

PARADOXICAL EFFECT OF CORTICOSTEROIDS ON PITUITARY ACTH/ β -ENDORPHIN RELEASE IN STRESSED ANIMALS

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(Received 17 July 1987; in final form 6 October 1987)

SUMMARY

We have previously demonstrated a number of changes in the anterior lobe proopiomelanocortin (POMC) system in chronically stressed rats. The purpose of the present experiments was to investigate whether chronically stressed rats demonstrate changes in pituitary sensitivity to glucocorticoid negative feedback. To study this question we compared the effects of glucocorticoids on ovine corticotropin releasing factor (oCRF)-stimulated ACTH and β -endorphin release from cell suspensions prepared from naive unhandled rats versus chronically stressed rats. After dexamethasone, there was a 50% decrease in oCRF-stimulated hormone release in control rats but no inhibition of oCRF-stimulated hormone release in anterior lobe suspension from chronically stressed rats. Rather, the chronically stressed group exhibited a 50% hormone increase above the oCRF-stimulated baseline. The same pattern was observed after the addition of corticosterone to the medium. These findings suggest that there may be a positive feedback effect of glucocorticoids at the pituitary level under some conditions of chronic stress.

INTRODUCTION

USING a chronic footshock stress model, we have demonstrated a number of changes in anterior lobe proopiomelanocortin (POMC) regulation. Following two weeks of 30 min daily footshock, there is an increase in mRNA for POMC (Shiomi *et al.*, 1986), a two-fold increase in POMC content (Young & Akil, 1985), and changes in the rate of biosynthesis and of post-translational processing of POMC to its end products. We observed no evidence of a subsensitive response to oCRF challenge in short-term anterior lobe suspensions from these chronically stressed animals; in fact, there was an increased sensitivity to CRF-stimulated β -endorphin release in anterior lobe suspensions from chronically stressed animals which were restressed prior to decapitation. Since a number of changes have been demonstrated in anterior lobe regulation of β -endorphin biosynthesis and release, it was of interest to test whether these animals demonstrated normal glucocorticoid inhibition of oCRF-stimulated hormone release from the pituitary. To evaluate

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this feedback, we compared the effects of glucocorticoids on oCRF-stimulated hormone release in short-term anterior lobe suspensions from control and chronically stressed rats.

METHODS

Two groups of rats were used. A control group consisted of animals which were unstressed and unhandled. A "chronic stress plus rest" (CS/R) group was exposed to the footshock stressor as previously described (Young & Akil, 1985) for 30 min daily for 14 days and then was allowed to rest for 24 hr. These two groups had similar plasma β -endorphin, ACTH and corticosterone concentrations at the time of decapitation. In addition, short-term anterior lobe suspensions from these animals showed the same response to oCRF (Young & Akil, 1985).

In order to study ACTH/ β -endorphin release, anterior lobe cell suspensions were prepared from these animals immediately following sacrifice. The methods for dispersing the cells and incubating them have been described previously (Young & Akil, 1985). In brief, pituitaries were dissected into anterior and neurointermediate lobe. The anterior lobes from two animals were pooled and incubated with collagenase (3 mg/ml) in Krebs Ringer bicarbonate to dissociate the cells. The cells were washed three times and incubated in Krebs Ringer bicarbonate at 37° C under 95% O₂:5% CO₂ for 90 min to stabilize the cells. The cells were centrifuged, fresh medium was added, and aliquots of each preparation were incubated with either medium alone, dexamethasone (50 nM) alone, oCRF (0.1 nM) alone, or a combination of oCRF and dexamethasone, under 95% O₂:5% CO₂ at 37° C. Following 60-min incubation, the medium was collected, acidified to pH 3.0, and subsequently extracted with Sep Pak C₁₈ cartridges prior to radioimmunoassay (RIA) for β -endorphin immunoreactivity. For each experiment, two anterior lobes were pooled. Eight anterior lobe suspensions were studied per group.

The β -endorphin antibody, Brenda, was used at a final dilution of 1:40,000. This antiserum recognizes β -endorphin and β -lipotropin equally well when ¹²⁵I β -endorphin is used as a radiolabeled tracer (Cahill *et al.*, 1983). Each sample was radioimmunoassayed for β -endorphin three times, and the mean values were used to calculate baseline and release. Stimulated release was calculated as total β -endorphin immunoreactivity minus baseline β -endorphin immunoreactivity. The intra-assay variability for the β -endorphin RIA was 7%, and the inter-assay variability ranged between 10% and 20%.

