Osmotic regulation of estrogen receptor-β expression in magnocellular vasopressin neurons requires lamina terminalis

Suwit J. Somponpun,1 Alan Kim Johnson,2 Terry Beltz,2 and Celia D. Sladek1

1Department of Physiology and Biophysics, University of Colorado Health Science Center, Denver, Colorado 80262; and 2Departments of Psychology and Pharmacology, University of Iowa, Iowa City, Iowa 52242

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Somponpun, Suwit J., Alan Kim Johnson, Terry Beltz, and Celia D. Sladek. Osmotic regulation of estrogen receptor-β expression in magnocellular vasopressin neurons requires lamina terminalis. Am J Physiol Regul Integr Comp Physiol 286: R465–R473, 2004.—Estrogen receptor-β (ER-β) expression in rat magnocellular vasopressin (VP) neurons of the supraoptic and paraventricular nuclei (SON and PVN, respectively) becomes undetectable after 72 h of 2% NaCl consumption. To test the hypothesis that osmosensitive mechanisms that originate in the region of the organum vasculosum lamina terminalis (OVLT) control ER-β expression in the SON and PVN, animals were deprived of water after electrolytic lesions were performed on the area anterior to the ventral third ventricle (AV3V). Such lesions prevent osmotic stimulation of VP release. Four weeks after surgery, male rats [lesioned (n = 16) or sham (n = 14)] were water deprived for 48 h or allowed water ad libitum. Water deprivation eliminated ER-β-immunoreactivity (-ir) in SON and magnocellular PVN of sham-lesioned animals. Fos-ir was evident in these neurons, and plasma osmolality (Posm) and hemocrit (Ht) were significantly elevated compared with the sham-hydrated rats (Posm 304 ± 1 vs. 318 ± 2 mosmol/kgH2O; P < 0.001; Ht 49.6 ± 0.6 vs. 55.0 ± 0.9%; P < 0.001). ER-β expression was comparable in sham-hydrated, AV3V-hydrated, and 6 of 8 AV3V-dehydrated rats despite significant increases in Posm in both groups (AV3V hydrated, 312 ± 2; AV3V dehydrated, 380 ± 10 mosmol/kgH2O; P < 0.001). OVLT was not ablated in the AV3V-dehydrated rats in which ER-β was depleted. Fos-ir was low or undetectable in SON in the AV3V-hydrated animals despite elevated Posm values. In AV3V-dehydrated rats, Fos-ir was significantly less than in sham-dehydrated animals but was significantly increased compared with the sham-hydrated group. This could reflect activation by nonosmotic parameters that do not inhibit ER-β expression. These data support the hypothesis that inhibition of ER-β expression in the SON by osmotic stimulation is mediated by osmoreceptive neurons in the lamina terminalis.

THE CONTROL OF BODY-FLUID homeostasis is achieved through a series of coordinated behavioral, autonomic, and endocrine responses. Vasopressin (VP), a hormone synthesized by the hypothalamic magnocellular neurosecretory cells (MNCs) in the supraoptic and paraventricular nuclei (SON and PVN, respectively) and secreted from the neurohypophysis, regulates water reabsorption by the kidney and is an important component of the endocrine homeostatic response. Although a variety of stimuli activate the MNCs and hence initiate VP release from the neurohypophysis, an increase in plasma osmolality (Posm) is the most potent physiological stimulus for VP release. Osmotic stimulation occurs as a result of interactions between the intrinsic osmosensitive properties of the MNCs and synaptic inputs from affenter osmosensitive pathways that arise from rostral forebrain structures anterior to the third ventricle. Electrolytic lesions of the tissue anterior to the ventral third ventricle (AV3V) disrupt osmotic regulation of VP secretion. These lesions typically destroy the organum vasculosum of the lamina terminalis (OVLT), the ventral median preoptic nucleus (vMnPO), and fibers of passage from the subfornical organ (SFO). Both the OVLT and SFO contain osmoreceptive neurons, and both project directly to the SON and PVN as well as indirectly via the vMnPO (5, 14, 23).

