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Effects of adrenocortical and gonadal steroids on the secretion *in vitro* of corticotrophin and its hypothalamic releasing factor

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SUMMARY

The effects of adrenocortical and gonadal steroids on the secretion *in vitro* of ACTH by adenohypophysial segments and corticotrophin releasing factor (CRF) by isolated hypothalami were studied in the rat. Corticosterone ($1{\cdot}25 \times 10^{-6}$ mol/l), betamethasone ($2{\cdot}5 \times 10^{-8}$ mol/l) and progesterone ($2{\cdot}5 \times 10^{-7}$ mol/l) reduced the hypothalamic extract-induced secretion of ACTH by pituitary tissue *in vitro* but aldosterone (2×10^{-7} mol/l), testosterone, androsterone, androstenedione (2×10^{-7} mol/l), oestradiol, oestriol and oestrone (10^{-6} mol/l) did not. Corticosterone ($2{\cdot}5 \pm 10^{-9}$ mol/l), aldosterone (2×10^{-8} mol/l) and betamethasone (2×10^{-10} mol/l) inhibited and oestradiol, oestriol and oestrone ($10^{-8} - 10^{-6}$ mol/l) potentiated the production of CRF by isolated hypothalami which occurred when acetylcholine or 5-hydroxytryptamine were added to the incubation medium but progesterone ($2{\cdot}5 \times 10^{-7}$ mol/l), testosterone, androsterone and androstenedione (2×10^{-7} mol/l) had no effects. The results indicate that hypothalamo-pituitary-adrenocorticotrophic activity may be modified not only by glucocorticoids but also by other steroids.

INTRODUCTION

The glucocorticoids, corticosterone and cortisol, are thought to influence the level of activity of the hypothalamo-pituitary-adrenocortical system by acting on specific receptors in the adenohypophysis (Arimura, Boyers, Schally, Saito & Miller, 1969; Bohus & Strashimirov, 1970; Buckingham & Hodges, 1977*a*; Jones, Hillhouse & Burden, 1977), in the hypothalamus (Jones *et al.* 1977; Buckingham, 1979) and in those centres in the brain which control the secretion of corticotrophin releasing factor (CRF) (Dallman & Yates, 1968; McEwen, Weiss & Schwartz, 1970; Feldman, 1973). Several reports suggest that other steroids may also contribute to the complex mechanisms which control the secretion of corticotrophin (ACTH) (Coyne & Kitay, 1969; Birmingham, Kraulis, Traikov, Bartova, Li, Chan, Oliver & Poffanza, 1974) but few attempts have been made to determine their site and mode of action. Accordingly, these experiments were carried out to study the effects of various adrenocortical and gonadal steroids on the secretion *in vitro* of ACTH by the pituitary gland and CRF by the hypothalamus.

MATERIALS AND METHODS

Animals

Male albino Sprague–Dawley rats (Charles River, SPF) weighing 100–125 g were housed four to a cage for at least 4 days before each experiment in a room with controlled lighting (lights on 07.00–19.00 h) in which the temperature was maintained at 22 °C. They were handled regularly (Hodges & Mitchley, 1970) and had free access to food and water. They

were killed by decapitation between 07.30 and 09.00 h and the tissues required (hypothalami or adenohypophyses) were removed immediately.

Incubation of hypothalami

Hypothalami were incubated separately at 37 °C in 1·0 ml of an artificial physiological medium exactly as described by Bradbury, Burden, Hillhouse & Jones (1974). After an initial 30-min equilibration period they were transferred to fresh medium in which they remained for a further 30 min (prestimulation period). The hypothalami were then challenged for 10 min with either acetylcholine (B. D. H., Enfield, Middlesex) or 5-hydroxytryptamine (B. D. H.) (stimulation period) or, in the case of controls, they were incubated in medium alone. The CRF contents of the hypothalami and the incubation medium were determined immediately.

