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# Estrogen influences osmotic secretion of AVP and body water balance in postmenopausal women

NINA S. STACHENFELD, LORETTA DiPIETRO, STEVEN F. PALTER, AND ETHAN R. NADEL  
*The John B. Pierce Laboratory and Departments of Epidemiology and Public Health, Cellular and Molecular Physiology, and Obstetrics and Gynecology, Division of Reproductive Endocrinology, Yale University School of Medicine, New Haven, Connecticut 06519*

**Stachenfeld, Nina S., Loretta DiPietro, Steven F. Palter, and Ethan R. Nadel.** Estrogen influences osmotic secretion of AVP and body water balance in postmenopausal women. *Am. J. Physiol.* 274 (Regulatory Integrative Comp. Physiol. 43): R187–R195, 1998.—To determine if estrogen upregulates osmotic secretion of arginine vasopressin (AVP) and alters body water balance, we infused hypertonic (3% NaCl) saline in 6 women ( $68 \pm 3$  yr) after 14 days of  $17\beta$ -estradiol (transdermal patch,  $\sim 0.1$  mg/day,  $E_2$ ) and placebo (control) administration. Hypertonic saline was infused at  $0.1 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 120 min, and after a 30-min equilibration period, the subjects drank water ad libitum for 180 min.  $E_2$  increased basal plasma estradiol concentration from  $\leq 12$  to  $80 \pm 12$  pg/ml and plasma AVP concentration ( $P_{[AVP]}$ ) from  $2.1 \pm 0.7$  to  $3.1 \pm 0.8$  pg/ml ( $P < 0.05$ ), but not plasma osmolality ( $P_{\text{osm}}$ ,  $288 \pm 1$  and  $287 \pm 1$ , for control and  $E_2$ , respectively). Hypertonic saline infusion increased  $P_{\text{osm}}$  by  $18 \pm 1$  and  $17 \pm 1$  mosmol/kgH<sub>2</sub>O and  $P_{[AVP]}$  by  $5.2 \pm 0.5$  and  $4.9 \pm 0.4$  pg/ml for control and  $E_2$  treatments, respectively. The  $P_{[AVP]} \cdot P_{\text{osm}}$  relationship shifted upward after  $E_2$ , with no change in sensitivity (slope,  $0.36 \pm 0.02$  and  $0.33 \pm 0.03$  pg  $\cdot$  ml<sup>-1</sup>  $\cdot$  mosmol<sup>-1</sup> for control and  $E_2$ , respectively). Water intake was similar between control and  $E_2$  (24 vs. 22 ml/kg), but by 180 min of drinking, urine output and free water clearance ( $C_{H_2O}$ ) were reduced by  $5.6 \pm 2.3$  ml/kg and  $2.6 \pm 2.0$  ml/min, respectively ( $P < 0.05$ ) after  $E_2$ . Plasma aldosterone concentration was unaffected by  $E_2$ , but fractional sodium excretion was reduced from  $2.7 \pm 0.5$  to  $1.7 \pm 0.4\%$  ( $P < 0.05$ ) at 180 min of drinking. Our data suggest that  $E_2$  augments osmotic AVP secretion, thereby implicating elevated AVP as a contributor to water retention in high  $E_2$  states; however, an increase in renal sodium reabsorption was a major component of the enhanced fluid retention.

renal osmoregulation; arginine vasopressin; hormonal regulation;  $17\beta$ -estradiol

BODY WATER RETENTION is common in high estrogen states, such as that immediately preceding ovulation (30), during pregnancy (11), and while taking estrogen ( $E_2$ ) (1) or  $E_2$ -dominant oral contraceptives (4). This body fluid retention could be due in part to enhanced osmotic stimulation of arginine vasopressin (AVP), the primary hormone involved in modulating renal water reabsorption. AVP is synthesized in the cell bodies of specific neurons (paraventricular and supraoptic nuclei) in the anterior hypothalamus, and axons from these areas project into the posterior pituitary, where it is stored and released because of stimulation of central osmoreceptors. These nerve endings lie in close proximity to capillary networks, so AVP release into the circulation occurs rapidly after elevations in plasma osmolality ( $P_{\text{osm}}$ ). There is evidence that  $E_2$  can modulate the osmotic regulation of AVP release in the brain

(15, 21), although the precise mechanism for this effect is not known.

Investigations of the influence of estrogen on AVP regulation have yielded variable results. A number of studies have demonstrated that basal plasma AVP concentration ( $P_{[AVP]}$ ) is elevated in the presence of high plasma concentrations of unopposed estrogen ( $P_{[E_2]}$ ), such as during the mid-follicular phase of the menstrual cycle in young women (12) and after exogenous  $E_2$  administration in postmenopausal women (13), whereas other studies have not found differences in basal  $P_{[AVP]}$  across the menstrual cycle (31) or between men and women (33). High plasma concentrations of sex steroids (estrogen, progesterone) may increase the slope (22) or threshold (11, 31) of the  $P_{[AVP]} \cdot P_{\text{osm}}$  relationship during hypertonic saline infusion, providing evidence that these hormones alter the osmotic regulation of AVP. During pregnancy, a period accompanied by elevated sex hormones and body water expansion, basal  $P_{\text{osm}}$  is reduced by  $\sim 6$  mosmol/kgH<sub>2</sub>O despite a constant  $P_{[AVP]}$ . Furthermore, the  $P_{[AVP]} \cdot P_{\text{osm}}$  relationship is shifted to a lower  $P_{\text{osm}}$ , and the threshold for thirst sensation is reduced during hypertonic saline infusion, indicating enhanced osmotic AVP secretion and thirst perception in pregnancy (11).

Examining the specific effects of  $E_2$  on AVP and body fluid regulation is complex in young women because circulating sex hormone and gonadotropin levels are constantly changing. Pregnancy is associated with increased circulating progesterone and vasopressinase (an enzyme that rapidly clears AVP from plasma), as well as alterations in blood volume and mean arterial pressure, all of which may alter  $P_{[AVP]}$  independent of  $E_2$ . Postmenopausal women, however, are naturally low in both progesterone and estrogen, so they provide an appropriate model with which the modulatory effects of  $E_2$  on the osmotic regulation of AVP can be isolated. The purpose of the present study was to determine the effect of estrogen administration on the osmotic control of AVP and the consequent adjustments in body water. We hypothesized that increased  $P_{[E_2]}$  would enhance the  $P_{[AVP]}$ , thirst, and drinking responses to an osmotic stimulus (3.0% NaCl saline infusion) and result in greater body fluid expansion during the infusion and subsequent ad libitum drinking recovery period.

