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Abstract

We examined the effect of food deprivation for three days on hypothalamic arginine vasopressin (AVP) mRNA in rats. Simultaneously the effect of water deprivation for the same period was examined as a model of dehydration. Levels of AVP mRNA in the supraoptic nucleus (SON) and the paraventricular nucleus (PVN) were determined by semiquantitative in situ hybridization histochemistry. Water deprivation increased AVP mRNA in both nuclei as previously reported. In contrast, food deprivation decreased AVP mRNA in these nuclei. The changes in AVP mRNA levels in the PVN were observed in the magnocellular subdivision of the nucleus. Plasma levels of ACTH and corticosterone were greatly increased in both treated groups of rats. Plasma AVP and osmolality levels were significantly elevated in water-deprived rats but not in food-deprived rats. These observations indicated that both food deprivation and water deprivation stimulated the pituitary-adrenal axis and that a reduction in AVP mRNA levels in food-deprived rats was caused by food deprivation but not by glucocorticoid feedback suppression nor by altered plasma osmolality.

KEYWORDS: food deprivation, arginine vasopressin mRNA, supraoptic nucleus, paraventricular uncleus, hypothalamo-pituitary-adrenal system, in situ hybridization histochemistry

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We examined the effect of food deprivation for three days on hypothalamic arginine vasopressin (AVP) mRNA in rats. Simultaneously the effect of water deprivation for the same period was examined as a model of dehydration. Levels of AVP mRNA in the supraoptic nucleus (SON) and the paraventricular nucleus (PVN) were determined by semiquantitative *in situ* hybridization histochemistry. Water deprivation increased AVP mRNA in both nuclei as previously reported. In contrast, food deprivation decreased AVP mRNA in these nuclei. The changes in AVP mRNA levels in the PVN were observed in the magnocellular subdivision of the nucleus. Plasma levels of ACTH and corticosterone were greatly increased in both treated groups of rats. Plasma AVP and osmolality levels were significantly elevated in water-deprived rats but not in food-deprived rats. These observations indicated that both food deprivation and water deprivation stimulated the pituitary-adrenal axis and that a reduction in AVP mRNA levels in food-deprived rats was caused by food deprivation but not by glucocorticoid feedback suppression nor by altered plasma osmolality.

Key words : **food deprivation, arginine vasopressin mRNA, supraoptic nucleus, paraventricular nucleus, hypothalamo-pituitary-adrenal system, *in situ* hybridization histochemistry**

Recently there have been a growing awareness of the hypothalamic neuropeptides participating in the regulation of appetite and feeding such as opioid peptides (1), corticotropin-releasing hormone (CRH) (2-4), neuropeptide Y (4) *etc.* CRH, the most potent secretagogue of ACTH, is known as a major anorexic agent to reduce both spontaneous or starvation-induced feeding (5). It is well established that arginine vasopressin (AVP) plays important roles in regulating the body fluid balance and the stress-induced ACTH secretion (6). In contrast to CRH, sparse data on the roles of AVP in feeding have been accumulated so far. Previous studies suggest that AVP is involved in hypothalamic regulation of feeding (7, 8), and there is evidence that abnormal secretion of AVP is associated with human dietary disorder such as anorexia nervosa (9).

In this study we examined the effect of food deprivation for three days on levels of AVP mRNA in hypothalamic nuclei as a consequence of abnormal feeding. To make a comparison with the effect of food deprivation, we simultaneously studied the effect of water deprivation for the same 3-day period as a model of dehydration on which the changes in AVP mRNA levels in the hypothalamus (10, 11) and AVP concentration in the plasma (12, 13), the posterior pituitary and the

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hypothalamus (14) have been well documented. *In situ* hybridization histochemistry using an oligonucleotide probe was performed to detect AVP mRNA expressed in the supraoptic (SON) and paraventricular (PVN) nuclei. In addition, the responses of the pituitary-adrenal axis and alterations of plasma AVP and osmolality levels were also examined following water or food deprivation.

Materials and Methods

Experiment I

Animals. Six male Wistar rats weighing approximately 200g were housed with free access to standard rat biscuits and water in an animal room (lights on 07.00–19.00 h) for three days before the experiment.

Treatments. Rats were weighed and randomly assigned to three groups; control, water-deprived and food-deprived groups (n = 2 per group). They were maintained individually in each cage for three days. The control rats had free access to food and water. The water-deprived rats were supplied only with food while the food-deprived rats were given only water.

Perfusion and tissue preparation. Three days after starting the experiment, the rats were anesthetized with sodium pentobarbital (45 mg per kg body weight, i.p.), weighed and sacrificed between 11.00 and 13.00 h by the transcardial perfusion of phosphate-buffered saline (about 100 ml per rat) followed by ice-cold 4 % paraformaldehyde in sodium phosphate buffer (about 500 ml per rat). The brains were quickly removed and post-fixed in the same fixative for 2 h, then immersed in 20 % sucrose solution overnight to prevent an ice crystal artifact formation. Eight-μm-thick coronal sections at the levels of the SON and the PVN were cut out on a cryostat at −20°C and directly thaw-mounted onto autoclaved gelatin-coated slides, then placed on a slide warmer at 45°C for 1–2 h and stored at −70°C until *in situ* hybridization.

Oligonucleotide probe. A ^{35}S-labeled synthetic oligonucleotide probe complementary to bases 286–315 of the rat prepro-AVP mRNA was purchased from DuPont/NEN Products (Boston, Mass., USA). According to the computer-based homology similarity search program (Soft Ware Developing Company, Tokyo, Japan) of all nucleic acid sequences in rodents stored in the Los Alamos Nucleic Acid Data Base, this oligomer has no homology with oligomer sequences of oxytocin and other hypothalamic peptides. Specific activity of the probe was 1.30 x 10^9 dpm/μg.

