Arginine vasopressin secretion in non-obese women with polycystic ovary syndrome

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Abstract. Arginine vasopressin responses to osmotic (0.1 ml · kg⁻¹ · min⁻¹ NaCl), orthostatic (standing upright and maintenance of orthostatic position for 20 min), and hypoglycemic (0.15 IU/kg insulin) stimuli were evaluated in women with polycystic ovaries and in normal subjects. Blood dehydroepiandrosterone, dehydroepiandrosterone sulphate, androstenedione, cortisol, and endogenous insulin levels were significantly higher in women with polycystic ovaries than in controls, whereas estrone, estradiol-17β, progesterone and 17OH-progesterone concentrations were normal in all subjects. Arginine vasopressin basal levels (mean ± SEM of 3 test days; women with polycystic ovaries: 2.8 ± 0.2 pmol/l; controls: 2.7 ± 0.2 pmol/l) and secretory responses to orthostatic (mean peaks 100% higher than baseline values) and to hypertonic (130% increments) stimuli were similar in the two groups. Arginine vasopressin responses to hypoglycemia were lower in women with polycystic ovaries (50% increment) than in controls (150% increment), although comparable blood glucose decrements and GH or cortisol increments were found in the two groups. Arginine vasopressin peak responses to hypoglycemia were negatively correlated to testosterone, androstenedione, and endogenous insulin levels, but did not correlate with basal and hypoglycemia-induced peak cortisol concentrations or with circulating levels of other steroids. These data indicate a hypothalamic posterior pituitary disorder affecting arginine vasopressin response to insulin-induced hypoglycemia in women with polycystic ovaries syndrome associated with elevated blood androgen and insulin concentrations.

A variety of studies indicate an involvement of circulating sex steroid levels in the regulation of vasopressin secretion. Both androgens and estrogens appear to play a role in maintaining vasopressin pathways in the rat central nervous system (1) and are capable of modifying vasopressin secretion when administered to castrated or to intact animals (2, 3). Studies in women indicate a stimulating effect of exogenously administered estrogens on vasopressin release in postmenopausal subjects (4); furthermore, in normally menstruating women the circulating concentrations of vasopressin rise at the time of ovulation and decrease at the onset of menstruation (5).

In view of these observations, we wondered whether the regulation of vasopressin secretion was altered in women with polycystic ovaries (PCO), a syndrome characterized by abnormal blood circulating concentrations of sex steroids (6). For this purpose, the AVP responses to osmotic, orthostatic and hypoglycemic stimuli (7) were tested in women with PCO. This protocol was chosen in order to have a wide evaluation of AVP secretion, because stimuli acting through osmoregulation, baroregulation and neuroglucopenia appear to increase AVP secretion through independent mechanisms and are considered primary stimulations for AVP release. Furthermore, elev-
ated basal insulin concentrations are a common finding in patients with PCO and have been related to hyperandrogenism (8, 9). In fact, hyperandrogenism is thought to influence hepatic removal of insulin (10) and peripheral insulin sensitivity (8–10), without modifying pancreatic insulin secretion (10). In a previous study, we found low AVP responses to insulin induced hypoglycemia in obese patients with blood concentrations of insulin higher than normal (11). These observations prompted us to evaluate the possible correlation between plasma androgens and endogenous insulin concentrations with AVP responses to insulin-induced hypoglycemia.

Finally, plasma cortisol and GH levels were measured during insulin-induced hypoglycemia in order to assess the anterior pituitary responsiveness to hypoglycemia and the possible occurrence of stress.

Patients and Methods

Ten women with PCO (age 22–28 years) and 9 age-matched (24–31 years) normal women participated in this study after giving informed consent. All women were defined non-obese since their body weight was within 20% of ideal body weight, according to the tables provided by the Metropolitan Life Insurance Co. Fat distribution was measured in all subjects with the Harpenden skinfold calipers, according to the method described by Bray (12), and was similar in the two groups. The normal women had regular menses and normal ovulatory function. In contrast, all women with PCO were amenorrhoic. The diagnosis of PCO was based on the clinical features of amenorrhea, hirsutism, absence of acanthosis nigricans, and a serum LH/FSH ratio higher than 2.0%. Bilateral polycystic ovaries were found with echography in all patients with PCO. Neither women with PCO, nor normal controls were taking any medication for at least 4 weeks before the study.

Experimental tests

The following tests were performed in all subjects, at weekly intervals in women with PCO and at monthly intervals (on the 22nd day of two following cycles) in the normal controls. All tests started at 8.00 h after an overnight rest in bed. In all tests, subjects were fasting/thirsting from at least midnight. The glucose tolerance test was the first to be performed; stimulatory tests for AVP were performed in random order.

