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Arginine vasopressin
antidiuretic hormone
hyponatremia
kidney
newborn
water metabolism

Control of Vasopressin Secretion in the Newborn Lamb

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Summary

The plasma sodium, osmolality, and arginine vasopressin (AVP) responses to phlebotomy, hypertonic saline, water loading and fluid restriction were studied in 2-49 day old lambs. Phlebotomy of 10 and 20% of the lamb's estimated blood volume produced 37- and 44-fold increments in plasma AVP, without a concomitant change in plasma sodium or osmolality. The infusion of 10 mEq/kg sodium chloride produced a 12% rise in plasma sodium concentration accompanied by a 7-fold rise in plasma AVP. Water loading with 100 ml/kg hypotonic fluid produced a significant fall in plasma sodium concentration (10.7%) and a decrease in plasma AVP. Eighteen hr of water deprivation evoked a 7-fold increase in AVP.

These results indicate that the newborn lamb is capable of responding appropriately to known stimuli for AVP secretion. The stimulus response ratio (SRR):

$$\text{SRR} = \frac{\text{Log [AVP]}_1 - \text{Log [AVP]}_2}{\Delta \text{ osmolality}}$$

of newborn lambs was nearly identical after hypertonic saline and water loading and was also quite similar to that of the adult ewe after a saline challenge. The SRR of water deprived lambs was greater than that after the other stimuli, presumably reflecting combined volume and osmolar stimuli. We conclude that the neurohypophysis and the volume receptor systems of the newborn lamb are capable of appropriate, mature AVP responsiveness during the first days of extrauterine life.

Speculation

The present studies indicate that AVP secretion in the newborn sheep is responsive to both volume and osmolar stimuli. Quantitative responses are equivalent to those of mature ewes. If the newborn human is both osmo- and volume sensitive, as seems likely, AVP secretion may be important in fluid and electrolyte homeostasis in the newborn period. Thus, lack of AVP secretion does not explain the limited ability to concentrate urine demonstrated by the newborn infant.

Earlier studies have shown that plasma antidiuretic activity and plasma AVP concentrations are low in random samples from human newborns after the first few days of life. Furthermore, the newborn is incapable of maximally concentrating urine to levels seen in dehydrated adults. These observations have led to the suggestion that the hypothalamic neurohypophysial system is immature in the neonatal period. However, it is known that newborn infants respond to the stress of delivery (2, 7, 8) or surgery (8, 16) with significant elevations of plasma AVP. Fisher *et al.* (5) have reported directional changes in plasma and urine osmolality and free water clearance after osmolar and volume stimuli which suggest appropriate changes in AVP secretion and renal responsiveness in the neonatal period. There are no data, however, quantifying endogenous AVP secretion in the newborn relative to the adult. The present studies were undertaken to characterize plasma AVP responses to phlebotomy, hypertonic saline infusion, water loading, and fluid deprivation in the newborn lamb and to relate these responses to those seen in adult ewes.

MATERIALS AND METHODS

ANIMALS

Jugular vein and carotid artery catheters were inserted under local anesthesia into 22 spontaneously delivered, full term, healthy, (Columbia-Suffolk) lambs. A heparin solution was used to maintain patency of the catheters. The animals were allowed to nurse *ad libitum* from the ewes. Each lamb was allowed at least 1 recovery day after catheter placement before study. Four study protocols were used to stimulate or suppress endogenous AVP release; these included phlebotomy, hypertonic saline, fluid deprivation, and water loading. No animal participated in more than two studies; sequential studies were performed in random order. Three ml base line blood samples were collected from the carotid artery at −6 min, −3 min, and immediately before each challenge.

