Estrogen receptor-α expression in osmosensitive elements of the lamina terminalis: regulation by hypertonicity

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Somponpun, Suwit J., Alan Kim Johnson, Terry Beltz, and Celia D. Sladek. Estrogen receptor-α expression in osmosensitive elements of the lamina terminalis: regulation by hypertonicity. Am J Physiol Regul Integr Comp Physiol 287: R661–R669, 2004. First published May 13, 2004; 10.1152/ajpregu.00136.2004.—The subfornical organ (SFO), median preoptic nucleus (MnPO), and organum vasculosum lamina terminalis (OVLT), which are associated with the lamina terminalis, are important in the control of body fluid balance. Neurons in these regions express estrogen receptor (ER)-α, but whether the ER-α neurons are activated by hypertonicity and whether hypertonicity regulates ER-α expression are not known. Using fluorescent, double-label immunocytochemistry, we examined the expression of ER-α-immunoreactivity (ir) and Fos-ir in control and water-deprived male rats. In control animals, numerous ER-α-positive neurons were expressed in the periphery of the SFO, in both the dorsal and ventral MnPO, and in the dorsal cap of the OVLT. Fos-positive neurons were sparse in euhydrated rats but were numerous in the SFO, MnPO, and the dorsal cap of the OVLT after 48-h water deprivation. Most ER-α-ir neurons in these areas were positive for Fos, indicating a significant degree of colocalization. To examine the effect of dehydration on ER-α expression, animals with and without lesions surrounding the anterior and ventral portion of the 3rd ventricle (AV3V) were water deprived for 48 h. Water deprivation resulted in a moderate increase in ER-α-ir in the SFO of sham-lesioned rats (P = 0.03) and a dramatic elevation in AV3V-lesioned animals (P < 0.05). This was probably induced by the significant increase in plasma osmolality in both dehydrated groups (P < 0.001) rather than a decrease in blood volume, because hematocrit was significantly increased only in the dehydrated sham-lesioned animals. Thus these studies implicate the osmosensitive regions of the lamina terminalis as possible targets for sex steroid effects on body fluid homeostasis.

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plasma estradiol concentration observed during the estrus cycle [e.g., 3–4 ng/ml for testosterone in males vs. 50 pg/ml for estradiol in females (43, 55)], even low to moderate aromatase expression may be sufficient to generate localized estradiol concentrations sufficient to activate ERs in male brain.

ERs are expressed in several regions important for regulation of fluid and electrolyte balance. ER-α is expressed in all of the osmoreceptive regions along the lamina terminals as well as in the perinuclear zone of SON (33, 36, 61), whereas ER-β is expressed in the magnocellular VP neurons in both SON and PVN (1, 11, 19, 40, 41). ER-β expression in the SON is regulated by changes in plasma osmolality, and this is dependent on afferents from the osmoreceptive elements in structures associated with the lamina terminals (46, 47).

The current studies were performed to determine if the ER-α-expressing neurons in the lamina terminalis-associated structures are the same neurons that are activated by increases in plasma osmolality associated with dehydration and to determine if chronic exposure to hypertonicity alters ER-α expression.

METHODS

Animals. Adult male Sprague-Dawley rats [Crl:CD(SD)BR; 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 and ambient temperature at 21 ± 1°C. Standard rat chow was available ad libitum throughout the entire experiment. All protocols used were performed in accordance with the National Institutes of Health Guidelines 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.

Water deprivation. After acclimation to the laboratory or after the post-surgery weaning period (see below), animals were either continued on ad libitum water or were water deprived for 48 h. In the experiment evaluating colocalization between ER-α-immunoreactivity (ir) and Fos-ir, n = 3 for both groups. In the AV3V lesion experiment, n = 6–7/group (hydrated sham-lesioned, dehydrated sham-lesioned, hydrated AV3V-lesioned, and dehydrated AV3V-lesioned rats).

