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The Influence of Gastrin on Plasma Calcium, Bile and Gastric Calcium Secretions in the Rat (40135)

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Whether gastrin plays a role in normal calcium homeostasis remains a controversial question. This gastrointestinal hormone has been implicated as a calcitonin secretagogue from which the hypocalcemic effect subsequently resulted in the pig (1, 2), while other investigators, using the rat, indicated that the hypocalcemic effect of gastrin was direct and independent of calcitonin release (3, 4). However, it is an attractive possibility that there could exist a calcium regulatory system involving gastrin acting as a rapid antihypercalcemic agent during the transitory calcium increment due to rapid calcium absorption. It is also known that circulating levels of gastrin increase during hypercalcemia in both man (5) and cat (6).

In the work reported here, evidence is presented to suggest that gastrin does indeed cause a hypocalcemic effect independent of calcitonin, and the preliminary search indicates that the effect is due, at least in part, to calcium excretion into the gastrointestinal lumen.

Methods. Measurement of plasma calcium, urinary calcium excretion, bile and gastric calcium secretions. Adult female (Fisher) rats weighing between 200–220 g and maintained on commercial rat pellets, were fasted overnight. After the rat was anesthetized with Nembutal sodium, the femoral artery and vein were cannulated, respectively, for blood samples (0.2 ml) collection and for infusion to maintain body fluid. Urine was collected via a urethra cannula and the body temperature was maintained with a rectal telethermometer probe and temperature regulator (YSI Telethermometer Controller). Porcine gastrin (Sigma Chem. Co.) at a dose of 50 μg/100 g rat in 0.5 ml or its vehicle (0.9% saline in the case of sham control) was administered intraperitoneally into both intact rats and rats which had been thyroparathyroidectomized (TPTX) by blunt dissection 2 hr previously. The dose used here was pharmacological and designed to achieve maximum gastric secretion. Intraperitoneal administration was chosen in an attempt to prolong absorption and action of gastrin. Thereafter, total calcium concentration in plasma (Ca^{2+}) and the rate of urine calcium excretion were measured at various time intervals for 4–5 hr. When the bile flow and the rate of calcium excretion in the bile were to be measured, the bile duct was cannulated and the flow of bile from the liver was collected. Gastric secretion was collected according to the methods of Shay (7) by which both the pylorus and esophagus were ligated to form a gastric pouch for a period of 60 min and the content was measured for its volume, pH and calcium concentration. The total calcium contents in plasma, urine, bile or gastric juices were assayed in duplicate according to the method of Pybus (8) and read with an Atomic Absorption Spectrophotometer (Beckman Co.) at 423 nm. The data were statistically computed as mean ± SE and compared by Student's paired *t* test using the Wang advanced programming calculator (700 series).

Chemicals. Porcine gastrin and Secretin (A grade) were obtained from Sigma Chem. Co. and Calbiochem., respectively.

Results. The hypocalcemic effect of gastrin independent of calcitonin. The sham operated animals maintained a normal plasma calcium concentration (9.9 ± 0.2 mg%), urinary calcium excretion (19.2 ± 7.1 μg/hr/100 g rat), and blood pressure (except for a transient, slight reduction during blood withdrawal) at stable levels throughout the experimental periods. Because of the relatively short period of experimentation (4–5 hr), a circadian fluctuation in plasma calcium as noted by Perault-Staub (9) was not observed. When gastrin was administered intraperitoneally to the intact rat, there was a fall in plasma calcium from 9.1 ± 0.2 to 7.9 ± 0.4 mg% ($P < 0.01$) within an hour and it was maintained at this

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subnormal level for hours, while there was no significant change in urinary calcium excretion (Fig. 1). In comparison, with thyroparathyroidectomy (TPTX), in which sources of both parathyroid hormone and calcitonin were removed, there was also a fall in plasma calcium concentration from 10.3 ± 0.4 to 8.1 ± 0.3 mg% ($P < 0.5$) which remained steady at this subnormal level. However, in this case urinary calcium excretion progressively increased with time (Fig. 2) in agreement with previous findings, (10). When gastrin was administered to the rat 2 hr after TPTX (Fig. 3), the subnormal plasma calcium level was further reduced from 8.2 ± 0.2 to 7.7 ± 0.2 mg% ($P < 0.05$) within 30 min and to 7.3 ± 0.3 mg% ($P < 0.5$) within 60 min but no marked increase in urinary calcium occurred. The results above thus suggested that the effect of gastrin on plasma calcium and urine calcium excretion was different from the effect produced by TPTX.

