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Synergistic interaction of corticotropin releasing factor and arginine vasopressin on adrenocorticotropin and cortisol secretion in *Macaca fuscata*.

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Abstract

Plasma immunoreactive CRF measured by radioimmunoassay decreased rapidly after intravenous injection of synthetic ovine corticotropin releasing factor (CRF) and showed a bi-exponential decay curve in five *macaca fuscata*s. Half lives of plasma immunoreactive CRF were 5.8 ± 1.4 (Mean \pm SEM) min for the fast component and 38.3 ± 2.4 min for the slow component. A bolus injection of 5 micrograms/kg CRF significantly increased the plasma cortisol level. CRF at 5 micrograms/kg induced a delayed response of ACTH and cortisol. Arginine vasopressin (AVP) at 0.5 micrograms/kg induced a slight increase in plasma ACTH and cortisol, but AVP at 0.1 micrograms/kg evoked no significant increase. When 0.5 micrograms/kg CRF and 0.1 micrograms/kg AVP were administered simultaneously, significant ACTH and cortisol responses occurred. The results indicate that CRF and AVP act synergistically to stimulate ACTH secretion in vivo.

KEYWORDS: corticotropin releasing factor, arginine vasopressin, adrenocorticotropin, cortisol, *macaca fuscata*

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**SYNERGISTIC INTERACTION OF CORTICOTROPIN
RELEASING FACTOR AND ARGININE VASOPRESSIN
ON ADRENOCORTICOTROPIN AND CORTISOL
SECRETION IN *MACACA FUSCATA***

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Abstract. Plasma immunoreactive CRF measured by radioimmunoassay decreased rapidly after intravenous injection of synthetic ovine corticotropin releasing factor (CRF) and showed a bi-exponential decay curve in five *macaca fuscata*s. Half lives of plasma immunoreactive CRF were 5.8 ± 1.4 (Mean \pm SEM) min for the fast component and 38.3 ± 2.4 min for the slow component. A bolus injection of $5 \mu\text{g}/\text{kg}$ CRF significantly increased the plasma cortisol level. CRF at $5 \mu\text{g}/\text{kg}$ induced a delayed response of ACTH and cortisol. Arginine vasopressin (AVP) at $0.5 \mu\text{g}/\text{kg}$ induced a slight increase in plasma ACTH and cortisol, but AVP at $0.1 \mu\text{g}/\text{kg}$ evoked no significant increase. When $0.5 \mu\text{g}/\text{kg}$ CRF and $0.1 \mu\text{g}/\text{kg}$ AVP were administered simultaneously, significant ACTH and cortisol responses occurred. The results indicate that CRF and AVP act synergistically to stimulate ACTH secretion *in vivo*.

Key words : corticotropin releasing factor, arginine vasopressin, adrenocorticotropin, cortisol, *macaca fuscata*.

Corticotropin releasing factor (CRF) was isolated from ovine hypothalamus and its amino acid sequence was determined in 1981 (1, 2). Synthetic ovine CRF has been shown to stimulate ACTH secretion in sheep (3, 4), rats (5), cynomolgus mokeys (6), rhesus monkeys (7) and humans (8, 9).

Vasopressin, although unlikely to be the principal regulator of ACTH secretion, may play a physiological role in ACTH secretion. It not only acts directly at the pituitary level to increase ACTH release (10-12), but also has been reported to evoke the release of endogenous CRF (13) and to potentiate the action of CRF on the pituitary (14-17).

The present study was undertaken to investigate the effect of the simultaneous administration of synthetic CRF and arginine vasopressin (AVP) on plasma ACTH and cortisol in *macaca fuscata*.