## METHODS

Six ( $>55$  yr) healthy, postmenopausal women, with no known cardiovascular, gynecological, or renal disease participated in this study. The subjects were cleared by their private physician and underwent physical and gynecological examina-

tions before participation. All women reported that >10 yr had passed since their last menstrual period. The reproductive hormonal status of the subjects was confirmed by measuring plasma estrogen concentration. 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 procedures.** Estrogen was administered through a percutaneous delivery system (Vivelle; Ciba Pharmaceuticals, Summit, NJ), designed to deliver 17 $\beta$ -estradiol continuously (0.1 mg/day) on application to intact skin. Estrogen administration lasted for 14 days, and the patch was replaced twice weekly. The placebo patches were identical to the E<sub>2</sub> patches but did not contain E<sub>2</sub>. Subjects were told to maintain their normal diet and exercise routines.

On *day 14* of E<sub>2</sub> administration the hypertonic saline infusion protocol was administered. This same protocol was also performed on *day 14* of placebo control. The order of treatment was assigned alternately as each subject entered the study in a placebo-controlled crossover design.

**Hypertonic saline infusion.** The subjects refrained from heavy exercise for 24 h and from alcohol and caffeine for 12 h before the experiment. Subjects ate a light breakfast (~300 kcal) and drank 5 ml/kg of water at home ~1 h before arriving at the laboratory at 8 AM. After arrival they voided and were seated for 60 min (25°C, <40% relative humidity). Hydration status was immediately assessed from urine specific gravity and was  $\leq 1.01$  in all subjects. During the 60-min control period a 20-gauge Teflon catheter was placed in an antecubital or forearm vein. Baseline arterial blood pressure (Dinamap; Critikon, Tampa, FL) and heart rate (electrocardiogram) were recorded every 10 min for the last 30 min. At the end of the 60-min control period, a control blood sample (20 ml) was taken, thirst perception was assessed (see *Thirst*), and a total urine volume was collected. Hypertonic (3.0% NaCl) saline was then infused at a rate of 0.1 ml·kg body wt<sup>-1</sup>·min<sup>-1</sup> for 120 min. Blood was sampled (8 ml) at 30, 45, 60, 85, 90, and 120 min during the infusion, and a urine void was obtained at the end of infusion. A 30-min seated equilibration period followed the infusion, after which time the subject was allowed to drink ad libitum for 180 min. Blood samples (8–13 ml) were obtained at 0, 5, 15, 30, 60, 120, and 180 min during drinking. Urine was collected at 60, 120, and 180 min of drinking. Thirst perception was assessed at all blood sampling times.

**Blood sampling.** Free flowing venous blood was obtained for the measurement of hematocrit (Hct), hemoglobin (Hb), total protein (TP), P<sub>osm</sub>, P<sub>[AVP]</sub>, plasma E<sub>2</sub> concentration (P<sub>[E<sub>2</sub>]</sub>), plasma creatinine (P<sub>[Cr]</sub>), plasma aldosterone (P<sub>[Aldo]</sub>), urea, glucose, and serum electrolyte concentrations. An aliquot (1 ml) was removed for immediate assessment of Hct, Hb, and TP in triplicate by microhematocrit, cyanomethemoglobin, and refractometry, respectively. An aliquot for P<sub>osm</sub>, P<sub>[Cr]</sub>, and P<sub>[Aldo]</sub> was transferred to a tube containing lithium heparin, and an aliquot for determination of P<sub>[AVP]</sub> and P<sub>[E<sub>2</sub>]</sub> was transferred into a tube containing EDTA. An aliquot for the determination of serum sodium (S<sub>[Na<sup>+</sup>]</sub>) and potassium (S<sub>[K<sup>+</sup>]</sub>) concentrations was placed into a tube without anticoagulant. Samples were centrifuged for 15 min at 4°C, after which plasma or serum was drawn off for the immediate analysis of P<sub>osm</sub> (freezing point depression, model 3D11 Advanced Instruments) and serum electrolytes (flame photometry, model 943, Instrumentation Laboratory, Lexington, MA). Whole blood used for serum electrolytes was allowed to clot before centrifugation. Plasma for analysis of P<sub>[Cr]</sub> was stored at -20°C. Plasma for the determination of P<sub>[AVP]</sub>, P<sub>[E<sub>2</sub>]</sub>, and P<sub>[Aldo]</sub> was stored at -70°C. P<sub>osm</sub>, Hct, Hb, TP, and P<sub>[AVP]</sub> were deter-

mined from all blood samples. P<sub>[Aldo]</sub> was determined at baseline, immediately after the infusion, and at 0, 60, and 180 min of ad libitum drinking. P<sub>[E<sub>2</sub>]</sub> was determined from baseline samples before each infusion. Serum solids concentration was determined from the regression equation relating TP and dried weight of serum (18). Serum electrolyte concentrations were represented as the concentrations in serum H<sub>2</sub>O (meq/kgH<sub>2</sub>O) after subtracting the solids fraction from the serum volume.

Plasma creatinine, urea, and glucose were assessed by colorimetric assay (Sigma kit, St. Louis, MO). P<sub>[AVP]</sub> was determined by radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA) after separation from binding proteins with ethanol extraction. Plasma concentration was corrected for recovery of AVP (average 70%) from plasma, determined by adding <sup>125</sup>I-labeled vasopressin to plasma before extraction. This assay is highly specific for AVP, with cross-reactivity of lysine vasopressin and oxytocin <0.01%. The sensitivity of this assay is 1.2 pg/ml, with intra- and interassay coefficients of variation for P<sub>[AVP]</sub> (4.4 pg/ml) of 2.93 and 5.23%. P<sub>[Aldo]</sub> and P<sub>[E<sub>2</sub>]</sub> were also determined in duplicate by radioimmunoassay. Intra- and interassay coefficients of variation for P<sub>[Aldo]</sub> (131 pg/ml) were 2.19 and 2.74% (Coat-A-Count, Diagnostic Products). All samples from a given subject were determined within the same assay. For one subject, P<sub>[Aldo]</sub> was below the sensitivity of our assay (<16.0 pg/ml) for three of five blood samples during E<sub>2</sub> and four of five blood samples during control, so all of her aldosterone data were excluded from further analysis. All samples for P<sub>[E<sub>2</sub>]</sub> were analyzed within one assay kit (Coat-A-Count, Diagnostic Products), with an intra-assay coefficient of variation for the mid-range standard (70.7 pg/ml) of 7.8%.

**Urinalysis.** Urine samples were analyzed immediately for osmolality (freezing point depression, model 3D11 Advanced Instruments) and electrolytes (flame photometry, model 943, Instrumentation Laboratory). An aliquot for the determination of urine creatinine (colorimetric assay, Sigma Kit) was frozen at -20°C until analyzed.