Phlebotomy was conducted in 10 lambs ranging in age from 1–7 wk (mean = 26 days). After collection of baseline samples, as above, 10 ml/kg of blood were removed over 1–2 min, after which 3 ml blood samples were obtained every 3 min for 15 min. A second 10 ml/kg phlebotomy was then performed and the sampling protocol repeated. Hypertonic saline stimulation was conducted in 11 lambs 2–35 days old (mean = 19 days). Base line blood samples were collected as described, and hypertonic NaCl (10 mEq/kg of a solution containing 4 mEq/ml) was injected over 1 min via the jugular vein. Ten min after the completion of the injection, 3 ml blood samples were collected via the carotid artery catheter at 3 min intervals for 30 min. Water loading was performed in seven lambs ranging in age from 2–42 days (mean = 14 days). Base line blood samples were collected after which 2.5% dextrose water (100 ml/kg) was infused via jugular vein over a 1 hr period. Four ml blood samples were obtained at 30 and 60 min via the arterial catheter. Fluid deprivation was performed in nine lambs ranging from 2–19 days old (mean = 7 days). After obtaining base line samples, the animals were fluid deprived for 18 hr; 3 ml samples were obtained every 2 hr during this period.

Blood samples were collected in chilled tubes containing 40 μliter 15% potassium EDTA; individual samples did not exceed 4 ml in volume. All samples were immediately chilled and plasma was separated using a refrigerated centrifuge. One ml aliquots were extracted with Bentonite as described by Skowsky *et al.* (23) and stored at −20° until assayed for AVP. The remaining plasma was stored at −20° for measurement of sodium concentration and osmolality. AVP concentration was measured by radioimmunoassay. Sodium was measured by flame photometry and osmolality by freezing point depression. Osmolality determinations were corrected for the contribution of the anticoagulant to the measured

values. Mean values for AVP, sodium, and osmolality for the various sampling periods were analyzed by Wilcoxon signed rank test. Previous studies (25) demonstrated that the rise in plasma AVP after osmolar stimulation is a logarithmic function of the change in plasma osmolality. A stimulus-response ratio (SRR) was, therefore, calculated as follows for each study protocol to quantify AVP release as described previously for fetal sheep (26):

$$SRR = \frac{Log[AVP]_1 - Log[AVP]_2}{\Delta \text{ osmolality}}$$

RESULTS

In the phlebotomy experiments, base line plasma osmolality (P_{Osm}) was 291 ± 4.6 (mean ± SEM) mOsm/kg and plasma sodium was 144 ± 2.4 mEq/liter. There was no change in mean sodium (144 ± 2.8 mEq/liter) or osmolality (291 ± 3.5 mOsm/kg) after phlebotomy. As shown in Figure 1, plasma AVP concentration (P_{AVP}) rose from a baseline of 1.9 ± 0.4 (mean ± SEM) μU/ml to an initial peak of 72 ± 40 μU/ml 3 min after the first phlebotomy ($P < 0.05$). The second peak response (85 ± 25 μU/ml, $P < 0.05$ *vs.* base line) occurred 9 min after the second phlebotomy. The P_{AVP} response to the second phlebotomy is probably underestimated because several values exceeded the upper limit of the radioimmunoassay curve; analyses could not be repeated because of limited sample size. In the hypertonic saline experiments, P_{Osm} rose from 291 ± 2.0–318 ± 4.3 mOsm/kg over a 30 min period ($P < 0.05$), and plasma sodium rose from 142 ± 2.7–159 ± 2.7 mEq/liter ($P < 0.05$). As shown in Figure 2, P_{AVP} increased from 2.9 ± 0.7–22.22 ± 9 μU/ml ($P < 0.05$) over the same 30-min interval.

