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The Mechanism of Action and Target Organ of Gastrin-Induced Hypocalcemia (41237)

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Abstract. The hypocalcemic effect of gastrin and the possible role of the hormone in calcium homeostasis have been demonstrated in our previous study. The mechanism involves neither the gastrointestinal absorption nor the removal of calcium from plasma but is possibly due to the suppression of the calcium influx into blood. In searching for the organ(s) involved in the action of gastrin, the following were tested and not found to be directly responsible: stomach, intestine, pancreas, liver, spleen, adrenal gland, kidney, lung, muscle, and red blood cell. After 17 hr of ^{45}Ca administration, the turnover of ^{45}Ca in the tibia was measured. Gastrin was found to suppress the release of ^{45}Ca by 25% within 1 hr. The suppressive effect of gastrin on ^{45}Ca release was also demonstrated in an *in vitro* preparation which showed that the ^{45}Ca released from prelabeled tibia into the incubating medium was also reduced by gastrin. It was thus concluded that the gastrin-induced hypocalcemia in rat was the result of a suppression of the release of calcium from bone.

Previous studies have demonstrated that gastrin could induce hypocalcemia in thyroparathyroidectomized (TPTX) rats (1–4), suggesting that gastrin-mediated hypocalcemia in the rat is not solely dependent upon the release and subsequent action of calcitonin (CT). Since hypocalcemia could have arisen from (i) a decrease in GI absorption of calcium, (ii) a decrease in release of calcium from various tissues, or (iii) an increased efflux of calcium from the plasma pool, we investigated each of these routes of calcium movement. Our previous work showed that gastrin suppressed the release of calcium into the plasma (5).

In the present studies, possible involvement of various tissues and organs in the hypocalcemic action of gastrin was investigated. The results show that gastrin reduced the plasma calcium concentrations primarily by suppressing the release of calcium from bone.

Materials and Methods. *Animals:* Female Fischer rats weighing between 180 and 200 g and maintained on commercial rat chow and tap water *ad libitum* were fasted overnight before the start of each experiment.

Test solutions. Porcine gastrin (Sigma Chemical 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 injected intraperitoneally. ^{45}Ca was obtained from Radiochemical Centre, Amersham, United Kingdom. For the *in vivo* prelabeling of bones, 50 μCi ^{45}Ca/100 g in 0.25 ml of 10 mg% $CaCl_2$ solution was injected intraperitoneally 17 hr prior to the experiment.

Surgical procedures. All surgical procedures including gastrectomy, enterectomy, pancreatectomy, spleenectomy, and nephrectomy were performed under sodium pentobarbital anesthesia. In some experiments the liver, lung, muscle, kidney, and red blood cells were removed after withdrawing blood and infusing saline via the femoral artery and vein.

Experimental procedures. a. Search for the tissue(s) responsive to hypocalcemic action of gastrin. After the rat was anesthetized and tracheotomized, a control blood sample (0.3 ml) was obtained via the femoral artery. Immediately after the removal of a certain organ or tissue, 50 μg/100 g gastrin or an equal amount of saline was injected intraperitoneally. Further blood collections were then made at time 30, 60, 90, and 120 min following the hormone administration.

In experiments involving measurement of the calcium contents, 60 min after an intraperitoneal administration of gastrin or an

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equal volume of saline and after the collection of the second blood sample, the animal was drained of blood via the femoral artery, then the liver, lung, kidney, and quadricep muscle were removed, digested and assayed for calcium.

b. In vivo study of the effect of gastrin on the calcium release from bone. The experiments involved an *in vivo* prelabeling of tibia with ^{45}Ca by an intraperitoneal injection of 50 μCi ^{45}Ca in 10 mg% calcium solution 17 hr prior to the experiment. After an overnight fast, the rat was anesthetized and tracheotomized, then the left tibia was removed to serve as a control. Gastrin or saline was given intraperitoneally and 60 min later the right tibia was dissected out. Tibias were rinsed in sucrose solution, blotted dry, and weighed before they were subjected to acid digestion and assay for ^{45}Ca.

c. In vitro study of the effect of gastrin on calcium release from bone. The tibias were similarly prelabeled *in vivo* with ^{45}Ca 17 hr preceding the experiment. The rat was sacrificed and both tibias were removed at once, weighed, and cut in half. One tibia of each pair served as a control. The tibias were incubated at 37° in Kreb–Henseleit bicarbonate buffer, pH 7.4, containing 4% glucose and 5 mg% calcium and gassed with 95% O_2 + 5% CO_2; this composition resembles rat extracellular fluid (6). Twenty-five micrograms of gastrin was added to 5 ml media of the experimental tibias at 0 min. One hundred-microliter samples of incubating media were removed at 1, 3, 5, and 8 hr for ^{45}Ca assay. In other sets of experiments the tibias were heat inactivated at 80° for 10 min before incubation.

Analyses. Blood was obtained from the femoral artery and centrifuged immediately. The plasma calcium concentration was determined with an atomic absorption spectrophotometer (Varian, Model AA575) according to the procedure of Pybus (7).