**Ad libitum fluid intake.** During the drinking period bottled water (15°C) was provided for 180 min. To monitor the rate of water consumption, the container (~900 ml) was weighed during each blood sampling period. The subjects were instructed to drink if they felt thirsty and were told that fluid intake was being monitored to determine fluid balance.

**Thirst.** Perceptions of thirst, mouth dryness, and stomach distension were assessed by three visual analog rating scales (17, 20). The subjects responded to the question "how thirsty do you feel right now?" on a scale 180 mm in length with intersecting lines at 0 mm "not at all" and at 125 mm "extremely thirsty." Two analogous scales were used to assess perception of stomach distension, "not at all" to "extremely full," and mouth dryness "not at all" to "extremely dry." The subjects were instructed to mark anywhere on the line, including beyond the "extreme" mark, and they could extend the line if they wished. These rating scales have been used extensively for psychophysical assessments in older men and women and have been shown to correspond to physiological determinants of thirst, such as P<sub>osm</sub> (20).

**Calculations.** Net fluid gain during infusion and drinking was determined by subtracting urine output from the volume infused and water ingested. Relative changes in plasma volume were calculated based on changes in Hct and Hb during the experiment (seated upright position) using the equation (14)

$$\Delta PV (\%) = 100$$

$$\times [(\text{Hb}_{\text{pre}}/\text{Hb}_{\text{post}}) \times (1 - \text{Hct}_{\text{post}}/100)/(1 - \text{Hct}_{\text{pre}}/100)] - 100$$

Fractional excretions of water ( $FE_{H_2O}$ ) and  $Na^+$  ( $FE_{Na^+}$ ) were calculated from the equations

$$FE_{H_2O} = (U_v/GFR)100$$

$$FE_{Na^+} = (U_v \times [Na^+]_u/GFR \times [Na^+]_f)100$$

$$[Na^+]_f = \text{the Donnan factor for cations } (0.95) \times S_{[Na^+]}$$

where the subscripts f and u are glomerular filtrate and urine, respectively,  $U_v$  is urine flow rate, and  $S_{[Na^+]}$  is  $S_{[Na^+]}$  in protein-free solution (meq/kgH<sub>2</sub>O). Glomerular filtration rate (GFR) was estimated from creatinine clearance.

**Statistics.** Data are expressed as means  $\pm$  SE. Repeated-measures analysis of variance models were performed to test differences in the dependent variables due to E<sub>2</sub> administration. Bonferroni's *t*-test was used to correct for multiple comparisons when appropriate. We used paired *t*-tests to determine the effect of E<sub>2</sub> administration on subject characteristics and baseline levels of  $P_{[AVP]}$  and  $P_{osm}$ . Pearson's product moment correlations were used to assess the relationships of  $P_{[AVP]}$  and thirst as functions of  $P_{osm}$  on individual data, excluding the baseline values. Paired *t*-tests were used to determine E<sub>2</sub>-related differences in the abscissal intercepts, which defined the "theoretical threshold" for AVP release (11). Analysis of covariance was applied to determine the E<sub>2</sub>-related differences in the  $P_{[AVP]}$ - $P_{osm}$  slope during hypertonic saline infusion, with E<sub>2</sub> status as the covariate (11). Based on an alpha level of 0.05 and a sample size of six our beta level (power) was  $\geq 0.80$  for detecting effect sizes of 35 mm, 2.0 pg/ml, and 0.67 ml/min for thirst,  $P_{[AVP]}$ , and renal  $C_{H_2O}$ , respectively (16, 23). Data were analyzed using BMDP<sup>®</sup> statistical software (BMDP Statistical Software, Los Angeles, CA).

## RESULTS

**Subject characteristics.** The mean age of the subjects was  $68 \pm 2$  yr (range 57–70). The subjects weighed  $72.3 \pm 5.0$  kg and were  $166 \pm 2$  cm tall.  $P_{[E_2]}$  was  $\leq 12$  pg/ml in all subjects before participation and after placebo control, and was undetectable in three subjects. Fourteen days of E<sub>2</sub> administration increased  $P_{[E_2]}$  in all subjects ( $79.7 \pm 12.4$  pg/ml, 50–124 pg/ml). Preinfusion body weight increased to  $73.4 \pm 4.9$  (mean increase

$1.0 \pm 0.3$  kg,  $P < 0.05$ ) after 14 days of E<sub>2</sub> administration but was unchanged after placebo control treatment [ $72.7 \pm 5.0$  kg, mean change  $0.3 \pm 0.4$ , not significant (NS)]. Compared with placebo control, E<sub>2</sub> administration reduced Hct by  $1.3 \pm 0.1\%$  and Hb by  $0.6 \pm 0.2$  g/dl (Table 1), indicating a plasma volume expansion of  $4.5 \pm 1.9\%$ . Baseline  $P_{osm}$  was unchanged, but  $P_{[AVP]}$  was increased from  $2.1 \pm 0.5$  to  $3.1 \pm 0.7$  pg/ml ( $P < 0.05$ ) after E<sub>2</sub> administration (Fig 1). Preinfusion  $S_{[Na^+]}$  was unchanged after E<sub>2</sub> administration (Table 1) as were plasma concentrations of urea ( $18 \pm 2$  and  $16 \pm 1$  g/dl for E<sub>2</sub> and control patches, respectively) and glucose ( $116 \pm 6$  and  $111 \pm 7$  mg/dl for E<sub>2</sub> and control patches, respectively). Mean arterial blood pressure was unaffected by E<sub>2</sub> administration ( $93 \pm 3$  vs.  $93 \pm 4$  mmHg for E<sub>2</sub> and control patches, respectively), as was baseline  $P_{[Aldo]}$  (Table 1).

Hypertonic saline infusion produced similar graded increases in  $P_{osm}$  from  $287 \pm 2$  to  $304 \pm 1$  mosmol/kgH<sub>2</sub>O during E<sub>2</sub> administration and from  $288 \pm 1$  to  $306 \pm 2$  mosmol/kgH<sub>2</sub>O during placebo control (Fig. 1). The increase in  $P_{osm}$  was accompanied by an increase in  $P_{[AVP]}$  of  $4.9 \pm 0.4$  pg/ml during E<sub>2</sub> administration and  $5.2 \pm 0.5$  pg/ml during placebo control treatment (Fig. 1,  $P < 0.05$ ). As shown in Table 1, Hct and Hb were reduced similarly during E<sub>2</sub> and placebo control treatments throughout hypertonic saline infusion and drinking, and therefore plasma volume expansion was also similar between the E<sub>2</sub> and control patches ( $16.3 \pm 2.8$  and  $16.9 \pm 2.0\%$ , respectively) after hypertonic saline infusion (Fig. 1).  $S_{[Na^+]}$ ,  $S_{[K^+]}$ , and  $P_{[Aldo]}$  also responded similarly to hypertonic saline infusion and drinking under both treatments (Table 1). After the hypertonic saline infusion, plasma concentrations of urea were unchanged ( $18 \pm 2$  and  $14 \pm 1$  g/dl for E<sub>2</sub> and control patches, respectively) and plasma glucose concentration fell slightly in both treatments to  $109 \pm 4$  mg/dl during E<sub>2</sub> administration and to  $101 \pm 9$  mg/dl during control patches (NS).

