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STRUCTURE-ACTIVITY RELATIONSHIP OF VARIOUS CORTICOSTEROIDS ON THE FEEDBACK CONTROL OF CORTICOTROPHIN SECRETION

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1 Several steroids occurring in the pathway of corticosteroid biosynthesis were investigated for their ability to exert a fast or delayed feedback inhibition of stress-induced release of corticotrophin. Rats were injected subcutaneously with vehicle or a steroid either 10 min (fast feedback) or 4 h (delayed feedback) before they were subjected to stress which consisted of a 2 min exposure to ether vapour.

2 Changes in plasma corticosterone concentration and *in vitro* corticosterone production by excised adrenal glands were used as indices of corticotrophin release.

3 Among the steroids tested only 11β, 21-dihydroxypregn-4-ene-3, 20-dione (corticosterone) and 11β, 17α, 21-trihydroxypregn-4-ene-3, 20-dione (cortisol) inhibited the stress response 10 min after their administration. Therefore, it appears that the fast feedback mechanism is limited to steroids with a 21-hydroxyl and a 11β-hydroxyl group.

4 In contrast, many steroids caused inhibition of the stress response 4 h after their administration. These steroids were corticosterone, cortisol, 21-hydroxypregn-4-ene-3, 20-dione (11-deoxycorticosterone), 17α, 21-dihydroxypregn-4-ene-3, 20-dione (11-deoxycortisol), 11β-hydroxypregn-4-ene-3, 20-dione (11β-hydroxyprogesterone) and 11β, 17α-dihydroxypregn-4-ene-3, 20-dione (11β, 17α-dihydroxyprogesterone). Thus, either the 21-hydroxyl group (e.g. 11-deoxycorticosterone) or the 11β-hydroxyl group (e.g. 11β-hydroxyprogesterone) is sufficient for delayed feedback activity. The 11α-hydroxyl group, e.g. 11α, 17α, 21-trihydroxypregn-4-ene-3, 20-dione (11-epicortisol) renders the steroid inactive on both feedback mechanisms.

5 18, 21-Dihydroxypregn-4-ene-3, 20-dione (18-hydroxydeoxycorticosterone) was found to be the only steroid that is secreted by the adrenal gland of the rat in quantities sufficient to cause exaggeration of the stress-induced release of corticotrophin. This steroid has been implicated as a possible hypertensive agent, and its role in the control of corticotrophin secretion is discussed here.

Introduction

There are two temporally distinct periods of inhibition of stress-induced release of corticotrophin (ACTH) following pretreatment with corticosteroids; these have been designated fast and delayed feedback (Dallman & Yates, 1969). Fast corticosteroid feedback occurs immediately after steroid administration and coincides with the time when the plasma steroid concentrations are rising (Dallman & Yates, 1969; Jones, Brush & Neame, 1972). Delayed corticosteroid feedback occurs an hour or more after steroid administration when the plasma steroid concentrations may have returned to basal values (Smelik, 1963; Hodges & Sadow, 1967; Dallman & Yates, 1969).

The steroids 11-deoxycorticosterone and 11-deoxycortisol have been found to have different actions on the fast and delayed feedback mechanisms; in fairly high doses they interfere with the fast feedback mechanism of corticosterone, but they cause inhibition of corticotrophin release when injected some hours before exposure to stress (Jones, Tiptaft, Brush, Fergusson & Neame, 1974). In view of the proposed separate receptors involved in these two feedback mechanisms, we have tested several steroids from the corticosteroid biosynthetic pathway for fast or delayed feedback actions. Some of the findings have been briefly reported previously (Tiptaft & Jones, 1975).

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Methods

Male Wistar-derived rats, weighing 140–200 g from a pathogen-free colony were kept three per cage in a sound insulated room for at least 12 h before each experiment. Lights were on from 07 h 00 min to 21 h 00 min and all experiments were performed between 08 h 00 min and 14 h 00 min, when the plasma corticosterone concentration was low and relatively constant.

Stress

The stress employed was a 2 min exposure to ether vapour; 25–30 min afterwards the animals were decapitated and blood was collected for the estimation of plasma corticosterone. At the same time one adrenal gland was removed, weighed, quartered and then incubated in 2 ml Krebs-Ringer solution at 37°C in an atmosphere of 95% O_2 and 5% CO_2 for one hour. The corticosterone content of the incubation medium was then measured.