AV3V lesion. The ablation procedure, histological evaluation, and effects of the lesion and water deprivation on blood parameters and ER-β expression in these rats have been described in a previous report (46). Briefly, the rats were allowed 4 days to acclimatize to their new surrounding before surgery. During this period they were also acclimated to the sucrose solution that would be used post-surgery to encourage drinking in rats with adipsia after the surgery, thus reducing the mortality rate. AV3V lesions were performed as previously described (12). Specifically, the animals were anesthetized with Avertin (2,2,2-tribromoethanol, Sigma; 40 mg/ml at 1 ml/100 g body wt). A nichrome electrode was placed stereotaxically in the AV3V region (midline, 0.2 mm posterior to bregma and 7.5 mm below dura; the skull was leveled between bregma and lambda), and 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 dura, and no current was applied. This protocol produced lesions that were limited to the OVL, ventral MnPO, and the immediately surrounding area. Representative pictures have been published previously (46). Although immediately after surgery, all lesioned rats were functionally adipsic, all rats had been weaned from the sucrose solution and had been drinking only water for a minimum of 11 days before the experiment. At the time of the experiment (4 wk post-surgery), the lesioned and sham-operated animals were consuming 54 ± 7 and 38 ± 2 ml of tap water/day, respectively.

Brain perfusion. As described previously (46), all rats were anesthetized with Avertin (2,2,2-tribromoethanol, Sigma; 40 mg/ml at 1 ml/100 g body wt) and perfused transcardially with physiological saline, followed by 3.75% acrolein (EM Grade, Electron Microscopy Sciences, Fort Washington, PA) in phosphate-buffered 4% parafomaldehyde (pH 6.7; Sigma, St. Louis, MO) between 0800 and 1300. Brains were collected and processed for immunocytochemistry (see below). Blood was collected via cardiac puncture for plasma osmolality (microvapor pressure osmometer; Wescor, Logan, UT), plasma sodium measurement (Flame photometry, Corning 435), and free testosterone (Active Free Testosterone Radioimmunoassay Kit, Diagnostic Systems Laboratories, Webster, TX). These data were reported previously (46).

Single immunolabeling for ER-α or Fos. The immunocytochemistry procedure was performed as previously described (47). After perfusion, brains were removed and allowed to sink in 30% aqueous sucrose for at least 72 h. Thirty-micrometer-thick cryostat brain slices were placed in cryoprotectant solution (30% sucrose, 30% ethylene glycol in 0.1 M PBS) until processed. Adjacent brain sections were used to localize either ER-α-ir or Fos-ir in the lamina terminals. After removal of cryoprotectant and treatment with 1% sodium borohydride (46), sections were incubated in primary antibody with either a rabbit antisem directed against the last 15 amino acids of rat ER-α (C1355; Upstate, Lake Placid, NY) at 1:60,000 dilution or a rabbit affinity-purified polyclonal antibody against α-Fos (sc-253; Santa Cruz Biotechnology, Santa Cruz, CA) at 1:120,000 dilution. Both primary antibodies were made up in 0.05 M potassium phosphate-buffered saline (KPBS) with 0.4% Triton X-100/1% normal donkey serum, and sections were incubated for 60 min at room temperature (RT) and then 72 h at 4°C. The sections were rinsed and incubated in avidin-biotin complex solution for 1 h 15 min at RT (Vector Elite Kit, Vector Laboratories, Burlingame, CA; with 45 μl of avidin and 45 μl of biotin in 10 ml of KPBS with 0.4% Triton X-100). 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 containing H2O2. This yielded a blue-black reaction product in the nuclear compartment.

Fluorescent double-immunolabeling for simultaneous detection of ER-α and Fos. All procedures were conducted in a similar fashion to the single-labeling method except for the following alterations. After pretreatment, sections containing areas of interest were incubated in a mixed solution of ER-α and Fos primary antibodies at a titer of 1:5,000 and 1:1,500, respectively, made up in KPBS with 0.4% Triton X-100/1% normal donkey serum. Higher antibody concentrations are required for fluorescence immunocytochemistry because the immunoperoxidase method includes an amplification step as well as nickel intensification of the DAB signal. Sections were incubated for 60 min at RT and then 72 h at 4°C. The ER-α primary antibody used in the double-labeling procedure was identical to that used in the single-labeling method while the Fos antibody used for the double labeling was a goat affinity-purified polyclonal antibody (sc-253-G; Santa Cruz Biotechnology, Santa Cruz, CA). After the incubation period, sections were rinsed with KPBS (10 times for 6 min each) and then incubated with a mixed solution of fluorescein-conjugated donkey anti-rabbit and rhodamine red-X-conjugated donkey anti-goat sera (Jackson ImmunoResearch, West Grove, PA) at 1:150 each, in KPBS with 0.4% Triton X-100 for 2 h 30 min at RT in the dark. Tissues were then rinsed in KPBS six times, for 5 min each, mounted on poly-l-lysine-treated slides, briefly air-dried, and coverslipped with ProLong Antifade Kit medium (P-7481, Molecular Probes, Eugene, OR) to reduce the photobleaching of fluorophore, thereby preserving the fluorescent signal.

