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Effect of Food Deprivation and Refeeding on the Concentration of Vasopressin and Oxytocin in Discrete Hypothalamic Sites

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BURLET, A. J., M. JHANWAR-UNIYAL, M. CHAPLEUR-CHATEAU, C. R. BURLET AND S. F. LEIBOWITZ. *Effect of food deprivation and refeeding on the concentration of vasopressin and oxytocin in discrete hypothalamic sites.* PHARMACOL BIOCHEM BEHAV 43(3) 897-905, 1992.—Recent evidence has implicated hypothalamic peptides, such as arginine vasopressin (AVP) and oxytocin (OT) in the control of feeding behavior. In this study, we investigated the impact of food deprivation (48 h) and subsequent refeeding (6 h) on the concentration of AVP and OT in discrete hypothalamic areas, as well as in the neurohypophysis. We also estimated in these rats certain peripheral measures, including hydroelectrolytic parameters, plasma and urine AVP, and plasma corticosterone. The results of this study revealed that food deprivation for 48 h produced little change in OT concentration in the various hypothalamic nuclei studied, including the paraventricular and supraoptic nuclei, with the exception of the median eminence (ME), where a significant decline (−36%; $p < 0.05$) was detected. This effect was not significantly reversed by 6 h of refeeding. With respect to AVP concentration, food deprivation caused a reliable decline exclusively in the parvocellular subdivision of the paraventricular nucleus (pPVN; −45%; $p < 0.01$) and in the supraoptic nucleus (SON; −45%; $p < 0.01$). No change in AVP was detected in the ME or in most other hypothalamic nuclei examined. Refeeding for 6 h actually potentiated the effect of food deprivation, decreasing further from baseline the content of AVP in the pPVN and SON. The only other hypothalamic area to exhibit a change in AVP content was the ventromedial nucleus, where AVP level increased ($p < 0.001$) after deprivation and declined to normal after 6 h of refeeding. The content of AVP and OT in the neurohypophysis was unaffected by food deprivation and subsequent refeeding. The AVP content of urine was significantly decreased after food deprivation; however, plasma AVP content remained unchanged. Plasma corticosterone levels were reliably enhanced after the food deprivation period. Refeeding for 6 h totally reversed these changes in peripheral hormone levels. Additional measurements of hydrolytic parameters suggested that these changes observed in hypothalamic concentration of AVP and OT are primarily due to alteration in nutritional state rather than to modifications in water balance.

Vasopressin Oxytocin Corticosterone Paraventricular nucleus Supraoptic nucleus Hypothalamus Feeding behavior Body weight Food deprivation Energy balance Water balance

THE paraventricular nucleus (PVN) of the hypothalamus is now believed to have a primary role in the control of food intake (24). Of more than 30 neuromediators isolated from this nucleus, arginine vasopressin (AVP) and oxytocin (OT) are perhaps the best known and most extensively studied. Immunocytochemical studies have shown that AVP is mainly synthesized in the magnocellular (mPVN) (9,34) and parvocellular (pPVN) (37) neurons of the PVN, as well as in the magnocellular neurons of the supraoptic nucleus (SON) and the parvocellular neurons of the suprachiasmatic nucleus (SCN) (38). Oxytocin is produced by a different neuronal population in both the PVN and SON (34,35).

The role of OT and AVP in the control of food intake or body weight has not been extensively studied. A few injection studies that exist, as well as reports using other experimental approaches, have suggested a function of OT and AVP in the control of satiety (3,21,39,40) and have also established an association between hypothalamic AVP and body weight gain (2,13).

To investigate further a possible link between these hypo-

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thalamic peptides and nutritional state, the present study, described in preliminary form (19), examined the concentration of AVP and OT in discrete hypothalamic nuclei and the neurohypophysis (NH) in rats exposed either to 48 h food deprivation or 6 h refeeding after 42 h food deprivation. Because numerous observations have shown that water intake, and consequently body fluid homeostasis, is strongly linked to food intake, we also focused our attention on plasma and urine AVP, a potent regulator of water conservation.

METHOD

Animals

Male, albino Sprague-Dawley rats (n = 8–16/group), weighing 350–380 g and singly housed, were used in the present study. Animals were given Purina lab chow pellets and water ad lib and maintained on a 12L : 12D cycle, with lights on at 0600 h. Rats were adapted to these conditions for at least 10–14 days before the start of the experiments. All rats were sacrificed 2 h after onset of the light period (0800 h).

Three groups of rats, one satiated and two food deprived, were tested in this study. In all groups, water was available ad lib. The first group, consisting of satiated rats with food freely available, served as a control group and was sacrificed at the same time as the other two groups. In group 2, subjects were deprived of food for 48 h prior to sacrifice. Rats of group 3, the "refed" subjects, were first deprived of food for 42 h and then given food ad lib for 6 h prior to sacrifice.

In addition to measurements of hypothalamic and pituitary AVP and OT taken in all subjects, a variety of other measurements were recorded, in approximately half the rats in each group, to provide additional information regarding animals' state of hydration. These measurements were: a) plasma and urine levels of AVP; b) plasma and urine NA+, K+, and osmolality; c) plasma levels of urea, hematocrit, glucose, and corticosterone (CORT); d) water and creatinine clearance; and e) food and water intake, body weight, and urine output, recorded at 24-h intervals during a 2-day pretest period as well as the 2-day period of deprivation and refeeding.

Tissue Preparation

All rats were sacrificed by decapitation and their brains were quickly removed, frozen on dry ice, and stored at −70°C. Trunk blood was collected for the estimation of CORT and AVP. The neural lobe of the hypophysis (NH) was immediately isolated and stored in 100 μl aprotinin (inhibitor of peptidase, Iniprol, Choay, France). Serial sections of 300 μm were cut and discrete hypothalamic sites micropunched. Eight hypothalamic areas were examined: pPVN and mPVN, SON, SCN, ventromedial nucleus (VMN), dorsomedial nucleus (DMN), perifornical hypothalamus (PFH), and median eminence (ME). Bilateral tissue samples were placed in 100 μl cold Iniprol in microfuge tubes (500-μl capacity) and stored at −70°C until assayed. The micropunched tissue samples from two rats were pooled in this study.

