The Renin-Angiotensin-Aldosterone System in the Newborn Lamb: Response to Furosemide

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Summary

Nine newborn lambs between 24 and 48 hr of age were studied before and after infusion of furosemide (2 mg/kg) over 1-2 min. Plasma renin activity (PRA) increased within 8 min after furosemide from a baseline value of 12.6 ± 3.5 ng/ml/hr (mean and SEM) to a level of 24.1 ± 8.6 ng/ml/hr ($P < 0.05$), and peaked 20 mins after the furosemide infusion at a level of 33.1 ± 8.0 ng/ml/hr. Plasma aldosterone concentration increased from a baseline of 12.2 ± 3.1 to 22.8 ± 9.1 ng/dl 35 min after the furosemide infusion, $P < 0.05$. There were no changes in plasma sodium or blood hematocrit and minimal changes in blood pressure and plasma protein concentrations during the first 35 min after the furosemide infusion. The results indicate that the renin-angiotensin-aldosterone system responds promptly to furosemide stimulation despite initially high PRA and aldosterone levels.

Speculation

The renin-angiotensin-aldosterone system responds promptly to stimulation with furosemide in the newborn lamb, despite initially high PRA and aldosterone concentrations. The high renin and aldosterone concentrations characteristic of the newborn could be due to a relative end organ insensitivity to aldosterone or to a renal glomerulotubular immaturity leading to salt wasting.

In the adult human, the renin-angiotensin-aldosterone system is important in the maintenance of blood pressure, blood volume, and sodium homeostasis. The role of the system in the fetus and newborn remains unclear. It is known that PRA (9) and plasma aldosterone levels (1, 15) are high during the neonatal period. The purpose of this study is to determine in the newborn lamb whether the system can respond to stimulation.

MATERIALS AND METHODS

Nine newborn lambs were studied between 24 and 72 hr of age. After a 30-min control period, furosemide (2 mg/kg) was infused over 1-2 min. Blood samples for PRA, plasma aldosterone, plasma sodium (Na), hematocrit, and plasma protein measurements were drawn 10 min before and 8, 20, 35, 65, and 125 min after the furosemide infusion. Urine volume and Na were measured 30 min before and at 2, 30, and 120 min after the infusion in the five female lambs. Four male lambs were not catheterized.

PRA (6, 13) and aldosterone (8) were measured by radioimmunoassay. Sodium was measured by flame photometry and plasma proteins by the biuret method (7). Blood pressure was measured with a water manometer (11) attached to an 8F catheter inserted into the carotid artery. All statistics were done by paired *t*-test.

RESULTS

Baseline PRA concentrations in the newborn lamb were 12.6 ± 3.5 ng/ml/hr (mean and SEM) compared to a mean value of 4.2 ± 0.78 ng/ml/hr in the nonpregnant ewe ($P < 0.05$). Plasma aldosterone levels were 12.2 ± 3.1 ng/dl in the newborn lamb and 5.6 ± 0.85 ng/dl in the ewe ($P < 0.05$; Table 1).

Plasma renin activity increased within 8 min after the furosemide infusion (Figure 1) from the 12.6 ng/ml/hr baseline value to a level of 24.1 ± 8.6 ng/ml/hr ($P < 0.05$). The mean value peaked 20 min after the furosemide infusion at a level of 33.1 ± 8.0 ng/ml/hr, $P < 0.01$ and remained elevated throughout the 120-min study period.

The mean plasma aldosterone concentration increased from the baseline of 12.2 ± 3.1 to 22.8 ± 9.1 ng/dl 35 min after the furosemide infusion ($P < 0.05$, Fig. 2). This level persisted for 65 min postinfusion, falling to 14.2 ± 3.9 ng/dl 2 hr after the infusion.

Blood pressure (Fig. 3) remained stable during the first 20 min after the furosemide infusion, dropped a mean of 3 mm Hg at 20 min ($P < 0.05$) and to 4 mm Hg by 35 min postinfusion. There were no changes in hematocrit or plasma sodium concentration during the first 30 min after the furosemide infusion.

There was a significant increase in plasma protein concentration (Fig. 4) 20 min after furosemide, from a base of 5.3 ± 0.46 g/dl to 5.8 ± 0.53 g/dl ($P < 0.01$); values remained stable through 60 min but increased further at 120 min.

The mean sodium concentration in the control urine samples in the five female animals (Table 2) increased from 7.3 mEq/liter 30 min before furosemide to a mean of 104 mEq/liter at 2 min and to 120 mEq/liter 30 min after the infusion. There was no change in urine potassium concentration. Urine volume increased from a mean of 1.5 ± 0.81 (mean and SEM) ml/kg:30 min to 18 ± 2.3 ml/kg:30 min after the infusion ($P < 0.01$). Sodium excretion increased from 0.02 mEq/kg:30 min in the control urine samples to 2.1 mEq/kg:30 min after the furosemide infusion ($P < 0.01$).

DISCUSSION

The newborn lamb has elevated PRA and aldosterone concentrations (Table 1) similar to the elevated values reported in human infants (1, 9, 15), and significantly higher than levels in the nonpregnant ewe. In response to stimulation with furosemide the mean PRA doubled within 8 min (Fig. 1) and increased about 300% by 20 min (Fig. 1). Mean plasma aldosterone levels doubled within 35 min after furosemide (Fig. 2). Individual values peaked between 35 and 65 min. These results indicate that the renin-angiotensin-aldosterone system responds promptly to stimulation despite the initially high renin and aldosterone concentrations.