Incubation of pituitary tissue

Anterior pituitary glands were divided into four pieces of approximately equal size and incubated in the artificial medium of Bradbury *et al.* (1974) under the conditions described by Buckingham & Hodges (1977*a*). One and a half hours later (equilibration period) the segments were transferred to fresh medium and incubated for 1 h (prestimulation period). The adenohypophysial pieces were then separated. Three were incubated individually in 1·0 ml medium containing hypothalamic extract (0·5 hypothalamic equivalents/ml) and the fourth (control) in a corresponding volume of medium alone. After a further 15 min (stimulation period), the media and pituitary tissue were separated and stored at −70 °C (Buckingham & Hodges, 1977*a*). Their ACTH contents were subsequently determined.

Hypothalamic extract

Hypothalami were homogenized in 10 µl 0·1 M-HCl/hypothalamus and stored at 4 °C for 1 h. One millilitre incubation medium/hypothalamus was then added and the mixture was shaken thoroughly and centrifuged at $1{\cdot}875 \times 10^3 g$ for 3 min. The supernatant fluid was stored on ice and used within 1 h.

Steroids

Steroids were added to the incubation medium of the hypothalami and the adenohypophysial segments during either the prestimulation or stimulation periods. The following were used: corticosterone (Organon Laboratories Ltd, Morden, Surrey), betamethasone disodium phosphate (Glaxo Laboratories Ltd, Greenford, Middlesex), aldosterone (MRC Blood Pressure Unit, Glasgow), progesterone (Organon), testosterone, androsterone, androstenedione, oestradiol, oestriol and oestrone (Sigma Chemical Company, Poole, Dorset). With the exception of betamethasone, the steroids were first dissolved in 10% ethanol and diluted subsequently in the incubation medium. The final concentration of ethanol was never greater than 0·01% and this did not influence the secretory activity of either the hypothalamus or adenohypophysis *in vitro*. Betamethasone was dissolved directly in the incubation medium and diluted appropriately.

Hormone assays

Corticotrophin was determined cytochemically (Alaghband-Zadeh, Daly, Bitensky & Chayen, 1974) using World Health Organization IIIrd corticotrophin I. W. S. as a standard, and CRF by a bioassay method which depends upon the ability of the hypothalamic hormone to stimulate segments of anterior pituitary tissue to secrete ACTH *in vitro* (Buckingham & Hodges, 1977*a*). Since there is no satisfactory standard preparation of CRF, corticotrophin releasing activity is expressed in terms of pituitary ACTH production.

Statistical analysis

The results were analysed using paired Student's *t*-test.

RESULTS

Table 1 shows the effects of corticosterone ($1{\cdot}25 \times 10^{-6}$ mol/l), betamethasone ($2{\cdot}5 \times 10^{-8}$ mol/l) and progesterone ($2{\cdot}5 \times 10^{-7}$ mol/l) on the adrenocorticotrophic activity of adenohypophysial tissue *in vitro*. Although none of these steroids affected the basal activity of the tissue they all reduced significantly ($P<0{\cdot}01$) the amount of ACTH released in response to hypothalamic extracts. Corticosterone and betamethasone also impaired the ability of the adenohypophysis to synthesize corticotrophin in response to trophic stimuli but only when added to the medium for the prestimulation period (Table 1). In contrast,

Table 1. *Effects of the addition of corticosterone ($1{\cdot}25 \times 10^{-6}$ mol/l), betamethasone ($2{\cdot}5 \times 10^{-8}$ mol/l) and progesterone ($2{\cdot}5 \times 10^{-7}$ mol/l) to the incubation medium during the stimulation period and the prestimulation period on the spontaneous production of ACTH by rat pituitary tissue* in vitro *and on the response to hypothalamic extract (0·5 hypothalamic equivalents/ml). Each value is the mean of five determinations and is shown with its standard error*

	Stimulation period		Prestimulation period	
	ACTH release (μu./mg pituitary tissue)	ACTH content (mu./mg pituitary tissue)	ACTH release (μu./mg pituitary tissue)	ACTH content (mu./mg pituitary tissue)
Basal	13·8±3·2	6·5±0·7	14·5±1·3	8·5±1·9
Corticosterone	9·9±2·6	7·1±0·4	10·6±4·2	7·0±0·8
Betamethasone	12·5±2·4	6·3±0·9	12·5±1·8	7·5±0·4
Progesterone	13·3±3·6	6·4±0·3	13·2±1·4	8·7±0·9
Hypothalamic extract	551±42	85±7	538±41	91±7
Hypothalamic extract+ corticosterone	216±20*	92±6	211±26*	66±7*
Hypothalamic extract+ betamethasone	103±10*	81±5	185±17*	69±5*
Hypothalamic extract+ progesterone	467±23*	86±3	444±26*	86±6

* $P<0{\cdot}01$ compared with basal levels (Student's *t*-test).

