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ADDITIVE EFFECTS OF EPINEPHRINE AND CORTICOTROPIN-RELEASING FACTOR (CRF) ON ADRENOCORTICOTROPIN RELEASE IN RAT ANTERIOR PITUITARY CELLS

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Summary: The ability of epinephrine to potentiate the action of corticotropin-releasing factor (CRF) was studied in rat anterior pituitary cells in primary culture. Increasing concentrations of epinephrine cause a maximal 6.5-fold stimulation of ACTH release while a 10-fold increase is induced by 5 nM CRF. The two substances exert an additive effect on ACTH release. Although epinephrine alone has no effect on cyclic AMP levels, it causes a maximal 4-fold increase in CRF-induced cyclic AMP accumulation. The stimulatory effect of epinephrine on both parameters is inhibited by the highly specific α_1-adrenergic antagonist prazosin but not by the β-adrenergic antagonist (-)propranolol. Dexamethasone causes a 75% inhibition of ACTH release induced by the combined action of CRF and epinephrine. The present data show that the additive effect of epinephrine on CRF-induced ACTH release is achieved through an α-adrenergic receptor and is accompanied by a marked stimulation of intracellular cyclic AMP levels.

The vital function of glucocorticoid secretion by the adrenal cortex is controlled by multiple factors which include the circadian rythm, various types of stresses and the inhibitory feedback action of glucocorticoids. It is also well demonstrated that multiple stimulatory agents can independently affect ACTH release by a direct action at the anterior pituitary level. These stimulatory agents include catecholamines which exert their effect through a highly specific α_1-adrenergic receptor (1, 2), vasopressin (1, 3) and the peptidic CRF isolated from ovine hypothalami (4).

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Abbreviations used: CRF, corticotropin-releasing factor; ACTH, adrenocorticotropin; DEX, dexamethasone; EPI, epinephrine.

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Recently, vasopressin has been found not only to stimulate ACTH release on its own but to potentiate the stimulatory effect of CRF on ACTH secretion (5-7). This potentiating action of vasopressin is probably mediated by the marked stimulatory effect of vasopressin on CRF-induced cyclic AMP accumulation in corticotrophs (5). Since, similarly to vasopressin, the specific stimulatory action of α-adrenergic agents could be involved in the physiological control of ACTH secretion in response to various stressful stimuli, we have investigated the possibility that catecholamines could also act as potentiators of the stimulatory action of CRF on ACTH release and cyclic AMP accumulation by a direct action at the pituitary level.

MATERIALS AND METHODS

Materials: (-)epinephrine (+)bitartrate was purchased from Sigma. Stock solutions of DEX obtained from Steraloids were prepared in 0.9% NaCl-1% ethanol and used at a 100-fold dilution in the incubation medium. Such a concentration of ethanol has no effect on spontaneous ACTH release. Prazosin and (-)propranolol were gifts from Pfizer and Ayerst Laboratories, respectively. CRF was prepared by solid-phase methods as described (8). Culture media and sera were purchased from Flow Laboratories and GIBCO.

Preparation of dispersed anterior pituitary cells. Adult female Sprague-Dawley rats (Crl:CD(SD)Br, Charles River Canada Inc.), at random stages of the estrous cycle were used for the preparation of primary cultures of anterior pituitary cells, as described (9). Cells ($4\text{-}6 \times 10^5$) in 1.0 ml DMEM containing 10% dextran-coated charcoal (DCC)-adsorbed horse serum and 2.5% DCC-adsorbed fetal calf serum were plated in Linbro multiwell petri dishes.

Incubation procedure. Three to 4 days after plating, cells growing as monolayers were washed four times with DMEM without serum, and the incubation was carried out in triplicate for the indicated time intervals after addition of the substance(s) to be tested. For measurement of the effect of DEX, cells were first incubated for 4h in the presence of the steroid alone, whereas the secretagogues were present only during the following 3-h incubation period. Ascorbic acid (10^{-4}M) was present in all incubations. At the end of the incubation, the medium was removed and centrifuged at 100 x g for 7 min at 4°C, and the supernatant was acidified with 100 µl of 1M HCl and frozen at -20°C until assayed for ACTH by radioimmunoassay. In order to measure cyclic AMP cell content, culture medium was aspirated at the end of the incubation, and intracellular cyclic AMP was extracted with 1.0 ml of 0.1M acetic acid for 60 min at 4°C. The extract was then lyophilized and resuspended in 50 mM sodium acetate (pH 6.2) before acetylation (10).

ACTH and cyclic AMP assays and calculations. ACTH and cyclic AMP were measured with radioimmunoassays developed in our laboratory (11, 12). Calculations and statistical analyses were performed as described (11).

