Vasopressin and oxytocin levels during normal pregnancy: effects of chronic dietary sodium restriction

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Abstract

Neurohypophysial hormones are thought to be involved in alterations in fluid balance during pregnancy and delivery. In the course of normal pregnancy intravascular volume is increased whereas sodium restriction is thought to reduce plasma volume and cardiac output. In the present study, we measured the effect of long-term severe sodium restriction on vasopressin (AVP) and oxytocin (OT) levels during normal pregnancy and after delivery.

Fifty-nine healthy nulliparous women were randomized either for a low sodium diet (20 mmol sodium daily) or for a normal diet from week 12 of pregnancy onwards. Circulating plasma levels and urinary excretion of AVP and OT, their neurophysins (Np-AVP and Np-OT) and AVP bound to platelets were determined at regular intervals during pregnancy and after delivery. After completion of the study, women on a sodium-restricted diet were compared with control women on a normal diet using repeated measurement ANOVA with adjustment for potentially confounding variables.

After randomization, a reduction in urinary sodium excretion of, on average, 40–82% was found. In general, no effect of sodium restriction could be demonstrated on the various parameters (0.53 < P < 0.98) with the exception of a significantly lower 24-h urinary AVP excretion by non-smokers with sodium restriction compared with non-smokers having a normal diet (P=0.018). For all parameters, clear changes were found in the course of pregnancy and puerperium (P<0.0001 to P<0.005). Platelet-bound AVP decreased and Np-OT increased during pregnancy. After birth, free plasma AVP, platelet-bound AVP, OT, osmolality, sodium and potassium increased, while Np-AVP and Np-OT decreased.

Although elevated Np-AVP and Np-OT levels during pregnancy seem to indicate increased release of neurohypophysial hormones, pregnancy up to 36 weeks of gestation is accompanied by low circulating AVP and OT levels.

Long-term severe sodium restriction diminishes urinary AVP excretion in (non-smoking) pregnant women, without changing circulating levels of AVP and OT, despite the known reduction in circulating volume. The reduced circulating (platelet-bound) AVP levels during pregnancy, whether or not in combination with severe sodium restriction, support the absence of significant non-osmotic stimulation of AVP during pregnancy.

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Introduction

Both the neurohypophysial hormones arginine vasopressin (AVP) and oxytocin (OT) are involved in volume regulation in human pregnancy (Whalley & Pritchard 1963, Schrier 1988). In normal pregnancy, a 30–40% increase in plasma volume is found, possibly as a reaction to early vasodilation. Because of the vasodilatation, baroreceptor activation occurs, which might lead to non-osmotic AVP release (Schrier 1988). However, the exact role of AVP and OT in volume expansion during pregnancy remains to be established and this work is hampered by difficulties in neuropeptide measurement leading to variations in reported plasma levels.

Sodium restriction during pregnancy is thought to reduce plasma volume (Brown 1988) and is considered to be an appropriate therapy in hypertensive pregnancy by some, on the assumption that hypertension is a consequence of salt retention and early volume expansion (Hamlyn & Mordecai 1986). On the other hand, a reduction of plasma volume is considered potentially dangerous for fetal well-being, especially in the case of...
hypertensive pregnancy complications like pre-eclampsia, associated with an already compromised placental circulation. Nevertheless, sodium restriction is reported to decrease vascular reactivity in pregnancy (Jaspers et al. 1983) and is apparently well tolerated by mother and fetus (Steegers et al. 1991). Conflicting views about the benefits of sodium restriction in pregnancy have led to the start of a multicentred trial in The Netherlands. It aims to evaluate the effects of chronic dietary sodium restriction on the incidence of hypertensive disorders in pregnancy and to examine possible maternal pathophysiological effects. Indeed, in women recruited earlier in the same trial, increased total peripheral resistance and reduced cardiac output were observed during long-term sodium restriction as compared with an unrestricted ad libitum diet (Steegers et al. 1991). To investigate which endocrine factors, besides those related to the renin-angiotensin-aldosterone system, are involved in these changes, AVP and OT maternal plasma levels were measured in pregnant women randomized for a low salt or a normal diet. This might not only confirm the involvement of both the neurohypo¬physial hormones AVP and OT in volume regulation in human pregnancy, but also establish the role of AVP in natriuresis (Brown 1988, Prat et al. 1993) and in modulating vascular tone (Crooke et al. 1988) during a physiological challenge such as sodium restriction.