Assay Technique

Brain AVP and OT were measured by radioimmunoassay. Briefly, the tissue samples were homogenized in 1 ml cold 0.2 N HCL. An aliquot was taken for protein analysis, according to the method of Lowry et al. (25). The remainder of the homogenate was centrifuged at 2,500 × g for 30 min at 4°C. The supernatant was then divided for the estimation of AVP and OT.

OT Assay

Standard or unknown samples were incubated in phosphate buffer (pH 7.4–0.15 M NaCl; 0.3% lysozyme) with antibody (L15; final dilution: 1/3,000, which has been raised in rabbits immunized with synthetic OT conjugated to thyroglobulin with carbodiimide) and [^{125}I]-labeled oxytocin [New England Nuclear Corp. (NEN), Newton, MA; 2,200 Ci/mmol; 5,000 CPM] at 4°C for 24 h. Final assay volume was 500 μl. Separation of free and antibody-bound OT was performed by addition of a dextran-charcoal solution followed by centrifugation at 2,500 × g for 10 min. The assays were performed in duplicate for each determination. Assay sensitivity was 1 pg/tube, and intra- and interassay variations were 3 and 8%, respectively. The cross-reactivity with OT (Sandoz, Inc., East Hanover, NJ) and mesotocin (Novabiochem, La Jolla, CA) was 100%. With isotocin (Novabiochem), it was less than 0.1% and less than 0.001% with AVP (Ferring) and tocinoic acid (Novabiochem).

AVP Assay

The assay procedure of AVP was similar ot that described for OT. The antibody P1 (dilution 1/25,000 final) was obtained by immunization with Lys-8-vasopressin conjugated to thyroglobulin. The tracer was [^{125}I]-labeled vasopressin (NEN; 2,200 Ci/mmol). Arg-8-Vasopressin (Ferring, AB, Malmö, Sweden) was used for standard. The cross-reactivity with Arg-vasopressin and Lys-vasopressin was 100%. It was 1.25% with vasotocin (Sigma Chemical Co., St. Louis, MO), 0.07% with pressinoic acid (C. R. Bard, Inc., Billerica, MA), and 0.002% with OT (Sandoz).

Plasma AVP was measured by the same radioimmunoassay. Fifty microliters of plasma were extracted with Amprep C8 microcoulombs (Amersham Corp., Arlington Heights, IL), dried under N_2, and stored at −28°C until assayed. Urine AVP was measured by the same radioimmunoassay, without extraction, from acidified urine. The sensitivity was 1 pg/tube; the intra- and interassay variations were 1.4 and 12%, respectively.

Plasma Corticosterone (CORT) was measured by radioimmunoassay (KIT SB-Cort, Oris, France). The intra- and interassay variations were 5 and 13%, respectively.

Plasma and urine creatinine was measured with the Jaffe method; plasma glucose and urea with enzyme method (glucoxydase and urease, respectively); Na+ and K+ with electrode-sensitive methods; and osmolality by -cryoscopic measurement.

Statistical Analyses

The data were subjected to a one-way analysis of variance (ANOVA) and then to appropriate posthoc tests (Newman-Keuls and Duncan's multiple-range test) for individual mean comparisons. A probability less than 0.05% was considered statistically significant.

RESULTS

OT in Hypothalamus and Neurohypophysis

Eight hypothalamic nuclei were examined for their concentration of OT (Fig. 1). In satiated control rats (open bars in Fig. 1), detectable levels of OT, ranging from 1.8–10.8 ng/mg protein, were observed in four of these hypothalamic areas, namely, the pPVN, mPVN, SON nuclei, which contain the OT perikarya, and the ME, which has dense OT-containing

