Metabolism and synthesis of arginine vasopressin in conscious newborn sheep

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Submitted 14 May 2008; accepted in final form 2 July 2008

Miao DC, Velaphi SC, Roy T, DeSpain K, Rosenfeld CR. Metabolism and synthesis of arginine vasopressin in conscious newborn sheep. Am J Physiol Endocrinol Metab 295: E672–E677, 2008. First published July 8, 2008; doi:10.1152/ajpendo.90441.2008.—Arginine vasopressin (AVP) is an important regulator of cardiovascular homeostasis in the fetus, but its role after birth is unclear. Although infused AVP increases mean arterial pressure (MAP) during the 1st mo after birth, pressor responses are unchanged, suggesting that vascular responsiveness is also unchanged. Alternatively, this could reflect increases in AVP metabolic clearance rate (MCRAVP). However, newborn AVP metabolism and synthesis are poorly studied. Therefore, we examined the pressor responses to infused AVP and the pattern of circulating AVP, AVP production rate (PRAVP), and MCRAVP in conscious newborn sheep (n = 5) at 9–38 days after birth. Basal MAP rose and heart rate (HR) fell during the study period (P ≤ 0.02), while circulating AVP was unchanged (P > 0.1), averaging 3.01 ± 0.86 pg/ml. Infused AVP elicited steady-state responses at 10–40 min, increasing plasma AVP and MAP and decreasing HR (P < 0.001). Although pressor responses were unchanged between 9 and 38 days, the rise in MAP correlated with increases in plasma AVP (R = 0.47, P = 0.02, n = 24). MCRAVP was unchanged throughout the 1st mo (P > 0.2), averaging 205 ± 17 ml·kg⁻¹·min⁻¹, and was associated with an elevated PRAVP, 973 ± 267 pg·kg⁻¹·min⁻¹, which was also unchanged (P > 0.1). After birth, MCRAVP and PRAVP are elevated, probably accounting for the stable plasma AVP levels. The former is also likely to account for the stable pressor responses to infused AVP during the 1st mo. The reason for the elevated PRAVP is unclear but may relate to increases in vascular volume associated with postnatal growth.

BLOOD PRESSURE (BP) rises immediately after birth, reflecting loss of the low-resistance placental vascular bed and closure of several fetal shunts (7). It then gradually increases during the 1st mo in newborn humans and sheep (17, 36). The level of basal BP immediately after birth and during the 1st wk is gestational age dependent and developmentally regulated (17, 36). However, the mechanisms that contribute to these processes and the subsequent rise in BP are poorly understood. They may include changes in circulating_pressors, e.g., ANG II, arginine vasopressin (AVP), or catecholamines (7), decreases in vasodilators, alterations in vascular responsiveness due to changes in vascular smooth muscle (VSM) maturation (13) or receptor expression (5), or alterations in the metabolism of endogenous constrictors (25, 32). For example, the angiotensin type 2 (AT2) receptor is the predominant ANG II receptor subtype in newborn sheep until after 1 mo of age (4, 5), resulting in attenuated peripheral ANG II-mediated vasoconstrictor responses in fetal and newborn sheep (16, 33). Thus ANG II may be an unlikely candidate in regulation of postnatal BP in the 1st mo of life.

Circulating levels of AVP, a potent vasoconstrictor and modulator of cardiovascular homeostasis in the fetus and newborn (8, 10, 14, 15, 23, 24, 29, 30, 35), are elevated soon after birth (7). However, the contribution of AVP to the regulation of newborn BP is unclear, and circulating levels beyond the 1st wk are not reported. Velaphi et al. (34) observed that infused AVP elicits systemic pressor responses and constricts the peripheral vasculature of conscious newborn sheep in the 1st mo after birth, but the magnitude of these responses was unchanged. Thus vascular responsiveness to AVP may be unchanged during this period. Alternatively, this could be explained by downregulation of the AVP V1 receptor in VSM due to increases in circulating AVP or an enhanced metabolic clearance of infused AVP (MCRAVP), as occurs with ANG II in fetal sheep (25). MCRAVP in full-term fetal sheep averages 60 ± 8.7 ml·kg⁻¹·min⁻¹ (35), which is 4 times greater than in adult animals (1, 31, 35). In contrast to ANG II, fetal MCRAVP is not due to placental clearance in humans or sheep (1, 8, 31, 35) but is primarily due to renal clearance (6, 12, 19, 22). Nonetheless, the metabolism and synthesis of AVP are not well studied after birth, and the effect of MCRAVP on the pressor responses to infused AVP is unknown. The purpose of the present study was to determine 1) the basal levels of circulating AVP and mean arterial pressure (MAP) and heart rate (HR) during the 1st mo after birth in conscious animals, 2) the availability of infused AVP by measurement of MCRAVP, which determines the removal rate, and 3) the neonatal AVP synthesis rate (PRAVP) to better understand the role of AVP in the regulation of postnatal MAP and newborn homeostasis. We hypothesized that MCRAVP is elevated in newborn sheep, which would modulate the pressor effects of endogenous and infused AVP while permitting it to contribute to other aspects of homeostasis during rapid neonatal growth.