In situ hybridization. Tissue sections mounted on gelatin-coated slides were denatured at 72°C for 30 min in a solution of 40 μl of diethylpyrocarbonate in 200 ml of autoclaved 2 x SSC (0.3 M sodium chloride, 0.03 M sodium citrate in autoclaved nanopure distilled water) titrated to pH 3.5 with acetic acid, and then deproteinated at 37°C for 15 min in a solution of 1 μg/ml pepsin in autoclaved 2 x SSC-acetic acid (pH 3.5). The slides were rinsed twice in a solution of autoclaved 0.2 x SSC for 1 min each and rinsed in 5 mM dithiothreitol (DTT) (77 mg/ml) in autoclaved nanopure distilled water for 1 min at room temperature. Slides were permitted to air dry. A microfuge tube containing prehybridization buffer (PHB; contents below) was heated at 100°C for 10 min and then cooled on ice. The buffer was diluted (1: 1) with 100 % formamide. A 20 μl of PHB-formamide solution was applied to each section and incubated for 2 h at room temperature to reduce the non-specific binding of the labeled probe to the nucleic acids in the tissue. The solution was then drained off the slides. The radioactive probe of 10^5cpm was dissolved in 20 μl of hybridization buffer (HB; contents also below) containing 50 % formamide, heat-denatured at 100°C for 10 min and cooled rapidly on ice. A 20 μl aliquot of labeled probe in HB-formamide solution was applied to each section and incubated overnight at 20°C. The post-hybridization procedure was performed as follows. The sections were washed in autoclaved 2 x SSC, then in autoclaved 0.5 x SSC containing 5 mM DTT at 20°C, dehydrated in graded (50, 70, 90 and 100 %) ethanol for 1 min each and allowed to air dry in a desiccator. Ethanol at concentrations of 50, 70 and 90 % was diluted with 300 mM ammonium acetate. The buffer used in the prehybridization (PHB) and hybridization (HB) procedures contained the following reagents per 5 ml autoclaved, nanopure distilled water: 1.2 ml of 5.0 M sodium chloride; 100 μl of 1.0 M Tris pH 7.6; 16.7 μl of 6 % (w/v) bovine serum albumin; 40 μl of 250 mM EDTA; 50 μl of 5 % sodium pyrophosphate; 33 μl of 6 % polyvinylpyrrolidone; 10 μl of 50 mg/ml yeast tRNA; 250 μl (PHB) or 50 μl (HB) of 20 mg/ml yeast total RNA (type III); 500 μl (PHB) or 100 μl (HB) of 10 mg/ml herring sperm DNA (type IV); and 5 μl of 20 mg/ml dithiothreitol. The HB also contained 1 g/5 ml of dextran sulfate and 33 μl of 15 mg/ml polyadenylic acid (15, 16).

Autoradiography and counterstaining. Dried slides were dipped in Kodak NTB-3 nuclear track emulsion

(diluted 1:1 with distilled water) and permitted to air dry. After exposing the slides for 11 days at 4°C, they were developed in Kodak D-19 developer, rinsed in water twice, fixed in Kodak fixer and washed in distilled water. Sections were counterstained with cresylviolet and covered with coverslips.

Image analysis. Anatomically level-matched sections at the rostral, middle and caudal parts of the SON and the PVN on the counterstained slides were selected from the control, water-deprived and food-deprived rats for image analysis. Semiquantitative analysis of autoradiographic silver grains was carried out using the computerized image analyzer as we previously reported (17). It was composed of a TV Image Processor Excel TVIP-4100 (Nippon Avionics Company, Ltd., Tokyo, Japan) and the TV Image Processor Excel Command 4198 (Ratoc System Engineering Company, Ltd., Tokyo, Japan). Silver grains projected onto the video monitor through the light microscope were converted to densography in which each grain was digitized. The threshold for subtracting the non-specific background was determined on densography. The outer margins of the SON and the PVN on the video monitor were traced manually based on their counterstains. The total area of each nucleus and the area occupied by the silver grains (hybridized area) within the nucleus (the SON and the PVN) were calculated automatically as μ m^2. The per cent (%) occupancy, presented as the ratio of the hybridized area to the total area of each nucleus, was compared among anatomically level-matched sections from the three groups.

Experiment II

Animals and treatments. Eighteen male Wistar rats weighing approximately 200 g were housed in an animal room under the same conditions as described for the experiment I. Before the experiment, the animals were weighed and randomly divided into three groups; control, water-deprived and food-deprived groups ($n = 6$ per group). They were treated for three days in the same way as in the experiment I.

Collection of blood. After three days' treatments the rats were decapitated between 11.00 and 14.00 h and weighed. The truncal blood was collected into chilled glass tubes containing EDTA and centrifuged at 3,000 g at 4°C for 10 min. The plasma was separated, frozen and stored at −20°C until the measurement of ACTH, corticosterone, AVP and osmolality.