There was a rapid sodium diuresis in response to the furosemide infusion. A sodium loss of 2 mEq/kg and water loss of 18 ml/kg occurred during the first 30 min. Previous studies have shown that the maintenance of sodium and water balance by continuous

Table 1. Baseline plasma renin activity (PRA) and aldosterone concentrations in lambs and nonpregnant ewes¹

Animals	PRA, $\mu\text{g/ml/hr}$	Aldosterone, ng/dl
Newborn lambs, $n = 9$	12.57 ² (3.51)	12.17 ² (3.0)
Adult ewes, $n = 9$	4.16 (0.78)	5.59 (0.85)

¹ Values are means (SEM).

² $P < 0.05$.

Fig. 1. Plasma renin activity (PRA) response to furosemide in the newborn lamb. Furosemide, 2 mg/kg, was infused intravenously over 1–2 min after two baseline PRA samples. PRA concentration increased at 8 min postinfusion ($P < 0.05$) and peaked at 20 min ($P < 0.01$).

Fig. 2. Aldosterone response to furosemide in the newborn lamb. Furosemide, 2 mg/kg, was infused intravenously over 1–2 min after two baseline PRA samples. Plasma aldosterone increased 35 min postinfusion ($P < 0.05$).

replacement of urinary losses with isotonic saline in dogs (17) or by shunting of urine into the femoral vein in rabbits (12) will not alter the furosemide-induced renin secretion, despite the constancy of blood pressure, plasma sodium concentration, and plasma volume. In the present study the renin response occurred within 8 min, before alterations in blood pressure or plasma protein concentrations were detected, and without changes in hematocrit or plasma sodium concentration. The early PRA response might have been mediated by alterations in intrarenal hemodynamics, by increase in intratubular sodium in the region of the macula densa, or by direct inhibition of macula densa sodium transport. It has been shown that furosemide in low doses does not significantly alter glomerular filtration rate (17) or renal plasma flow. In high doses (2.5 mg/kg) renal plasma

flow increases. The drug increases blood flow to the pars radiata of the kidney cortex and decreases flow to the juxtaglomerular cortex and medulla (2). Since most renal renin resides in the outer cortex, these data suggest that altered renal hemodynamics may not evoke the early renin release. Vander and Miller (18), and White (19) showed that increasing the tubular sodium load resulted in reduction of the secretion of renin induced by clamping the renal artery. A direct inhibitory effect on macula densa sodium transport thus seems more likely. The present data do not clarify the mechanism further.

The reason for the high PRA and plasma aldosterone levels in the newborn also remains obscure. They might be due to the stress or metabolic changes associated with parturition, to high baseline fetal and newborn renin and aldosterone secretion rates, to decreased metabolic clearances of the hormones, to

Fig. 3. Blood pressure before and after furosemide in the newborn lamb. Blood pressure dropped a mean of 3 mm Hg at 20 min ($P < 0.05$) and 4 mm Hg by 35 min postfurosemide (2 mg/kg) infusion.

Fig. 4. Plasma protein concentrations before and after furosemide in the newborn lamb. The furosemide infusion, 2 mg/kg, was begun at zero time. The plasma protein concentration was significantly increased above baseline ($P < 0.01$) 20 min after the infusion was begun.

Table 2. Urine sodium and volume changes in response to furosemide in newborn lambs¹

Urine	During 30 min before furosemide	During 30 min after furosemide
Sodium (mEq/liter/30 min)	7.3 (0.94)	120 (0.47)
Sodium (mEq/kg/30 min)	0.017 (0.004)	2.12 (0.24)
Volume (ml/kg/30 min)	1.5 (0.41)	18 (2.3)

¹ Values are means SEM; $n = 5$.

relative end organ insensitivity to aldosterone, or to increased renal salt loss due to glomerulotubular immaturity. The fact that PRA (9) and plasma aldosterone concentration (10) remain high during the first month of life suggests that events related to parturition are not involved. Kowarski *et al.* (10) have shown that metabolic clearance and production rates of aldosterone are higher on a body surface area basis in infants than in adults. The present results show that the renin-angiotension-aldosterone system is appropriately linked to a juxtaglomerular cell stimulus. Finally, earlier data indicate that plasma aldosterone levels in the newborn are related to urine sodium excretion (15). Thus, relative end organ insensitivity to aldosterone and/or renal glomerulotubular immaturity leading to salt wasting would seem to be possible explanations for the altered renin and aldosterone metabolism in the newborn.

Several lines of evidence would support this view. Greenberg (5) observed that normal newborn infants failed to show an increase in renal tubular sodium reabsorption in response to exogenous aldosterone; the newborn continues to excrete sodium in the face of a salt-free intake (4); the preterm infant excretes excessive sodium (16) and can develop hyponatremia within a few weeks (14); the rate of sodium transport in the proximal tubule of the infant rat is suppressed (3). Further study is necessary to clarify the defect(s).

CONCLUSION

Furosemide stimulated the renin-angiotensin system and promptly evoked aldosterone secretion in the newborn lamb. There were no changes in plasma sodium or hematocrit and minimal changes in blood pressure and plasma protein during the first 35 min following the furosemide infusion. This data, plus other studies in the literature, suggest that the early PRA response might have been mediated by a direct inhibitory effect on macula densa sodium transport. The present study also confirmed high baseline PRA and aldosterone concentrations in the newborn period. The mechanism for these alterations remains obscure.

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20. The authors wish to thank Dr. C. Dennis Ashby and Robert MacPhee of Nichols Institute for their help with the radioimmunoassays. We thank Hoechst-Roussel Pharmaceuticals for providing the Lasix.
21. This research was supported by Grants 5-S01-RR05551-14 and HD-06335 from the National Institutes of Child Health and Human Development, National Institutes of Health, Bethesda, Md.
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23. Received for publication October 26, 1976.
24. Accepted for publication December 21, 1976.