Potassium: Aldosterone Relationships in Pregnant Ewes and Chronically Cannulated Ovine Fetuses

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Summary

As maternal hyperkalemia quickly induces fetal hyperkalemia, it was decided to investigate the effect of small elevations of plasma $[K^+]$ on the peripheral blood aldosterone concentration in chronically catheterized ovine fetuses and to compare this response to that obtained in the adult ewe both pregnant and nonpregnant. For elevations of plasma $[K^+]$ of 0.1 to 0.3 mmole/liter blood aldosterone concentrations were elevated by 6.7 ± 4.9 (9) ng/dl in nonpregnant sheep, 6.3 ± 5.0 (6) in pregnant ewes, but only 0.3 ± 1.7 (4) in fetal sheep. For increments in plasma $[K^+]$ of 0.4 – 1.0 mmole/liter aldosterone increments in nonpregnant ewes

were 9.5 ± 4.5 (4) ng/dl compared with 10.1 ± 4.7 (5) in pregnant ewes and 1.5 ± 2.6 (7) in fetuses. There was no obvious correlation between the age of the fetus and the response to increased plasma $[K^+]$. In effect, the blood aldosterone concentration of the fetus was not elevated by increasing plasma $[K^+]$.

Speculation

The ovine fetus is not able to cope with hyperkalemia by increasing blood aldosterone concentration. Until the fetus is close to term, hyperkalemia may jeopardize the pregnancy by causing placental hemorrhages.

It has been reported that during experimentally-induced potassium deficiency in pregnant rats (4, 9) and bitches (8) the fetus is substantially protected from hypokalemia at the expense of the mother. On the other hand, maternal hyperkalemia quickly induces fetal hyperkalemia. In all species studied so far an elevation in the plasma potassium of the adult elicits a number of compensatory mechanisms designed to lower the extracellular fluid potassium concentration (10) including an increase in aldosterone secretion, stimulation of insulin and glucagon secretion, and, in the long term, increased renal secretory capacity. The kidney of the chronically cannulated ovine fetus can excrete potassium in increasing quantities when the peripheral blood aldosterone concentration is elevated by aldosterone infusion (6) from at least 90 days gestation (term 147 $\pm$ 5). Increased plasma K⁺ concentration is a potent stimulus to aldosterone secretion in the non-pregnant sheep (1, 5). It was therefore decided to investigate the effect of small elevations of plasma K⁺ on the peripheral blood aldosterone concentration in chronically-catheterized ovine foetuses, and to compare this response to that obtained in the adult ewe both pregnant and non-pregnant.

METHODS

Animals: Five pregnant ewes of known mating dates were used for the studies on the adult sheep.

They were studied between 100-138 days of gestation and again, non-pregnant, after their lambs had been weaned. Seventeen experiments were performed on the chronically-cannulated fetuses of nine ewes of 109-145 days gestation. The fetuses had been cannulated with silastic cannulae in one carotid artery and one jugular vein (9) 10-50 days prior to the K infusion experiment.

Experimental

Adult sheep: Two cannulae were introduced into the jugular veins twenty-four hours before the experiment commenced. Following collection of a control sample, KCl, in isotonic saline, was infused at the rates of 4, 8, 12, 16, 24 mmol/h (for one hour at each rate), at the end of which time a blood sample was collected for plasma Na⁺ and K⁺ determination and blood aldosterone, corticosterone and cortisol determinations.

Fetal sheep: KCl, in isotonic saline, was infused at 0.4 mmol/h (1ml/hr) or 1.6 mmol/hr (4 ml/h) for two hours via the fetal venous cannulae, and carotid arterial blood samples collected before and just prior to the conclusion of the infusion. At the same time as the control fetal sample was being collected a 25 ml sample of maternal venous blood was also collected. 13 ml of this sample was transfused to the fetus immediately after the collection of the control fetal sample, the rest was kept for blood steroid and plasma electrolyte measurements. The transfusion of maternal blood to the fetus has been shown previously not to alter significantly adrenal steroid concentrations in fetal blood or to harm the fetus in any other detectable way (2). Ten ml of fetal blood was frozen immediately and kept at -20°C for steroid analysis, whilst 3 ml was used to determine hematocrit and plasma sodium and potassium concentrations. The second fetal blood sample was replaced by an equivalent volume of saline.

