Acta Medica Okayama

Volume 38, *Issue* 4 1984 *Article* 4

AUGUST 1984

Effect of angiotensin II, catecholamines and glucocorticoid on corticotropin releasing factor (CRF)-induced ACTH release in pituitary cell cultures.

Kazuharu Murakami* Kozo Hashimoto†

Zensuke Ota‡

*Okayama University,

†Okayama University,

‡Okayama University,

Effect of angiotensin II, catecholamines and glucocorticoid on corticotropin releasing factor (CRF)-induced ACTH release in pituitary cell cultures.*

Kazuharu Murakami, Kozo Hashimoto, and Zensuke Ota

Abstract

The effects of angiotensin II, catecholamines and glucocorticoid on CRF-induced ACTH release were examined using rat anterior pituitary cells in monolayer culture. Synthetic ovine CRF induced a significant ACTH release in this system. Angiotensin II produced an additive effect on CRF-induced ACTH release. The ACTH releasing activity of CRF was potentiated by epinephrine and norepinephrine. Dopamine itself at 0.03-30 ng/ml did not show any significant effect on ACTH release, but it inhibited CRF-induced ACTH release. Corticosterone at 10(-7) and 10(-6)M inhibited CRF-induced ACTH release. These results indicate that angiotensin II, catecholamines and glucocorticoid modulate ACTH release at the pituitary level.

KEYWORDS: corticotropin releasing factor(CRF), ACTH release, angiotensin ?, catecholamines, glucocorticoid

---

*PMID: 6093444 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **38,** (4), 349-355 (1984)

EFFECT OF ANGIOTENSIN II, CATECHOLAMINES AND GLUCOCORTICOID ON CORTICOTROPIN RELEASING FACTOR (CRF)-INDUCED ACTH RELEASE IN PITUITARY CELL CULTURES

Kazuharu MURAKAMI, Kozo HASHIMOTO and Zensuke OTA

Third Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan

Received March 22, 1984

Abstract. The effects of angiotensin II, catecholamines and glucocorticoid on CRF-induced ACTH release were examined using rat anterior pituitary cells in monolayer culture. Synthetic ovine CRF induced a significant ACTH release in this system. Angiotensin II produced an additive effect on CRF-induced ACTH release. The ACTH releasing activity of CRF was potentiated by epinephrine and norepinephrine. Dopamine itself at 0.03-30 ng/ml did not show any significant effect on ACTH release, but it inhibited CRF-induced ACTH release. Corticosterone at 10^{-7} and 10^{-6} M inhibited CRF-induced ACTH release. These results indicate that angiotensin II, catecholamines and glucocorticoid modulate ACTH release at the pituitary level.

Key words : Corticotropin releasing factor (CRF), ACTH release, Angiotensin II, Catecholamines, Glucocorticoid.

Many investigators have suggested that several kinds of biologically active peptides and neurotransmitter substances have effects on the release of ACTH from the pituitary. We have examined the effect of neurogenic peptides and neurotransmitter substances on ACTH release (1) and hypothalamic extract-induced ACTH release (2). However, crude hypothalamic extracts contain some substances besides CRF which may affect ACTH release. Therefore, authentic CRF instead of hypothalamic extracts should be used to examine the effects of substances on CRF-induced ACTH release. In 1981, Vale and co-workers (3, 4) reported the isolation, sequence analysis and synthesis of a 41-residue ovine hypothalamic peptide which potently stimulated the secretion of ACTH and β-endorphin *in vivo* and *in vitro.* In the present investigation, we examined the role of angiotensin II, catecholamines and corticosterone on synthetic CRF-induced ACTH release at the pituitary level using rat pituitary cells in culture.