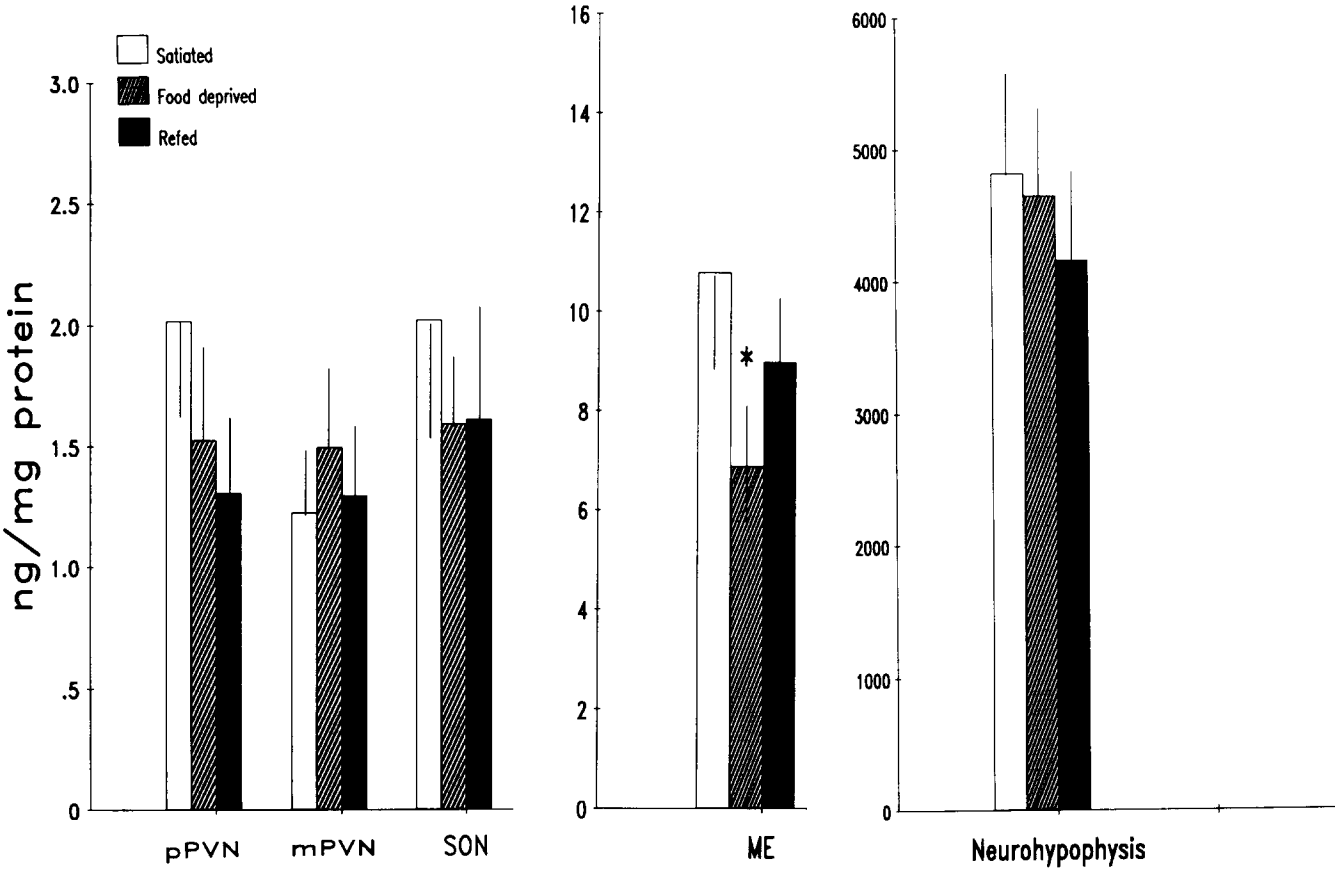

FIG. 1. Effect of food deprivation and subsequent refeeding on brain oxytocin concentration [(OT), ng/mg protein]. Rats that were satiated (open bar), 48 h food deprived (hatched bar), and 6 h refed after 42 h food deprivation (solid bar) were sacrificed during the light cycle. Statistical comparisons between the group means revealed a significant difference only in the ME, where food-deprived rats showed a reliable decrease in OT (*$p < 0.02$ relative to satiated rats). pPVN, parvocellular division of the paraventricular nucleus; mPVN, magnocellular division of the PVN; SON, supraoptic nucleus; ME, median eminence.

fibers. There were nondetectable levels of OT in the VMN, DMN, and PFH, where OT fibers cross, and also in the SCN. The NH exhibited the expected very high content of OT.

After 48 h of food deprivation, OT levels remained stable in the NH and the hypothalamic areas with measurable OT (Fig. 1). Only in the ME was there a significant change in OT ($p < 0.05$), a decline from 10.8 to 6.0 ng/mg protein (−36%) following food deprivation. Six hours of refeeding in 42-h food-deprived rats did not significantly reverse this deprivation-induced decline in OT levels in the ME. Thus, OT levels in the ME of the refed group remained statistically insignificant from the satiated group as well as from the food-deprived group. Furthermore, refeeding had no additional impact on OT levels in any of the hypothalamic nuclei studied (Fig. 1), including in areas where baseline OT concentration was undetectable in satiated animals.

AVP in the Hypothalamus and Neurohypophysis

As with OT, hypothalamic levels of AVP in satiated control rats were generally highest in nuclei containing AVP perikarya (open bars of Fig. 2). The lowest quantity was observed in the SCN (1.6 ng/mg protein), moderate levels in both subdivisions of the PVN (13–16 ng/mg protein), and highest concentration in the SON (23 ng/mg protein). The steady-state levels of AVP in other hypothalamic areas, namely, the VMN, DMN, and PFH, were in a relatively low range of 0.3–1.2 ng/mg protein. The ME, which accommodates both fibers and terminals of AVP neurons, demonstrated considerably higher levels of AVP (73.2 ng/mg protein) than the other hypothalamic nuclei, while the NH was highest at over 7,000 ng/mg protein.

Following 48 h food deprivation, only three of the eight hypothalamic sites demonstrated a significant change in AVP content (Fig. 2). A strong decline (−45%, $p < 0.01$) in AVP levels was observed in the pPVN, while no change was detected in the mPVN. The SON responded similarly to the pPVN, also showing a 45% decrease ($p < 0.01$) in AVP after deprivation. However, the VMN responded differently, exhibiting an increase in AVP from its low baseline levels of 0.45 ng/mg protein to 2.01 ng/mg protein in response to deprivation ($p < 0.001$). Like the mPVN, no change in AVP was detected in the four remaining hypothalamic sites, namely, the SCN, DMN, PFH, and ME.

Refeeding for 6 h after 42 h food deprivation failed to reverse the effect of food deprivation. In fact, it significantly potentiated the decrease in AVP levels in the pPVN ($p < 0.05$) and SON ($p < 0.001$) from the satiated baseline. This pattern of change was most dramatic in the SON, which, relative to satiated control rats, showed a 45% decline in AVP

