Estrogen effects on osmotic regulation of AVP and fluid balance

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Stachenfeld, Nina S., and David L. Keefe. Estrogen effects on osmotic regulation of AVP and fluid balance. Am J Physiol Endocrinol Metab 283: E711–E721, 2002; 10.1152/ajpendo.00192.2002.—To determine estrogen effects on osmotic regulation of arginine vasopressin (AVP) and body fluids, we suppressed endogenous estrogen and progesterone using the gonadotropin-releasing hormone (GnRH) analog leuprolide acetate (GnRHa). Subjects were assigned to one of two groups: 1) GnRHa alone, then GnRHa + estrogen (E, n = 9, 25 ± 1 yr), 2) GnRHa alone, then GnRHa + estrogen with progesterone (E/P, n = 6, 26 ± 3). During GnRHa alone and with hormone treatment, we compared AVP and body fluid regulatory responses to 3% NaCl infusion (HSI, 120 min, 0.1 ml-min⁻¹·kg body wt⁻¹), drinking (30 min, 15 ml/kg body wt), and recovery (60 min of seated rest). Plasma [E₂] increased from 23.9 to 275.3 pg/ml with hormone treatments. Plasma [P₄] increased from 0.6 to 5.7 ng/ml during E/P and was unchanged (0.4 to 0.6 ng/ml) during E. Compared with GnRHa alone, E reduced osmotic AVP release threshold (275 ± 4 to 271 ± 4 mosmol/kg, P < 0.05), and E/P reduced the AVP increase in response during HSI (6.0 ± 1.3 to 4.2 ± 0.6 pg/ml at the end of HSI), but free water clearance was unaffected in either group. Relative to GnRHa, pre-HSI plasma renin activity (PRA) was greater during E (0.8 ± 0.1 vs. 1.2 ± 0.2 ng ANG I·ml⁻¹·h⁻¹) but not after HSI or recovery. PRA was greater than GnRHa during E/P at baseline (1.1 ± 0.2 vs. 2.5 ± 0.6) and after HSI (0.6 ± 0.1 vs. 1.1 ± 1.1) and recovery (0.5 ± 0.1 vs. 1.3 ± 0.2 ng ANG I·ml⁻¹·h⁻¹). Baseline fractional excretion of sodium was unaffected by E or P but was attenuated by the end of recovery for both E (3.3 ± 0.6 vs. 2.4 ± 0.4%) and E/P (2.8 ± 0.4 vs. 1.7 ± 0.4%, GnRHa alone and with hormone treatment, respectively). Fluid retention increased with both hormone treatments. Renal sensitivity to AVP may be lower during E due to intrarenal effects on water and sodium excretion. E/P increased sodium retention and renin-angiotensin-aldosterone stimulation.

Body fluid regulation; arginine vasopressin; osmolality; thirst; gonadotropin-releasing hormone agonist; leuprolide acetate.

CARDIOVASCULAR DISEASE-RELATED morbidity and mortality are lower in women than in men throughout middle age (8, 22), and this risk “advantage” is often attributed to estrogen. Arginine vasopressin (AVP) may be another key hormone involved in these gender differences. AVP is an important hormone involved in fluid balance and blood pressure regulation. AVP is synthesized in the cell bodies of the paraventricular (PVN) and supraoptic (SON) nuclei, located in the anterior hypothalamus. Axons from these areas project into the posterior pituitary, where AVP is stored and released in response to stimulation of central osmoreceptors. Estrogen receptors are present in the PVN and SON of both animals (17, 33) and humans (19), and a number of studies have demonstrated sex differences in AVP neuron size in these nuclei (20, 21).

Resting plasma AVP levels are greater in males than in females (34, 37), and men have greater AVP sensitivity and blood pressure responses to hypertonic saline infusion (41). On the other hand, in postmenopausal women, estrogen administration increases resting plasma AVP and shifts the osmotic threshold for AVP release to a lower plasma osmolality (36). This same shift to a lower osmotic threshold occurs in young women in the midluteal phase of the menstrual cycle (40, 44, 46) and during combined estrogen/progesterone oral contraceptive pill administration (40).

Despite these findings, the direct effects of estrogen on the osmotic regulation of AVP are difficult to define in young women. Progesterone also affects body water and sodium regulation (29, 36, 40) and is usually increased concurrently with estrogen in women of reproductive age. Data from postmenopausal women should not be applied to younger women, because estrogen receptor function in the hypothalamus is affected by aging (20, 21). In the present investigation, we suppressed endogenous production of estrogen and progesterone in young women by use of the gonadotropin-releasing hormone (GnRH) analog (GnRHa) leuprolide acetate. We then administered estrogen to test the hypothesis that estrogen lowers the osmotic threshold for AVP release during hypertonic saline infusion. We also tested the hypothesis that estrogen administered with progesterone increases overall body water and sodium retention above that of estrogen alone. Blood...
METHODS

We recruited seventeen healthy nonsmoking women with no contraindications to GnRHAs or reproductive hormone administration to participate in these experiments. All subjects were interviewed about their medical history and provided written confirmation of a negative Papanicolaou smear and normal physical examination within 1 yr of being admitted to the study. They gave written informed consent to participate in the study, which had prior approval by the Human Investigation Committee of Yale University School of Medicine.