Various factors hamper reliable AVP and OT assay. The presence of cystyl-aminopeptidase (CAP; vasopressinase or oxytocinase) disturbs reliable measurement of AVP and OT in human pregnancy (Lauzon 1974, van der Post et al. 1994) and changes the metabolic clearance rate (MCR) of the peptides (Davison et al. 1989). Immediate inactivation of CAP during blood sampling is mandatory for a reliable assay. Even with optimal inactivation of CAP, free AVP levels in pregnancy during normal hydration are around the detection limit of the assay (Davison et al. 1989, Thornton et al. 1990, van der Post et al. 1994) and therefore subject to large assay variation. More information about OT and AVP metabolism can be obtained by measuring prohormone products. The precursor molecules of AVP and OT, neurophysylin-vasopressin (Np-AVP) and neurophysylin-oxytocin (Np-OT), are metabolized more slowly (Lauzon 1974). They provide, therefore, complementary information on OT and AVP release and/or clearance. The same purpose is served by the measurement of urinary immunoreactive AVP. Immunoreactive AVP and OT in 24-h collections of urine are reported to reflect AVP and OT release over a 24-h time-span (Moses & Steciak 1986, Amico et al. 1987). The variation in plasma levels due to pulsatile, intermittent peptide release (Fuchs et al. 1991) is bypassed in this way. Finally, platelet-associated AVP is reported to change as a result of chronic osmotic and non-osmotic stimuli (Preibisz et al. 1983, Bichet et al. 1986), like reduced cardiac output, in non-pregnant subjects. In third trimester pregnancy, platelet-associated AVP levels are two to five times higher than free circulating AVP levels (van der Post et al. 1993). AVP is bound to the same type of AVP receptor (van der Post et al. 1993) present on the vascular smooth muscle cell. This is analogous to platelet angiotensin-II (All) binding (Baker et al. 1990). Platelet-associated AVP therefore might reflect haemodynamic alterations and altered vascular reactivity.

In describing AVP and OT plasma levels, excretion and AVP binding to platelets, this study aims to provide insight into AVP and OT release during physiological challenges such as pregnancy and low salt diets.

Subjects and Methods

Patients

All patients participated in a multicentred study (Steegers et al. 1991, van Buul et al. 1995). Some data from subgroups of the current study population have contributed to previous publications (van Buul et al. 1995). In the University Hospital Nijmegen, a total of 59 healthy nulliparous women with singleton pregnancies were enrolled from May 1990 onwards in a randomized study from 12 weeks gestational age onwards. All pregnancies were dated by the last menstrual period and ultrasound. After giving informed consent, women were randomly allocated, by a closed envelope system, to using a low sodium (20 mmol/day) diet or to continue their unrestricted sodium intake. The 29 women allocated to the low sodium group started with the diet in week 14 of gestation and stopped immediately after delivery. All women received iron supplementation (65–130 mg Fe2+ daily). Compliance to dietary restrictions was checked by measurement of sodium excretion and sodium/creatinine ratios in 24-h urine samples at regular intervals during pregnancy.

Study protocol

The study protocol has been described previously (Steegers et al. 1991). Briefly, at 13, 16, 20, 24, 28, 32 and 36 weeks of gestation and 1 and 6 weeks postpartum the following parameters were determined in each patient. Sodium excretion, sodium/creatinine ratio and urinary osmolality were evaluated in 24-h urine samples. Free and platelet-bound AVP, OT, platelet number, osmolality and sodium were measured in plasma.

Furthermore, in the first 21 women, Np-AVP and Np-OT were measured in plasma and AVP and OT in 24-h urine samples. Blood was taken after 30 min in the left lateral tilt position. All blood samples were taken between 1400 and 1600 h. Women refrained from cigarette smoking, and food and fluid intake, except for water, for at least 2 h before plasma sampling. Blood pressure measurements were taken with a DINAMAP 1846 SX (Kritikon, Tampa, FL, USA). Patients were labelled

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hypertensive when a diastolic blood pressure level of at least 90 mmHg was found on two occasions with an interval of at least 4 h. Proteinuria in pregnancy was defined as a total protein excretion of ≥ 0.3 g/24 h. The study protocol was approved by the Ethic Medical Committees of both hospitals involved.