Quantification of ER-α- or Fos-ir in SFO in the single-labeling experiment. ER-α and Fos expression was quantified by counting the number of blue-black DAB-nickel reactive nuclei in digitized images of two to three sections of SFO from each rat (approximately bregma –1.0 mm) using image-analysis software (NIH Image 1.55, available free online at http://rsb.info.nih.gov/nih-image/). Only intensely
stained nuclei were counted to avoid interference of background staining, and the same threshold was applied to count all sections of ER-α-ir or Fos-ir. The intensity of ER-α staining was also evaluated by measuring optical density (gray level/pixel) on digitized images of the SFO. The mean optical density was determined following thresholding of the image to limit evaluation to the ER-α-ir regions. The same threshold was applied to all images. Background measurements were made over appropriate adjacent tissue with no evident expressing neurons and subtracted to obtain the net optical density. For each variable, the mean value from multiple measurements from each animal was calculated, and these values were used to calculate group means.

Statistical analysis. Student’s t-test (or rank sum test) and two-way ANOVA (or Kruskal-Wallis analysis on ranks) with post hoc test (Student-Newman-Keuls) were used (SigmaStat software; SPSS, Chicago, IL) as appropriate to determine the statistical significance between groups. The α-value was set at $P < 0.05$. Results are expressed as group means ± SE.

RESULTS

The immunocytochemical expression of ER-α in lamina terminalis structures was consistent with previous reports (33, 36, 61). In the SFO, the majority of ER-α-ir neurons is concentrated around the periphery with only a few positive cells found in the central core (Fig. 1, A and C). In the OVLT, many cells in the dorsal cap region exhibited immunoreactivity for ER-α, with almost no labeled cells found in the lateral margins of the OVLT (Fig. 1, E and G). Within the MnPO, ER-α-ir was localized in both dorsal and ventral parts of the nucleus (Fig. 1, I and K). Because all three of these regions have been implicated in regulation of body fluid balance, the localization of ER-α in these regions suggests the possibility that gonadal steroids may act on these neurons to modify fluid balance.

Expression of Fos-ir and its colocalization with ER-α in neurons of the lamina terminalis after 48-h water deprivation. The possibility that the ER-α-expressing neurons in the lamina terminalis may participate in fluid homeostasis was further supported by the observation that Fos expression is induced in these same regions after 48 h of water deprivation. As reported previously (5, 29, 32), although Fos-positive neurons were only occasionally observed in any of these regions in euhydrated control animals and those exhibited very weak expression (Fig. 1, B, F, and J), Fos-ir was significantly upregulated in all three regions after 48 h of water deprivation (Fig. 1, D, H, and L). In the SFO, Fos-ir induction was observed primarily in the outer annular ring where most of the ER-α-positive neurons were found (Fig. 1D). Occasional cells in the central core did exhibit some Fos-ir. Similarly, Fos-ir was significantly upregulated in neurons in the OVLT, particularly in the dorsal cap region (Fig. 1H) where ER-α-positive neurons were localized. Fewer Fos-positive cells were observed in the lateral margin of the OVLT. Fos-ir was also increased in the MnPO, in both dorsal and ventral portions (Fig. 1L), compared with controls (Fig. 1J).

Given the similar spatial distribution of ER-α and Fos expression in stimulated animals, it is likely that ER-α-ex-
pressing neurons in the lamina terminalis responded to an increase in osmolality, and thus expressed Fos, after water deprivation. To verify this, a separate group of animals was subjected to 48 h of water deprivation and examined for the presence of colocalization between ER-α and Fos using fluorescent double-labeling immunocytochemistry. As shown in Fig. 2, the pattern of staining for both ER-α-ir and Fos-ir (Fig. 2, columns 1 and 2, respectively) in all three nuclei observed with fluorescent labeling was comparable to that seen at the light microscopic level (Fig. 1). As shown in the overlay images, a significant degree of colocalization between ER-α and Fos was observed in the outer annular ring of the SFO, in the dorsal cap of the OVLT as well as in the MnPO (Fig. 2, column 3). At high magnification, many neurons were positive for both ER-α and Fos, indicative of colocalization (Fig. 2, column 4, white arrowheads). Note the presence of independent staining for ER-α and Fos in neurons lateral to the third ventricle in the OVLT in the overlay image (Fig. 2G) as well as those observed in the overlay detail images (Fig. 2, column 4), demonstrating the specificity of the staining procedure.