Tissue extraction. The median eminence (ME), the remaining tissue of the hypothalamus (rHy), the anterior pituitary (AP) and the posterior pituitary (PP) were quickly dissected out and placed in plastic tubes on dry ice. The block of the hypothalamic tissue obtained was bounded by the optic chiasm and mammillary bodies rostrocaudally and by the anterior commisure dorsally (18). ME and rHy for CRH and AVP assays were extracted by the acetone-petroleum ether methods previously reported (19). The dried extracts were stored at −20 °C until assays. AP for ACTH and PP for CRH and AVP assays were homogenized in a 2 ml solution of 0.1 N HCl with a glass homogenizer. After centrifugation at 7,000 g at 4 °C for 10 min, the supernatant of AP was stored at −20 °C, and the supernatant of PP was lyophilized overnight and stored at −20 °C until assays. Tissue protein was measured by a protein assay kit (Bio-Rad Laboratories, Richmond, VA., USA).

Hormone assays. Concentration of ACTH in the anterior pituitary and the plasma was measured using a commercially available immunoradiometric assay kit (ACTH-IRMA kit, Mitsubishi Yuka Petrochemical Company, Tokyo, Japan). Assay sensitivity of plasma ACTH was 5 pg/ml. Plasma corticosterone was measured with a cortisol radioimmunoassay kit (SPAC Cortisol Radioimmunoassay Kit, Daiichi Radioisotope Laboratories, Tokyo, Japan) with corticosterone standard. Although the cross-reactivity of the antiserum to corticosterone in this kit was low, the values obtained were mostly ascribed to corticosterone, as the levels of cortisol and 11-deoxycortisol are negligible in rat blood. Plasma AVP was measured by AVP-RIA kit (Mitsubishi Yuka Petrochemical Company,Tokyo, Japan). Tissue concentrations of CRH (19) and AVP (20) were measured by the radioimmunoassay methods established in our laboratory. Assay sensitivities for CRH and AVP were 2 pg/tube and 0.5 pg/tube, respectively. Plasma osmolality was measured by the descending freezing point method.

Statistical analysis. Values are presented as the mean ± SEM. The differences between the values were conducted using unpaired Student's t-test, with $P < 0.05$ established as significant.

Results

Body weight changes. Before starting the experiment II there was no difference in body weight among the control, water-deprived and food-deprived groups. After three days' treatments the body weight was significantly lower in

Table 1 Body weight changes following deprivation of water or food for three days in the experiment II.

Groups of rats	Control (n = 6)	Water-deprived (n = 6)	Food-deprived (n = 6)
Weight at start (g)	225.8 ± 2.0	215.0 ± 3.7	217.3 ± 3.4
Weight at end (g)	250.4 ± 3.1	186.3 ± 3.7*	189.0 ± 2.3*
Weight change (%)	10.9 ± 0.8	−13.4 ± 0.8*	−13.0 ± 0.9*

Values are presented as the mean ± SEM. Numbers in parentheses refer to the number of rats used.
* $p < 0.001$, compared to controls.

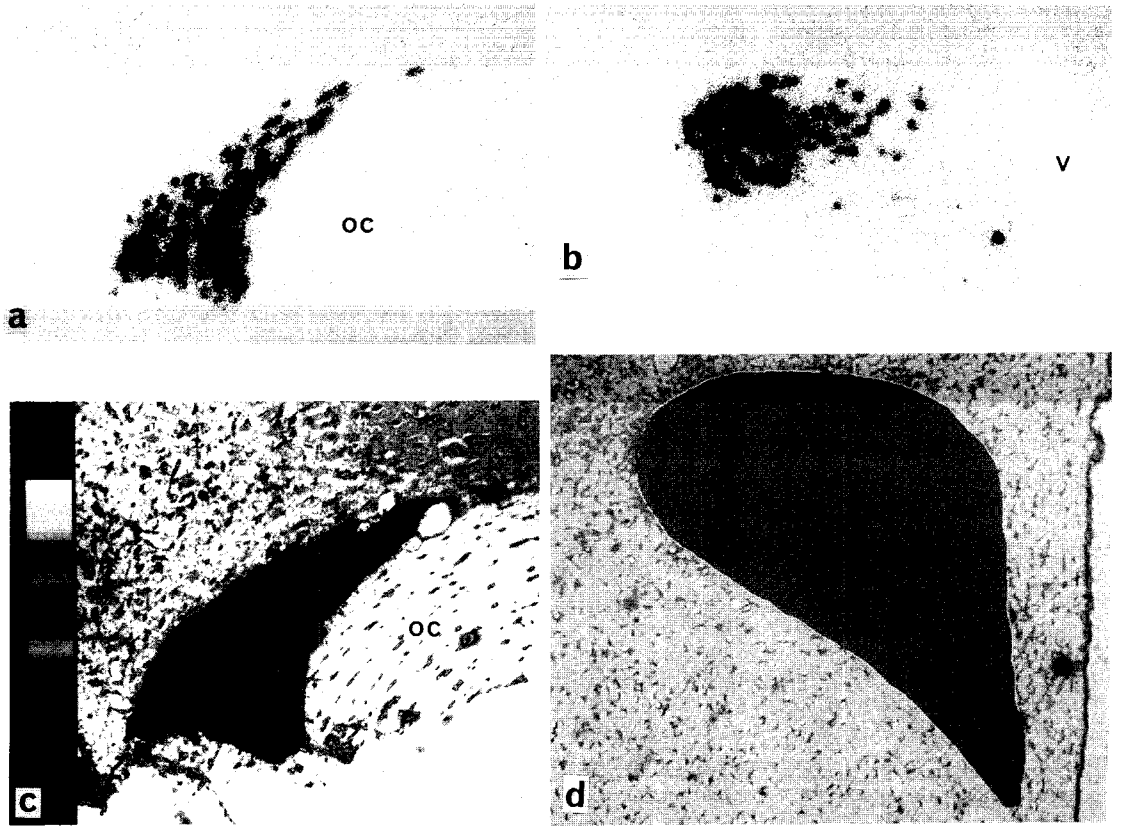

Fig. 1 Autoradiographic silver grains observed by *in situ* hybridization histochemistry (a,b) and the defined areas of the SON (c) and the PVN (d) in a control rat. The SON and the PVN were outlined by tracing on the video monitor on the basis of the counterstaining with cresylviolet. SON: the supraoptic nucleus, PVN: the paraventricular nucleus, V: the third ventricle, OC: the optic chiasm.

both the water-deprived and the food-deprived groups than that of the control group. Body weight was reduced to a similar extent in the two treated groups (Table 1). Similar changes were seen in the mean body weight in the experiment I.