Glucose tolerance test (OGTT). At 08.00 h of the experimental day, an iv catheter was inserted into a forearm vein. The catheter was kept patent by a slow infusion of normal saline and was used for blood sampling; 20 min later, a 75 g glucose load was administered after withdrawal of a basal blood sample (25 ml) (time 0). Further blood specimens (5 ml each) were taken at 30-min intervals for 2 h after glucose ingestion. Plasma insulin and glucose concentrations were measured in all samples.

Hypertonic saline infusion test. Subjects remained supine from 08.00 to 12.00 h. At 08.00 h two iv catheters were inserted into forearm veins of each arm; one was used for infusion, the other for blood sampling. Blood specimens were taken just before the hypertonic saline (0.51 mol/l NaCl) infusion (time 0), which started at 09.00 h and every 30 min during the next 2 h. Hypertonic saline was infused at the constant rate of 0.1 ml·kg⁻¹·min⁻¹ for 2 h. Plasma AVP, osmolality and hematocrit were measured in all samples. In this and in the following tests, 25 ml of blood were taken at each sampling time.

Orthostatic test. At 08.00 h of the experimental day, an iv catheter was inserted into the left antecubital vein and was maintained patent by a slow infusion of normal saline. Blood pressure was measured at −30, −20, −10 and 0 min; the women were then requested to stand without moving aside their beds for 20 min. Blood pressure and heart rate were monitored at 2-min intervals for 40 min. During this period, starting from time 0, blood samples were taken at 10-min intervals. Serum AVP levels were measured in all specimens.

Insulin tolerance test. A 19 gauge scalp vein needle was inserted at 08.00 h into an antecubital vein kept patent by a slow normal saline infusion. Baseline blood samples were withdrawn 30 min later, just before the iv injection of 0.15 IU/kg insulin (Actrapid® MC, Novo, Denmark). During all tests, blood pressure and heart rate were monitored before and 15, 30, 45 and 60 min after insulin injection. Serum AVP levels, plasma GH, cortisol and glucose concentrations, osmolality and hematocrit were measured in all samples.

Besides the specific measurements indicated for each individual test, blood samples taken at time 0 in all tests were used for the following assays: glucose, insulin, testosterone (T), androstenedione (A), dehydroepiandrosterone (DHEA), DHEA sulphate (DHEAS), estrone (E1), estradiol (E2), 17OH-progesterone (17OHP), and cortisol.

Specimens taken at time 0 in the OGTT were used also for GH, PRL, LH and FSH assays.

Laboratory assays

Serum AVP concentrations were measured with a specific RIA (13). The intra- and inter-assay coefficient of variation was 7.2% and 12.8%, respectively; the sensitivity of the RIA was 1.2 ng/l. Blood osmolality was determined with an advanced osmometer (Osmette S Sedas s.r.l.); blood hematocrit was measured with a Drummond microhematocrit (Drummond Scientific Co, Broomal,
Table 1. Clinical and laboratory data of patients with PCO and normal women.

<table>
<thead>
<tr>
<th></th>
<th>PCO</th>
<th>Controls</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>25.6 ± 0.7</td>
<td>27.4 ± 0.9</td>
</tr>
<tr>
<td>% Ideal Body Weight</td>
<td>103.9 ± 2.6</td>
<td>101.5 ± 1.9</td>
</tr>
<tr>
<td>Serum LH (IU/l)</td>
<td>28.8 ± 4.3*</td>
<td>12.8 ± 0.7</td>
</tr>
<tr>
<td>Serum FSH (IU/l)</td>
<td>9.8 ± 1.2</td>
<td>9.0 ± 1.0</td>
</tr>
<tr>
<td>Serum LH/FSH</td>
<td>2.9 ± 1.2</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Serum testosterone (nmol/l)</td>
<td>2.8 ± 0.5*</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>Serum androstenedione (nmol/l)</td>
<td>10.5 ± 1.4*</td>
<td>4.2 ± 0.7</td>
</tr>
<tr>
<td>Serum DHEA (nmol/l)</td>
<td>28.8 ± 1.1*</td>
<td>19.0 ± 1.7</td>
</tr>
<tr>
<td>Serum DHEA-sulphate (µmol/l)</td>
<td>10.6 ± 0.7*</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td>Serum 17OH-progesterone (nmol/l)</td>
<td>5.1 ± 1.8</td>
<td>3.9 ± 1.3</td>
</tr>
<tr>
<td>Serum E₁ (pmol/l)</td>
<td>281.9 ± 43.3</td>
<td>258.2 ± 45.9</td>
</tr>
<tr>
<td>Serum E₂ (pmol/l)</td>
<td>432.8 ± 38.9</td>
<td>355.3 ± 27.5</td>
</tr>
<tr>
<td>Serum cortisol (nmol/l)</td>
<td>543.5 ± 30.4*</td>
<td>325.6 ± 24.8</td>
</tr>
<tr>
<td>Serum GH (µg/l)</td>
<td>1.3 ± 0.3</td>
<td>1.4 ± 0.3</td>
</tr>
</tbody>
</table>