MATERIALS AND METHODS

Experiments. In the first experiments five monkeys (7-9 kg) were anesthetized with ketamine HCl (30 mg/kg). One h later saline was infused into the forearm, and 15 min later the first blood sample was withdrawn and synthetic ovine CRF (5 μ g/kg) was given as a bolus injection. Blood samples were taken at 10, 20, 30, 45, 60, 90 and 120 min after the injection. Plasma was separated and stored for CRF and cortisol assay. Plasma from 5 monkeys was collected and pooled 10, 45 and 90 min after CRF injection and extracted with acetone-petroleum ether. Lyophilized extracts were resuspended in 0.1 N HCl and chromatographed on a Sephadex G-50 fine column (0.9 \times 60 cm) in 0.1 N HCl. Each 2 ml eluate was lyophilized for CRF radioimmunoassay. Several days later a similar experiment was carried out, but vehicle was injected instead of synthetic CRF. Several days later, CRF was injected again 4 h after 2 mg dexamethasone administration to examine the effect of dexamethasone on CRF-induced ACTH release. In these experiments ketamine HCl (15 mg/kg) was given to the monkeys 30 and 90 min after CRF or vehicle injection.

In the second experiments, the same monkeys were anesthetized with sodium pentobarbital (30 mg/kg) instead of ketamine HCl. CRF and AVP were administered simultaneously as a bolus injection and blood samples were taken at 30 min intervals. Plasma samples were stored for ACTH and cortisol radioimmunoassay.

Synthetic ovine CRF and AVP were purchased from Protein Research Foundation (Osaka) and stored in 0.01 N HCl-0.9% NaCl containing 0.1% bovine serum albumin. They were diluted with 0.9% NaCl just before use.

Hormone assays. Plasma CRF was extracted by acetone-petroleum ether extraction. Namely, one milliliter acetone was added to 0.5 ml plasma and mixed. After centrifugation the supernatant was transferred to another tube, and 2 ml petroleum ether was added and mixed. The upper layer was discarded and the lower layer was lyophilized for CRF radioimmunoassay. CRF was measured by radioimmunoassay using anti-ovine CRF serum prepared in our laboratory (18). Synthetic CRF (10 μ g) was serially diluted with hormone free serum, and then extracted along with samples and used for determination of standard curves.

Plasma ACTH was measured using ACTH assay kits (C.I.S., France) and plasma cortisol was measured using cortisol assay kits (SPAC Cortisol, Daiichi Isotope Inc.).

Statistical analysis was conducted using Duncan's multiple range test.

RESULTS

Disappearance of plasma immunoreactive CRF. Most CRF immunoreactivity in plasma collected at 10, 45 and 90 min coeluted with synthetic ovine CRF from the Sephadex G-50 fine column (Fig.1). Therefore, the measured immunoreactive CRF represented ovine CRF concentration in plasma.

Plasma immunoreactive CRF decreased rapidly after injection of ovine CRF and showed a bi-exponential decay curve in the five monkeys (Fig. 2). Half lives of plasma immunoreactive CRF were 5.8 ± 1.4 min (mean \pm SEM) for the fast component and 38.3 ± 2.4 min for the slow component.

Effect of CRF on cortisol secretion. Plasma cortisol levels increased from 29.6 ± 5.6 μ g/dl to 85.2 ± 12.0 μ g/dl 45 min after CRF administration (Fig. 3). In the vehicle-injected group, cortisol levels at 45 and 60 min seemed to be higher than basal levels, but the differences were not statistically significant. Two milli-

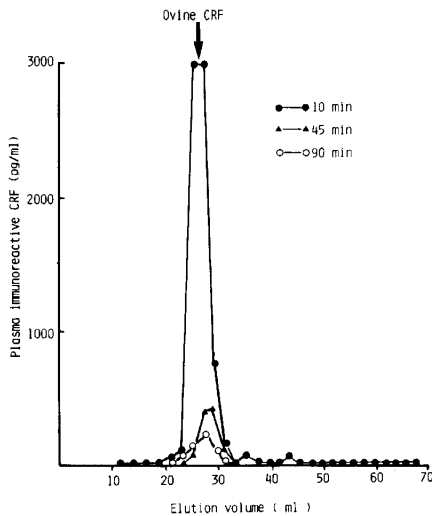


Fig. 1. Gel filtration pattern of plasma immunoreactive CRF on Sephadex G-50 fine column (0.9 \times 60 cm) in 0.1 N HCl. Plasma samples were collected and pooled 10, 45 and 90 min after an injection of synthetic ovine CRF in *macaca fuscata* and extracted.

