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Effects of stress and betamethasone on the production of corticosterone by the rat adrenal gland *in vitro*

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Summary

1. Production of corticosterone *in vitro* was studied using adrenal glands from rats which had been subjected to stress or treated with betamethasone.

2. An increase in corticosterone production occurred in the first but not the second hour of incubation in adrenals removed immediately after stress. When the interval between stress and adrenalectomy was longer, the increased corticosterone production persisted.

3. Betamethasone had no effect on corticosteroidogenesis *in vitro* when added directly to the incubation medium or when administered to rats shortly before removal of their adrenal glands.

4. Corticosteroidogenesis *in vitro* was considerably reduced in the adrenals of rats given prolonged treatment with betamethasone.

5. The significance of the results and the reliability of *in vitro* corticosteroid production as an index of hypothalamo-pituitary-adrenal activity is discussed.

Introduction

Production of corticosteroids by adrenal glands *in vitro* is extensively used in assay methods for corticotrophin (ACTH) (Saffran & Bayliss, 1953; Saffran & Schally, 1955; Bakker & de Wied, 1961; de Wied, Witter, Versteeg & Mulder, 1969). Van der Vies, Bakker & de Wied (1960) demonstrated in the rat the parallelism between *in vitro* corticosterone formation and plasma corticosterone changes after stress or hypophysectomy. However, the method has not been widely adopted as an index of hypothalamo-pituitary-adrenal (HPA) function except by Smelik (1963a & b), Bohus (1969) and Flack (1970).

Long term administration of corticosteroids is followed by a reduction in adrenal cortical activity which can be attributed mainly to lack of stimulation by ACTH. However, steroids may also have a direct effect on the adrenal cortex, and corticosterone, cortisol, prednisolone and dexamethasone all inhibit steroid production by rat adrenals *in vitro* (Birmingham & Kurlents, 1958; Péron, Moncloa & Dorfman, 1960; Fekete & Görög, 1963).

These experiments were done to extend our investigation (Hodges & Mitchley, 1970a, b) of HPA function in the betamethasone treated rat, to determine whether betamethasone can inhibit corticosterone production by a direct action on the adrenal cortex and to assess the usefulness of adrenal corticoidogenesis *in vitro* as an index of HPA activity.

Methods

Approximately 120 male Sprague-Dawley rats (Fisons Pharmaceuticals Ltd.), weighing 150–250 g, were used. They were housed and fed as described previously (Hodges & Mitchley, 1970a & b).

Betamethasone (Betnesol, Glaxo) was administered in the drinking water, subcutaneously or intra-arterially (under ether anaesthesia into the abdominal aorta). Tetracosactrin (Cortrosyn, Organon) was injected intra-arterially in a similar way. When betamethasone or tetracosactrin was added to the incubation flasks it was dissolved in volumes of 0·1 ml of the medium.

Adrenal glands were removed from rats anaesthetized with ether, or killed rapidly by decapitation. The glands were cleaned, weighed, quartered and the production of corticosterone *in vitro* for 1 h periods was measured by the method of Bakker & de Wied (1961), with the modifications described by Hodges & Mitchley (1970b). None of the materials used interfered with the assay method for corticosterone (Zenker & Bernstein, 1958).