Table 1. Responses of blood variables to hypertonic (3.0% NaCl) saline infusion and 180 min of ad libitum drinking during E<sub>2</sub> administration and placebo control conditions

	Preinfusion	Infusion	Ad Libitum Drinking		
	0 min	120 min	210 min	270 min	330 min
Hct, %					
E <sub>2</sub>	$39.5 \pm 0.8$	$35.7 \pm 1.2$	$36.8 \pm 1.0$	$36.4 \pm 1.1$	$36.4 \pm 1.1$
Control	$40.7 \pm 0.8$	$36.6 \pm 1.0$	$38.0 \pm 1.1$	$37.7 \pm 1.0$	$37.6 \pm 1.0$
Hb, g/dl					
E <sub>2</sub>	$13.0 \pm 0.2$	$12.0 \pm 0.3$	$12.2 \pm 0.3$	$12.0 \pm 0.4$	$11.9 \pm 0.3$
Control	$13.4 \pm 0.3$	$12.3 \pm 0.3$	$12.6 \pm 0.3$	$12.4 \pm 0.3$	$12.3 \pm 0.3$
$S_{[Na^+]}$ , meq/kgH <sub>2</sub> O					
E <sub>2</sub>	$150.1 \pm 1.2$	$159.0 \pm 1.4$	$152.4 \pm 1.0$	$149.9 \pm 0.8$	$148.8 \pm 0.7$
Control	$151.3 \pm 0.7$	$160.6 \pm 0.5$	$153.3 \pm 1.1$	$150.9 \pm 0.8$	$150.1 \pm 0.8$
$S_{[K^+]}$ , meq/kgH <sub>2</sub> O					
E <sub>2</sub>	$4.61 \pm 0.14$	$4.77 \pm 0.15$	$4.66 \pm 0.08$	$4.69 \pm 0.08$	$4.43 \pm 0.05$
Control	$4.66 \pm 0.14$	$4.76 \pm 0.10$	$4.74 \pm 0.11$	$4.63 \pm 0.14$	$4.40 \pm 0.07$
$P_{[Aldo]}$ , pg/ml					
E <sub>2</sub>	$113.8 \pm 30.6$	$27.2 \pm 5.9$	$32.9 \pm 8.9$	$26.7 \pm 5.2$	$23.2 \pm 4.4$
Control	$96.3 \pm 4.9$	$41.0 \pm 7.3$	$25.5 \pm 4.1$	$26.8 \pm 6.6$	$24.4 \pm 4.9$

Values are means  $\pm$  SE;  $n = 6$  subjects, data on  $P_{[Aldo]}$  were analyzed on 5 subjects. E<sub>2</sub>, estrogen; Hct, hematocrit; Hb, hemoglobin;  $S_{[Na^+]}$  and  $S_{[K^+]}$ , serum concentrations of Na<sup>+</sup> and K<sup>+</sup>;  $P_{[Aldo]}$ , plasma concentration of aldosterone.

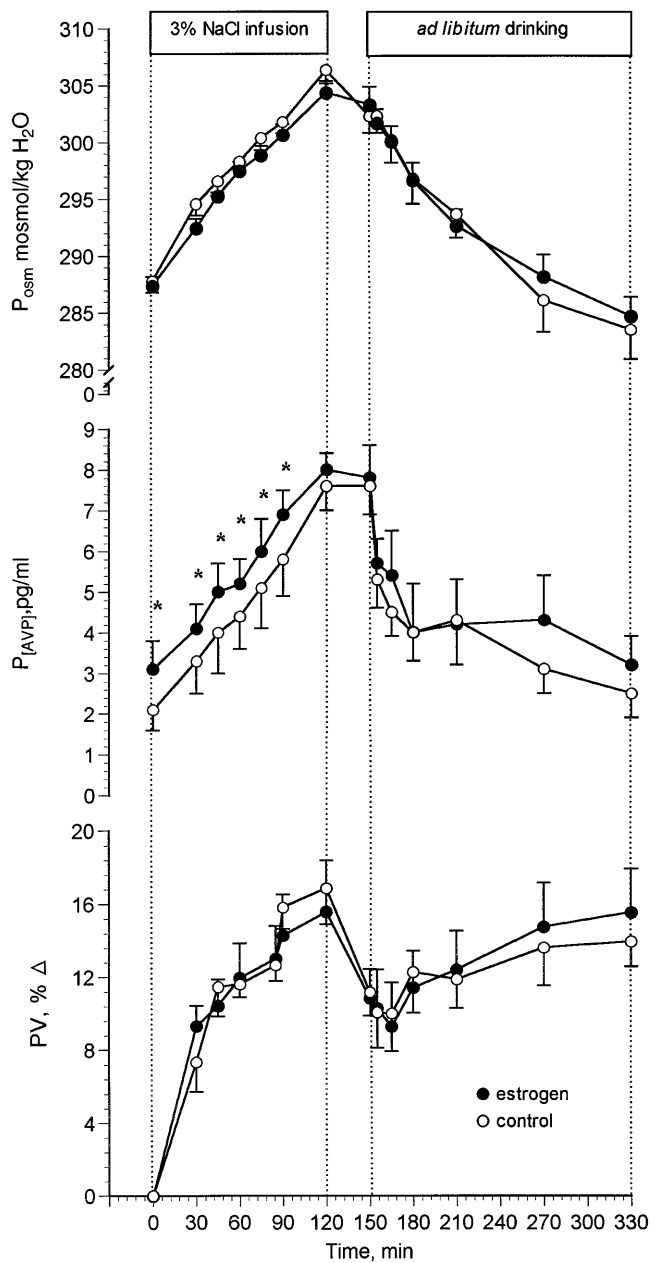


Fig. 1. Plasma osmolality ( $P_{osm}$ , top), plasma arginine vasopressin concentration ( $P_{[AVP]}$ , middle), and percent change plasma volume (PV, bottom) during hypertonic (3.0% NaCl) saline infusion and at 5, 15, 30, 60, 120, and 180 min of ad libitum fluid intake during estrogen and placebo control conditions. \*Different from placebo control,  $P < 0.05$ . Data are expressed as means  $\pm$  SE.