METHODS

Animal preparation. Five chronically instrumented newborn singleton sheep were studied at 9–38 days after birth. The animal preparation has been described previously (32–34). Briefly, pregnant ewes were brought to the University of Texas Southwestern Medical Center at Dallas at ~145 days of gestation (full term = 150 days) and allowed to deliver, and surgery was performed at 3–5 days after birth. Preoperatively, animals received subcutaneous atropine (0.088 mg/kg) followed by intravenous pentobarbital sodium (7.5–10 mg/kg) and 1% ketamine hydrochloride (1–2 mg/kg) via a percutaneous jugular catheter. During surgery, anesthesia was maintained with 1% ketamine hydrochloride (1 mg/kg iv). Two animals underwent surgery using isoflurane gas anesthesia. Polyvinyl catheters were implanted to re-
12.5 and 7.5 cm in the femoral artery for cardiovascular monitoring and blood sampling, respectively, and 15 cm in the femoral vein for systemic AVP infusions. Catheters were flushed with heparinized saline (250 U/ml), closed with sterile metal pins, brought to the flank through a subcutaneous tunnel, and stored in a canvas pouch attached to the skin with sterile steel pins. The skin incision was closed with sterile surgical staples. During surgery, animals received 10% dextrose and isotonic saline at 20 ml·kg⁻¹·h⁻¹. Postoperatively, they received intravenous flunixin meglumine (Banamine, 0.1 ml) for pain, penicillin (60,000 U) and gentamicin (7.5 mg) for prophylaxis, and 70 ml/kg of intravenous 5% dextrose and isotonic saline. The animals were kept under a heated warmer until they fully recovered from anesthesia, at which time they were returned to their mothers, but they were removed on the 1st night and kept in a postoperative recovery facility, where they were monitored and bottle fed. Subsequently, animals were kept with their mothers, except during experiments. Catheters were flushed daily with sterile heparinized saline (250 U/ml). Animals received intramuscular penicillin (60,000 U) and gentamicin (7.5 mg/kg) at the completion of each study for prophylaxis. The studies were approved by the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center.

Experimental protocol. Animals were not studied until 3–4 days postoperatively to allow for recovery, which was judged by consistent weight gain, normal arterial blood gases, and stable hemodynamic parameters (32–34). At the time of each study, animals were removed from the mother and placed in a sling, and hemodynamic parameters were allowed to return to baseline before AVP infusion. Once the infusion was stopped, hemodynamic parameters were allowed to return to baseline. Studies were performed weekly. Animals were awake and alert, and they accommodated to the sling rapidly. There was no evidence of stress or dehydration. Weight increased from 6.1 to 8.5 kg (±0.86 pg/ml) during the steady state, and I is the rate of AVP infusion corrected for weight (in pg·kg⁻¹·min⁻¹) (18, 20, 25, 32, 35).

Metabolic clearance rate. After it was determined that a steady state was established, MCRAVP was calculated using the following equation: MCRAVP = 1/Cp, where MCRAVP is the AVP clearance (in ml·kg body wt⁻¹·min⁻¹), Cp is the AVP plasma concentration (in pg/ml) during the steady state, and I is the rate of AVP infusion corrected for weight (in pg·kg⁻¹·min⁻¹) (18, 20, 25, 32, 35).

Endogenous AVP production. To estimate PRAVP, the equation for MCRAVP was rearranged as follows: PRAVP = MCRAVP × Cbasal, where MCRAVP was obtained at 40 min of infusion (in ml·kg⁻¹·min⁻¹) and Cbasal is the basal arterial AVP level (in pg/ml) before AVP infusion.

