Acta Medica Okayama

Volume 43, *Issue* 3 1989 *Article* 4

JUNE 1989

Effect of acute ether or restraint stress on plasma corticotropin-releasing hormone, vasopressin and oxytocin levels in the rat.

Kozo Hashimoto* Kazuharu Murakami† Toshihiro Takao‡
Shinya Makino** Masanori Sugawara†† Zensuke Ota‡‡

*Okayama University,
†Okayama University,
‡Okayama University,
**Okayama University,
††Okayama University,
‡‡Okayama University,

Effect of acute ether or restraint stress on plasma corticotropin-releasing hormone, vasopressin and oxytocin levels in the rat.*

Kozo Hashimoto, Kazuharu Murakami, Toshihiro Takao, Shinya Makino, Masanori Sugawara, and Zensuke Ota

Abstract

Ether and restraint stress-induced peripheral plasma corticotropin releasing hormone (CRH), arginine vasopressin (AVP), oxytocin (OXY) and adrenocorticotropin (ACTH) levels were measured by radioimmunoassays. Plasma CRH, AVP, OXY and ACTH rose to approximately twice the level of control rats 2 min after the onset of a 1-min exposure to ether. Plasma CRH rose further 5 min after the onset of ether stress, while plasma AVP and OXY returned to the baseline levels at 5 min. Plasma CRH, OXY and ACTH showed significant elevation 2 min after the onset of restraint stress, while plasma AVP did not show a significant change. Plasma OXY and ACTH rose further 5 min after the onset of restraint stress, whereas plasma CRH returned to baseline levels. CRH and OXY concentrations in the hypothalamic median eminence decreased 5 min after the onset of ether exposure and restraint, while the AVP concentration did not differ from control levels. The results, including the discrepancy between plasma CRH and ACTH 5 min after stress, suggest that CRH in the peripheral plasma is derived from both hypothalamic and extrahypothalamic tissues. The levels of stress-induced CRH in the peripheral plasma were sufficient to stimulate ACTH release. These results suggest that ether and restraint stress elevate plasma CRH shortly after the onset of the stress, and that this elevation in the plasma CRH level is at least partly responsible for stress-induced ACTH secretion.

KEYWORDS: ether stress, restraint stress, corticotropin-releasing hormone, vasopressin, oxytocin

---

*PMID: 2548372 [PubMed - indexed for MEDLINE]

Acta Med Okayama 43 (3) 161-167 (1989)

Effect of Acute Ether or Restraint Stress on Plasma Corticotropin-Releasing Hormone, Vasopressin and Oxytocin Levels in the Rat

Kozo Hashimoto*, Kazuharu Murakami, Toshihiro Takao, Shinya Makino, Masanori Sugawara and Zensuke Ota

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

Ether and restraint stress-induced peripheral plasma corticotropin releasing hormone (CRH), arginine vasopressin (AVP), oxytocin (OXY) and adrenocorticotropin (ACTH) levels were measured by radioimmunoassays. Plasma CRH, AVP, OXY and ACTH rose to approximately twice the level of control rats 2 min after the onset of a 1-min exposure to ether. Plasma CRH rose further 5 min after the onset of ether stress, while plasma AVP and OXY returned to the baseline levels at 5 min. Plasma CRH, OXY and ACTH showed significant elevation 2 min after the onset of restraint stress, while plasma AVP did not show a significant change. Plasma OXY and ACTH rose further 5 min after the onset of restraint stress, whereas plasma CRH returned to baseline levels. CRH and OXY concentrations in the hypothalamic median eminence decreased 5 min after the onset of ether exposure and restraint, while the AVP concentration did not differ from control levels. The results, including the discrepancy between plasma CRH and ACTH 5 min after stress, suggest that CRH in the peripheral plasma is derived from both hypothalamic and extrahypothalamic tissues. The levels of stress-induced CRH in the peripheral plasma were sufficient to stimulate ACTH release. These results suggest that ether and restraint stress elevate plasma CRH shortly affer the onset of the stress, and that this elevation in the plasma CRH level is at least partly responsible for stress-induced ACTH secretion.

Key words : ether stress, restraint stress, corticotropin-releasing hormone, vasopressin, oxytocin

ACTH secretion is under multifactorial regulation, mainly by CRH, and partly by additional hypothalamic factors. Vasopressin, OXY, catecholamine and angiotensin II seem to play at least modulating roles in ACTH secretion (1, 2). It has been reported that CRH, vasopressin and catecholamines are involved in acute stress-induced ACTH secretion and that CRH has the most important role among these factors (3-5).