Table 2. *Effects of corticosterone ($2{\cdot}5 \times 10^{-9}$ mol/l) and betamethasone (2×10^{-10} mol/l) added to the incubation medium during either the stimulation or prestimulation periods on the spontaneous production of corticotrophin releasing factor (CRF; expressed as ACTH (μu./ml per mg pituitary tissue)) by isolated rat hypothalami* in vitro. *Each value is the mean of five determinations and is shown with its standard error*

	CRF release	CRF content
Stimulation period		
Basal	48±5	53±6
Corticosterone	52±7	61±7
Betamethasone	32±1*	46±8
Prestimulation period		
Basal	62±8	48±9
Corticosterone	43±1*	45±5
Betamethasone	60±14	46±2

* $P<0{\cdot}01$ compared with basal levels (Student's *t*-test).

aldosterone (2×10^{-7} mol/l) and the gonadal steroids (oestradiol, oestriol, oestrone (10^{-6} mol/l), testosterone, androsterone, androstenedione (2×10^{-7} mol/)) did not influence the capacity of pituitary segments to secrete ACTH *in vitro*.

Table 2 and Figs 1–5 illustrate the effects of the same steroids on the secretion of CRF by isolated hypothalami *in vitro*. Corticosterone ($2{\cdot}5 \times 10^{-9}$ mol/l in the final incubation medium) did not affect the basal corticotrophin releasing activity of the hypothalamus (Table 2) but reduced significantly ($P < 0{\cdot}01$) the amount of CRF released when the organ was challenged with acetylcholine or 5-hydroxytryptamine. It also caused a concomitant

