

Calcitonin Secretion in Ovine, Porcine, and Bovine Fetuses (36159)

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(Introduced by R. W. Dougherty)

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Hypercalcemia induced by infusion of calcium has been reported to cause increases in immunoreactive calcitonin (ICT) in plasma of the pig (1), rabbit (2), and cow (3). The concentration of ICT present in plasma of pigs was reported (1) to be directly proportional to the concentration of calcium in plasma during sustained infusion of calcium. Because of this finding and because the hypocalcemic action of calcitonin has been well established in some species, it was suggested (1) that calcitonin is potent, rapidly acting hormone which suppresses oscillations of plasma calcium above physiologic levels.

There are no reports concerning the ability of the fetus to release ICT in response to hypercalcemia induced by infusion of calcium. This report describes increases in ICT in plasma of fetuses of several species in response to calcium infusion. It also compares the response of fetal pigs to newborn pigs following the administration of calcitonin.

Materials and Methods. Three crossbred ewes in the last 30 to 40 days of gestation, one Holstein cow in the last third of gestation, two crossbred sows, and six newborn pigs from a crossbred sow were included in this study. One sow (No. 1) was approximately 60 days and the other (No. 2) approximately 90 days into gestation.

Both ewes and fetal lambs had femoral arterial and venous catheters implanted (4) 2 to 7 days before the experimental period. The cow, the fetal calf, and the sows had femoral arterial and venous catheters implanted several hours previously.

All infusions were done through the venous catheters using a Harvard peristaltic pump except in the fetal pigs. Infusions in the fetal

pigs were done using 1-ml tuberculin syringe attached to 26-gauge needles and the solutions were infused directly into the umbilical veins. The cow and the sheep were conscious and under minimal restraint during the infusions; the sows were anesthetized (halothane¹).

Solutions of calcium borogluconate were made as calcium gluconate (23 g/100 ml), boric acid (4 g/100 ml), plus sodium chloride (0.85 g/100 ml) in distilled water. The rates of calcium borogluconate infusion into the fetuses were 0.0045 ml/kg/min in lambs and 0.0046 ml/kg/min in calves. A solution of calcium gluconate (2.3 g/100 ml), boric acid (0.4 g/100 ml), and sodium chloride (0.85 g/100 ml) in distilled water was infused into the fetal pigs (0.354 ml/kg/min). Calcitonin with a specific activity of 150 MRC units/mg produced by the procedure previously reported (5) was injected into the umbilical vein of fetal pigs or injected subcutaneously in newborn pigs.

Blood samples (heparinized syringes) were obtained from femoral arterial catheters in all cases except with fetal pigs. With fetal pigs, blood was obtained directly from the umbilical arteries. All blood except that used for blood gas and pH analysis was cooled and centrifuged immediately after collection. The plasma was separated and frozen (-15°) within 15 min after collection.

The condition of fetal lambs was monitored at the start and the end of each experiment by recording blood pressure. Blood CO₂ and O₂ were also determined at each sam-

¹ Fluothane, Ayerst Laboratories, 685 Third Avenue, New York, NY.

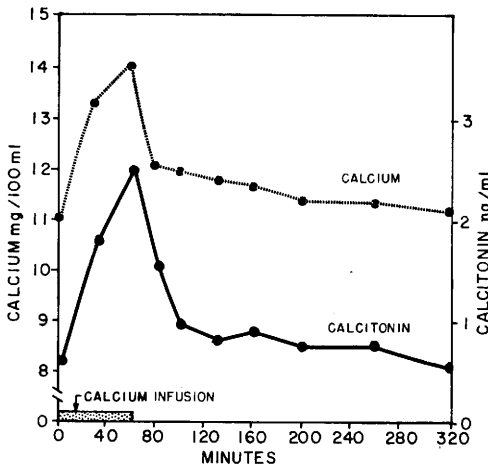


FIG. 1. Mean calcium (● - -) and calcitonin (—) concentration in plasma of three fetal sheep infused with calcium borogluconate (23%) at 0.0045 ml/kg/min.

pling interval.

Plasma ICT concentrations were determined by radioimmunoassay (6, 7). Plasma calcium and magnesium concentrations were determined by atomic absorption spectrophotometry (8). Ionized plasma calcium was determined using the Orion flow-through system² attached to a Beckman Expandomatic pH meter³ and a Photovolt Varicord recorder

(Model 43)⁴ (1–100 mV). Blood CO₂ and O₂ were determined using a Loenco⁵ Model AD-200 blood gas extractor and the gases were analyzed on a Loenco Model AD-2000 gas chromatograph. Measurement of blood pH was done on a Beckman Model 1019 research pH meter³ attached to a micro-blood pH assembly, and a thermomatic constant temperature block.