*To whom correspondence should be addressed.

Acute stress causes CRH secretion from the hypothalamic median eminence (ME) into the pituitary portal vessels to stimulate the pituitary-adrenocortical system. Vasopressin and OXY are secreted into the systemic circulation from the posterior pituitary, and they are also secreted into the hypophyseal portal vessels from the neurons which project from the paraventricular nucleus and supraoptic nucleus to the external layer of the median eminence. Both vasopressin and OXY have ACTH-releasing activity *in vitro* and *in vivo* (6-13). Stress-induced CRH and arginine vasopressin (AVP) changes in the rat portal plasma, and stress-induced changes in peripheral plasma AVP, OXY and ACTH have been reported (4, 14, 15). However, stress-induced CRH changes in peripheral plasma have not been reported. In the present study, we examined the acute stress-induced changes of CRH, AVP and OXY in rat peripheral plasma and hypothalamic tissue in relation to plasma ACTH changes in order to investigate the role of peripheral plasma CRH in stress-induced ACTH secretion.

Materials and Methods

Male Wistar rats, weighing approximately 250 g were used. They were maintained in an animal room (lights on 0600h to 1800h) at room temperature (20-26°C) with food and water *ad libitum*. The experiments were carried out several days after their arrival.

The rats were stressed by exposing them to ether vapor for 1 min in a jar, or immobilizing them in a wire mesh made of stainless steel and kept in a supine position for 2 or 5 min. The rats were decapitated 2 or 5 min after the onset of the stress. Control rats were decapitated without the stress. They were decapitated as soon as possible to minimize decapitation-induced changes in hormones. The experiments were carried out from 1300h to 1500h to avoid diurnal variation of hormones. Truncal blood was collected into chilled glass tubes. Then, plasma was divided into 3 samples and frozen at −20°C until hormone assays. One 2-ml plasma sample was used for CRH extraction; one 1-ml sample was used for AVP and OXY extraction, and the rest was used for the ACTH assay. The median eminence (ME) and the remaining tissue of the hypothalamus (rHy) were quickly dissected out into plastic tubes on dry ice. The blocks of the hypothalamic tissue were bordered by the optic chiasm and mammillary bodies rostrocaudally and by the anterior commissure dorsally.

Tissue and plasma extraction. ME and rHy were extracted by a previously reported method for CRH assay (16). Fifty and 10 μl out of 2 ml homogenates of ME and rHy were dried and stored for protein measurement, respectively. Tissue protein was measured using a protein assay kit (Bio-Rad Laboratories, Richmond, CA, USA). The rest of the homogenate was centrifuged at 10,000 × *g* at 4°C for 10 min. The supernatant was frozen and stored until CRH, AVP and OXY assays.

Plasma CRH was extracted using disposable SEP-PAK C18 cartridges (Waters Associates, USA). Two milliliters of plasma was mixed with 8 ml of acid-saline (containing 0.1 N HCl), and the mixture was applied to the column. The column was washed twice with 10 ml of 0.1 N HCl and eluted with 4 ml of a mixture of 80% CH_3CN-20% 0.2 N HCl. The eluate was evaporated to dryness under N_2 gas at 40°C. The dried eluate was resuspended in CRH assay buffer (0.02 M phosphate buffer containing 0.15 M NaCl, 0.5% bovine serum albumin (BSA), 1 mM ascorbic acid and 25 mM EDTA, pH 7.4) prior to assay. Plasma AVP and OXY were extracted using the acid acetone-petroleum ether method. Namely, 2 ml of acetone was added to 1 ml of plasma, and the mixture was centrifuged. The supernatant was transferred to another glass tube, and 3 ml of petroleum ether was added. The mixture was centrifuged, and the lower layer was transferred to another tube and evaporated to dryness. The extracts were resuspended in 1 ml of AVP and OXY assay buffer (0.02 M phosphate buffer containing 0.15 M NaCl, 0.1% BSA and 0.1% NaN_3, pH 7.4). Duplicate 200 μl samples were used for the AVP or OXY radioimmunoassay. Synthetic AVP and OXY were diluted with hormone free rat plasma to make standard plasma for AVP and OXY radioimmunoassays and they were extracted along with the samples.