Experimental Design

Plasma concentrations of 17β-estradiol (P\(_{E2}\)) and progesterone (P\(_{P4}\)) were determined in the early follicular phase (days 1–5) of a normal menstrual cycle. If the subject was already taking oral contraceptive pills (n = 5, 2 in the estrogen group, 3 in the E\(_2/P_4\) group), we determined P\(_{E2}\) and P\(_{P4}\) on days 25–28 of the normal pill cycle (i.e., the end of the week of placebo pills). Blood volume using Evans blue dye (see Blood Volume) was also determined on this day.

To suppress reproductive function for the duration of the study, the subjects received the GnRHa leuprolide acetate (0.5 mg/day, Lupron; TAP Pharmaceuticals, Deerfield, IL) each day for 5 wk. At the end of the 5th wk, the women received either 4 days of estrogen administration (17β-estradiol, transdermal patch, 0.1 mg/day, n = 9, 25 ± 1 yr) or 4 days of estrogen with progesterone administration (vaginal gel, 90 mg P\(_4\) twice per day, n = 7, 28 ± 3 yr). One subject was excluded from the study (see RESULTS). Group assignment was randomized. Experimental protocols were performed at the end of the 4th (GnRHa alone) and 5th wk (GnRHa with hormone) of administration. This design permitted within-subject comparisons concerning hormone effects on osmotic regulation of AVP, thirst, and sodium and fluid regulation without concern for slow hormone washout between trials.

The GnRHa analog leuprolide acetate possesses greater receptor binding and decreased degradation than endogenous GnRH, and it acts as a potent inhibitor of gonadotropin secretion. When leuprolide acetate is given continuously, it downregulates the hypothalamic-pituitary-ovarian axis, with internalization and uncoupling of the GnRH receptors at the hypothalamic level. After an initial stimulation, chronic administration suppresses steroidogenesis via GnRH secretion, leading to low or undetectable estrogen and progesterone concentrations within 14 days. The GnRHa administration began 2–7 days after the subject’s luteinizing hormone peak. This peak precedes ovulation, usually days 12–14 of a 28-day menstrual cycle, and was determined individually by the use of ovulation prediction kits (OvuQuick; Quidel, San Diego, CA). Some of the subjects were already taking oral contraceptives, so they ceased taking the pills and began GnRHa administration in the 3rd wk of the pill cycle. The subjects self-administered daily subcutaneous injections of the GnRHa (0.5 mg/day) after training by qualified medical personnel. This method of GnRHa administration was chosen because it is easily discontinued in the event of uncomfortable side effects, such as headaches or “hot flashes,” and the suppression of the hypothalamic-pituitary-ovarian axis is reversed upon cessation of drug therapy.

For estrogen treatment alone, the subjects received 17β-estradiol, administered by two transdermal patches delivering 0.1 mg/day (Vivelle; CIBA Pharmaceuticals, Summit, NJ) for 4 days (38). During combined estrogen-progesterone (E\(_2/P_4\)) administration, the subjects wore the estradiol patches and also used a vaginal gel containing 90 mg progesterone twice per day (2.250 g of gel at 8% concentration; Crinone; Wyeth-Ayerst Pharmaceuticals, Philadelphia, PA) for 4 days (9, 38).

Hypertonic Saline Infusion Protocol

For each experiment, the subjects arrived at the laboratory at ~7:00 AM, after having eaten a light (~300 kcal) breakfast, having drunk 10 ml/kg body wt of water, and having refrained from alcohol or coffee for the previous 12 h. Upon arrival at the laboratory, the subjects voided their bladders and were weighed to the nearest 10 g on a beam balance. The subjects were then seated in a semirecumbent position for a 60-min control period in an environmental chamber (27°C, 30% relative humidity) to ensure a steady state in plasma volume and constituents. During this control period, a 22-gauge Teflon intravenous catheter was placed in an antecubital or forearm vein in each arm with a heparin block (20 U/ml) to maintain catheter patency. A blood pressure cuff was positioned for automatic readings by a sphygmomanometric device (Colin Medical Instruments, Komaki, Japan) to monitor changes in blood pressure. A three-lead electrocardiogram (Colin Medical Instruments) provided continuous heart rate monitoring.