The MNC VP neurons express estrogen receptor-β (ER-β; Refs. 1, 12, 19, 28, 29). Although the precise role of ER-β in MNCs remains to be established, evidence from this laboratory suggests an inhibitory role that includes modulation of osmotic responses. Physiological concentrations of estradiol (E2) inhibit both osmotically stimulated and NMDA-induced VP release from explants of the hypothalamo-neurohypophysial system (31, 34). Genistein, which is an ER-β agonist, mimics the effects of E2 on NMDA-induced VP release, whereas a specific ER-β antagonist blocks E2 inhibition (31). We have also shown that ER-β expression is inversely correlated with the osmotic state of the animal. Thus ER-β is depleted in MNCs after animals are subjected to 72 h of 2% saline drinking and is upregulated by the presence of chronic hyponatremia (32). This inverse correlation with Posm supports an inhibitory role for ER-β that is removed during osmotic stimulation of VP release.

In the present study, we used AV3V-lesioned rats to evaluate the role of osmosensitive mechanisms that originate in the lamina terminalis on the osmotically stimulated inhibition of ER-β expression in the SON and PVN. Water deprivation (for 48 h) was used to induce a chronic increase in Posm.

METHODS

Animals. Adult male Sprague-Dawley rats [Crl:CD(SD)BR; body wt, 250–300 g; Charles Rivers Laboratories, Wilmington, MA] were singly housed and maintained on a 12:12-h light-dark cycle (with lights on at 0600) at an ambient temperature of 21 ± 1°C. Male rats were used in this study; ER-β is expressed in males as well as females, and testosterone can be metabolized to E2 as well as to other steroids that represent potential ligands for ER-β. In addition, using only male rats allowed us to avoid potential variation in E2 secretion across the female estrus cycle. Standard rat chow was available ad libitum throughout the entire experiment. Body weight and fluid intake measurements from each rat were determined daily between 0700 and 0900.


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1000. All protocols used were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Colorado Health Science Center.

**AV3V lesions and pre- and postsurgery drinking regimens.** Rats were allowed 4 days to acclimate to their new surroundings before surgery. They had ad libitum access to tap water for the first day, followed by a 10% (wt/vol) sucrose solution on the second and third days, and had tap water again the night before the surgery. The purpose of the presurgery drinking regimen was to prime the rats to the sucrose solution that would be used to encourage drinking in rats with adipsia after the surgery and thus reduce the mortality rate. The rats were given tap water the night before the surgery to prevent association of the surgical trauma with the sucrose solution. AV3V lesions were performed as previously described (13). Specifically, the animals were anesthetized with avertin (2,2,2-tribromoethanol, 40 mg/ml at 1 ml/100 g of body wt; Sigma, St Louis, MO). A nichrome electrode was placed stereotaxically in the AV3V region (midline, 0.2 mm posterior to the bregma and 7.5 mm below the dura; skull was leveled between bregma and lambda), and an anodal direct current of 2.5 mA was passed through the electrode for 20 s. For sham lesions, the electrode was lowered 6.5 mm ventral to the dura, and no current was applied. All lesioned rats were functionally adipsic (i.e., overnight fluid intake was <10 ml) immediately following surgery. Adipsic animals were given ad libitum access to a 10% sucrose solution to provide adequate hydration, whereas sham-lesioned animals continued to receive tap water. The rats were gradually weaned from the sucrose solution to tap water, and the experiment began 4 wk after the surgery, at which point the lesioned and sham-operated animals consumed comparable amounts of tap water.

**Water deprivation.** After the postsurgery weaning period, animals were either continued on ad libitum water (AV3V hydrated or sham hydrated, n = 7 each) or were water deprived (AV3V dehydrated or sham dehydrated, n = 8 each) for 48 h. All rats were anesthetized with avertin and perfused transcardially with physiological saline solution followed by 3.75% acrolein (electromicroscopy grade; Electron Microscopy Sciences, Fort Washington, PA) in phosphate-buffered 4% paraformaldehyde (pH 6.7; Sigma) between 0800 and 1300. Brains were collected and processed for immunocytochemistry. Blood was collected via cardiac puncture for measurement of hematocrit (via microcapillary method), P_{sm} (using a microvapor pressure osmometer; Wescor, Logan, UT), plasma sodium (P_{Na}; via flame photometry; Corning 435), plasma glucose (P_{glucose}; using Accu-Chek; Roche Diagnostics, Indianapolis, IN), blood urea nitrogen (BUN; using BUN Analyzer 2; Beckman Instruments, Fullerton, CA), and free testosterone (Active Free Testosterone Radioimmunoassay Kit; Diagnostic Systems Laboratories, Webster, TX).