AVP mRNA levels in the SON and the PVN. Fig. 1 shows autoradiographic silver grains indicating the localization of AVP mRNA (a,b) and the traced areas of the SON (c) and the PVN (d) of a control rat. The hybridized areas in the SON (Fig. 2; b) and the PVN (Fig. 3; b) were expanded and the density of silver grains increased in the water-deprived rats. By contrast, in the food-deprived rats the hybridized areas and the density of AVP mRNA signals were reduced in both nuclei (Fig. 2; c and Fig. 3; c). The changes in AVP mRNA signals in the PVN were observed in the magnocellular subdivision and no apparent changes were seen in the medial parvocellular subdivision (mp). Semiquantitative image analysis showed that the per cent occupancy of AVP mRNA signals expressed in the SON and the PVN tended to increase in the water-deprived rats but to decrease in the food-deprived rats as compared with controls (Table 2).

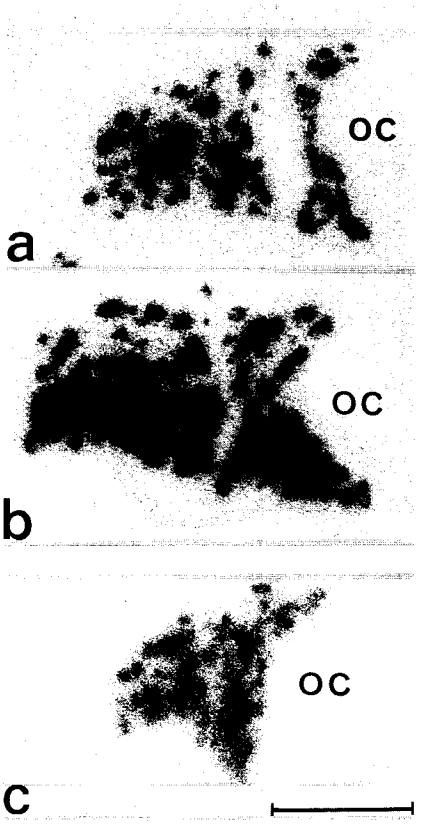

Fig. 2 Comparable series of autoradiographic silver grains showing the localization of AVP mRNA within the SON obtained in the control (a), water-deprived (b) and food-deprived (c) rats. Each section was rostrocaudally level-matched according to the countrstaining. Water deprivation increased AVP mRNA signals in the SON, while food deprivation decreased AVP mRNA signals in the SON. OC: the optic chiasm. Bar = 200 μm.

Plasma ACTH, corticosterone, AVP and osmolality levels. In both the water-deprived and the food-deprived rats the plasma ACTH levels showed marked increases; 70.1 $\pm$ 12.3 pg/ml and 148.2 $\pm$ 23.0, respectively, compared with control values (24.8 $\pm$ 6.7). The plasma levels of ACTH were significantly higher in the food-deprived rats than in the water-deprived rats. Plasma corticosterone levels were greatly elevated in both the water-deprived (20.0 $\pm$ 2.4 μg/dl) and the food-deprived (26.5 $\pm$ 2.7) rats as compared with control values (1.3 $\pm$ 0.1). Plasma AVP and osmolality levels were significantly elevated in the water-deprived (17.3 $\pm$ 2.7 pg/ml, 304.8 $\pm$ 3.1 mOsm/kg, respectively) rats, but not in the food-deprived (1.4 $\pm$ 0.2, 288.3 $\pm$ 4.8) rats, compared to the controls (1.3 $\pm$ 0.2, 290.3 $\pm$ 3.4) (Fig. 4).

AVP concentration in the pituitary and the hypothalamus. The concentration of AVP was lower in the posterior pituitary of the water-deprived rats and in the median eminence of the food-deprived rats than in those of the controls, while AVP concentration in the rest of the hypothalamus did not differ significantly among the three groups (Fig. 5).

ACTH and CRH concentrations in the pituitary and the hypothalamus. The ACTH concentration in the anterior pituitary was significantly reduced in the water-deprived rats

