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CHANGES IN RELEASABILITY OF ACTH AND BETA-ENDORPHIN WITH CHRONIC STRESS

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ABSTRACT

The activation of the hypothalamic pituitary adrenal axis by stress is well-known. Using inescapable intermittent footshock as a stressor in rats, we have previously demonstrated a rise in circulating plasma Beta-endorphin/Beta-LPH which parallels the rise in plasma ACTH, the primary POMC derived peptides released by anterior lobe. In addition, the rise in ACTH is accompanied by approximately a tenfold rise in plasma corticosteroids. Short term anterior lobe pituitary cultures from rats who have received inescapable intermittent footshock for 30 minutes show a blunted dose response curve to the ACTH releasing secretagogues arginine vasopressin (AVP) and ovine corticotropin releasing factor (oCRF). Similarly a blunted dose response curves to secretagogues can be seen by either the addition of dexamethasone (0.5 nM) to the culture medium or pretreatment of the rats with 1 mg dexamethasone intraperitoneally 90 minutes prior to decapitation. Thus, glucocorticoids may play a role in the blunted response to secretagogues seen in anterior lobe cultures from acutely stressed rats. We now report that chronically stressed rats exhibit increased releasability of ACTH and Beta-endorphin/Beta-LPH products by oCRF, suggesting an increase of the peptides in the releasable pool.

INTRODUCTION

In male rats, it has been previously demonstrated that the history of chronic stress does not affect the subsequent glucocorticoid rise to a new stress administered more than 8 hours after a previous stress (1; and our own data). In addition, the resting corticosteroid levels are often normal in chronically stressed rats. Similarly, we found low resting plasma values for ACTH in naive rats and rats who were chronically stressed with thirty minutes daily intermittent footshock (30 $\pm$ 10 fmoles/ml plasma for control, 20.2 $\pm$ 2 fmoles/ml plasma for chronic stress). When these chronically stressed rats are stressed again, rises in plasma ACTH similar to that of naive control rats are seen in chronically stressed rats (166 $\pm$ 18 fmoles/ml plasma for control; 148 $\pm$ 8 fmoles/ml plasma in chronic stress). Thus, it would appear that no adaptation to stress has occurred. However, when we examine anterior lobe pituitary content of either Beta-Endorphin (BE)/Beta-Lipotropin (B-LPH), we see a twofold increase in anterior lobe stress of these peptides in chronically stressed rats. Thus, we examined the sensitivity of the pituitary

to ovine corticotropin releasing factor (oCRF) in naive control versus chronically stressed rats, using a short term pituitary culture system (2,3).

METHODS

Male albino rats were obtained from Charles River and were allowed to adjust to the new environment for two weeks prior to the start of the experiment. Inescapable intermittent footshock, of 1.5 mAmp, one second duration, and 10 per minute frequency was delivered to each experimental animal for 30 minutes. Chronically stressed rats were stressed for 30 minutes daily for 14 days, then decapitated 24 hours after the last stress session. Control animals were naive, unhandled rats.

Pituitaries were removed and dissected into anterior and neurointermediate lobes. For the content studies, the pituitary were frozen immediately on dry ice, then stored at -80°C until extraction. For the release studies, the anterior pituitaries were placed in oxygenated calcium free Krebbs Ringer lactate buffer with 0.2% glucose, 0.5% bovine serum albumin (Sigma, Fraction V), 0.2% sodium bicarbonate with 0.01% limabean trypsin inhibitor, 0.01% soybean trypsin inhibitor and 0.1% Kanamycin (RGBAlb). In order to process them into single cell suspensions, anterior lobes from the same groups were pooled, then placed in calcium free KRGBAlb with 3mg/ml of crude collagenase, and incubated for one hour at 37°C under 95% 02 and 5% C02 in a Dubnoff Shaking Metabolic Incubator. The dispersed tissue was filtered through 30 micron nylon mesh, then washed three times with low speed centrifugation (100xg). The single cell suspension was then re-suspended in calcium-containing KRGBAlb, then incubated at 37°C for 90 minutes to stabilize the cells (preincubation).

The cells were then centrifuged and resuspended in fresh medium at a concentration of 500,000 cells/ml KRGBAlb. One half milliliter aliquots were incubated with buffer or with varying doses of oCRF under the same conditions as the preincubation. At the end of 60 minutes, the cells were centrifuged, and the media removed and pooled for subsequent extraction.

