treated animals. The results are shown as increments in plasma corticosterone concentration in Fig. 3. In the untreated controls the stress-induced increments were the same in the morning as in the afternoon, despite the diurnal difference in

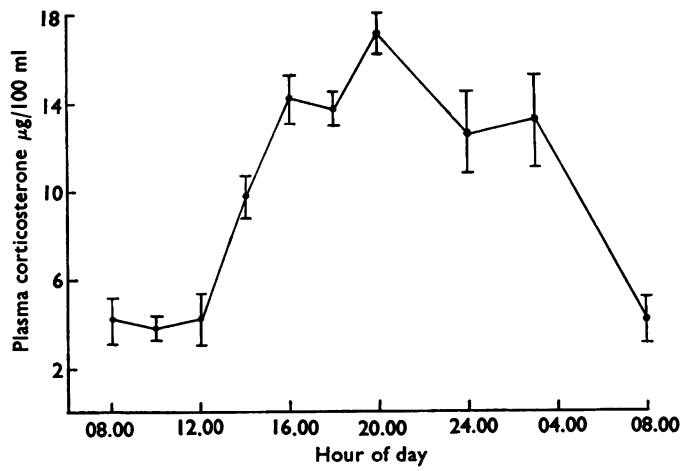

FIG. 1. Circadian rhythm in plasma corticosterone concentration in male rats. Each point is the mean (±S.E.) of six or more determinations.

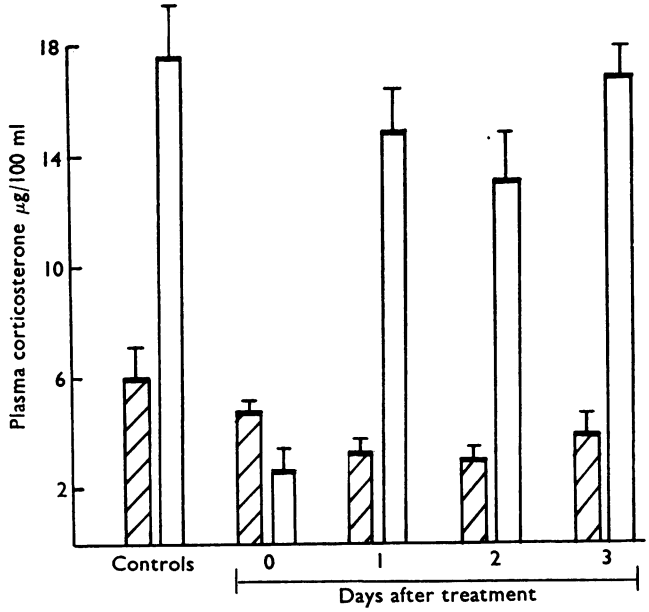

FIG. 2. Morning (hatched columns) and afternoon (open columns) plasma corticosterone concentrations in male rats after treatment with betamethasone. Each column shows the mean (±S.E.) of at least six determinations.

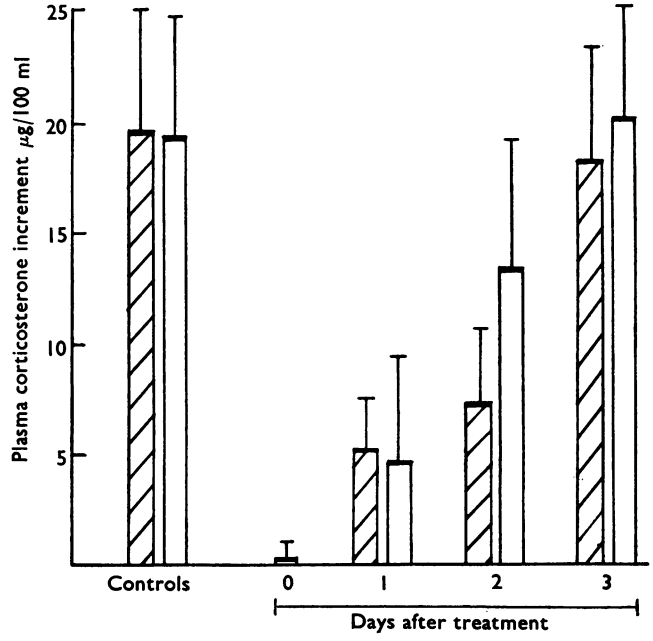

FIG. 3. Plasma corticosterone increments 0·5 h after stress, in the morning (hatched columns) and afternoon (open columns), in rats treated with betamethasone. Each column represents the mean (±S.E.) of at least six determinations (twelve rats).

the pre-stress level of the steroid. Betamethasone blocked completely the stress-induced rise in plasma corticosterone and this response was still absent one day after withdrawing the steroid. The stress response did not return to normal until the third day after treatment.

