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Blood
fetus
hyperphosphatemia

kidney
renal phosphate clearance

Renal Phosphate Clearance in Fetal Lambs

EDDIE S. MOORE,[24] ELLIS E. CHUNG, EDUARDO E. CEVALLOS, AND BARBARA J. McMANN

WITH THE TECHNICAL ASSISTANCE OF MARGARITA OCAMPO AND ELIZABETH LYONS

Department of Pediatrics, Michael Reese Hospital and Medical Center, Chicago, Illinois 60616, USA

Summary

The purpose of this study was to investigate the possible role of diminished phosphate clearance by the fetal kidney in production of relative fetal hyperphosphatemia. Stimuli known to affect renal phosphate clearance in adults were investigated in young fetal lambs. Our studies confirm that the fetal lamb kidney responds to exogenous and endogenous parathyroid hormone (PTH) with inhibition of tubular phosphate reabsorption. Renal tubular phosphate reabsorption in the fetus is in part related to sodium reabsorption. These studies indicate that so-called "immaturity" of renal phosphate clearance *in utero* is not a significant factor in production of fetal hyperphosphatemia.

Speculation

The fetal kidney responds early in gestation to stimuli that influence renal clearance of phosphate in adults.

RENAL PHOSPHATE CLEARANCE IN FETAL LAMBS

The concentration of inorganic phosphate (P_i) in the blood of infants is high and renal excretion of phosphate low when compared to adults in both human and animal models (11, 14, 20). Studies *in utero* have demonstrated that these differences are even more exaggerated in the fetus (1, 13). The role of relatively low clearance of phosphate in production of hyperphosphatemia during this period of life has not been adequately studied. Many stimuli are reported to affect renal clearance of phosphate (7). The purpose of this study was to investigate the response of the fetal kidney to certain stimuli known to influence renal phosphate clearance by the adult kidney.

MATERIALS AND METHODS

Studies were performed on pregnant ewes and their fetal lambs at 85–100 days of gestation; term pregnancy in sheep is 150 days. The preparation of the ewes for cesarean section and delivery of the intact fetus for study was similar to that previously described by our laboratory (15).

GROUP 1: ACUTE EXTRACELLULAR FLUID (ECF) EXPANSION WITHOUT ADDED CALCIUM (Ca^{++}) (n = 4)

[^{125}I]Sodium iothalamate (Abbott Laboratories, North Chicago, IL) in 0.9 N sodium chloride was infused at 0.5 ml/min in this and all subsequent groups to measure glomerular filtration rate. After equilibrium and collection of three to four 10-min control periods, Ringer's lactate was infused at 15–20 ml/min for 30 min. Urine and blood samples were collected for an additional 90–120 min after volume expansion. In order to produce a fall in serum Ca^{++} due to hemodilution, Ca^{++} was not added to the infusate.

GROUP 2: ACUTE ECF EXPANSION WITH ADDED Ca^{++} (n = 3)

After collection of control samples, Ringer's lactate with 750 mg calcium gluconate/1000 ml was infused at 15–20 ml/min for 30 min. Urine and blood samples were collected for an additional 90–120 min after stopping the infusate.

GROUP 3: EXOGENOUS BOVINE PTH INFUSION (n = 5)

Ten units of bovine PTH (Eli Lilly Co., Indianapolis, IN) in 0.5 ml 0.9 N NaCl was infused/min. The small fluid volume was used to minimize ECF expansion. The PTH was infused for 45 min and blood and urine samples were collected for an additional 120 min after the infusion was stopped.

GROUP 4: CALCIUM DISODIUM EDETATE (EDTA) (ABBOTT LABORATORIES) INFUSION (n = 5)

After appropriate control periods, EDTA in saline (20 mg/ml) was infused at 1 ml/min while simultaneously measuring plasma Ca^{++} concentration ($P_{Ca^{++}}$) at 10- to 15-min intervals. The infusion was continued until the $P_{Ca^{++}}$ fell at least 50% from the control value.

[^{125}I]Sodium iothalamate activity in blood and urine samples was measured using a Nuclear Chicago gamma counter. Total calcium in blood and urine was measured by atomic absorption spectrophotometry. Inorganic phosphate was measured by the method of Fiske and Subbarow (4). Sodium and potassium was measured using an Instrument Laboratories flame photometer. Immunoreactive PTH (iPTH) in fetal blood was assayed using methodology previously described (17) (kindly performed by Doctors L. M. Sherwood and A. S. Schneider). Statistical analysis for significance of paired data was by use of Student's *t*-test. Regression analysis was done by the method of computing least square estimates.