**Immunocytochemistry for ER-β or Fos.** The immunocytochemistry procedure was performed as previously described (32). After fixation, brains were removed and allowed to sink in 30% aqueous sucrose for at least 72 h. Cryostat brain slices (30 μM thick starting rostrally at the level of OVLT and ending caudally at the lateral hypothalamic area) were collected in a one-in-four series and placed in culture wells with cryoprotectant solution (30% sucrose and 30% ethylene glycol in 0.1 M PBS) until processing. Each set of adjacent sections was used to localize either ER-β or Fos immunoreactivity (-ir) in brain areas of interest. Cryoprotectant was removed from freely floating tissue sections with multiple rinses of 0.05 M potassium phosphate-buffered saline (KPBS, pH 7.4). Tissues were then treated with 1% sodium borohydride for 20 min followed by several rinses with KPBS and were incubated with 0.2% Triton X-100 (twice for 10 min each) and 0.1 M glycine for 30 min. All solutions were prepared in KPBS, and multiple rinses with KPBS were used between each treatment. The sections were then incubated in blocking solution (20% normal donkey serum, 1% BSA, and 1% H_{2}O_{2} in KPBS) for 30 min. All procedures were carried out at room temperature (RT). The tissues were subsequently incubated in primary antibody with an affinity-
purified rabbit polyclonal antiserum raised against amino acids 468–485 of ER-β (ZBP; Zymed Laboratories, San Francisco, CA) at a 1:5,000 dilution made up in KPBS with 0.4% Triton X-100 and 1% normal donkey serum for 60 min at RT then 72 h at 4°C. The sections were rinsed with KPBS (10 times for 5 min each), incubated with a biotinylated donkey anti-rabbit serum (Jackson ImmunoResearch, West Grove, PA) at a 1:600 dilution in KPBS with 0.4% Triton X-100. Tissues received avidin-biotin complex solution for 1 h at RT (Vector Elite Kit; Vector Laboratories, Burlingame, CA; with 50 µl each of avidin and biotin in 10 ml of KPBS with 0.4% Triton X-100). Tissues received three 5-min rinses in KPBS followed by three 5-min rinses in 0.175 M sodium acetate. Primary antibody was localized using a conventional immunoperoxidase method with a 15-min exposure to nickel sulfate (25 mg/ml) plus diaminobenzidine-HCl (DAB, 0.2 mg/ml) in sodium acetate solution that contained 0.83 µl of 3% H2O2. This yielded a blue-black reaction product in the nuclear compartment. After three 5-min rinses in sodium acetate, samples received three 5-min rinses in KPBS. Sections were mounted on poly-l-lysine-treated slides, air-dried overnight, dehydrated in a series of graded alcohols, cleared in Histoclear (National Diagnostics, Atlanta, GA), and coverslipped with mounting medium (Fisher Diagnostics, Middletown, VA).

For Fos immunolabeling, all procedures were conducted in an identical manner to the ER-β-immunolabeling method except that the ER-β primary antibody was replaced with a rabbit affinity-purified polyclonal antibody against c-Fos (sc-253; Santa Cruz Biotechnology, Santa Cruz, CA) at a 1:120,000 dilution in KPBS with 0.4% Triton X-100 and 1% normal serum. Sections were incubated for 60 min at RT and then for 72 h at 4°C.

### RESULTS

#### Characterization of AV3V lesions.
Postoperative adipsia provided an initial indication of lesion success. All AV3V-lesioned animals demonstrated postsurgical adipsia (defined as <10 ml of fluid intake overnight). Decreased drinking was also observed in the sham-lesioned animals immediately postsurgery (presurgery, 43.1 ± 3.1; postsurgery, 25.2 ± 2.2 ml), but rats resumed their presurgery drinking patterns within 24–48 h.

Histological analysis of the lesions was performed on sections stained for ER-β and Fos. As shown in Fig. 1, a typical lesion ablated the OVLT as well as the vMnPO but left the SFO as well as the SON and PVN intact. However, as discussed below, incomplete lesions were observed in two animals assigned to the AV3V-lesioned group.