Calcium in bile secretion. The bile calcium excretion probably had no relationship with the hypocalcemic effect of gastrin. Both bile flow (from 0.4 ± 0.03 in the control to 0.4 ± 0.08 ml/hr/100 g rat 1 hr after gastrin was administered, $P > 0.05$), and total amount of calcium (from 26.5 ± 3.0 in the control to 26.1 ± 4.6 μg/hr/100 g rat after gastrin, $P > 0.5$) in the bile did not increase.

Calcium in gastric secretion. Figure 4 compares the values for gastric calcium excretion in controls (14 ± 1.4 μg/hr/100 g rat), after TPTX, gastrin administration, and gastrin with concurrent injection of a potent gastric

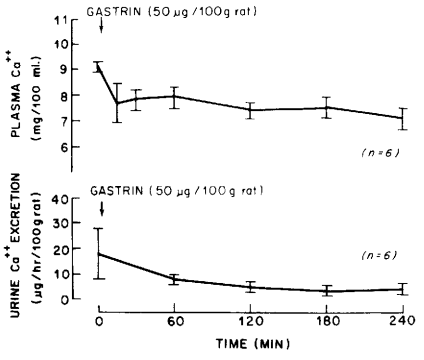

FIG. 1. The effect of gastrin administration (50 μg/100 g rat) intraperitoneally, on plasma calcium concentrations and the rate of urine calcium excretion in the intact rats. The values at each point were means ± SE.

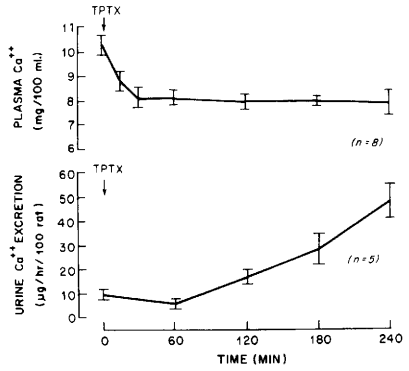

FIG. 2. The effect of thyroparathyroidectomy (TPTX) on plasma calcium concentrations and the rate of urine calcium excretion. Each point represents the mean ± SE.

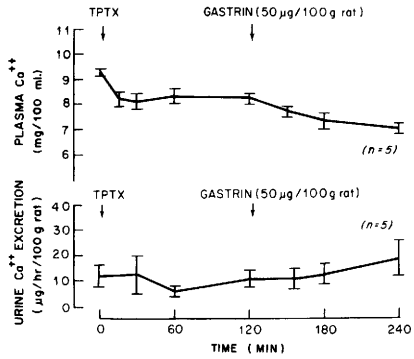

FIG. 3. The effect of thyroparathyroidectomy (TPTX) and the subsequent gastrin administration (50 μg/100 g rat) at the time indicated by an arrow (2 hr later), on plasma calcium concentrations and the rate of urine calcium excretion.

secretory inhibitor, secretin (2 secretin units). TPTX alone did not significantly alter the rate of calcium excretion in the gastric juice (17 ± 3.1 μg/hr/100 g rat, $P > 0.05$), whereas with gastrin the calcium content in gastric secretions increased significantly (22 ± 2.4 μg/hr/100 g rat, $P < 0.01$). In addition, when secretin was given with gastrin, calcium excretion (14 ± 2.6 μg/hr/100 g rat, $P > 0.05$) was similar to the calcium excretion of the control. It was noted that the increase in calcium content by gastrin was due mainly to an augmented gastric calcium concentration (from 5.4 ± 0.7 to 7.5 ± 1.1mg%, $P < 0.05$). In addition, it could be seen from the Fig. 5 that the hypocalcemic effect of gastrin was almost abolished by secretin except for a slight, but significant fall in plasma calcium

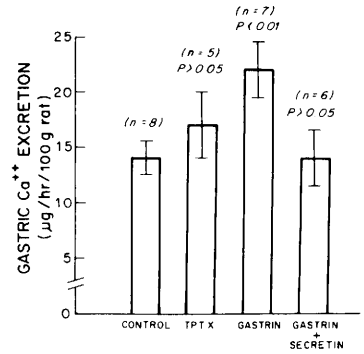

FIG. 4. A composite figure showing the effects of TPTX, gastrin (50 μg/100 g rat) and gastrin plus a gastric inhibitor, secretin (2 units), on the rates of gastric calcium excretion (μg/hr/100 g rat ± SE) in the gastric pouches of Shay's rats. *P*-values are the statistical comparison (unpaired t-test) with the control.