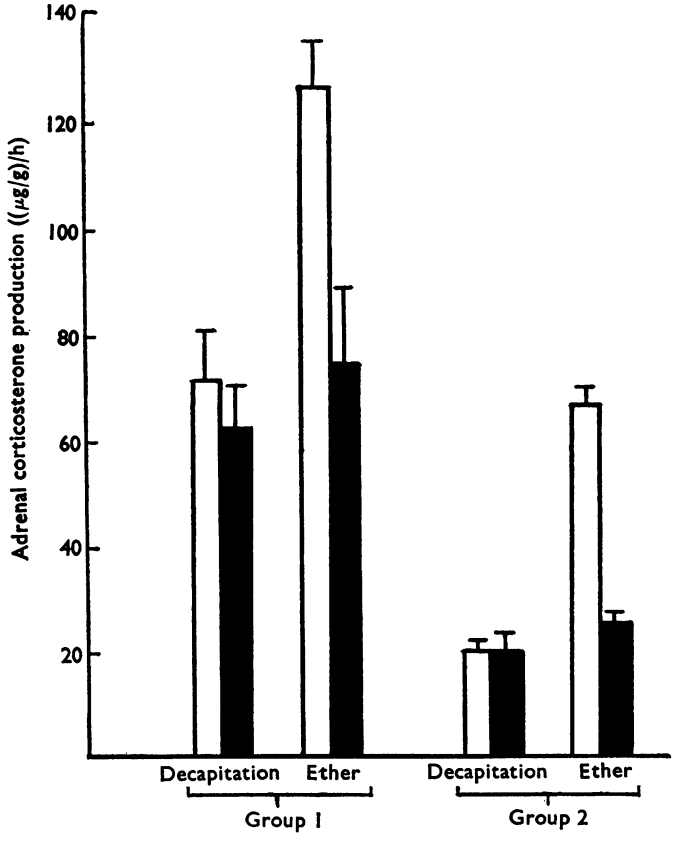

FIG. 1. Corticosterone production *in vitro* during the first (□) and second (■) hour of incubation in adrenal glands removed from rats under ether anaesthesia or after decapitation. Animals in group 1 were unaccustomed to handling and subjected to the stresses of a strange environment and frequent disturbance. Rats in group 2 were in a familiar quiet environment and accustomed to handling. Each column is the mean of eight or ten determinations and is shown ± the S.E. of the mean.

Results

Production of corticosterone *in vitro* was estimated using adrenals removed from rats after decapitation or under ether anaesthesia. Rats from one group, unaccustomed to handling, were removed serially from a large stock cage placed in a different laboratory 24 h previously. Rats from a second group, accustomed to handling, were kept two to a cage in the same room all the time. Corticosterone