Base digestion of tissues involved the incubation of tissues in 1–2 ml of 1 *N* NaOH at 75° for 60 min. The digested sample was then acidified to pH 7.0 and made up to a final volume with sucrose solution and centrifuged and the supernatant was analyzed for calcium.

Acid digestion of bones involved the incubation of bone in 2 ml of 2 *N* HCl at 75–80° for 2 hr. The digested samples were neutralized, made up to a volume with sucrose solution, and centrifuged. The supernatant, 0.1 ml, was added to the scintillation fluid. The radioactivity of ^{45}Ca was estimated by the standard liquid scintillation technique using a Beckman liquid scintillation spectrometer (Model LS-100).

All data were statistically computed as mean ± SEM and compared by Student's *t* test, using a Wang programming calculator (700 series).

Results. *a. The effect of gastrin on plasma in rat after tissue resections.* Table I shows that gastrectomy did not abolish the hypocalcemic action of gastrin. Neither enterectomy, pancreatectomy, nor spleenectomy had any effect on the gastrin-

TABLE I. THE EFFECT OF GASTRIN (50 μg/100 g RAT) ip ON PLASMA CALCIUM (mg% ± SEM) IN RATS AFTER VARIOUS ORGAN RESECTIONS

Condition	Time (min) after injection of gastrin or saline				
	0	30	60	90	120
Gastrectomy + NaCl	10.1 ± 0.2	9.8 ± 0.3	9.5 ± 0.2	9.6 ± 0.3	9.3 ± 0.2
Gastrectomy + gastrin	10.0 ± 0.2	9.1 ± 0.2**	8.7 ± 0.2*	8.9 ± 0.2**	8.9 ± 0.2
Nephrectomy + NaCl	10.3 ± 0.2	9.8 ± 0.3	9.9 ± 0.3	10.4 ± 0.4	10.8 ± 0.4
Nephrectomy + gastrin	10.3 ± 0.8	10.0 ± 0.2	10.1 ± 0.2	10.4 ± 0.2	11.2 ± 0.2

Note. The results are expressed as mg plasma calcium/100 ml. Statistical comparisons (Student's paired *t* test) are between the gastrin-treated and the corresponding NaCl control values.

* $P < 0.01$.

** $P < 0.05$.

induced hypocalcemia. The plasma calcium concentrations in nephrectomized rats receiving either saline or gastrin did not change but showed a tendency to increase from their corresponding time zero values.

b. The effect of gastrin on the calcium contents of various tissues. The calcium contents in the following named tissues were not increased after gastrin administration when compared to the saline controls ($P > 0.05$): lung, muscle, liver, kidney, and RBC. In fact, there was a tendency for a decrease in the calcium content in some tissues due to the hypocalcemic action of gastrin.

c. The effect of gastrin on bone calcium release. In the studies related to the effect of gastrin on the release of calcium from bone, it was established that the two tibias of the same rat had a similar weight (0.32 ± 0.02 g of the left tibia vs 0.32 ± 0.1 g of the right tibia) and the same distribution of ^{45}Ca content (2993 ± 239 cpm × 10^3/g bone in the left tibia vs 3024 ± 236 cpm × 10^3/g bone of the right tibia, $P > 0.05$) after 17 hr of ^{45}Ca prelabeling. Thus, one tibia was used as a control and the other as an experimental tibia.

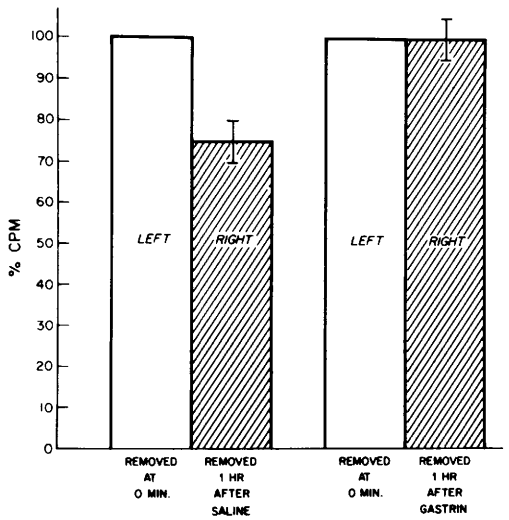

FIG. 1. The radioactivity of the whole tibias of the rats which were prelabeled with 50 μCi ^{45}Ca 17 hr earlier. The left tibia was removed at the beginning of the experiment and expressed as 100% cpm. The right tibia was removed 1 hr after saline or gastrin (50μg/100 g rat, ip) administration and its radioactivity was expressed as percentage of the left tibia.

As demonstrated in Fig. 1, 60 min after removal of the left tibia and normal saline administration, the radioactivity of the right tibia dropped to 75 ± 6% of the control left tibias, reflecting the net release of ^{45}Ca from the right tibia during a 1-hr period. In contrast, 60 min after gastrin injection, the radioactivity of the right tibia remained the same as that of the control left removed 1 hr earlier. This result can be interpreted in two ways: there was an increase in the deposition of ^{45}Ca in bone or there was a decrease in the release of calcium from bone. Since the previous experiment (5) has shown that gastrin had no effect on the removal of ^{45}Ca from plasma, it is more likely that the unaltered radioactivity of the right tibia was due to a suppression of the calcium efflux from bone.

