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Hypocalcemia and Increased Serum Calcitonin in Baby Rats Given Glucose Orally[1,2] (40056)

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Recently, we reported that 14-day-old suckling rat pups exhibit high levels of circulating immunoreactive calcitonin (CT) (1). In normocalcemic pups suckling *ad lib.*, serum CT levels as high as 4000–5000 pg/ml were observed. If the babies were fasted 12 hr or longer, serum CT fell to <150 pg/ml. When babies fasted for 12 hr were allowed to suckle again, both serum calcium and serum CT rose at 30 and 60 min; by 90 min serum calcium had returned to normal but serum CT remained high (1).

Because serum CT was high in normocalcemic suckling baby rats, we hypothesized that, in addition to a rise in blood calcium, some gastrointestinal factor might be involved in stimulating the baby rat to secrete CT during ingestion and absorption of milk (1). As a first approach to testing this hypothesis, we decided to orally administer a calcium-free solution of glucose to rat pups and see whether or not increased CT release occurred. Under these conditions, any increase in CT could not be secondary to a rise in serum calcium during absorption of calcium from the gut.

Materials and methods. Animals. Female Zivic-Miller rats (Zivic-Miller Co., Allison Park, PA), with litters of eight to ten pups each were received in the laboratory when the babies were 6 days old. Experiments were performed when the babies were 15 days old.

Procedures and analyses. All babies were separated from the mother 13–16 hr (overnight) before each experiment. Half of the babies in each litter were assigned to different treatment groups. Babies given glucose were administered 1.5 ml isotonic (5% w/v) glucose in distilled water. Control babies received either no solution or 1.5 ml isotonic (0.9% w/v) NaCl. Solutions were delivered directly into the stomach by gavage using a marked length of Intramedic polyethylene tubing (PE50, inner diameter 0.023 in.) attached to a syringe. Babies were lightly anesthetized with ether during the gavage procedure.

Thyroparathyroidectomy was performed by blunt dissection under ether anesthesia. Sham operation involved simply exposure of the thyroparathyroid complex.

At the desired time interval after treatment, blood was obtained from each baby under ether anesthesia. In selected experiments the tip of the tail was severed and blood was collected in a heparinized microhematocrit tube. After centrifugation of the tubes for 2 min in a Model MB microhematocrit centrifuge (International Equipment Co., Needham Heights, MA), hematocrit values were read using a Model CR microhematocrit tube reader (International Equipment Co.).

Blood also was obtained from each baby by cardiac puncture using a 27 gauge, ½ in. needle. Serum was collected by centrifugation within 1 hr of blood collection, and each sample was subdivided, one portion being analyzed freshly for serum calcium and inorganic phosphorus and the other being stored at −20° until subjected to radioimmunoassay. Both calcium (2) and inorganic phosphorus (3) were analyzed by automated procedures using an Autoanalyzer (Technicon, Inc.).

CT in serum samples was measured by radioimmunoassay using an homologous system for rat CT. The procedure has been described earlier in detail and both intra- and interassay variations have been reported (4–6).

Glucose in serum was analyzed using a colorimetric procedure (7).

Statistical analyses. For serum calcium, phosphorus, glucose and hematocrit, data

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from individual experiments were subjected to analysis of variance (8), and standard errors were calculated from the residual error term of the appropriate analysis. Significance of differences between groups was determined using either the F test or the multiple comparisons test of Hartley (8).

In one experiment where all values for CT were detectable (Fig. 3), data were subjected to analysis of variance and comparisons made as just described. In other experiments where some values for CT were below the limits of detectability of the immunoassay (Fig. 2) mean values could not be calculated. Therefore, individual values are shown and the significance of differences between groups was evaluated using the nonparametric test of Wilcoxon (8).

In Table I and Figs. 1 and 2, results of identical experiments conducted on separate days were combined because the variances were homogeneous and statistical analyses showed that the results were not significantly different.

Results. Figure 1 and Table I (Groups A and B) summarize the combined results of six separate experiments which showed that 1.5 ml of 5% glucose given orally by gavage to thyroid-intact baby rats produced a significant fall in serum calcium 60 min later. The fall in serum calcium was accompanied by a fall in serum phosphorus as well (Fig. 1). No decrease in either serum calcium or serum phosphorus occurred when the same volume

TABLE I. EFFECT OF ORAL GLUCOSE ON SERUM CALCIUM IN ACUTELY THYROIDECTOMIZED AND SHAM OPERATED BABY RATS.