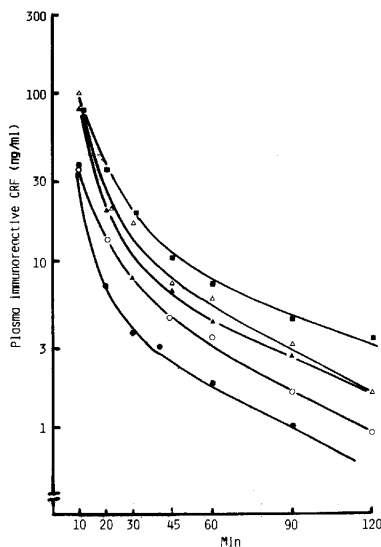


Fig. 2. Disappearance of plasma immunoreactive CRF after an injection of synthetic ovine CRF (5 μ g/kg) in *macaca fuscata*.

grams of dexamethasone administered 4 h before CRF was injected suppressed both the basal cortisol level and the CRF-induced ACTH response.

Effect of simultaneous CRF and AVP administration on ACTH and cortisol. In this experiment ACTH and cortisol changes were evaluated using percentage of the basal level due to individual variability. CRF at 0.5 μ g/kg induced delayed ACTH and cortisol responses in sodium pentobarbital anesthetized monkeys. The ACTH change was more significant than the cortisol change (Fig. 4, 5). AVP at 0.5 μ g/

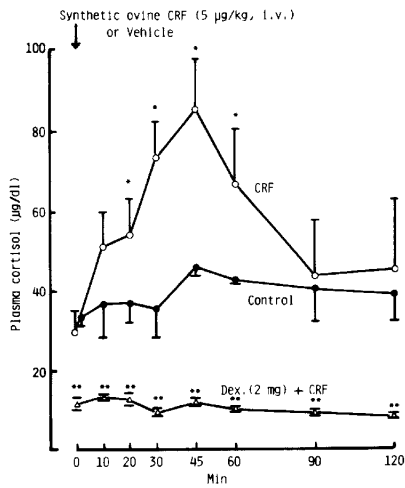


Fig. 3. Effect of synthetic ovine CRF and dexamethasone on plasma cortisol in *macaca fuscata*. Means \pm SEMs are given. * $p < 0.05$ vs control group, ** $p < 0.01$ vs CRF-injected group.

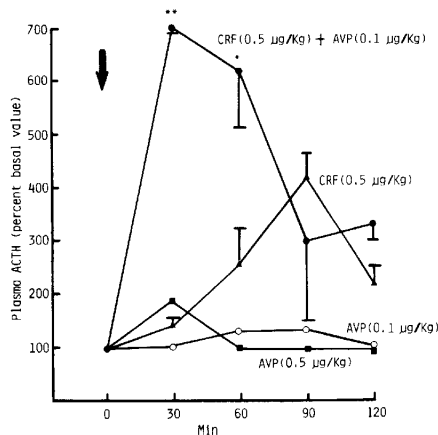


Fig. 4. Effect of synthetic ovine CRF and arginine vasopressin (AVP) on plasma ACTH in *macaca fuscata*. In AVP-injected groups (0.1 µg/kg or 0.5 µg/kg), plasma ACTH was measured in one case only due to the limited amount of plasma. The arrow shows the time of sample injection. Means and SEMs are given. Statistical analysis was carried out using the original values of plasma ACTH. * $p < 0.05$, ** $p < 0.01$, vs CRF (0.5 µg/kg) -injected group.