Results. The mean response of three fetal lambs infused for 60 min with calcium borogluconate is shown in Fig. 1. Plasma ICT concentration increased to 2.5 ng/ml at the end of the infusion period. The markedly increased levels of ICT returned to near pre-infusion levels within 60 min of cessation of infusion of calcium. Several hours were required for plasma calcium concentration to return to preinfusion levels. This prolonged hypercalcemia was reflected in sustained moderate increased concentrations of ICT in the plasma.

Fetal blood pH in the lambs included in the experiments reported were between 7.2 and 7.3 and the vol % of blood CO₂ was between 68 and 72 and the blood O₂ was between 2.0 and 4.5 vol %. Little change was noted in blood pressure between the initial and final sampling intervals.

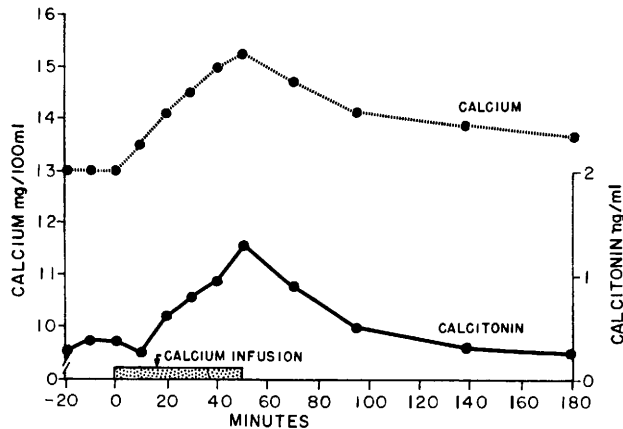


FIG. 2. Calcium (● - -) and calcitonin (● —) concentration in plasma of a fetal calf infused with calcium borogluconate (23%) at 0.0045 ml/kg/min.

² Orion Research Company, Inc., 11 Blackstone Street, Cambridge, MA.

³ Beckman Instruments, Inc., 2500 Harbor Boulevard, Fullerton, CA.

⁴ Photovolt Corporation, 1115 Broadway, New York, NY.

⁵ Info-tronic Instrument Corporation, 1062 Linda Vista, Mountainview, CA.

TABLE I. Concentration of Calcium, Calcium Ion Activity, Calcitonin, and Magnesium in the Plasma of Fetal Pigs of Sow No. 1 Given Either Calcium, Saline, or Calcitonin.

Fetus	Material injected	Time of sample (min)	Plasma calcium (mg/100 ml)	Plasma calcium ion activity (mg/100 ml)	Plasma calcitonin (ng/ml)	Plasma magnesium (mg/ml)
A	Calcium ^a	0	11.3	5.0	0.1	1.99
		5	23.7	9.2	4.5	1.94
		15	17.4	7.4	7.0	2.08
B	Calcium	0	11.7	5.6	0.4	2.13
		5	22.1	9.0	5.5	2.20
		15	15.8	7.8	6.8	2.23
C	Calcium	0	11.5	5.4	0.3	1.95
		5	32.0	10.1	2.6	1.98
		15	16.4	7.5	2.3	2.05
D	0.85% Saline	0	12.1	5.6	0.1	2.01
		5	12.2	5.6	0.1	2.10
		15	12.2	5.6	0.2	2.05
E	0.85% Saline	0	11.8	5.4	0.2	1.95
		5	11.8	5.4	0.3	2.05
		15	11.9	5.4	0.2	2.10
F	Calcitonin ^b (0.1 mg/ml)	0	12.1	5.8	0.2	2.13
		30	12.6	5.9	15.0 <	2.25
G	Calcitonin (0.1 mg/ml)	0	12.1	5.6	0.1	2.28
		30	12.4	5.7	15.0 <	2.48
		65	13.1	6.1	10.2	2.15

^a Given as 0.1 ml/min of 2.3% calcium borogluconate in 0.85% saline infused into the umbilical vein.

^b Given in 1.0 ml of 0.01 M acetic acid with 10 mg of albumin-human, grade III (Sigma Chemical Co., St. Louis, MO) infused into the umbilical vein over a 5-min period.