Radioimmunoassay. Plasma ACTH levels in

100 μl samples were measured using by radioimmunoassay kits (CEA-IRE-SORIN, France). Tissue and plasma AVP levels were radioimmunoassayed by a previously reported method (17). Tissue CRH was assayed according to a CRH radioimmunoassay method previously reported (16). Plasma CRH was assayed by a similar method except that anti-rat CRH serum given by Mitsubishi Petrochemical Co., Ltd., Tokyo, Japan was used. The sensitivity of the assay was 4 pg/ml plasma. The antiserum recognizes the C-terminal portion of the CRH (1-41) molecule and does not cross react with other neuropeptides (18). The recovery rate was 87% and 113.3% for 10 and 100 pg/ml plasma, respectively. The intra-assay coefficient of variation was 26.5% for 8.7 pg/ml

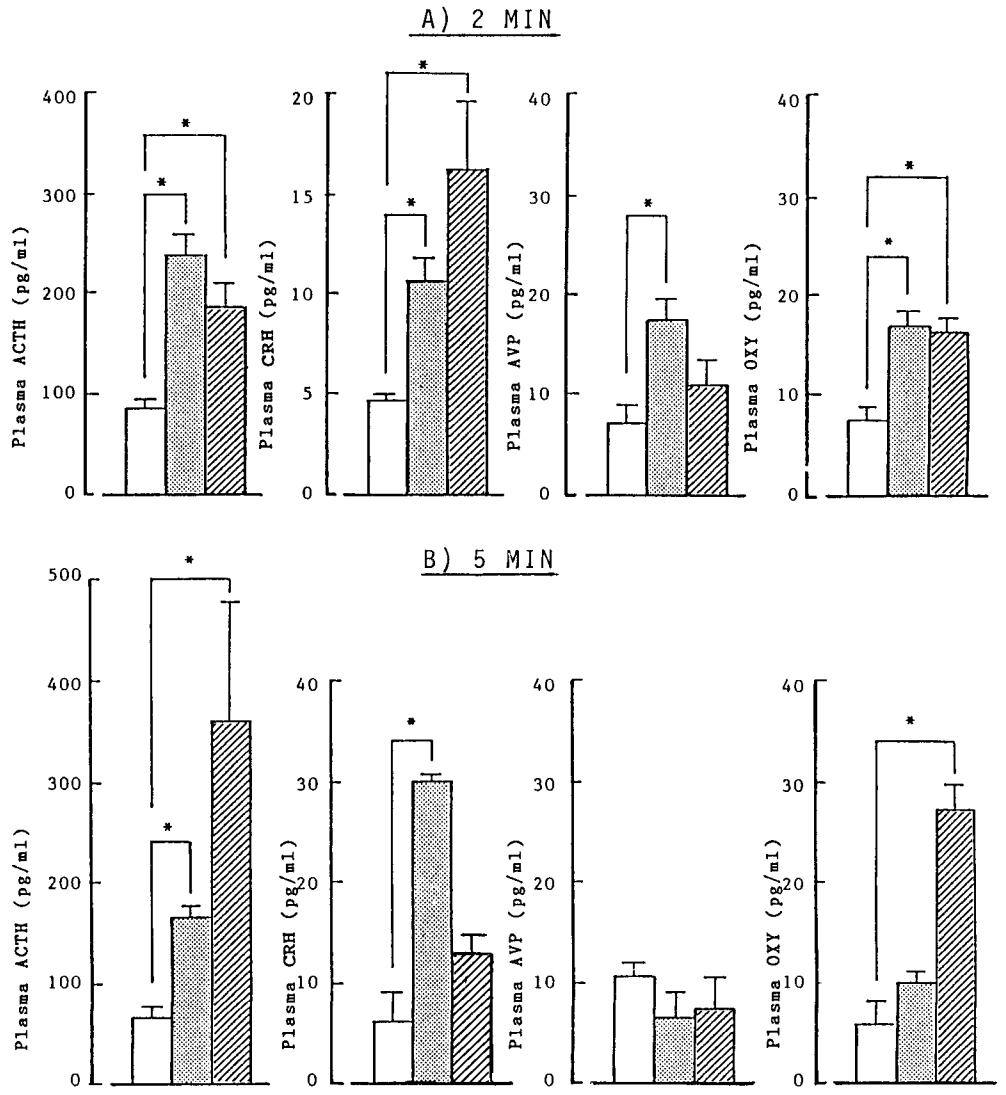

Fig. 1 A) Plasma adrenocorticotropic hormone (ACTH), corticotropin-releasing hormone (CRH), arginine vasopressin (AVP) and oxytocin (OXY) levels 2 min after the onset of 1-min ether (▨, n = 6) and restraint (▧, n = 6) stress. B) Plasma ACTH, CRH, AVP and OXY levels 5 min after the onset of 1-min ether (n = 5) and restraint (n = 