Acta Medica Okayama

Volume 35, *Issue* 1 1981 *Article* 4

FEBRUARY 1981

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Abstract

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KEYWORDS: corticotropin releasing factor (CRF), arginine vasopressin (AVP), hypothalamic nucler.

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*PMID: 6266205 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 35, (1), 37—43 (1981)

A DIFFERENT DISTRIBUTION OF CORTICOTROPIN RELEASING FACTOR AND ARGININE VASOPRESSIN CONTENTS IN THE HYPOTHALAMIC NUCLEI AFTER ESTROGEN ADMINISTRATION

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Received November 18, 1980

Abstract. The distribution of corticotropin releasing factor (CRF) and arginine vasopressin (AVP) in hypothalamic nuclei were examined in control and estrogen-treated female rats. CRF activity was measured using monolayer cultured rat anterior pituitary cells and AVP by radioimmunoassay. Hypothalamic nuclei were punched out according to the method of Palkovits. The distribution of CRF activity in 5 different hypothalamic nuclei was similar to that of AVP in intact female rats. CRF activity in hypothalamic nuclei, pituitary ACTH content and plasma ACTH levels in estrogen-treated rats were not significantly different from those in control rats. However, significant elevation of AVP content was observed in the supraoptic and paraventricular nuclei of estrogen-treated rats. These results indicate that CRF and AVP are distributed in similar hypothalamic nuclei, but that they are not identical.

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Recently, there is a revival of the idea that vasopressin is a corticotropin releasing factor (CRF)-like substance, although many investigators have suggested that vasopressin differs from authentic corticotropin releasing hormone (1-3). Vasopressin has been found in the external layer of the median eminence (4, 5), the portal vessel (6), and the anterior pituitary (7), and it is assumed that vasopressin has an important role in ACTH secretion (5, 7). Beny and Baertschi (8) reported that vasopressin is the predominant CRF released from neurohypophyseal axons. Gillies *et al.* (9), suggested that hypothalamic CRF might have a vasopressin-like structure. They also proposed that CRF is vasopressin modulated by other hypothalamic factor(s) released into the hypothalamo-hypophyseal portal system (10, 11). In this investigation we compare CRF content with vasopressin content in the hypothalamic nuclei of control and estrogen-treated rats.

It has been reported that estrogen not only regulates gonadal function but also modifies the pituitary-adrenal and hypothalamo-neurohypophyseal function. However, there have been controversies among the reports regarding the ef-

fects of estrogen on ACTH and vasopressin secretion. In this paper we also discuss the effects of estrogen on the CRF-ACTH and hypothalamo-neurohypophyseal systems.

MATERIALS AND METHODS

Estrogen administration. Adult female Wistar rats weighing approximately 200 g were provided with food and water *ad libitum*. Rats were injected daily with estradiol benzoate (100 μg/rat, Teikoku Zoki Co., Tokyo) for 2 weeks. Control rats were injected with 0.1 ml saline for 2 weeks. All animals were sacrificed by decapitation in the morning.

Punch-out method for hypothalamic nuclei. Brains were immediately removed after decapitation and frozen on dry-ice. The anterior and posterior pituitaries were separated, weighed and frozen at −40°C. Hypothalamic nuclei were punched out according to the method of Palkovits (12) with slight modifications. Frozen serial sections were cut alternately at 100 and 400 μm in the frontal plane by an electrofreezing micrtome. The 100 μm sections were stained with toluidin blue and photographs were taken for identification of hypothalamic nuclei. The hypothalamic nuclei identified by photography were removed from 400 μm sections with stainless steel needles, having an inside diameter of 400 - 1100 μm, according to the size of nuclei. Each tissue sample (25-180 μg protein equivalent) was homogenized in 1.0 ml of 0.2 N-HCl by ultrasound. An aliquot (0.1 ml) was taken for protein determination by the micromethod of Lowry *et al.* (13), and the remainder of the homogenate was centrifuged at 10,000 rpm for 10 min at 4°C and 0.6 and 0.1 ml aliquots were lyophilized for CRF and arginine vasopressin (AVP) assay, respectively.