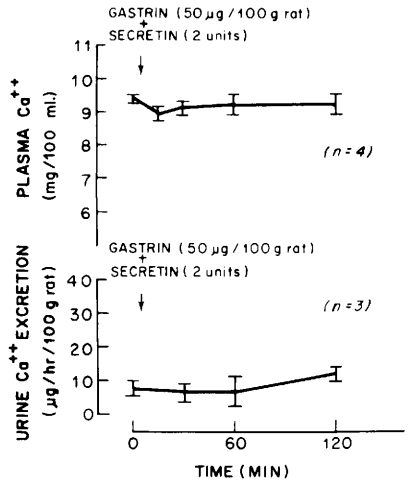

FIG. 5. The effect of gastrin (50 μg/100 g rat) and a gastric inhibitor, secretin (2 units/rat) on plasma calcium concentrations and the rate of urine calcium excretion in the intact rats.

(from 9.4 ± 0.1 to 8.9 ± 0.2 mg%, $P < 0.05$), which occurred 15 min after the administration of the two agents.

Discussion. The hypocalcemic effect of TPTX as shown in Fig. 2 was similar to the classical observation and the effect was due to the lack of parathyroid hormone. However, when gastrin was administered to the intact rats (Fig. 1), a hypocalcemic effect was also observed. This gastrin-induced hypocalcemia could possibly be due to the release of calcitonin, whose action led to the lowering of plasma calcium as proposed by Cooper *et al.* (1) and Care *et al.* (2). Cooper and his group (1) showed that gastrin and its structurally related compounds like pentagastrin and cholecystokinin acted as calcitonin secretagogues in pig (11). Furthermore, Cooper's results in the rat (12) also showed that, with multiple intravenous injections of pentagastrin, calcitonin apparently could only be transiently released once, and both calcitonin and the hypocalcemic effect returned to the normal level within 60 min, even with subsequent injections of pentagastrin. The present result (Fig. 1) demonstrates a more prolonged hypocalcemic response and this could perhaps be explained by the possibility that gastrin may act through a mechanism quite independent of the stimulation of calcitonin. In fact, a more recent *in vitro* study involving actual measurement of calcitonin directly showed gastrin to be ineffective as a calcitonin secretagogue in the rat (13). In addition, the present results on urinary calcium excretion are in contrast to the effect of calcitonin whose actions include an increase in urinary calcium (14).

When gastrin was administered 2 hr after TPTX, the result confirms the hypothesis that the hypocalcemic effect was due to a direct action of gastrin since the source of calcitonin had been abolished by TPTX and its half life in the plasma has been reported to be only 5–10 min (15). However, in contrast to the results of Schulak *et al.* (3, 4), which showed only a transient reduction in plasma calcium 30 min after gastrin and the plasma calcium returned to the post TPTX level at 60 min, our result indicated a prolonged effect. This could be due to the variations in experimental design e.g., Schulak *et al.* (3, 4) used younger rats, chronic TPTX (18–20 hr) and iv gastrin administration in which the half life of gastrin in the circulation would be approximately 5–10 min (16). In our study, a slower release of gastrin from the peritoneum could also contribute to the prolonged effect observed. Our results do not explain why the calcium in urine decreased after gastrin. However, it is possible that the fall in plasma calcium might result in a reduced filtered load of calcium through the kidney.

The possible cause of gastrin-induced hypocalcemia. Since, in contrast to the TPTX rats, urinary calcium excretion in gastrin injected animals (17.8 ± 10.1 μg/hr/100 g rat) was