5) stress. The blood samplings were carried out by decapitation. The control rats (□, n = 6 in experiment A; n = 5 in experiment B) were decapitated without these stresses. The data are expressed as the mean ± SEM. *, $p < 0.01$.

and 19.1% for 113.3 pg/ml. Plasma OXY was assayed using anti-OXY serum provided by Dr. M. Morris (19). The sensitivity was 1 pg/ml plasma. Iodination of OXY and subsequent radioimmunoassay were carried out by the same procedure used for the AVP radioimmunoassay (17). The intra-assay coefficient of variations for plasma AVP and OXY assays were 10.4% (17) and 8.4%, respectively.

Statistics. Statistical analyses were conducted by a multiple range test after analysis of

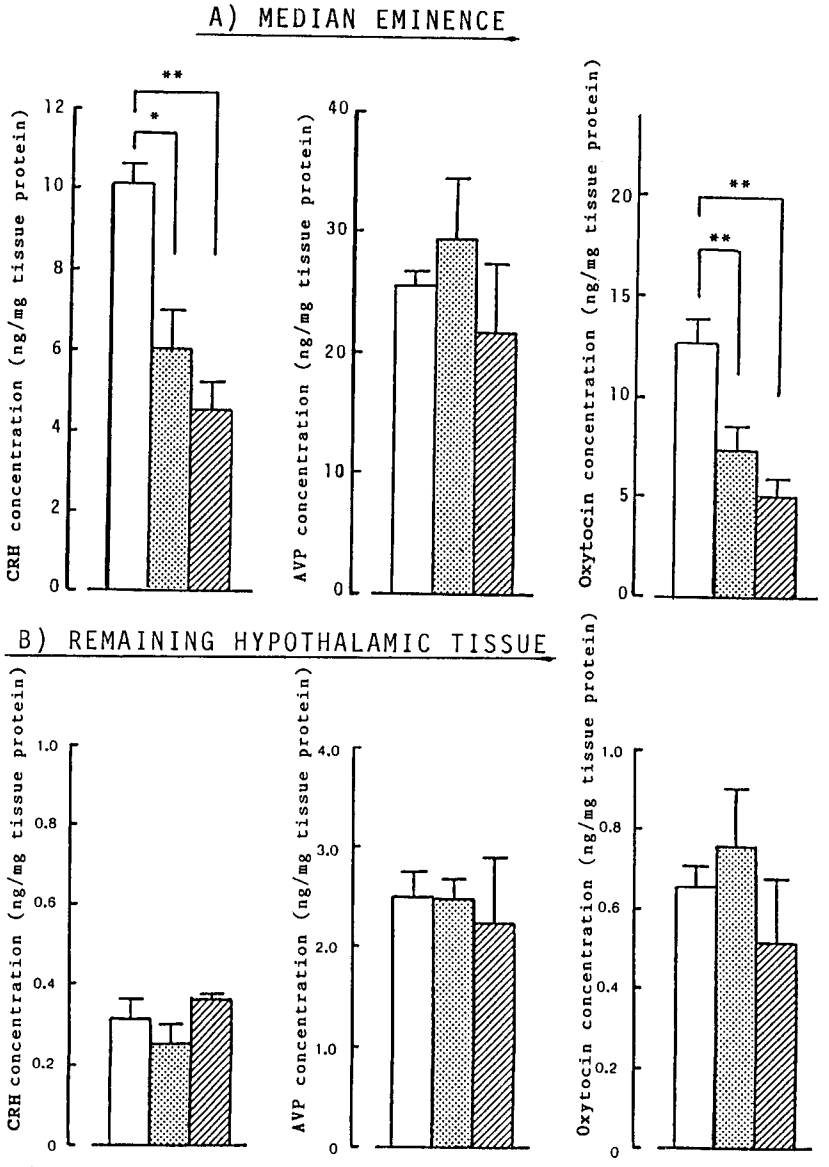

Fig. 2 Corticotropin-releasing hormone (CRH), arginine vasopressin (AVP) and oxytocin (OXY) concentrations in the median eminence (A) and the remaining tissue of the hypothalamus (B) 5 min after the onset of 1-min ether (▨, n = 5) and restraint (▧, n = 5) stress. The control rats (□, n = 5) were sacrificed without these stresses. The data are expressed as the mean ± SEM. *, $p < 0.05$; **, $p < 0.01$.

variance.