The response of a fetal calf to a 60-min infusion of calcium borogluconate is shown in Fig. 2. The ICT increased throughout the infusion period to 1.3 ng/ml and returned to near preinfusion levels approximately 60 min after cessation of infusion. A prolonged period of hypercalcemia followed infusion of calcium. Little, if any, increase in ICT was detected after 120 min in spite of an approximately 1 mg/100 ml increase in plasma calcium concentration.

The response of the fetal pigs (av wt 283 g) in sow No. 1 is shown in Table I. Infusion of calcium solution for 5 min increased plasma calcium to peak concentrations from 23.7 to 32.0 mg/100 ml, calcium ion activity to 9.0–10.0 mg/100 ml, and ICT to 2.6–5.5 ng/ml. Ten minutes after calcium infusion was stopped, plasma calcium decreased (15.8–17.4 mg/100 ml) as did the calcium ion activity (7.4–7.8 mg/100 ml) but calcitonin in two of the pigs increased

further (6.8–7.0 ng/ml). A slight increase in plasma magnesium occurred following calcium infusion. Infusion of saline (0.85%) as a control elicited no marked change other than a slight increase in plasma magnesium.

When the same amount of calcium and saline solutions were infused into fetal pigs of sow No. 2 (Table II), which were much larger (av wt, 450 g) than those of sow No. 1, a smaller more uniform rise in plasma calcium was present at the end of the infusion period (15.6–15.9 mg/100 ml). Calcium ion activity was increased to 6.8 mg/100 ml and plasma calcitonin levels increased to 2.3–2.5 ng/ml. Plasma magnesium levels were variable. Plasma calcium, plasma calcium ion activity, and ICT remained high 25 to 95 min after the infusion stopped. Little or no change in calcium, magnesium, or calcitonin concentrations were detected in plasma of pigs infused with saline.

The response of the fetal pigs in sow No. 1

TABLE II. Concentration of Calcium, Calcium Ion Activity, Calcitonin, and Magnesium in the Plasma of Fetal Pigs of Sow No. 2 After a Calcium or Saline Infusion.

Fetus	Material injected	Time of sample (min)	Plasma calcium (mg/100 ml)	Plasma calcium ion activity (mg/100 ml)	Plasma calcitonin (ng/ml)	Plasma magnesium (mg/ml)
H	Calcium	0	11.7	5.5	0.1	1.64
		5	15.6	6.8	2.5	1.63
		100	14.2	6.0	1.8	1.90
I	Calcium	0	11.5	5.4	0.3	1.88
		7	13.5	5.9	2.4	1.77
J	Calcium	0	11.8	5.4	0.1	1.77
		5	15.9	6.8	2.5	1.83
		50	14.0	6.0	1.7	1.93
K	Calcium	0	11.6	5.4	0.1	1.68
		5	15.8	6.8	2.3	1.69
		30	14.0	5.9	1.7	1.93
M	0.85% Saline	0	11.8	5.5	0.1	1.77
		5	11.7	5.5	0.1	1.83
		50	11.9	5.6	0.2	1.90
N	0.85% Saline	0	11.7	5.5	0.2	1.85
		5	11.8	5.6	0.2	1.87
		100	11.8	5.6	0.2	1.90

^a Given as 0.1 ml/min of 2.3% calcium borogluconate in .85% saline infused into the umbilical vein.

following infusion of calcitonin (0.1 mg/min over a 5-min period), are shown in Table I. Plasma calcium tended to rise as did calcium ion activity. High levels of calcitonin were detected in plasma up to 60 min following the infusion. Plasma magnesium concentrations were variable.

The response of newborn pigs (2 hr old) to calcitonin injections is shown in Table III. A marked decrease (1.5–2.1 mg/100 ml) in plasma calcium occurred following subcutaneous injection of calcitonin (0.1 mg). There was a tendency for plasma magnesium to decrease also following injection of calcitonin. Little or no decrease in plasma calcium or magnesium occurred in the pig 15 min after the control solution was injected.

Little change in calcium, calcium ion activity, magnesium, or calcitonin was observed in the uninfused dams. Throughout the infusions, calcium concentrations in all adult sheep were 9.0–9.8 mg/100 ml; calcium ion activity was 3.5–3.8 mg/100 ml; magnesium concentrations were 1.7–1.9 mg/100 ml; and ICT concentrations were 0.3–0.8 ng/100 ml. Plasma calcium concentration in the cow was

8.0–8.5 mg/100 ml; calcium ion activity was 3.1–3.4 mg/100 ml; magnesium, 1.9–2.2 mg/ml, and ICT concentrations, 0.6–0.9 ng/100 ml. Plasma calcium concentration in the sows was 9.6–9.8 mg/100 ml; calcium ion activity, 4.2–4.5 mg/100 ml; ICT concentrations were 0.1–0.3 ng/ml, and magnesium concentrations were 1.85–1.95 mg/100 ml.