Regulation of ER-α-ir and Fos-ir in SFO neurons by hypertonicity. Water deprivation induced an increase in ER-α expression as well as Fos in the lamina terminalis neurons. This was most evident in the SFO of the dehydrated AV3V-lesioned rats. Due to disruption of osmotic control of VP secretion and natriuretic control of oxytocin release by the AV3V lesion (13, 28), the increase in plasma osmolality induced by water deprivation is exaggerated in these animals [380 ± 10 vs. 304 ± 1

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Fig. 2. Simultaneous immunocytochemical localization of ER-α and Fos in the SFO, OVLT, and MnPO in 48 h water-deprived rats. Fluorescent images of the ER-α and Fos staining from the same tissue section were collected independently. Boxes in columns 1–3 indicate the area shown at higher magnification in column 4. The white arrowheads in column 4 indicate examples of neurons coexpressing ER-α and Fos. Scale bars, 50 μm.
mosmol/kgH_{2}O in hydrated sham-lesioned animals, \( P < 0.001 \) (46)] without a significant decrease in blood volume as monitored by hematocrit [hematocrit: 49.6 \pm 0.6% in hydrated sham-lesioned rats vs. 52.0 \pm 1.4% in dehydrated AV3V-lesioned rats (46)]. This allows the clear demonstration of stimulation of ER-\( \alpha \) expression by hypertonicity in the SFO. There was a significant increase in the optical density of ER-\( \alpha \)-ir (\( F = 9.2, P = 0.006 \), Fig. 3A), as well as an increase in the number of ER-\( \alpha \)-expressing neurons (\( F = 5.1, P = 0.034 \), Fig. 3B) in the AV3V-lesioned animals by water deprivation, reflecting both an increase in the population of neurons expressing ER-\( \alpha \) as well as increased protein expression within individual neurons. As depicted in Fig. 4D, an increase in the number of ER-\( \alpha \)-expressing neurons was confined mainly to the peripheral margin of the SFO, although an increase in expression was also occasionally observed in the central core. In the sham-lesioned animals, water deprivation also induced a significant, albeit smaller, increase in ER-\( \alpha \)-ir per neuron, as measured by optical density (\( F = 9.2, P = 0.006 \), Fig. 3A).

This, however, was accompanied by no change in the number of ER-\( \alpha \)-positive neurons (\( P > 0.05 \), Fig. 3B; also see Fig. 4B). Interestingly, an increase in ER-\( \alpha \) expression was not detected in the hydrated AV3V-lesioned animals (\( P > 0.05 \), Figs. 3, A and B, and 4C) despite a significant increase in plasma osmolality compared with the hydrated sham-lesioned animals [312 \pm 2 vs. 304 \pm 1 mosmol/kgH_{2}O, \( P < 0.01 \), respectively (46)]. Because the AV3V lesion destroyed the OVLT and vMnPO, no attempt was made to quantify the effect of water deprivation on ER-\( \alpha \) expression in these nuclei.

Fos-ir was also induced in SFO neurons in response to the increase in plasma osmolality associated with either the AV3V lesion or water deprivation (Fig. 3C and Fig. 4, middle row). The number of Fos-positive neurons was significantly induced in both dehydrated sham-lesioned and hydrated AV3V-lesioned animals compared with hydrated sham-lesioned controls (\( P = 0.001 \) and \( P = 0.026 \), respectively; independent Student’s \( t \)-test or rank sum test, Fig. 3C). The increase was restricted primarily to the periphery of the SFO (Fig. 4, F and G). In contrast, as reported previously (65), not only was there a much greater response in the number of Fos-positive neurons after water deprivation in the AV3V-lesioned animals (Fig. 3C), but also Fos-positive neurons were no longer confined to the peripheral margin of the SFO but were now prominent also in the central core of SFO (Fig. 4H).

As shown in Fig. 4, I–L, neither water deprivation, AV3V lesion, nor the combination of both induced ER-\( \alpha \) expression in the SON.