Results

Effect of ether and restraint stress on plasma CRH, AVP, OXY and ACTH. In ether-stressed rats, plasma CRH, AVP, OXY and ACTH rose approximately 230-280% (mean CRH level, 10.5 pg/ml ; AVP, 17.3 pg/ml ; OXY, 16.8 pg/ml ; ACTH, 237.5 pg/ml) of the control rats' levels 2 min after the onset of a 1-min exposure to ether (Fig. 1A). In immobilized rats, plasma CRH, OXY and ACTH showed significant elevation (CRH, 16.3 pg/ml ; OXY, 16.2 pg/ml ; ACTH, 185.6 pg/ml) 2 min after the onset of the stress, while plasma AVP (11.1 pg/ml) did not show a significant change. Plasma ACTH also showed a significant elevation (165.8 pg/ml) 5 min after the onset of ether stress (Fig. 1B). Plasma CRH elevated further at 5 min after the onset of ether stress (29.8 pg/ml, 476.8% of the control rats' levels) compared with the levels at 2 min, while plasma AVP and OXY did not show a significant elevation at 5 min. Five min after the onset of restraint stress, plasma ACTH showed a greater elevation (359.4 pg/ml, 529.4% of the control rats' levels) than that at 2 min. Plasma OXY rose further (27.5 pg/ml, 424.3% of the control rats' levels) at 5 min compared with the levels at 2 min, while the plasma CRH and AVP levels were not significantly different from the control levels.

CRH, AVP and OXY concentrations in ME and rHy. CRH, AVP and OXY concentrations in the median eminence 2 min after the onset of a 1-min exposure to ether or restraint were not different from those in the control rats. CRH and OXY concentrations decreased 5 min after the onset of a 1-min exposure to ether or restraint, while the AVP concentration did not differ from the control levels (Fig. 2A). In the remaining tissue of the hypothalamus, CRH, AVP and OXY did not show significant changes either 2 or 5 min (Fig. 2B) after the onset of ether or restraint stress.

Discussion

CRH levels in portal and peripheral blood of rats under ether or restraint stress have not been reported, even though the prime importance of CRH in stress-induced ACTH release has been demonstrated from the fact that anti-CRH serum inhibited ether and restraint stress-induced ACTH release (20, 21). In the present study, plasma ACTH, CRH, AVP and OXY levels rose 2 min after the onset of ether stress. CRH levels in peripheral blood were also elevated 5 min after the onset of the stress, but AVP and OXY levels returned to control levels. Plasma ACTH, CRH and OXY rose 2 min after the onset of restraint stress. Five min after onset of the stress, CRH levels were not different from the control levels, but ACTH and OXY levels rose higher. Plasma AVP did not show any significant change. CRH and OXY concentrations in the ME decreased 5 min after exposure to ether or immobilization stress. It has been reported that CRH in the ME decreased shortly after stress and adrenalectomy when CRH levels in the portal blood were supposed to be elevated (22, 23). However, ME depletion of these peptides is difficult to interpret as CRH secretory rates seem to be small as determined from portal blood collection experiments (24) and from *in vitro* hypothalamic fragment studies (25). Yokoe *et al.* (26) reported recently that CRH levels in the peripheral plasma and hypothalamus vary in parallel with changes in the pituitary-adrenal axis. The depletion of CRH and OXY in the ME at 5 min suggests

that the release of CRH and OXY into the portal blood increased shortly after the onset of the stress.

In our experiments, we found continued secretion of CRH 5 min after the onset of the ether stress when plasma ACTH levels appeared to have stabilized. However, 5 min after the onset of the restraint stress, plasma ACTH continued to rise, while CRH levels had returned to the baseline level. It is difficult to explain this discrepancy between CRH and ACTH. CRH in portal blood is secreted from the median eminence, while sources of peripheral plasma CRH are not only the hypothalamus but also extrahypothalamic tissue (27). The dissociation between plasma CRH and ACTH after stress might indicate that CRH in plasma is derived from both hypothalamic and extrahypothalamic tissues.

