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Possible Role and Mode of Action of Gastrin on Calcium Homeostasis in the Rat (41236)

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Abstract. The hypocalcemic effect of gastrin and its possible mode of action were studied in rats. Gastrin (13 μg/100 g rat), injected intravenously led to a significant reduction in the plasma calcium concentrations. The release of endogenous gastrin by an intragastric phenylalanine instillation similarly led to a significant hypocalcemia in intact rat but not in antrectomized rat. Moreover, the protective role of endogenous gastrin against hypercalcemia induced by an intraduodenal infusion of $CaCl_2$ (10 mg/100 g rat) was demonstrated. Gastrin (50 μg/100 g rat) seems to have no influence on the net intestinal absorption of ^{45}Ca. The removal of ^{45}Ca from plasma was also unaffected by gastrin administration. The disappearance rate from plasma of ^{45}Ca administered 17 hr previously was compared in sham, thyroidectomized (TX) and parathyroid-autotransplanted rats receiving saline or gastrin. The faster rate of disappearance of plasma ^{45}Ca from plasma in gastrin-treated TX autoparathyroid-transplanted rats indicated the suppressive action of gastrin on the release of ^{45}Ca from as yet unknown source(s).

Several investigators have demonstrated the hypocalcemic actions of gastrin and pentagastrin in the pig and rat (1–3). The response has been shown by Cooper and his group (3) to be attributed to the gastrin-induced calcitonin (CT) release. However, the hypocalcemic effect of gastrin has also been demonstrated in the thyroparathyroidectomized rat (4, 5) suggesting that gastrin, at least in the rat, may exert its action independently of calcitonin. Our previous study (5) showed that administration of gastrin led to hypocalcemia and a significant increase in the total gastric calcium excretion which, nevertheless, could not totally account for the observed hypocalcemia.

The present studies were designed to examine the response of the plasma calcium to a small dose of exogenous gastrin and the endogenous gastrin released from gastric antrum in an attempt to explore the role of gastrin and its mechanism in calcium regulation. The study includes such aspects as the gastrin action against hypercalcemia induced by intestinal calcium absorption and the removal of calcium from the plasma pool.

Materials and Methods. *Animal.* Adult female Fischer rats weighing between 180 and 200 g and fed *ad libitum* with regular rat chow (Gold-Coin LTD., Singapore) were used. The rats were fasted overnight with free access to tap water before the experiments.

Surgical procedures. As previously described in detail (5), after being anesthetized with nembutal sodium, the rat was tracheotomized and the femoral artery and vein were cannulated with polyethylene tube (PE 50). In the study of the effect of endogenous gastrin, 0.2 *M* phenylalanine (Sigma Chemical Co.) which was used to induce the release of gastrin from the gastric antrum (6) or 0.9% NaCl was injected into the stomach through the pylorus which was subsequently ligated. In a group of experimental rats on which antrectomy had been performed, the same amount of 0.9% NaCl or phenylalanine was introduced directly into the body of the stomach. The gastric secretion was collected at the end of the 90-min experiment according to the method of Shay (7). For the study involving the intestinal absorption of calcium, the duodenum was exposed and calcium solution (10 mg/100 g rat) was infused into the proximal end of duodenum. The rectal temperature was monitored and maintained at 37° by a thermostatically controlled heating lamp.

Experimental procedures. A. The effects

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of exogenous and endogenous gastrin. The response of the plasma calcium to gastrin (13 μg/100 g rat (8), Sigma Chemical Co.) administered intravenously, was studied through a 90-min period. Two control blood samples (0.3 ml each) were obtained at 15-min intervals before administration of gastrin. Thereafter blood collections were made at time 10, 20, 30, 45, and 60 min.