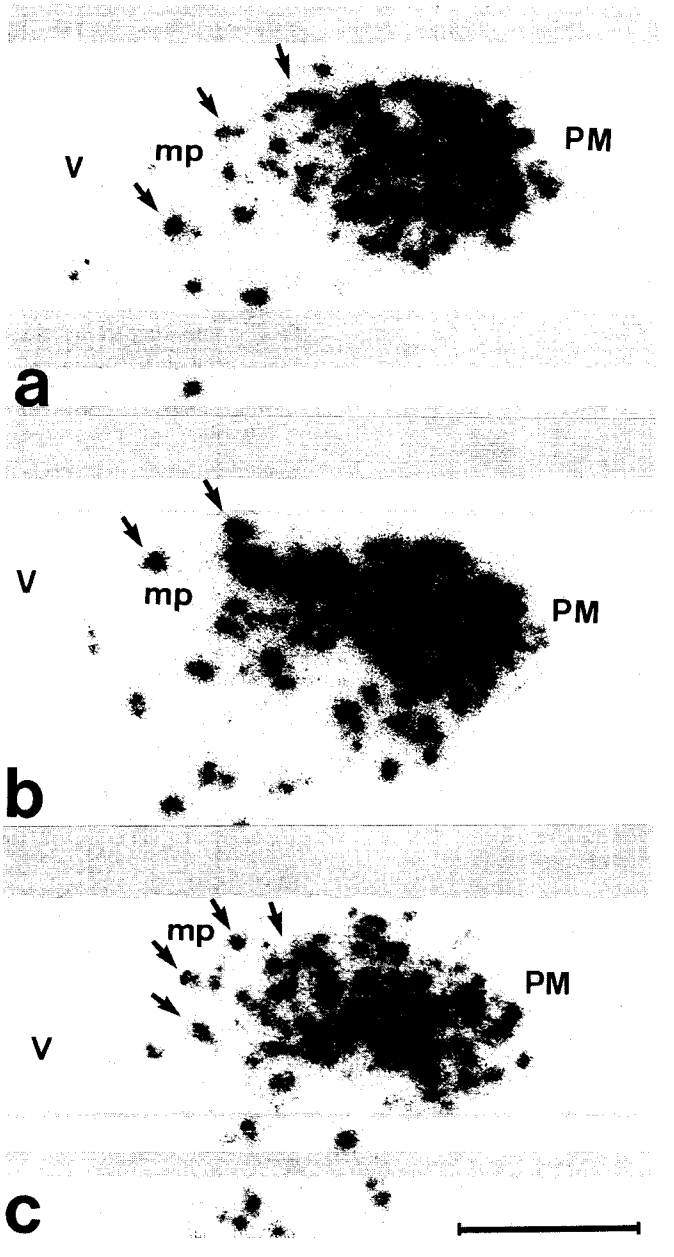

Fig. 3 Autoradiographs of AVP mRNA localization within the PVN obtained in the control (a), water-deprived (b) and food-deprived (c) rats. Each section was anatomically level-matched according to the counterstaining. Water deprivation increased AVP mRNA signals in the magnocellular subdivision of the PVN, while food dprivation decreased AVP mRNA signals in this region. No apparent changes were seen in the medial parvocellular subdivisions of the PVN among the three groups. Clusters of silver grains in the medial part of the PVN are localized within the magnocellular neurons normally scattered in this region (*arrows*). PM: the posterior magnocellular subdivision. mp: the medial parvocellular subdivision. V: the third ventricle. Bar = 200 μm.

Table 2 Semiquantitative analysis of AVP mRNA signals in the SON and the PVN of the control, water-deprived and food-deprived rats.

A. Per cent (%) occupancy of AVP mRNA in the SON

Anatomical level	Treatments		
	Control	Water-deprived	Food-deprived
Rostral	62.8	75.6	41.7
Middle	68.7	79.4	55.2
Caudal	62.7	75.2	55.3

B. Per cent (%) occupancy of AVP mRNA in the PVN

Anatomical level	Treatments		
	Control	Water-deprived	Food-deprived
Rostral	36.4	41.0	32.8
Middle	35.2	42.0	29.1
Caudal	33.1	38.8	24.0

Anatomically level-matched sections at the rostral, middle and caudal parts of the SON and the PVN were selected from the three groups of rats (n = 2 per group). Per cent occupancy is expressed as the ratio of the hybridized area to the total area of the supraoptic nucleus (SON) or the paraventricular nucleus (PVN). The hybridized areas tended to increase in the water-deprived rats but to decrease in the food-deprived rats at the three rostrocaudal levels tested, compared with controls.

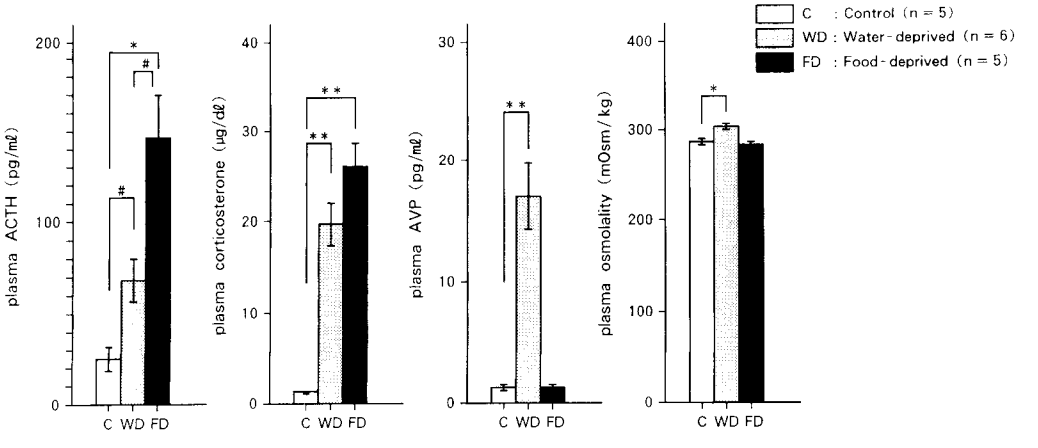

Fig. 4 Effect of deprivation of water or food for three days on the plasma levels of ACTH, corticosterone, AVP and osmolality. Values are presented as the mean ± SEM. Numbers in parentheses refer to the number of determinations.
* $p < 0.01$ ** $p < 0.001$ # $p < 0.02$