Stressed levels of peripheral plasma CRH (around 15-30 pg/ml) are sufficient to stimulate ACTH release *in vitro* (12). Therefore, stress-induced peripheral plasma CRH levels might be at least partly responsible for plasma ACTH elevation, although ACTH secretion is mainly stimulated by CRH in portal blood. Peripheral AVP and OXY levels after these stresses are not sufficient to stimulate ACTH secretion. However, it is possible that AVP and OXY were also elevated in portal plasma after ether stress, and OXY was elevated in portal plasma after restraint stress, and these elevations were enough to stimulate ACTH secretion directly or by potentiating CRH-induced ACTH secretion.

Dissociation of CRH, vasopressin and OXY responses to different types of stress has been reported. Plotsky *et al.* (4) reported that hemorrhagic stress resulted in increased secretion of CRH as well as AVP and OXY into portal vessels. However, in hypoglycemic stress, increased secretion of ACTH was not associated with a change in CRH secretion, but rather with increased release of AVP into the portal blood (28). Gibbs (15) reported that ACTH, AVP and OXY levels were reduced in peripheral plasma of rats stressed with hypothermia compared with euthermic controls and that hypothalamic secretions of OXY and AVP into portal blood were also inhibited in hypothermic rats, while that of CRH was unchanged. OXY secretion was shown to be enhanced by certain stresses, such as restraint, swimming and ether stress (14, 29). On the other hand, AVP was stimulated by ether, but not by restraint or swimming. ACTH levels were increased by all these stresses. The present results confirm these reports.

In conclusion, the present study shows that ether stress elevates plasma CRH, AVP and OXY levels shortly after the onset of stress, and that restraint stress elevates plasma CRH and OXY levels. The results suggest that the stress-induced elevation in peripheral plasma CRH is at least partly responsible for stress-induced ACTH secretion.

Acknowledgments. We thank Miss Naomi Yano and Mr. Makoto Aoi for their technical assistance. We are grateful to Dr. Mariana Morris and Mitsubishi Yuka Petrochemical Co., Ltd. for providing anti-OXY serum and anti-CRH serum, respectively.

References

1. Vale W, Vaugham J, Smith M, Yamamoto G, Rivier J and Rivier C: Effects of synthetic ovine corticotropin-releasing factor, glucocorticoids, catecholamines, neurohypophysial peptides, and other substances on cultured corticotrophic cells. Endocrinology (1983) **113**, 1121-1131.
2. Murakami K, Hashimoto K and Ota Z: Effect of angiotensin II, catecholamines and glucocorticoid on corticotropin releasing factor (CRF)-induced ACTH release in pituitary cell cultures. Acta Med Okayama (1984) **38**, 349-355.
3. Antoni FA: Hypothalamic control of adrenocorticotropic hormone secretion: advances since the discovery of 41-residue corticotropin-releasing factor.