Statistical analysis. Two-way ANOVA for repeated measures was used to examine changes in hemodynamic parameters and AVP levels across infusion times and doses and between age groups. Linear regression analysis was used to examine changes in baseline hemodynamic parameters and control levels of AVP, MCRAVP, and PRAVP across postnatal ages. Paired t-test was used to compare control AVP levels between doses. Values are means ± SE.

RESULTS

Baseline parameters. Basal measurements of MAP and HR were determined from values obtained before AVP infusion. Basal MAP increased (R = 0.65, P = 0.02, n = 12), while HR fell (R = 0.72, P = 0.009, n = 12), with increasing postnatal age (Fig. 1, A and B). Blood samples for the measurement of baseline AVP levels before AVP infusion were available from 10 studies. There was no change during the study period (Fig. 1C; R = 0.54, P > 0.1, n = 10); basal levels averaged 3.01 ± 0.86 pg/ml.

Cardiovascular responses to infused AVP. Both doses of infused AVP increased MAP and achieved steady-state pressor responses by 10 min of infusion that were maintained throughout the 40-min infusion in all age groups (Fig. 2; P < 0.001, by ANOVA). A dose-dependent response (P < 0.05) was only seen at ≤14 days of age. Importantly, the pressor response did not change with increasing postnatal age (P > 0.1). Since basal MAP increased with age, it was necessary to examine determination of assay variability and recovery. At the time of assay, plasma samples were extracted and measured using procedures provided by the manufacturer with the radioimmunoassay kit (Buhlmann Laboratories, Allschwil, Switzerland). Samples were counted using a Packard Cobra II Auto Gamma Counter (Perkin-Elmer, Waltham, MA). For determination of recovery, 10 μl of 125I-AVP were added to adult plasma samples; values averaged 83.6%. Intra- and interassay variability was 4.8 and 10.0%, respectively. The coefficient of variation was 7.6%.

Fig. 1. Changes in basal mean arterial pressure (A), heart rate (B), and arterial plasma levels of arginine vasopressin (AVP, C) in conscious newborn sheep in the 1st mo after birth. Lines represent linear regressions.
and compare the relative increases in MAP during AVP infusion, i.e., the percent change from baseline, at each age and for both doses to determine whether there are alterations in vascular responsiveness. As seen with the absolute increase in MAP (in mmHg), there was no difference in the percent increase in MAP at each age or between doses during the study period ($P > 0.1$, by 2-way ANOVA); the rise in MAP averaged $12 \pm 2.1\%$.

Since the steady-state pressor responses to AVP were similar for the two doses of infused AVP and across age groups, we examined the relationship between the rise in MAP and the plasma AVP concentrations at 40 min of infusion, which corrects for any effect of MCRAVP (25). There was a significant, although modest, correlation between the rise in MAP and arterial AVP concentrations ($R = 0.47$, $P = 0.02$, $n = 24$).

Both doses of AVP decreased HR and achieved steady-state responses within 10 min that were maintained throughout the 40-min infusion (Fig. 4; $P < 0.001$, by ANOVA). There was no dose effect at any age ($P > 0.1$, by ANOVA). Because basal HR progressively falls after birth, we also examined the relative change or decrease in HR at 40 min of infusion. There were no differences between age groups ($P > 0.05$, by ANOVA); the fall in HR averaged $28 \pm 1.7\%$.

**Plasma AVP.** Arterial AVP levels rose during the infusion of both doses, achieving steady state at 30 and 40 min (Table 1; $P < 0.001$, by ANOVA) that did not differ from each other ($P > 0.1$, by ANOVA). Although the mean plasma levels achieved during infusion of 6.96 $\mu$g·kg$^{-1}$·min$^{-1}$ were modestly higher, there was no significant dose effect ($P > 0.05$, by ANOVA).