In response to water loading, plasma sodium and osmolality fell from 140 ± 2.4–125 ± 3.2 mEq/liter ($P < 0.05$) and 283 ± 5.4–262 ± 4.0 mOsm/kg ($P < 0.05$), respectively over a 60-min period. As shown in Figure 3, P_{AVP} fell during water loading from 3.4 ± 1.2–1.1 ± 0.3 μU/ml by 30 min ($P < 0.05$) and to 0.7 ± 0.3 μU/ml by 60 min ($P < 0.05$). During 18 hr of fluid deprivation, plasma sodium rose from 135 ± 1.3–141 ± 2.0 mEq/liter ($P < 0.05$). As shown in Figure 4, P_{AVP} increased from 0.6 ± 0.1–4.8 ± 1.8 μU/ml ($P < 0.05$).

The stimulus response ratios (SRR) for the various study protocols are listed in Table 1. The SRR of lambs to hypertonic saline stimulation and to water loading are nearly identical to the values measured in adult ewes (26). The fluid deprivation SRR in the lamb is greater than that after other stimuli. The greatest AVP response occurred after phlebotomy; a vigorous response unassociated with an osmolar change. Figure 5 represents these data graphically.

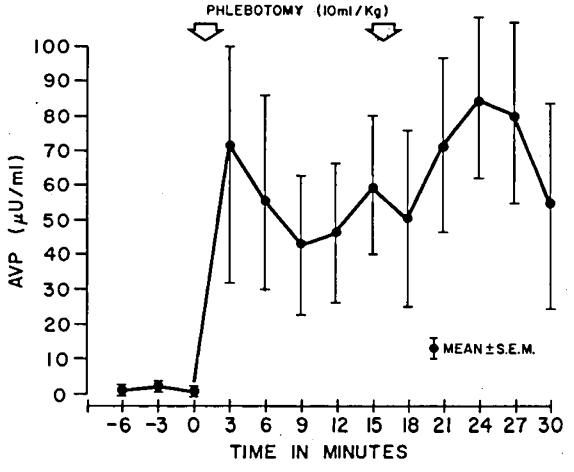

Fig. 1. The effect of sequential phlebotomies on plasma AVP concentration in 10 newborn lambs.

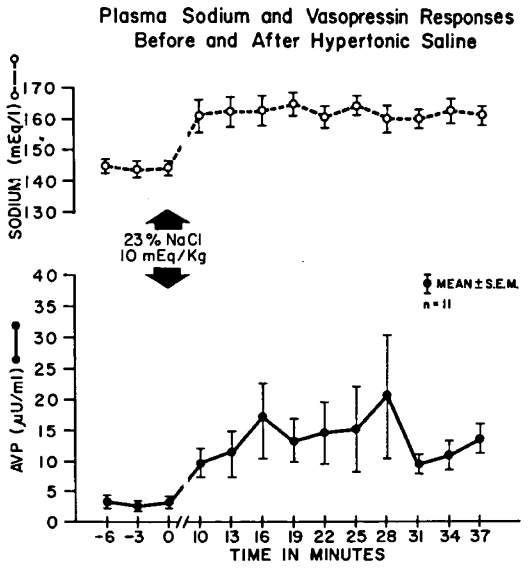

Fig. 2. The effect of hypertonic saline infusion on AVP concentration and sodium concentrations in 11 newborn lambs.

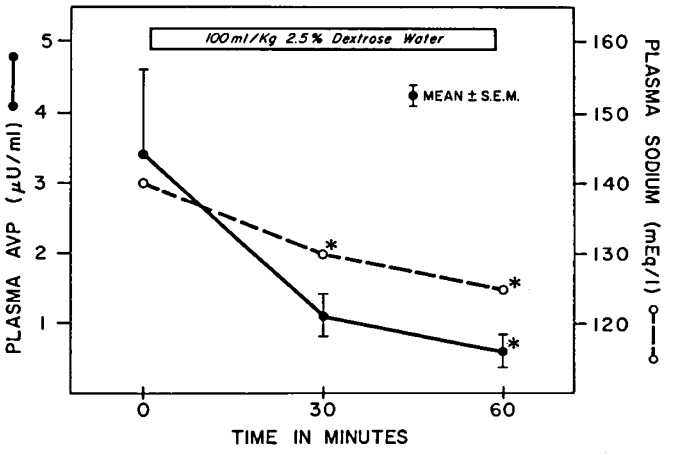

Fig. 3. The effect of water loading on AVP concentrations (*closed circles*) and sodium concentrations (*open circles*) in 7 newborn lambs (*$P < 0.05$).