Analytical methods

AVP and OT in plasma were measured as described earlier (van der Post et al. 1994). Special care was taken during sample preparation to inhibit CAP activity with enzyme inhibitors o-phenantrolin (o-PHE; Sigma Chemical Co., St Louis, MO, USA) and Na₂EDTA. Whole blood was taken from an antecubital vein into silicon-coated vacuum tubes (Venoject, Teruma, Leuven, Belgium) precooled on ice and containing 100 μl of a 125 mmol o-PHE/l solution. These samples were immediately transferred to identical tubes containing 100 μl of a 1 mol EDTA/l solution on melting ice. Centrifugation at 90 g for 15 min at 4 °C to obtain platelet-rich plasma (PRP) and at 2000 g for 15 min at 4 °C to obtain platelet-poor plasma (PPP) resulted in less than 1% of the PRP number in the PPP.

Extraction of AVP or OT from preparations was performed within 1 h over octadecasilyl-silica packed SepPak-C18 columns (Waters Associates Inc., Milford, USA) using 2 ml aliquots for AVP and 1 ml aliquots for OT, acidified with 3 ml 0·1 mol HCl to pH 1–2. Inhibitors were sufficiently removed by a wash with 2 ml CHCl₃ and subsequently AVP and OT were eluted with 4 ml 100% CH₃OH. No CAP activity was detectable in this extract. Preliminary experiments showed that AVP and OT were stable for at least 3 weeks of storage at room temperature in methanol. All samples were assayed within 3 weeks of extraction. AVP and OT tracers were iodinated by the chloramine T method and purified by fractionation on a Sephadex G-25 column. Specific activity was >64·8 TBq/mmol. Polyclonal anti-AVP antibody (M160480) was raised in a rabbit and used in a final dilution of 1/100 000. Anti-OT antibody was kindly donated by T Higuchi (Fukui Medical School, Matsuoka, Japan) and was used in a final dilution of 1/350 000. For OT antibody, cross-reactivity with lysine-vasopressin, AVP, vasotocin and desamino-ν-AVP was below 0·01%. The detection limit, defined as B/B₀ ≤ 90%, was 0.25 pg/tube for AVP and 0.125 pg/tube for OT. Intra-assay coefficient of variation (C.V.) was 9·7% and the interassay C.V. in ten different assays was 13·4%. Recovery of 3·2 pg AVP/OT per ml added to whole blood of 22 pregnant women and nine non-pregnant controls was 57% and 59% and from plasma 76% and 80% respectively. In plasma from pregnant women, kept at 20 °C for several minutes without CAP inhibitors, AVP levels were equal to the detection limit. Platelet-bound AVP was defined as the difference between PRP and PPP values corrected for the number of platelets. The detection limit of our platelet-associated AVP assay in this study was 0.6 pg/ml, which corresponds to approximately 0.24 pg/10⁸ platelets (see Fig. 2).

Np-AVP and Np-OT assays were performed at the Neuroendocrinology Section, CHU Sart Tilman, Université de Liège, Belgium (Legros & Franchimont 1972, Pullan et al. 1979). Briefly, plasma samples were obtained after centrifugation of 10 ml venous blood (3 mmol/l). Small aliquots of plasma were stored at −20 °C until analysis. Direct assays were performed in 10 μl plasma volumes. For Np-AVP, the detection limit was 0·02 ng/ml and the intra- and interassay variations were 6 and 10·7% respectively. Np-OT was defined as total neurophysin immunoreactivity minus Np-AVP levels. Detection limit and assay variation of total neurophysin immunoreactivity were 0·2 ng/ml and 6.1% respectively.

Urinary analysis for AVP and OT

Isoelectric focusing (IEF) was performed using thin large-pore polyacrylamide gels. Urine samples (10 ml), acidified with 1 mol HCl to pH 4·0, from the pregnant women under study were stored at −20 °C, extracted over SepPak-C18 columns and dried in a vacuum rotator. The residue was redissolved in 25 μl IEF medium, 10% (v/v) dimethyl-formamide (BDH, Poole, Dorset, UK; for gas chromatography), 10% (v/v) glycerol (BDH) and 2·5% (v/v) Nonidet P-40 (Sigma), and 5 μl was taken for AVP assay. The same volume containing 0, 10, 20, 30, 40 and 50 pg synthetic AVP was used for focusing. After IEF, a sheet of filter paper was applied onto the gel and 5 mm gel paper strips of the separation tracks were cut. Each fraction was eluted by incubation for 1 h in 1 ml 1·8 diluted RIA assay buffer and dried. Each fraction was redissolved in 125 μl distilled water for AVP and OT assay. With this procedure, median recovery of 128 pg synthetic AVP/OT added to 10 ml urine was 60% and 70% respectively. All urine samples of one patient were focused in one run. Without prior separation of peptides by IEF, dilutions of AVP/OT extracted by SepPak columns from pregnancy urine did not parallel standard curves.