At the end of the control period, a baseline blood sample was taken, thirst perception was assessed (see Thirst Ratings), and a urine sample was collected. After the baseline sample was taken, 3.0% NaCl was infused at a rate of 0.1 ml/kg body wt \(-1\cdot\text{min}^{-1}\) for 120 min (hypertonic saline infusion (HSI)). Blood was sampled at 15, 25, 35, 45, 60, 75, 90, 105, and 120 min during the infusion. After the HSI, the subject rested seated for the next 90 min. The recovery period consisted of a 30-min drinking period during which the subject drank 15 ml water/kg body weight, followed by a 60-min rest period. A blood sample was obtained at the end of the 60-min rest period. Urine was collected at baseline, immediately postinfusion, and at the end of the recovery period. Thirst perception was assessed at all blood sampling times. The subjects were weighed at the end of the infusion, after the drinking period, and at the end of the protocol. Blood pressure and heart rate were recorded every 15 min throughout infusion and every 30 min postinfusion.

All blood samples were analyzed for hematocrit (Hct), hemoglobin (Hb), total protein (TP), osmolality (P\(_{Osm}\)), plasma concentrations of creatinine (P\(_{Cr}\)), and arginine vasopressin (P\(_{AVP}\)), and serum concentrations of sodium (S\(_{Na^+}\)) and potassium (S\(_{K^+}\)). Blood samples at baseline, at the end of the infusion, and at 90 min postinfusion were analyzed for the concentrations of atrial natriuretic peptide (P\(_{ANP}\)), aldosterone (P\(_{ALD}\)), and plasma renin activity (PRA). The baseline blood sample was also analyzed for P\(_{E2}\) and P\(_{P4}\). Urine volume, osmolality (U\(_{Osm}\)), sodium (U\(_{Na^+}\)), potassium (U\(_{K^+}\)), prostaglandin E\(_2\) (U\(_{PG\(_2\)}\)), and creatinine (U\(_{Cr}\)) concentrations were measured from all urine samples.

Blood Sampling

Blood samples were separated into aliquots. One aliquot was immediately analyzed for Hb, Hct, and TP in triplicate by cyanomethemoglobin, microhematocrit, and refractometry, respectively. A second aliquot was transferred to a heparinized tube to be analyzed for P\(_{Osm}\), P\(_{Cr}\), and P\(_{ALD}\). A third aliquot, for the determination of S\(_{Na^+}\) and S\(_{K^+}\), was placed into a tube without anticoagulant. The remaining aliquots were placed in tubes containing EDTA for analysis.
of P[AVP], P[ANP], and PRA. The tubes were centrifuged at 4°C, and the plasma was taken off. After centrifugation, the EDTA samples were frozen immediately at −70°C until analysis.

Plasma and urine sodium and potassium concentrations were measured by flame photometry (model 943, Instrumentation Laboratory). Plasma and urine osmolalities were measured by freezing point depression (Advanced Instruments 3DII), and P[Cl] was determined by colorimetric assay (Sigma Diagnostic Products). P[AVP], P[ALD], P[ANP], P[Ep], P[Ep], and U[PGE2] were measured by radioimmunoassay. P[ANP] was determined after extraction from plasma on octadecysilane cartridges (SEP-PAK C18, Waters Associates, Needham, MA), and the eluate was collected with 4% acetic acid and 86% ethanol. P[AVP] was determined after extraction from plasma by the methods described by Freund and colleagues (13, 14) on octadecysilane cartridges (SEP-PAK C18, Waters Associates). Extracted samples were assayed using a disequilibrium assay with the extracts incubated with the antiserum at 4°C for 72 h, followed by the addition of 125I-labeled AVP (New England Nuclear, Boston, MA). Bovine albumin-coated charcoal was used for separation of free and antibody-bound labeled AVP. This assay is highly specific for AVP, with the antiserum prepared against a lysine vasopressin-thyroglobin conjugate, and has a sensitivity of 0.6 pg/ml. Extraction recovery of AVP was determined using plasma spiked with a known concentration of AVP (Peninsula Laboratories, Belmont, CA). The recovery sample was extracted and analyzed along with the subjects’ samples. The extraction recovery was 87%.

Intra- and interassay coefficients of variation for the midrange standards were, respectively, as follows: P[AVP] (2.3 pg/ml), 9.2 and 4.6%; PRA (4.7 ng ANG I·ml⁻¹·h⁻¹), 8.4 and 6.1% (Diasorin; Stillwater, MN); P[ALD] (145 pg/ml), 9.0 and 8.6% (Diagnostic Products, Los Angeles, CA); P[ANP] (16.1 pg/ml), 8.9 and 7.6% (Alpeco Diagnostic, Windham, NH); P[Ep] (82 pg/ml), 5.5 and 5.2% (Diagnostic Products); P[Ep] (2.3 ng/ml), 3.4 and 1.7% (Diagnostic Products); and U[PGE2] (23.7 pg/ml), 3.7 and 1.7% (Amersham, Chicago, IL).

Blood Volume

Absolute blood volume was measured by dilution of a known amount of Evans blue dye (New World Trading, DuBarry, FL). An accurately determined volume of dye (by weight, because the specific density is 1.0) was injected into an arm vein, and a blood sample was taken at 10, 20, and 30 min for determination of dilution after complete mixing had occurred (10 min). Blood was also sampled at 20 and 30 min to ensure that complete mixing had occurred by the 10-min sample. If not, the 20-min sample was used for analysis. However, if complete mixing had not occurred by the 20-min sample, the blood volume measurement would not have been used. Plasma volume was determined from the product of the concentration and volume of dye injected divided by the concentration in the plasma after mixing, with account taken of 1.5% lost from the circulation within the 10 min. Blood volume was calculated from plasma volume and Hct concentration, corrected for peripheral sampling.