The effect of endogenous release of antral gastrin upon plasma calcium involved the injection of phenylalanine or 0.9% NaCl into the stomach prior to the collection of blood samples. Immediately after the last collection, the gastric secretory volume was measured, titrated to pH 5, and expressed as microequivalents of H^+ per gram of stomach per hour. The increase in gastric secretion was implicated to be an indirect indication of endogenous gastrin release (6). In this series of experiments the animals were divided into three groups as follows: (i) control group receiving 0.5 ml of 0.9% NaCl, (ii) sham group receiving 0.5 ml of 0.2 *M* phenylalanine, and (iii) antrectomized group receiving 0.5 ml of 0.2 *M* phenylalanine. All the test solutions were at pH 5. The response of plasma calcium concentrations to the intestinal calcium loading was also studied in sham and antrectomized rats.

B. The investigation of the mode of action of gastrin-induced hypocalcemia. 1. The effect of gastrin on the amount of $^{45}CaCl_2$ (Radiochemical Centre, Amersham, U.K.) which remained in the plasma after being absorbed from the intestine. After a control blood sample was collected, 0.2 μCi ^{45}Ca/100 g body wt in 0.5 ml of 10 mg% $CaCl_2$ solution was introduced into the duodenum, and gastrin at a dose of 50 μg/100 g body wt or an equal amount of saline was given intraperitoneally. Further blood collections were then made at 30-min intervals for 3 hr.

2. The effect of gastrin on the disappearance rate of intravenously administered ^{45}Ca from plasma in the presence or absence of intestinal absorption of calcium. A solution of 2 μCi ^{45}Ca/100 g body wt in 0.25 ml 10 mg% $CaCl_2$ was injected intravenously. A period of 30 min was allowed for the distribution of isotope in blood before a control blood sample was collected. In the group with intestinal absorption of calcium, a solution of $CaCl_2$ (10 mg/100 g body wt in 0.5 ml) was injected into the duodenum prior to an intraperitoneal administration of gastrin (50 μg/100 g body wt) or the same amount of 0.9% NaCl. Blood samples were then obtained at time 30, 60, 120, and 180 min.

3. The effect of gastrin on the disappearance rate of plasma ^{45}Ca administered intraperitoneally 17 hr prior to the experiment. Three groups of rats were used: (1) intact rats receiving 0.9% NaCl, (2) parathyroid-autotransplanted rats which were thyroidectomized (TX) and receiving 0.9% NaCl, and (3) as (2) but receiving 50 μg/100 g body wt gastrin.

Autotransplantation of the parathyroid glands into the thigh was performed 14 days before the TX. Autotransplanted rats whose fasting plasma calcium concentrations were above 9.5 mg% on the experimental day and still remained above 9.5 mg% 45–60 min after TX were assumed to have been successfully autotransplanted and only those fulfilling such criteria were experimented upon as follows: The rats were injected with an ip dose of 50 μCi ^{45}Ca in 0.25 ml of 10 mg% $CaCl_2$ solution; 17 hr later, the first control blood sample was collected and TX or sham operation was performed. The second control blood collection was made 45 min after this operation to check whether the transplantation was successful and to serve as time zero value. Gastrin (50 μg/100 g body wt, ip) was then administered and further blood samples were taken at an interval of 1 hr for 5 hr.

Analysis. Blood was obtained from the femoral artery and centrifuged immediately. Concentration (mg%) of calcium in the plasma was determined with atomic absorption spectrophotometer (Varian, Model AA 575). Measurement of the radioactivity of ^{45}Ca was made using a Beckman liquid scintillation spectrometer (Model LS-100). At time zero, both the plasma calcium concentration (x mg%) and the radioactivity of ^{45}Ca (a cpm) were determined. The counts per minute of ^{45}Ca at time zero was then taken to represent the amount of calcium concentration (mg%) at that time, and

counts per minute of ^{45}Ca (b cpm) of subsequent plasma samples could then be calculated in such a way as to represent proportionally the calcium of time zero pool which still remained in the plasma (y mg%), i.e., $y = xb/a$ mg%. In this way, the level of calcium (mg%) of time zero which still remained in the plasma was shown to decrease gradually with time. Similar calculation was also made when measuring the amount of calcium absorbed from the intestine by determination of the total calcium and the ^{45}Ca introduced into the duodenum. All data were statistically computed as mean ± SEM and compared by Student's t test using a Wang programming calculator (700 series).