RESULTS

We first investigated the possible ability of epinephrine to potentiate the stimulatory action of CRF on ACTH release in rat anterior pituitary cells in culture. As illustrated in Fig. 1A, increasing concentrations of epinephrine alone cause a maximal 6.5-fold stimulation of ACTH release at an ED_{50} value of 20 nM while 50 nM CRF alone causes a 10-fold increase in ACTH release. It can be seen in Fig. 1A that increasing concentrations of the catecholamine lead to a further increase in ACTH release up to 18-fold at an ED_{50} value of 6 nM. It can be seen in Fig. 1B that although epinephrine, in the presence of (-)propranolol, has no significant effect on cyclic AMP accumulation, it can dramatically increase the 5-fold stimulation of cyclic AMP accumulation induced

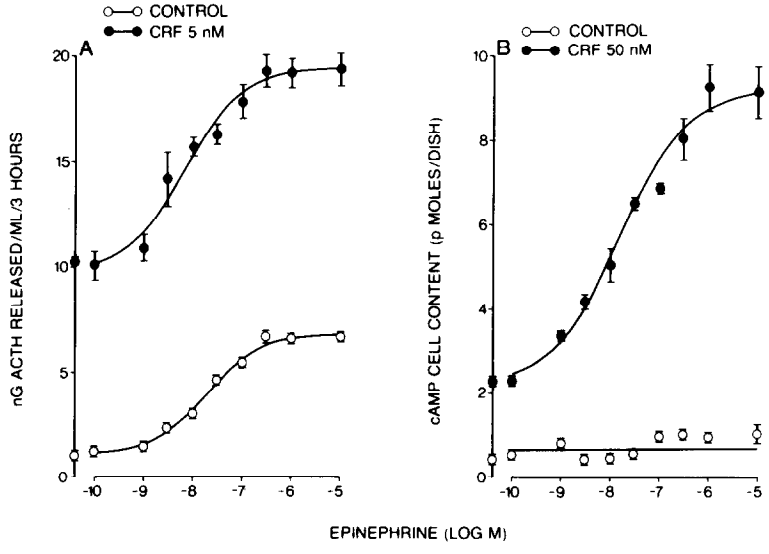


Fig. 1. Effects of epinephrine on CRF-induced ACTH release (A) and cyclic AMP accumulation (B) in rat adenohypophysial cells in culture. Epinephrine alone has no significant effect on cyclic AMP levels. (-)propranolol (1 μM) was present in the incubation medium to avoid an increase in cyclic AMP levels through interaction of the catecholamine with a β-adrenergic receptor. Cyclic AMP was measured after 10 min of incubation.

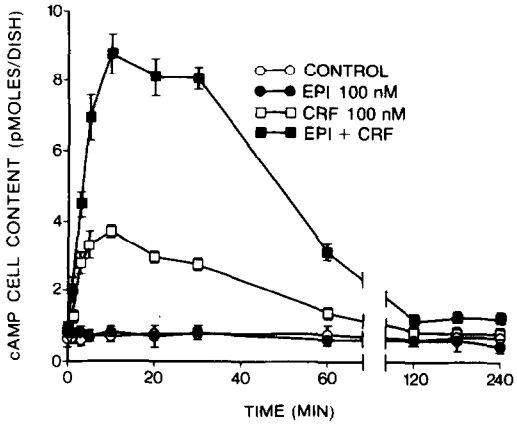
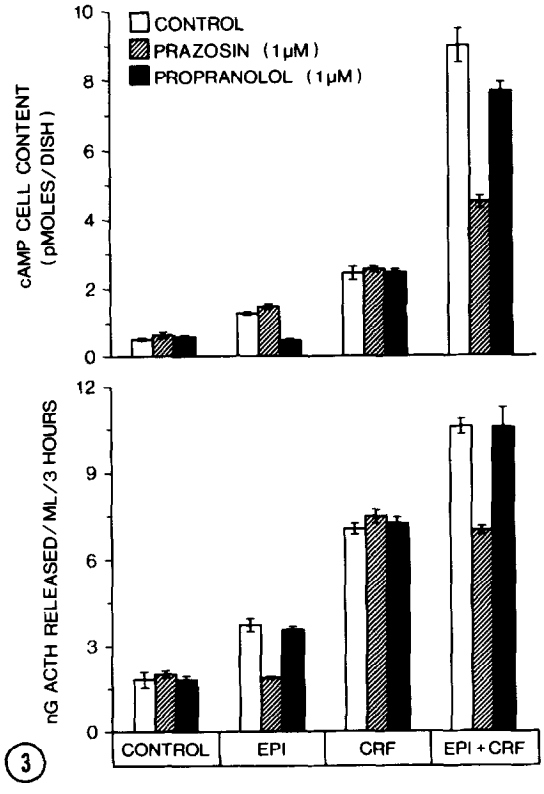


Fig. 2. Time course of the effect of 100 nM epinephrine and 100 nM CRF alone or in combination on cyclic AMP accumulation in rat anterior pituitary cells. (-)propranolol (1 μM) was present in the incubation medium.

by CRF alone up to a 20-fold stimulation. This effect of epinephrine is exerted at an ED_{50} value of 15 nM.