Urine sodium was measured by flame photometry and creatinine was measured spectrophotometrically after colorimetric reaction with alkaline picrate. Urine protein was estimated using the sulphasalicylic acid test.

Plasma osmolality was established by freezing-point depression, plasma sodium and potassium with standard clinical laboratory automatres.

Statistical analysis

Data were analyzed using repeated measurement ANOVA with missing data on the values measured at the second visit up to and including the seventh prenatal control visit. The effect of diet was adjusted for smoking.
hypertension, age and the pretreatment value of the studied parameter, all regarded as potentially confounding variables. $P$ values were calculated using Wald tests. The residuals from the resulting model were studied both on a between- and a within-patient level in order to identify outliers as well as the need for a logarithmic transformation. If a logarithmic transformation was deemed useful, the analytic process described above was repeated after such a transformation. First, an analysis was made for the randomized group who completed the study (48 patients) without correction for dietary failure. Secondly, the analysis was repeated after correction for dietary failure in which patients who failed to keep to the diet during the study were not included (39 patients). The magnitude of the changes in the mean study parameters during pregnancy were estimated. Differences between the diet groups in urinary sodium excretion or sodium/creatinine ratio during and after pregnancy were tested with two-way ANOVA. Differences between postpartum and prepregnancy values for the diet as well as for the normal group were tested with Student’s $t$-tests for paired data. Correlations between the studied parameters were established at the between- and within-patient level.

**Results**

**Patient characteristics and sodium excretion**

Of the 59 randomized women 48 completed the study, 11 women completely stopped the project, seven with salt restriction and four without. Consequently, data were obtained from 22 women randomized for salt restriction and 26 women randomized for a free diet, together forming the ‘intention to treat’ group. In this group, nine women allocated to sodium restriction continued the study using a normal diet early in the course of pregnancy. This implied that one could distinguish a ‘compliant to diet’ group consisting of 26 women without dietary restrictions and 13 women actually adhering to the severe sodium restriction. Patient characteristics are given in Table 1.

Table 1 Characteristics of the women and the outcome of their pregnancies. Values are means with s.e.s in parentheses for age (years), gestational age (GA; days) and birthweight (g). Represented here are the women randomized for salt restriction who completed the study ($n=48$). Diet: those who were taking the salt-restricted diet; PIH= pregnancy-induced hypertension. There were no neonatal deaths or handicaps.

<table>
<thead>
<tr>
<th></th>
<th>Diet ($n=22$)</th>
<th>No diet ($n=26$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (s.d.)</td>
<td>29±6 (0-9)</td>
<td>28±2 (1-0)</td>
<td>0-90</td>
</tr>
<tr>
<td>Smoking</td>
<td>8</td>
<td>10</td>
<td>0-48</td>
</tr>
<tr>
<td>PIH</td>
<td>2</td>
<td>2</td>
<td>0-55</td>
</tr>
<tr>
<td>GA at delivery</td>
<td>275 (3)</td>
<td>274 (3)</td>
<td>0-48</td>
</tr>
<tr>
<td>Birthweight</td>
<td>3060 (94)</td>
<td>3077 (104)</td>
<td>0-77</td>
</tr>
</tbody>
</table>

$P$ value for comparison of birthweight percentiles (birthweight corrected for gestational age and sex) between both groups was 0-98.

Figure 1 shows the 24-h sodium excretion and sodium/creatinine ratio after randomization (‘intention to treat’, $n=48$) as well as after correction for dietary failure (‘compliant to diet’, $n=39$). The average urinary sodium excretion during dietary sodium restriction (113–251 days gestational age) was 80±9 (s.d. 83±9) mmol/24 h and 133 (s.d. 42±9) mmol/24 h for women without sodium restriction, while the sodium/creatinine ratio averaged 7±46 (s.d. 6±89) and 13±7 (s.d. 4±3) respectively. This means that a mean reduction of 40% in sodium excretion was obtained by the prescription of sodium restriction. In the ‘compliant to diet’ group, the reduction amounted to 82%, with an average sodium excretion of 28±8 (s.d. 33±8) mmol/24 h and a sodium/creatinine ratio of 2±5 (s.d. 2±4). The differences between the diet groups in sodium excretion in both analyses were highly significant during the period of salt restriction ($P=0-0023$, $P<0-0001$). However, there were no significant differences between both groups with respect to urinary sodium excretion before (93 days gestational age, $P=0-29$) and after (7 and 42 days postpartum, $P=0-25$) the dietary period.