Results. The blood pressure and plasma calcium concentration after injection of saline and subsequent blood samples collections, were found to be steady ($P > 0.05$) throughout the experiment. However, an intravenous administration of gastrin (13 μg/100 g body wt) resulted in a significant drop ($P < 0.05$) in plasma calcium concentration at 20 min. The hypocalcemia remained through the 60-min period (Fig. 1), and it usually returned to normal level in approximately 2 hr. The gastric acid secretion after intragastric instillation of 0.2 M phenylalanine increased significantly from 3.8 ± 1.7 to 9.1 ± 0.1 μeq H^+/stomach/hr in the intact rat. The response of the gastric acid secretion provided an indirect indication of the phenylalanine-stimulated endogenous gastrin release (6). The intact rats receiving phenylalanine exhibited marked hypocalcemia at 60 and 90 min while both intact rats receiving saline and antrectomized rats receiving phenylalanine showed no significant change in either the gastric acid secretion or the plasma calcium concentrations when compared to the zero time values (Fig. 2).

The plasma calcium concentrations of sham and antrectomized rats after intestinal absorption of calcium are demonstrated in Fig. 3. Normocalcemia was observed in the sham group but in the antrectomized group, the plasma calcium levels were significantly increased from 9.6 ± 0.1 to 10.1 ± 0.2 mg/100 ml at 30 min and to 10.5 ± 0.4 mg/100 ml at 60 min ($P < 0.05$), after which the level dropped to normal level of 9.9 ± 0.2 mg/100 ml at 120 min. This increase in the plasma calcium concentration was reproducible.

Studies of the mode of action of gastrin-induced hypocalcemia. 1. The effect of 50 μg/100 g rat gastrin on ^{45}Ca which has been absorbed from the intestine and remained in the plasma. ^{45}Ca absorbed and present in the plasma of sham and gastrin-treated rats was not different through the 3-hr period.

2. The effect of gastrin on the disappearance of ^{45}Ca from the plasma in the presence and absence of the intestinal absorption of calcium. In the absence of intestinal calcium absorption, the plasma calcium concentrations were markedly reduced 30, 60, 90, and 180 min after gastrin administration (Fig. 4). However, despite the different response of the total plasma calcium levels in sham and gastrin-treated groups, the plasma calcium of the time zero pool was lost at the same rate ($P > 0.05$, when the results of the same time interval were compared) in both groups. Similarly in the presence of intestinal calcium absorption, the plasma calcium concentration of the gastrin-treated group dropped from 9.6 ± 0.2 to 9.0 ± 0.2 mg% at 30 min and 8.7 ± 0.1 mg% at 180 min. The disappearance rate of plasma calcium of the time zero pool, was also similar in the sham and the gastrin-treated group.

3. The effect of gastrin on the disappearance of ^{45}Ca administered 17 hr prior to experiment, from the plasma of intact and thyroparathyroidectomized autoparathyroid-transplanted rats. An estimation of

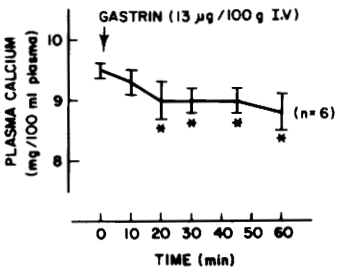

FIG. 1. The plasma calcium concentration in the intact rat after an intravenous administration of 13 μg/100 g rat gastrin. The vertical bars indicate the standard error of mean. An asterisk denotes value significantly different from time zero value. ($P < 0.05$).