**DISCUSSION**

Although ER-\( \alpha \) expression in the osmosensitive regions of the lamina terminalis has been observed previously (33, 36, 42, 61), the current studies provide further anatomic evidence that the majority of these ER-\( \alpha \)-expressing neurons is indeed activated by an increase in osmolality associated with water deprivation (as identified by a high degree of ER-\( \alpha \) and Fos colocalization). Furthermore, water deprivation induced a significant increase in the expression of ER-\( \alpha \)-ir in the SFO. Because the increase in ER-\( \alpha \) expression was most prominent in the dehydrated AV3V-lesioned animals, it was likely due to the increase in plasma osmolality induced by water deprivation as opposed to the decrease in blood volume because these animals had a profound increase in plasma osmolality without a detectable decrease in blood volume (as reflected by hematocrit) (46). Thus the AV3V lesion resulted in conservation of...
blood volume at the expense of a greater increase in plasma osmolality. Although the mechanism by which hyperosmolality induced an increase in ER-α remains to be elucidated, possible mechanisms include an increase in ER-α gene expression and/or a decrease in degradation of either ER-α mRNA or protein. A change in ER-α gene expression might have occurred in response to induction of the immediate early gene, c-fos, or fos may regulate expression of other genes involved in altered protein or mRNA turnover. However, because ER-α gene expression is regulated by multiple promoters in a tissue-specific manner (17), further speculation about the mechanism of hypertonicity-induced ER-α expression is not warranted at this time.

The hyperosmolality-induced increase in ER-α expression reflected both an increase in the number of ER-α-expressing neurons as well as an increased amount of ER-α protein per cell (as indicated by the significant increase in optical density). In the dehydrated sham-lesioned animals, the modest increase in plasma osmolality resulted in an upregulation of receptor protein content but without further increase in the number of ER-α-expressing neurons. In contrast, the greater increase in plasma osmolality induced by water deprivation in the AV3V-lesioned animals resulted in a significant increase in both the number of ER-α-expressing neurons as well as the amount of ER-α expression within individual neurons.

The increase in the number of Fos-expressing neurons in the SFO provoked by water deprivation in the AV3V-lesioned animals was much more extensive than the increase in ER-α expression. While the increase in ER-α-positive neurons was confined to the peripheral margin of the SFO, the increase in Fos-positive cells included neurons in the central core as well as the periphery, indicating that additional non-ER-α-positive neurons were being activated. Therefore, although water deprivation in the AV3V-lesioned animals activated a large population of neurons in the central core of the SFO, only a small proportion of these neurons was ER-α positive.

Aside from its osmosensitive property, the SFO is also the site where the peptide ANG II acts to promote drinking and VP secretion (24, 59). Previous studies have reported a functional compartmentalization between the periphery and the central core of the SFO in terms of its neural connectivity as well as its sensitivity to different stimuli. Specifically, neurons in the central core of the SFO are activated by treatments that generate relatively moderate increases in plasma and/or central ANG II levels. These include, for instance, intravenous and intracerebroventricular exogenous ANG II administration (23, 26, 52, 64), polyethylene glycol-induced hypovolemia (44), and subcutaneous injection of isoproterenol (52), all of which lead to an increase in endogenous ANG II levels. Additionally, this innermost group of neurons in the SFO also contains the highest density of ANG II receptors (exclusively of AT1 subtype) (9, 20, 23, 25). Furthermore, these central neurons of the SFO send afferents to the bed nucleus of the stria terminalis (BNST) (52). In contrast, neurons in the peripheral ring of the SFO, although they too express ANG II receptor (albeit in fewer numbers) and are activated by ANG II (but at a higher concentration than that required to activate neurons in the central core), appear to be more sensitive to hypertonicity (and

Fig. 4. ER-α-immunoreactivity (ir) in SFO and supraoptic nucleus (SON) and Fos-ir in SFO of sham-lesioned (Sham) and AV3V-lesioned (AV3V) rats that were either hydrated or water deprived (dehydrated). Note the increase in ER-α-ir in SFO (top row) of Sham + dehydrated and AV3V + dehydrated animals. Fos-ir was also increased in SFO in these groups (middle row). As reported previously, ER-α-ir is present in a few scattered cells in the perinuclear region. There was no change in ER-α-ir in either the SON or perinuclear region with the current manipulations. OC, optic chiasm. Scale bars, 100 μm.
relaxin) (23, 27, 34, 44, 51). Many of these neurons project, directly and indirectly, to the magnocellular neurons in the SON and PVN (29, 31, 35, 51, 63), as opposed to the BNST.