MATERIALS AND METHODS

Pituitary cell cultures. Wistar rats, weighing 200-250 g, were decapitated, and the anterior pituitaries were immediately removed, minced into small pieces and placed in Hanks-HEPES

buffer (pH 7.4). The pituitaries were then dispersed with collagenase using a previously described procedure (2). Dispersed pituitary cells were washed 3 times with Dulbecco's modified Eagle medium (DMEM, GIBCO) containing 10 % horse serum, 2.5 % fetal calf serum, 1 % non-essential amino acid (GIBCO), fungisone 2.5 μg/ml, penicillin 50 U/ml and streptomycin 50 μg/ml. The dispersed cells were resuspended in an appropriate volume of medium and were placed in sterile Petri dishes (35 × 10 mm). Usually 2-5 × 10^5 cells per 1.5 ml medium were placed into a single dish, and four dishes were usually cultured from one pituitary. The dishes were cultured at 37℃ for 4 days in an incubator with 95 % O_2 and 5 % CO_2 and continuously supplied with water vapor.

Experiments using cultured cells. After 3-5 days of culture, the cells firmly attached to the dishes were washed twice with non-serum-containing DMEM. DMEM containing the test substances was then added for 3 h of incubation. Corticosterone (Sigma) was first dissolved in ethanol and further diluted with DMEM just before use. Other test substances and their sources were : angiotensin II (CIBA, LTD), norepinephrine (Wako Pure Chemical Industries, LTD), epinephrine (Wako pure Chemical Industries, LTD), and dopamine (Nakarai Chemicals). Synthetic ovine CRF was a gift from Drs. W. Vale and J. Rivier (Salk Institute). All test substances were dissolved and diluted with DMEM just before use. Test incubations were carried out at 2.0 ml/dish for 3 h. Three or four dishes were employed in each test group. The amount of ACTH in the culture medium was assayed by a sensitive radioimmunoassay employing an anti-porcine ACTH serum which reacted with ACTH, but did not cross react significantly with other pituitary hormones and test substances employed in the present investigation (5).

Statistical analysis was conducted by Duncan's new multiple range test.

RESULTS

Effect of angiotensin II on CRF-induced ACTH release. Angiotensin II at 0.03 and 30 ng/ml induced a slight ACTH release ($p<0.05$), but not in a dose-dependent manner. Angiotensin II at 0.3 and 3 ng/ml showed an additive effect on CRF-

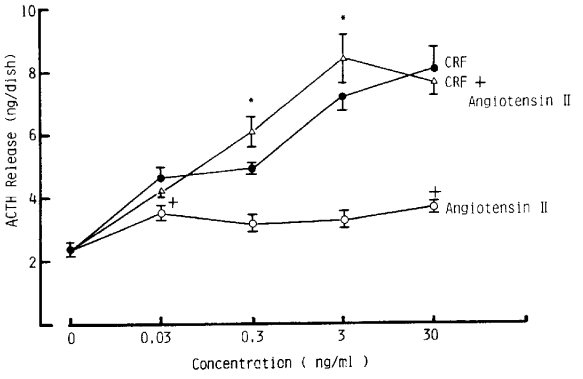

Fig. 1. Effect of angiotensin II on basal and CRF-induced ACTH release in pituitary cell cultures. Angiotensin II at 0.03 and 30 ng/ml induced slight ACTH release (+ $p<0.05$). *$p<0.05$ vs the amount of ACTH released by CRF alone. All data are presented as the mean ± SEM.

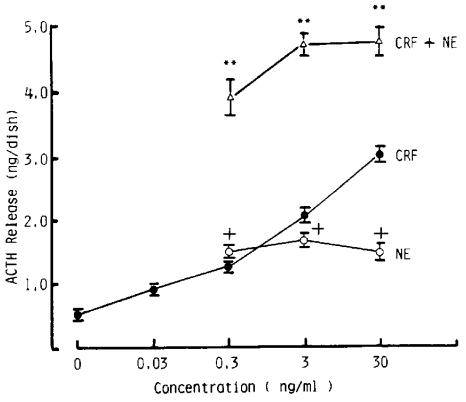

Fig. 2. Effect of norepinephrine (NE) on basal and CRF-induced ACTH release in pituitary cell cultures. Norepinephrine at 0.3-30 ng/ml induced statistically significant ($+ p<0.05$) ACTH release compared to the control group. $**p<0.01$ vs the amount of ACTH released by CRF alone.

