Atrial natriuretic peptide in pregnant and lactating goats

K. Olsson¹, B. E. Karlberg² and L. Eriksson³

Department of Animal Physiology¹, Swedish University of Agricultural Sciences, Uppsala; Clinical Research Centre², Department of Internal Medicine, University Hospital, Linköping, Sweden, and Department of Physiology³, College of Veterinary Medicine, Helsinki, Finland

Abstract. Plasma concentrations of atrial natriuretic peptide (ANP) were measured in 6 goats during pregnancy, lactation and a nonpregnant, nonlactating (= control) period before and during a rapid iv load of 0.9% NaCl. The volume of the load was 20% of blood volume. The infusions increased central venous pressure by 7 ± 1 mmHg during pregnancy and 8 ± 1 mmHg during lactation. Before infusions plasma ANP concentrations were 5.7 ± 0.7 pmol/l (control period), 10.8 ± 1.8 pmol/l (pregnancy; $P < 0.05$), and 6.5 ± 1.5 pmol/l (lactation; NS). ANP increased significantly in all periods. Maximal values were 12.5 ± 1.5 (control period), 25.5 ± 2.3 (pregnancy; $P < 0.01$ vs control period, $P < 0.05$ vs lactation), and 13.0 ± 1.6 (lactation; NS). Renal Na excretion increased similarly during pregnancy and control period, but slightly more during lactation. In 4 of the goats iv infusions of ANP (1 μg/min, 60 min) were given. The infusions caused natriuresis during the control period, but not during pregnancy and lactation, despite more than 10-fold increases of plasma ANP levels. In conclusion, our results indicate that although plasma ANP concentration rose to high levels during acute NaCl loading in pregnant goats, this effect was not important for the natriuresis. Instead, the natriuretic response to ANP appears attenuated during pregnancy, and also during lactation.

Atrial natriuretic peptide (ANP) is a recently discovered cardiac hormone which is intimately involved in blood pressure and body fluid homeostasis (de Bold et al. 1981; Cantin & Genest 1985; Needleman & Greenwald 1986). Volume expansion with saline results in significant natriuresis and diuresis mediated by multiple regulatory mechanisms. There is good evidence that ANP plays a role in this response, although the casual relationship between atrial stretch, the release of ANP, and the natriuretic response has recently been questioned (see Goetz 1988).

Pregnancy is a state which involves adjustments of several homeostatic mechanisms. In goats, blood volume increases in parallel with the increasing body weight and the blood volume remains expanded during lactation (Hoversland et al. 1974; Olsson et al. 1987). This could be expected to stimulate the release of ANP, but whether this is the case is a matter of controversy in women (Cusson et al. 1985; Otsuki et al. 1987; Rutherford et al. 1987), whereas no change has been reported in pregnant rats (Nadel et al. 1988). Similarly, the effects of injected ANP in pregnant vs nonpregnant rats appear inconsistent (Corwin & Solomon 1985; Kristensen et al. 1986).

In order to further elucidate the physiological role of ANP in the regulation of fluid balance, we have studied the changes in plasma ANP and aldosterone concentration, and the natriuretic response to rapid intravenous infusions of isotonic saline in the same goats during their pregnancy, lactation and nonpregnant, nonlactating (= control) periods. The effects were compared with those obtained during intravenous infusions of ANP during the three periods.

Material and Methods

Six female goats were used. They all delivered one kid. The goats were fed hay and grain (with 3 g of NaCl added)
twice daily at 07.30 and at 15.30 h. During lactation the animals were milked at feeding times. Their milk production at the time of the experiments was (1.3 ± 0.2 l/day).

The NaCl infusions were performed 20–36 days before parturition (pregnancy), 7 weeks after parturition (at peak lactation), and 3 months after the goats had been dried (control period). At the day of the NaCl experiment the body weight was 32 ± 1 (pregnancy), 29 ± 2 (lactation), and 30 ± 1 kg (control), respectively.