Linear regression analysis of the individual data indicated significant correlations between  $P_{osm}$  and  $P_{[AVP]}$ , with  $r$  values ranging from 0.88 to 0.98. The plots in Fig. 2 demonstrate that the  $P_{[AVP]}-P_{osm}$  relationship was shifted upward relative to placebo control in five of six subjects; the mean abscissal intercepts for the  $P_{[AVP]}-P_{osm}$  relationships were  $280 \pm 4$  mosmol/kgH<sub>2</sub>O and  $285 \pm 4$  mosmol/kgH<sub>2</sub>O for E<sub>2</sub> and placebo control treatments, respectively ( $P < 0.05$ ). The slopes of these relationships were unaffected by E<sub>2</sub> administration ( $0.33 \pm 0.02$  and  $0.36 \pm 0.03$  pg·ml<sup>-1</sup>·mosmol<sup>-1</sup> for E<sub>2</sub> and placebo control, respectively).

Hypertonic saline infusion led to an increase in the ratings of thirst to  $70 \pm 14$  and  $81 \pm 11$  mm on the line rating scale and resulted in water intake of  $24 \pm 3$  and  $22 \pm 3$  ml/kg by 180 min during ad libitum drinking for E<sub>2</sub> and placebo control patches, respectively. Individual linear regression analyses of the thirst- $P_{osm}$  relationship revealed a mean slope of  $6.85 \pm 0.98$  mm/mosmol and a threshold for the onset of thirst perception of  $290 \pm 1$  mosmol/kgH<sub>2</sub>O during E<sub>2</sub> administration, which were not different from placebo control treatment ( $6.65 \pm 1.56$  mm/mosmol and  $292 \pm 4$  mosmol/kgH<sub>2</sub>O). Ratings of mouth dryness were increased during hypertonic saline infusion, but the responses were unaffected by E<sub>2</sub> treatment. Individual linear regression analysis of the mouth dryness-thirst relationship during saline infusion and the early part of drinking yielded correlation coefficients exceeding 0.90 ( $P < 0.05$ ) for all subjects. Stomach fullness ratings were unaffected by saline infusion or by E<sub>2</sub> treatment. Hypertonic saline infusion led to significant increases in mean arterial pressure ( $12 \pm 3$  mmHg,  $P < 0.05$ ) during placebo control but not during E<sub>2</sub> treatment ( $7 \pm 6$  mmHg, NS).

**Fluid balance.** Figure 3 shows the cumulative fluid balance over the infusion and ad libitum drinking periods, with zero time denoting the beginning of drinking. There were no E<sub>2</sub>-related differences in fluid intake, but urine output was lower after E<sub>2</sub> ( $9.1 \pm 1.7$  vs.  $13.2 \pm 2.3$  ml/kg,  $P < 0.05$ ), resulting in a net fluid gain during the drinking period ( $18.2 \pm 3.3$  vs.  $12.5 \pm 2.7$  ml/kg,  $P = 0.05$ , for E<sub>2</sub> and control conditions, respectively).

**Renal water and electrolyte regulation.** Hypertonic saline infusion reduced  $U_v$ ,  $FE_{H_2O}$ ,  $C_{H_2O}$ , and increased urine osmolality ( $U_{osm}$ ),  $FE_{Na^+}$ , and renal osmolar clearance in a similar fashion during E<sub>2</sub> administration and placebo control (Table 2). However, E<sub>2</sub> administration reduced  $C_{H_2O}$ ,  $U_v$ ,  $FE_{H_2O}$ , and  $FE_{Na^+}$  by 180 min of drinking ( $P < 0.05$ ), indicating greater renal water and sodium retention. Urine potassium excretion was greater during the last 60 min of infusion and through the first 60 min of drinking during E<sub>2</sub> administration (Table 2,  $P < 0.05$ ).

## DISCUSSION

In postmenopausal women, 14 days of E<sub>2</sub> administration increased basal  $P_{[AVP]}$ , thus shifting the  $P_{[AVP]}-P_{osm}$  relationship upward during hypertonic saline infusion, suggesting that E<sub>2</sub> modulates AVP release by the hypothalamus. During ad libitum drinking, water and sodium reabsorption rates were enhanced by E<sub>2</sub> treatment, whereas thirst and water intake were unaffected, leading to greater water retention. The greater water retention was due in part to an AVP-mediated fall in  $C_{H_2O}$ , but primarily to increased renal sodium reabsorption. An increase in sodium retention occurred in the presence of a constant  $P_{[Aldo]}$ , suggesting that E<sub>2</sub> may directly influence electrolyte handling in the kidney.