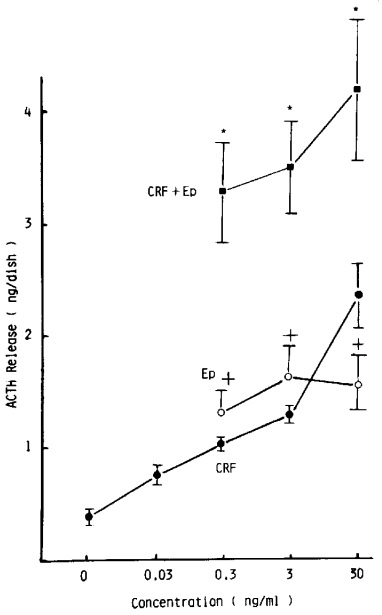

Fig. 3. Effect of epinephrine (Ep) on basal and CRF-induced ACTH release in pituitary cell cultures. Epinephrine at 0.3-30 ng/ml induced statistically significant ($+ p<0.05$) ACTH release compared to the control group. $*p<0.05$ vs the amount of ACTH released by CRF alone.

induced ACTH release (Fig. 1).

Effect of catecholamines on CRF-induced ACTH release. Norepinephrine and epinephrine at 0.3-30 ng/ml induced significant ACTH release ($p<0.05$), but the

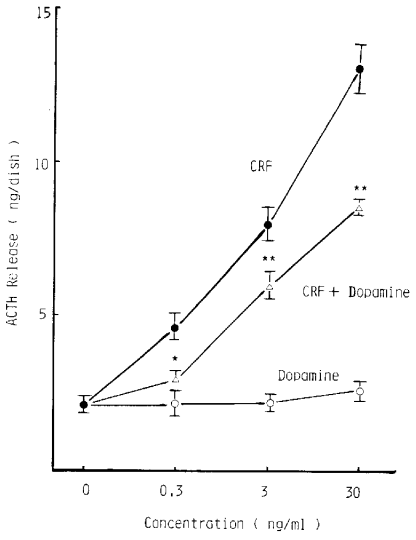

Fig. 4. Effect of dopamine on basal and CRF-induced ACTH release in pituitary cell cultures. Dopamine at 0.3-30 ng/ml did not induce ACTH release. *$p<0.05$, **$p<0.01$ vs the amount of ACTH released by CRF alone.

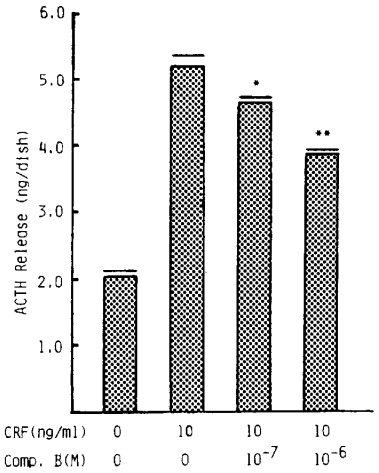

Fig. 5. Effect of corticosterone (Comp. B) on synthetic ovine CRF (10 ng/ml)-induced ACTH release in pituitary cell cultures. Histograms and bars represent the mean + SEM. *$p<0.05$, **$p<0.01$ vs the amount of ACTH released by CRF alone.

responses were not dose-related. They greatly potentiated CRF-induced ACTH release (Figs. 2, 3). Dopamine at 0.03-30 ng/ml did not induce a significant ACTH release, but suppressed CRF-induced ACTH release (Fig. 4).

Effect of corticosterone on CRF-induced ACTH release. Corticosterone at 10^{-7} and 10^{-6} M suppressed CRF-induced ACTH release in a dose-related manner (Fig. 5).

