Progesterone does not alter osmotic regulation of AVP

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Calzone, Wendy L., Celso Silva, David L. Keefe, and Nina S. Stachenfeld. Progesterone does not alter osmotic regulation of AVP. Am J Physiol Regulatory Integrative Comp Physiol 281: R2011–R2020, 2001.—To test the hypothesis that progesterone, independent of estrogen, decreases the plasma osmotic threshold for arginine vasopressin (AVP) release and thirst onset, we compared AVP and thirst responses to hypertonic saline infusion (HSI) during administration of oral contraceptives (OCs) containing progesterone (OCP) with responses to infusion of OCs containing progesterone and estrogen (OCEP). Eight women (29 ± 2 yr) were infused with 3% NaCl (120 min, 0.1 ml·kg body wt·min−1) and consumed fluid (90 min, 15 ml/kg body wt) in the early follicular and midluteal phases of a 28-day menstrual cycle and also after 4 wk of OCP and after 4 wk of OCEP in a randomized crossover design. Baseline plasma osmolality (P_{osm}) was lower in the luteal phase (280 ± 1 mosmol/kgH2O) and during OCEP (283 ± 1 mosmol/kgH2O) than in the follicular phase (286 ± 1 mosmol/kgH2O, P < 0.05) but was unaffected by OCP (284 ± 1 mosmol/kgH2O). P_{osm} remained lower in the follicular phase than in the luteal phase and with OCEP throughout the first 50 min of HSI. The mean abscissal plasma AVP concentration-P_{osm} intercept was unaffected by OCP (267 ± 1 mosmol/kgH2O) but was greater in the follicular phase (273 ± 2 mosmol/kgH2O) than in the luteal phase (266 ± 4 mosmol/kgH2O) and with OCEP (268 ± 2 mosmol/kgH2O, P < 0.05). There were no differences in osmotic thresholds for thirst onset across experimental days. Despite the lower osmotic threshold for AVP release during the luteal phase and with OCEP, fluid balance, renal free water clearance, and Na+ regulation during HSI were unaffected by menstrual phase or OC treatment, indicating a lower osmotic operating point for body water balance. OCP did not affect osmotic AVP regulation, suggesting that progesterone does not affect osmotic fluid regulation through a mechanism independent of estrogen.

Progesterone does not alter osmotic regulation of AVP because of a lower osmotic threshold for AVP release during OCP and OCEP, which may be due to changes in body fluid regulation. These changes in body fluid regulation are usually attributed to estrogen (1, 2, 6, 25). Progesterone may also have independent effects on water regulation, however. Water retention and edema occur during early pregnancy (9) and during oral contraceptive use (30); under both conditions, progesterone is elevated without elevations in estrogen. Progesterone can influence neuronal function in the hypothalamus (10), and progesterone receptors are found in the paraventricular nucleus (3), a primary area involved in AVP synthesis and release. In young women taking OCP, we found a small reduction in the osmotic thresholds for AVP release and thirst onset during exercise-induced dehydration (30). However, this shift may have been influenced by exercise effects (14) or plasma volume (PV) loss that occurs concomitantly with the increases in plasma osmolality (P_{osm}) during dehydration (23).

The purpose of this study was to determine progesterone effects on osmotic regulation of AVP release and thirst sensation, as well as the impact of estrogen on those effects. To this end, we administered oral contraceptives containing estrogen and progesterone (OCP) or OCP to young women and evaluated their responses to HSI (3% NaCl). HSI provides a strong osmotic stimulation of AVP and thirst occurs with no changes in body fluid balance, indicating a downward resetting of the osmoreceptors (30).

HIGH PLASMA ESTROGEN AND PROGESTERONE levels can lead to fluid retention (9, 28, 32, 33, 36), hypertension (20), and edema (21, 32–34). One proposed mechanism for these changes in body fluid regulation may be due to changes in the osmotic regulation of arginine vasopressin (AVP) and thirst sensation (27, 35, 37). Studies using hypertonic saline infusion (HSI) have demonstrated a lower osmotic threshold for AVP release when plasma estrogen and progesterone are elevated, such as during the luteal phase of the menstrual cycle (27, 37) and late pregnancy (9). In a recent study using dehydration, we demonstrated that this shift in osmotic stimulation of AVP and thirst occurs with no change in body fluid balance, indicating a downward resetting of the osmoreceptors (30).