**Osmotic AVP regulation.** Earlier studies have demonstrated increased basal  $P_{[AVP]}$  (5, 13) and plasma vol-

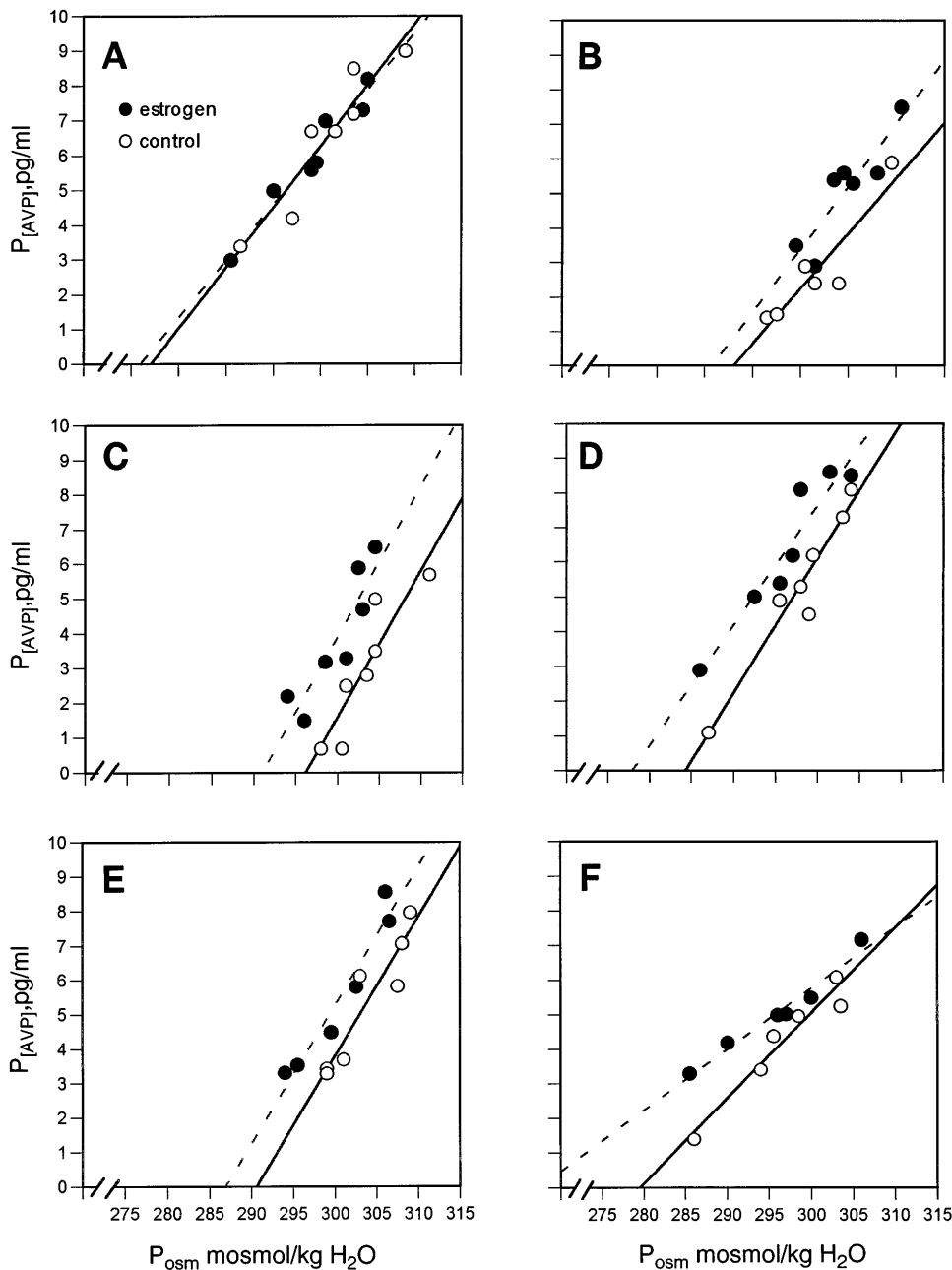


Fig. 2. Individual subjects' relationships between  $P_{\text{osm}}$  and  $P_{[\text{AVP}]}$  during hypertonic (3.0% NaCl) saline infusion during estrogen administration and placebo control. Mean linear regression equations were  $P_{[\text{AVP}]} = 0.33 (P_{\text{osm}} - 280)$  and  $P_{[\text{AVP}]} = 0.36 (P_{\text{osm}} - 285)$  for estrogen and placebo control treatments, respectively.

ume expansion (32) in women after  $E_2$  administration. Our data confirm and extend these findings, demonstrating an upward shift of the  $P_{[\text{AVP}]}-P_{\text{osm}}$  relationship during osmotic stimulation. Although  $P_{[\text{AVP}]}$  increased concomitantly with  $P_{\text{osm}}$  with both  $E_2$  and placebo control patches,  $P_{[\text{AVP}]}$  was greater through the physiological range of  $P_{\text{osm}}$  (i.e.,  $\sim 287-297$  pg/ml) during  $E_2$  administration, with no difference between  $E_2$  and control in the slope (sensitivity) of the  $P_{[\text{AVP}]}-P_{\text{osm}}$  relationship.

Whether  $P_{[\text{AVP}]}$  is elevated in the presence of high endogenous estrogen, such as during pregnancy (11) and the mid-follicular and mid-luteal phases of the menstrual cycle (12, 31), is still controversial. Vokes et al. (31) did not find differences in basal  $P_{[\text{AVP}]}$  over the course of the menstrual cycle. However, basal  $P_{\text{osm}}$  was

lowest ( $\sim 4$  mosmol/kg $H_2O$ ) in the mid-luteal phase, when estrogen and progesterone peak, indicating an earlier osmotic threshold for AVP secretion. These findings are similar to those reported in pregnant women (11) but are in contrast to those in young, cycling women (12), in whom  $P_{\text{osm}}$  was unaffected by the menstrual cycle, but  $P_{[\text{AVP}]}$  was increased in the mid-luteal phase. The findings in our present study in which unopposed  $E_2$  was administered, are consistent with earlier studies of exogenous  $E_2$  administration (13), in which basal  $P_{[\text{AVP}]}$  was elevated, whereas  $P_{\text{osm}}$  was unaffected. The cause of the discrepant findings between endogenous and exogenous  $E_2$  cannot be determined from our data but may be related to differences in bioavailability or binding characteristics between the synthetic and natural forms of this hormone.

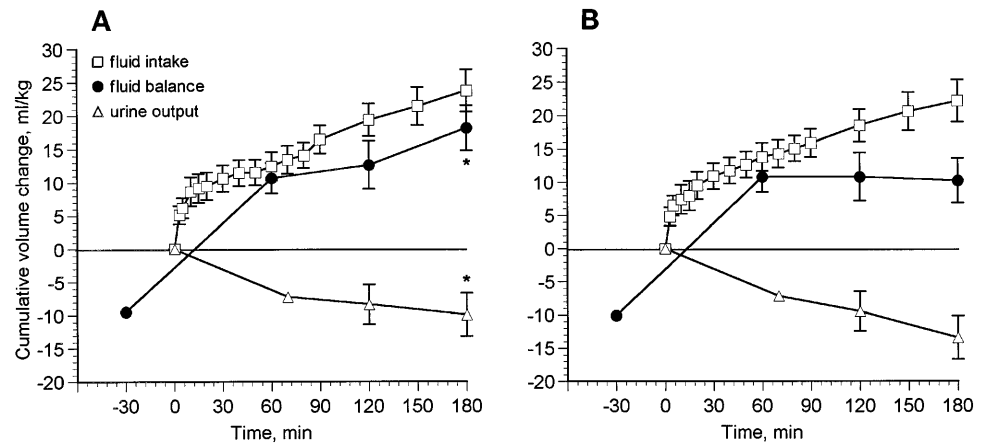


Fig. 3. Fluid balance during estrogen administration (A) and placebo control (B) after hypertonic (3.0% NaCl) saline infusion. \*Different from placebo control,  $P < 0.05$ . Data are expressed as means  $\pm$  SE.