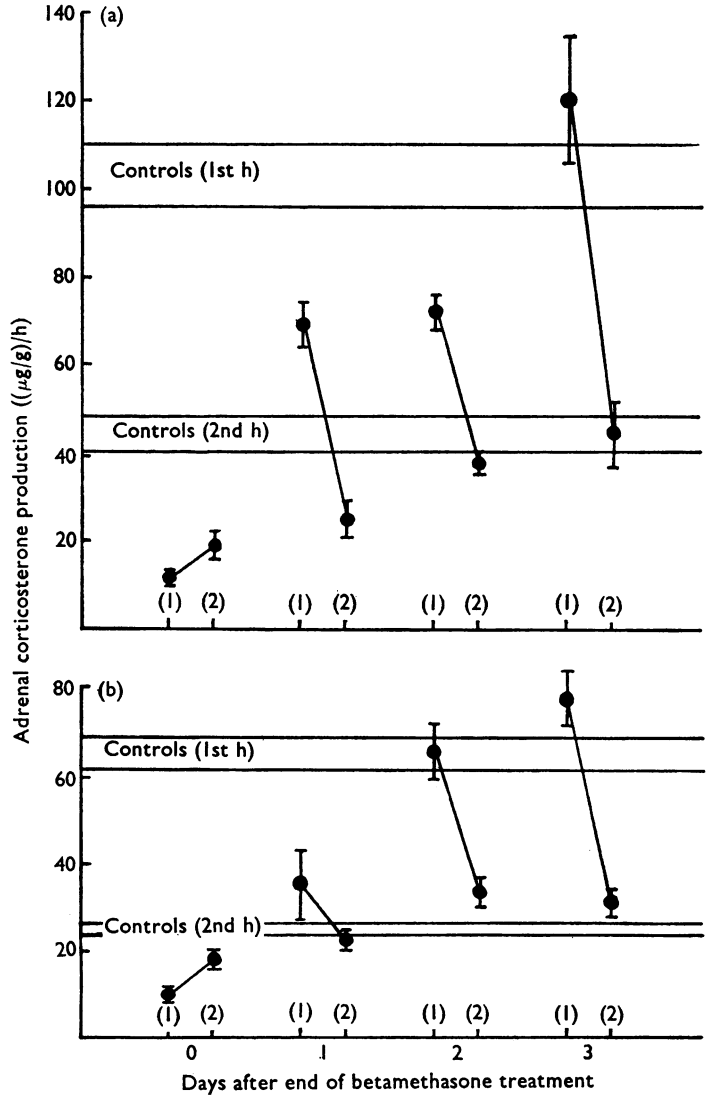

FIG. 2. Corticosterone production *in vitro* during the first (1) and second (2) hour of incubation in adrenals removed from rats at various times after the inclusion of betamethasone (20 μg/ml) in the drinking water for 24 hours. Adrenals were removed under ether anaesthesia. Some rats were subjected to the stress of exposure to ether vapour for 1 min (Fig. 2a) 15 min before removal of the adrenal glands; others were not (Fig. 2b). Each point is the mean of four determinations and is shown with the S.E. of the mean. Control values (mean ± 2 S.E.) are indicated by the pairs of horizontal lines and are the results of ten or twelve determinations.

produced during the first and second hour of incubation was measured and the results are shown in Fig. 1. Adrenals removed from rats under ether anaesthesia produced more corticosterone than the glands from decapitated animals during the first hour of incubation but not during the second hour. Considerably more corticosterone was produced at all times by the adrenals from rats subjected to the stresses of a change of environment and frequent disturbances (that is removal of other rats from the cage).