Each sample was also radioimmunoassayed for ACTH with the ACTH assay described previously (Young & Akil, 1985). The ACTH antibody was raised against ACTH 11-24 and was used at a final dilution of 1:80,000. Synthetic human ACTH 1-39 was used as the standard and radiolabeled tracer. The intra-assay variability for the ACTH RIA was 5%, and the inter-assay variability for ACTH ranged between 10 and 30%. The ACTH and β -endorphin data showed the same pattern, with similar percent stimulation by oCRF and similar percent suppression by dexamethasone. However, because ACTH was assayed only once, there was more variability in ACTH across samples. Therefore, we report only the β -endorphin values.

oCRF was obtained from Bachem (Torrance, CA) and was dissolved in a solution of 0.9% NaCl with 0.1% BSA (Fraction V, Sigma) and 0.01% ascorbic acid (oCRF diluent) added. Dexamethasone sodium phosphate solution (Decadron, Merck, Sharp and Dohme) was obtained at a concentration of 24 mg/ml from the hospital pharmacy and diluted in oCRF diluent. Corticosterone was obtained from Sigma (St. Louis, MO), dissolved at a concentration of 2 mg/ml in 100% ethanol, and diluted in oCRF diluent to the appropriate concentration.

The effects of dexamethasone and corticosterone on oCRF-stimulated release (total release minus baseline release) were compared by two-way analysis of variance (ANOVA). Because the data were not normally distributed, all values were log transformed prior to the ANOVA.

RESULTS

In cell suspensions from control rats, with 10⁻¹⁰ M oCRF as a secretagogue, an approximately two-fold increase in β -endorphin in the medium was observed. The data are expressed as percent of unstimulated baseline. When control and chronic stress rest groups were compared, there were no differences in baseline hormone release or oCRF-stimulated release between groups. Dexamethasone had no effect on baseline (unstimulated) release in control or chronic stress cultures. Dexamethasone (50 nM) produced a 40% inhibition of oCRF-stimulated hormone release in control cell suspensions (Fig. 1). In contrast, in cell suspensions from chronically stressed rats, dexamethasone induced a 50% increase above the

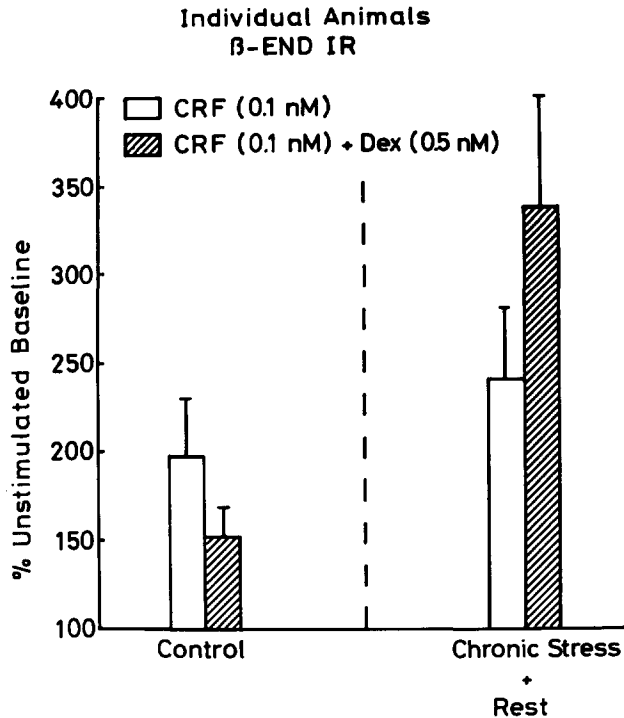


FIG. 1: The effect of dexamethasone (50 nM) on oCRF-stimulated β -endorphin release in pituitary cell suspensions from control and chronically stressed rats (eight rats per group, two rats per suspension). Values are expressed as per cent oCRF-stimulated hormone release. There is no difference between groups in either unstimulated baseline hormone release or in oCRF stimulated release (total release minus baseline). In control cell suspensions from stressed rats, dexamethasone produced a 50% decrease in oCRF stimulated release (suppression). In contrast, dexamethasone produced an approximately 50% increase in oCRF-stimulated release in pituitary cell suspensions from chronically stressed rats (potentiation).

oCRF-stimulated baseline (Fig. 1). There also was a significant interaction between stress and dexamethasone ($F=4.54$; $df=1,32$; $p<.04$). We have found this same stimulatory effect of dexamethasone at 1 nM oCRF in pooled pituitary suspensions from chronically stressed rats. However, since we do not have data from individual animals and therefore have no estimate of variance, we cannot state that this effect is significant at the 1 nM dose of oCRF.