Despite the large differences in sodium excretion, neither plasma osmolality (276 mosmol/kg s.d. 5 vs 276 mosmol/kg s.d. 5) nor plasma sodium (138±6 mmol/l s.d. 1 vs 138±6 mmol/l s.d. 0-7) nor potassium (3±8 mmol/l s.d. 0-23 vs 3±8 mmol/l s.d. 0-18) concentration differed significantly between the low sodium and normal diet groups; levels increased significantly after pregnancy: mean differences between pre- and postpartum values were osmolality, 9 mosmol/kg ($P<0-0001$), sodium, 3±2 mmol/l ($P<0-0001$) and potassium, 0±27 mmol/l ($P<0-0001$).

Of the 48 women followed until postpartum, four developed hypertension (8%).

There were no significant differences in birthweight between the diet groups. No perinatal deaths occurred nor were neonates born with major or minor handicaps.

**AVP and OT excretion: effects of salt restriction (see Table 2)**

There appeared to be no overall differences nor time-specific differences between the two groups for any of the measured parameters, not even after correction for dietary failure. However, a statistically significant difference in 24-h urinary AVP excretion was found during sodium restriction when adjusted for smoking. In non-smoking women, sodium restriction resulted in a significantly lower AVP excretion ($P<0-018$, see Table 2) from day 196 onwards. Urinary OT excretion was below the detection limit of 1±6 pg/ml, which equals about 2-4 ng/24 h. After correction for dietary failure (‘compliant to diet’ analysis) the 13 women with salt restriction appeared to have lower platelet-bound AVP values at 141 days but higher levels at
168 days gestational age. Even so, Np-OT was lower at 113 days but higher at 251 days gestational age.

Patients with high peptide levels at the beginning of pregnancy tended to have high levels later on: peptide levels measured before randomization significantly determined levels later in pregnancy after randomization (P=0.04 to P<0.001), except for 24-h urinary AVP (P=0.26) and free AVP plasma levels (P=0.67).

Apart from the effect on urinary AVP excretion, smoking had no further effect on circulating peptide levels. Hypertension influenced the time-course of platelet-bound AVP values; there was a decrease only at 113 days gestational age (P=0.003).

**AVP and OT levels: effects of pregnancy (see Fig. 2)**

Plasma levels were not corrected for recovery. Free plasma AVP, platelet-bound AVP, OT and their associated neurophysins showed clear changes over time. Free AVP values appeared very stable during pregnancy and they increased after labour (P=0.011). Platelet-bound AVP levels decreased during pregnancy and were found to be increased at the 6-weeks postpartum time-point (P<0.001). No difference was found between (42 days) postpartum values and values before dietary restriction was started (93 days gestational age). Np-OT increased steadily during pregnancy with a maximum at 7 days postpartum, and a drop at 42 days postpartum to 25% of the first pregnant value (P<0.0001). Np-AVP decreased after pregnancy (P<0.0001). OT plasma levels were stable during pregnancy but showed a significant increase after delivery (P=0.039).

**Correlations**

At the between-patient level, free plasma AVP and platelet-bound levels were significantly correlated (r=0.45,
Table 2 Changes (±SD) in plasma levels of OT, free AVP and platelet AVP, their neurophysins, Np-OT and Np-AVP, and urinary AVP measured in women randomized for salt restriction ('intention to treat', n=22) compared with those without dietary restriction (n=26) at various days of gestational age. Urinary AVP and neurophysin data from seven salt-restricted and fourteen women on a free diet were analysed.