#### Effects of AV3V lesions and water deprivation on body weight.
The initial weights and the rates of weight gain of the rats before surgery were comparable among all animals. After the surgery, body weight in sham-lesioned animals decreased slightly but then rapidly recovered and continued to increase throughout the recovery period. In contrast, the AV3V-lesioned animals had a dramatic drop in body weight that persisted for several days before they began to gain weight.
The body weight of AV3V-lesioned animals remained significantly lower than the sham-lesioned animals throughout the experiment (Fig. 2). Water deprivation for 48 h induced a significant decrease in body weight in both AV3V- and sham-lesioned animals compared with their respective water-replete controls (Fig. 2).

**Effects of AV3V lesions and water deprivation on $P_{osm}$ and hematocrit.** As shown in Table 1, $P_{osm}$ was increased by both the lesion ($F = 28.04; P < 0.001$) and dehydration ($F = 30.85; P < 0.001$). The AV3V lesion induced a rise of 8 mosmol/kg H$_2$O in $P_{osm}$ in nondehydrated animals. Dehydration increased $P_{osm}$ by 14 in sham-lesioned rats but further increased $P_{osm}$ by 76 mosmol/kg H$_2$O in AV3V-lesioned rats from the level in sham-hydrated controls ($P < 0.001$). $P_{Na}$, $P_{glucose}$, and BUN were analyzed to determine the relative contributions of these agents to the large increase in $P_{osm}$ observed in the AV3V-dehydrated animals (Table 1). Similar to $P_{osm}$, $P_{Na}$ was increased by both the lesion ($F = 9.7; P < 0.005$) and
dehydration \((F = 12.24; P < 0.002)\). Interestingly, \(P_{\text{glucose}}\) was significantly reduced by the AV3V lesion regardless of hydramonal state \((F = 37.15; P < 0.001)\). There was also a significant increase in BUN in the AV3V-dehydrated group. However, the changes in \(P_{\text{osm}}\) primarily reflected changes in these agents; using the formula \((17)\) \(P_{\text{osm}} = [2(P_{\text{Na}}) + P_{\text{glucose}}/18 + \text{BUN}/2.8]\) predicted \(P_{\text{osm}}\) values that did not differ significantly from measured \(P_{\text{osm}}\) values in any of the groups (Table 1).

Hematocrit was significantly increased only in sham-dehydrated animals compared with other groups \((F = 13.102; P < 0.001; \text{Table 1})\). Thus AV3V-lesioned rats remained capable of regulating their blood volume but failed to monitor changes in their \(P_{\text{osm}}\).

**Expression of ER-\(\beta\) in SON and PVN magnocellular neurons.** As previously reported \((1, 12, 28, 32)\), numerous intensely labeled ER-\(\beta\)-ir neurons were found throughout the rostrocaudal extent of the SON (Fig. 3A) and posterior magnocellular PVN neurons (Fig. 4A) in sham-hydrated controls. Water deprivation for 48 h virtually eliminated ER-\(\beta\)-ir from SON (Fig. 3B) and magnocellular PVN neurons (Fig. 4B) in sham-dehydrated rats. SON and magnocellular PVN were virtually devoid of ER-\(\beta\) positive nuclei in all seven sham-lesioned dehydrated rats, although ER-\(\beta\) staining persisted in parvocellular regions of PVN. In contrast, the AV3V-hydrated animals continued to express ER-\(\beta\) in SON (Fig. 3C) and magnocellular PVN (Fig. 4C) despite an increased \(P_{\text{osm}}\) that was comparable to the dehydrated sham-lesioned rats. All eight animals in this group retained prominent ER-\(\beta\) staining in MNCs. The majority of AV3V-dehydrated rats (6 of 8 animals) exhibited prominent expression of ER-\(\beta\) in the SON (Fig. 3D). Again, this was despite an extreme increase in \(P_{\text{osm}}\) \((380 \pm 10\) mosmol/kgH\(\text{O}, n = 6)\) following water deprivation in these animals. ER-\(\beta\) expression was absent in SON neurons in two of the AV3V-dehydrated rats, but histological examination demonstrated an incomplete ablation of the OVLT in these animals (see Fig. 6). These two rats also had the lowest \(P_{\text{osm}}\) values \((332\) and \(340\) mosmol/kgH\(\text{O}\)) and highest hematocrit values \((56\) and \(58\)%\) among the rats in the AV3V-dehydrated group. Thus based on histology, \(P_{\text{osm}}\), and hematocrit, these animals likely had incomplete AV3V lesions. Nevertheless, \(\chi^2\)-analysis for the presence or absence of ER-\(\beta\) staining in SON indicated a significant difference between the groups \((\chi^2 = 22.86; P < 0.001)\), and the Fisher exact test for individual group comparisons demonstrated a significant difference between sham-dehydrated and AV3V-dehydrated rats \((P = 0.007)\) even when the two animals with ER-\(\beta\) expression were included. Therefore, we conclude that the AV3V region is required for inhibition of ER-\(\beta\) expression in SON in response to water deprivation. As shown in Fig. 5A, not only was ER-\(\beta\) expression not suppressed in SON in the AV3V-lesioned animals, the number of SON cells that exhibited intense ER-\(\beta\)-ir was significantly greater in the AV3V-hydrated than as AV3V-dehydrated rats compared with the sham-hydrated rats \((P = 0.008)\). This suggests that ER-\(\beta\) expression is partially inhibited in ehydrated, intact rats.