The media was extracted using SepPak C18 cartridge with the manufacturer's recommended procedure for arginine vasopressin. The tissue was extracted using a 1:3 mixture of 0.2N hydrochloric acid and acetone. Samples were lyophilized then stored at -80°C until assayed. Beta endorphin was assayed using the radioimmunoassay procedure previously described (4). The antibody (Brenda) is 100% cross reactive with B-LPH and the precursor, POMC. ACTH was assayed using the ACTH radioimmunoassay previously described. The ACTH antibody is an antibody to ACTH 11-24 used at a 1:200,000 dilution with ^{125}I ACTH 1-34 (human) as a radiolabelled tracer. The antibody cross reacts with ACTH 18-39 (CLIP) 10%, but does not cross react with alpha-MSH. ACTH 1-39 (Peninsula) is used as a standard. For both BE and ACTH assays, all standards and samples are dissolved in 0.1% human serum albumin solution, pH 3.0.

RESULTS

The results are shown in Table 1. As can be seen, the pituitary cultures from control rats show an increase in BE/B-LPH and ACTH release in a dose dependent fashion, with an approximate three-fold elevation over baseline in response to 1nM oCRF.

TABLE 1

BETA-ENDORPHIN AND ACTH RELEASE INTO THE MEDIUM
WITH STIMULATION BY oCRF

(Results expressed as percent of unstimulated release)

Beta-Endorphin-IR

	Control	Chronic Stress
unstimulated	100	100
0.01nM CRF	176	251
0.1nM CRF	280	480
1nM CRF	340	420

ACTH-IR

	Control	Chronic Stress
unstimulated	100	100
0.01nM CRF	185	200
0.01nM CRF	250	310
1nM CRF	300	400

In anterior lobe cultures, from chronically stressed rats, there is a 4.5 to 5 fold increase over baseline in ACTH or BE/B-LPH release (1.5-2 fold greater than control) at the 0.1nM an 1nM doses of oCRF. This is in agreement with the increased content (Table 2) of either ACTH or BE/B-LPH in the anterior lobe after chronic stress.

TABLE 2

ANTERIOR LOBE PITUITARY CONTENT OF
POMC PRODUCTS (pmoles/pituitary)

	Control	Chronic Stress
ACTH-IR	223 $\pm$ 25	463 $\pm$ 130
BE-IR	191 $\pm$ 21	489 $\pm$ 50

DISCUSSION

Previous in vivo studies in chronic stress have shown either decreased or normal corticosteroid response to another stress the following day (1,5,). Our in vivo studies are in complete agreement. Thus, examining either plasma corticosterone or ACTH values, we see no difference in the response to stress in either a naive or chronically stressed rat. However, when we directly examine anterior lobe pituitary content of ACTH and related peptides, we see a twofold increase in these peptides with chronic stress. This elevation occurs in both final products (ACTH, BE, and B-LPH) as well as precursors and intermediates (data not shown). In addition to these increased stores, we see an increased releasability of ACTH and BE/B-LPH with oCRF stimulation. Thus

the increase in ACTH stores is translated into an increase in ACTH release with a given dose of oCRF.

Such an increase in POMC derived peptides stores and in releasability of these peptides could be an adaptive mechanism to maintain both high circulating levels and adequate stores of ACTH during a situation of high demand (severe stress). That is, during stress in a chronically stressed rat, the anterior pituitary can release the same amount (pmoles) of ACTH with less depletion of ACTH stores. The increased releasability would assure that the chronically stressed organism is able to meet the demand for ACTH with greater ease than a naive animal. Since oCRF, in addition to its role as an ACTH releaser, has been shown to increase the content of ACTH in anterior pituitary lobe cultures, the increase in content may be secondary to the trophic influence of endogenous CRF released during the course of stress.

Whether the increased releasability of ACTH from the pituitary in vitro, is accompanied by a similar increased releasability of ACTH to oCRF in vitro remains to be tested. However, the ability of chronically stressed animals (during the course of a new stress) to maintain plasma ACTH and corticosteroid levels similar to naive animals in the face of increased pituitary stores and releasability of ACTH, suggests that other adaptations occur to maintain homeostasis. Such compensatory adaptation might occur in the amount of endogenous CRF released during stress, in changes in sensitivity to glucocorticoid feedback or in the modulatory influences of other hormones, such as sex steroids, on ACTH release. At the current time it is unclear which of these mechanisms are critical for maintaining homeostasis in chronic stress.

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