<table>
<thead>
<tr>
<th></th>
<th>113 days</th>
<th>141 days</th>
<th>168 days</th>
<th>196 days</th>
<th>224 days</th>
<th>251 days</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVP (ng/24 h)</td>
<td>-11 (23)</td>
<td>-4 (23)</td>
<td>-20 (23)</td>
<td>-7 (23)</td>
<td>-8 (21)</td>
<td>1 (22)</td>
<td>0·64</td>
</tr>
<tr>
<td>Platelet AVP (%)</td>
<td>-2 (41)</td>
<td>-39 (41)</td>
<td>56 (48)</td>
<td>17 (42)</td>
<td>14 (40)</td>
<td>9 (41)</td>
<td>0·92</td>
</tr>
<tr>
<td>Np-AVP (pg/ml)</td>
<td>2 (68)</td>
<td>7 (69)</td>
<td>74 (68)</td>
<td>-18 (68)</td>
<td>-10 (68)</td>
<td>-59 (68)</td>
<td>0·71</td>
</tr>
<tr>
<td>Urinary AVP (%)</td>
<td>-10 (6)</td>
<td>-3·8 (8)</td>
<td>-3·6 (6)</td>
<td>-28 (8)</td>
<td>-21 (8)</td>
<td>-17 (6)</td>
<td>0·018*</td>
</tr>
<tr>
<td>(non-smokers)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OT (%) change</td>
<td>37 (22)</td>
<td>10 (22)</td>
<td>-14 (22)</td>
<td>-45 (23)</td>
<td>-1 (22)</td>
<td>31 (22)</td>
<td>0·89</td>
</tr>
<tr>
<td>Np-OT (%) change</td>
<td>2 (15)</td>
<td>-13 (15)</td>
<td>-6 (15)</td>
<td>2 (15)</td>
<td>-11 (15)</td>
<td>2 (16)</td>
<td>0·98</td>
</tr>
</tbody>
</table>

For urinary AVP (ng/24 h) and Np-AVP (pg/ml) absolute changes from average normal diet values are given, for all others percentage changes because of logarithmic transform of data. Differences are corrected for the effects of confounding factors. P values are given for the comparison by means of ANOVA of measured parameters from women with and without sodium restriction (P<0·05). Comparative analysis of women who kept to their severe sodium-restricted diets revealed P values ranging from 0·53 to 0·94.

P=0·001). Both free circulating (r=0·46, P<0·001) and platelet-bound (r=0·40, P=0·004) AVP values were also significantly correlated with urinary osmolality.

Discussion

This is the first time that circulating AVP and OT levels have been described during long-term severe sodium restriction in human pregnancy. The decrease in platelet-bound AVP levels in human pregnancy has not been described previously.

No overall difference or time-specific differences between the diet groups appeared to be present for both circulating peptides or prohormone products, but a difference was demonstrated for urinary AVP excretion. No significant differences in plasma sodium, potassium and osmolality were found between the diet groups at any of the time-points during pregnancy. First of all one might argue that these results are biased by the non-compliance to sodium-restricted diets of a considerable number of women. Although women who actually kept to their salt-restricted diets had indeed a huge reduction in sodium excretion (see Fig. 1) it was clearly difficult to keep to a severe sodium-restricted diet as the reduction in sodium excretion or sodium/creatinine ratio, on average, was less than 40% (see Fig. 1). From the 29 women allocated to sodium restriction, seven stopped the study and nine shifted early in pregnancy to a non-sodium-restricted diet, making a total of a 55% (16 out of 29) dropout. As this might lead to considerable selection bias, analysis was performed for the 'intention to treat' group (n=48) as well as for the 'compliant to diet' group (n=39). Hereby, we corrected for non-compliance as well as selection bias. The two analyses revealed no differences in the results (see Table 2).