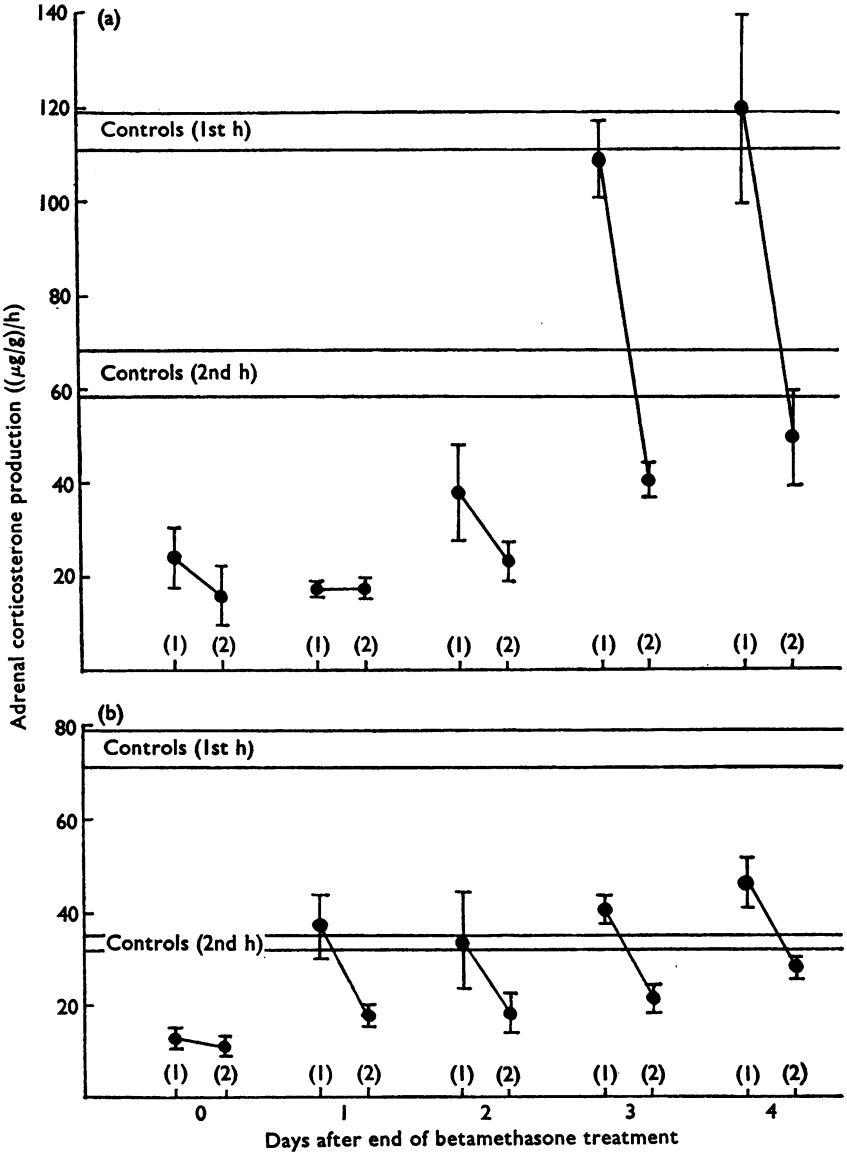

FIG. 3. Corticosterone production *in vitro* in adrenal glands removed from rats at various times after the inclusion of betamethasone (2 μg/ml) in the drinking water for 2 weeks. The results are expressed as in Fig. 2.

Corticosterone production in the first hour of incubation by the adrenal glands from rats pretreated with betamethasone (20 μg/ml in the drinking water for 24 h) is shown in Fig. 2. The glands removed under ether anaesthesia produced corticosterone in similar amounts to controls (Fig. 2b) on the second day after stopping the betamethasone treatment, whereas adrenals from rats subjected to ether stress 15 min beforehand produced less than the controls until the third day (Fig. 2a). In both cases, however, corticoidogenesis during the second hour of incubation equalled that of the controls sooner than the first hour's.

TABLE 1. *Adrenal corticosterone production* ((*µg/g*)/*h*) in vitro *in the presence of betamethasone*

Hour of incubation	With betamethasone (2 μg/3 ml)	Without betamethasone
1	16·0±0·5*	16·9±3·8
2	18·1±1·6	20·7±0·5
3	18·1±0·7	21·3±3·0
1	20·1±3·1	23·9±3·8
2	20·1±4·1*	16·4±6·3
3	18·3±2·1	19·7±2·3

* Betamethasone added to medium. Each result is the mean of at least four determinations± standard error.

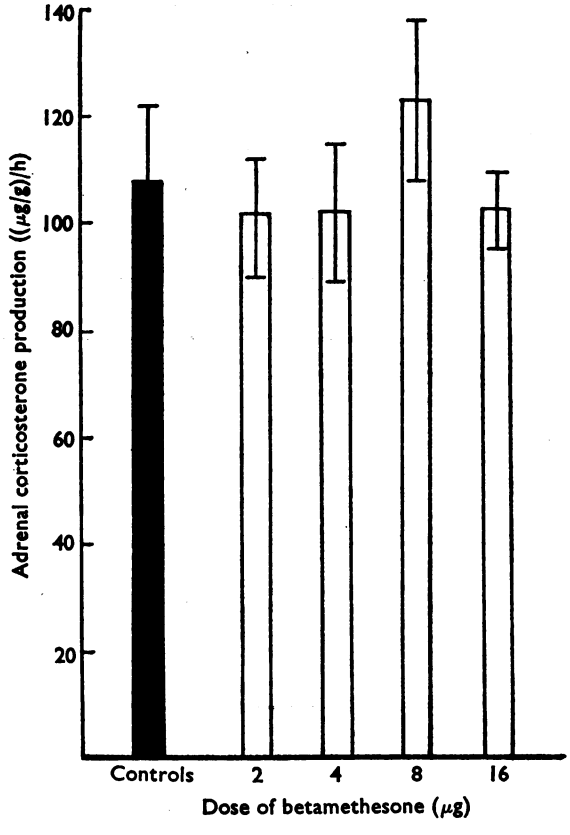

FIG. 4. Corticotrophin-stimulated adrenal corticosteroidogenesis *in vitro* with betamethasone in the incubation medium. Each column indicates the mean of at least eight determinations±the S.E.

The results from a similar experiment using betamethasone solution in a concentration of 2 μg/ml for 2 weeks are shown in Fig. 3. Corticoidogenesis by the adrenals of rats subjected to ether stress 15 min beforehand (Fig. 3a) reached the control values on the third and fourth days after withdrawing betamethasone. Adrenals from animals which were not subjected to the stress produced less corticosterone at all times up to 4 days after the end of the treatment (Fig. 3b).