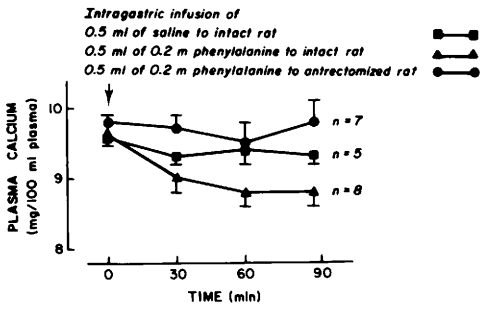

FIG. 2. The plasma calcium in response to the endogenous gastrin released by intragastric infusion of phenylalanine in intact and antrectomized rats. A significant ($P < 0.05$) hypocalcemia was observed in intact rat receiving phenylalanine at 30, 60, and 90 min whereas no significant hypocalcemia was observed in both saline-treated and antrectomized rats at all time intervals.

the disappearance of ^{45}Ca in different groups was made by measuring the radioactivity of the plasma drawn at hourly intervals beginning at 17 hr (time zero) following ip administration of ^{45}Ca. As shown in Fig. 5, the disappearance of ^{45}Ca, expressed as percentage of the time zero value, of TX parathyroid-autotransplanted rats which received gastrin was significantly faster than that of intact rats and TX parathyroid-transplanted rats receiving 0.9% saline at 180, 240, and 300 min. However, the disappearance rate of ^{45}Ca of the latter two groups was not significantly different ($P > 0.05$).

Discussion. In our previous report (5), a pharmacological dose of gastrin was found to result in a plasma calcium reduction in intact and TPTX rats, suggesting that gastrin could act independently of CT. In the present studies, a significant hypocalcemia was demonstrated to be induced by both a small dose of exogenous gastrin and endogenous gastrin. Although gastrin and its structurally related compounds are potent CT secretagogues in the pig (2) and man (9), they are weak or ineffective in stimulating the CT release in the rat (10). In addition, the hypocalcemic action of gastrin was also observed in TPTX rat (5). Thus it is unlikely that the observed gastrin-induced hypocalcemia is caused by stimulation of CT release.

A possible physiological role of gastrin was further demonstrated by the effect of endogenous gastrin on the plasma calcium concentrations. Since an intragastric instillation of phenylalanine was reported to increase both serum gastrin and gastric acid secretion (6, 11, 12), we used the increase in gastric acid secretion after phenylalanine instillation as an indication of an endogenous gastrin release. The possibility that the amino acid solution may stimulate gastric acid secretion simply through the neural reflex caused by distension may be discarded since phenylalanine-stimulated acid secretion was much greater than the secretion initiated by the same volume of normal saline. However, it is recognized that it cannot be ascertained whether the blood level of gastrin produced by injection of 13 μg gastrin/100 g rat and after phenyl-

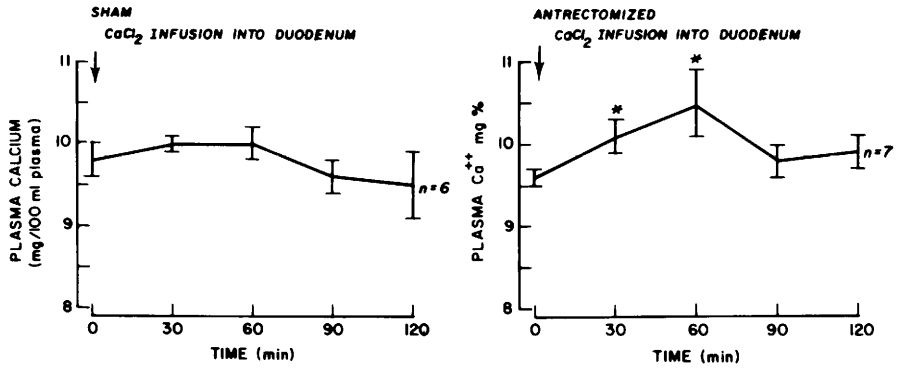

FIG. 3. The effect of the presence (sham) and absence (antrectomized rats) of endogenous gastrin on the plasma calcium during intestinal absorption of 10 mg $CaCl_2$/100 g rat in 0.5 ml solution. An asterisk denotes value significantly different from time zero value. ($P < 0.05$).