Because we could not find any differences between the diet groups in circulating AVP or Np-AVP plasma levels, any elevation of AVP release due to non-osmotic stimulation during severe sodium restriction seems unlikely. Moreover, we found reduced urinary excretion of AVP in non-smoking women on a sodium-restricted diet. Theoretically, reduced circulating AVP levels might be anticipated during salt restriction. As reported by Davison et al. (1981), decreased solute diuresis, as in restricted-salt diets, lowers distal tubular flow. Therefore, the fraction of solute escaping reabsorption and the residual volume to be concentrated are decreased, requiring decreased tubular binding of AVP. Since AVP is also natriuretic (Torres et al. 1966), by regulating the amiloride-sensitive apical sodium channel in the kidney tubules (Prat et al. 1993), the mandatory diminished natriuresis during very low sodium intake can be achieved in part by decreased AVP binding. This fits well with our finding of diminished urinary AVP excretion in sodium-restricted non-smoking women, but not with the unchanged plasma levels. Perhaps, in our study, diminished AVP plasma levels might have escaped detection because of assay sensitivity (see Introduction) or because patients in our study were positioned in the left lateral tilt position, necessary for simultaneous Doppler flow measurements of the fetal and uterine circulation. In this position, the maximal concentrating ability of the kidney is diminished probably because of intravascular mobilized interstitial fluids which will consequently suppress AVP release (Davison et al. 1981). Possible differences in neurophysin or AVP levels due to dietary restrictions might therefore have been blunted. This would, however, not influence the differences due to pregnancy or gestation length demonstrated in this study, since concentrating ability is equally suppressed postpartum (Davison et al. 1981). Nonetheless, no difference in plasma AVP levels in pregnant women before and after a 7-day period of a comparable severe (20 mmol/24 h) reduction in sodium intake was found by Brown (1988), which is in agreement with our results.
Figure 2 Median values of peptide levels with s.d. (dotted lines) for all women (n=48) completing the study. No correction for recovery is made. The shaded area represents the perinatal period. (a) AVP: stable during pregnancy with a significant increase afterwards (P=0.011). (b) Platelet-bound AVP: a significant decrease during pregnancy (P<0.0001) with no significant difference between the value at 42 days postpartum and the first measurement in pregnancy. (c) Np-AVP (n=21): stable during pregnancy with a significant drop thereafter (P<0.0001) between time-points 7 (251 days of gestational age) and postpartum values. (d) 24-h urinary AVP excretion (n=21): stable during and after pregnancy. (e) OT: stable during pregnancy with a significant (P=0.039) increase postpartum. (f) Np-OT (n=21): a significant steady increase during pregnancy with a maximum at 7 days postpartum, and a drop at 42 days postpartum to 25% of the value at the first pregnant value (P<0.0001).
This study was designed to overcome technical difficulties in peptide measurements by simultaneous determination of peptides, neurophysins and excretion in urine (see Introduction). Peptide excretion in 24-h collections of urine are more likely a reflection of AVP release over long time-periods (Moses & Steciak 1986) and therefore not subject to short-term variation because of position changes. Of interest is that non-smoking women on a sodium-restricted diet were found to excrete less AVP in 24-h urine collections from day 196 gestational age onwards, compared with non-smoking women who were not sodium restricted (Table 2). This is in concert with studies by van den Horn (1981) in non-pregnant subjects. van den Horn (1981) found, in 13 healthy males, 52 ng AVP excretion/24 h in cases of 185 mmol sodium excretion/24 h while in cases of 14 mmol sodium excretion/24 h, 36:6 ng AVP/24 h was excreted in urine (P<0.05). This decrease in AVP excretion might be explained by a slight decrease in circulating plasma levels of AVP, not detectable in our study, but resulting over a 24-h period in a detectable decrease in urinary AVP excretion. Another explanation could be altered renal clearance (Robertson 1972) through a reduction in renal plasma flow. We cannot differentiate between these two possibilities. Smoking was found to be a significant confounder in analyzing the urinary AVP excretion. Smoking cigarettes temporarily increases AVP levels in non-pregnant subjects (Nussey et al. 1986). It is very likely therefore that AVP excretion in urine is increased proportionally to the number of cigarettes smoked. Since data on smoking habits obtained by questionnaire are unreliable we did not analyze the urinary AVP data from women who smoked further. In any case, since women in our study refrained from smoking at least 2 h before blood sampling, smoking did not influence circulating peptide levels, keeping in mind that AVP metabolized by CAP activity in plasma is not detected by our AVP antibody (van der Post et al. 1994).

No lowering of AVP binding to platelets was demonstrated by us during sodium restriction. This again indicates unaltered circulating levels of AVP since free plasma AVP and platelet-bound AVP are positively correlated (see Results). On the other hand, the reduction in plasma volume (Brown 1988) or cardiac output during sodium restriction (Steegers et al. 1991) might normalize a dietary-induced decrease in platelet-bound AVP, since non-osmotic stimuli like reduced cardiac output increase platelet-bound AVP in non-pregnant subjects (Preibisz et al. 1983). It indicates that reduction in effective circulating volume during sodium restriction in pregnancy (Steegers et al. 1991) does not lead to non-osmotic AVP release. Interestingly, in the course of normal pregnancy, we even found a gradual decrease in platelet-bound AVP levels (see Fig. 2), which contradicts any underfilling hypothesis of pregnancy (Schrier 1988). The steadily reduced circulating platelet-bound AVP levels found during the course of pregnancy might indeed reflect the increase in blood volume during pregnancy. Decreased peptide platelet binding has also been described for All (Baker et al. 1990) and parallels the decreased vascular reactivity to vasopressor agents found during normal pregnancy. An identical AVP (and All) receptor type (V1a) is expressed on the vascular smooth muscle cell and platelet and earlier we described a (not significant) decrease in platelet AVP maximal binding capacity during third trimester pregnancy (van der Post et al. 1993), compared with outside pregnancy. During sodium restriction in human pregnancy, an increase in vascular peripheral resistance is found (Steegers et al. 1991). The unaltered AVP platelet binding found by us does not indicate a role for the V1a-AVP receptor type in the increased vascular resistance found during sodium restriction.