In other acute experiments plasma [K⁺] was measured at four half-hourly intervals after commencement of a similar infusion and was significantly elevated from 30 minutes until completion of infusion.

Chemical

Plasma sodium and potassium concentrations were determined in a Technicon Autoanalyser. Whole blood samples were assayed for aldosterone, corticosterone and cortisol by the double isotope dilution derivative method of Coghlan and Scoggins (1967) (3). Specificity of the method for ovine fetal blood had been established previously (2).

Statistical analysis

Results were compared using Student's t'-test.

RESULTS

The potassium infusions into the ewe raised plasma K⁺ by 0.1 - 2.3 mmol/l. The potassium infusions into the fetus raised fetal plasma K⁺ by 0.3 to 1.5 mmol/l. The control plasma K⁺ in the non-pregnant ewe was 4.4 $\pm$ 0.2 (5) mmol/l - mean $\pm$ standard deviation (number of samples) which is not different from that of the ewe during the last third of pregnancy 4.3 $\pm$ 0.4 (4) mmol/l. Whole blood aldosterone concentrations were not significantly different, being 3.8 $\pm$ 1.6 (5) ng/dl in the non-pregnant ewes and 5.9 $\pm$ 2.3 (4) in the pregnant ewes.

The control plasma K⁺ in 17 plasma samples from fetuses 109-145 days gestation was 4.4 $\pm$ 0.5 (17) mmol/l and peripheral blood aldosterone concentration was 4.8 $\pm$ 3.6 (17) ng/dl. The effect of increasing plasma potassium concentrations on blood aldosterone concentrations in non-pregnant, pregnant, and fetal sheep is shown in figure 1. For elevations of plasma K⁺ of 0.1 - 0.3 mmol/l blood aldosterone concentrations were elevated by 6.7 $\pm$ 4.9 (9) ng/dl in non-pregnant sheep 6.3 $\pm$ 5.0 (6) in pregnant ewes but only 0.3 $\pm$ 1.7 (4) ng/dl in fetal sheep. For increments in plasma [K⁺] of 0.4 - 1.0 mmol/l aldosterone increments in non-pregnant ewes were 9.5 $\pm$ 4.5 (4) ng/dl compared with 10.1 $\pm$ 4.7 (5) in pregnant ewes and 1.5 $\pm$ 2.6 (7) in fetuses. Even greater increments in fetal plasma K⁺ produced no change in plasma aldosterone concentration. There was no obvious correlation between the age of the foetus and the response in increased plasma [K⁺].

When responses in non-pregnant and pregnant ewes were compared it was seen that pregnancy did not alter the magnitude of the change in peripheral blood aldosterone concentration at various increased plasma [K⁺]. In effect the blood aldosterone concentration of the fetus was not elevated by increasing plasma [K⁺].

Increases in plasma K⁺ had no effect on peripheral blood values

of corticosterone or cortisol in the non-pregnant or pregnant ewe, Table 1, or in the fetus of less than 138 days of age but may have had an effect in the four fetuses of 138 days or more gestation (P < 0.05).

TABLE 1.

EFFECT OF INCREASED PLASMA [K⁺] ON BLOOD STEROID VALUES

	<u>Corticosterone</u>		<u>Cortisol</u>	
	<u>µg/100 ml blood</u>			
	Control	+ K	Control	+ K
Non-pregnant ewe	0.09±0.06	0.08±0.05	0.49±0.48	0.38±0.26
	(5)	(17)	(5)	(18)
Ewes 100-140 days pregnant	0.14±0.11	0.12±0.09	0.58±0.30	0.55±0.35
	(5)	(20)	(5)	(20)
Fetuses 109-137 days gestation	0.03±0.03	0.03±0.03	0.21±0.26	0.18±0.17
	(11)	(11)	(13)	(13)
Fetuses 138-145 days gestation	0.04±0.05	0.15±0.08	1.33±1.29	3.0 ±2.2
	(4)	(4)	(4)	(4)

Effects of K⁺ infusion on fetal well-being:

Three fetuses which each received 2 potassium infusions on or after the 136th day of gestation were born alive on day 146 and survived. Two fetuses which each received two potassium infusions at younger ages (117-136 days) died 1 or 4 days respectively after the second potassium infusion and were aborted. Daily pH and blood gas analysis on fetuses showed no deviation from normal values until day of death. In two other fetuses which received their first infusion on days 109 or 134 were delivered alive by caesarian section 6-7 days later in order to study the placenta for potential pathological changes. Neither fetus lived after delivery and autopsy showed no pathological changes in the fetus. Table 2 shows the results for pH and pCO₂ during and after potassium infusions in one foetus which died at 140 days (Verlene) and one which was delivered alive at 140 days (Keva).