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arterial pressure is essential for a complete evaluation of body fluid regulation, so we also determined the effects of our oral contraceptive regimen on Na"+ regulation and the Na"-regulating hormones. Finally, we measured plasma cortisol concentration (P[Cort]) during the infusion to evaluate potential stress responses to the intense thirst induced by HSI.

**METHODS**

**Study Design**

Subjects were nine healthy nonsmoking women (age 29 ± 2 yr, range 22–35 yr) with no contraindications to oral contraceptive use. The subjects underwent medical and gynecological examinations and provided written confirmation of a negative Papanicolaou smear within 1 yr of being admitted to the study. The subjects gave written informed consent to participate in the study, which had prior approval by the Human Investigations Committee of Yale University School of Medicine.

The study design employed four HSI tests, which included an infusion conducted in the early follicular phase, 2–4 days after the beginning of menstrual bleeding (low estrogen and progesterone), and an infusion in the midluteal phase, 7–9 days after the luteinizing hormone peak (high estrogen and progesterone). Each woman tested her urine using an ovulation prediction kit (OvuQuick, Quidel, San Diego, CA) to determine the monthly peak in luteinizing hormone, which occurs ~48 h before ovulation. After completing the two baseline tests, the women again underwent HSI after 4 wk of OCEP or OCP treatment (single-blind, random assignment). After a 4-wk “washout” period, the subjects crossed over to the other pill treatment. During OCEP, subjects received 0.035 mg of ethinyl estradiol and 1 mg of the progestin norethindrone daily. During OCP, subjects received 1 mg/day of the progestin norethindrone. To verify phase of the menstrual cycle, plasma levels of estrogen and progesterone were assessed from the baseline blood sample before infusion.

**HSI Protocol**

For each experiment, the subjects arrived at the laboratory at ~8 AM, after having eaten a light (~300 kcal) breakfast, having refrained from alcohol or coffee for the previous 12 h, and having drunk 10 ml H2O/kg body wt that morning before arriving at the laboratory.

On reporting to 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 PV and plasma constituents. During this control period, a 20-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, and a urine sample was collected. After these baseline samples, 0.1 ml kg body wt" -1 min" -1 for 120 min. Blood was sampled at 10, 20, 30, 40, 50, 60, 75, 105, and 120 min during the infusion. After the infusion, the subject rested seated for the next 120 min. This recovery period consisted of a 30-min rest period followed by a 30-min drinking period (15 ml/kg body wt of water) followed by another 60-min rest period. Blood was sampled at 30, 90, and 120 min of recovery, and an extra 5 ml of blood were drawn at 10-min intervals during the final 30 min of recovery for PV determination (Evans blue dye; see Blood Volume). In total, 200 ml of blood were drawn from each subject during each experiment. Urine was collected at baseline, immediately after infusion, and 60 min after the drinking period. Thirst perception was assessed at every 15 min throughout infusion and every 30 min after infusion.

All blood samples were analyzed for hematocrit (Hct), hemoglobin (Hb), total protein (TP), plasma osmolality (P(osm)), plasma concentration of creatinine (P[E2]), plasma AVP concentration (P[AVP]), P[Cort], and serum concentrations of Na"+ (S[Na+]i) and K"+ (S[K+]i). The baseline blood sample was also analyzed for concentrations of 17α-estradiol (P[E2]) and progesterone (P[P4]). Blood samples at baseline, the end of the infusion, and 60 and 120 min after infusion were analyzed for concentrations of atrial natriuretic peptide (P[ANP]) and aldosterone (P[AlD]) and plasma renin activity (PRA). Volume, osmolality (U(osm)), and concentrations of Na"+ (U[Na+]i), K"+ (U[K+]i), and creatinine (U[Cr]) were measured from all urine samples.

**Blood sampling.** Blood samples were separated into aliquots. One aliquot was immediately analyzed for Hb and Hct. A second aliquot was transferred to a heparinized tube to be analyzed for P(osm), P[Cort], and P[AlD]. A third aliquot, for the determination of S[Na+]i and S[K+]i, was placed into a tube without anticoagulant. The remaining aliquots were placed in tubes containing EDTA for analysis of P[AVP], P[Cort], P[ANP], and PRA. The tubes were centrifuged at 4°C, and the plasma was removed. After centrifugation, the EDTA samples were frozen immediately at −80°C until analysis.