RESULTS

Composite data for results of studies in all four groups are shown in Table 1.

GROUP 1: ECF EXPANSION WITHOUT ADDED CALCIUM

Urine flow rate and GFR increased significantly from the mean control value after ECF expansion. Mean control $P_{Ca^{++}}$ was 11.7 mg/dl and decreased significantly to 10.5 mg/dl after ECF expansion ($P < 0.05$). As a result of hemodilution with phosphate-free fluids, mean control plasma P_i concentration decreased significantly from 6.77 mg/dl to 5.70 mg/dl. Fractional tubular reabsorption of phosphate decreased significantly with ECF expansion from the mean control of 92.2% to 81% ($P < 0.01$). After ECF expansion, phosphate excretion (E_P) increased significantly from the control mean of 7.80 μg/min to 22.70 μg/min ($P < 0.05$). Sodium excretion (E_{Na}) also increased significantly from the control mean of 22.43 to 52.00 μEq/min ($P < 0.001$). As shown in Figure 1, as a result of ECF expansion, there was a significant linear correlation between the increase in E_P and the increased E_{Na}. There was no measurable increase in fetal iPTH in blood samples up to 120 min after ECF expansion.

Table 1. *Mean control and experimental values for all studies*[1]

Mean	Values	(I) ECF expansion without Ca^{++}	(II) ECF expansion with Ca^{++}	(III) PTH infusion	(IV) EDTA infusion
UV. (ml/min)	Control	0.59 (0.09)[1]	0.96 (0.20)	0.31 (0.04)	0.70 (0.06)
	Experimental	2.00 (0.22)	2.56 (0.38)	1.49 (0.18)	1.24 (0.12)
	P	<0.001	<0.005	<0.001	<0.001
GFR (ml/min)	Control	2.40 (0.30)	2.23 (0.82)	2.06 (0.25)	2.60 (0.34)
	Experimental	4.81 (0.45)	4.36 (0.36)	2.42 (0.24)	3.15 (0.41)
	P	<0.001	<0.001	N.S.	<0.01
$P_{Ca^{++}}$ (mg/dl)	Control	11.70 (0.32)	10.50 (0.27)	10.79 (0.50)	12.80 (0.27)
	Experimental	10.50 (0.54)	10.10 (0.31)	10.16 (0.52)	5.60 (0.24)
	P	<0.05	N.S.	N.S.	<0.001
Pi (mg/dl)	Control	6.77 (0.22)	4.53 (0.30)	6.26 (0.25)	6.88 (1.43)
	Experimental	5.70 (0.14)	3.54 (0.08)	6.19 (0.19)	6.67 (0.17)
	P	<0.001	<0.001	N.S.	N.S.
TRP (%)	Control	92.20 (1.50)	92.93 (2.01)	95.90 (0.92)	92.2 (3.39)
	Experimental	81.00 (0.30)	74.72 (4.77)	79.00 (6.02)	79.4 (2.97)
	P	<0.01	<0.05	<0.001	<0.05
E_P (μg/min)	Control	7.80 (1.70)	10.17 (1.29)	2.22 (0.51)	2.88 (2.02)
	Experimental	11.70 (2.02)	13.30 (1.16)	7.78 (1.22)	18.14 (0.22)
	P	<0.05	<0.05	<0.005	<0.05
E_{Na} (μEq/min)	Control	22.43 (2.25)	22.97 (2.18)	13.95 (2.51)	9.35 (1.65)
	Experimental	52.00 (6.52)	71.65 (6.04)	23.93 (3.03)	20.31 (2.35)
	P	<0.001	<0.001	<0.02	<0.01

[1] UV: urine flow; GFR: glomerular filtration rate; TRP: fractional tubular phosphate reabsorption.
[2] ±SE.