Treatment	No.	Serum Ca (mg/dl)	P^b
A. SHAM + NaCl p.o.	15	10.2 ± 0.12	<0.001
B. SHAM + Glucose p.o.	14	9.5 ± 0.13	
C. TPTX + NaCl p.o.	14	9.8 ± 0.13	N.S. (>0.1)
D. TPTX + Glucose p.o.	13	9.3 ± 0.14	

[a] Baby rats, 15-days old, were fasted overnight before surgery (see Methods). Immediately after surgery (sham op. or thyroidectomy by thyroparathyroidectomy), babies were given 1.5 ml of 0.9% NaCl or 5% Glucose by gavage. Blood was collected 1 hr after gavage.

[b] Values are presented as mean ±SE and represent the combined results from three separate experiments.

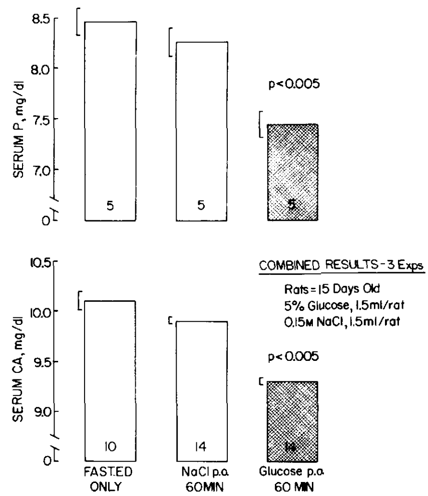

FIG. 1. Hypocalcemia and hypophosphatemia in baby rats given glucose orally. Babies were fasted overnight before the experiment and either simply bled (Fasted Only) or given NaCl or glucose as indicated and bled 1 hr later. Each bar represents the mean value for the number of babies shown within the bar, and the brackets show the SE.

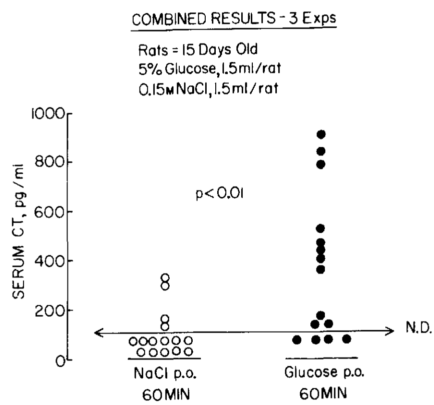

FIG. 2. Increased serum CT in baby rats given glucose orally. Babies were fasted overnight before the experiment, given NaCl or glucose as indicated and bled 1 hr later. Each point represents an individual baby, and the horizontal arrow shows the lower limit of detectability of the radioimmunoassay for rat CT (N.D. = Not Detectable).

of isotonic saline was administered (Fig. 1, saline vs. fasted only).

The fall in serum calcium which occurred in baby rats 60 min after oral glucose was accompanied by a significant increase in immunoreactive serum CT (Fig. 2).

The results suggested that the fall in serum calcium after glucose might, at least in part, be due to the increase observed in circulating CT. To test this idea glucose was administered to baby rats with intact thyroid glands and to babies deprived of endogenous CT by acute thyroidectomy (Table I). The results showed that glucose produced a significant fall in thyroid intact pups but not in the babies subjected to thyroidectomy. A small decrease in the mean value for serum Ca (C vs D) was apparent in the thyroidectomized pups given glucose in each of three experiments but it did not reach statistical significance in any of them ($P > 0.1$). Likewise, a small but insignificant fall in the mean serum calcium was observed after acute thyroid removal by thyroparathyroidectomy (A vs C) which probably can be attributed to the loss of the parathyroid glands for 1 hr.

In order to examine the time course of the changes in serum calcium and CT after glucose, the experiment illustrated in Fig. 3 was conducted. The results showed that serum CT was significantly elevated and serum calcium was significantly decreased only at 60 min after glucose. Values had not changed at 30 min after glucose and had returned to control levels by 90 min. In contrast, the serum glucose was highest at the earliest time period examined (30 min) and had declined toward the control level by 60 and 90 min. As observed in this experiment (Fig. 3), as well as in several others not shown, no change in plasma hematocrit was observed after administration of glucose.