Thirst Ratings

Asking the subject to make a mark on a line rating scale in response to the question “How thirsty do you feel now?” assessed the perception of thirst. The line is 175 mm in length and is marked “not at all” on one end and “extremely thirsty” at the 125-mm point. We tell subjects that they can mark beyond the extremely thirsty point if they wish and may even extend the line if they feel it necessary. This method was developed by Marks et al. (26) and has been used with great success in the evaluation of several sensory systems. We have found an extraordinarily good relationship between the perception of thirst and plasma osmolality during HSI and dehydration in young volunteers (39, 40).

Calculations

Changes in plasma volume were estimated from changes in Hct and Hb concentration from the baseline sample according to the equation

\[
\% \Delta PV = 100 \left( \frac{[(\text{Hb}_1/\text{Hct})] - [(1 - \text{Hct}_a \cdot 10^{-2})]}{(1 - \text{Hct}_a \cdot 10^{-2})} \right)-100
\]

where subscripts a and b denote measurements at time a and before HSI, respectively. (15). These changes in plasma volume between baseline samples of GnRHa and GnRHa with hormone (38, 40, 42).

Fractional excretions of water (FEH₂O) and Na⁺ (FENa⁺) were calculated from the following equations

\[
\text{FEH}_2\text{O} = \left( \frac{U_v}{GFR} \right) \cdot 100
\]

\[
\text{FENa}^+ = \left( \frac{U_v \cdot U_{[\text{Na}^+]}/GFR \cdot [\text{Na}^+]}{[\text{Na}^+]_t} \right) \cdot 100
\]

where the subscript f is glomerular filtrate, U_v is urine flow rate, and S_{[\text{Na}^+]} is S_{[\text{Na}^+]} in protein-free solution (meq/kg H₂O). Glomerular filtration rate (GFR) was estimated from creatinine clearance.

Data Analysis

For each subject in the estrogen group, osmotic regulation of AVP and thirst was determined by plotting P[AVP] and thirst as linear functions of P Osm during HSI. The slope of P[AVP] vs. P Osm provides the sensitivity of this relationship, and the intercept provides the threshold for P[AVP] release or thirst onset. Body water handling was determined through the assessment of overall fluid balance and the renal clearance of free water, osmoles, and sodium.

Statistics. The variables over time (control tests, hormone intervention tests) were analyzed by conditions (estrogen vs. GnRHa alone, combined E₂/P₄ vs. GnRHa alone) with ANOVA for repeated measures. When significant differences were found, orthogonal contrasts tested differences between specific means related to the hypothesis of interest. Data are expressed as means ± SE. Differences were considered statistically significant when P < 0.05 (SPSS; SPSS, Chicago, IL).

Sample size calculation. Expected P[AVP] responses within and between groups were derived from data from our laboratory during HSI (5). In an earlier study, E₂/P₄ administration decreased the P[AVP] threshold by 5 mosmole, with an estimated pooled standard deviation for the group of 2 mosmole during HSI.

The desired statistical test is two-sided at an α-level of 0.05 with 80% power to detect a difference. On the basis of our previous work, 80% power is sufficient to detect a significant alteration in P Osm. For a two-sided test, Z(α) = 1.96, and for 80% power, Z(β) = 0.84. The formula for calculating sample size for continuous response variables is

\[
N = 2[(Z(\alpha) + Z(\beta))^2/s^2/(d)^2]
\]
Substituting the values, the calculated sample size is eight subjects per group.

RESULTS

Three subjects reported occasional, and one subject reported frequent, hot flashes during GnRHa treatment. Many subjects reported mild breast tenderness. The subjects reported no other adverse effects due to the GnRHa or hormone administration, and the side effects did not cause any of the subjects to leave the study. One subject in the E2/P4 group did not complete the second infusion study, so all of her data were excluded from analysis. The data reported here are from nine subjects in the estrogen alone group and six subjects in the combined E2/P4 group.

Baseline P [E2] and P [P4] during GnRHa treatment alone were reduced, and P [E2] and P [P4] during GnRHa with hormone treatment were increased (Table 1, P < 0.05). These data demonstrate a high compliance with, and effectiveness of, the GnRHa/hormone add-back protocol.

Estrogen-Only Group

The reductions in preinfusion Hct and Hb during estrogen administration (Table 2, P < 0.05) suggest a calculated increase in plasma volume (~6.3 ± 1.9%). Furthermore, baseline P [Osm] was reduced during estrogen treatment compared with GnRHa-alone control (Fig. 1, P < 0.05). Renal excretory variables were unchanged at baseline between the two treatments (Table 3, Figs. 2 and 3). Finally, compared with GnRHa alone, PRA was increased during estrogen administration, although no other fluid-regulating hormones were different between trials (Table 4, P < 0.05).