Injections

In the experiments in which the fast feedback mechanism was tested, the animals were injected 10 min before ether inhalation with a 0.9% sodium chloride solution (saline s.c.), or a suspension of a steroid in saline. In the experiments on the delayed feedback mechanism steroids were injected in larger doses subcutaneously as suspensions in arachis oil 4 h before stress. The volumes injected were 0.25 ml/100 g body weight. The injections were given into an axilla, and when two injections were given to the same animals the second axilla was used to avoid leakage.

Estimation of corticosterone

Corticosterone was measured fluorimetrically by a technique similar to that described by Zenker & Bernstein (1958) using a Hitachi Perkin-Elmer spectrophotofluorimeter.

In the experiments in which corticosterone was injected the basal plasma corticosterone concentration was elevated (see Table 1). In all experiments the magnitude of the response to the inhalation of ether was expressed as the difference in plasma corticosterone concentration or adrenal corticosterone production between stressed and unstressed rats treated with the same steroid (for basal values see Table 1). The significance of the difference in the values between groups of rats receiving different treatments was assessed by Student's *t*-test.

Results

Inhalation of ether caused a rise in plasma corticosterone concentration from 138 ± 24 (s.e. mean) nmol/litre to 1032 ± 19 nmol/litre ($n = 100$) 25–30 min later, whilst *in vitro* corticosterone production rose from 25 ± 8 nmol 100 mg^{-1} h^{-1} to 161 ± 8 nmol 100 mg^{-1} h^{-1} ($n = 100$).

Table 1 Plasma corticosterone concentrations (nmol/litre) in unstressed, vehicle-treated control and in steroid-treated rats killed either 40 min or 4.5 h after injection.

Treatment	*Plasma corticosterone concentration (nmol/litre)*	
Vehicle control (saline)	96 ± 19*	
(arachis oil)		141 ± 19
	100 μg steroid/100 g body wt. 40 min before death	*2 mg steroid/100 g body wt. 4.5 h before death*
Pregnenolone	—	130 ± 14
17α-Hydroxypregnenolone	—	190 ± 30
Progesterone	116 ± 6	86 ± 22
11β-Hydroxyprogesterone	88 ± 14	119 ± 14
11α-Hydroxyprogesterone	—	104 ± 8
11β, 17α-Dihydroxyprogesterone	75 ± 17	149 ± 22
17α-Hydroxyprogesterone	121 ± 25	116 ± 19
11-Deoxycorticosterone	110 ± 11	149 ± 14
18-Hydroxydeoxycorticosterone	97 ± 14	135 ± 14
Cortisol	154 ± 19	122 ± 11
Corticosterone	518 ± 30*	113 ± 22

Each result is the mean ± s.e. of the mean ($n = 6$).
Vehicle control *vs.* corticosterone: * $P < 0.001$.