All results are expressed as the mean ± SEM; *p < 0.001 vs controls.

CA). Plasma glucose was measured with an IL 918 autoanalyser (Instrumentation Laboratory, Milan, Italy). Insulin and cortisol concentrations were assayed in the plasma; T, A, E₁, E₂, DHEA, DHEAS, 17OHP, GH, PRL, LH and FSH were measured in the serum. All the above-mentioned hormones were measured by specific RIA, using commercial kits.

Data were analysed statistically with the two-way analysis of variance (ANOVA), paired or unpaired Student's t-test, and linear correlation coefficients, as appropriate. Correlations were calculated using data obtained in the same day. The cortisol curves of the insulin tolerance test were evaluated for parallelism (profile analysis).

Results are reported as mean ± SEM.

![Fig 1](image_url)

Circulating levels of glucose and insulin (mean ± SEM) in normal control (N = 9) and PCO subjects (N = 10) in response to oral glucose administration.
Results

Basal hormone concentrations
The basal hormone concentrations in normal controls and women with PCO are shown in Table 1 (the data presented in this table were found in the samples collected at time 0 of the OGGT; similar values were observed at time 0 in the other tests). In both groups, serum FSH, E₂, E₃ and 17OHP levels were in the normal range. In contrast, serum concentrations of LH, DHEA, DHEAS, A and T, and plasma cortisol levels were significantly higher in women with PCO than in normal subjects. The LH/FSH ratio was 1.4 ± 0.2 in normal controls and 2.9 ± 1.2 in women with PCO.

OGTT
The basal glucose concentrations and the incremental pattern after oral glucose ingestion were similar in normal subjects and women with PCO (Fig. 1 left side). On the other hand, the basal insulin levels were significantly higher in women with PCO (143.6 ± 10.0 mean ± SEM pmol/l) than in normal controls (80.4 ± 3.6; p < 0.01). Furthermore, the insulin response during OGGT was significantly higher in patients with PCO than in control subjects (F = 91.95; p < 0.001) (Fig. 1 right side).

The basal AVP concentrations observed in all tests were similar in normal controls and women with PCO (mean ± SEM of 3 test days (time 0): women with PCO = 2.8 ± 0.2; controls = 2.7 ± 0.2).

Fig. 2.
The effect of upright posture in 9 normal and 10 PCO women on plasma AVP levels and hemodynamic parameters (mean ± SEM) (mean blood pressure (mmHg) and heart rate (beats/min)). AVP rose significantly at 10 and 20 min in both control and PCO women (p < 0.001 vs time 0).

Fig. 3.
Effect of insulin-induced hypoglycemia on AVP levels of control (N = 9) and PCO (N = 10) women. Each point represents the mean ± SEM of the observations.
**Hypertonic saline infusion test**

The increase in plasma osmolality owing to hypertonic saline infusion was followed by a significant rise in plasma AVP concentrations in both women with PCO (regression equation: $0.07 \text{ Bosmol} - 15.49$; threshold (expressed as the $x$ axis intercept of the regression line) = 279; $r = 0.80$ $p < 0.01$) and normal controls (regression equation: $0.06 \text{ Bosmol} - 16.37$; threshold: 278; $r = 0.85$ $p < 0.01$). Regression lines for the PCO and control women were similar. The two groups showed a similar AVP rise ($F = 1.97$ NS). Basal hematocrit values were similar in normal controls (43.4 ± 0.3%) and women with PCO (43.2 ± 0.3%). During tests, hematocrit values decreased slightly, but not significantly in both groups (at 120 min - controls: 42.6 ± 0.4%; women with PCO: 42.5 ± 0.5%).

**Orthostatic test**

Upright posture induced a significant increase in plasma AVP levels in both the control and the experimental group. A similar AVP response was observed in normal women and in patients with PCO ($F = 0.78$, NS) (Fig. 2).