During the first 50 min of HSI, P [Osm] remained lower with estrogen treatment relative to GnRHa alone. Linear regression analysis of the individual subjects’ data during HSI indicated significant correlations between P [AVP] and P [Osm] (r = 0.71 ± 0.03) and thirst and P [Osm] (r = 0.81 ± 0.06). The mean abscissal intercept for the P [AVP]-P [Osm] relationship was greater during GnRHa alone than during GnRHa with estrogen (Fig. 4, P < 0.05), but the mean slope of the P [AVP]-P [Osm] relationship during HSI was unaffected by estrogen treatment (Fig. 4). Estrogen treatment did not affect the mean abscissal intercept for the thirst-P [Osm] relationship (286 ± 3 and 285 ± 1 mosmol/kgH2O for GnRHa alone and with estrogen, respectively) or the mean slope of the thirst-P [Osm] relationship (6.7 ± 1.0 and 5.9 ± 1 mm/mosmol for GnRHa alone and with estrogen, respectively; data not shown). Renal responses to hypertonic saline indicate that the primary difference between estrogen administration and GnRHa alone was lower sodium excretion during the estrogen treatment.

Table 1. Baseline subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>GnRHa + Estrogen</th>
<th>GnRHa + Estrogen/Progesterone</th>
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<tbody>
<tr>
<td></td>
<td>Pre-GnRHa</td>
<td>GnRHa</td>
</tr>
<tr>
<td>Body wt, kg</td>
<td>64.4 ± 3.9</td>
<td>65.6 ± 4.2</td>
</tr>
<tr>
<td>PV, ml</td>
<td>2,718 ± 141</td>
<td>4,055 ± 209</td>
</tr>
<tr>
<td>BV, ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV, ml/kg</td>
<td>51 ± 14</td>
<td>18 ± 7</td>
</tr>
<tr>
<td>P[E2], pg/ml</td>
<td>51 ± 1.5</td>
<td>0.4 ± 0.0</td>
</tr>
<tr>
<td>P[P4], pg/ml</td>
<td>2.0 ± 0.2</td>
<td>0.8 ± 0.0</td>
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</tbody>
</table>

GnRHa, gonadotropin-releasing hormone analog leuprolide acetate; PV, plasma volume; BV, blood volume; P [E2] and P [P4], plasma concentrations of estrogen and progesterone. Values are means ± SE. *P < 0.05, significant hormone effect.

Table 2. Blood responses at rest and during HSI and recovery

<table>
<thead>
<tr>
<th></th>
<th>GnRHa + Estrogen</th>
<th>GnRHa + Estrogen/Progesterone</th>
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<tbody>
<tr>
<td></td>
<td>Pre-GnRHa</td>
<td>GnRHa</td>
</tr>
<tr>
<td>Hct, %*†</td>
<td>37.0 ± 0.6</td>
<td>34.3 ± 0.5</td>
</tr>
<tr>
<td>E2</td>
<td>35.8 ± 0.9</td>
<td>32.9 ± 0.8</td>
</tr>
<tr>
<td>Hb, g/dl*†</td>
<td>12.9 ± 0.3</td>
<td>11.7 ± 0.2</td>
</tr>
<tr>
<td>GnRHa</td>
<td>12.3 ± 0.2</td>
<td>11.3 ± 0.3</td>
</tr>
<tr>
<td>S[Na+], meq/l†</td>
<td>137.1 ± 0.9</td>
<td>141.9 ± 0.9</td>
</tr>
<tr>
<td>S[K+]†</td>
<td>3.66 ± 0.09</td>
<td>3.79 ± 0.08</td>
</tr>
</tbody>
</table>

Blood responses at rest and during hypertonic saline infusion (HSI) and recovery during GnRHa alone and with estrogen (E2) or estrogen and progesterone (E2/P4) treatment. Hct, hematocrit; Hb, blood hemoglobin concn; S[Na+] and S[K+], serum concns of sodium and potassium, respectively. Values are means ± SE. *P < 0.05, significant hormone effect (ANOVA). †P < 0.05, significant change over time (ANOVA).
GnRHa alone (Fig. 1, Table 4). There were no hormone treatment-by-time interactions for any of the blood, thirst, or renal variables during HSI or recovery.

For the estrogen-only group, baseline heart rate (76 ± 4 and 75 ± 3 beats/min for GnRHa alone and GnRHa with estrogen, respectively) and mean blood pressure (84 ± 2 and 84 ± 4 mmHg for GnRHa alone and GnRHa with estrogen, respectively) were unaffected by estrogen treatment. There were no heart rate or mean blood pressure differences between treatments in response to HSI (71 ± 6 and 77 ± 6 beats/min and 84 ± 3 and 84 ± 4 mmHg for GnRHa alone and GnRHa with estrogen, respectively) at the end of HSI. Finally, there was no effect of estrogen administration on heart rate (71 ± 5 and 75 ± 4 beats/min) or mean blood pressure (80 ± 4 and 79 ± 3 mmHg for GnRHa alone and GnRHa with estrogen, respectively) during recovery. There were no hormone treatment-by-time interactions for any of the cardiovascular variables during HSI or recovery.