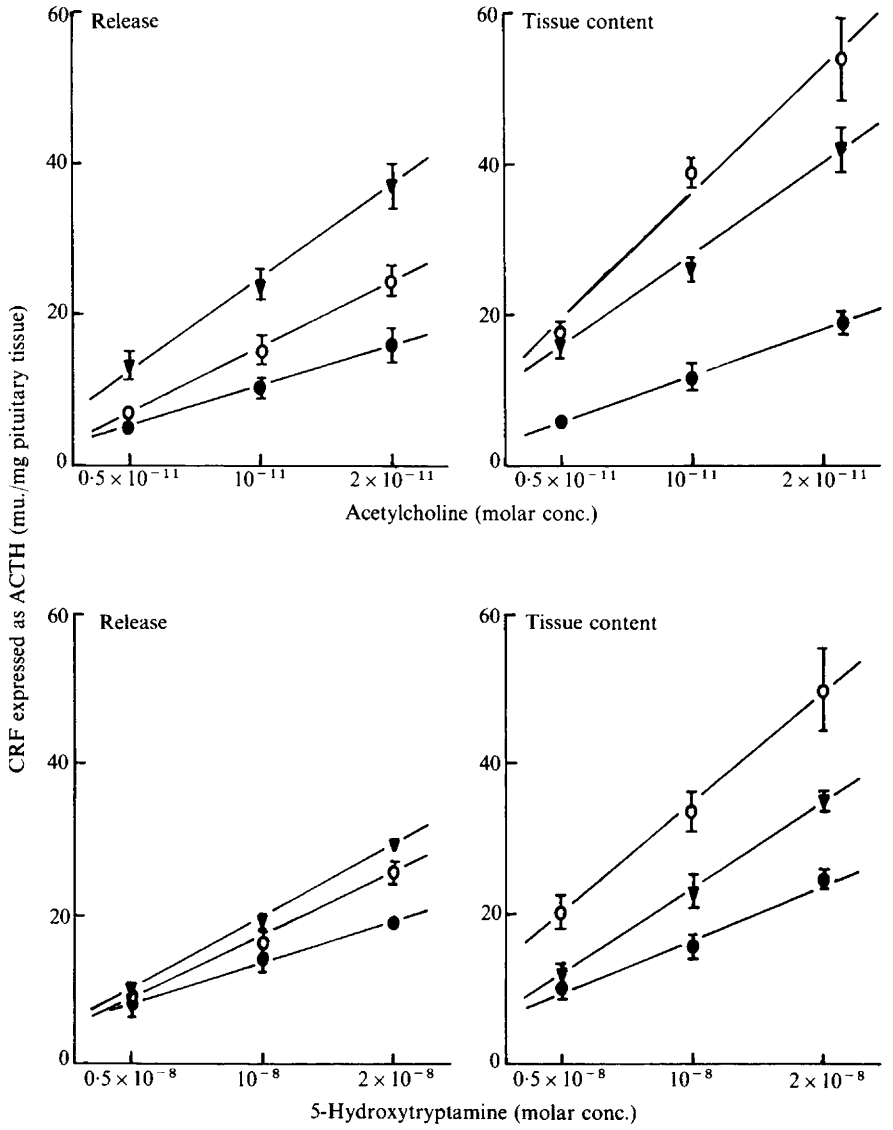

Fig. 1. Effects of corticosterone on the acetylcholine- and 5-hydroxytryptamine-induced production of corticotrophin releasing factor (CRF) by isolated rat hypothalami *in vitro*. Values shown are control (▼), corticosterone ($2{\cdot}5 \times 10^{-9}$ mol/l) during the stimulation period (○) and corticosterone ($2{\cdot}5 \times 10^{-9}$ mol/l) during the prestimulation period (●). Each point is the mean ± S.E.M. of five determinations. Standard errors omitted were within ± 10% of the mean.

increase in the tissue content of the releasing factor which was probably merely a reflection of the steroid-induced inhibition of CRF release (Fig. 1). When corticosterone was present in the same concentration during the prestimulation period it reduced significantly ($P<0{\cdot}01$) both the spontaneous activity of the tissue (Table 2) and the rises in CRF release and content evoked by acetylcholine or 5-hydroxytryptamine (Fig. 1).

Betamethasone (2×10^{-10} mol/l) also inhibited the basal secretion of CRF (Table 2) and the responses to trophic stimuli (Fig. 2) but, in contrast to corticosterone, it was more effective when present during the final period of incubation. Neither progesterone ($2{\cdot}5\times10^{-7}$ mol/l) nor aldosterone (2×10^{-9}–2×10^{-6} mol/l) affected spontaneous CRF production. Progesterone also failed to influence the neurotransmitter-stimulated secretion of CRF but aldosterone, like betamethasone, reduced significantly ($P<0{\cdot}001$) the amount