TABLE 2.

EFFECT OF INTRAVENOUS POTASSIUM CHLORIDE INFUSION ON FOETAL BLOOD ACIDITY

<u>Experiment</u>	<u>Time</u>	<u>Verlene</u>		<u>Keva</u>	
		pH	pCO ₂	pH	pCO ₂
FIRST INFUSION					
I.V. KCl 0.4 mM/L	0	7.42	41.6	7.39	37.8
	+ 30 mins	7.44	35.0	7.39	45.0
	+ 60 mins	7.38	37.1	7.38	36.2
	+ 90 mins	7.38	42.2	7.37	33.0
	+120 mins	7.38	33.0	7.38	35.0
24 h after infusion ceased					
		7.39	41.6	7.42	31.9
+ 48 h					
		7.38	38.0	7.37	37
Second K infusion					
+ 24 h after second infusion					
		Lamb dead		7.42	38
+ 48 h					
				7.42	37.5
+ 72 h					
				7.42	45.0
				Live lamb delivered	
				placental deterioration observed.	

In the placenta of the two sacrificed fetuses approximately one quarter of the cotyledons showed evidence of haemorrhage which appeared to originate in the hylar region at the site of insertion of fetal vessel into the cotyledon. In the more advanced cases the blood extravasated from the hylar over the surface of the cotyledon and spread under the membranes towards the uterine wall. Microscopically the haemorrhages were multifocal occurring within the first two millimetres of the foetal surface immediately adjacent to the hylar. There was a tendency for confluence and extravasation into the loose connective tissue of the hylar and from there over the surface of the cotyledon. The large foetal vessels were intact and the remainder of the cotyledon was normal. There were no general haemorrhagic changes within the cotyledon. The position, size and number of the haemorrhages suggest that they originated from medium sized fetal vessels (arterioles) rather than capillaries or arteries and veins.

DISCUSSION

Increasing plasma [K⁺] in the ewe during the last third of pregnancy produced similar elevations in peripheral blood aldosterone concentration to those seen in the non-pregnant ewe. It has already been reported that peripheral blood concentration of aldosterone, cortisol and corticosterone are similar in pregnant and non-pregnant sheep. In contrast is the relatively poor response to infused potassium in the fetus. There are at least two possible explanations for this: (a) the fetal adrenal is relatively insensitive to variations in plasma [K⁺], or (b) most of the aldosterone in the fetal circulation originates from the mother by trans-placental passage, and a significant alteration in the fetal adrenal aldosterone secretion rate would not alter the peripheral blood concentration to such a large extent. Preliminary results

suggest up to 50% of the fetal aldosterone may come from the mother prior to 130 days but that more than 80% is of fetal adrenal origin thereafter (Wintour - unpublished results). This then leaves us with the conclusion that the fetal adrenal is relatively unresponsive to increased plasma $[K^+]$.

This would confirm previously reported work on fetal adrenal secretion in acute preparations, and on in vitro incubation studies (11). In this earlier study adrenals from fetuses of less than 20 days gestation were incubated in a buffer containing $[K^+]$ at 1 mmol/l and no effect on aldosterone was measured, although in older fetuses there was a stimulation. However, this value of $[K^+]$ is outside the normal physiological range achieved in the present paper. It is interesting to note that in the previous study in which adrenal venous blood was collected during K^+ infusion in acutely cannulated fetuses the adrenal secretion rate of aldosterone was only elevated in one fetus of 140 days and both corticosterone and cortisol secretion rates were similarly elevated, which correlates with an increased peripheral blood value of corticosterone and cortisol in fetuses of 138 days gestation in the present series.