Plasma and urine Na"+ and K"+ concentrations were measured by flame photometry (model 943, Instrumentation Laboratory). P(osm) and U(osm) were measured by freezing-point depression (model 3DII, Advanced Instruments), TP by refractometry, and P[Cort] by colorimetric assay (Sigma Diagnostic Products). P[AlD], P[Cort], P[ANP], P[E2], and P[P4] were measured by radioimmunoassay. P[AVP] was determined by radioimmunoassay after extraction from plasma by the methods described by Freund et al. (12, 13) on octadecylsilylane cartridges (Sep-Pak C18, Waters Associates, Needham, MA). 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-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. The extraction recovery was 87%.

Intra- and interassay coefficients of variation for the midrange standards were, respectively, as follows: 7.1% and 10.7% for P[AVP] (3.1 pg/ml), 3.6% and 4.5% for P[Cort] (12.1 g/dl; Diagnostic Products, Los Angeles, CA), 2.8% and 5.0% for P[AlD] (4.8 ng·ml ANG" -1·h" -1; Immuno Biological Laboratories), 3.8% and 5.2% for P[E2] (181 pg/ml; Diagnostic Products), 6.0% and 8.2% for P[ANP] (61.8 pg/ml; Diasorin, Stillwater, MN), 5.6% and 8.3% for P[P4] (81 pg/ml; Diagnostic Products), and 2.1% and 2.7% for P[P4] (1.5 ng/ml; Diagnostic Products).

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**Blood Volume**

Absolute blood volume was measured by dilution of a known amount of Evans blue dye. An accurately determined volume of dye (by weight, since 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 was not used. PV was determined from the product of the concentration and volume of dye injected divided by the concentration in the plasma after mixing, taking into account 1.5% lost from the circulation within the 10 min. Blood volume was calculated from PV and Hct concentration, corrected for volume peripheral sampling.

**Thirst Ratings**

Perception of thirst was assessed by asking the subject to make a mark on a line rating scale in response to the question, “How thirsty do you feel now?” The line is 175 mm long and is marked “not at all” on one end and “extremely thirsty” at the 125-mm point. The subjects could mark beyond the “extremely thirsty” point if they wished and could even extend the line if they felt it was necessary. This method was developed by Marks et al. (18) 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 P osm during HSI and dehydration in young volunteers (29, 30).

**Calculations**

Changes in PV were estimated from changes in Hct and Hb concentration from the baseline sample according to the following equation (17)

\[
\% \Delta PV = 100 \frac{[(Hb_b) - (Hb_a)]\times[1 - Hct_a \times 10^{-2}]}{[(1 - Hct_b \times 10^{-2}) - 100}
\]

where subscripts \( a \) and \( b \) denote measurements at time \( a \) and before HSI, respectively.

**Table 1. Baseline subject characteristics and mean slope and intercepts of thirst-P osm and P[AVP]-P osm relationships during HSI**

<table>
<thead>
<tr>
<th></th>
<th>Follicular Phase</th>
<th>Luteal Phase</th>
<th>OCP</th>
<th>OCEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, kg</td>
<td>59.9 ± 2.3</td>
<td>59.6 ± 2.6</td>
<td>60.6 ± 2.9</td>
<td>60.3 ± 2.7</td>
</tr>
<tr>
<td>PV, ml</td>
<td>2,529 ± 183</td>
<td>2,437 ± 113 ±§</td>
<td>2,533 ± 227</td>
<td>2,706 ± 99</td>
</tr>
<tr>
<td>BV, ml/kg body wt</td>
<td>59.8 ± 3.3</td>
<td>58.7 ± 2.4</td>
<td>56.2 ± 3.8</td>
<td>60.0 ± 3.1</td>
</tr>
<tr>
<td>P glomerular, pg/ml</td>
<td>16.1 ± 2.8</td>
<td>18.0 ± 1.14 ±§</td>
<td>12.9 ± 4.4</td>
<td>20.9 ± 5.5</td>
</tr>
<tr>
<td>P[AVP], ng/ml</td>
<td>0.8 ± 0.1</td>
<td>12.7 ± 0.7 ±§</td>
<td>0.7 ± 0.1</td>
<td>1.0 ± 0.5</td>
</tr>
<tr>
<td>P osm, mosmol/kgH2O</td>
<td>286 ± 1</td>
<td>280 ± 1*</td>
<td>284 ± 1</td>
<td>283 ± 1‡</td>
</tr>
<tr>
<td>Thirst-P osm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope, mm/mosmol</td>
<td>5.9 ± 0.9</td>
<td>7.0 ± 0.8</td>
<td>7.6 ± 0.9</td>
<td>6.3 ± 0.7</td>
</tr>
<tr>
<td>x-Intercept, mosmol</td>
<td>286 ± 1</td>
<td>283 ± 1</td>
<td>286 ± 2</td>
<td>284 ± 2</td>
</tr>
<tr>
<td>P[AVP]-P osm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope, pg/mosmol</td>
<td>0.13 ± 0.04</td>
<td>0.12 ± 0.04</td>
<td>0.09 ± 0.02</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>x-Intercept, mosmol</td>
<td>273 ± 2‡</td>
<td>266 ± 4‡</td>
<td>267 ± 2‡</td>
<td>268 ± 2‡</td>
</tr>
</tbody>
</table>