DISCUSSION

The present data indicate that the newborn lamb manifests qualitatively appropriate plasma AVP responses to phlebotomy, hypertonic saline, water loading and fluid deprivation. In several of the study protocols, base line AVP values tended to be somewhat higher than those of adult nonpregnant ewes (1.5 ± 0.36 μU/ml) (25). The reason for the higher baseline values is not clear; any painful procedure was followed by a recovery period of at least 1 day to minimize the effect of painful stimuli on AVP secretion (22). However, the stress of the physiologic studies might be somewhat greater for lambs than for adult ewes. In those studies in which multiple sequential samples were obtained after the stimulus (phlebotomy, hypertonic saline), pulsatile AVP secretion was demonstrated.

When the responses to all the stimuli for AVP secretion were compared (hypertonic saline, water deprivation, phlebotomy, and water loading), it was clear that phlebotomy evoked the highest plasma AVP concentration. After a phlebotomy of 10 ml/kg

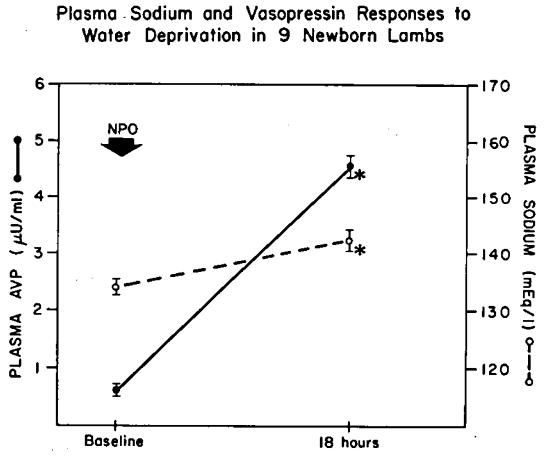

Fig. 4. The effect of 18 hr of water deprivation on AVP concentrations (*closed circles*) and sodium concentrations (*open circles*) in 9 lambs (*$P < 0.05$).

Table 1. *Stimulus response ratios for various study protocols*

$$SRR = \frac{\text{Log [AVP]}_1 - \text{Log [AVP]}_2}{\Delta \text{ osmolality}}$$

	Lambs	Ewes
H_2O loading	0.02 ± 0.01[1]	
Hypertonic saline	0.02 ± 0.01	0.02 ± 0.01
Fluid deprivation	0.06 ± 0.02	

[1] Mean ± SEM.

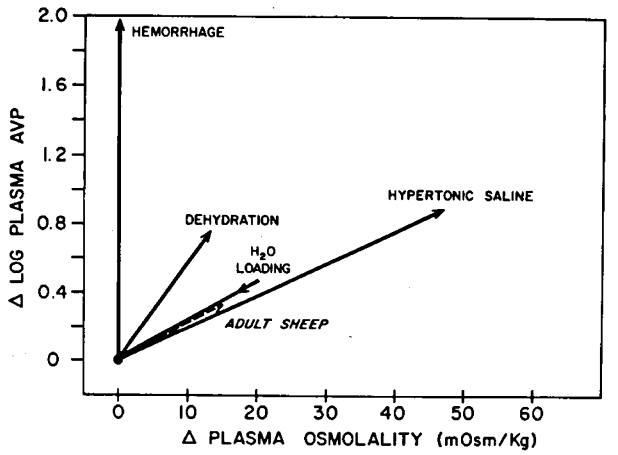

Fig. 5. Comparison of stimulus response ratios,

$$\frac{\text{Log [AVP]}_1 - \text{Log [AVP]}_2}{\Delta \text{ osmolality}},$$

after similar stimuli to AVP secretion in the lamb and ewe.