The death of two fetuses following potassium infusions, and the pathological changes in the placenta of the two fetuses which were delivered by caesarian section suggest that hyperkalemia may be dangerous to the pregnancy. Whilst the effects cannot be assigned, unequivocally, to the potassium experiment, it is worthwhile to note that in at least thirty other chronically cannulated fetuses in this laboratory, no such placental changes or deaths have occurred. These other fetuses have been subjected to repeated experimentation, involving collection of fetal blood, transfusion of maternal blood, and infusion of substances such as aldosterone (6), antidiuretic hormone (7) and ACTH (2). The survival of the older fetuses could be due to the elevation of blood cortisol and corticosterone concentrations upon K^+ infusion. Alternatively their survival may have been due to the fact that they were born before placental damage had occurred. In conclusion, potassium infused into the fetal circulation of chronically cannulated ovine fetuses does not produce significant increments in fetal peripheral blood aldosterone concentration, and may cause abortion after 1-5 days, dependent on the level of placental haemorrhage.

REFERENCES

1. Blair-West, J. R., Coghlan, J. P., Denton, D. A., Goding, J. R., Munro, J. A., Peterson, R. E., and Wintour, E. Marelyn.: Humoral stimulation of adrenal cortical secretion. *J. Clin. Invest.* 41 : 1606 (1962).
2. Brown, Elizabeth H., Coghlan, J. P., Hardy, K. J., and Wintour, E. Marelyn.: Aldosterone, corticosterone, cortisol, 11-deoxycorticosterone, 11-deoxycortisol concentrations in the blood of chronically-cannulated ovine fetuses. *Acta Endocrin. (Kbh)* 88 : 364 (1978).
3. Coghlan, J. P., and Scoggins, B. A. : Measurement of aldosterone in peripheral blood of man and sheep. *J. Clin. Endocrin.* 27 : 1470 (1967).
4. Dancis, J. and Springer, Dawn.: Fetal Homeostasis in maternal malnutrition : Potassium and sodium deficiency in rats. *Ped. Res.* 4 : 345 (1970).
5. Funder, J. W., Blair-West, J. R., Coghlan, J. P., Denton, D. A., Scoggins, B. A., and Wright, R. D.: Effect of Plasma $[K^+]$ on the secretion of aldosterone. *Endocrinol.* 85 : 381 (1969).
6. Lingwood, B. L., Coghlan, J. P., Hardy, K. J., and Wintour, E. M.: Effect of aldosterone on urine composition in the chronically-cannulated ovine fetus. *J. Endocrinol.* 76 : 553 (1978).

7. Lingwood, Barbara, Hardy, K. J., Horacek, I., McPhee, M. L., Scoggins, B. A. and Wintour, E. Marelyn.: The effect of antidiuretic hormone on urine flow and composition in the chronically-cannulated ovine fetus. *Quart. J. Exp. Physiol.* 63 : 315 (1978).
8. Serrano, C. V., Talbert, L. M., and Welt, L. G.: Potassium deficiency in the pregnant dog. *J. Clin. Invest.* 43 : 27 (1964).
9. Stewart, E. L. and Welt, L. G.: Protection of the fetus in experimental potassium depletion. *Amer. J. Physiol.* 200 : 824 (1961).
10. Stockigt, J. R.: Review - Potassium homeostasis. *Aust. & N.Z. J. Med.* 7 : 66 (1977).
11. Wintour, E. Marelyn, Brown, Elizabeth H., Denton, D. A., Hardy, K. J., McDougall, J. G., Oddie, Catherine J., and Whipp, G. T.: The ontogeny and regulation of corticosteroid secretion by the ovine foetal adrenal - In vitro and in vivo studies. *Acta Endocrinol. (Kbh.)* 79 : 301 (1975).
12. Wintour, E. Marelyn, Blair-West, J. R., Brown, Elizabeth, J., Coghlan, J. P., Denton, D. A., Nelson, J., Oddie, C. J., Scoggins, B. A., Whipp, G. T. and Wright, R. D.: The effect of pregnancy on mineralo- and gluco-corticoid secretion in the sheep. *Clin. Exp. Physiol.* 3 : 331 (1976).

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Figure 1. Increment in blood aldosterone concentrations, with alteration in plasma $[K^+]$ in (a) non-pregnant ewes (b) ewes during the last third of gestation and (c) ovine fetuses between 109 and 145 days of gestation.