Discussion. The fetal lamb, calf, and pig all appear to be able to increase ICT in response to hypercalcemia induced by infusion of calcium borogluconate. Thus it appears that calcitonin is available to fetuses of sheep, cattle, and pigs to aid in the regulation of their blood calcium concentration if the immunologically reactive material being detected is biologically active calcitonin. However, when calcitonin was infused *in utero* to fetal pigs, no hypocalcemia was observed. In fact, an increase in plasma calcium concentration was observed after calcitonin administration. This was directly opposite to the results in newborn pigs where calcitonin caused a marked hypocalcemia in all pigs in which it was injected. The reason for this difference between neonatal and fetal pigs is un-

TABLE III. Concentration of Calcium and Magnesium in the Plasma of Newborn Pigs Which Received Calcitonin (CT).

Pig	Material injected	Time of sample (min)	Plasma calcium (mg/100 ml)	Plasma calcium change (mg/100 ml)	Plasma magnesium (mg/100 ml)	Plasma magnesium change (mg/100 ml)
P	CT, ^a 0.1 mg	0	10.8	—	1.71	—
		65	9.6	-1.2	1.45	-0.26
		120	9.3	-1.5	1.45	-0.26
Q	CT, 0.1 mg	0	11.1	—	1.63	—
		65	9.3	-1.8	1.70	+0.07
		120	9.0	-2.1	1.35	-0.28
R	CT, 0.1 mg	0	10.8	—	1.75	—
		65	9.5	-1.3	1.56	-0.19
		120	9.1	-1.7	1.50	-0.25
S	Control	0	10.4	—	1.38	—
		65	10.3	-0.1	1.43	+0.05
		120	10.4	0.0	1.41	+0.03
T	Control	0	11.6	—	1.68	—
		65	11.3	-0.3	1.99	+0.31
		120	11.2	0.4	1.68	0.00
U	Control	0	10.0	—	1.59	—
		65	9.7	-0.3	1.35	-0.24
		120	9.8	-0.2	1.49	-0.10

^a Each pig received 0.1 mg of calcitonin dissolved in 1.0 ml of 0.01 M acetic acid with 10 mg of albumin-human, grade III (Sigma Chemical Company, St. Louis, MO).

known. Similar results were found in preliminary studies of lamb and calf fetuses which received calcitonin infusions. It has been shown that the serum calcium and phosphate of fetal rats *in utero* near term decrease very little, if at all, following porcine CT administration compared to the newborn or cesarean delivered rats (12-14). Since numerous reports have been published (9-11) which show that fetal bone is responsive to calcitonin *in vitro*, it may be that placental influences prevent hypocalcemia following calcitonin administration in the fetus.

The fetal lamb appeared to be the animal of choice for this type of experiment. It was possible to maintain a large percentage of the fetal lambs in apparent good condition *in utero* for several days following the catheterization procedures judged by blood pressure, pH, blood pCO₂, PO₂ levels. In preliminary studies, fetal calves tended to be aborted within 1 to 2 days of the catheterization procedure. No progesterone was used in any animals to maintain pregnancy. Instead the

calf was infused within a few hours of the time the cow had been anesthetized. Fetal pigs were too small to make catheterization practical for the purpose of these experiments.

No change in maternal calcium or calcitonin was observed during infusion of either calcium or calcitonin into their fetuses. Apparently little, if any, calcium or calcitonin is returned to the maternal system or the relative differences between the maternal volumes and fetal volume prevented a detectable difference from occurring. Other workers (12-15) have shown that labeled or unlabeled CT does not cross the placenta. In preliminary experiments, we have demonstrated that large changes (up to 40 mg/100 ml of calcium) in calcium concentrations in the plasma of pregnant sheep produced no detectable difference in calcium in the fetus, and no detectable change in fetal ICT occurs at this time. Thus, the placental membranes in sheep appear to prevent acute changes in calcium or calcitonin in the maternal or fetal

system from being reflected in the other.

Summary. Intravenous infusion of calcium into the fetus increased the concentration of ICT in plasma of sheep, cow, and pig fetuses. In preliminary studies, calcitonin was not effective in lowering the plasma calcium of the fetus, but it was effective in the newborn pig.

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