**Metabolic clearance rate.** To calculate MCR$_{AVP}$, a steady-state plasma level must be achieved and the rate of AVP infusion should exceed endogenous PR$_{AVP}$ (18, 20, 25, 32, 35). As noted above, steady-state levels of AVP were established at 30 min of infusion (Table 1); therefore, we used plasma levels obtained at 30 and 40 min of infusion to calculate MCR$_{AVP}$. MCR$_{AVP}$ was similar at both time points ($P > 0.05$) and was not dose dependent ($P > 0.1$, by ANOVA) for the doses studied. To determine whether MCR$_{AVP}$ changed during the postnatal period, we examined MCR$_{AVP}$ obtained at 40 min for each dose of AVP studied. There was no change during the study period ($P > 0.1$, $R = 0.25$, $n = 23$); MCR$_{AVP}$ averaged $205 \pm 17$ ml·kg$^{-1}$·min$^{-1}$.

**Endogenous PR$_{AVP}$.** By rearranging the equation for MCR$_{AVP}$, we can estimate PR$_{AVP}$. PR$_{AVP}$ was also unchanged during the postnatal period ($R = 0.33$, $P > 0.1$, $n = 11$); it averaged $973 \pm 267$ pg·kg$^{-1}$·min$^{-1}$. Importantly, AVP infusion rates exceeded PR$_{AVP}$.

**DISCUSSION**

BP rises in newborn humans and sheep during the 1st mo after birth (17, 33, 34, 36), but the mechanisms remain unclear. AVP is a potent vasoconstrictor in fetal sheep and contributes to vascular homeostasis (8, 10, 14, 15, 23, 29, 30, 35); however, its role after birth remains unclear. Although infused AVP constricts the peripheral vasculature throughout the 1st mo after birth (34), the response is unchanged, suggesting that the postnatal rise in MAP is not due to increases in vascular responsiveness to endogenous AVP. However, this could be impacted by AVP metabolism. To address this issue, we measured baseline levels of circulating AVP and examined the relationships between basal AVP, MAP, MCR$_{AVP}$, and PR$_{AVP}$ in conscious newborn sheep during the 1st mo of life. We observed that after birth 1) basal plasma AVP is unchanged and unrelated to the rise in MAP, 2) infused AVP increases MAP in a non-age-dependent manner, and 3) MCR$_{AVP}$ and PR$_{AVP}$ are elevated compared with fetal and adult sheep and unchanged throughout the study period. These observations demonstrate that the absence of a change in vascular responsiveness to infused AVP is probably due to an elevated MCR$_{AVP}$. Furthermore, elevated MCR$_{AVP}$ may also contribute to the
stable plasma concentrations of the peptide in the face of an elevated \( \text{PR}_{\text{AVP}} \). Thus AVP appears to actively contribute to neonatal homeostasis and, possibly, BP regulation in the 1st mo after birth.

Plasma AVP concentrations increase in response to hypoxemia, asphyxia, and hemorrhage in fetal, newborn, and adult animals, demonstrating its role in stress responses (2, 3, 8, 9, 30). However, it is unknown whether basal circulating AVP levels increase after birth and contribute to the postnatal rise in MAP. In prior studies, basal AVP concentrations were examined in newborn sheep over relatively short time periods, but no attempt was made to determine whether there was an association with the postnatal rise in MAP (21, 31). In the present study, we measured basal plasma AVP throughout the 1st mo of life in conscious newborn animals that were considered to be minimally stressed after acclimating to the sling over 30–45 min and did not exhibit elevations in MAP or HR. If the level of circulating AVP contributes to the postnatal rise in MAP, concentrations might have risen accordingly. However, it is unknown whether basal circulating AVP levels were unchanged throughout the study period and did not differ from levels observed in fetal and adult sheep (21, 31). Thus it is possible that vascular responsiveness to the peptide increases with age and that basal plasma AVP levels do not accurately depict the contribution of AVP to the regulation of postnatal MAP.

Since the rise in newborn MAP was not associated with increases in circulating AVP concentrations, there might be an increase in vascular responsiveness to the peptide or alterations in its metabolism, as previously observed for ANG II in fetal sheep (25). In the present study, the same dose of AVP increased MAP and decreased HR similarly at all ages, suggesting that vascular responsiveness or sensitivity to AVP is unchanged during the 1st mo after birth. Since basal MAP rose during the study period, it is essential to examine the relative rise in MAP from baseline, i.e., the percent increase, which takes into account the progressive change in baseline MAP. When this was done, there also was no difference in the pressor responses at any age or between doses, with values increasing \(-12\%\). Thus there is no evidence of a change in vascular sensitivity in the present study. We have no explanation for the dose response seen only in the early study period.