GROUP 2: ECF EXPANSION WITH ADDED CALCIUM

Urine flow and GFR increased significantly from control values. Mean $P_{Ca^{++}}$ after ECF expansion was 10.1 mg/dl and was not significantly different from the mean control value of 10.5 mg/dl. With ECF expansion, plasma P_i concentration decreased significantly from the mean control value. Fractional tubular reabsorption of phosphate fell significantly from a mean control of 92.9% to 74.7% ($P < 0.05$). E_P increased significantly from the control of 10.17 to 13.30 μg/min as a result of ECF expansion ($P < 0.04$). After ECF expansion, E_{Na} increased significantly from the control mean of 22.97 to 71.65 μEq/min ($P < 0.001$). As was true for group 1, there was again a significant linear correlation between the increase in E_P and E_{Na}. There was no detectable increase in fetal iPTH from control values. The percentage decrease in hematocrit and total protein as a result of hemodilution was similar in groups 1 and 2.

GROUP 3: INFUSION OF EXOGENOUS PTH

Urine flow increased significantly from the control mean of 0.31 ml/min to 1.49 ml/min as a result of infusion of PTH. However, there was no significant increase in GFR, and no significant decrease in serum hematocrit or total protein. Since ECF expansion was avoided, there was no significant change in $P_{Ca^{++}}$ or plasma P_i concentration. Fractional tubular phosphate reabsorption fell significantly from the mean control of 95.9% to 79.0% ($P < 0.001$). E_P increased significantly from the control mean of 2.22 to 7.78 μg/min with PTH infusion ($P < 0.005$). There was a small but significant increase in E_{Na} from the control mean of 13.95 μEq/min ($P < 0.02$). There was no significant correlation between E_P and E_{Na}.

GROUP 4: EDTA INFUSION

Urine flow rate and GFR increased significantly as a result of hemodilution produced by infusing a large volume of fluid with EDTA to produce significant hypocalcemia. During EDTA infusion mean control $P_{Ca^{++}}$ fell significantly to a mean value of 5.60 mg/dl ($P < 0.001$). Fractional tubular phosphate reabsorption decreased significantly from the control mean of 92.2% to 79.4% ($P < 0.05$). E_{Na} rose significantly from the control mean of 9.35 μEq/min to 20.31 μEq/min ($P < 0.01$); there was no significant

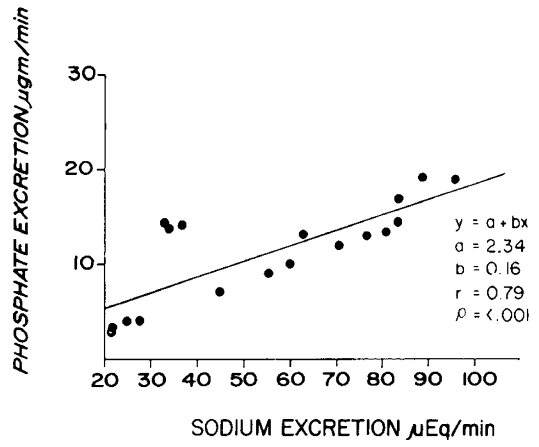

Fig. 1. Correlation between phosphate and sodium excretion after acute ECF volume expansion with Ringer's lactate without added Ca^{++}.

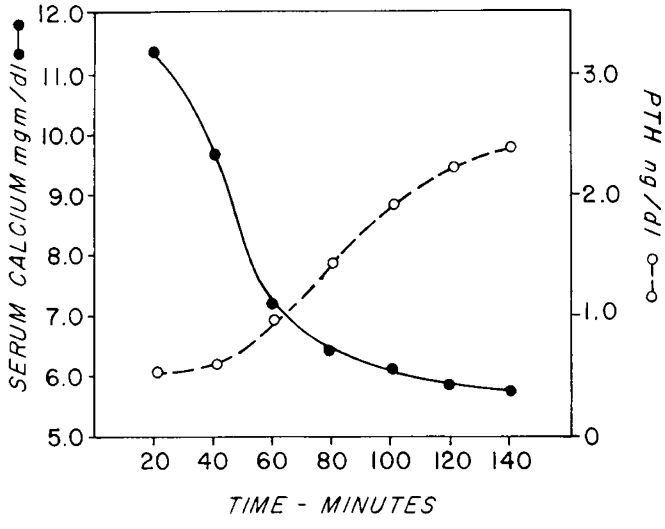

Fig. 2. The relationship of fetal hypocalcemia to fetal plasma iPTH activity.

correlation between E_P and E_{Na}. As shown in Figure 2, fetal iPTH increased to a high of 2.4 µm/ml when the serum calcium was 5.6 mg/dl.