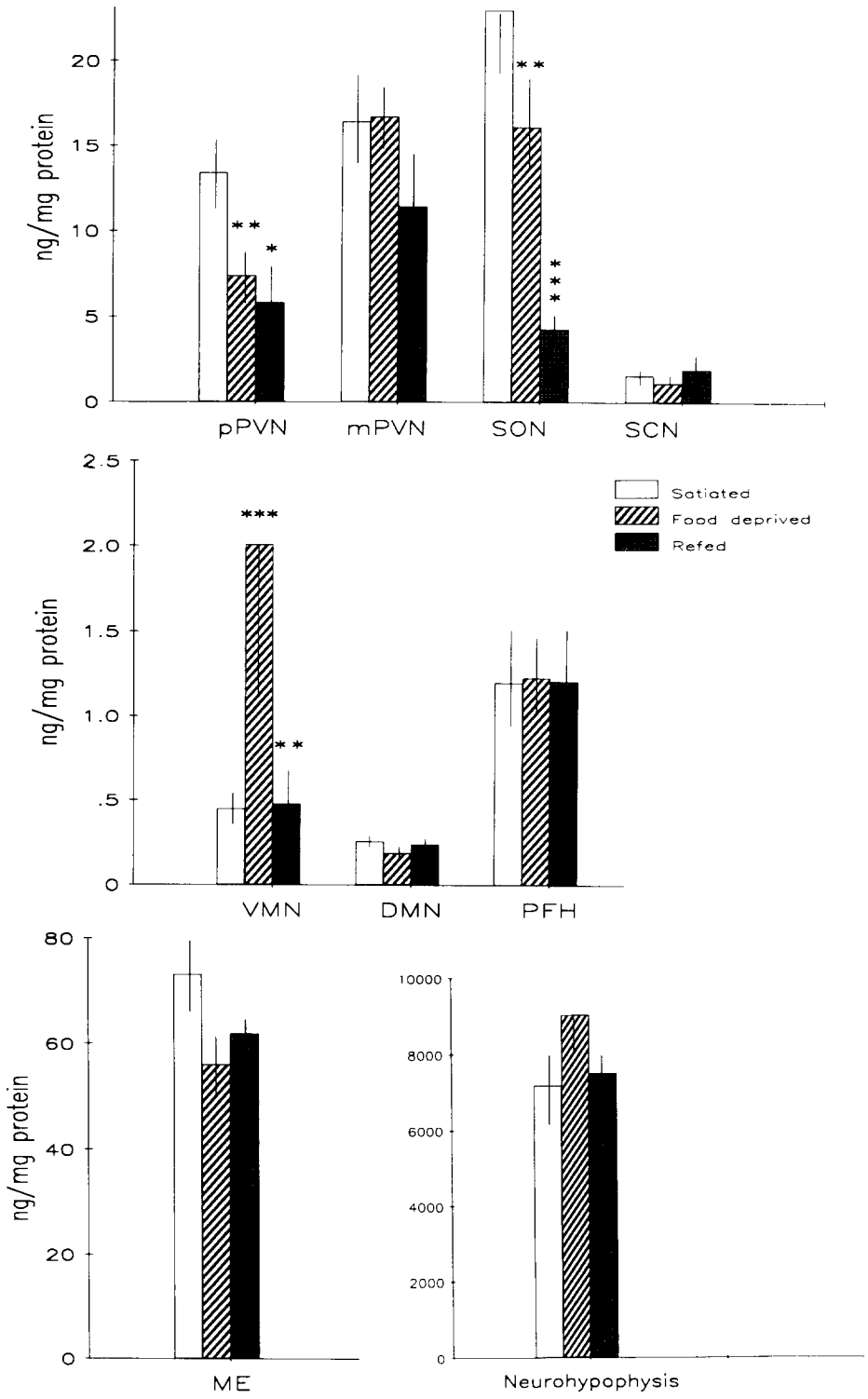

FIG. 2. Effect of food deprivation and subsequent refeeding on brain vasopressin concentration [(AVP), ng/mg protein]. Rats that were satiated (open bar), 48 h food deprived (hatched bar), and 6 h refed after 42 h food deprivation (double hatched bar) were sacrificed during the light cycle. Top panel presents the data for the hypothalamic nuclei that synthesize AVP; the middle panel presents the hypothalamic areas through which AVP-containing fibers pass; and the bottom panel shows the AVP content of areas where the AVP axons pass through [median eminence (ME)] as well as terminate (ME and neurohypophysis). Statistical comparisons between the group means revealed significant differences in the parvocellular division of the paraventricular nucleus (pPVN), supraoptic nucleus (SON), and ventromedial nucleus (VMN) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for comparisons between satiated and food-deprived rats and between food-deprived and refed rats). mPVN, magnocellular division of the PVN; SCN, suprachiasmatic nucleus; DMN, dorsomedial nucleus; PFH, perifornical hypothalamus.

TABLE 1

MEASURES OF Na+, K+, AND OSMOLALITY AND AVP IN URINE AND PLASMA AND MEASURES OF UREA, HEMATOCRIT, AVP, CORT, AND GLUCOSE IN PLASMA

	Satiated	Food Deprived	Refed
Urine			
Na+ (mEQ)	179.1 ± 18.0	48.0 ± 7.5*	83.9 ± 21.2*†
K+ (mEq)	298 ± 25	43 ± 8*	218 ± 15*†
Osmolality (mOsm)	1,797 ± 203	728 ± 138*	1,662 ± 100†
AVP (pM/h)	192 ± 25	116 ± 20*	256 ± 39†
Plasma			
Na+ (mEq)	141.3 ± 0.6	142.4 ± 0.1	141.0 ± 0.7
K+ (mEq)	6.6 ± 0.1	6.1 ± 0.1	6.4 ± 0.9
Osmolality (mOsm)	299.6 ± 1	295 ± 1.2*	304 ± 0.9*†
AVP (pM/l)	5.4 ± 0.6	6.9 ± 1.8	4.8 ± 0.5
Urea (mM/l)	7.04 ± 0.34	5.61 ± 0.20*	8.04 ± 0.38†
Hematocrit (%)	46.60 ± 0.5	46.00 ± 0.40	44.6 ± 0.30*†
Corticosterone (pM/l)	83.13 ± 20	665 ± 102*	69.5 ± 9.7†
Glucose (mM/l)	7.67 ± 0.02	5.04 ± 0.03*	8.94 ± 0.05†‡

Values are given as mean ± SEM (n = 8/group).