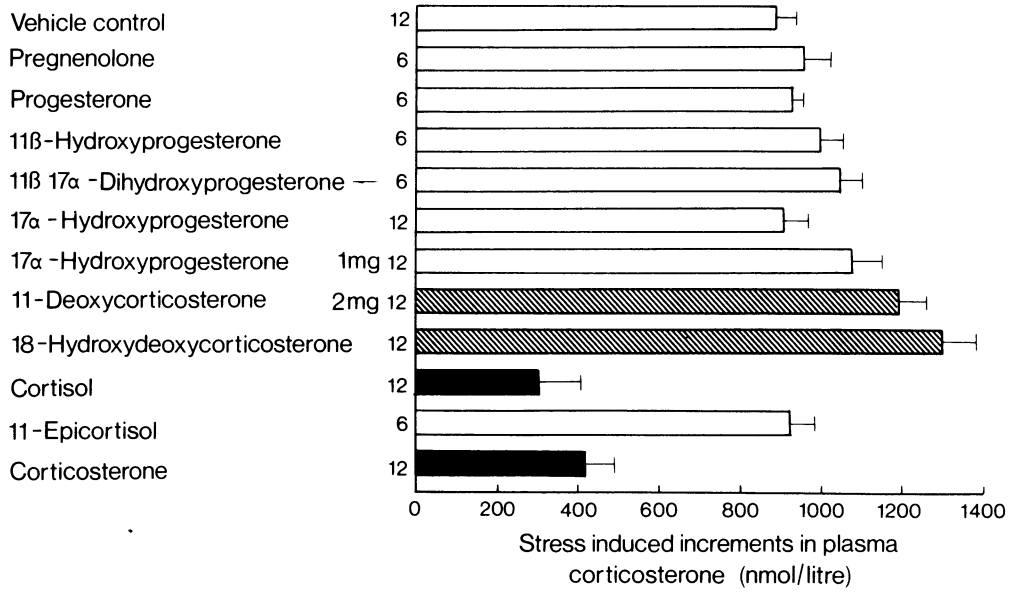

Figure 1 Ether-induced increases in plasma corticosterone concentrations (nmol/litre) in rats injected (s.c.) with either saline (vehicle) or a steroid (100 μg/100 g body wt. unless otherwise stated) 10 min before ether inhalation and the plasma corticosterone measured 25–30 min after stress. Each column is the mean + s.e. mean of 6 to 12 animals. Solid columns show a significant reduction ($P < 0.001$) and hatched columns a significant increase in the reaction to ether inhalation ($P < 0.01$) when compared with the vehicle control.

Plasma corticosterone concentrations of unstressed animals injected subcutaneously with various steroids in a dose of either 100 μg or 2 mg/100 g body wt. are shown in Table 1. It can be seen that any possible conversions of these steroids to corticosterone were not reflected in plasma corticosterone concentrations.

Fast feedback

The effect of various steroids in a dose of either 100 μg or 1–2 mg/100 g body wt. injected 10 min before stress upon the stress-induced increments in plasma corticosterone concentrations is shown in Figure 1. Corticosterone and cortisol in doses of 100 μg/100 g body wt. caused significant reductions in the stress response ($P < 0.001$), whereas 11-deoxycorticosterone (2 mg/100 g body wt.) and 18-hydroxydeoxycorticosterone (100 μg/100 g body wt.) caused significant exaggeration of the stress response ($P < 0.01$). 3β-Hydroxypregn-5-en-20-one (pregnenolone), pregn-4-ene-3, 20-dione (progesterone), 11β-hydroxyprogesterone, 11β, 17α-dihydroxyprogesterone, 17α-hydroxypregn-4-ene-3, 20-dione (17α-hydroxyprogesterone) and 11-epicortisol were found to exert no fast feedback effect. Similar results with these steroids were obtained when *in vitro* corticosterone production was used as the index of ACTH release.

The possibility that 18-hydroxydeoxycorticosterone pretreatment interfered with the fast feedback effect of corticosterone, causing a significant exaggeration of the corticotrophic response to stress was investigated (Figure 2). Corticosterone in a dose of 100 μg/100 g body wt. administered 10 min before stress reduced the corticotrophin release as assessed by the stress-induced increments in plasma corticosterone ($P < 0.001$) and the *in vitro* corticosterone production ($P < 0.01$). When 18-hydroxydeoxycorticosterone (100 μg/100 g body wt.) was given 10 min before the corticosterone the response of corticosterone production and release to ether stress was no longer inhibited by the corticosterone injection.

Delayed feedback

The effects of various steroids injected 4 h before ether inhalation upon the stress-induced increments in plasma corticosterone are shown in Figure 3. The stress-induced increments in plasma corticosterone were reduced in rats injected with corticosterone, cortisol, 11-deoxycorticosterone, 11-deoxycortisol, 11β, 17α-dihydroxyprogesterone or 11β-hydroxyprogesterone 2 mg/100 g body wt. ($P < 0.01$). After the same dose of 17α-hydroxyprogesterone or 18-hydroxydeoxycorticosterone, however, there was exaggeration of the stress response at this time interval