DISCUSSION

Many investigators have reported that synthetic ovine CRF is a potent stimulator of ACTH release *in vivo* and *in vitro* (4, 6, 7). On the other hand, some neurogenic peptides and neurotransmitter substances, such as arginine vas-

opressin, oxytocin, angiotensin II, epinephrine and norepinephrine, have been shown to have slight CRF-like activity (8-10). A variety of *in vitro* and *in vivo* studies have also suggested that the action of physiological CRF may be modulated by glucocorticoid (8, 11, 12). Vasopressin, which has CRF activity (8, 13, 14), appears to potentiate the effect of synthetic ovine CRF on ACTH release by isolated pituitary cells (15). We have shown that arginine vasopressin produces a synergistic effect on CRF-induced ACTH release and that the preincubation of pituitary cells with synthetic ovine CRF enhances subsequent AVP-induced ACTH release (16).

Maran and Yates observed that intrapituitary infusion of angiotensin II caused an increase in cortisol in dogs (17). In the present examination, angiotensin II was shown to be a slight ACTH secretagogue and at 0.3 and 30 ng/ml showed an additive effect on CRF-induced ACTH release. Angiotensin II may have some effect on ACTH release *in vivo* under the influence of authentic CRF.

Previous reports showed that peripheral administration of epinephrine increased plasma ACTH levels in rats (18). A stimulatory effect of catecholamines on ACTH release at the pituitary level was demonstrated in *in vitro* experiments (19). In the present examination, epinephrine and norepinephrine stimulated ACTH release in pituitary cell cultures, but less than synthetic ovine CRF. However, they produced a synergistic effect on CRF-induced ACTH release. Vale *et al.* also reported that both catecholamines acted additively with CRF to stimulate ACTH release (20).

Dopamine did not show any significant effect on ACTH basal release at 0.3, 3, 30 ng/ml, but suppressed CRF-induced ACTH release. We previously reported (2) that dopamine at 10 ng/ml reduced ACTH release induced by median eminence extracts and that its suppressive effect was not dose-dependent. The discrepancy between our present and previous results may be ascribed to the difference in the CRF materials used.

Corticosterone at 10^{-7} and 10^{-6} M suppressed CRF-induced ACTH release. We previously reported that corticosterone at 10^{-6} M inhibited ACTH release induced by median eminence extracts or arginine vasopressin in a superfusion system (21). Vale *et al.* also reported that dexamethasone and corticosterone inhibited ACTH/β-endorphin secretion induced by synthetic ovine CRF *in vivo* and *in vitro* (6, 20). It is probable that glucocorticoids exert at least a part of their negative feedback effects directly on the anterior pituitary corticotropic cells.

The present results suggest that pituitary ACTH secretion is controlled principally by CRF and glucocorticoid. Our present and former results also suggest that ACTH secretion is modified by other substances such as vasopressin, angiotensin II and catecholamines at the pituitary level.

Acknowledgment. The authors are grateful to Dr. W. Vale and Dr. J. Rivier for their gift of synthetic ovine CRF. The ACTH standard for the ACTH assay was the hormone distribution program, NIAMDD National Pituitary Agency. We thank Miss. R. Akiyama for her competent technical assistance.