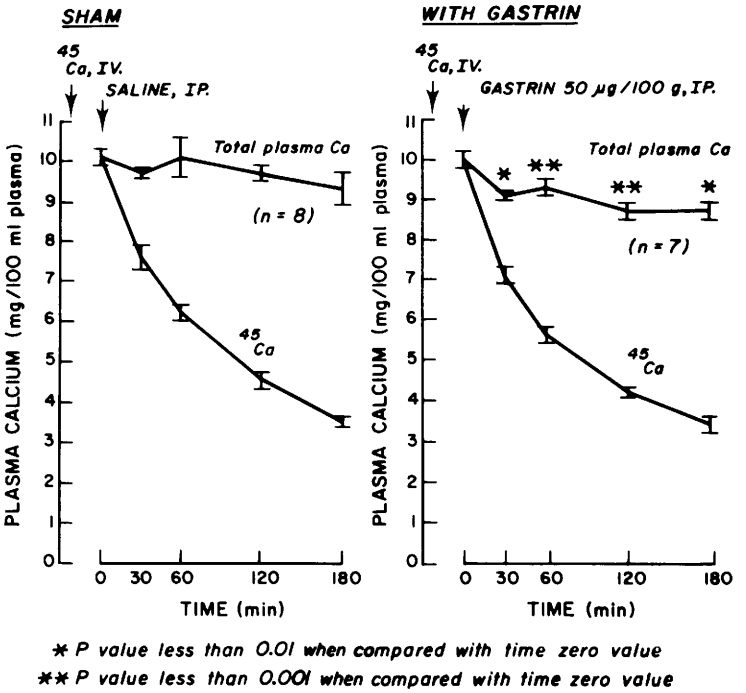

FIG. 4. The effect of gastrin on total plasma calcium (mg%) at various time intervals and the turnover rate of calcium of the time zero pool (labeled as "^{45}Ca" on the figure since the plasma calcium of the time zero was prelabeled with ^{45}Ca and the cpm of ^{45}Ca of the subsequent samples thus represent proportionally the calcium of time zero pool which still remained in plasma, unit = mg%) in the absence of GI absorption of $CaCl_2$.

alanine would correspond with those achieved postprandially. Nevertheless, the technique used has the support of previous reports cited above that it will release endogenous gastrin. Therefore, hypocalcemia, observed in intact rats receiving phenylalanine and not in intact rats receiving the same volume of saline or the antrectomized rats receiving phenylalanine (Fig. 2), strongly suggests that the reduction of the plasma calcium levels was the consequence of the endogenous gastrin release.

Since the hypocalcemic action of the endogenous gastrin has been demonstrated, it is of interest to study the protective role of gastrin against a condition of calcium ingestion. Oral ingestion of calcium carbonate in man was reported to cause an increase of serum gastrin and gastric acid secretion, accompanied by normal or slightly increased serum calcium levels (13–15). Our results similarly demonstrated normocalcemia in sham rats after an intraduodenal $CaCl_2$ infusion. On the other hand a transient hypercalcemia was found to develop in the antrectomized rats which points to the possible importance of the presence of antrum in the protection against $CaCl_2$-induced hypercalcemia. It has been suggested that intraluminal calcium can act to release gastrin (15). Thus, it is possible that the absorbed calcium stimulates the release of gastrin which in turn acts to prevent hypercalcemia. Under the present experimental condition, the involvement of CT in preventing development of hypercalcemia induced by calcium loading cannot be ruled out since rats with intact thyroparathyroid complex were used. However, the occurrence of a transient but significant hypercalcemia in antrectomized rats in which the thyroid glands were intact pointed to the possibility that although CT is an essential antihypercalcemic agent, its presence cannot completely prevent the transient rise in plasma calcium due to a high calcium