Similarly, a functional and anatomic segregation also exists in the OVLT. While the dorsal cap neurons in the OVLT appear to be most responsive to hypertonicity (34), those in the lateral margins are activated by circulating ANG II. The lateral margins also contain the greatest density of the AT1 receptor while the dorsal part shows a much lower expression (25).

Because hyperosmolality and increased ANG II elicit many similar physiological responses (e.g., VP secretion, natriuresis, pressor response, and drinking behavior), an interesting question to ask is whether the increase in ER-α as well as Fos expression observed in the current study was due to plasma hypertonicity, an increase in ANG II production, or a combination of both. Indeed, the plasma ANG II level induced by 48 h of water deprivation in intact animals exceeds the dipsogenic threshold (13). However, based on the differential anatomic and functional regionalization of the SFO and OVLT mentioned above, it is likely that activation of ER-α (indicated by Fos expression) in the periphery of the SFO and the dorsal OVLT in response to 48 h of water deprivation was due to plasma hypertonicity, rather than an increased ANG II production, for the following reasons. First, a moderate increase in ANG II stimulates Fos production initially in the central core of the SFO, whereas neurons in the peripheral margin only become activated later with higher concentrations of ANG II (23). Similarly, in the OVLT, an increase in Fos production in response to ANG II occurs predominantly in the lateral margin and not in the dorsal cap region. These observations are in stark contrast with the pattern of Fos-ir and ER-α expression observed in the present study where Fos activation of ER-α-positive neurons was primarily observed in the periphery of the SFO and the dorsal cap of the OVLT (see Fig. 1, columns 3 and 4).

In support of this, Fos-ir expression observed in the SFO of hydrated AV3V-lesioned animals exhibited a similar pattern to that found in the dehydrated sham-lesioned animals. These groups of rats had comparable increases in their plasma osmolality [hydrated AV3V-lesioned animals 312 ± 2 vs. dehydrated sham-lesioned animals 318 ± 2 mosmol/kgH2O, P > 0.05 (46)]. However, it is unlikely that they had similar increases in plasma ANG II because hematocrit was not significantly increased in the hydrated AV3V-lesioned group (46), and in a previous study, plasma renin activity remained unaltered in hydrated AV3V-lesioned rats despite a significant increase in plasma renin concentration (39). However, because there was no detectable increase in the expression of ER-α in the hydrated AV3V-lesioned rats, the increase in ER-α expression in the dehydrated sham-lesioned rats must reflect a yet unidentified influence in addition to hypertonicity.

In contrast, in dehydrated AV3V-lesioned animals, the prominent expression of Fos-ir throughout the entire SFO structure suggests activation of SFO neurons by both hypertonicity and ANG II. However, because blood volume was conserved in these rats [as evidenced by unaltered hematocrit (46)], it is, once again, unlikely that plasma ANG II was elevated. Therefore, it is possible that 1) the increase in Fos-ir in the central core of the SFO might have been a result of the extreme increase in plasma osmolality, or 2) if there was, indeed, an increase in ANG II level in the AV3V-lesioned rats after water deprivation, then the increase in ER-α-ir in the periphery and Fos-ir in both the central core and the periphery of SFO in these animals reflects the combination of the extreme hypertonicity and increased ANG II level. Future experiments should help clarify these observations.

ER-α is also expressed in the MnPO. Previous reports found that neurons in the MnPO were heavily labeled with Fos-ir in response to hypertonic saline injection but essentially devoid of Fos-ir after induced volume depletion (10, 44). This lends further credence to the role of hypertonicity as opposed to ANG II after volume depletion in regulation of ER-α in the osmosensitive circuitry.