ER-\(\beta\) expression in magnocellular PVN was more variable than in SON in the AV3V-dehydrated group. As in the SON, it was virtually absent in the two animals with incomplete AV3V lesions (Fig. 6). However, it was also noticeably diminished in four of the remaining six animals in the AV3V-dehydrated group in contrast to the strong expression of ER-\(\beta\) in SON in the same animals (Fig. 7). ER-\(\beta\) expression was comparable to sham-hydrated rats in the remaining two AV3V-dehydrated animals (see Fig. 4). As shown in Fig. 5B, cell counts in magnocellular PVN corroborated the partial decrease in ER-\(\beta\) expression in the AV3V-lesioned animals. The number of ER-\(\beta\)-positive cells was significantly reduced in the AV3V-dehydrated group in comparison to the sham-dehydrated group but it was significantly greater than in the sham-dehydrated group.

The decrease in ER-\(\beta\)-ir in the sham-dehydrated groups was not due to receptor regulation by circulating gonadal steroids, because the free plasma testosterone concentration was not significantly different among the groups (see Table 1); thus there was no correlation between ER-\(\beta\) and free testosterone.
Expression of Fos in magnocellular neurons. Fos-ir was increased in SON (see Fig. 3F) and magnocellular PVN (see Fig. 4F) neurons after water deprivation in sham-dehydrated rats compared with sham-hydrated controls (see Figs. 3E and 4E). Thus as was observed after chronic saline consumption (32), an inverse correlation exists between Fos and ER-β expression in MNCs in the sham-lesioned groups. As reported previously (24, 35), it was low or undetectable in SON (see Fig. 3G) and magnocellular PVN (see Fig. 4G) neurons in the AV3V-dehydrated animals despite the elevated $P_{\text{osm}}$ levels. In AV3V-dehydrated rats, however, Fos expression was variable. In both SON and magnocellular PVN, the number of Fos-positive cells in the AV3V-dehydrated group was significantly increased compared to the sham-hydrated group, but was significantly less than in the sham-dehydrated group (excluding the two animals with incomplete lesions; two-way ANOVA: SON, $F = 53.00$ and $P < 0.001$; PVN, $F = 22.07$ and $P < 0.001$). In PVN, Fos expression was inversely correlated with ER-β staining (Fig. 7), which suggests that the lesions caused variable destruction of osmotic afferents to PVN. In SON, the continued expression of ER-β in the presence of Fos activation suggests that in AV3V-lesioned animals, water deprivation activated neurons by pathways that were ineffective in inhibiting ER-β expression (Fig. 7).