CRF bioassay. CRF-bioassay was performed with cultured rat anterior pituitary cells as described previously (14). Briefly, the rat anterior pituitaries were removed, dispersed with Hank's buffer containing collagenase and bovine serum albumin (BSA) at 37°C for 45 min. The dispersed cells were washed 4 times with sterile Dulbecco's modified Eagle Medium (DMEM) containing horse serum, fetal calf serum, non-essential amino acids, penicillin, streptomycin and fungisone. The cells were resuspended in an appropriate volume of medium, placed in sterile plastic Petri dishes, and cultured at 37°C in an incubator with 95% air and 5% CO_2. After 4 days of culture the cells were washed twice with fresh DMEM and used for experiments. Lyophilized tissue extracts were reconstituted with DMEM and pooled. Pooled extracts equivalent to 0.5 hypothalamic nuclei were added to cultured pituitary cells in triplicate. Incubation was carried out in a total volume of 2.0 ml/dish for 4 h. Trasylol (1000 U/0.1 ml) was added to each tube to prevent possible breakdown of CRF and ACTH. Sample was also incubated in dishes without cells to determine the amount of ACTH present in sample, which was later subtracted from the total ACTH amount. CRF activity was expressed by net ACTH released by sample.

AVP and ACTH radioimmunoassay (RIA). AVP was radioimmunoassayed using anti-AVP serum raised in rabbits by multiple intradermal injections of synthetic AVP-albumin conjugate. The antiserum was highly specific for AVP, the cross-reaction with oxytocin and lysine-vasopressin being less than 0.01% and 0.66%, respectively (15). Synthetic AVP for iodination and the reference preparation were purchased from Sigma Chemical Co. (Grade VI, 1 IU = 2.725 μg). Plasma AVP was extracted from 0.5 ml plasma with 1 ml acetone, washed with 2 ml petroleum ether and evaporated to dryness.

Just prior to RIA, samples were reconstituted with 500 μl RIA buffer (0.01 M phosphate buffer, 0.15 M NaCl, 0.1% NaN_3, 0.05% BSA; pH 7.2) and duplicate 200 μl samples were employed for RIA. The sensitivity of the assay was 0.5 pg/tube for tissue AVP and 2 pg/ml for plasma AVP assay. The intra-assay coefficient of variations was 10.4%. The amounts of ACTH were determined by RIA with anti-porcine ACTH serum which reacted specifically with ACTH (16). Plasma ACTH was measured by more sensitive RIA with anti-ACTH serum provided from NIAMDD (Baltimore, USA). The sensitivity of the assay was 25 pg/tube for tissue and medium ACTH assay and 20 pg/ml for plasma ACTH assay. The intra-assay coefficient of variations averaged 8.4%.

Statistical analysis was conducted with Duncan's new multiple range test (17).

RESULTS

Effect of estrogen on AVP and CRF contents in hypothalamic nuclei. In saline injected control rats, the highest concentration of AVP was found in the median eminence (ME), with appreciable amounts in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) (Table 1). In the estrogen-treated group, significant elevation of AVP content was observed in SON and PVN, whereas the AVP contents in the median eminence, suprachiasmatic nucleus and arcuate nucleus were not significantly changed.

TABLE 1. EFFECT OF ESTRADIOL-TREATMENT (14 DAYS) ON AVP CONTENT IN HYPOTHALAMIC NUCLEI

Treatment	AVP content (ng/0.5 nucleus)				
	Median eminence	Supraoptic nucleus	Paraventricular nucleus	Suprachiasmatic nucleus	Arcuate nucleus
Control	3.17±0.36	1.99±0.52	0.78±0.29	0.32±0.07	0.47±0.28
(N)	(6)	(7)	(5)	(5)	(4)
Estradiol	2.98±0.12	4.16±0.49**	2.66±0.56*	0.41±0.08	0.48 + 0.08
(N)	(8)	(8)	(7)	(8)	(7)

Means ± S.E.M. are indicated. *P<0.05; **P<0.01 v.s. control group

In control rats, the highest CRF content was found in the median eminence with appreciable contents in SON and PVN (Table 2). CRF contents in these hypothalamic nuclei were not significantly changed by estrogen administration, a finding which was quite different from AVP changes.

Effect of estrogen on AVP and ACTH in pituitary and peripheral blood. In estrogen-treated rats, anterior pituitaries were greatly enlarged and the weights (19.3 ±1.7 mg) were approximately double those of control rats (10.1 ±0.7 mg). The ACTH content in the anterior pituitary was not affected by estrogen administration but its concentration in estrogen-treated rats (30.2 ±2.0 ng/mg wet tissue) was lower than that in control rats (61.5 ±6.5 ng/ml wet tissue) (Table 3). Mean plasma ACTH levels in estrogen-treated rats were lower than those in control rats, but not statistically significant. Plasma AVP levels in these two groups were not significantly different.