Because dexamethasone, a synthetic steroid, has properties somewhat different from those of naturally occurring glucocorticoids, including a preference for type II glucocorticoid receptors and a lack of binding to corticosteroid binding globulin (CBG) (De Kloet & McEwen, 1976), we next examined the effect of corticosterone, the predominant naturally occurring glucocorticoid in rats, on oCRF-stimulated β -endorphin release in cell suspensions from control and chronically stressed animals (Fig. 2). The dose of corticosterone was 0.2 $\mu\text{g/ml}$: or 0.5 μM . In this experiment, the same hormone release pattern occurred: In control cell suspensions, corticosterone inhibited oCRF-stimulated hormone release by about 60%, whereas it stimulated release in cell suspensions from chronically stressed rats. There again was a significant steroid-stress interaction ($F=5.82$, $df=1.58$, $p<.02$).

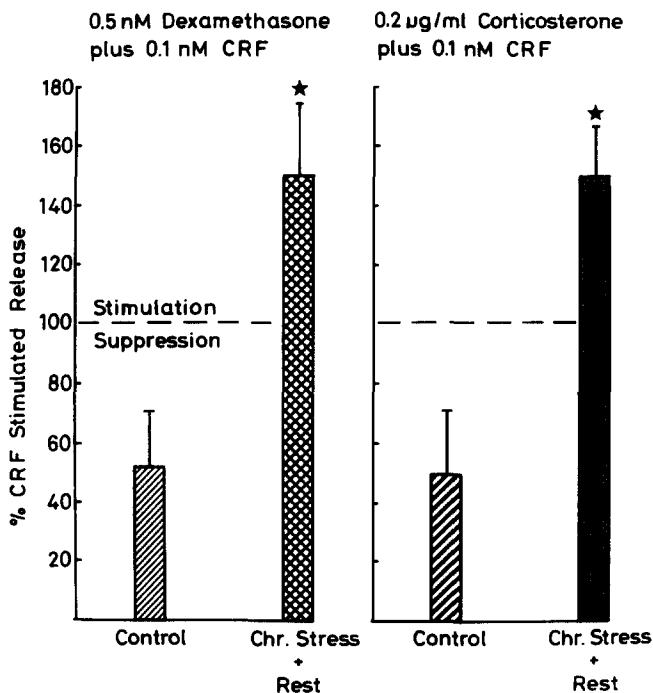


FIG. 2: Comparison of the effect of dexamethasone (50 nM) and corticosterone (500 nM) on CRF-stimulated β -endorphin release in anterior pituitary suspensions from control or chronically stressed rats. Because the steroids affected only stimulated release and not basal secretion, steroid effects are expressed as percent of CRF-stimulated hormone release for each cell suspension. In control anterior pituitary suspensions from unstressed rats, both dexamethasone and corticosterone produced a 50% decrease in CRF-stimulated hormone release (suppression). In contrast, dexamethasone and corticosterone produced an increase in CRF-stimulated release in anterior pituitary cell suspensions from chronically stressed rats (42% increase for dexamethasone, 55% increase for corticosterone). Pituitary cells from chronically stressed rats showed a significantly different response to steroids compared to the response of cells from normal rats.

DISCUSSION

The hypothalamo-pituitary adrenal cortical (HPA) axis was long regarded as showing little adaptation to chronic stress. However newer evidence no longer supports this view, particularly for the pituitary. The pituitary content of ACTH and β -endorphin increases dramatically with adrenalectomy (Birnborg *et al.*, 1983) and chronic footshock (Young & Akil, 1985). The increased hormone content with chronic footshock does not appear to be accompanied by decreased sensitivity to oCRF; in fact, there appears to be an increase in sensitivity to this releasing factor in animals which are restressed before decapitation. The current data suggest that these animals have abnormal glucocorticoid feedback at the anterior pituitary. Because we did not test other doses of dexamethasone or corticosterone, it is possible that higher doses of steroids would have demonstrated normal suppression of β -endorphin in the anterior lobe suspensions from chronically stressed rats. However, the doses used in this study achieved tissue concentrations similar to those in studies demonstrating positive feedback in man (Fehm *et al.*, 1977; 1979; Carey, 1980; Carr *et al.*, 1984).