Estrogen-Progesterone Group

Before HSI, there was a statistically significant reduction in Hct and Hb during E2/P4 treatment (Table 2, P < 0.05). However, these reductions were inconsistent among subjects, which led to large variability in the calculated percent change in plasma volume (7.2 ± 5.1%) when the relative changes in Hct and Hb were used. Plasma osmolality was reduced at baseline during E2/P4 treatment compared with the GnRHa-alone control value (Fig. 1, P < 0.05). Renal excretory variables were unchanged at baseline between the two treatments (Table 3, Figs. 2 and 3). Compared with GnRHa alone, PRA and P[ALD] were increased at baseline with combined E2/P4 treatment (Table 4, P < 0.05).

Plasma osmolality was lower during the combined E2/P4 treatment than during GnRHa alone throughout HSI (Fig. 1, P < 0.05). Both Hct and Hb decreased in response to HSI, and these changes were similar in magnitude between treatments (Table 2). Linear regression analysis of the individual subjects’ data during HSI indicated significant correlations between thirst and P[Osm] (r = 0.84 ± 0.03). Combined E2/P4 treatment did not significantly affect the mean abscissal intercept for the thirst-P[Osm] relationship (286 ± 2 and 281 ± 2 mosmol/kg H2O for GnRHa alone and with E2/P4, respectively) or the mean slope of the thirst-P[Osm] relationship (5.9 ± 1.3 and 5.2 ± 1.0 mm/mosmol for GnRHa alone and with E2/P4, respectively). Renal sodium excretion was lower during the E2/P4 treatment than during GnRHa alone (Table 3 and Fig. 3, P < 0.05). Although both CH2O and COsm changed over time with the HSI, these variables were similar between the GnRHa-alone and E2/P4 treatments, despite the lower P[AVP] at the end of the infusion with E2/P4 treatment (Table 4, P < 0.05).

(Table 3 and Fig. 2, P < 0.05). Although both free water (CH2O) and osmotic (COsm) clearances changed over time with the HSI, there was no effect of estrogen treatment (Table 3). The baseline differences in PRA between treatments were no longer apparent after HSI (Table 4).

Plasma osmolality was lower at the end of the recovery period during estrogen administration than during GnRHa alone (Fig. 1, P < 0.05). Moreover, the similar changes in Hct and Hb (Table 2) suggest that plasma volume change during the recovery period was similar between the two conditions. Renal sodium excretion remained lower during recovery with estrogen treatment compared with GnRHa alone (Table 3 and Fig. 3, P < 0.05). Overall urine output through the HSI and recovery periods was reduced relative to GnRHa alone (Fig. 5, P < 0.05). This reduction in urine output led to greater overall fluid retention (i.e., HSI + drinking − urine output) during estrogen treatment (Fig. 5, P < 0.05). The fluid- and sodium-regulating hormones responded similarly under both hormonal conditions during recovery (Table 4). There were no hormone treatment-by-time interactions for any of the blood, thirst, or renal variables during HSI or recovery.

![Fig. 1. Plasma osmolality in response to hypertonic saline infusion and recovery during gonadotropin-releasing hormone analog (GnRHa) alone and GnRHa with estrogen administration (A) and with GnRHa with estrogen/progesterone administration (B). Values are means ± SE. *P < 0.05, GnRHa alone compared with hormone treatment.](image-url)
Plasma osmolality remained lower at the end of the recovery period during E2/P4 administration compared with GnRHa alone (Fig. 1, \( P < 0.05 \)). Moreover, Hct and Hb indicated a similar plasma volume response in the recovery period between the two hormone conditions (Table 2). Sodium and urine excretions were also reduced over the recovery period with the E2/P4 treatment compared with GnRHa alone (Figs. 2 and 3, \( P < 0.05 \)), and overall fluid retention (i.e., HSI + drinking – urine output) was greater during the E2/P4 than during the GnRHa alone treatment (Fig. 5, \( P < 0.05 \)). Both PRA and P_{(ALD)} remained elevated during E2/P4 administration through recovery (Table 4). Finally, there were no hormone treatment-by-time interactions for any of the blood, thirst, or renal variables during HSI or recovery.

PRA and P_{(ALD)} were greater with E2/P4 than with estrogen treatment at rest, during HSI, and at the end of recovery (Table 4, \( P < 0.05 \)).