*Significant difference (at least $p < 0.05$) between satiated and deprived or refed rats.

†Significant difference (at least $p < 0.05$) between deprived and refed rats.

‡Significant difference (at least $p < 0.05$) between satiated and refed rats.

after deprivation and a considerably larger (82%) decline after refeeding. In the VMN, the potentiation of AVP content produced by deprivation was totally reversed by 6 h refeeding ($p < 0.01$). The other hypothalamic sites, namely, the mPVN, SCN, DMN, PFH, and ME, that maintained normal AVP levels after deprivation also remained unchanged following refeeding. The concentration of AVP in the NH (Fig. 2) failed to show any alteration following 48 h food deprivation or 6 h refeeding after 42 h food deprivation.

AVP Content in Plasma and Urine

As shown in Table 1, the concentration of AVP in plasma remained unaltered after food deprivation and refeeding. Excretion of AVP (Table 1) fell dramatically after food deprivation (−40%, $p < 0.05$), while refeeding for 6 h reversed this effect ($p < 0.001$).

NA+, K+, and Osmolality in Plasma and Urine

As shown in Table 1, urine Na+, K+, and osmolality were significantly reduced in food-deprived rats. While refeeding for 6 h totally reversed this change in osmolality, the concentration of Na+ and K+ showed only a partial reversal after refeeding, remaining significantly lower than the satiated baseline scores ($p < 0.05$). Whereas food deprivation and refeeding failed to alter plasma concentration of Na+ and K+, plasma osmolality declined significantly after food deprivation and was increased after refeeding (Table 1).

Water and Creatinine Clearance and Plasma Content of Urea and Hematocrit

Table 1 presents the data for plasma urea and hematocrit and Table 2 presents the data for water and creatinine clearance in satiated, deprived, and refed rats. Water clearance and urea content in plasma dropped significantly after food deprivation, and these changes were reversed totally by refeeding. Plasma hematocrit levels remained unaltered after food deprivation, but plasma hematocrit levels were significantly lower in refed rats from the satiated and food-deprived groups. The creatinine clearance was significantly decreased by food deprivation and 6 h of refeeding increased it above the baseline value.

CORT and Glucose Content in Plasma

Food deprivation for 48 h caused an eightfold increase in circulating levels of CORT (Table 1). Refeeding for 6 h in deprived rats produced a total reversal of this enhancement. As expected, glucose content in the plasma (Table 1) declined reliably after deprivation and rose significantly higher, even rose higher than the satiated baseline, after 6 h of refeeding.

Twenty-Four Hour Food Intake and Body Weight

As shown in Table 3, daily food intake of ad lib-fed rats ranged from 26.9 and 29.0 g/day during the 2-day pretest period, as well as during the 2 test days. In rats given food for a 6-h period after 42 h deprivation, food intake during this brief interval was a total of 18.2 g, more than half the rat's 24-h food intake under ad lib-fed state.

While the body weight of ad lib-fed rats increased approximately 5% over the 48-h pretest period (from 376–394 g, 9 g/day), 48 h food deprivation caused a continuous decline of 9–10% during the first 24 h and 13–15% (total of 57 g loss) over the entire 48-h period. Six hours of refeeding to 42-h food-deprived rats significantly attenuated ($p < 0.05$) this decline in body weight; however, it did not fully restore body weight to the level of ad lib-fed rats ($p < 0.05$).

Water Intake and Urine Output

Table 3 also presents the data for water intake and urine output in the three groups of rats. During the 2-day pretest

TABLE 2

MEASURES OF WATER AND CREATININE CLEARANCE

	Satiated	Food Deprived	Refed
Water cleareance (μl/min)	−47.23 ± 2.55	−15.53 ± 3.42*	−40.42 ± 9.76†
Creatinine clearance (μl/min)	1,930 ± 166	766 ± 75*	2,998 ± 724†

Values are given as mean ± SEM (n = 8/group).
*Significant difference (at least at $p < 0.05$) between satiated and deprived rats.
†Significant difference (at least at $p < 0.05$) between deprived and refed rats.

period, daily water intake of the three groups of rats was approximately 48.0 ml. This value remained constant during the subsequent 48-h test period for satiated rats but declined significantly (20–40%, $p < 0.001$) during 48 h food deprivation. After 6 h refeeding, water intake increased reliably, to a level significantly higher than that of deprived ($p < 0.01$) as well as satiated ($p < 0.01$) rats.

The daily output of urine (Table 3), approximately 17.0 ml in satiated rats, significantly increased during the first 24 h of food deprivation ($p < 0.001$) and then declined somewhat during the second 24-h period of deprivation. A similar pattern was observed in refed rats, and in both groups urine output at the end of the last 24-h interval remained significantly higher ($p < 0.01$) than that of satiated rats.

DISCUSSION

The results of this study demonstrate that food deprivation caused: a) a significant decline in OT concentration exclusively in the ME; b) a reliable decline in AVP concentration specifically in the pPVN and SON, an effect not reversed by refeeding; c) a significant increase in AVP concentration within the VMN that was totally reversed by 6 h refeeding; d) no change in NH levels of OT and AVP; and e) no evidence of dehydration in measures of water clearance, as well as plasma and urine AVP.

It is of interest that OT levels in the hypothalamus are in general resistant to the effects of food deprivation and refeeding. This is in contrast to a number of other potential neurotransmitters involved in feeding behavior, such as norepinephrine (20), serotonin (15), and neuropeptide Y (5, 32), which are clearly altered in specific hypothalamic nuclei by similar changes in nutritional status. The only significant change in OT levels was detected in the ME, where OT-containing fibers from the PVN are known to terminate as well as traverse on their course to the NH (9,35). These fibers in the ME exhibited a 36% decrease in hormone levels after deprivation.