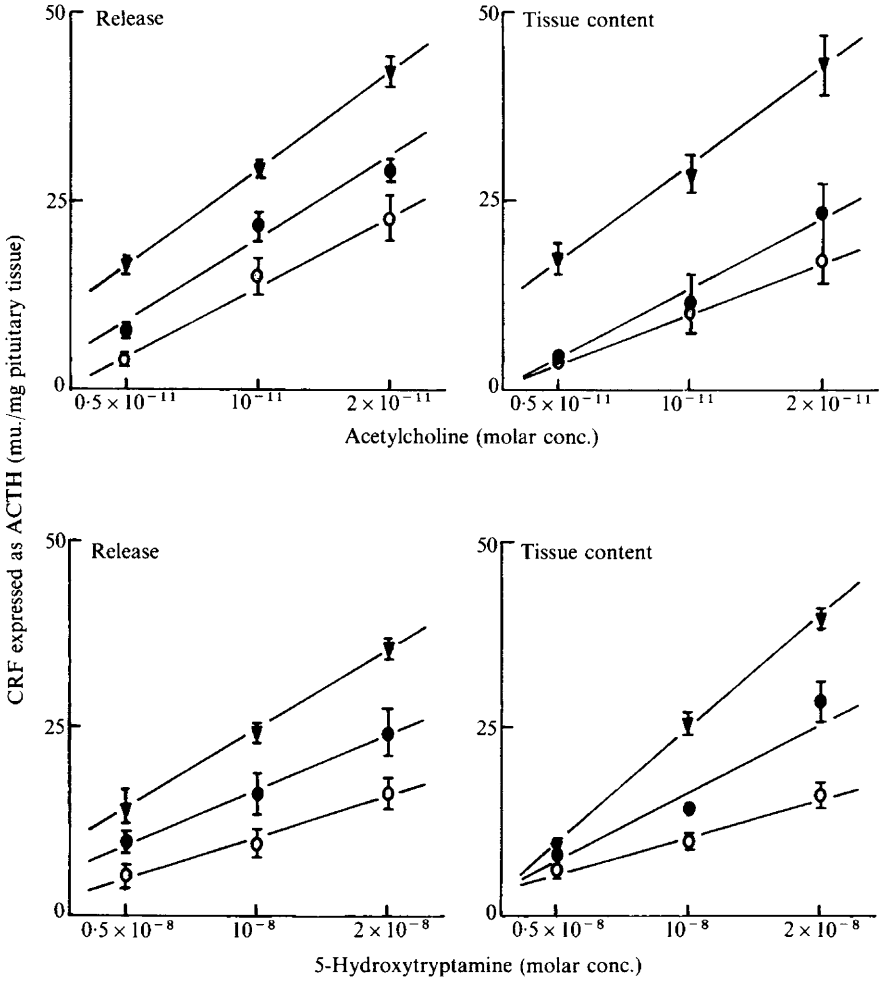

Fig. 2. Effects of betamethasone on the acetylcholine- and 5-hydroxytryptamine-induced production of corticotrophin releasing factor (CRF) by isolated rat hypothalami *in vitro*. Values shown are control (▼), betamethasone (2×10^{-10} mol/l) during the stimulation period (○) and betamethasone (2×10^{-10} mol/l) during the prestimulation period (●). Each point is the mean ± S.E.M. of six determinations. Standard errors omitted were within ± 10% of the mean.

of CRF produced in response to trophic stimuli when present in the medium during either the prestimulation or stimulation periods. These results are shown in Fig. 3.

The presence of the androgenic steroids, testosterone, androsterone or androstenedione (2×10^{-8}–2×10^{-7} mol/l) in the incubation medium during either the prestimulation or stimulation periods did not affect significantly ($P > 0{\cdot}1$) the basal secretion of CRF or the responses to acetylcholine or 5-hydroxytryptamine. The spontaneous corticotrophin releasing activity of the hypothalamus was also unaffected by oestrogens (oestradiol, oestriol, oestrone (10^{-8}–10^{-6} mol/l)). However, these steroids, when added in the final incubation medium, potentiated the effects of acetylcholine and 5-hydroxytryptamine. The responses were generally dose-related and, in some cases, exaggerated when the hormones were present during the prestimulation period (Figs 4 and 5).