For the E2/P4 group, hormone administration did not affect baseline heart rate (67 ± 4 and 76 ± 4 beats/min) and mean blood pressure (88 ± 3 and 86 ± 5 mmHg for GnRHa alone and GnRHa with E2/P4, respectively). Furthermore, heart rate was unaffected by hormone status at the end of HSI (69 ± 5 and 72 ± 3 beats/min). There was no effect of HSI on mean blood pressure (84 ± 5 and 83 ± 4 mmHg for GnRHa alone and GnRHa with E2/P4, respectively) under either hormone condition. Similarly, there was no effect of E2/P4 administration on heart rate (71 ± 4 and 73 ± 5 beats/min) or mean blood pressure (85 ± 3 and 79 ± 2 mmHg for GnRHa alone and GnRHa with E2/P4, respectively) in recovery. Finally, there were no treatment-by-time interactions for any of the cardiovascular variables during HSI or recovery.

**DISCUSSION**

Forty weeks of GnRHa administration reduced endogenous 17\beta-estradiol and progesterone blood levels of healthy young women to those of postmenopausal women. With selective hormone administration, we then measured the effects of estrogen, with and without progesterone, on body fluid regulation in young women. These are the first data to isolate estrogen effects on the osmoregulation of AVP in young women. Our findings demonstrate that estrogen lowers the osmotic threshold for the release of AVP. Estrogen administration also led to greater sodium and fluid retention in response to HSI, although these changes appeared to be independent of the changes in AVP or changes in the primary sodium-regulating hormones, renin, aldosterone, or ANP. Taken together, these findings suggest that the greater water and sodium retention during estrogen administration was related to intrarenal mechanisms. Compared with GnRHa alone, progesterone administration along with estrogen administration reduced P_{AVP} release during HSI and led to renin-angiotensin-aldosterone system (RAAS) activation and subsequent water and sodium retention.

The estrogen-related shift in osmotic regulation of AVP release most likely occurs via the central nervous system. Estrogen readily crosses the blood-brain barrier and can modulate osmotic AVP regulation via its...
action within the hypothalamus. Estradiol receptors have been identified in the nuclei of neurophysin- and AVP-producing cells in the mouse (33), rat (25), and human SON (19–21). Moreover, osmotic stimulation of vasopressinergic neuronal activity is upregulated by estrogen in the SON of brain slices of ovariectomized rats (2).

The mechanism for the lower osmotic AVP threshold in the hypothalamus during estrogen administration relative to GnRHα alone cannot be determined from our data, although the effect of estrogen administration on the activity of AVP neurons in the hypothalamus could be related to the particular subtype of estrogen receptor activated. Two primary subtypes of estrogen receptors have been identified, with opposite effects on AVP neurons in the SON (19). In humans, estrogen receptor-β (ERβ) inhibits, whereas estrogen receptor-α (ERα) stimulates, AVP neuronal activity in SON (31). Young women express primarily ERβ in the AVP-producing neurons in the SON (19), which should have caused a later, rather than an earlier, osmotic threshold for AVP release during 17β-estradiol administration. However, the ERβ receptors possess a much lower affinity for 17β-estradiol than the ERα receptors (23), so stimulation of the AVP neurons in the SON may have predominated when we administered 17β-estradiol. In addition, the GnRHα may have interfered with ERβ function (4). This interpretation is consistent with previous data demonstrating greater plasma AVP levels at rest and during HSI in postmenopausal women in response to 17β-estradiol administration (36), in whom the ERα predominates (19) and GnRH levels are typically high.

Regardless of the mechanism, the lower osmotic threshold for AVP release does not appear to be a primary cause for the greater fluid retention in the
women during estrogen administration. Renal free water clearance was unaffected by estrogen administration despite the greater AVP stimulation, suggesting that estrogen may alter renal sensitivity to AVP or even interfere with AVP action in the kidney (6, 16, 47). In rats, estrogen attenuates the antidiuretic action of AVP in the collecting duct (6, 16, 47), and this AVP modulation most likely occurs at the receptor level (43). We recently observed little change in CH2O in young women during combined estrogen and progesterone administration despite greater P[AVP], again indicating a reduced renal response to AVP. Moreover, although osmotic secretion of AVP is greater in the midluteal phase of the menstrual cycle (5, 10, 40) (concomitant with increases in estrogen and progesterone), the luteal phase is usually not accompanied by greater water retention (5, 11, 12, 40).

Rather than the AVP-induced increases in free water retention, the greater overall water retention after HSI with estrogen administration appears associated with sodium retention. However, the increase in sodium retention was independent of the RAAS system. Although PRA was increased at baseline during estrogen administration, the greater PRA did not persist through HSI, and there was no change in aldosterone, suggesting minimal RAAS contribution to the greater sodium retention. On the other hand, there are a number of reports that estrogen stimulates the RAAS (18, 24). Estrogen administration can activate the RAAS by enhancing angiotensinogen synthesis, inhibiting angiotensin-converting enzyme activity, and augmenting plasma and tissue levels of renin. The increase in renin will cause a decrease in renal blood flow, but not necessarily a change in angiotensin II or aldosterone (18, 24). Because circulating angiotensinogen is synthesized primarily in the liver, increases in plasma angiotensin II and aldosterone may only be a consequence of oral estrogen administration. Thus the greater fluid and sodium retention that occurred in the present study may be a function of lower renal blood flow, direct estrogen effects on the proximal or distal tubule, effects on aldosterone metabolism or protein binding, or effects on the RAAS actions within the kidney (18, 24).