TABLE 3

MEASUREMENT OF FOOD INTAKE, BODY WEIGHT, WATER INTAKE, AND URINE OUTPUT

	Pretest Period	Test Day 1	Test Day 2
Body weight (g)			
Ad lib fed	394 ± 4	400 ± 3	412 ± 3
Food deprived	391 ± 4	350 ± 2*	334 ± 2*†
Refed	387 ± 6	353 ± 4*	372 ± 7†
Food intake (g/24 h)			
Ad lib fed	26.9 ± 0.5	27.3 ± 1.3	28.6 ± 1.5
Food deprived	26.5 ± 1.2	0	0
Refed	29.0 ± 2.0	0	18.2 ± 0.5*‡
Water intake (ml/24 h)			
Ad lib fed	48.0 ± 1.7	50.1 ± 2.8	50.0 ± 2.5
Food deprived	47.7 ± 2.4	39.1 ± 4.2*	29.2 ± 5.4*
Refed	48.3 ± 1.6	32.7 ± 4.3*	60.3 ± 5.4*†
Urine output			
Ad lib fed	17.2 ± 2.0	17.3 ± 2.5	16.0 ± 2.5
Food deprived	17.8 ± 1.3	35.6 ± 3.1*	25.2 ± 4.0*†
Refed	14.4 ± 1.7	35.1 ± 6.1*	23.0 ± 4.1*†

Values are given as mean ± SEM (n = 8/group).
*Significant difference (at least at $p < 0.05$) between pretest period and test day 1 or 2.
†Significant difference (at least at $p < 0.05$) between test days 1 and 2.
‡Food intake during 6-h refeeding period.

The absence of any alteration in OT content within the PVN itself, where the OT-containing cell bodies are concentrated (9,35), may suggest that the equilibrium between the synthesis and transport of this hormone is not directly affected by deprivation. However, the changes in the ME, a decline in response to food deprivation, may reflect a disturbance in either the transport or release of OT in fibers coursing via the ME to the NH. This link, between the OT content of these projections and the animal's nutritional state, may be related to the proposed function of peripheral and possibly central OT in the inhibition of food intake or the production of satiety (3,21,39,40). Moreover, an altered level of OT was observed in the cerebrospinal fluid of patients with eating disorders, specifically, anorexia nervosa (14).

With regard to AVP, a similar response to food deprivation, namely, a decline in peptide levels, was detected exclusively in the pPVN and SON but not in the ME or any other hypothalamic nuclei. Additional measures recorded suggest that this change in hypothalamic AVP content reflects an alteration in the animal's nutritional state as opposed to a change in water balance. This is supported by the physiological and endocrine measures presented in Tables 1 and 2. These data show, after deprivation, a decrease in plasma and urine osmolality, urine electrolyte and AVP concentration, water clearance, and plasma urea; no change in NH or plasma AVP or plasma electrolytes; and an increase in urine volume and plasma hematocrit. These effects, consistent with other reports in food- or water-deprived animals (6,10,11,23,26,27,29, 36,41,42), give no indication that animals were dehydrated and actually provide some evidence that they may be overhydrated. Moreover, the findings that AVP in specific nuclei declines after food deprivation, while it increases after hypertonic saline, (43) and that AVP levels in the mPVN and NH remain unaltered, are also consistent with the suggestion that these AVP changes in the pPVN and SON are not attributed directly to modifications in osmotic stimuli.

The decline in pPVN and SON AVP concentration after deprivation may reflect either a decrease in AVP synthesis within the peptidergic neurons localized in these nuclei (9,35) or an increase in AVP release from the hypothalamic–pituitary axis that is inadequately paced by hormone synthesis. A recent study of Ogasa et al. (30) demonstrated a decrease in AVP mRNA in the SON and PVN of food-deprived rats, suggesting a decrease in AVP synthesis under this condition. It is noteworthy that these changes in hypothalamic AVP occur in the absence of any changes in ME or NH AVP, where vasopressinergic fibers from the hypothalamus are known to project (35). Thus, the disturbance in the equilibrium between synthesis and release of this neurohormone appears to be expressed exclusively in the region of the hypothalamic cell bodies, specifically in the pPVN and SON, as opposed to other hypothalamic areas.

While the evidence supports the proposal that hypothalamic AVP is shifting in relation to the animal's nutritional state, rather than to water balance, this possibility may be questioned in light of the finding that refeeding fails to reverse this effect of food deprivation. One possible explanation for this lack of reversal may be that the duration of the refeeding period, only 6 h, was too short to adequately stabilize specific physiological parameters that may contribute to the AVP response. This is reflected in the physiological measures given in Table 3, which show only a partial restoration of food intake and body weight after refeeding. Another possible explanation is that, during the period of refeeding, there occurred certain changes in water balance, as reflected in the excessive water intake (Table 3), that preclude the restoration of a normal concentration of hypothalamic AVP.

An additional measure, namely, plasma levels of CORT (Table 1), also needs to be considered in relation to the deprivation-induced changes in brain AVP. The neurons of the pPVN simultaneously synthesize AVP and corticotropin-releasing factor, and the expression of AVP in these neurons is directly subjected to the negative feedback action of the glucocorticoid (7,18,33,37). Thus, the decline in pPVN concentration of AVP may reflect the sharp rise detected in circulating levels of CORT after deprivation. This relationship, however, is not confirmed by the additional finding that refeeding totally reverses this rise in CORT, without affecting pPVN AVP concentration. The possibility that the changes in AVP concentration in the pPVN or SON are attributed to the effects of stress associated with deprivation, rather than to changes in energy balance, is not supported by the evidence that stress causes an increase in AVP synthesis within these hypothalamic nuclei (8).