REFERENCES

1. Hashimoto, K., Yunoki, S., Hosogi, H., Takahara, J. and Ofuji, T.: The effect of biologically active peptides and neurotransmitter substances on ACTH release in pituitary cell cultures. *Acta Med. Okayama* **33**, 81-90, 1979.
2. Hashimoto, K., Yunoki, S., Takahara, J. and Ofuji, T.: ACTH release in pituitary cell cultures. Effect of neurogenic peptides and neurotransmitter substances on ACTH release induced by hypothalamic corticotropin releasing factor (CRF). *Endocrinol. Jpn.* **26**, 103-109, 1979.
3. Spiess, J., Rivier, J., Rivier, C. and Vale, W.: Primary structure of corticotropin releasing-factor from ovine hypothalamus. *Proc. Natl. Acad. Sci. USA.* **78**, 6517-6521, 1981.
4. Vale, W., Spiess, J., Rivier, C. and Rivier, J.: Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. *Science* **213**, 1394-1397, 1981.
5. Hashimoto, K., Takahara, J., Hosogi, H., Ofuji, N., Yasuhara, Y., Mori, M. and Ofuji, T.: *In vitro* assay for ACTH-releasing activity using ACTH radioimmunoassay : ACTH releasing activities by various drugs. *Folia Endcrinol. Jpn.* **52**, 114-124, 1976 (in Japanese).
6. Rivier, C., Brownstein, M., Spiess, J., Rivier, J. and Vale, W.: *In vivo* corticotropin-releasing factor-induced secretion of adrenocorticotropin, β-endorphin and corticosterone. *Endocrinology* **110**, 272-278, 1982.
7. Turkelson, C.M., Arimura, A., Culler, M.D., Fishback, J.B., Groot, K., Kanda, M., Luciano, M., Thomas, C.R., Chang, D., Chang, J.K. and Shimizu, M.: *In vivo* and *in vitro* release of ACTH by synthetic CRF. *Peptides* **2**, 425-429, 1981.
8. Fleisher, N. and Vale, W.: Inhibition of vasopressin-induced ACTH release from the pituitary by glucocorticoids in vitro. *Endocrinology* **83**, 1232-1236, 1968.
9. Vale, W. and Rivier, C.: Substances modulating the secretion of ACTH by cultured anterior pituitary cells. *Fed. Proc.* **36**, 2094-2099, 1977.
10. Giguere, V., Cote, J. and Labrie, F.: Characteristics of the α-adrenergic stimulation of adrenocorticotropin secretion by rat anterior pituitary cells. *Endocrinology* **109**, 757-762, 1981.
11. Kraicer, J. and Milligan, J.V.: Suppression of ACTH release from adenohypophysis by corticosterone. *Endocrinology* **87**, 371-376, 1970.
12. Sayers, G. and Portanova, R.: Secretion of ACTH by isolated anterior pituitary cells : Kinetics of stimulation by corticotropin-releasing factor and of inhibition by corticosterone. *Endocrinology* **94**, 1723-1730, 1974.
13. Yasuda, N., Greer, M.A., Greer, S.E. and Panton, P.: Studies on the site of vasopressin in inducing adrenocorticotropin secretion. *Endocrinology* **103**, 906-911, 1978.
14. Portanova, R. and Sayers, G.: Isolated pituitary cells : CRF-like activity of neurohypophyseal and related peptides. *Proc. Soc. Exp. Biol. Med.* **143**, 661-666, 1973.
15. Gillies, G.E., Linton, E.A. and Lowry, P.J.: Corticotropin releasing activity of the new CRF is potentiated several times by vasopressin. *Nature* **299**, 355-357, 1982.
16. Murakami, K., Hashimoto, K. and Ota, Z.: Interaction of synthetic ovine corticotropin releasing factor and arginine vasopressin on in vitro ACTH release by the anterior pituitary of rats. *Neuroendocrinology* **39**, 49-53, 1984.
17. Maran, W.M. and Yates, F.E.: Cortisol secretion during intrapituitary infusion of angiotensin II in conscious dogs. *Am. J. Physiol.* **233**, R 273-R 285, 1977.
18. Tilders, F.J.H., Berkenbosch, F. and Smelik, P.G.: Adrenergic mechanisms involved in the control of pituitary-adrenal activity in the rat.: A β-adrenergic stimulatory mechanism. *Endocrinology* **110**, 114-120, 1982.

19. Giguere, V., Cote, J. and Labrie, F.: Specific inhibition by glucocorticoids of theα_1-adrenergic stimulation of adrenocorticotropin release in rat anterior pituitary cells. *Endocrinology* **110**, 1225-1230, 1982.
20. Vale, W., Vaughan, J., Smith, M., Yamamoto, G., Rivier, J. and Rivier, C.: Effects of synthetic ovine corticotropin-releasing factor, glucocorticoid, catecholamines, neurohypophysial peptides, and other substances on cultured corticotropic cells. *Endocrinology* **113**, 1121-1131, 1983.
21. Murakami, K., Hashimoto, K. and Takahara, J.: Secretion pattern of ACTH by superfused rat anterior pituitary. *Hormone to Rinsho* **31**, 61-64, 1983 (in Japanese).