Betamethasone (2 μg) added to the *in vitro* media during the first or second hour of incubation of adrenals from decapitated rats had no effect on steroid production (Table 1). Some adrenals were stimulated by the addition of 2·5 μg tetracosactrin to the *in vitro* medium during the second hour of incubation (the products of the first hour of incubation were discarded (Saffran & Bayliss, 1953 ; Bakker & de Wied, 1961)), and 2, 4, 8 or 16 μg betamethasone were added. Again, there was no significant reduction in corticoidogenesis (Fig. 4).

Since betamethasone given in this way may not reach the steroid producing sites, in another experiment it was injected (50 μg in 1 ml 0·9% saline) intra-arterially 1·5 min before adrenalectomy. Adrenal corticoidogenesis during the first hour of incubation ($(83 \pm 7$ μg/g)/h) did not differ significantly from that in controls ($(81 \pm 5$ μg/g)/h). Since adrenals removed immediately after tetracosactrin injected similarly showed an increase in corticosteroidogenesis of $(79 \pm 11$ μg/g)/h, 1·5 min should have been ample time for the betamethasone to reach the corticoid producing cells. However, the time of exposure to the betamethasone may still have been too short for it to produce an effect. Therefore, betamethasone was injected subcutaneously in a large dose (1 mg/100 g) 1·25 h before adrenalectomy. The glands were incubated in the usual way but with 2·5 μg tetracosactrin added to the first hour's incubation medium. Again corticosterone production in adrenals from betamethasone treated rats ($(101 \pm 12$ μg/g)/h) did not differ significantly from controls ($(105 \pm 20$ μg/g)/h).

Discussion

Adrenal production of corticosteroids *in vitro* provides an index of stress-induced-corticotrophin release as do changes in plasma corticosterone concentrations (Knigge, Penrod & Schindler, 1959 ; van der Vies *et al.*, 1960). Minor stressful procedures such as alterations in environment or handling, cause corticotrophin release which is shown both by a marked increase in adrenal corticosteroidogenesis *in vitro* and by a rise in plasma corticosterone concentration. The usefulness of plasma corticosterone determinations is limited because of the delay between ACTH release and the resultant rise in plasma corticosterone concentration. We have found that adrenals removed under ether anaesthesia show significantly higher levels of corticoid production than glands from decapitated rats in the first hour but not in the second hour of incubation. In contrast, the elevated corticoid production of glands from animals subjected to stress 15 min or more beforehand persists into the second hour. The observations suggest that the difference between corticoid production in the first and second hour of incubation is a measure of ACTH released immediately before the removal of the adrenal glands. The amount produced during the second hour is an indication of ACTH released some time previously.

Corticoidogenesis *in vitro* was used to assess the return of HPA activity in rats treated with betamethasone and our previous findings (Hodges & Mitchley, 1970a,

b), using plasma corticosterone changes as the index of HPA function, were confirmed. However, the *in vitro* corticosterone formation revealed some residual defect in function of the HPA axis (Fig. 3b) which was not shown by plasma corticosterone changes. Our results also suggest that betamethasone, like dexamethasone (Landon, Wynn, James & Wood, 1965), has no direct action on the adrenal cortex. It had no effect on adrenal corticoid production *in vitro* when it was added directly to the incubation medium or when injected either immediately or 75 min before adrenal removal. Our work does not preclude the possibility that its long term administration may result in a direct action of the steroid on the adrenal cortex, but in this event, such an action is overshadowed by the effect on the hypothalamo-pituitary complex.

The use of *in vitro* corticosteroidogenesis as an index of HPA activity has the advantage over other indices, such as plasma corticosterone changes, that it requires smaller numbers of animals for precise results. However, when adrenal sensitivity to corticotrophin is impaired the method is subject to the same limitations as is the plasma corticosterone index of HPA activity (Hodges & Mitchley, 1970b). These include marked variations with haemodynamic and metabolic changes and the method reflects only adrenocorticotrophic activity which took place some time previously. On the other hand, the production of corticosteroids by adrenal glands *in vitro* is not influenced by these variables and also reflects corticotrophin released immediately before the glands are removed. The method which can be used with little difficulty under carefully controlled conditions appears to provide a very useful and reliable index of HPA activity (van Goch, de Wied & Schönbaum, 1963).

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