The only other hypothalamic area to exhibit a change in AVP content after deprivation was the VMN. In contrast to the pPVN and SON, levels of AVP in this nucleus were significantly enhanced by deprivation, and this effect was totally reversed by refeeding. The significance of this change needs to be evaluated cautiously in light of the relatively low basal concentration of AVP that exists in the VMN. While the change observed may simply reflect an alteration in the transport of AVP to the ME or NH, electrophysiological studies demonstrate a clear excitatory effect of AVP on neural firing in the VMN (22), indicating that this peptide may in fact be released within this nucleus to modulate behavioral or physiological systems. In light of the well-known role of the VMN in control of satiety (31), this stimulatory effect of AVP on neurons in this nucleus may reflect an inhibitory effect of AVP on feeding behavior. It is of interest that this electrophysiological effect of AVP in the VMN is correlated with neuronal responses to norepinephrine and glucose, two substances that have been closely linked to the control of feeding behavior (17,24).

These changes in hypothalamic AVP, in relation to nutritional state, support the work of other studies indicating a possible role for hypothalamic AVP in the control of feeding. Brattleboro rats, which are deficient in AVP synthesis, have a reduced feeding response to glucoprivation (1), a stimulus known to release AVP (4). In rats maintained on palatable diets (2), an increase in body weight has also been associated with a decrease in AVP content in the SON and PVN, along with no change in the ME and blood. This effect, which does not occur after chronic dehydration, supports a direct link between hypothalamic AVP and the control of food intake and body weight. Consistent with this proposal is the evidence relating AVP to carbohydrate and lipid metabolism (1,12) and the finding that peripheral AVP injection suppresses feeding behavior in the goat (28). Abnormal levels of pituitary AVP have been detected in genetically obese Zucker rats (13), and, furthermore, brain AVP has been associated to human eating disorders, specifically, anorexia nervosa (16).