If \( \text{MCR}_{\text{AVP}} \) is elevated, circulating AVP levels might be unchanged and the pressor responses to infused AVP attenuated, resulting in inappropriate conclusions about the role of the peptide in the regulation of MAP. To examine the metabolic clearance rate of any agent, the infusion rate must equal the removal rate and exceed the endogenous production rate (18, 20, 25, 32, 35). \( \text{PR}_{\text{AVP}} \) averaged 973 ± 267 pg·kg\(^{-1} \)·min\(^{-1} \) and is severalfold less than the lowest infusion rate studied, satisfying one requirement for measuring MCR. Furthermore, elevated plasma AVP levels induce a negative feedback upon its release, taking into account the progressive change in baseline MAP. When this was done, there also was no difference in the pressor responses at any age or between doses, with values increasing \(-12\%\). Thus there is no evidence of a change in vascular sensitivity in the present study. We have no explanation for the dose response seen only in the early study period.

### Table 1. Changes in plasma AVP during continuous AVP infusions in conscious newborn sheep

<table>
<thead>
<tr>
<th>Dose of AVP, ( \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} )</th>
<th>Plasma AVP, pg/ml</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>( \leq 14\text{d} )</td>
<td></td>
</tr>
<tr>
<td>3.48</td>
<td>7.5±4.0</td>
</tr>
<tr>
<td>6.96</td>
<td>7.0±1.1</td>
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<tr>
<td>( 15–28\text{d} )</td>
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</tr>
<tr>
<td>3.48</td>
<td>5.6±2.3</td>
</tr>
<tr>
<td>6.96</td>
<td>6.0±1.7</td>
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<tr>
<td>( &gt;28\text{d} )</td>
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</tr>
<tr>
<td>3.48</td>
<td>10.7±2.8</td>
</tr>
<tr>
<td>6.96</td>
<td>3.1±1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE (\( n = 4 \)). *\( P < 0.05 \) vs. control. There was no difference at 30 and 40 min.
response to the high PR\textsubscript{AVP}; this would explain why basal circulating AVP levels are unchanged and do not parallel the postnatal rise in MAP. The enhanced MCR\textsubscript{AVP} also would explain why the pressor responses to infused AVP are unchanged and do not parallel the increase in blood volume. Thus AVP may contribute to newborn BP regulation.

To examine the activity of the AVP system after birth, we measured AVP synthesis. Since basal plasma AVP in newborn sheep does not differ from that reported in fetal and adult sheep and PR\textsubscript{AVP} is proportional to MCR\textsubscript{AVP}, PR\textsubscript{AVP} in the newborn must exceed that in the fetus and adult. This suggests that PR\textsubscript{AVP}, similar to MCR\textsubscript{AVP}, is developmentally regulated. However, it is unclear when either transitions to adult values. The elevation in endogenous AVP synthesis and metabolism throughout the newborn period suggests that AVP contributes to some important aspect of neonatal homeostasis, e.g., fluid and electrolyte homeostasis. This is not surprising, since the blood volume of the rapidly growing newborn parallels the increase in body weight, which was \(\sim 40\%\) during the course of the study. Thus AVP may contribute to newborn BP regulation through its renal effects and the increase in blood volume.

To the best of our knowledge, these are the first studies to examine postnatal AVP metabolism and synthesis in depth and their relationship with newborn BP regulation. We have demonstrated that basal plasma AVP, MCR\textsubscript{AVP}, and PR\textsubscript{AVP} are unchanged in the 1st mo after birth and that MCR\textsubscript{AVP} and PR\textsubscript{AVP} in the newborn exceed those previously reported in the fetus and adult. The elevated MCR\textsubscript{AVP} can explain the stable pressor responses to infused AVP in conscious newborn sheep during the 1st mo after birth (34). Thus the age-dependent differences in AVP synthesis and metabolism suggest that the peptide contributes to postnatal homeostasis, which may include BP regulation and the expanding blood volume associated with postnatal growth. Studies of VSM AVP \(V_1\) receptor expression and function are needed.

ACKNOWLEDGMENTS

S. C. Velaphi was a Fellow in Training and is presently affiliated with the Department of Neonatology, Chris Hani Baragwanath Hospital, University of Witwatersrand, Johannesburg, South Africa.

GRANTS

These studies were supported by the George L. MacGregor Professorship in Pediatrics and National Institute of Child Health and Human Development Grant HD-08783.

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