The figure of 5% per kg body weight was used to estimate the plasma volume during pregnancy and the control period, and that of 6% per kg body weight during lactation (Hoversland et al. 1974; Olsson et al. 1987). The blood volume in each goat was calculated by using its body weight on the experimental day and the hematocrit value obtained from the blood sample taken before the infusion of NaCl.

Before the NaCl infusions, the goats were provided with catheters inserted into the jugular veins; one was used for infusion and the other for blood sampling and for recording of the central venous pressure (CVP). The tip of the latter catheter was positioned close to the right atrium and the other end was connected to a Statham pressure transducer (Hato Rey, Puerto Rico, Statham). The CVP was displayed on a Grass polygraph (Polygraph MA, USA). Isotonic saline was infused (0.2 ml/min) via the catheter to prevent clotting. For blood sampling the infusion was stopped temporarily, the stopcock turned, blood withdrawn, whereafter the recording continued. For technical reasons CVP could be measured only during pregnancy and lactation.

Isotonic saline (20% of the calculated blood volume) was infused by gravity from a bottle positioned 1.5 m above the back of the animal. One blood sample was taken before the infusion of saline, and one immediately at the end of the infusion. Additional blood samples were taken 1–2, 10, 20 and 30 min post-infusion.

For urine sampling, a Foley catheter was inserted into the urinary bladder.

Within 6–28 days before parturition 4 of the goats were subjected to iv infusions of ANP (human μ-ANP, 1–28, Bachem, Bubendorf, Switzerland; 2 μg/ml, 0.5 ml/min for 60 min). Identical experiments were made during lactation (8–12 weeks after parturition), and in the control period.

In the morning, before the infusions of ANP, the goats were prepared with catheters in both jugular veins and a Foley catheter in the urinary bladder. One blood sample was taken before the ANP infusion, three samples at 20-min interval during the infusion, and then 10, 20, 40, and 60 min after end of the infusion.

**Analyses**

Blood aimed at determinations of plasma hematocrit, total plasma proteins, osmolality, and Na and K analyses was drawn in heparinized tubes. Blood for determinations of plasma concentrations of ANP and aldosterone was collected in ice-chilled tubes containing K$_2$EDTA. The tubes were centrifuged at 4°C, the plasma separated and stored at −80°C until analysed.

For hematocrit (Ht) determinations blood was spun in triplicate capillary tubes for 5 min. Total plasma protein concentration was estimated by use of refractometry. Urine Na and K concentrations were measured using ion-selective electrodes (model E2A Electrolyte Analyzer; Beckman Instruments). The plasma and urine osmolality was determined by freezing point depression using and Adv. Instruments Inc. osmometer (Hermann Roebling Meßtechnik, Berlin). The plasma osmolality was 296 ± 2 mosm/kg during pregnancy and lactation, and 299 ± 1 mosm/kg during anestru (NS) and these values did not change during the infusions. The mean values were therefore used to calculate renal free water clearance.

Plasma ANP concentration was analysed by RIA using a

**Table 1.**

Urine flow and renal K excretion in response to an intravenous load of 0.9% NaCl during pregnancy, lactation and a control period in 6 goats.

<table>
<thead>
<tr>
<th></th>
<th>Urine flow (ml/min)</th>
<th>K excretion (μmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Pregnancy</td>
</tr>
<tr>
<td>Before infusion</td>
<td>1.0 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>During infusion</td>
<td>2.4 ± 0.3*</td>
<td>2.2 ± 0.4*</td>
</tr>
<tr>
<td>Post-infusion (min)</td>
<td>10–20</td>
<td>3.2 ± 0.5***</td>
</tr>
<tr>
<td></td>
<td>20–30</td>
<td>2.7 ± 0.5***</td>
</tr>
<tr>
<td></td>
<td>30–40</td>
<td>2.3 ± 0.5*</td>
</tr>
<tr>
<td></td>
<td>40–50</td>
<td>1.3 ± 0.2</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *P < 0.005; **P < 0.01; ***P < 0.001 vs the pre-infusion value.
Plasma ANP concentrations and renal Na excretion during intravenous infusions of isotonic saline in 6 goats during a): a nonpregnant, nonlactating period (= control), b): pregnancy, and c): lactation. The infusions started at time 0 and continued for 10 ± 1 min (control period and lactation) and 7 ± 1 min (pregnancy; \( P < 0.05 \)). \( *P < 0.05; **P < 0.01; ***P < 0.001 \) vs pre-infusion values. \( /P < 0.05; //P < 0.001; ///P < 0.001 \) vs pregnancy.