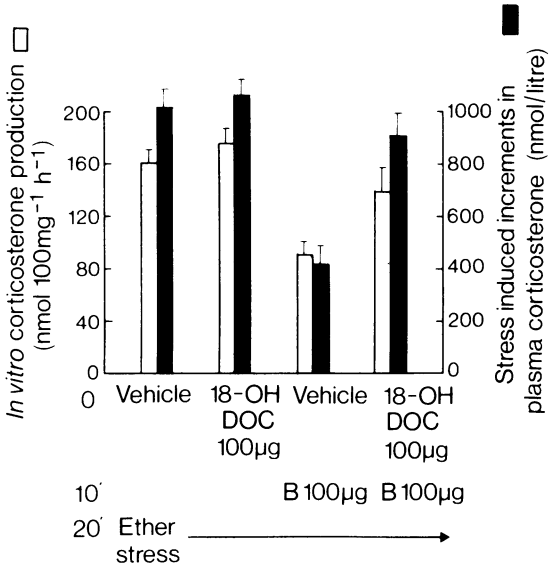

Figure 2 Ether-induced increase in plasma corticosterone concentration (nmol/litre) and *in vitro* corticosterone production (nmol 100 mg^{-1} h^{-1}) in rats injected (s.c.) at zero time with saline (vehicle) or 18-hydroxydeoxycorticosterone (18-OH DOC, 100 µg/100 g body wt.) and at 10 min with corticosterone (B, 100 µg/100 g body wt.). The animals were exposed to ether at 20 min and killed 25 to 30 min later. Each column is the mean + s.e. of the mean of 6 animals. Steroid secretion in the 18-OH DOC + B-treated rats was significantly higher than in vehicle + B-treated rats ($P < 0.01$).

($P < 0.01$). Similar results were obtained using *in vitro* corticosterone production.

The possibility that 18-hydroxydeoxycorticosterone or 17α-hydroxyprogesterone administered some hours before stress altered corticosterone-induced fast feedback was investigated (Table 2, experiment 1). Corticosterone injected in a dose of 100 µg/100 g body wt. 10 min before stress reduced significantly the stress-induced increments in plasma corticosterone and the *in vitro* corticosterone production compared to vehicle control ($P < 0.05$). 18-Hydroxydeoxycorticosterone or 17α-hydroxyprogesterone (2 mg/100 g body wt.) given 4 h before the corticosterone reduced the inhibitory action of corticosterone on the response to stress applied 10 min after the corticosterone administration.

Finally the possibility that 18-hydroxydeoxycorticosterone or 17α-hydroxyprogesterone administered several hours before stress may alter corticosterone-induced delayed feedback activity was investigated (Table 2, experiments 2 and 3). Experiment 2 shows the effect of combining a dose of 18-hydroxydeoxycorticosterone (2 mg/100 g body wt. which was capable of exaggerating stress-induced ACTH release) administered 2 h beforehand, with an inhibitory dose of corticosterone (400 µg/100 g body wt.). There was a reduced response to stress applied 4 h after the first injection as assessed by both indices, indicating that 18-hydroxydeoxycorticosterone did not interfere with the delayed feedback action of corticosterone. Neither did 17α-hydroxyprogesterone (experiment 3).

Discussion

It is evident from the results in Table 1 that the steroids tested do not act at the adrenal level by becoming involved in the biosynthetic pathway or by substrate inhibition (see also: Kraulis, Traikov, Li & Birmingham, 1973). Furthermore, Kraulis *et al.* (1973) found that adrenal glands removed from rats treated with 11β-hydroxyprogesterone, 11-deoxycorticosterone, 18-hydroxydeoxycorticosterone or corticosterone (1 mg/100 g body wt. for 3 days) responded to ACTH stimulation *in vitro* with a similar increase in *in vitro* corticosterone production to that in control animals. More important, therefore, is the conversion or metabolism of steroids at the hypothalamic and pituitary levels. The dependence of the feedback response on conversion of steroids in other parts of the body (e.g. liver) has been excluded as these steroids have a similar activity *in vitro* on CRF release when added to the hypothalamus *in vitro* (Jones, Hillhouse & Burden, 1976).

The proposed theory that two different receptors are involved in the fast and delayed corticosteroid feedback control of ACTH secretion (Jones *et al.*, 1974) is supported by the experiments described here. The fast feedback receptor mechanism appears to be highly specific for corticosterone and cortisol, requiring the 11β-hydroxyl group (as 11-epicortisol is not active) and the 21-hydroxyl group (as progesterone and 11β-hydroxyprogesterone are inactive). Interestingly, replacing the 11β-hydroxyl group of corticosterone and cortisol by a hydrogen atom increases the affinity but not the efficacy, and the corresponding steroids are antagonists to corticosterone and cortisol (Jones *et al.*, 1974). The delayed feedback receptor mechanism appears to be less specific since corticosterone, cortisol, 11-deoxycorticosterone, 11-deoxycortisol, 11β-hydroxyprogesterone and 11β-, 17α-dihydroxyprogesterone all exert a feedback action. The presence of either the 11β-hydroxyl group (as 11β-hydroxyprogesterone is effective) or the 21-hydroxyl group (as 11-deoxycorticosterone is effective) appears to be necessary for delayed feedback activity. Again the 11α-hydroxyl group renders the steroid inactive, as in the case of 11-epicortisol and 11α-hydroxypregn-4-ene-3, 20-dione (11α-hydroxyprogesterone).