Values (means ± SE) represent baseline subject characteristics and responses during hypertonic saline infusion (HSI) in follicular and midluteal menstrual phases and during oral contraceptive treatment with combined estrogen and progesterone (OCP) and progesterone only (OCP). Baseline body weight, plasma (PV) and blood (BV) volumes, plasma concentrations of endogenous 17β-estradiol (P[estradiol]) and progesterone (P[progesterone]), and plasma osmolality (P osm). Thirst-P osm and plasma arginine vasopressin (P[AVP]-P osm) slopes and intercepts and P[AVP]-P osm x-intercepts are mean slopes and abscissal intercepts of individual subjects’ thirst-P osm and P[AVP]-P osm relationships during HSI. *Significantly different from follicular phase, \( P < 0.05 \). †Significantly different from OCP, \( P < 0.05 \). ‡Significantly different from follicular phase, \( P < 0.05 \). §Significantly different from OCP, \( P < 0.05 \).
80% power, \( Z_{\alpha} = 0.84 \). The formula for calculating sample size for continuous response variables is (4)
\[
n = \frac{(Z_{\alpha} + Z_{\beta})^2}{d^2}
\]
Substituting the values, the calculated sample size is eight subjects per group.

**RESULTS**

**Baseline**

One subject did not have estrogen and progesterone peaks in the midluteal phase, so her data were excluded from all analyses. Data on eight subjects are presented here.

Baseline \( P_{[E_1]} \) and \( P_{[P]} \) confirm that subjects were tested in the follicular and midluteal phases (Table 1; \( P < 0.05 \)). Endogenous levels of 17\( \beta \)-estradiol and progesterone were at follicular phase levels during oral contraceptive administration, as would be expected during exogenous estrogen-progesterone administration. During OCEP and OCP, ethinyl estradiol and norethindrone would be elevated to pharmacological levels in blood and tissue but were not measured by our assays. Preinfusion Hct was increased and estimated PV was reduced during the luteal phase compared with the follicular phase, OCP, and OCEP (Tables 1 and 2; \( P < 0.05 \)). \( P_{[osm]} \) was lower in the luteal phase and during OCEP than in the follicular phase (Fig. 1; \( P < 0.05 \)). However, \( P_{[AVP]} \) was unaffected by menstrual phase or either oral contraceptive pill (Fig. 1). Baseline \( P_{[Cort]} \) was greater during OCEP than in all other conditions (Fig. 1; \( P < 0.05 \)).

Preinfusion \( P_{[Adl]} \) was increased in the luteal phase relative to the follicular phase, although PRA was greater in the luteal phase, OCEP, and OCP than in the follicular phase (Fig. 2; \( P < 0.05 \)). \( U_V, U_{osm}, Na^+ \) excretion, \( C_{H2O} \), and \( C_{osm} \) were unaffected by phase or pill treatment, indicating that baseline hydration levels were similar among the treatment days (Table 3). Although mean baseline \( U_{osm} \) is greater than mean \( P_{[osm]} \) during the follicular phase, OCP, and OCEP, \( C_{H2O} \) is slightly above zero under these three conditions. These apparently inconsistent findings may be the result of variability in the subjects’ \( U_{osm} \) within each condition at baseline (82–858 mosmol/kgH2O) concomitant with only small mean differences in \( U_{osm} \) among the conditions (Table 3). This anomaly led to fairly wide variability in \( U_V \) (0.29–6.00 ml/min), \( C_{H2O} \) (−2.02–4.31 ml/min), and \( C_{osm} \) (3.46–5.57 ml/min) across the four conditions. Perhaps because of differences in the subjects’ habitual water intake, the subjects’ water intake (10 ml/kg body wt) on the morning of the tests did not fully hydrate some women but “over-