Endocr Rev (1986) **7**, 351-378.
4. Plotsky PM, Bruhn TD and Vale W: Evidence for multifactor regulation of the adrenocorticotropin secretory response to hemodynamic stimuli. Endocrinology (1985) **116**, 633-639.
5. Makara GB: Mechanism by which stressful stimuli activate the pituitary-adrenal system. Fed Proc (1985) **44**, 149-153.
6. Pearlmutter AF, Rapino E and Saffran M: Semi-automated *in vitro* assay for CRF: Activities of peptides related to oxytocin and vasopressin. Neuroendocrinology (1974) **15**, 106-119.
7. Gillies G, Greidanus VW and Lowry PJ: Characterization of rat stalk median eminence vasopressin and its involvement in adrenocorticotropin release. Endocrinology (1978) **103**, 528-534.
8. Yasuda N, Greer MA, Greer SE and Panton P: Studies on the site of action of vasopressin inducing adrenocorticotropin secretion. Endocrinology (1978) **103**, 906-911.
9. Beny JL and Baertschi AJ: Oxytocin: Major corticotropin-releasing factor secreted from diabetes insipidus rat posterior pituitary *in vitro*. Neuroendocrinology (1980) **31**, 261-264.
10. Antoni FA, Holmes MC and Jones MT: Oxytocin as well as vasopressin potentiate ovine CRF in heterozygous and homozygous Brattleboro rats. Peptides (1983) **4**, 411-415.
11. Gibbs DM, Vale W, Rivier J and Yen SSC: Oxytocin potentiates the ACTH-releasing activity of CRF (41) but not vasopressin. Life Sci (1984) **34**, 2245-2249.
12. 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 (1984) **39**, 49-53.
13. Gibbs DM: High concentrations of oxytocin in hypophysial portal plasma. Endocrinology (1984) **114**, 1216-1218.
14. Gibbs DM: Measurement of hypothalamic corticotropin-releasing factors in hypophyseal portal blood. Fed Proc (1985) **44**, 203-206.
15. Gibbss DM: Inhibition of corticotropin release during hypothermia: The role of corticotropin-releasing factor, vasopressin and oxytocin. Endocrinology (1985) **116**, 723-727.
16. Hashimoto K, Hattori T, Murakami K, Suemaru S, Kawada Y, Kageyama J and Ota Z: Reduction in brain immunoreactive corticotropin-releasing factor (CRF) in spontaneously hypertensive rats. Life Sci (1985) **36**, 643-647.
17. Hashimoto K, Yunoki S, Kageyama J, Ohno N, Takahara T and Ofuji T: Vasopressin and CRF-ACTH in adrenalectomized and dexamethasone-treated rats. Neuroendocrinology (1981) **32**, 87-91.
18. Takao T, Hashimoto H and Ota Z: Effect of atrial natriuretic peptide on acetylcholine-induced release of corticotropin-releasing factor from rat hypothalamus *in vitro*. Life Sci (1988) **42**, 1199-1203.
19. Morris M, Stevens SW and Adams MR: Plasma oxytocin during pregnancy and lactation in the cynomolgus monkey. Biol Reprod (1980) **23**, 782-787.
20. Rivier C and Vale W: Modulation of stress-induced ACTH release by corticotropin-releasing factor, catecholamines and vasopressin. Nature (1983) **305**, 325-327.
21. Linton EA, Tilders FJH, Hodgkinson S, Berkenbosch F, Vwemes I and Lowry PJ: Stress-induced secretion of adrenocorticotropin in rats is inhibited by administration of antisera to ovine corticotropin-releasing factor and vasopressin. Endocrinology (1985) **116**, 966-970.
22. Suemaru S, Hashimoto K and Ota Z: Brain corticotropin-releasing factor (CRF) and catecholamine responses in acutely stressed rats. Endocrinol Jpn (1985) **32**, 709-718.
23. Suda T, Tomori N, Tozawa F, Mouri T, Demura H and Shizume K: Effects of bilateral adrenalectomy on immunoreactive corticotropin-releasing factor in the rat median eminence and intermediate-posterior pituitary. Endocrinology (1983) **113**, 1182-1184.
24. Plotsky PM, Otto S and Sapolsky RM: Inhibition of immunoreactive corticotropin-releasing factor secretion into the hypophysial-portal circulation by delayed glucocorticoid feedback. Endocrinology (1986) **119**, 1126-1130.
25. Suda T, Yajima F, Tomori N, Sumitomo T, Nakagami Y, Ushiyama T, Demura H and Shizume K: Inhibitory effect of adrenocorticotropin on corticotropin-releasing factor release from rat hypothalamus *in vitro*. Endocrinology (1986) **118**, 459-461.
26. Yokoe T, Audhya T, Brown C, Hutchinson B, Passarelli J and Hollander CS: Corticotropin-releasing factor levels in the peripheral plasma and hypothalamus of the rat vary in parallel with changes in the pituitary-adrenal axis. Endocrinology (1988) **123**, 1348-1354.
27. Sasaki A, Sato S, Murakami O, Go M, Inoue M, Shimizu Y, Hanew K, Andon N, Sato I, Sasano N and Yoshinaga K: Immunoreactive corticotropin-releasing hormone present in human plasma may be derived from both hypothalamic and extrahypothalamic sources. J Clin Endocrinol Metab(1987) **65**, 176-182.
28. Bruhn TD and Vale W: Hypophysiotropic regulation of adrenocorticotropin secretion in response to insulin-induced hypoglycemia. Endocrinology (1985) **117**, 323-329.
29. Lang RE, Heil JWE, Ganten D, Hermann K, Unger T and Rascher W: Oxytocin unlike vasopressin is a stress hormone in the rat. Neuroendocrinology (1983) **37**, 314-316.

Received December 26, 1988; accepted March 7, 1989