DISCUSSION

In this study, we examined the contributions of neural input from the AV3V region in regulating the expression of ER-β in MNCs during water deprivation. Although VP and OT neurons themselves are osmoreceptive and respond directly to changes in the osmotic pressure of their extracellular environment, they require excitatory synaptic input to fire in response to increases in $P_{\text{osm}}$ levels (21, 26). Osmosensitive neurons also reside in the OVLT and communicate osmotic information to the MNCs via glutamatergic synapses (26). Thus interaction between the intrinsic properties of the MNCs and osmosensitive synaptic inputs dictates the magnitude of the hormonal responses. AV3V lesions destroy the osmosensitive neurons in the OVLT and disrupt afferents to the MNCs from the SFO and vMnPO. This results in impaired release of VP (and oxytocin) from the neurohypophysis following osmotic stimulation in animals with AV3V lesions (20, 25). The present study demonstrates that AV3V lesions also disrupt inhibition of ER-β expression in SON MNCs after water deprivation. This supports the hypothesis that osmotically sensitive afferents from the AV3V region are responsible for inhibition of ER-β expression during chronic dehydration and demonstrates that the intrinsic osmosensitive properties of the MNCs are not sufficient to inhibit ER-β expression.

The continued expression of ER-β in SON of AV3V-dehydrated rats also suggests that afferent pathways other than those from the lamina terminalis to the MNCs are not effective in inhibiting ER-β expression under these conditions. This is based on the presence of Fos-ir in the SON of AV3V-dehydrated rats, which indicates that these neurons are being activated by excitatory afferents. The excitatory input probably elicits VP release, because AV3V-lesioned animals excrete urine that is concentrated compared with sham-lesioned rats after 3 days of water deprivation (11). Specific candidates for stimulation of VP release during water deprivation in AV3V-lesioned animals are pathways that carry information from peripheral osmoreceptors (or sodium receptors) and pathways that carry information about blood volume. Extensive evidence for regulation of VP secretion by visceral osmoreceptors or sodium receptors exists (14). Specifically, water and sodium are more effective in eliciting diuresis and antidiuresis, respectively, when administered into the portal vein than when given intravenously (10), and hyperosmotic solutions administered intragastrically or into the portal vein increase plasma VP without altering systemic osmolality (2, 7, 8, 33). However, information from these receptors is then transmitted to the vMnPO in the hypothalamus via multisynaptic pathways, where it is integrated with information from the central osmoreceptors (15). Thus AV3V lesions not only destroy central osmoreceptive mechanisms but also interrupt the pathways that carry information from peripheral osmoreceptors. Therefore, the presence of Fos-ir in MNCs of the AV3V-dehydrated rats cannot be due to peripheral osmoreceptors.

Blood volume is another important regulator of VP secretion during dehydration in intact rats. Information about blood
any effects on ER-β expression that might have been detected with the 2% NaCl drinking paradigm following AV3V lesion could, arguably, have been due to differences in the amounts of fluid intake. Therefore, water deprivation provided a uniform manipulation in the sham- and AV3V-lesioned groups. However, water deprivation not only causes hypertonicity but also induces hypovolemia, both of which potently activate MNCs. Had ER-β been eliminated in the AV3V-lesioned rats, it might have reflected activation of the MNCs by hypovolemia rather than a sustained effect of hypertonicity. However, ER-β was not depleted in the animals with complete AV3V lesions, which suggests that hypovolemia did not contribute to the regulation of ER-β expression in MNCs in these animals. This could indicate that the brain stem afferents that carry cardiovascular information do not regulate ER-β expression in MNCs, or it could indicate that the hypovolemia in AV3V-lesioned animals was too small to suppress ER-β expression. The latter possibility is supported by the lack of evidence for hypovolemia in the AV3V-dehydrated rats. Hematocrit was used as an index of blood volume, and although it was significantly elevated in the sham-dehydrated rats (indicating water deprivation-induced hypovolemia), it was not significantly elevated in the AV3V-lesioned rats. However, a prior study did report a significant decrease in both blood and plasma volume without a detectable increase in hematocrit in AV3V-lesioned rats (4). Thus there may have been a decrease in blood volume that was not detected as a change in hematocrit. Nevertheless, during water deprivation, the AV3V-lesioned rats conserved blood volume better than the sham-lesioned animals, and therefore stimulation of VP release by hypovolemia may have been inadequate to suppress ER-β expression. The conservation of blood volume may be due to decreased dehydration-induced natriuresis in the AV3V-lesioned animals. In sheep, lesions of the OVLT/MnPO attenuate dehydration-induced natriuresis and result in extreme hypernatremia as was observed here (22). The SON also receives numerous afferent projections from other brain stem, limbic, and hypothalamic regions (30) that could contribute to the Fos response in AV3V-dehydrated animals. In addition, although the endogenous osmoreceptivity of MNCs is not normally sufficient to elicit VP release without excitatory input from the AV3 region (21, 26), given the extreme increase in P osm induced by water deprivation in the AV3V-dehydrated rats, it is possible that direct osmotic activation of the MNCs occurred in these animals, which could have contributed to Fos expression without inhibiting ER-β expression. Regardless of the source of stimulation of the SON MNCs in the AV3V-dehydrated rats, there clearly was a disruption in the previously observed inverse correlation between Fos and ER-β in SON (32). The conservation of ER-β expression in Fos-expressing neurons supports the hypothesis that ER-β expression is selectively regulated by specific afferents to SON.