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

<table>
<thead>
<tr>
<th></th>
<th>Pre-HSI (0 min)</th>
<th>End HSI (120 min)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>180 min</td>
</tr>
<tr>
<td>Hct, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>36.8 ± 0.6</td>
<td>32.2 ± 0.8</td>
<td>33.3 ± 0.7</td>
</tr>
<tr>
<td>Luteal</td>
<td>37.7 ± 0.6†‡</td>
<td>33.2 ± 0.5*</td>
<td>34.2 ± 0.5*</td>
</tr>
<tr>
<td>OCP</td>
<td>37.3 ± 0.6</td>
<td>33.0 ± 0.5</td>
<td>33.6 ± 0.5</td>
</tr>
<tr>
<td>OCEP</td>
<td>36.7 ± 0.6</td>
<td>33.5 ± 0.5</td>
<td>33.0 ± 0.5</td>
</tr>
<tr>
<td>Hb, grdl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>12.4 ± 0.3</td>
<td>12.0 ± 0.3</td>
<td>11.4 ± 0.3</td>
</tr>
<tr>
<td>Luteal</td>
<td>12.5 ± 0.3</td>
<td>11.3 ± 0.3</td>
<td>11.5 ± 0.3</td>
</tr>
<tr>
<td>OCP</td>
<td>12.5 ± 0.3</td>
<td>11.3 ± 0.3</td>
<td>11.6 ± 0.3</td>
</tr>
<tr>
<td>OCEP</td>
<td>12.3 ± 0.2</td>
<td>11.1 ± 0.2</td>
<td>11.4 ± 0.2</td>
</tr>
<tr>
<td>PV, %change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>21.0 ± 1.5</td>
<td>15.0 ± 1.1</td>
<td>16.0 ± 1.0</td>
</tr>
<tr>
<td>Luteal</td>
<td>18.9 ± 2.5</td>
<td>14.2 ± 1.3</td>
<td>15.4 ± 1.6</td>
</tr>
<tr>
<td>OCP</td>
<td>18.0 ± 1.5</td>
<td>14.8 ± 1.5</td>
<td>17.5 ± 1.2</td>
</tr>
<tr>
<td>OCEP</td>
<td>18.7 ± 0.8</td>
<td>14.3 ± 0.6</td>
<td>17.1 ± 1.7</td>
</tr>
<tr>
<td>( S_{[Na^+]_{[1]}} ), meq/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>137.7 ± 0.6</td>
<td>144.1 ± 0.7</td>
<td>143.3 ± 0.8</td>
</tr>
<tr>
<td>Luteal</td>
<td>137.0 ± 0.6</td>
<td>144.6 ± 0.6</td>
<td>142.2 ± 1.5</td>
</tr>
<tr>
<td>OCP</td>
<td>137.7 ± 0.7</td>
<td>144.2 ± 0.8</td>
<td>143.6 ± 1.0</td>
</tr>
<tr>
<td>OCEP</td>
<td>137.2 ± 0.7</td>
<td>144.0 ± 1.0</td>
<td>142.2 ± 0.9</td>
</tr>
<tr>
<td>( S_{[K^+]_{[1]}} ), meq/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>3.81 ± 0.13</td>
<td>4.17 ± 0.06</td>
<td>4.26 ± 0.06</td>
</tr>
<tr>
<td>Luteal</td>
<td>3.83 ± 0.09</td>
<td>4.08 ± 0.06</td>
<td>4.18 ± 0.07</td>
</tr>
<tr>
<td>OCP</td>
<td>3.82 ± 0.06</td>
<td>4.10 ± 0.09</td>
<td>4.27 ± 0.11</td>
</tr>
<tr>
<td>OCEP</td>
<td>3.77 ± 0.06</td>
<td>4.22 ± 0.06</td>
<td>4.35 ± 0.07</td>
</tr>
<tr>
<td>Thirst, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>74 ± 12</td>
<td>76 ± 12</td>
<td>70 ± 7</td>
</tr>
<tr>
<td>Luteal</td>
<td>97 ± 10</td>
<td>95 ± 10</td>
<td>15 ± 5</td>
</tr>
<tr>
<td>OCP</td>
<td>100 ± 5</td>
<td>105 ± 5</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>OCEP</td>
<td>82 ± 6</td>
<td>94 ± 9</td>
<td>18 ± 6</td>
</tr>
</tbody>
</table>

Values (means ± SE) represent blood responses at rest and during HSI and recovery in follicular and midluteal menstrual phases and during OCEP and OCP. Hct, hematocrit; [Hb], blood concentration of hemoglobin; \( S_{[Na^+]_{[1]}} \) and \( S_{[K^+]_{[1]}} \), serum concentrations of \( Na^+ \) and \( K^+ \).