We next measured the effect of the catecholamine on the time course of the stimulatory action of CRF on cyclic AMP accumulation. As shown in Fig. 2, epinephrine has no effect at any time interval on cyclic AMP accumulation whereas CRF causes a maximal 6-fold stimulation of cyclic AMP cellular content between 10 and 30 min after its addition. When both epinephrine and CRF are present in the incubation medium, the rise in cyclic AMP cell content induced by CRF is more than doubled at all time intervals.

We then investigated the specificity of the effect of epinephrine on ACTH release and cyclic AMP accumulation. Fig. 3 shows that prazosin, a highly specific α_1-adrenergic antagonist, but not (-)propranolol, a β-adrenergic antagonist, can inhibit ACTH release induced by epinephrine alone or in combination with CRF. Furthermore, it can be seen in Fig. 3 that although epinephrine alone causes a small increase of cyclic AMP accumulation in rat anterior pituitary cells through interaction with a β-adrenergic receptor, the potentiating effect of epinephrine on CRF-induced cyclic AMP



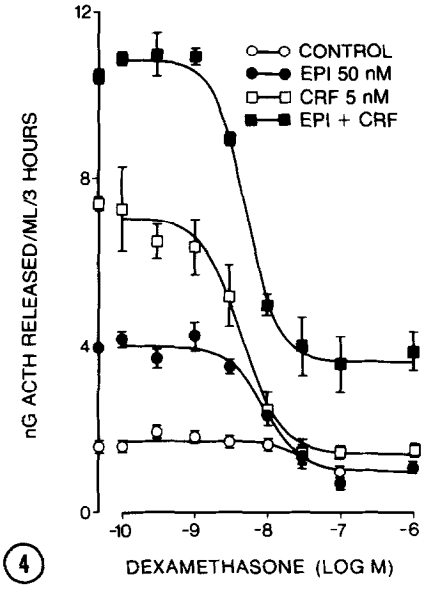


Fig. 3. Specificity of the action of 50 nM epinephrine on 5 nM CRF-induced ACTH release (lower panel) and cyclic AMP accumulation (upper panel). The times of incubation are as in Fig. 1.

Fig. 4. Inhibitory effect of increasing concentrations of DEX on ACTH release induced by epinephrine, CRF or a combination of both agents.

accumulation is reversed only by the α-adrenergic antagonist prazosin.

Finally, we have studied the ability of the potent glucocorticoid DEX to inhibit ACTH release induced by epinephrine, CRF or the combination of both substances. Fig. 4 shows that increasing concentrations of DEX cause a complete inhibition of ACTH release stimulated by epinephrine or CRF at an ED_{50} value of 5 to 10 nM. However, when both epinephrine and CRF are present, DEX could only achieve a maximal 75% inhibition of ACTH release with no change in the ED_{50} value of glucocorticoid action.

DISCUSSION

The present study provides the first evidence for a stimulatory action of epinephrine on CRF-induced ACTH release in rat anterior pituitary cells. In addition, the present data provide information about the mechanisms involved in this α_1-adrenergic effect, namely a marked potentiation by epinephrine of the rise in cyclic AMP levels induced by CRF. The mechanisms responsible for the potent stimulatory effect of epinephrine on CRF-induced cyclic AMP accumulation remain to be investigated.

Glucocorticoids have previously been shown to completely inhibit ACTH release induced by maximal concentrations of epinephrine (11) and CRF (13). The present findings demonstrate that the potent glucocorticoid DEX is unable to completely abolish ACTH release induced by the combined action of epinephrine and CRF. This observation could be of physiological importance, since it has been observed that increased serum glucocorticoid levels induced by repeated stress did not block the plasma ACTH response to a subsequent stressful stimulus (14).

A physiological role for epinephrine in the adrenocortical response to stress has been proposed many years ago (15). However, it is only recently that a potent and highly specific stimulatory effect of catecholamines on ACTH release at the anterior pituitary level could be demonstrated (1, 2). The present data indicate that in addition to its own stimulatory action, epinephrine can markedly influence the stimulatory effect of CRF on ACTH secretion. It is thus likely that elevated peripheral epinephrine concentrations observed during immobilization stress (16) or released by hypothalamic nerve terminals (17) could not only stimulate ACTH release independently but could well exert a predominant effect by increasing the action of CRF on ACTH release.

An important conclusion arising from the present study is that not only vasopressin (5-7) but also epinephrine can exert a

potent stimulatory effect on CRF-induced ACTH release. These three positively interacting mechanisms are likely to be involved in the pituitary adrenocortical response to various stressful stimuli.

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