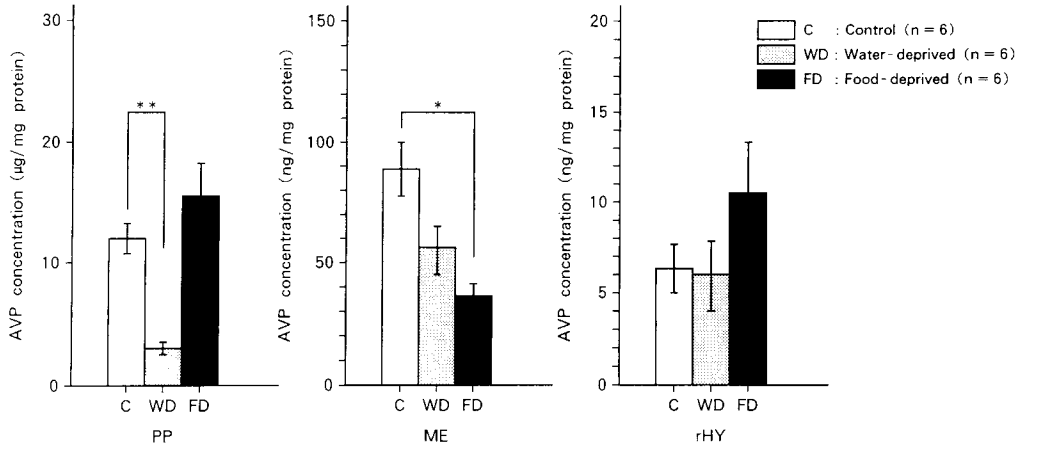

Fig. 5 Effect of deprivation of water or food for three days on the brain concentration of AVP. Values are presented as the mean ± SEM. PP: the posterior pituitary, ME: the median eminence, rHy: the remaining tissue of the hypothalamus. Numbers in parentheses refer to the number of determinations. * $p < 0.01$ ** $p < 0.001$

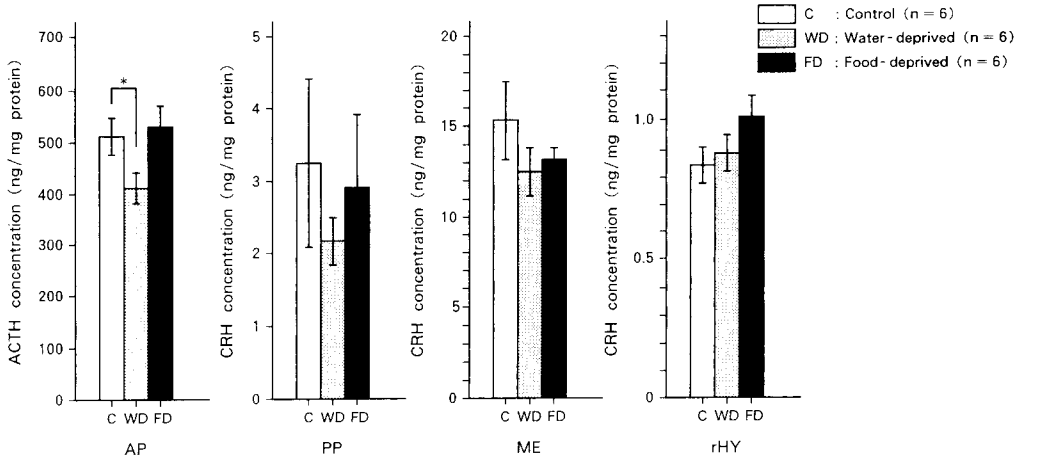

Fig. 6 Effect of deprivation of water or food for three days on the brain concentrations of ACTH and CRH. Data are expressed as the mean ± SEM. AP: the anterior pituitary, PP: the posterior pituitary, ME: the median eminence, rHy: the remaining tissue of the hypothalamus. Numbers in parentheses refer to the number of determinations. * $p < 0.05$

(413.4 ± 30.8 ng/mg protein), but not in the food-deprived rats (527.5 ± 38.6) as compared to the controls (515.0 ± 34.1). The concentration of CRH in the posterior pituitary, the median eminence and the remaining hypothalamic tissue did not differ significantly among the three groups (Fig. 6).

Discussion

In this study we investigated the changes in hypothalamic AVP mRNA levels and the CRH-ACTH-corticosterone axis induced by food or water deprivation. AVP mRNA in the magnocellular neurons of the SON and the PVN was increased by water deprivation. The marked reduction of AVP concentration in the posterior pituitary of the water-deprived rats may be ascribed to the excess release of AVP by hyperosmotic and hypovolemic stimuli. On the other hand, Young *et al.* recently reported that CRH mRNA was decreased in both the magnocellular and the parvocellular neurons of the PVN after three days of water deprivation in rats (21). It is well established that CRH is the most potent secretagogue of pituitary ACTH, and that AVP originating from the parvocellular subdivision of the PVN predominantly potentiates the action of CRH to secrete ACTH from the corticotrophs but AVP from the magnocellular subdivision only partly does (6, 22, 23). Therefore, it seems likely that AVP, instead of CRH, is at least partly involved in the secretion of ACTH during water deprivation.