*Significantly different from follicular phase, \( P < 0.05 \). †Significantly different from OCP, \( P < 0.05 \). ‡Significantly different from OCP, \( P < 0.05 \).
hydrated" others. However, within each woman, the hydration levels were similar at baseline across all four tests.

Heart rate (73 ± 3, 71 ± 2, 70 ± 2, and 69 ± 3 beats/min) and mean blood pressure (82 ± 2, 80 ± 3, 76 ± 2, and 76 ± 3 mmHg) in the follicular and luteal phases, OCP, and OCEP, respectively, were also unaffected by menstrual cycle phase or by oral contraceptive treatment before HSI.

**HSI**

\( \text{P}_{\text{osm}} \) during the luteal phase remained below that of the follicular phase and OCEP through the first 50 min of HSI (Fig. 1; \( P < 0.05 \)), although \( \text{P}_{[\text{AVP}]} \), percent PV change, and thirst were similar under all four conditions (Fig. 1, Table 2). Linear regression analysis of the individual subjects’ data during HSI indicated significant correlations between \( \text{P}_{[\text{AVP}]} \) and \( \text{P}_{\text{osm}} \) (\( r = 0.80 \pm 0.03 \)) and thirst and \( \text{P}_{\text{osm}} \) (\( r = 0.90 \pm 0.02 \)). The mean abscissal intercept for the \( \text{P}_{[\text{AVP}]}-\text{P}_{\text{osm}} \) relationship was greater in the follicular phase than in the luteal phase and OCEP (Fig. 3, Table 1; \( P < 0.05 \)). In contrast, the slopes of the \( \text{P}_{[\text{AVP}]}-\text{P}_{\text{osm}} \) and thirst-\( \text{P}_{\text{osm}} \) relationships during HSI were unaffected by menstrual phase or oral contraceptive pills (Table 1). The regression line indicating the osmotic regulation of thirst and the slope of this relationship was unaffected by menstrual phase or oral contraceptive treatment (Table 1, Fig. 3).

\( \text{PRA} \) and \( \text{P}_{[\text{Ald}]} \) decreased during HSI in all conditions, but only \( \text{P}_{[\text{Ald}]} \) remained greater in the luteal phase than in the follicular phase (Fig. 2; \( P < 0.05 \)). During HSI, \( \text{P}_{[\text{Cort}]} \) fell steadily in all conditions but...
remained greater with OCEP (Fig. 1; \( P < 0.05 \)). Renal \( \text{Na}^+ \) and \( \text{K}^+ \) excretion increased similarly during HSI in all conditions, as did \( C_{\text{osm}} \) and \( C_{\text{H}_2\text{O}} \) (Table 3). HSI had no effect on heart rate (69 ± 2, 72 ± 3, 72 ± 2, and 68 ± 2 beats/min) or mean blood pressure (83 ± 4, 83 ± 4, 83 ± 3, and 81 ± 4 mmHg) for follicular and luteal phase and OCP and OCEP, respectively under any of the four conditions. There were no treatment-by-time interactions for any of the blood, renal, or cardiovascular variables during HSI.

Recovery

\( P_{\text{osm}} \) and percent change in PV were similar across conditions throughout recovery from HSI (Fig. 1, Table 1). Again, \( P_{[\text{Cort}]} \) fell in all conditions but remained greater in OCEP than in the other three conditions (Fig. 1; \( P < 0.05 \)). Although PRA was similar across menstrual cycle phases and oral contraceptive treatment, \( P_{[\text{Ald}]} \) remained greater in the luteal phase than in the follicular phase, OCP, and OCEP (Fig. 2; \( P < 0.05 \)).

During the recovery period, neither renal function nor electrolyte excretion was affected by menstrual phase or oral contraceptive administration (Table 3). Moreover, overall fluid balance (i.e., HSI + drinking − urine output) was unaffected by either phase of the menstrual cycle or oral contraceptive administration (19 ± 1, 19 ± 1, 20 ± 1, and 20 ± 1 ml/kg body wt for follicular phase, luteal phase, OCP, and OCEP, respectively). Heart rate (68 ± 3, 71 ± 3, 75 ± 2, and 70 ± 3 beats/min) and mean blood pressure (78 ± 2, 78 ± 3, 78 ± 3, and 76 ± 3 mmHg) for follicular phase, luteal phase, OCP, and OCEP, respectively, during recovery were also not affected by menstrual cycle phase or oral
contraceptive treatment. There were no treatment-by-time interactions for any of the blood, renal, or cardiovascular variables during recovery.