Although the mechanism for the  $E_2$ -associated change in  $P_{[AVP]}$  response cannot be determined from our data, it is likely that  $E_2$  influences the central regulation of AVP synthesis and release. Estrogen readily crosses the blood-brain barrier and thereby gains access to sites in the brain that control AVP release. In lower animals,  $E_2$  acts directly on the central nervous system through neurons that bind  $E_2$  and increase hypothalamic AVP synthesis and/or release (2, 3, 9, 10, 21). Sar and Stumpf (21) identified estradiol receptors in the

nuclei of neurophysin- and AVP-producing cells in the mouse supraoptic nucleus. Furthermore, the threshold for osmotic stimulation of vasopressinergic neuronal activation is lower in the supraoptic nucleus of brain slices of ovariectomized rats given  $E_2$ , compared with untreated rats (2). It is likely that sex steroids play a modulating role in AVP gene expression in the paraventricular and supraoptic nuclei because osmotically stimulated hypothalamic AVP mRNA concentration is reduced after gonadectomy in the rat (9).

Table 2. Renal function, fluid, and osmoregulatory responses to hypertonic (3% NaCl) saline infusion and 180 min of ad libitum drinking during  $E_2$  administration and placebo control conditions

	Preinfusion		Infusion		Ad Libitum Drinking	
	0 min	120 min	210 min	270 min	330 min	
GFR, ml/min						
$E_2$	96.6 $\pm$ 8.3	111.0 $\pm$ 10.9	118.5 $\pm$ 11.8	110.2 $\pm$ 14.2	99.3 $\pm$ 15.1	
Control	98.6 $\pm$ 8.3	105.3 $\pm$ 10.9	125.9 $\pm$ 14.5	100.6 $\pm$ 12.8	111.9 $\pm$ 8.3	
$U_v$ , ml/min	2.0 $\pm$ 0.7	1.5 $\pm$ 0.3	2.7 $\pm$ 0.6	1.4 $\pm$ 0.3	1.9 $\pm$ 0.6*	
Control	2.6 $\pm$ 0.7	1.7 $\pm$ 0.4	2.7 $\pm$ 0.7	2.9 $\pm$ 1.0	5.1 $\pm$ 1.9	
$U_{osm}$ , mosmol/kgH <sub>2</sub> O						
$E_2$	398 $\pm$ 137	523 $\pm$ 56	511 $\pm$ 34	625 $\pm$ 52	630 $\pm$ 33*	
Control	378 $\pm$ 83	527 $\pm$ 53	623 $\pm$ 17	589 $\pm$ 68	381 $\pm$ 81	
$U_{osm}/P_{osm}$						
$E_2$	1.10 $\pm$ 0.48	1.75 $\pm$ 0.23	2.12 $\pm$ 0.13	2.18 $\pm$ 0.22	2.20 $\pm$ 0.14*	
Control	1.07 $\pm$ 0.18	1.75 $\pm$ 0.21	2.14 $\pm$ 0.06	2.02 $\pm$ 0.28	1.13 $\pm$ 0.22	
$C_{H_2O}$ , ml/min						
$E_2$	0.3 $\pm$ 0.7	-1.2 $\pm$ 0.2	-2.5 $\pm$ 0.4	-1.5 $\pm$ 0.2	-1.8 $\pm$ 0.5*	
Control	0.5 $\pm$ 0.6	-1.2 $\pm$ 0.3	-2.6 $\pm$ 0.6	-1.3 $\pm$ 0.4	0.7 $\pm$ 1.1	
$C_{osm}$ , ml/min						
$E_2$	1.7 $\pm$ 0.2	2.6 $\pm$ 0.4	5.3 $\pm$ 1.0	2.9 $\pm$ 0.4	3.7 $\pm$ 1.1	
Control	2.1 $\pm$ 0.4	2.9 $\pm$ 0.6	5.3 $\pm$ 1.3	4.2 $\pm$ 0.7	4.4 $\pm$ 1.0	
$FE_{Na^+}$ , %						
$E_2$	0.50 $\pm$ 0.09	1.70 $\pm$ 0.58	2.63 $\pm$ 0.62	1.71 $\pm$ 0.45*	1.64 $\pm$ 0.36*	
Control	0.79 $\pm$ 0.20	2.00 $\pm$ 0.54	2.88 $\pm$ 0.50	2.83 $\pm$ 0.54	2.47 $\pm$ 0.56	
$FE_{H_2O}$ , %						
$E_2$	2.10 $\pm$ 0.70	1.69 $\pm$ 0.50	2.29 $\pm$ 0.46	1.49 $\pm$ 0.42	1.79 $\pm$ 0.34*	
Control	2.70 $\pm$ 0.72	1.70 $\pm$ 0.48	2.05 $\pm$ 0.38	2.60 $\pm$ 0.67	4.39 $\pm$ 1.56	
Urine K <sup>+</sup> , meq						
$E_2$	4.2 $\pm$ 1.2	16.1 $\pm$ 5.2*	34.2 $\pm$ 15.2*	7.3 $\pm$ 2.2	8.19 $\pm$ 3.3	
Control	3.5 $\pm$ 0.7	8.72 $\pm$ 1.2	12.3 $\pm$ 3.0	8.3 $\pm$ 2.2	10.7 $\pm$ 3.6	
$U_{[Na^+]}/U_{[K^+]}$						
$E_2$	1.8 $\pm$ 0.6	2.7 $\pm$ 1.1*	2.3 $\pm$ 0.8*	2.3 $\pm$ 0.6	2.4 $\pm$ 0.6	
Control	2.3 $\pm$ 0.5	5.1 $\pm$ 1.1	4.8 $\pm$ 0.8	3.7 $\pm$ 0.9	3.2 $\pm$ 0.9	

Values are means  $\pm$  SE;  $n = 6$  subjects. GFR, glomerular filtration rate;  $C_{H_2O}$ , free water clearance;  $C_{osm}$ , osmolar clearance;  $FE_{Na^+}$  and  $FE_{H_2O}$ , fractional excretions of  $Na^+$  and water;  $U_{[Na^+]}/U_{[K^+]}$ , ratio of urine  $Na^+$  and  $K^+$  concentrations;  $U_v$ , urine flow;  $U_{osm}$ , urine osmolality. \* Different from placebo control,  $P < 0.05$ .

Estrogen could modify AVP release directly through  $E_2$  receptors in the hypothalamus or indirectly by acting on catecholaminergic (15) and/or angiotensinergic (26) neurons, which bind estrogen and project to the paraventricular and supraoptic nuclei. Using [ $^3$ H]estradiol, Heritage et al. (15) identified estradiol binding sites in the nuclei of catecholamine cell bodies, as well as the presence of catecholaminic nerve terminals near estradiol target sites in the paraventricular and supraoptic nuclei. Crowley et al. (10) noted parallel changes in brain norepinephrine and AVP in normally cycling rats and that ovarian steroids modulated norepinephrine turnover in the paraventricular nucleus, indicating that  $E_2$  may act on the osmoregulatory system through catecholamines. There also is evidence for cholinergic and angiotensinergic innervation of vasopressinergic cells in the paraventricular and supraoptic nuclei, both of which are modulated by sex steroids (26). Alternatively, the effect of  $E_2$  on osmoregulation could be due to an indirect effect through luteinizing or follicle-stimulating hormones, which may indirectly elevate AVP through an adenylate cyclase mechanism. We did not measure these substances, but gonadotropins are slightly reduced after  $E_2$  administration in postmenopausal women (27), making this mechanism unlikely.