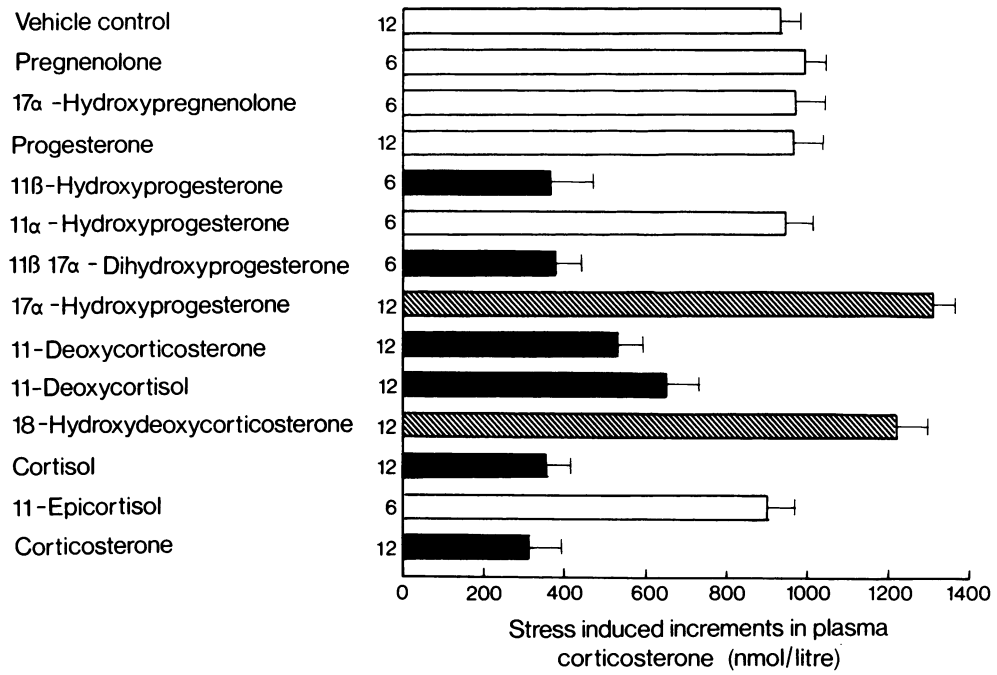

Figure 3 Ether-induced increases in plasma corticosterone concentration (nmol/litre) in rats injected (s.c.) with either arachis oil (vehicle) or a steroid (2 mg/100 g body wt.) 4 h before stress. Each column is the mean+s.e. of the mean of 6 to 12 animals. Solid columns are significantly lower ($P<0.01$, or less) and hatched columns are significantly higher ($P<0.01$) than the vehicle control.

Table 2 Ether-induced increments in plasma corticosterone concentration (nmol/litre) and in the *in vitro* corticosterone production (nmol 100 mg^{-1} h^{-1}) in rats injected (s.c.) with either arachis oil, 18-hydroxydeoxycorticosterone (18-OH DOC), 17α-hydroxyprogesterone (17α-OH Prog), corticosterone (B) or a combination of these steroids at different time intervals before ether inhalation.