DISCUSSION

Our major finding was that administration of oral contraceptive pills containing estrogen with progesterone, but not pills containing progesterone alone, decreased the osmotic threshold for AVP release during HSI in young women. Our findings support previous studies in which AVP secretion persists during HSI at lower P_{osm} at periods in the menstrual cycle corresponding to high P_{E2}, leading to lower renal free water excretion and maintenance of the lower plasma tonicity (27, 35, 37). The present investigation extends these earlier findings demonstrating no independent effect of progesterone and indicates that this shift in osmotic regulation of AVP during OCEP and the luteal phase is primarily an estrogen-mediated effect. The shift in osmotically mediated AVP release occurred without excess fluid retention during HSI or recovery. Thus the estrogen-induced shift in osmotic regulation of AVP represents a shift in body water regulation to a lower P_{osm} operating point or a resetting of osmoreceptors (30). Moreover, although elevated estrogen and progesterone levels were associated with greater PRA and aldosterone, these increases in the Na⁺-regulating hormones did not drive up blood pressure or Na⁺ retention. Finally, OCEP led to increases in P_{[Cort]} before, during, and after HSI, although without any measurable metabolic or cardiovascular effects; this P_{[Cort]} increase was likely due to changes in cortisol-binding protein induced by the synthetic hormones.

The estrogen-related shift in osmotic regulation of AVP release most likely occurs via the central nervous system. Estrogen acts directly on AVP-synthesizing neurons in the hypothalamus (1, 2, 6, 25), and estrogen receptors have been identified in the nuclei of neurophysin- and AVP-producing cells in the mouse supraoptic nucleus (25). There is also evidence of angiotensinergic innervation of vasopressinergic cells in the paraventricular and supraoptic nuclei, which are modulated by estrogen (31). In addition, estrogen may impact AVP release indirectly via catecholamines (7).

Progesterone administration without estrogen seemed to have little effect on osmotic regulation of AVP. In an earlier study, progesterone treatment increased the threshold and decreased the slope of the P_{[AVP]}-P_{osm}
regression lines in women with premenstrual syndrome (38). In our study, osmotic threshold for AVP release in OCP was similar to that in the luteal phase and OCEP. In addition, during OCP, there was a shift to a lower osmotic threshold for AVP release relative to the follicular phase in some, but not all, subjects. Taken together, these observations suggest an inconsistent effect of OCP on osmotic AVP regulation. There is evidence that progesterone receptor activity in the brain stimulates AVP production from the paraventricular nucleus, although this AVP stimulation by progesterone appears to depend on the presence of estrogen (3). During the midluteal phase and during OCEP, estrogen concentrations in blood and tissue are greatly increased over follicular phase levels. Even though blood and tissue estrogen concentrations remained at follicular phase levels during OCP, follicular phase tissue levels of endogenous estrogen can vary considerably among individual women. Thus we can only speculate that subtle differences in hypothalamic endogenous estrogen levels among the subjects during OCP led to the variability in the $P_{AVP}$ response.

The different $P_{AVP}$ responses to osmotic stimulation during the luteal phase vs. OCP may be due to structural or metabolic differences in endogenous progesterone vs. the synthetic progestin in OCP, norethindrone. Actions of progesterone in the brain may respond only to progesterone, and not to norethindrone, a gestational derivative of testosterone, which differs in structure from endogenous progesterone. Unlike endogenous progesterone, norethindrone does not produce the metabolite 3α-hydroxydehydroprogesterone (11), a primary regulator of the GABA$\alpha$ receptor, which modulates a number of neural pathways (24). Norethindrone metabolites may have similar effects on GABA$\alpha$, and there is evidence indicating that oral contraceptives similar in structure to norethindrone increase GABA$\alpha$ levels in rats (8, 26). However, progestin effects on GABA$\alpha$ have not been demonstrated in humans or in the hypothalamus of animals, so the impact on AVP release needs to be investigated further.

Although the most likely mechanism for the sex hormone-related shift in osmotic regulation of AVP is the central nervous system, peripheral mechanisms, such as changes in PV, also play an important role in the regulation of AVP release (23). However, although changes in PV shift the $P_{AVP}$-$P_{osm}$ response curve, in our study PV was decreased in the luteal phase but was increased during OCEP, despite similar reductions in the osmotic thresholds for AVP release. Furthermore, the difference in PV between the follicular phase and the luteal phase and between the follicular phase and OCEP ($<10\%$) would have increased central venous pressure by $<1$ mmHg (15), an insufficient change in central venous pressure to load cardiopulmonary baroreceptors enough to cause changes in the $P_{osm}$-$P_{AVP}$ response curve (23).