Although the sodium and fluid retention appears independent of the RAAS during estrogen administration, when progesterone is administered with the estrogen, the RAAS may have a more important role in electrolyte and fluid regulation. PRA and P[ALD] were increased with E2/P4 compared with GnRHa alone and compared with GnRHa plus estrogen. The RAAS activation was consistent with previous findings in the midluteal phase, when a progesterone-mediated inhibition of aldosterone-dependent sodium reabsorption 
at distal sites in the nephron produces a transient natriuresis and a compensatory stimulation of the renin-aldosterone system (29). We also found that E2/P4 administration increased sodium retention throughout HSI and recovery compared with GnRHa alone. This RAAS activation during concomitant elevations of plasma estrogen and progesterone seems to be a component of a system evolved to maintain blood pressure and plasma water and sodium content during changes in sex hormones over the menstrual cycle and pregnancy (3, 28, 32). For example, increases in plasma progesterone stimulate the RAAS, but the vasoconstrictive/antinatriuretic actions of the RAAS are counterbalanced by the vasodilatory/natriuretic effects of natriuretic peptides (32) and vasodilation (3, 28), which can be stimulated by increases in plasma estrogen levels.

Finally, ANP can have a role in this homeostatic feedback system that regulates sodium balance, that is, sodium- and volume-retainiing stimuli increase ANP, which in turn antagonizes renin and aldosterone (3, 35, 49). In humans, high plasma progesterone levels are associated with lower plasma concentrations of ANP during sodium loading (48) and dehydration (40). In animals, estrogen and progesterone modulate ANP receptor activity (27, 28, 30, 45). For example, studies have indicated that progesterone may interfere with the inhibitory effects of ANP on aldosterone secretion, whereas estrogen appears to promote ANP inhibition (27). Thus estrogen and progesterone may have impacted sodium retention via direct or indirect effects on ANP intrarenal actions.

This study is the first to isolate estrogen effects on osmoregulation of AVP and water regulation in young women by suppressing reproductive function and adding back estrogen and progesterone in controlled doses. Our finding that estrogen lowers the osmotic threshold for the stimulation and release of AVP confirms earlier studies with estrogen administration to postmenopausal women (36), during E2/P4 administration, and during the menstrual cycle in young women (5, 40). Despite this earlier release of AVP, the primary changes in water retention during estrogen treatment were more closely related to sodium retention. Further studies using kidney tracers to measure renal blood flow and distal tubule sodium handling will help to elucidate the specific mechanism for the greater sodium and water retention during estrogen administration. Sodium retention during combined estrogen and progesterone administration was increased compared with the GnRHa alone, most likely related to transient RAAS stimulation.

Perspectives

The GnRHa add-back protocol enabled us to do in humans what has thus far only been done in animals: to temporaril pharmacologically “oophorectomize” young, healthy women to isolate specific effects of estrogen and/or progesterone on a physiological system. This type of incisive intervention is necessary to provide the basis for understanding the etiology of disturbances in body fluid regulation during hormone administration to humans, an area that is poorly understood. Inappropriate regulation of body fluids has a role in chronic disease etiology. It has long been known that susceptibility to, and the progression of, chronic disease differ between men and women and are drastically changed in women after menopause. Characterizing the actions of the female sex hormones in humans on the systems that regulate cardiovascular and renal function is important to our understanding and eventual sex-specific treatment of these chronic debilitating diseases. Our findings are also relevant to acute conditions resulting from inappropriate regulation of body water and electrolytes. For example, menstruating women are more susceptible to postoperative hyponatremia with associated neurological involvement (i.e.,

Fig. 5. Overall body fluid balance per kilogram body wt in response to hypertonic saline infusion and recovery during GnRHa alone and GnRHa with estrogen administration (A) and with GnRHa with estrogen/progesterone (B) administration. Fluid intake represents fluid infused + fluid drunk. Urine output represents total urine output after hypertonic saline infusion and recovery. Fluid balance = fluid intake – urine output. Values are means ± SE. *P < 0.05, GnRHa alone vs. hormone treatment.
hyponatremic encephalopathy) compared with men and postmenopausal women (1). Finally, there is a large body of evidence to indicate that hormone status plays a role in adaptation to the heat.

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REFERENCES


33. Stachenfeld NS, Mack GW, Takamata A, DiPietro L, and Nadel ER. Thirst and fluid regulatory responses to hypertonic-


44. Trigoso WF, Wesly JM, Meranda DL, and Shenker Y. Vasopressin and atrial natriuretic peptide hormone responses to hypertonic saline infusion during the follicular and luteal phases of the menstrual cycle. Hum Reprod 11: 2392–2395, 1996.