DISCUSSION

Relatively low phosphate clearance by the fetal lamb kidney was previously reported by Moore *et al.* (15) and Smith *et al.* (19). These studies demonstrated that under basal conditions, the kidney *in utero* has low phosphate clearance which may theoretically contribute to the relative hyperphosphatemia in the fetus. In this study, we investigated whether the fetal kidney was incapable of increasing phosphate excretion as a function of developmental "immaturity."

In groups 1 and 2, acute ECF expansion resulted in significant phosphaturia and an increase in urine flow and GFR. Several studies in the dog, in the rat, and in man have demonstrated significant phosphaturia with acute ECF expansion (5, 12, 18). Phosphaturia produced by ECF expansion is thought to be PTH dependent as studies with saline infusion immediately after thyroid-parathyroidectomy in the rat and the dog produced either no phosphaturia or a minimal rise in phosphate excretion despite a significant natriuresis (8, 9). A fall in plasma calcium concentration resulting from hemodilution could be the stimulus for increased PTH production.

The increase in phosphate clearance in group 1 was associated with a significant fall in plasma total calcium concentration but not with a rise in iPTH. In group 2, significant phosphaturia occurred without a fall in plasma calcium concentration with no detectable increase in iPTH activity. Although the fall in plasma total calcium in group 1 was significant, it apparently was not of a magnitude that would elicit PTH response. An alternative explanation for our failure to demonstrate increased iPTH with the significant fall in $P_{Ca^{++}}$ in group 1 is related to the methodology for assay of iPTH used in our studies. The relative merits of the different assays for iPTH have been adequately discussed elsewhere (2); this would be a possible explanation for the results in group 1 but not in group 2 where there was no significant change in $P_{Ca^{++}}$ and no increase in iPTH. The phosphaturia with ECF expansion in our studies significantly correlated with sodium excretion. It has been demonstrated that proximal tubular reabsorption of phosphate is in part related to sodium reabsorption in that part of the nephron (10). Inhibition of proximal tubular reabsorption of sodium should lead therefore to natriuresis as we demonstrated in groups 1 and 2. Our results can be interpreted to suggest early maturation of sodium-dependent phosphate reabsorption.

Infusion of bovine PTH in our studies resulted in significant phosphaturia. This resulted from inhibition of primary tubular phosphate reabsorption since glomerular filtration rate, filtered load of phosphate, and plasma P_i concentration did not change significantly. Although there was a 70% increase in sodium excretion with PTH infusion, phosphate excretion increased by 250% and was not correlated with sodium excretion. Since the glomerular filtration rate and filtered load of sodium did not change, the increase in sodium excretion may represent suppression of PTH dependent tubular reabsorption of sodium. The far greater phosphaturia compared to sodium excretion is evidence for both proximal and distal tubular inhibition of phosphate reabsorption by PTH, and demonstrates the need to relate changes in phosphate excretion to sodium excretion. Similar studies demonstrating the response of the fetal lamb kidney to exogenous PTH were reported by Smith *et al.* (21). The studies by Smith *et al.* (21) were in near term fetuses; our studies were performed at an earlier age, and would suggest earlier maturation of fetal renal tubular responsiveness to PTH.

Considerable quantities of EDTA were required to lower plasma total calcium levels to those that elicited a PTH response and slight volume expansion could not be avoided. After EDTA produced hypocalcemia and increased iPTH, fractional tubular reabsorption of phosphate decreased and resulted in significant phosphaturia. Although sodium excretion increased as a result of ECF expansion, the phosphaturia was independent of sodium excretion suggesting inhibition of both proximal and distal tubular phosphate reabsorption by endogenous PTH. The level of iPTH with fetal hypocalcemia is similar to that demonstrated in fetal lambs by Buckle *et al.* (3).

These studies demonstrate that certain factors that influence renal phosphate clearance may be present and operative very early in intrauterine development. Our studies confirm that the fetal lamb parathyroid glands respond to fetal hypocalcemia by increased production of iPTH. The fetal lamb kidney is responsive to endogenous as well as exogenous PTH with inhibition of proximal and distal tubular phosphate reabsorption. Fetal lamb renal tubular reabsorption of phosphate is in part related to sodium reabsorption and is depressed by inhibition of proximal reabsorption of sodium. Our studies indicate that "immaturity" of renal phosphate clearance *in utero* is not a significant factor in production of fetal hyperphosphatemia.

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24. Requests for reprints should be addressed to: E. S. Moore, M.D., Department of Pediatrics, Michael Reese Medical Center, 2929 South Ellis Ave., Chicago, IL 60616 (USA).
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