Corticotropin-releasing hormone and AVP interact at the level of the anterior pituitary (16), so we determined whether HSI induced a $P_{Cort}$ response. $P_{Cort}$ was greater only during OCEP relative to the other three experimental days, which is consistent with earlier studies and has been attributed to increases in cortisol-binding globulin (CBG) and associated with changes in the binding characteristics of cortisol to CBG during estrogen administration (5). Circulating cortisol is usually $\sim93–96\%$ bound: $\sim83\%$ to CBG and $\sim10\%$ to albumin. However, during estrogen administration, cortisol binding can increase to $\sim99\%$, with the greater binding primarily to CBG. We attempted to measure differences in free $P_{Cort}$ across our four conditions using filters (Pall Filtron, Ann Arbor, MI) designed to separate free cortisol from its bound form. Once filtered, we found no differences in the free, biologically active portion of circulating cortisol among the different conditions. Thus the greater circulating cortisol should have had no significant physiological effects on our subjects. The decrease in $P_{Cort}$ over the course of the experiment in all four conditions was
likely due to the normal circadian fall in cortisol release from its early morning peak to its later-day nadir.

Our data did not show a strong effect of estrogen or progesterone on the osmotic regulation of thirst. A study by Vokes et al. (37) demonstrated a 5 mosmol/kgH₂O fall in this threshold in the midluteal vs. the early follicular phase. We did not see any statistically significant effect of menstrual cycle or oral contraceptive pills on osmotic thirst regulation; however, we found trends showing that the thirst threshold fell from 286 mosmol/kgH₂O in the follicular phase to 283 and 284 mosmol/kgH₂O in the luteal phase and OCP, respectively. Moreover, an earlier study also demonstrated a nonstatistically significant 3 mosmol/kgH₂O fall in thirst onset during osmotic stimulation between the follicular and luteal phases of women with normal menstrual cycles (35). Taken together, these studies suggest a small shift in the osmotic regulation of thirst. The difficulty in detecting these differences consistently may be a result of variability in the subjects' subjective interpretation of the thirst scales or subtle differences in the scales themselves (22).

Arterial pressure also plays an important role in body fluid regulation, primarily by modulating Na⁺ excretion. During the luteal phase, a progesterone-mediated inhibition of aldosterone-dependent Na⁺ reabsorption at distal sites in the nephron produces a transient natriuresis and a compensatory stimulation of the renin-aldosterone system (19). The renin and aldosterone stimulation is a component of a system that evolved to maintain blood pressure and plasma water and Na⁺ content during the progesterone peak in the luteal phase. The progestin in OCEP and OCP (norethindrone), in contrast to endogenous progesterone, lacks antimineralocorticoid properties and did not increase P[Ald], alter Na⁺ excretion, or increase blood pressure.

Our studies found a decrease in the osmotic threshold for AVP release during the administration of estrogen with progesterone, although there were no changes in osmotic regulation of AVP with progesterone administration alone. These data support the hypothesis that the observed changes in osmotic AVP regulation during the luteal phase and combined oral contraceptive administration are mediated by estrogen, most likely acting in the central nervous system. Thus mechanisms for the water retention and edema during periods of increased progesterone need to be addressed. Finally, the progestin in oral contraceptive pills differs structurally from endogenous progesterone; therefore, studies examining changes in body fluid distribution using the endogenous form of progesterone should be considered.

Perspectives

Alterations in the compartmentalization of fluid have important implications for a number of clinical conditions such as edema, hyponatremia, or hypertension. The relationships between sex hormones, fluid balance, and Na⁺ regulation play an important role in chronic conditions such as hypertension and other cardiovascular diseases. Progesterone fluctuations have been implicated as a primary cause of the cramping, water retention, and edema associated with premenstrual syndrome. In addition, estrogen and progesterone-one impact physiological responses to acute stress, such as heat stress and hyponatremia. Thus understanding the independent and combined mechanisms by which progesterone and estrogen affect body water regulation and Na⁺ regulation has implications for the treatment of a broad number of diseases.

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In conduct of research where humans are the subjects, the investigators adhered to the policies regarding the protection of human subjects as prescribed by 45 CFR 46 and 32 CFR 219 (Protection of Human Subjects).

REFERENCES