about the same as in the control intact groups (19.2 ± 7.1 μg Ca^{2+}/hr/100 g rat, $P > 0.05$), gastrin induced hypocalcemia could not be explained by an increase in renal excretion of calcium. This result was thus in accord with the finding of Schulak and Kaplan (4) that the fall in plasma calcium due to gastrin was not modified by nephrectomy. It is possible that calcium sequestration in bone or other tissues could well be responsible for the changes observed; however, the present preliminary search was designed to examine actions of gastrin on the gastrointestinal tract. Schulak and Kaplan (4) demonstrated that a fall in serum calcium after gastrin was not affected by small and large bowel resection but was abolished totally by gastrectomy. Bile and gastric secretory juices were known to contain considerable amounts of calcium and both are stimulated by gastrin. The result from the gastric secretion of Shay rats, as shown in Fig. 4, indicated that in TPTX rats gastric calcium excretion did not differ significantly from control ($P > 0.05$) and this was in agreement with the findings of Wilson *et al.* (17), who found that gastric acid output and the gastrin level were not affected by TPTX. On the other hand, gastrin administration led to an increase in gastric volume, a drop in pH, a significant increase in calcium concentrations as well as the total calcium excretion. Interestingly, in the most recent report of Kaplan *et al.* (18) it was demonstrated that histamine, another gastric stimulant, could induce hypocalcemia. Thus, the present results and those of Kaplan *et al.* (18) would suggest that the calcium loss in the gastric secretions may, in part, be responsible for the hypocalcemic response to gastrin. In addition, this confirmed the fact that gastrin-induced hypocalcemia was not via calcitonin, since calcitonin has been reported to reduce gastric secretion (6, 18). The increase in total calcium in the gastric juice may be the result of the direct action of gastrin on calcium transport mechanisms of the gastric mucosa *per se* or the purely physical properties of an increase in calcium solubility in a more acidic medium.

Since the average hypocalcemic effect of gastrin in the intact rat reduced plasma calcium from 9.1 ± 0.2 to 7.9 ± 0.4 mg% (Fig. 4) or a reduction of about 1.2 mg% within 60 min and, taking the plasma volume as 5% of body weight, this would correspond to approximately 60 μg/hr/100 g rat. However, the increase in calcium excretion via gastric secretion when gastrin was administered, was from 14 ± 1.4 to 22 ± 2.4 μg/hr/100 g rat. Therefore, gastric calcium excretion alone could not totally account for the decrease in plasma calcium concentration induced by gastrin, although it is possible that gastric calcium excretion was involved, at least in part, in the hypocalcemic effect of gastrin. However, when gastrin and secretin were given together the gastric calcium excretion was not different from the control, but there was a transient drop of plasma calcium observed in the first 15 min, after which the level returned to normal (Fig. 5). This slight transient lowering of plasma calcium could be explained by the possibility of gastrin getting to the parietal cells and stimulating the gastric secretion before secretin could reach the cells and exert its inhibitory effect. It is possible that the gastric response to gastrin here is indeed solely responsible for the degree of hypocalcemic effect since it was totally abolished by gastrectomy (4). However, the calcium in gastric juice only represents part of calcium removed from the blood; some may remain in the gastric tissue, or it is also probable that other calcium excretory routes which are affected by gastrin are also inhibited by secretin. These two possibilities are being explored.

Gastrin and bile flow. Gastrin has been reported to stimulate bile secretion and change the bile composition in dogs (19). However, our results showed that there were no changes in the bile calcium content or bile flow after gastrin administration. Therefore, it is unlikely that, at least in the rat, the bile calcium excretion could be responsible for the hypocalcemic effect of gastrin.

It can be concluded from our results that gastrin, in addition to being a calcitonin secretagogue, can directly result in hypocalcemia. As for the mechanism, it can be stated on the basis of our findings that calcium was removed from the blood, at least in part, by stimulated gastric secretory activities. Other routes of calcium exit remaining to be explored are the bone, the rest of gastrointestinal tract and the calcium sequestration in

other tissues. Thus, the present studies suggest that gastrin may constitute another calcium control system, especially in the case of hypercalcemia resulting from an excess calcium absorption due to high calcium intake or other causes. Although calcitonin has been reported to be able to decrease calcium absorption from the gut, this effect only began after 48 hr of infusion (20). Therefore, the direct hypocalcemic effect of gastrin may play a role in preventing this plasma calcium fluctuation.

Summary. The influence of gastrin on calcium (Ca) homeostasis was investigated in anesthetized rats. When administered intraperitoneally, gastrin was found to lower plasma Ca concentration in both intact rats and rats which had their source of calcitonin removed (thyroparathyroidectomized) but to exert no significant effect on the rates of urine Ca excretion in the intact rats. Thus, its hypocalcemic effect was independent of calcitonin. In search of possible routes of Ca removal from the blood, both the Ca concentration in gastric juice and the total gastric Ca excretion were measured. Total gastric calcium excretion increased from 14 ± 1.4 to 22 ± 2.4 μg/hr/100 g rat by the administration of gastrin. The increase in gastric Ca secretion and the hypocalcemic effect were abolished by concurrent administration of secretin (2 units). The bile volume and the Ca content in the bile secretion were not affected by gastrin. These findings were compatible with the hypothesis that the hypocalcemic effect of gastrin was partly caused by an increased Ca excretion into the gastrointestinal lumen.

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