Treatment		*Dose/100 g body wt.*	*Ether-induced increments in plasma B (nmol/litre)*	In vitro *B production (nmol 100 $mg^{-1}h^{-1}$)*
Expt. 0:	Vehicle control		1021 ± 58**	152 ± 14*
	18-OH DOC	2 mg	1223 ± 66	240 ± 14
	17α-OH Prog	2 mg	—	226 ± 8
Expt. 1:	Vehicle + B	100 µg	588 ± 97** **	97 ± 14* * ***
	18-OH DOC + B	2 mg + 100 µg	999 ± 69 **	174 ± 25 *
	17α-OH Prog + B	2 mg + 100 µg	—	177 ± 8 ***
Expt. 2:	Vehicle + B	400 µg	566 ± 91	108 ± 22
	18-OH DOC + B	2 mg + 400 µg	765 ± 99	105 ± 25
Expt. 3:	Vehicle + B	400 µg	—	50 ± 22
	17⁺-OH Prog + B	2 mg + 400 µg	—	69 ± 17

In Expt. 1 the steroids were injected 3 h 50 min apart, in Expt. 2, 2 h apart and in Expt. 3, 1 h apart. All groups of animals were stressed 4 h after the initial injection. Each result is the mean ± s.e. mean from six animals. The position of the asterisks to the right of each column of figures indicate which experimental values are being compared.
Student's *t*-test; *$P<0.05$; **$P<0.01$; ***$P<0.001$.

The fact that such a wide variety of steroids are agonists on the delayed feedback mechanism is surprising, particularly since many of these steroids have little glucocorticoid or anti-inflammatory activity. This reinforces our previous suggestion that an analysis of the structure-activity relationship may enable the design of a steroid with strong anti-inflammatory activity and little feedback suppression, or a steroid that specifically antagonizes the feedback action of potent anti-inflammatory steroids (Jones *et al.*, 1974). An antagonist to the delayed feedback action of corticosteroids would be the most useful since the latter must be the main mechanism of the unwanted ACTH suppression following therapeutic use of steroids.

The rat adrenal *in vitro* has the capacity to 17α-hydroxylate progesterone, but this system is inhibited in the presence of the 11β-hydroxylating system (Young & Sweat, 1967). However, cortisol and 17α-hydroxyprogesterone are not found in the adrenal vein blood of the rat, although they have been shown in this study to have interesting feedback actions. The steroid 18-hydroxydeoxycorticosterone however, is an adrenal steroid in the rat (Birmingham & Ward, 1961; Péron, 1961) and its secretion is increased by ACTH (Ward & Birmingham, 1960). This steroid has actions on electrolyte and water excretion in this species (Birmingham, MacDonald & Rochefort, 1968) and it has been implicated as a possible hypertensive agent in rats (Birmingham, Rochefort & Traikov, 1965; Rapp & Dahl, 1971) and in man (Melby, Dale & Wilson, 1971). In a recent study, unilaterally nephrectomized male rats maintained on 1% saline were injected with vehicle, corticosterone, 11-deoxycorticosterone or 18-hydroxydeoxycorticosterone 200 µg/daily for 21 days (Oliver, Birmingham, Bartova, Li & Chan, 1973). Oliver *et al.* (1973) showed that not only 11-deoxycorticosterone but 18-hydroxydeoxycorticosterone contributes to the aetiology of hypertension possibly by a mechanism involving stress-induced ACTH release. The same laboratory had previously shown that rats treated for 3 days with 18-hydroxydeoxycorticosterone (1 mg/100 g body wt. twice a day) responded on the third day with abnormally high plasma corticosterone concentrations and *in vitro* corticosterone production in response to ether stress (Kraulis *et al.*, 1973). Our experiments confirm that 18-hydroxydeoxycorticosterone is capable of exaggerating stress-induced ACTH release, and this is probably due to an interference with the fast feedback mechanism of corticosterone. 18-Hydroxydeoxycorticosterone may therefore play a role influencing ACTH secretion in the rat and other species.

The current work and that reported previously (Jones *et al.*, 1974) reinforce our contention that high circulating levels of adrenal corticosteroid precursors found in adrenogenital syndrome (11-deoxycortisol, 11-deoxycorticosterone and 17α-hydroxyprogesterone) antagonize the fast feedback action of cortisol. This antagonism might provide another mechanism whereby ACTH levels are elevated in this disease, and explain why treatment with physiological doses of glucocorticoids often fails to reduce the high circulating levels of ACTH (Jacobs, Abraham, Glasser, Hopper & Kondon, 1972). The high levels of 17α-hydroxyprogesterone at mid cycle during the human menstrual cycle may also account for the elevated ACTH levels at this time (Burns, 1975).

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References

BIRMINGHAM, M.K., MacDONALD, M.L. & ROCHEFORT, J.G. (1968). Adrenal function in normal rats and in rats bearing regenerated adrenal glands. In *Functions of the Adrenal Cortex*, ed. McKerns, K.W. Vol. **2**, pp. 647–689. New York: Appleton.