Basal plasma levels of free circulating AVP after correction of recovery (1 pg/ml) are in concert with the findings of Davison et al. (1988, 1989) and are very near to the detection limit of the AVP assay of 0.6 pg/ml plasma. The same holds for OT with its detection limit in our assay of 0.6 pg/ml plasma (see also Fig. 2). Increased MCR of AVP (Davison et al. 1989) probably accounts for the lower plasma AVP levels during pregnancy compared with after delivery. This is in spite of increased Np-AVP levels during pregnancy which indicate increased release. This is in agreement with activation of the neurosecretory nuclei in the rat brain during pregnancy (Swaab & Jongkind 1970). From these data it might be concluded that in fact there is increased release of AVP leading to elevated Np-AVP levels but, because of the increased MCR of the circulating peptide, AVP levels are decreased.

The basal OT plasma levels (Fig. 2) found in this study are within the range of the low predelivery values found in most recent studies (Leake et al. 1981, Fuchs et al. 1991, Thornton et al. 1992). OT plasma levels seem to rise only during delivery by an increase in pulse frequency and duration. Np-OT, measured in this study, was determined by subtraction assay and is considered to be an index of endogenous oestrogens (Legros & Grau 1973). From the very steep increase of Np-OT during pregnancy one would expect a strong increase in release of OT during pregnancy. Surprisingly, circulating OT levels remained low during pregnancy up to day 253 of gestation. Furthermore, after pregnancy, Np-OT declined while circulating OT increased. This again may be explained by an increased CAP breakdown of OT during pregnancy and consequently an elevated MCR (Thornton et al. 1992). In addition, Amico & Hempel (1990) supplied evidence for altered post-translational processing of OT-prohormone during oestrogenic stimulation (pregnancy), leading to non-immunoreactive OT-glycine instead of OT. Possibly, our Higuchi OT antibody does not cross-react with OT-glycine and therefore immunoreactive OT levels remained low during pregnancy. This could also explain the undetectable OT urinary excretion in our study during
pregnancy. Since OT and Np-OT did not change during salt restriction we considered this issue, interesting though it may be, to be beyond the scope of this study and we did not investigate this further. A total of 21 mothers were breastfeeding. Plasma values for both Np-OT ($P=0.06$) and OT ($P=0.99$) were not different between women who were breastfeeding and those who were not, although Np-OT values in breastfeeding mothers tended to be higher. Median (range) plasma Np-OT values in mothers who were breastfeeding compared with those who were not were $1.3 \text{ ng/ml (0.7–5.5)}$ vs $0.6 \text{ (0.55–0.95)}$. Finally, one must consider the possibility that increased Np-OT is of uterine origin as can be inferred from studies by Chibbar et al. (1993).

We could not demonstrate an effect of hypertension on any of the parameters measured. About 5–10% of the women enrolled in the study were expected to develop hypertension. In our study group, four women developed hypertension (8%), which is too low a number to enable any conclusion to be drawn. On the other hand, an earlier study did not support a role for AVP in pregnancy-induced hypertension (van der Post et al. 1993).

In conclusion, this study provides evidence for decreased renal AVP excretion during chronic severe sodium restriction in human pregnancy without a significant change in circulating AVP and OT levels or platelet-bound AVP, despite the known decrease in circulating volume. No evidence was found for non-osmotic AVP release during pregnancy or chronic dietary sodium restriction.

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References


Swaab DF & Jongkind JF 1970 The hypothalamic neurosecretory activity during the oestrous cycle, pregnancy, parturition, lactation, and persistent oestrus, and after gonadectomy in the rat. Neuroendocrinology 6 133–145.


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