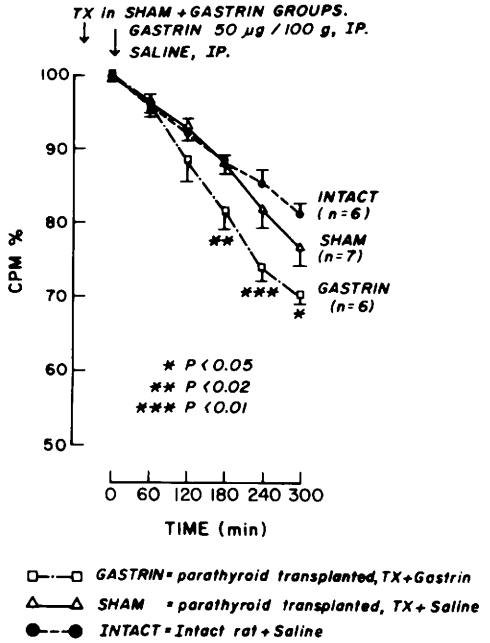

FIG. 5. The rate of disappearance from plasma of ^{45}Ca which was administered intraperitoneally 17 hr prior to the experiment. The plasma ^{45}Ca samples were obtained at the beginning of experiment and subsequent intervals from (a) intact rat, (b) thyroidectomized and autoparathyroid-transplanted rats receiving saline (sham), and (c) thyroidectomized and autoprathyroid-transplanted rats receiving 50 μg gastrin/100 g rat.

load. The results agree with those of Coen and Palagi (16) who reported that although CT could counteract the hypercalcemia resulted from excess calcium absorption, the transitory calcium increment was not abolished fast enough. In addition, variations in the levels of gastrin were reported to be most commonly related to feeding in man (17) and in rats (18, 19). The serum gastrin markedly decreased during food deprivation and increased during hyperphagia in rat (18, 19). It is also interesting to note that gastrin level peaks in the prandial period during which hypocalcemia is usually present. From the above studies, the role of gastrin in calcium homeostasis is very likely.

Since gastrin was found to have no effect on the amount of ^{45}Ca absorbed and present in the plasma, it could mean either that gastrin might increase or decrease simultaneously both the net intestinal absorption and net removal of ^{45}Ca from the plasma thus resulting in no net change in plasma ^{45}Ca or gastrin might affect neither. By injecting ^{45}Ca tracer intravenously at time zero and allowing a few minutes for an even distribution, the radioactivity of ^{45}Ca in the plasma would represent the amount of calcium of the time zero pool. The radioactivity of plasma subsequently taken at intervals would reflect the amount of calcium of the time zero pool which still remained in the plasma. The results (Fig. 4) show that gastrin significantly reduced the level of the total plasma calcium concentrations but had no effect on the rate of removal of the calcium of the time zero pool of plasma calcium under both conditions. Thus, the hypocalcemic action of gastrin is mediated neither through the removal of calcium from the plasma nor the inhibition of intestinal absorption of calcium.

The plasma calcium concentration at any one time is a result of a complex balance between the influx into plasma of calcium from the GI tract or from various tissues including bone and the removal of calcium from the plasma pool. Thus a possibility of gastrin suppressing the release of calcium from other tissues was investigated in intact and thyroidectomized autoparathyroid-transplanted rats. The results which were presented as percentage counts per minute thus represent the change in ^{45}Ca transfer between the various tissues and the plasma. Gastrin administration led to a steeper disappearance rate of ^{45}Ca in the thyroidectomized autoparathyroid-transplanted rats. Considering the radioactivity of ^{45}Ca at any time being a result of influx into and efflux of ^{45}Ca from the plasma, a faster rate of disappearance thus indicates either a greater efflux from or a decreased influx of calcium into plasma. As gastrin has been shown to have no effect on the efflux of calcium from the plasma, it leaves a possibility of suppression of the calcium influx into plasma from as yet unknown sources. The suppressive action of gastrin is independent of CT for the autoparathyroid-transplanted rats were thyroidectomized prior to the experiment.

In conclusion, the ability of gastrin to lower blood calcium and protect against hypercalcemia due to a calcium load has been demonstrated. The hypocalcemic action of gastrin is possibly due to a suppression of the influx of calcium into plasma from as yet unknown site(s). Studies are being undertaken to find the tissues or organs responsive to this suppressive action of gastrin.

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