The effect of betamethasone on both the circadian and the stress-induced rise in plasma corticosterone was entirely due to the inhibition of corticotrophin release. This is evident from Fig. 4, which shows plasma corticosterone concentrations in control and betamethasone-treated rats before and after the subcutaneous injection of 0·9% sodium chloride solution or corticotrophin (Porcine ACTH, Organon—0·05 i.u./100 g body weight, Hodges & Sadow, 1969). Unlike the circadian rhythm and the response to stress, the rise in plasma corticosterone caused by exogenous ACTH was unchanged by betamethasone.

Discussion

The mechanisms controlling ACTH release under stress and non-stress conditions appear to be functionally dissociable, because small doses of corticosteroids suppress the marked circadian rise in plasma corticosterone without affecting the stress response (Zimmerman & Critchlow, 1965, 1969). Our experiments have shown that betamethasone (administered to rats as first described for dexamethasone by Purves &

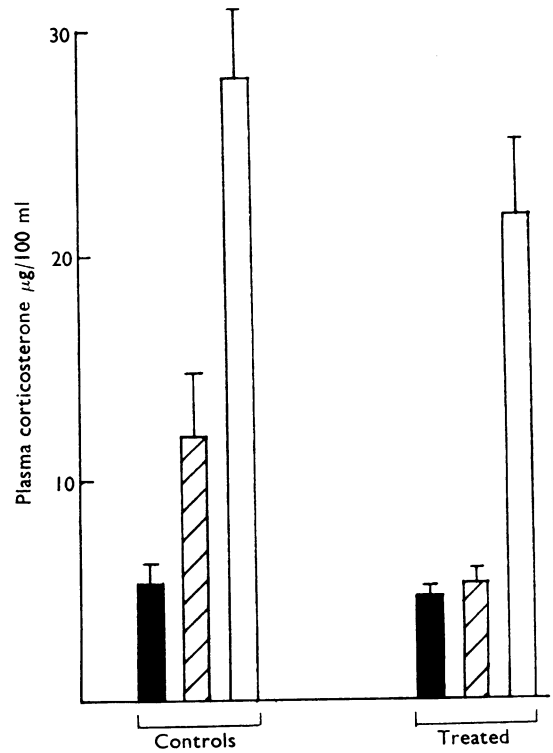

FIG 4. Plasma corticosterone concentrations in control and betamethasone-treated rats before (solid columns) and 0·5 h after subcutaneous saline (hatched columns) or corticotrophin (open columns) 0·05 i.u./100 g body weight. Each column is the mean (±S.E.) of at least six determinations.

Sirett, 1965) blocked completely the diurnal rise and the stress-induced increment in plasma corticosterone concentration. Since the sensitivity of the adrenal glands to exogenous ACTH was not impaired, the action of betamethasone was due to inhibition of ACTH release. After inhibition by betamethasone, circadian ACTH rhythm recovered before the ability of the hypothalamo-pituitary-adrenocortical system to respond to stress. These observations agree with the suggestion that the mechanisms controlling circadian ACTH rhythm and stress-induced ACTH release are dissociated, but indicate that the former is less sensitive to corticoids than the latter, in contrast to the findings of Zimmerman & Critchlow (1965, 1969). However, some differences between their experimental approach and ours might account for the apparent contradiction between the results. They injected female rats with a single small dose of dexamethasone and studied its acute effects upon the circadian rhythm in plasma corticosterone and the changes in the blood concentration of the steroid which occur in response to stress. Zimmerman & Critchlow (1965, 1967, 1969) discounted the possibility that the results could be largely dependent on the time of day at which the experiments were performed (Haus, 1963; Clayton, Librik, Gardner & Guillemin, 1963; Nichols, Nugent & Tyler, 1965; Ader & Friedman, 1968). We studied the inhibition and subsequent recovery of hypothalmo-pituitary-adrenal activity in male rats after a large dose of betamethasone had been ingested over a relatively long period of time.

Unimpaired pituitary adrenocorticotrophic activity is dependent on the functional integrity of the hypothalamus and its afferent nervous pathways (Szentágothai, Flerko, Mess & Halasz, 1968). The circadian ACTH rhythm is controlled by nervous pathways different from those regulating the release of the hormone in response to stress (Slusher, 1964; Halasz, Slusher & Gorski, 1967; Halasz, Vernikos-Danellis & Gorski, 1967). The difference in sensitivity of the two mechanisms to the inhibitory action of corticosteroids suggests that the corticosteroids do not act on a final common pathway for ACTH release. Therefore, it is unlikely that they act on the adenohypophysis itself, or on the cells which elaborate corticotrophin releasing factor. The findings could be explained by the existence of corticoid sensitive controllers in parts of the central nervous system other than the hypothalamus. Such a possibility is in agreement with the marked dissociation between blood (and presumably hypothalamic) corticosteroid concentrations and the degree of inhibition of ACTH release (Smelik, 1963a, 1963b; Hodges & Sadow, 1967).

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