**Insulin tolerance test**

Administration of insulin induced a similar decrease in blood glucose concentrations of both normal controls and women with PCO (Fig. 3). In both groups, AVP rose sharply in response to hypoglycemia, with a mean peak response at the time of the glycemic nadir. However, the AVP response was significantly lower in women with PCO than in normal controls ($F = 14.30$; $p < 0.001$). The mean AVP peak was 171% higher than basal value in control subjects, but only 75% higher than baseline in women with PCO.

Basal and hypoglycemia-stimulated GH levels were similar in both groups (Fig. 4 upper part). Basal cortisol concentrations were higher in women with PCO than in normal controls; however, the two groups presented comparable cortisol increments in response to hypoglycemia. In fact, the test of parallelism revealed no significant differences in the cortisol curves between the groups in response to hypoglycemia (Fig. 4 lower part).

**Correlation studies**

When data obtained in normal women and in patients with PCO were combined, significant positive correlations were found between plasma endogenous insulin levels and serum concentrations of $T$ ($r = 0.79$; $p < 0.01$) and of $A$ ($r = 0.88$; $p < 0.01$). Furthermore, a negative correlation was found between maximal AVP peaks in response to insulin-induced hypoglycemia and endogenous insulin concentrations ($r = 0.78$; $p < 0.01$). In contrast, AVP peak responses during the insulin tolerance test were not correlated with basal cortisol levels, peak cortisol responses to hypoglycemia or the circulating concentrations of $E_1$, $E_2$, and the other examined parameters.

**Discussion**

The data of the present study showed normal basal AVP concentrations and normal AVP responses to hypertonic and orthostatic stimuli in women with PCO, even though these subjects presented high circulating concentrations of androgens. These re-
results failed to provide evidence for a role of androgens in modulation of AVP secretion in basal conditions and in response to its major determinants, i.e. osmoregulation and baroregulation. In contrast, our data showed impaired AVP responses to insulin-induced hypoglycemia in women with PCO. This phenomenon appears to be specific for AVP, since women with PCO showed comparable GH and cortisol responses to hypoglycemia. The lower AVP rise during the insulin tolerance test in our patients cannot be attributed to a lower hypoglycemic response. In fact, despite the presence of some degree of insulin resistance in women with PCO (as shown by elevated basal insulin levels and elevated insulin responses during OGTT), all subjects had normal blood glucose decrements during the insulin tolerance test. This latter finding is not inconsistent with the hypothesized insulin resistance in women with PCO, because evaluation of blood glucose decrements in response to a relatively large bolus of insulin is a particularly poor and insensitive method to distinguish subjects with slight insulin resistance. In fact, similar findings have been described in obesity with normal glucose tolerance (14), another condition characterized by slight insulin resistance (14).

Cortisol is known to counteract AVP secretion (15); however, it is unlikely that the lower AVP response to hypoglycemia found in women with PCO was due to cortisol inhibition. In fact, even though basal plasma cortisol levels were significantly higher in women with PCO than in normal controls, their correlation with AVP peak responses was not significant. Furthermore, hypoglycemia-stimulated cortisol peak responses were comparable in both groups of women and were not significantly correlated with AVP peaks during the insulin tolerance test. Also circulating estrogens do not seem to play a role in the mechanism underlying the lower AVP response to hypoglycemia in women with PCO, because they did not correlate with AVP peak responses. In contrast, blood concentrations of A and TT presented a significant negative correlation with AVP peak levels during the insulin tolerance test; that is, lower AVP responses to insulin-induced hypoglycemia were found in women with higher plasma TT and A levels.

In view of the elevated endogenous insulin concentrations found in women with PCO, we suspected a role of hyperinsulinemia in the mechanism underlying the lower AVP response to hypoglycemia (see Introduction). Results showed a significant positive correlation between plasma A, TT and endogenous insulin concentrations and a significant negative correlation between the basal concentrations of endogenous insulin and the AVP peak responses to exogenous insulin. These data indicated that hyperinsulinemic women affected by PCO have a lower AVP responsiveness during the insulin tolerance test.

As in hyperinsulinemic obese subjects (11), the AVP secretory system of non-obese women with PCO seems to be affected by a reduced sensitivity to exogenous insulin or to insulin-induced hypoglycemia. Therefore, the PCO syndrome might represent another clinical condition where elevated blood insulin levels are associated with a hypothalamic posterior pituitary disorder which affects the AVP response to insulin-induced hypoglycemia.

References


Received October 27th, 1988.
Accepted August 9th, 1989.

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