Results

Infusions of 0.9% NaCl
The calculated blood volumes were 2.1 ± 0.1 l (control period), 2.3 ± 0.1 l (pregnancy), and 2.6 ± 0.1 l (lactation). The animals were given 445 ± 16 ml isotonic NaCl during pregnancy, 498 ± 7 ml during lactation and 430 ± 6 ml during anestrous. These volumes were infused during 7 ± 1 min during pregnancy and 10 ± 1 min during lactation and the control period (\( P < 0.01 \)). The CVP increased by 7 ± 1 mmHg during pregnancy and by 8 ± 1 mmHg during lactation.

The basal plasma ANP concentration was almost two times higher during pregnancy than dur-

commercially available specific antibody to rabbit α-ANP (Amersham human α-ANP RIA System, code RPA, 512). The antibody cross-reacts 100% with human 1-28 α-ANP and 70% with rat ANP (which is identical to human α-ANP except for one amino-acid residue in position 12). Cross-reactions with smaller peptides, i.e. 5-28 compounds, are less than 1%. After extraction, samples were purified by passing through C18-octadecyl silica cartridges (SEP-Pak, Waters, Milford, MA). Recovery was always over 85%. Separation of antibody bound α-ANP was performed with the Amerlex M second antibody reagent with magnetic separation. The limit of detection for the method is 3 pmol/l. The inter-assay coefficient of variation for a control sample with a mean value of 7.4 is 17.4% (N = 20). Plasma aldosterone concentration (PAC) was measured by RIA according to the method described by McKenzie & Clemens (1974). The limit of detection of the method is 20 pmol/l. The method has been validated in our laboratory (Tolagen & Karlberg 1978).

Statistics
The values are given as means ± SEM. Analysis of variance was used to test statistical differences. The statistical model which was applied regarded reproductive period, individual animal, sample, and the interaction sample × reproductive period as the prime sources of variation. Calculations were executed using the Statistical Analysis System (SAS), procedure GLM (SAS Institute, Inc 1985).
ing the control period. The iv saline load caused plasma ANP to increase significantly higher during pregnancy as compared with the control period and lactation (Fig. 1).

The renal Na excretion rose in a similar manner during pregnancy and the control period in response to the saline load, but more Na was excreted 40 to 60 min post-infusion in lactating goats (P < 0.05 vs control period). Urine flow and renal K excretion first increased, but then gradually decreased (Table 1).

The total plasma protein concentration was significantly lower during pregnancy (52 ± 2 g/l; $P < 0.001$) compared to control period (62 ± 2 g/l) and lactation (63 ± 2 g/l). After the saline load, protein concentration was significantly ($P < 0.001$) lowered in all periods. The values immediately after loading were 42 ± 2, 52 ± 2, and 52 ± 1 g/l, respectively.

The PAC was 82 ± 19, 85 ± 17 and 50 ± 15 pmol/l before the saline load in the control period, pregnancy and lactation, respectively. It fell immediately after the saline load in all animals (in three of them below the detection limit of the method; values are therefore not given). PAC had not returned to pre-infusion values 30 min post-infusion.

**Infusion of ANP**

Before ANP infusions the plasma ANP concentrations were 5.4 ± 0.8 pmol/l (control period), 12.7 ± 2.3 pmol/l (pregnancy; $P < 0.05$), and 8.7 ± 3.2 pmol/l (lactation; NS). After 40 min of ANP infusion the values were 95.9 ± 11.2, 122.5 ± 6.7 (NS vs control period; $P < 0.05$ vs lactation), and 82.4 ± 8.5 pmol/l, respectively.