Behaviorally, E2 has been shown to have a profound effect on the drinking response in female rats. Both spontaneous and stimulus-induced water intake vary over the course of the estrous cycle while ovariectomy abolishes this variation (8, 58). Daily water intake was found lowest on the day of estrus when circulating E2 is at its peak (14), suggesting an inhibitory influence of E2 on the drinking behavior. Similarly, treatment of ovariectomized animals with E2, either centrally or peripherally, reduced the stimulated water intake (14, 15, 18). However, the inhibitory effect of E2 on the stimulated dipsogenic response appears specific to drinking induced by extracellular dehydration associated with an increase in ANG II. For example, administration of E2 to ovariectomized female rats results in a reduction in water intake induced by water deprivation (18), polyethylene glycol (14, 60), or isoproterenol (18) treatments. Conversely, thirst provoked by intracellular dehydration, such as hypertonic NaCl stimulation, is not affected by E2 treatment (8, 14). Therefore, not only is the inhibitory effect of E2 selective to ANG II induction but, more importantly, E2 does not inhibit the dipsogenic response to hypertonic stimulation. ER-α expression in SFO neurons may mediate this inhibitory effect of E2 on drinking in response to ANG II stimulation, because a recent study (36) reported extensive colocalization between ER-α and AT1 receptors in the SFO. These investigators also reported that E2 treatment of ovariectomized rats reduced AT1 receptor expression (36). Thus E2 inhibition of ANG II-induced drinking in females may reflect an E2-induced decrease in AT1 receptor expression in the periphery of the SFO, leaving these cells primarily responsive to changes in plasma osmolality and not to ANG II action. However, because the SFO neurons that are most responsive to ANG II do not express ER-α, an alternative possibility is that the inhibitory effect of E2 on ANG II-induced drinking is not mediated by ER-α in SFO.

The demonstration that chronic water deprivation increases ER-α expression in osmoreceptive neurons in the SFO is in marked contrast to the effect of chronic hypertonicity on ER-β expression in the magnocellular system (46, 47). Chronic water deprivation or saline consumption inhibits ER-β expression in the magnocellular neurons of the SON and PVN with both protein and mRNA becoming undetectable after 72 h of saline consumption (47). In fact, ER-β expression is depleted in the magnocellular neurons as early as 22 h of water deprivation (48). The disappearance of ER-β in the magnocellular VP neurons with hyperosmolality is consistent with an inhibitory role for ER-β in these neurons, because both water deprivation and saline consumption are potent stimuli for VP secretion. In vitro studies with explants of the hypothalamoneurohypophyseal system (HNS) have corroborated an inhibitory function of ER-β in this system. Both estradiol and dihydrotestosterone

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inhibit osmotically induced VP secretion from HNS explants (55), and the inhibition by estradiol is prevented by a specific ER-β antagonist (45). Thus chronic hypotonicity induces expression of one type of ER (ER-α) in the osmoreceptive regions along the lamina terminalis but decreases another type of ER (ER-β) in one of the important effector pathways (the VP neurons) for regulation of body fluid homeostasis.

Given the fluctuation in ER-β expression in the magnocellular neurons after changes in plasma osmolality, one might speculate that there could be an induction in ER-α expression in these same neurons by water deprivation. The dynamic nature of steroid receptor expression has also been demonstrated with the glucocorticoid receptor (GR). While not normally expressed in the magnocellular neurons, GR becomes detectable in response to chronic hyponatremia, and expression decreases after adrenalectomy (3, 16). However, there was no increase in ER-α expression in the SON in our studies. Thus the increase in ER-α expression in the osmosensitive forebrain nuclei after hypotonicity appears to be specific to neurons along the lamina terminals.

The precise function of ER-α in the lamina terminalis region remains to be determined. As mentioned in the preceding section, neurons in the dorsal cap region of the OVLT and those in the periphery of the SFO have efferent projections to the magnocellular neurons in the SON and have been shown to mediate VP secretion in response to hypotonicity (34); thus, based on its distribution, the expression of ER-α in these nuclei, coupled with those in the MnPO, could potentially coordinate with ER-β in regulating the VP magnocellular neurons. Indeed, as shown previously, at least 15% of all neurons in the lamina terminalis retrogradely labeled by injections of a tracer into the SON were ER-α positive (61).

Perspectives

The expression of ER-α among the osmosensitive neurons of the lamina terminalis along with ER-β in the magnocellular neurons places gonadal steroids at a strategic position to influence the osmoregulatory function in the magnocellular neurons. Although in the current studies, the effects of hyperosmolarity were observed in adult male rats, it is well established that the female gender experiences profound changes in fluid balance throughout their lifetime. Of particular interest, reproduction in the female poses heavy demands on body fluid homeostasis to accommodate the developing fetus(es) in utero as well as during the ensuing lactation. These conditions are conspicuously associated with prominent changes in the steroid milieu. Although it remains to be examined, it is tempting to speculate that changes in circulating steroid concentrations, such as during pregnancy, across the estrous or menstrual cycle, and even in males during stress or gonadal dysfunction, working through ER-α at these sites, could bring about changes in plasma osmolality and drinking responses resulting in altered body water homeostasis.

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