By contrast, food deprivation reduced magnocellular AVP mRNA levels in both the SON and the PVN, indicating a reduction in the synthesis of AVP. No apparent changes could be detected in the parvocellular neurons of the PVN. The significant reduction of AVP concentration in the median eminence may reflect a decreased axonal transport of AVP from the SON and the PVN to the posterior pituitary via the internal layer of the median eminence. By the way, several immunohistochemical studies revealed the heavy noradrenergic innervation to the SON and the PVN from the brain stem (24, 25). Norepinephrine (NE) has been reported to stimulate AVP release through α_1-adrenergic receptors (26, 27) and to inhibit AVP release through α_2-adrenergic receptors (27). Brooks *et al.* indicated that α_2-adrenergic receptors exert their tonic inhibitory effect on AVP release (27). Jhanwar-Uniyal *et al.* reported that food deprivation for 48 hours decreased α_1-adrenergic receptor binding and increased NE turnover in the rat PVN (28). Together with their reports, the present observations suggest that a food deprivation-induced reduction in AVP mRNA levels in the PVN and the SON neurons are presumably mediated by the decreased α_1-adrenergic receptor binding and relatively predominant α_2-adrenergic activity.

It has been found that hyperphagia induced by NE injection into the PVN is mediated by α_2-adrenergic receptors (29). Kow *et al.* reported that AVP stimulated the function of the ventromedial hypothalamus (VMH), the satiety center of feeding, leading to a reduction in appetite and feeding (7). Aravich *et al.* carried out an experiment using Brattleboro rats and reported that AVP could have a role in the central regulation of feeding as a satiety factor (8). These data, along with the present finding that AVP mRNA levels in the PVN and the SON were decreased by food deprivation, lead us to the hypothesis that the appetite is increased in the food-deprived rats by relatively predominant α_2-adrenergic receptors in the PVN and by the attenuated stimulation of the VMH function.

In the water-deprived rats plasma AVP and osmolality levels were significantly elevated, but not in the food-deprived rats. And plasma ACTH and corticosterone levels were greatly elevated in both the food-deprived and the water-deprived rats. These results indicated that a reduction in AVP mRNA levels was induced by food deprivation, but not by the feedback suppression due to the increased levels of circulating corticosterone, nor by the altered plasma osmolality. This notion

is compatible with the several previous studies using *in situ* hybridization (30, 31) and immunohistochemistry (32, 33) that show the magnocellular vasopressinergic neurons were not under the negative feedback regulation by glucocorticoid.

The present observation of the greatly elevated ACTH levels in the plasma, despite the unchanged ACTH concentration in the anterior pituitary of the food-deprived rats, suggested that the synthesis of ACTH was accelerated to compensate for its release into the peripheral circulation. Therefore, it was likely that CRH was responsible for the food deprivation-induced secretion of ACTH. The present data showed, however, no significant changes in the concentration of CRH in the median eminence, the remaining hypothalamic tissue, and the posterior pituitary of the food-deprived rats. As the CRH concentration in the hypothalamus is expressed as the integration of CRH synthesis, storage and release, the data on the tissue CRH concentration did not give evidence as to whether CRH was unaffected, or whether CRH synthesis and release were equivalently increased to restore balance in the food-deprived rats. Very recently, however, Brady *et al.* observed that 4 -day food restriction or deprivation decreased CRH mRNA levels in the PVN (4). As for their results, the plasma levels of ACTH and corticosterone were not affected by 4 days' food deprivation in male rats. Their results on plasma ACTH and corticosterone levels were quite different from our results and other previous reports (3, 34, 35). Suemaru *et al.* reported the reduction of the hypothalamic CRH concentration and the greatly elevated plasma corticosterone levels in the rat after 7 days' food deprivation, and suggested that food deprivation might accelerate the CRH turnover in the hypothalamus (3). The influence of food deprivation on the CRH-ACTH-corticosterone axis must be elucidated by further examination of hypothalamic CRH mRNA levels following food deprivation.

In conclusion, our finding of decreased AVP mRNA levels in the supraoptic and paraventricular nuclei in the food-deprived rats suggests a possible role of AVP in the inhibition of feeding and appetite, and the disinhibition leading to an appetite increase during food deprivation.

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References

1. Vaswani KK and Tejwani GA : Food deprivation-induced changes in the level of opioid peptides in the pituitary and brain of rat. Life Sci (1986) **38**, 197–201.
2. Britton D, Koob G, Rivier J and Vale W: Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. Life Sci (1982) **31**, 363–367.
3. Suemaru S, Hashimoto K, Hattori T, Inoue H, Kageyama J and Ota Z. Starvation-induced changes in rat brain corticotropin-releasing factor (CRF) and pituitary-adrenocortical response. Life Sci (1986) **39**, 1161–1166.
4. Brady LS, Smith MA, Gold PW and Herkenham M: Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. Neuroendocriology (1990) **52**, 441–447.
5. Morley JE and Levine AS: Corticotropin releasing factor, grooming and ingestive behavior. Life Sci (1982) **31**, 1459 –1464.
6. Rivier C and Vale W: Modulation of stress-induced ACTH release by corticotropin-releasing factor, catecholamines and vasopressin. Nature (1983) **305**, 325–327.
7. Kow LM and Pfaff DW: Vasopressin excites ventromedial hypothalamic glucose-responsive neurons *in vitro*. Physiol Behav (1986) **37**, 153–158.
8. Aravich PF and Sladek CD: Deficiencies in hypothalamic hyperphagia, norepinephrine-induced feeding and glucoprivic feeding in Brattleboro rats lacking vasopressin. Soc Neurosci Abstr (1983) **9**, 185.
9. Gold PW, Kaye W, Robertson GL and Ebert M: Abnormalities in plasma and cerebrospinal-fluid arginine vasopressin in patients with anorexia nervosa. N Engl J Med (1983) **308**, 1117–1123.
10. Uhl GR, Zingg HH and Habener JF: Vasopressin mRNA *in situ* hybridization: localization and regulation studied with oligonucleotide cDNA probes in normal and Brattleboro rat hypothalamus. Proc Natl Acad Sci USA (1985) **82**, 5555–5559.
11. Nojiri H, Sato M and Urano A: Increase in the vasopressin mRNA levels in the magnocellular neurosecretory neurons of water-deprived rats: *In situ* hybridization study with the use of synthetic oligonucleotide probe. Zool Sci (1986) **3**,