BIRMINGHAM, M.K., ROCHEFORT, G. & TRAIKOV, H. (1965). Steroid fractions from incubated normal and regenerated adrenal glands of male and female rats. *Endocrinology*, **76**, 819–827.

BIRMINGHAM, M.K. & WARD, P.J. (1961). The identification of the Porter-Silber chromogen secreted by the rat adrenal. *J. biol. Chem.*, **236**, 1661–1667.

BURNS, J.K. (1975). Variation in plasma ACTH levels during the human menstrual cycle. *J. Physiol., Lond.*, **249**, 36P.

DALLMAN, M.F. & YATES, F.E. (1969). Dynamic asymmetries in the corticosteroid feedback path and distribution-metabolism-binding elements of the adrenocortical system. *Ann. N.Y. Acad. Sci.*, **156**, 696–721.

HODGES, J.R. & SADOW, J. (1967). Impairment of pituitary adrenocorticotrophic function by corticosterone in the blood. *Br. J. Pharmac. Chemother.*, **30**, 385–391.

JACOBS, H.S., ABRAHAM, G.E., GLASSER, E.J., HOPPER, K. & KONDON, J.J. (1972). 17α-hydroxyprogesterone and testosterone levels in congenital adrenal hyperplasia: effect of treatment with cortisone acetate, dexamethasone and corticotrophin. *J. Endocr.*, **53**, xxxvi abstract.

JONES, M.T., BRUSH, F.R. & NEAME, R.L.B. (1972). Characteristics of fast feedback control of corticotrophin release by corticosteroids. *J. Endocr.*, **55**, 489–497.

JONES, M.T., HILLHOUSE, E. W. & BURDEN, J.L. (1976). Secretion of corticotrophin-releasing hormone *in vitro*. In *Frontiers in Neuroendocrinology*, ed. Martini, L. & Ganong, W.F., Vol. **4**, pp. 195–227. New York: Raven Press.

JONES, M.T., TIPTAFT, E.M., BRUSH, F.R., FERGUSSON, D.A.N. & NEAME, R.L.B. (1974). Evidence for dual corticosteroid-receptor mechanisms in the feedback control of adrenocorticotrophin secretion. *J. Endocr.*, **60**, 223–233.

KRAULIS, I., TRAIKOV, H., LI, M.P. & BIRMINGHAM, M.K. (1973). The effects of corticosterone, 18-OH DOC, DOC and 11β-hydroxyprogesterone on the adrenal pituitary axis of the stressed rat. *J. Steroid Biochem.*, **4**, 129–137.

MELBY, J.C., DALE, S.L. & WILSON, T.E. (1971). 18-hydroxydeoxycorticosterone in human hypertension. *Circulation Res.*, **28/29** Suppl. II, 143–153.

OLIVER, J.T., BIRMINGHAM, M.K., BARTOVA, A., LI, M.P. & CHAN, T.H. (1973). Hypertensive action of 18-hydroxydeoxycorticosterone. *Science*, **182**, 1249–1251.

PÉRON, F.G. (1961). Isolation of 18-hydroxydeoxycorticosterone from rat adrenals. *Endocrinology*, **69**, 39–45.

RAPP, J.P. & DAHL, L.K. (1971). 18-hydroxydeoxycorticosterone secretion in experimental hypertension in rats. *Circulation Res.*, **28/29**. Suppl. II, 153–159.

SMELIK, P.G. (1963). Relation between blood levels of corticoids and their inhibiting effect on the hypophyseal stress response. *Proc. Soc. exp. Biol. Med.*, **113**, 616–619.

TIPTAFT, E.M. & JONES, M.T. (1975). Structure-activity relationship of various steroids in the feedback control of corticotrophin secretion. *J. Endocr.*, **64**, 9P.

WARD, P.J. & BIRMINGHAM, M.K. (1960). Properties of the ultra-violet absorbing lipids produced by rat adrenals *in vitro*. *Biochem. J.*, **76**, 269–278.

YOUNG, R.B. & SWEAT, M.L. (1967). Steroid 17α-hydroxylation in the rat adrenal gland. *Arch Biochem. Biophys.*, **121**, 576–579.

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

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