whole blood (10% of the lamb's estimated total blood volume), mean plasma AVP rose from 1.9–72 μU/ml within 3 min. A second phlebotomy of an additional 10% of the estimated blood volume produced a second rise in plasma AVP concentration. The precise magnitude of this second response is difficult to estimate because the values for many of the samples were above the limits of the assay and limited sample size prohibited repeat measurements. Nonetheless, it is clear that the mean response to phlebotomy in newborn lambs is of considerably greater magnitude than that seen in adult ewes (10) or adult humans (20), after a comparable reduction in blood volume.

Because of a relatively low urine osmolality in the neonatal period and the failure to show significant AVP bioassay responses to stress or dehydration, it was felt for many years that the human infant was incapable of AVP secretion (9, 14). More recent evidence developed with more sensitive bioassays or with radioimmunoassay methods has suggested that the newborn can secrete AVP under some circumstances. Plasma AVP levels are increased in cord blood after vaginal delivery (2, 7, 8), and urinary AVP increases after fluid restriction during the first 3 days of life (1). Also, cold exposure produces a water diuresis in the newborn presumably via inhibition of AVP secretion, although in these studies AVP was not measured directly (4). Surgical stress results in elevated AVP levels (8, 16) during the early weeks of life, and newborns with sepsis (19), pneumonia (15), and after patent ductus arteriosus ligation (24) have been shown to have increased plasma AVP concentrations. There are no data, however, regarding sensitivity or maturity of neonatal osmoreceptor and volume receptor responses. The present results provide such information for the sheep (Table 1).

Hypertonic saline infusion increased the mean plasma sodium concentration from 142–149 mEq/liter and plasma osmolality from 291–318 mOsm/kg and evoked a 7-fold increase in plasma AVP concentration (2.9–22.2 μU/ml). The log AVP response per mOsm change in plasma osmolality (SRR) was identical to that of the adult ewe (0.02 ± 0.01) after a similar challenge (26). Water loading resulted in a decrease in plasma sodium concentrations from 140–125 mEq/liter, and mean P_{AVP} levels fell from 3.4 ± 1.2–1.1 ± 0.3 μU/ml. The resultant SRR (0.02 ± 0.01) was identical in magnitude, although opposite in sign to that achieved by infusion of hypertonic saline. The SRR after water deprivation (0.06 ± 0.02) in lambs was greater than that after hypertonic saline or water loading (0.02 ± 0.01).

These data indicate that the volume and the osmoreceptor systems in the newborn lamb are at least as sensitive to stimulation as are the adult receptors. The fact that the SRR after water deprivation exceeded the SRR response to hypertonic saline most likely reflects the fact that dehydration produces both an osmolar and a volume stimulus for AVP secretion. Robertson and Athar (21) have previously shown that hypovolemia potentiates the AVP response to hypertonicity. There was no difference in AVP responsiveness at postnatal ages varying from 2–49 days for any of the study protocols.

Indirect data suggest that the newborn human infant also manifests quantitatively mature osmolar and volume receptor control of AVP secretion: the infusion of 8 ml/kg isotonic dextran has been shown to produce minimal urine osmolality and a 3% increase in plasma osmolality induced by hypertonic saline produces maximal urine osmolality and a negative free water clearance without a change in glomerular filtration (5). Thus, the explanation of the relatively low urine osmolality characteristic of newborn infants during the first days of extrauterine life (5) is not yet clear. Moreover, the inability of the newborn to excrete a water load as rapidly as the adult (13, 17) could not be due to unresponsiveness of the osmoreceptors to hypotonicity. Rather, it appears that the kidney is unable to excrete a water load due to the reduced glomerular filtration rate present in the first few days of life (6, 12, 18), especially in the premature infant (11).

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