Although a central mechanism for the increase in AVP secretion with  $E_2$  seems the most plausible, peripheral effects cannot be ruled out. To provide several examples, a reduction in the metabolic clearance rate of AVP, a decrease in blood pressure, or an alteration in plasma concentration of atrial natriuretic peptide could elevate  $P_{[AVP]}$ . The first explanation seems unlikely because  $E_2$  would more likely increase, rather than decrease metabolic clearance of AVP, as plasma volume expansion would be expected to increase blood flow to the liver and kidneys, where most AVP breakdown occurs. Thus expanded blood volume should have diminished the AVP response, making the rise in AVP even more remarkable. Estrogen administration attenuated the rise in blood pressure during hypertonic saline infusion and therefore may have contributed to the elevation in  $P_{[AVP]}$ . However, this small difference in blood pressure ( $\sim 5$  mmHg) was probably insufficient to affect  $P_{[AVP]}$  (19). Finally, it is unlikely that changes in circulating levels of atrial natriuretic peptide played a role in the enhanced AVP response. In young women, the follicular phase of the menstrual cycle (when  $P_{[E_2]}$  is high) is associated with increased plasma levels of atrial natriuretic peptide at baseline and during hypertonic saline infusion (29). Furthermore, atrial natriuretic peptide has been shown to suppress the osmotically induced rise in AVP (8). Preliminary data on two of our six subjects showed  $E_2$  administration increased plasma atrial natriuretic peptide concentration both at baseline (76.7 and 59.4 pg/ml) and after hypertonic saline infusion (163.7 and 92.0 pg/ml, for  $E_2$  and placebo control administration, respectively).

*Renal water and electrolyte handling.* Our subjects were in positive fluid balance during hypertonic saline

infusion in both  $E_2$  administration and placebo control conditions. Despite the greater elevation of  $P_{[AVP]}$  during  $E_2$  treatment during the early part of hypertonic saline infusion, renal excretion of free water and renal concentrating capacity ( $U_{osm}/P_{osm}$ ) were similar in both conditions, perhaps indicating that antidiuretic action of AVP in the collecting duct was already at maximal level. Although little is known about the influence of  $E_2$  in the human kidney, some evidence suggests that  $E_2$  attenuates the antidiuretic action of AVP in the rat (7), and that  $E_2$  modulates AVP action in the collecting duct at the level of the receptors (28). However, the supposition that  $E_2$  downregulated renal AVP action in our subjects is complicated by our observation of an increased renal concentrating response (greater  $U_{osm}/P_{osm}$ ) during ad libitum drinking, despite similar  $P_{[AVP]}$ . This would suggest that  $E_2$  enhances, rather than attenuates renal free water retention. Perhaps this relationship is affected by plasma volume status, which was elevated at the end of drinking, or reflects a sluggish renal response to the recovery of  $P_{[AVP]}$ . In addition, because the subjects drank ad libitum, drinking patterns may have transiently affected individual  $P_{[AVP]}$  measurements (23). Clearly, more studies are needed to elucidate the impact of  $E_2$  on AVP modulation of renal water regulation. Nonetheless, estrogen administration increased overall body water retention over placebo control (18.6 vs. 12.5 ml/kg) during the ad libitum drinking period, and this fluid retention was due in part to increased free water reabsorption (negative  $C_{H_2O}$ ).

Although  $C_{H_2O}$  was reduced during drinking with  $E_2$  administration, the primary cause of the greater water retention was a reduction in sodium and total osmol excretion. Our observation that sodium retention during ad libitum drinking was enhanced after  $E_2$  administration is consistent with earlier findings in postmenopausal women during long-term estrogen therapy (1), as well as in young women during estrogen-dominant oral contraceptive administration (4). Although there is evidence to suggest that  $E_2$ -related sodium retention is mediated through aldosterone (7),  $E_2$  administration did not alter  $P_{[Aldo]}$  at baseline or in response to hypertonic saline infusion. Furthermore, an increase in potassium excretion did not accompany the sodium retention during most of the ad libitum drinking period, which also argues against an aldosterone-dependent mechanism. We speculate that the alteration in electrolyte handling with  $E_2$  is due to an effect of  $E_2$  on aldosterone distal tubule binding sites (7) or a direct effect of  $E_2$  on the proximal tubule (24, 28). There are no data that directly address this question, but Stock et al. (25) have demonstrated altered renal mineral metabolism in postmenopausal women after  $E_2$  treatment.

In summary, we found that  $E_2$  administration augmented  $P_{[AVP]}$  at baseline and in response to hypertonic saline infusion in postmenopausal women. The elevated plasma AVP levels may be due to a direct action of  $E_2$  on structures in the anterior hypothalamus that secrete AVP or through catecholaminergic or angiotensinergic neuronal action on these same structures.

Although thirst, drinking, and  $P_{[Aldo]}$  responses were unaffected, total body water and sodium retention were increased after  $E_2$  administration. The AVP-mediated reduction in renal  $C_{H_2O}$  during  $E_2$  only partially explains the greater body water retention, suggesting that  $E_2$  may have directly affected water and electrolyte handling in the renal tubule.

### Perspectives

Body water retention is common following estrogen replacement therapy and administration of oral contraceptive pills, as well as during pregnancy and the periods of the menstrual cycle when endogenous estrogens are at their peaks in the blood. However, it is difficult to isolate estrogen as the cause of the water retention in young women because the levels of the sex hormones (estrogen, progesterone) and gonadotropins (luteinizing or follicle-stimulating hormones) are constantly fluctuating. This study uses postmenopausal women as a low estrogen model to isolate the effects of estrogen on an important fluid regulating hormone, AVP. AVP is primarily responsible for the retention of free water by the kidney and is highly sensitive to changes in  $P_{osm}$ . We found that AVP was increased at baseline and throughout infusion of hypertonic saline, despite similar osmolality throughout the protocol. However, we also found that the primary mechanism for the enhanced body fluid expansion was not due to free water retention, i.e., to the effects of AVP. The body water retention was primarily the result of increased salt retention, suggesting a role for estrogen in renal electrolyte handling.

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Address for reprint requests: N. S. Stachenfeld, The John B. Pierce Laboratory, 290 Congress Ave., New Haven, CT 06519.

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