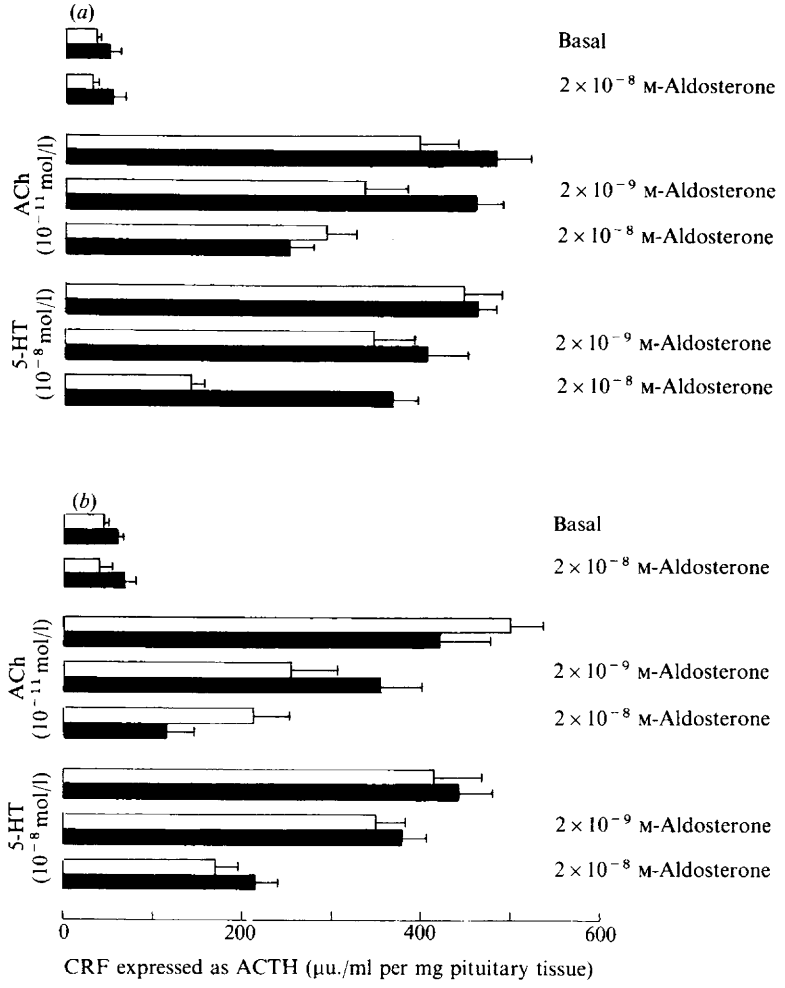

Fig. 3. Effects of aldosterone present during (*a*) the stimulation period and (*b*) the prestimulation period on the acetylcholine- (ACh) and 5-hydroxytryptamine- (5-HT) induced production of corticotrophin releasing factor (CRF) by isolated rat hypothalami *in vitro*. The open bars show CRF release and the solid bars hypothalamic CRF content. Each column is the mean ± S.E.M. of five determinations.