Plasma Na and K concentrations did not change during these infusions. There were no significant difference in the renal Na excretion before the ANP infusions in the three periods (Fig. 2). The Na excretion increased in response to the ANP infusions during the control period, but during pregnancy and lactation only a small gradual increase in the Na excretion was seen. The Na excretion then remained at the same level for 60 min post-infusion during pregnancy, but became significantly higher than the pre-infusion values within 50 min post-infusion in lactating goats. Renal K excretion tended to increase in response to ANP infusions during pregnancy, but not in the other periods (Fig. 2).

Urine flow rose in response to the infusion during the control period and tended to do so also during pregnancy, whereas no clear change was

![Fig. 2.](image_url)

*Effects on renal Na and K excretions of intravenous infusions of ANP (1 µg/min; 60 min) in 4 goats during a): a nonpregnant, nonlactating period (= control), b): pregnancy, and c): lactation. *$P < 0.05$; **$P < 0.001$ vs pre-infusion values.*
Urine flow and renal free water clearance \((C_{H_2O})\) in 4 goats during intravenous ANP infusions (1 \(\mu g \cdot min^{-1}\); 60 min) during pregnancy, lactation, and a control period.

<table>
<thead>
<tr>
<th></th>
<th>Urine flow (ml/min)</th>
<th>(C_{H_2O}) ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Pregnancy</td>
</tr>
<tr>
<td>Before infusion</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Infusion (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.3 ± 0.3</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>40</td>
<td>1.7 ± 0.3</td>
<td>1.6 ± 1.0</td>
</tr>
<tr>
<td>60</td>
<td>2.2 ± 0.4</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Post-infusion (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.8 ± 0.1</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>40</td>
<td>1.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>60</td>
<td>0.9 ± 0.1</td>
<td>0.7 ± 0.2</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *\(P<0.05\) vs the pre-infusion value; \# \(P<0.05\) vs the control period.

Table 2.

observed during lactation (Table 2). In two lactating and one pregnant goat, renal free water clearance became positive at the end of the infusion, but the difference in renal free water clearance did not become significant.

Before the ANP infusions basal PAC was 43 ± 11 pmol/l during the control period and 95 ± 36 pmol/l (NS) during pregnancy. During lactation the PAC was below the detection limit of the method in two of the animals before the start of the infusions. PAC fell during the ANP infusions and in most cases below the detection limit of the method (therefore no values are given). Not until 60 min post-infusion was PAC detectable in all animals; the values being 69 ± 12 pmol/l (control), 114 ± 21 pmol/l (pregnancy), and 154 ± 62 pmol/l (lactation).

The total plasma protein concentration was 64 ± 3 g/l before, and 66 ± 1 g/l (NS) after the ANP infusions during the control period, 58 ± 1 g/l and 61 ± 1 g/l (NS), respectively, during lactation, and 52 ± 1 g/l both before and at the end of the infusions during pregnancy.

Discussion

Pregnancy and lactation are conditions characterized by an expanded blood volume. It is therefore reasonable to expect stimulation of the ANP release during both these conditions. In our study, we have shown that the basal plasma ANP concentration in goats was elevated during the last month of pregnancy, but not during lactation. Furthermore, the response to a saline load was most pronounced during pregnancy. It could be argued that this was due to the more rapid infusion. We have no obvious explanation of the fact that the infusion was faster when the goats were pregnant, but one possibility may be that the venous capacitance was larger during pregnancy. Whatever the cause, the more rapid infusion does not seem to explain the higher ANP levels achieved, since the CVP rose to the same level both in pregnant and lactating goats. The observation that plasma ANP levels did not rise more during lactation than in the control period in response to the saline load indicates that expansion of the blood volume per se was not the cause of the rise in pregnant goats. Instead, our results indicate a higher sensitivity of the ANP release mechanisms, or a slower metabolic clearance rate during pregnancy.