The partial depletion of ER-β in magnocellular PVN among the AV3V-dehydrated animals as well as the presence of strong Fos expression in these animals suggests that the AV3V lesions did not completely eliminate osmoreceptive afferents to the PVN. A differential effect of AV3V lesions on the response to water deprivation in SON and PVN was observed in early ultrastructural studies (6). Although axonal degeneration occurred in both SON and PVN following an AV3V lesion, by 5
wk postlesion. PVN neurons of water-deprived, AV3V-lesioned rats exhibited fine structural changes that are characteristic of increased secretory activity (e.g., cellular hypertrophy and increased numbers of Golgi stacks and neurosecretory granules). In contrast, SON neurons appeared smaller and contained fewer neurosecretory granules than control animals (6). Similarly and comparable to our results, Xu and Herbert (36) reported that 24 h of water-deprivation-induced Fos expression was less-completely suppressed in PVN than in SON by AV3V lesions. The likely explanation for this difference is that some PVN afferents from the SFO exit the lamina terminals above the lesion site, whereas SFO afferents to SON continue to descend in the lamina terminals and therefore are completely disrupted by the lesion (6, 36). Therefore, afferents to the SON are more completely destroyed than those to the PVN.

The observation that ER-β-ir is actually increased in SON of the AV3V-lesioned rats suggests that ER-β is partially suppressed in normally hydrated rats. This is consistent with our previous observation that ER-β mRNA is increased by chronic hyponatremia (32). It is also consistent with the early ultrastructural observation that the MNCs in AV3V-lesioned rats appear quiescent and contain fewer neurosecretory granules than control animals (6).

In conclusion, we have demonstrated for the first time that ER-β expression in rat MNCs is depleted by 48 h of water deprivation. Because this response is lost in SON and is attenuated in PVN by lesions of the AV3V region, it occurs in response to osmoreceptive elements and/or fibers of passage in the AV3V region.

Perspectives

The physiological significance of ER-β expression in the MNCs remains an important question. Although the evidence to date supports an inhibitory role for ER-β during osmotic stimulation of VP release, it is evident that euvhymed adult animals retain the ability to release VP in response to increases in P_{Osm} despite exposure to gonadal steroids. Possible explanations for this as follows. 1) In the whole animal, the gonadal steroids may have multiple offsetting actions such that the overall responsiveness of the neurohypophyseal system to osmotic control is retained. This possibility is supported by the fact that the results of numerous studies evaluating the impact of gender and gonadal steroid hormone production on VP secretion have been inconsistent (see Refs 10, 32, and 34 for review of the literature). 2) Gonadally produced steroid hormone may not be the primary endogenous ligand for the receptor. There is evidence for de novo neural production of steroids (3), and several enzymes required for steroidogenesis are present in the hypothalamus including steroidogenic acute regulatory protein and P-450 aromatase (16, 27). Furthermore, in addition to estrogen, the androgenic metabolite 5α-androstane-3α,17β-diol has affinity for ER-β (18). 3) ER-β may inhibit transcription of genes that are important for responses to chronic or intense stimulation rather than those involved in responses to small fluctuations in water balance. Thus complete understanding of the physiological role of ER-β in the hypothalamo-neurohypophysial system awaits elucidation of the genes regulated by ER-β.

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