TABLE 2. EFFECT OF ESTRADIOL-TREATMENT (14 DAYS) ON CRF ACTIVITY IN HYPOTHALAMIC NUCLEI

Treatment	CRF activity Net ACTH release (ng/dish/0.5 nucleus)				
	Median eminence	Supraoptic nucleus	Paraventricular nucleus	Suprachiasmatic nucleus	Arcuate nucleus
Control	5.08±0.50	3.41±0.63	3.92±0.48	1.53±0.32	0.64±0.60
(N)	(6)	(7)	(6)	(7)	(5)
Estradiol	6.60±1.10	2.66±0.50	4.69±0.21	1.23±0.27	1.07±0.31
(N)	(8)	(8)	(8)	(8)	(8)

Means ± S.E.M. are indicated.

TABLE 3. EFFECT OF ESTRADIOL-TREATMENT (14 DAYS) ON ACTH AND AVP LEVELS IN PITUITARY AND PERIPHERAL PLASMA

Treatment (N)	Pituitary ACTH (ng/anterior pituitary)	Plasma ACTH (pg/ml)	Pituitary AVP (ng/posterior pituitary	Plasma AVP (pg/ml)
Control (7)	595±48	143±28	661±52	20.5±2.6
Estradiol (8)	617±38	87±26	556±19	22.8±2.1

Mean ± S.E.M. are indicated.

DISCUSSION

It was previously considered that CRF accounted for the enhanced "Gomori-positive" granules after adrenalectomy (18), and the changes of "CRF-granules" were examined by immunohistochemical method compared with "classical" neurosecretory material in the rat neurohypophysis (19 - 21). However, more recent studies suggested that neurophysin and vasopressin may be partly or totally responsible for the "CRF-granules" (6, 22).

There has been no report measuring both CRF activity and AVP content in the same sample from several hypothalamic nuclei by bioassay and radioimmunoassay, respectively. Lang *et al.* (23) measured CRF activity in rat hypothalamic nuclei using acutely dispersed pituitary cells and found that its distribution was similar to the AVP distribution reported by George and Jacobovitz (24). Our results also showed that the distribution of CRF activity in hypothalamic nuclei was similar to AVP distribution in control rats except in PVN where the CRF content was relatively high. The results seem to indicate that CRF is identical with AVP. However, the distributions of CRF and AVP contents were not identical in estrogen-treated rats. We observed that 1 - 10 ng of AVP showed CRF activity in monolayer cultured anterior pituitary cells but its CRF activity accounted for only a part of total median eminence CRF activity (25). These results indicate that CRF and AVP are distributed in similar places, but that they are not identical.

Coyne and Kitay (26) reported that replacement doses of estradiol increased the responsiveness of rat corticotrophs to median eminence CRF and pituitary ACTH synthesis. They also observed that estradiol administration resulted in a significantly decreased hypothalamic CRF content. In the present study, we found that excessive estrogen evoked no significant change in plasma ACTH levels, pituitary ACTH content and CRF content in several hypothalamic nuclei. The discrepancy between our results and those of Coyne and Kittay may be ascribed to the difference in estrogen dosage used. In our experiments, estrogen was given daily to intact rats for 14 days, but in their experiments it was injected in the form of a single injection to ovariectomized rats.

Our results showed that vasopressin contents in SON and PVN were significantly elevated in estrogen-treated rats, whereas no significant changes were observed in other hypothalamic nuclei. From the findings of the present study alone, we can not decide whether the increase of AVP in SON and PVN was caused by direct action of estrogen or via some other mechanism. The increase in AVP content may only indicate the accumulation of AVP in the perikaryon of AVP neurons, because the posterior pituitaries were oppressed by tumor-like enlarged anterior pituitary. Pituitary AVP contents and plasma AVP levels in estrogen-treated rats were not significantly different from those in control rats in spite of the high tissue levels of AVP. Skowsky *et al.* (27) reported that estrogen administration produced an increase in plasma vasopressin levels of rats. However, Yamaguchi *et al.* (28) observed no significant elevation in plasma vasopressin levels after estrogen treatment in spite of significant elevation of plasma oxytocin. Plasma AVP levels in the present investigation were higher than levels reported in the literature (27, 28). Skowsky *et al.* (27) have demonstrated marked changes in plasma AVP throughout the estrus cycle, apparently dependent on alterations in endogenous estadiol secretion. The high plasma values in the present examination might reflect proestrus values. We did not quantitate the stage of the estrus cycle in control female rats. Therefore, plasma AVP levels in estradiol-treated rats might be higher than those in diestrus female rats; it is also likely that AVP synthesis in SON and PVN, and AVP release from posterior pituitary, are enhanced by estrogen administration.

Acknowledgment. ACTH standard preparation and anti-ACTH serum for plasma ACTH assay were gifts of the hormone distribution program, NIAMDD National Pituitary Agency.

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