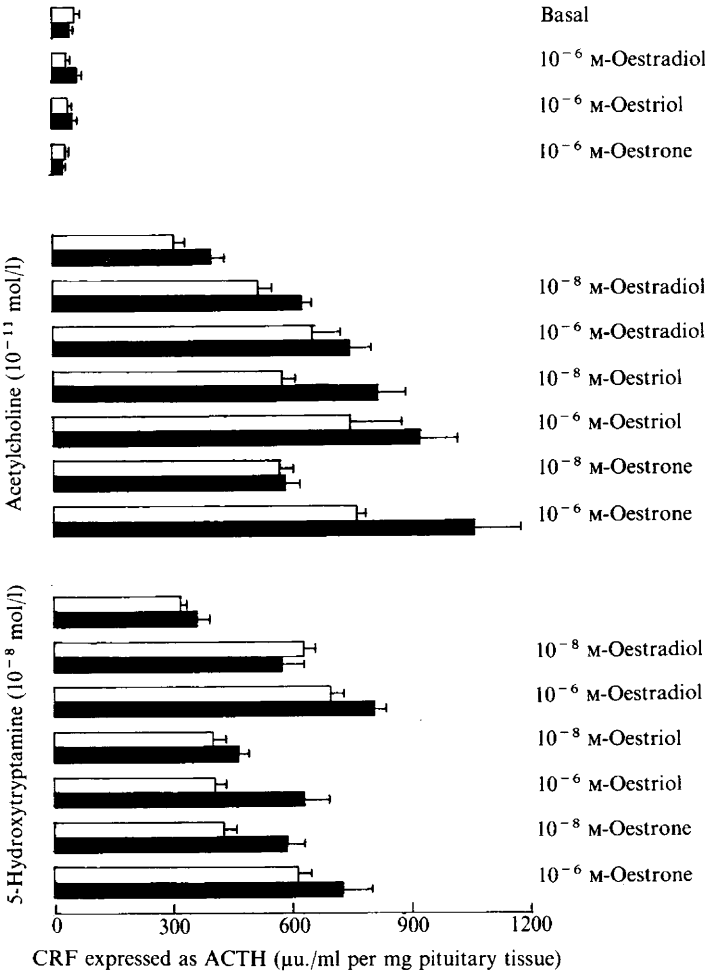

Fig. 4. Effects of the addition of oestradiol, oestriol or oestrone to the incubation medium during the stimulation period on acetylcholine- and 5-hydroxytryptamine-induced secretion of corticotrophin releasing factor (CRF) by isolated rat hypothalami *in vitro*. The open bars show CRF release and the closed bars hypothalamic CRF content. Each column is the mean ± S.E.M. of six determinations.

DISCUSSION

The results described in this paper confirm previous reports (Buckingham & Hodges, 1977*b*; Jones *et al.* 1977; Mahmoud & Jones, 1977; Buckingham, 1979) that corticosterone, in low physiological concentrations, inhibits the secretion of CRF by hypothalami *in vitro* and show that its potent semi-synthetic analogue, betamethasone, is similarly effective. Both steroids also reduce the adrenocorticotrophic activity of the pituitary gland *in vitro* but, as earlier studies have illustrated (Mahmoud & Jones, 1977; Buckingham, 1979), only when present in relatively high concentrations. The data with corticosterone support suggestions (Jones, Tiptaft, Brush, Fergusson & Neame, 1974; Buckingham & Hodges, 1977*a*, *b*; Buckingham, 1979) that the inhibitory response, in both tissues, consists of two components, an immediate effect which influences hormone release only and a delayed effect, evident after prolonged contact of the steroid with the tissue, which results in impairment of hormone formation. A 'silent period' between the responses (Jones *et al.* 1977) was not apparent but may well have been observed had different incubation times

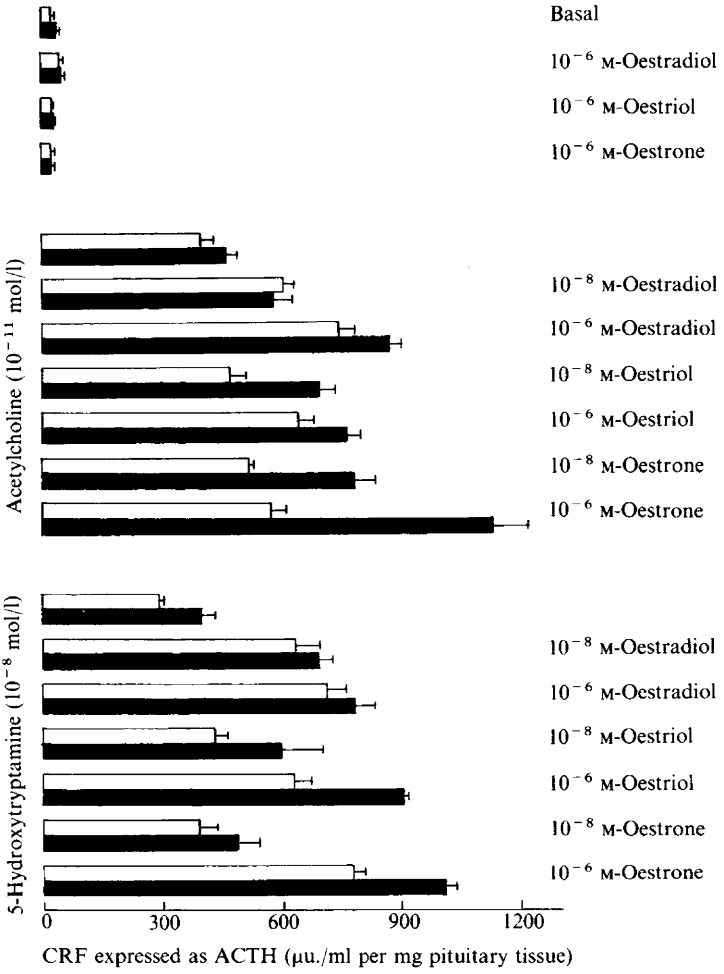

Fig. 5. Effects of the addition of oestradiol, oestriol and oestrone to the incubation medium during the prestimulation period on the acetylcholine- and 5-hydroxytryptamine-induced secretion of corticotrophin releasing factor (CRF) by isolated rat hypothalami *in vitro*. The open bars show CRF release and the closed bars hypothalamic CRF content. Each column is the mean ± S.E.M. of six determinations.