345-350.
12. Dunn FL, Brennan TJ, Nelson AE and Robertson GL: The role of blood osmolality and volume in regulating vasopressin secretion in the rat. J Clin Invest (1973) **52**, 3212-3219.
13. Woods RL and Johnston CI: Contribution of vasopressin to the maintenance of blood pressure during dehydration. Am J Physiol (1983) **245**, F615-F621.
14. Dohanics J, Linton EA, Lowry PJ and Makara GB: Osmotic stimulation affects neuohypophysial corticotropin releasing factor-41 content: Effect of dexamethasone. Peptides (1990) **11**, 51-57.
15. McCabe JT, Morrel JI, Ivell R, Schmale H, Richter D and Pfaff DW: *In situ* hybridization technique to localize rRNA and mRNA in mammalian neurons. J Histochem Cytochem (1986) **34**, 45-50.
16. Kawata M, McCabe JT and Pfaff DW: *In situ* hybridization histochemistry with oxytocin synthetic oligonucleotide: Strategy for making the probe and its application. Brain Res Bull (1988) **20**, 693-697.
17. Suemaru S, Hashimoto K, Ogasa T, Takao T, Ota Z, Hirakawa M and Kawata M: Effects of hyperosmotic stimulation and adrenalactomy on vasopressin mRNA levels in the paraventricular and supraoptic nuclei of the hypothalamus: *In situ* hybridization histochemical analysis using a synthetic oligonucleotide probe. Acta Med Okayama (1990) **44**, 233-241.
18. Glowinski J and Iversen LL: Regional studies of catecholamines in the rat brain-I. The disposition of (^{3}H) norepinephrine, (^{3}H) dopamine and (^{3}H) DOPA in various regions of the brain. J Neurochem (1966) **13**, 655-669.
19. 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.
20. Ohno N, Hashimoto K, Yunoki S, Takahara J and Ofuji T: A perifusion method for examining arginine vasopressin (AVP) release from the hypothalamo-neurohypophyseal system. Acta Med Okayama (1981) **35**, 27-35.
21. Young WS III: Corticotropin-releasing factor mRNA in the hypothalamus is affected differently by drinking saline and by dehydration. FEBS Lett (1986) **208**, 158-162.
22. 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.
23. 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.
24. Swanson LW, Sawchenko PE, Berod A, Hartman BK, Helle KB, and Van Orden DE: An immunohistochemical study of the organization of catecholaminergic cells and terminal fields in the paraventricular and supraoptic nuclei of the hypothalamus. J Comp Neurol (1981) **196**, 271-285.
25. McNeill TH and Sladek JR: Simultaneous monoamine histofluorescence and neuropeptide immunocytochemistry. II. Correlative distribution of catecholamine varicosities and magnocellular neurosecretory neurons in the rat supraoptic and paraventricular nuclei. J Comp Neurol (1980) **193**, 1023-1033.
26. Randle JCR, Bourque CW and Renaud LP: α-Adrenergic activation of rat hypothalamic supraoptic neurons maintained *in vitro*. Brain Res (1984) **307**, 374-378.
27. Brooks DP, Share L and Crofton JT: Central adrenergic control of vasopressin release. Neuroendocrinology (1986) **42**, 416-420.
28. Jhanwar-Uniyal M, Fleischer F, Levin BE and Leibowitz SF: Impact of food deprivation on hypothalamic α-adrenergic receptor activity and norepinephrine (NE) turnover in rat brain. Soc Neurosci Abstr (1982) **8**, 711.
29. Marino LA, DeBellis MD and Leibowitz SF: α_2-Adrenergic receptors in the paraventricular nucleus mediate feeding induced by norepinephrine and clonidine. Soc Neurosci Abstr (1983) **9**, 467.
30. Swanson LW and Simmonds DM: Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: A hybridization histochemical study in the rat. J Comp Neurol (1989) **285**, 413-435.
31. Wolfson B, Manning RW, Davis LG, Arentzen R and Baldino F Jr.: Co-localization of corticotropin releasing factor and vasopressin mRNA in neurones after adrenalectomy. Nature (1985) **315**, 59-61.
32. Kiss JZ, Mezey E and Skirboll L: Corticotropin-releasing factor-immunoreactive neurons of the paraventricular nucleus become vasopressin positive after adrenalectomy. Proc Natl Acad Sci USA (1984) **81**, 1854-1858.
33. Sawchenko PE, Swanson LW and Vale WW: Co-expression of corticotropin-releasing factor and vasopressin immunoreactivity in parvocellular neurosecretory neurons of the adrenalectomized rat. Proc Natl Acad Sci USA (1984) **81**, 1883-1887.
34. Chowers I, Einat R and Feldman S: Effects of starvation on levels of corticotrophin-releasing factor, corticotrophin and plasma corticosterone in rats. Acta Endocrinol Cph (1969) **61**, 687-694.
35. Malatova Z and Ahlers I: Diurnal rhythm of corticosterone in fasted rats. Endocrinol Exp (1977) **11**, 241-247.

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