Two possible explanations to account for the changes in hormone release in anterior lobe suspensions from chronically stressed rats are: an increase in CRF receptor number and/or a decrease in glucocorticoid receptor number. The identical response to oCRF stimulation in control and chronic stress/rest anterior lobe suspensions (Fig. 1; Young & Akil, 1985) suggests that changes in CRF receptor number would not explain the increased response to oCRF plus glucocorticoids in these suspensions. Previous reports of increased CRF stimulation, adrenalectomy (Wynn *et al.*, 1985), and chronic stress (Hauger *et al.*, 1986) have indicated decreases in CRF receptors rather than increases. The evidence for pituitary glucocorticoid receptor down-regulation is also unclear.

Pituitary steroid receptor numbers have been shown to increase *in vivo* after adrenalectomy and to decrease *in vitro* in cultures continuously exposed to corticosteroids in the medium (Svec & Rudis, 1981). Demonstration of pituitary steroid receptor down-regulation *in vivo* has been more difficult. Using a number of daily stressors as well as daily injections with corticosterone in a long acting preparation, Sapolsky *et al.* (1984) have shown a decrease in steroid receptor numbers in several brain regions, but no changes in the number of pituitary steroid receptors. Certainly, the footshock-endured increase in corticosterone in this stress paradigm is of a smaller magnitude than that of the chronic corticosterone injection paradigm.

However, a change in steroid receptor number is not necessary to explain our finding. Nor is it clear that these effects are mediated by the classic glucocorticoid receptor. Schwartz *et al.* (1986) have shown that glucocorticoids appear to bind directly to the CRF receptor, and Childs *et al.* (1986) have shown that glucocorticoids can directly affect CRF receptor number. It is quite possible that the effects of either antagonism or potentiation of CRF-stimulated hormone release *in vitro* are mediated through a non-glucocorticoid receptor mechanism. The potentiation of oCRF-stimulated hormone release can be explained by a positive feedback mechanism in the pituitary for glucocorticoids. Such positive feedback effects of glucocorticoids have been reported in patients with Cushing's disease and severe depression (James *et al.*, 1965; Krieger *et al.*, 1975; Fehm *et al.*, 1977; 1979; Carey, 1980; Carr *et al.*, 1984). In other systems, such as the gonadal steroid system, positive feedback effects of sex steroid hormones have been noted (Yamaji *et al.*, 1971; Nakai *et al.*, 1978). Likewise, the effects of glucocorticoids are not always the same at different levels of the HPA axis. Mahmoud *et al.* (1984) reported that 11-deoxycorticosterone or 11-deoxycortisol can antagonize the negative feedback effect of corticosterone fast feedback in intact rats, while these same steroids show negative feedback effects on pituitary ACTH release *in vitro* in delayed feedback paradigms and in fast feedback studies on brain-lesioned rats. Thus, there is some indication that the positive or negative coupling of different steroids may vary across levels of the HPA axis as well as across experimental conditions and feedback paradigms. If such a change in coupling occurs to elicit positive feedback at the pituitary, then one would not necessarily expect a decrease in steroid receptor number, because such a decrease would lead to a blunted negative feedback response rather than a positive response. Finally, it is possible that a decrease in the classic glucocorticoid inhibitory response leads to an uncovering of an already existing stimulatory effect of glucocorticoids. Our data do not allow us to distinguish between this hypothesis and a "switch" hypothesis. In either case, glucocorticoids can have stimulatory effects at the pituitary. The lack of effect of glucocorticoids alone (in the absence of oCRF) in this experimental paradigm suggests that this stimulatory effect is not a simple one but requires an interaction with oCRF.

We conclude that daily phasic stimulation of the HPA axis can uncover a positive feedback effect of glucocorticoids on the pituitary. This stimulatory effect of steroids on the pituitary provides a possible explanation for our previously reported paradoxical response to an acute stress in anterior lobe cell suspensions from chronically stressed rats (Young & Akil, 1985). These studies provide *in vitro* evidence of a proposed positive feedback loop occurring in

Cushing's patients and in some patients with endogenous depression (James *et al.*, 1965; Krieger *et al.*, 1975; Fehm *et al.*, 1977; 1979; Carey, 1980; Carr *et al.*, 1984). It is unclear whether this change results from a change in the coupling of the receptor to a second messenger system or from a different and unknown mechanism. Although such changes may seem counter-intuitive in a chronically driven system, they may enable the organism to meet continually the demands imposed by repeated chronic stress without the risk of steroid negative feedback exerting too great a clamp on the system. In addition, they may be coupled to counterbalancing feedback influences at the suprapituitary level, permitting the system to adapt to recurring demands with a different set of regulatory mechanisms. The physiological or environmental events which would foster such mechanisms *in vivo* need to be explored.

Acknowledgements: We would like to acknowledge the support of ADAMHA grants DA02265 to HA and MH00427 to EY.

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