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REFERENCES

1. Aravich, P. F.; Sladek, C. D. Vasopressin and glucoprivic-feeding behavior: A new perspective on an "old" peptide. Brain Res. 385:245–252; 1986.
2. Aravich, P. F.; Sladek, C. D.; Forbes, G. B. The dietary obesity syndrome and vasopressin secretion. Soc. Neurosci. Abstr. 10: 651; 1984.
3. Arletti, R.; Benneli, A.; Bertolini, A. Influence of oxytocin on feeding behavior in the rat. Peptides 10:89–93; 1989.
4. Baylis, P. H.; Robertson, G. L. Rat vasopressin response to insulin-induced hypoglycemia. Endocrinology 107:1975–1978; 1980.
5. Beck, B.; Jhanwar-Uniyal, M.; Burlet, A.; Chapleur, M.; Leibowitz, S. F.; Burlet, C. Rapid and localized alterations of neuropeptide Y (NPY) in discrete hypothalamic nuclei with the feeding status. Brain Res. 528:245–249; 1990.
6. Bennett, T.; Gardiner, S. M. Water deprivation: Effects on fluid and electrolyte handling and plasma biochemistry in Long-Evans and Brattleboro rats. J. Physiol. 385:35–48; 1987.
7. Beyer, H. S.; Shannon, G. M.; Sharp, B. M. Regulation of the messenger ribonucleic acid for corticotropin-releasing factor in the paraventricular nucleus and other brain sites of the rat. Endocrinology 123:2117–2123; 1988.
8. Burbach, J. P. H.; DeHoop, M. J.; Richter, D.; DeKloet, E. R.; Haaf, J. A. T.; DeWied, D. Differential responses to osmotic stress of vasopressin-neurophysin mRNA in hypothalamic nuclei. Neuroendocrinology 39:582–584; 1984.
9. Burlet, A.; Chateau, M.; Dreyfus, F. Distribution of immunoreactive neurohypophysial peptides in the rat brain. Differential response of hypothalamic nuclei synthesizing vasopressin under various experimental conditions. In: Ajmone Marsan, C.; Traczyk, W. Z., eds. Neuropeptides and neural transmission. New York: Raven; 1980:315–332.
10. Cizek, L. J.; Simchon, S.; Nocenti, M. R. Effects of fasting on plasma volume and fluid and sodium exchanges in male rabbits. Proc. Soc. Exp. Biol. Med. 154:229–303; 1977.
11. Cowley, A. W., Jr.; Merrill, D. C.; Smith, M. J., Jr.; Skelton, M. M. Interaction of vasopressin and drinking in osmoregulation. In: Cowley, A. W., Jr.; Liard, J.; Ausiello, D. A., eds. Vasopressin: Cellular and integrative functions. New York: Raven; 1988: 157–167.
12. Crine, A. F. On the possible role played by vasopressin in paraventricular nucleus–feeding behavior relationship. Horm. Behav. 17:342–343; 1983.
13. Crowley, W. R.; George, J. M.; Jacobowitz, D. M. Levels of arginine-vasopressin and oxytocin in the pituitary gland and individual hypothalamic nuclei of the genetically obese Zucker rat. J. Endocrinol. 77:417–418; 1978.
14. Demitrack, M. A.; Lesem, M. D.; Listwak, S. J.; Brandt, H. A.; Jimerson, D. C.; Gold, P. W. CSF oxytocin in anorexia nervosa and bulimia nervosa: Clinical and pathophysiologic considerations. Am. J. Psychiatry 147:882–886; 1990.
15. Fuenmayor, L. D.; Garcia, S. The effect of feeding on 5-hydroxytryptamine metabolism in brain regions of the albino rat. Br. J. Pharmacol. 83:357–362; 1984.
16. Gold, P. W.; Kaye, W.; Robertson, G. L.; Ebert, M. Abnormalities in plasma and cerebrospinal-fluid arginine vasopressin in patients with anorexia nervosa. N. Engl. J. Med. 308:1117–1123; 1983.
17. Grossman, S. P. The role of glucose, insulin, and glucagon in the regulation of food intake and body weight. Neurosci. Biobehav. Rev. 10:295–315; 1986.
18. Itoi, K.; et al. Suppression by glucocorticoid of the immunoreactivity of corticotropin-releasing factor and vasopressin in the paraventricular nucleus of rat hypothalamus. Neurosci Lett. 73: 231–236; 1987.
19. Jhanwar-Uniyal, M.; Chapleur-Chateau, M.; Burlet, A. J.; Leibowitz, S. F. Vasopressin and oxytocin content in hypothalamic nuclei: Relation to food deprivation and subsequent refeeding. Soc. Neurosci. Abstr. 14:148; 1988.
20. Jhanwar-Uniyal, M.; Darwish, M.; Levin, B. E.; Leibowitz, S. F. Alterations in catecholamine levels and turnover in discrete brain areas after food deprivation. Pharmacol. Biochem. Behav. 26:271–275; 1987.
21. Kirchgessner, A. L.; Sclafani, A.; Nilaver, G. Histochemical identification of a pvn–hindbrain feeding pathway. Physiol. Behav. 42:529–543; 1988.
22. Kow, L. M.; Pfaff, D. W. Vasopressin excites ventromedial hypothalamic glucose-responsive neurons in vitro. Physiol. Behav. 37:153–158; 1986.
23. Kutscher, C. L. Hematocrit, plasma osmolality, and plasma protein concentration as estimators of plasma volume in hooded rats during food and water deprivation. Physiol. Behav. 7:283–285; 1971.
24. Leibowitz, S. F. Hypothalamic paraventricular nucleus: Interaction between α2-noradrenergic system and circulating hormones and nutrients in relation to energy balance. Neurosci. Biobehav. Rev. 12:101–109; 1988.
25. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
26. McKinley, M. J.; Allen, A.; Congiu, M.; Denton, D. A.; Mendelsohn, F. A. O.; Oldfield, B. J.; Weisinger, R. S. Central integration of osmoregulatory vasopressin secretion, thirst, and sodium excretion. In: Cowley, A. W., Jr.; Liard, J.; Ausiello, D. A., eds. Vasopressin: Cellular and integrative functions. New York: Raven; 1988:185–191.
27. Metzler, C. H.; Thrasher, T. N.; Keil, L. C.; Ramsay, D. J. Endocrine mechanisms regulating sodium excretion during water deprivation in dogs. Am. J. Physiol. 251:R560–R568; 1986.
28. Meyer, A. H.; Langhans, W.; Scharrer, E. Vasopressin reduces food intake in goats. Q. J. Exp. Physiol. 74:465–473; 1989.
29. Negro-Vilar, A.; Samson, W. K. Dehydration induced changes in immunoreactive vasopressin levels in specific hypothalamic structures. Brain Res. 169:585–589; 1979.
30. Ogasa, T.; Hashimoto, K.; Ota, Z. Food deprivation decreases vasopressin mRNA in the supraoptic and paraventricular nuclei of the hypothalamus in rats. Acta Med. Okayama 45:283–293; 1991.
31. Oomura, T. Input–output organization in the hypothalamus relating to food intake behavior. In: Morgane, P. J.; Panksepp, J., eds. Physiology of the hypothalamus. Handbook of the hypothalamus. vol. 2. New York: Marcel Dekker; 1980:557–620.
32. Sahu, A.; Kalra, P. S.; Kalra, S. P. Food deprivation and ingestion induce reciprocal changes in neuropeptide Y concentrations in the paraventricular nucleus. Peptides 9:83–86; 1988.
33. Sawchenko, P. E. Evidence for a local site of action for glucocorticoids in inhibiting CRF and vasopressin expression in the paraventricular nuclei. Brain Res. 403:213–224; 1984.
34. Swaab, D. F.; Pool, C. W.; Nijveld, F. Immunofluorescence of vasopressin and oxytocin in rat hypothalamo-neurohypophysial system. J. Neural Trans. 36:195–215; 1975.
35. Swanson, L. W.; Sawchenko, P. E. Hypothalamic integration: Organization of the paraventricular and supraoptic nuclei. Annu. Rev. Neurosci. 6:269–324; 1983.
36. Thrasher, T. N.; Wade, C. E.; Keil, L. C.; Ramsay, D. J. Sodium balance and aldosterone during dehydration and rehydration in the dog. Am. J. Physiol. 247:R76–R83; 1984.
37. Tramu, G.; Croix, C.; Pillez, A. Ability of the CRF immunoreactive neurons of the paraventricular nucleus to produce a vasopressin-like material. Neuroendocrinology 37:467–469; 1983.
38. Vandesande, F.; Dierickx, K.; DeMey, J. Identification of the vasopressin-neurophysin producing neurons of the rat suprachiasmatic nuclei. Cell Tiss. Res. 156:377–380; 1975.
39. Verbalis, J. G.; McCann, M. J.; Mchale, C. M.; Stricker, E. M. Oxytocin secretion in response to cholecystokinin and food: Differentiation of nausea from satiety. Science 232:1417–1419; 1986.
40. Verbalis, J. G.; Richardson, D. W.; Stricker, E. M. Vasopressin

release in response to nausea-producing agents and cholecystokinin in monkeys. Am. J. Physiol. 252:R749–R753; 1987.
41. Wright, J. W.; Gubernick, D. J.; Reynolds, T. J. Intravascular, carcass and gut fluid changes induced by food deprivation in xeric and mesic adapted rodents. Comp. Biochem. Physiol. 58A:137–142; 1977.
42. Wright, J. W.; Reynolds, T. J.; Kenny, J. T.; Donlan, K. Inhibition of food deprivation induced hypovolemia by saline consumption in rats. Physiol. Behav. 21:215–221; 1978.
43. Zerbe, R. L.; Palkovits, M. Changes in the vasopressin content of discrete brain regions in response to stimuli for vasopressin secretion. Neuroendocrinology 38:285–289; 1984.