It has been convincingly shown that ANP is of importance in promoting excretion of a saline load (Schwab et al. 1986; Khraibi et al. 1987; Hirth et al. 1987), but also that atrial stretch may increase the Na excretion independently of release of ANP (Goetz et al. 1986). Here, the degree of atrial stretch (as judged from CVP) was similar during pregnancy and lactation, and probably also during the control period. The magnitude and duration of the natriuresis did not differ significantly dur-
ing pregnancy and the control period, and was only slightly larger during lactation. The latter was by all probability due the larger amount of NaCl infused during this period. This indicates that the augmented increase in plasma ANP concentration during pregnancy was not large enough to cause excessive Na excretion. Instead, it may be questioned whether the plasma ANP levels obtained were important for the natriuresis in any of the three periods.

Few studies have been performed in which plasma values of ANP during saline loading have been compared with ANP levels necessary to elicit a natriuretic response during infusions of endogenous ANP. The dose of ANP (60 μg/h) causing natriuresis in our goats during their control period was somewhat lower than the 100 μg/h reported in sheep (Parkes et al. 1987). However, the dose of ANP used in our goats raised plasma ANP levels about 7 times more than the rise in endogenous ANP observed during the load of saline. A similar relationship between plasma ANP levels during natriuresis in response to saline loading vs infusions of ANP has been observed also in rats (Kaneko et al. 1987). During pregnancy and lactation we did not see any significant natriuretic response, although the plasma ANP concentration was about 5 times higher relative to values during saline loading. Furthermore, the absolute value tended to be highest during pregnancy. Thus, the concentration ought to have been high enough to cause natriuresis. Instead, our results indicate that the natriuretic response to exogenous ANP is blunted both in pregnant and lactating goats, which is in agreement with findings in rats by Corwin & Solomon (1985). As judged from the plasma ANP levels during the infusions the smaller natriuretic response was not due to dilution of the infused ANP during pregnancy as has been suggested by Kristensen et al. (1986).

At present it cannot be excluded that the elevated plasma ANP concentration during pregnancy, as read by our immunoassay, could have been due to interference by some enzyme systems, proteases, or scavenger molecules circulating in the blood during this period. However, work is in progress in order to solve this problem.

It has been claimed that intravenous infusions of ANP inhibits the release of vasopressin (Samson 1985; Fujio et al. 1986), whereas others found no such effect (Ogawa et al. 1987; Cameron et al. 1988). The water turnover increases during pregnancy and lactation in small ruminants (Olsson et al. 1982; Benlamlih et al. 1985). In the present study the animals occasionally excreted a diluted urine regardless of whether an infusion was performed or not, and renal free water clearance turned positive also at the end of the infusions in one pregnant and two lactating goats. However, on the whole, the ANP infusion caused no significant changes in the renal free water clearance. This is in agreement with our previous findings for this dose of ANP (Olsson & Eriksson 1987).

Infusions of ANP have been shown to cause increased total plasma protein concentrations, indicating hemoconcentration in many species, including goats (Olsson & Eriksson 1987), but this effect was not obvious in the present study. We have no ready at hand explanation for this, but if the complete lack of change during pregnancy can be verified, the cause deserves investigation.

After the infusions of ANP, PAC increased slightly above control levels in all periods, a result which has been observed previously (Olsson & Eriksson 1987; Metzler et al. 1985). This could be a reaction to the Na loss during ANP infusions, although hardly in the present study during pregnancy and lactation. It seems more likely that the effect was due to withdrawal of a direct inhibition of the aldosterone release from the adrenal cortex (Goodfriend et al. 1984).

In conclusion, the results of this study show that the plasma ANP concentration is elevated during pregnancy, but not during lactation. The rise in endogenous ANP concentrations during a saline load appears not to be of importance for excretion of the load. Rather, the natriuretic response to ANP seems attenuated both during pregnancy and lactation.

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Prof Kerstin Olsson,
Institutionen för djurfysiologi,
Sveriges Lantbruksuniversitet,
Box 7045,
S-75007 Uppsala, Sweden.