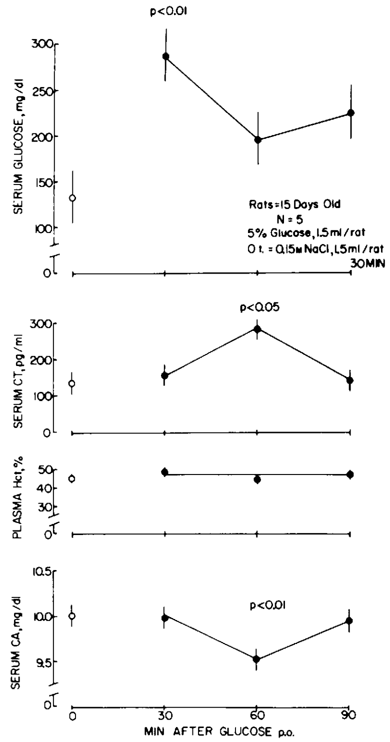

FIG. 3. Time course of increase in serum CT and decrease in serum calcium in baby rats given glucose orally. Babies were fasted overnight, given glucose as indicated and bled 30, 60 or 90 min later. Values for 0 time represent fasted babies given NaCl orally and bled 30 min later. Each point represents the mean value for a separate group of five pups, and the vertical line shows the SE.

Discussion. These experiments were designed to determine whether or not a calcium-free solution of glucose given orally could increase release of CT in the baby rat. The results clearly show that serum CT was elevated 60 min after a gavage with isotonic glucose in modest amounts (Fig. 2). The increase in CT was accompanied by a fall in both serum Ca (Figs. 1 and 3 and Table I) and serum phosphorus (Fig. 1). The changes induced by glucose were not nonspecific, e.g., simply due to gastrointestinal distension, because control gavages with saline were without effect (Fig. 1).

The glucose-induced decrease in serum calcium probably was, at least in part, due to the increase in serum CT. This conclusion is based on the observation that glucose produced a significant decrease in serum calcium only in the presence of the thyroid gland and endogenous CT (Table I).

The decrease in serum phosphorus after glucose also may partly be produced by CT which is well known to cause hypophosphatemia as well as hypocalcemia. However, it has long been known that hypophosphatemia results from glucose administration and this has been attributed to phosphorylation and cellular uptake of glucose (9). Hypophosphatemia also occurs together with hypoglycemia when young adult rats are injected with insulin (10). We have routinely observed significant ($P < 0.01$) hypophosphatemia 30 min after injection of 5 units of insulin iv in 35-day-old rats. This high dose of insulin, which lowers serum glucose from ~120 mg/dl to ~30 mg/dl ($P < 0.001$) does not lower serum calcium. Since glucose does lower serum calcium in these young adult rats ($P < 0.05$) just as it does in the babies but insulin does not, it seems unlikely that insulin mediates the increase in CT and decrease in serum calcium observed after oral glucose. Also, the present results show that the changes in CT and calcium in blood are not secondary to changes in plasma volume, since hematocrit was unaffected by oral glucose.

The mechanisms by which oral glucose elevates blood CT and lowers serum calcium remain to be elucidated. However, our findings do agree with the observations reported by Heynen and Franchimont (11) that, in man, CT increased after an oral glucose tolerance test at a time when the concentration of blood sugar was falling.

We think that some as yet unidentified gastrointestinal factor or hormone may well be involved in the CT response of baby rats to suckling and to oral glucose. Although gastrin and its structural analogs, e.g. CCK, are potent CT secretagogues in the pig (12–14) and man (15), they are weak or ineffective in causing release of rat CT (5, 16). Further studies will be required to pursue and identify the factor or events involved in CT release during feeding and absorption of milk constituents in the baby rat. However, our present results show clearly that even simple sugar components of ingested milk may constitute part of the stimulus for CT secretion.

Summary. Baby rats, 15 days old, were fasted >13 hr and given 1.5 ml 5% glucose by stomach tube. Control babies were gavaged with 1.5 ml 0.9% NaCl which itself did not affect serum calcium or CT.

Gavage with glucose produced a fall in serum calcium of 0.5–1.0 mg/dl 60 min later ($P < 0.05$–<0.005). Glucose produced a fall in serum phosphorus at 60 min similar to that in serum calcium. Measurement of immunoreactive CT in serum showed a significant rise from ~120 pg/ml or less up to as much as 900 pg/ml ($P < 0.01$). A time course study showed that serum CT increased and serum calcium decreased only at 60 min after oral glucose; values had not changed at 30 min and had returned to control levels by 90 min. In contrast, serum glucose was high (~300 mg/dl) at 30 min after gavage and had returned toward the control level (~130 mg/dl) by 60–90 min. The decrease in Ca after glucose must, at least in part, be due to CT since after thyroidectomy glucose did not produce a significant fall in serum calcium.

The findings show that oral administration of a calcium-free solution can increase CT release in the absence of a rise in blood calcium.

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