been employed. Betamethasone, like corticosterone, also caused a biphasic response in the pituitary gland. However, in contrast to the naturally occurring steroid, its effects on the hypothalamus were most pronounced after only a short period of contact with the tissue. This discrepancy may reflect differences in the solubility, receptor-binding affinity and efficacy of the steroids and emphasizes the hazards of using synthetic steroid analogues to study physiological problems.

Adrenocortical steroids other than corticosterone (cortisol in appropriate species) possess weak glucocorticoid-like activity (Deane, 1962) and it was not surprising therefore to find that progesterone and aldosterone inhibit the secretion *in vitro* of ACTH and CRF respectively. Indeed, similar effects have been described previously *in vivo* and *in vitro* (Birmingham *et al.* 1974; Buckingham & Hodges, 1977*a*). These responses are probably not of any physiological significance for, in this study, the effective concentrations of both steroids were undoubtedly supra-physiological. However, interestingly, Birmingham *et al.* (1974) have suggested that the influence of aldosterone on ACTH secretion may be greater

than that of corticosterone. This is surprising since severe pituitary-adrenocortical dysfunction does not appear to have been described in subjects with primary hyperaldosteronism.

Differences in the circulating levels of corticosteroids in males and females have led to suggestions (Critchlow, Liebelt, Bar-Sela, Mountcastle & Lipscomb, 1963) that gonadal steroids influence the activity of the hypothalamo-pituitary-adrenocortical system. The results described here, like those reported previously, indicate that oestrogens (Kitay, 1963) and not androgens (Jones & Hillhouse, 1976) are important in this respect. They do not support proposals that oestrogens act at the pituitary level either to stimulate the synthesis of ACTH or to potentiate the activity of CRF (Kitay, 1963; Coyne & Kitay, 1969) for none of the steroids tested influenced either the spontaneous adrenocorticotrophic activity of adenohypophysial segments *in vitro* or the increases in ACTH release and content which occurred in response to hypothalamic extracts. However, the possibility cannot be excluded that effects of the steroids may have been demonstrated had their contact time with the pituitary tissue been increased for it is well known that the biochemical expression of steroid action often takes several hours (Munck, 1968). Nevertheless, the present data show clearly that, in the limited period studied, oestrogenic steroids act directly on the hypothalamus and enhance its responsiveness to trophic stimuli. A 'positive' effect of this nature may be of some physiological significance for parallel changes in blood oestradiol and hypothalamo-pituitary-adrenocortical activity often occur. For example, in the female, the mid-cycle surge in oestrogen production is accompanied by rises in the concentrations of CRF in the hypothalamus (Hiroshige & Wada-Okada, 1973), ACTH in the pituitary gland (Critchlow *et al.* 1963) and plasma (Buckingham, Döhler & Wilson, 1978) and corticosterone in the plasma (Raps, Barthe & Desaulles, 1971; Buckingham *et al.* 1978). Furthermore, the blood corticosteroids are also raised in the later stages of pregnancy (Robinson, Bernhard, Brugin, Wanner, Sewekow & Silber, 1955) and in subjects taking oral contraceptive steroids (Bulbrook, Herian, Tong, Hayward, Swain & Wang, 1973).

Although the validity of translating results from experiments performed *in vitro* to the situation *in vivo* must always be questioned, the techniques employed in this study have obvious advantages over conventional in-vivo methods which, almost invariably, involve the destruction of less discreet, ill-defined areas of the brain. The results suggest that the secretion of corticotrophin and its hypothalamic releasing factor is modified not only by glucocorticoids but also by oestrogens. Other steroids may also have significant effects in this respect particularly in pathological states.

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