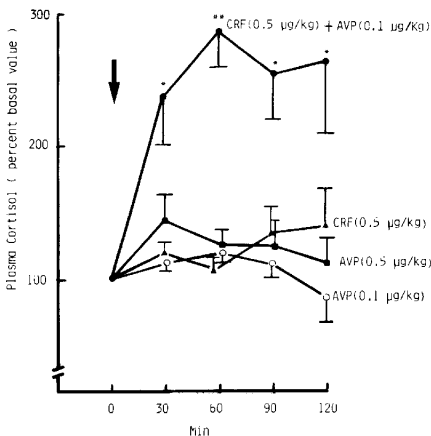


Fig. 5. Effect of synthetic ovine CRF and AVP on plasma cortisol in *macaca fuscata*. The arrow shows the time of sample injection. Means and SEMs are given. Statistical analysis was carried out using the original values of plasma cortisol. * $p < 0.05$, ** $p < 0.01$ vs CRF injected group.

kg induced a slight increase in plasma ACTH and cortisol 30 min after the injection. AVP at 0.1 $\mu\text{g}/\text{kg}$ did not evoke any significant increase in plasma ACTH and cortisol. When 0.5 $\mu\text{g}/\text{kg}$ CRF and 0.1 $\mu\text{g}/\text{kg}$ AVP were administered simultaneously, significant ACTH and cortisol responses occurred. Plasma ACTH increased from 41 ± 9 pg/ml to 290 ± 5 pg/ml 30 min after the injection, and plasma cortisol increased from 18 $\mu\text{g}/\text{dl}$ to 50 $\mu\text{g}/\text{dl}$ 60 min after the injection.

DISCUSSION

Both the fast and slow component half-lives of CRF were longer than those of TRH (19) and LH-RH (20). However, Schulte et al. (21) reported longer CRF half-lives than those in our experiment. They used 125 I-ovine CRF, and the discrepancy might be due to the difference in the methods used.

Control of ACTH secretion is considered to depend on a number of substances including CRF, glucocorticoids, vasopressin, oxytocin (17, 22), catecholamines (17, 23) and angiotensin II (24, 25). In this study synthetic ovine CRF at 5 $\mu\text{g}/\text{kg}$ significantly stimulated ACTH and cortisol secretion in *macaca fuscata*, but 0.5 $\mu\text{g}/\text{kg}$ seemed to be a little higher than the threshold dose to stimulate ACTH secretion. The *in vivo* ACTH releasing activity of synthetic ovine CRF in *macaca fuscata* coincides with those reported in rats (5), sheep (3, 4), cynomolgus monkeys (6) and human (9). Dexamethasone suppressed both the basal cortisol level and the CRF-induced cortisol response. Therefore, CRF and glucocorticoid are likely to be the principal regulators of ACTH secretion.

Exogenously administered vasopressin stimulates ACTH secretion by acting directly on the pituitary, causing the release of endogenous CRF and potentiating the action of CRF on the pituitary. The synergistic effect of synthetic CRF and vasopressin has been reported mainly in *in vitro* experiments (15, 17). We also reported the synergistic effect of CRF and AVP in an *in vitro* study and found that ACTH releasing activity of AVP in pituitary cell cultures was potentiated by preincubating the pituitary cells with synthetic CRF (26). AVP at 0.5 $\mu\text{g}/\text{kg}$ caused only a slight increase in ACTH and cortisol, and AVP at 0.1 $\mu\text{g}/\text{kg}$ seemed to be subthreshold for the stimulation of ACTH release in pentobarbital-anesthetized monkeys. Simultaneous administration of CRF and AVP at threshold concentrations significantly increased plasma ACTH and cortisol levels. Thus, marked synergistic interaction of CRF and AVP was confirmed in this *in vivo* experiment.

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