The inhibitory action of gastrin on calcium efflux from bone was confirmed by the results of the *in vitro* experiment. As shown in Table II, the tibias which were incubated in the medium to which gastrin was added released less ^{45}Ca than the paired controls. The radioactivity of the medium containing bone and gastrin compared with that of the controls was 868±40 versus 966 ± 33 cpm/0.1 ml ($P < 0.05$) at 1 hr, 986 ± 156 versus 1237 ± 61 ($P < 0.05$) at 3 hr, and 1109 ± 48 vs 1394 ± 78 cpm/0.1 ml ($P < 0.05$) at 5 hr. At the end of 8 hr, however, the cpm/0.1 ml medium of both gastrin-treated and controls became more variable and the mean values were not significantly different from each other being 1244 ± 88 vs 1429 ± 210 cpm/0.1 ml ($P > 0.05$), respectively.

On the other hand, the release of ^{45}Ca by the heat-inactivated tibias showed a different result (Table II). Although both the gastrin-treated and control groups exhibited an increase in the net release of ^{45}Ca, the radioactivity of the media of both groups did not differ from each other at all sampling periods. The overall radioactivity released by the heat-inactivated tibias was much less than that released by bones which had not been inactivated.

Discussion. In our studies gastrectomy,

TABLE II. THE *IN VITRO* STUDY OF THE EFFECT OF 25 μg/5 ml GASTRIN IN 5 ml OF INCUBATING MEDIUM, ON THE RELEASE OF ^{45}Ca FROM TIBIAS WHICH WERE PRELABELED *IN VIVO* 17 hr PRECEDING THE EXPERIMENT

	No. of bones	Accumulative radioactivity (cpm/0.1 ml medium) of released ^{45}Ca in the incubating medium			
		1 hr	3 hr	5 hr	8 hr
Control	8	966 ± 33	1237 ± 61	1394 ± 78	1429 ± 210
Gastrin treated	8	868 ± 40*	986 ± 156*	1109 ± 48*	1244 ± 88
Heat inactivated	5	381 ± 81	474 ± 89	543 ± 121	743 ± 112
Heat inactivated + gastrin	5	352 ± 68	494 ± 99	495 ± 96	606 ± 139

Note. The data are the accumulative radioactivity of ^{45}Ca (mean cpm/0.1 ml medium ± SEM) in the medium sampled at 1, 3, 5, and 8 hr.
* $P < 0.05$.

enterectomy, pancreatectomy, and spleenectomy had no effect on the gastrin-induced hypocalcemia. In contrast, Klementschitsch *et al.* (4) found that the gastrin or histamine-induced hypocalcemia can be abolished by vagotomy or gastrectomy and by the gastric secretion inhibitors, atropine and secretin, suggesting the importance of the stomach in serum calcium regulation. Our previous work (3) showed that although gastrin increased the gastric calcium secretion, such an increase could not totally account for the hypocalcemia observed. With an intact stomach, a mild increase in blood pH (the alkali tide) usually associated with the increase in acid secretion by gastrin, may account for some of the decrease in plasma ionized calcium. In gastrectomized rat, the degree of hypocalcemia may not be as great as in intact animal. Moreover, the hypocalcemia observed in our gastrectomy experiment but not in Klementschitschs' could also be explained partly by the different age group of animal and the different dose of gastrin. We used older animals and the dose of porcine gastrin was twice as high as the dose of human gastrin used in their experiment. In their study, nephrectomy on the previous day did not abolish the hypocalcemic response of plasma calcium to gastrin. In contrast, our results showed that the plasma calcium concentrations in acutely nephrectomized rats receiving saline and gastrin were increased slightly but the values of plasma calcium levels of the two groups were not different from each other. We explain this phenomenon in terms of a direct result of an acute abolishment of the urinary excretion of calcium. This disturbance in the plasma calcium concentrations may have overshadowed the hypocalcemic action of gastrin.

Since the method of resection could not be applied to certain vital organs, the calcium content of the tissues was measured and compared between the control and that of the gastrin-treated rats. If a tissue were responsible for the hypocalcemia observed after gastrin injection, there should be an accumulation of calcium content in the tissue whether due to the suppression of release or increase in accumulation of calcium. The kidney was also included in this experiment because the earlier results of nephrectomy casted doubt on the role of kidney in the gastrin-mediated hypocalcemia. We found that the calcium contents in the various tissues were not increased after gastrin administration. However, it was possible that a slight increase in calcium content may have occurred throughout the body soft tissues to an extent as to account for the detectable decrease in blood calcium concentration yet be undetected by analysis of each individual organ. Nevertheless, there was, in fact, a tendency for a decrease in the calcium content in many organs, possibly as the consequence of hypocalcemia. It is unlikely that the kidney is the target for gastrin since there was no calcium accumulation and since urinary excretion of calcium after gastrin administration was not increased (3).

These studies demonstrated that those tissues under investigation were not involved in the hypocalcemic action of gastrin but the effect was primarily due to the suppression of calcium efflux from bone. The use of paired tibias provided convenient paired controls since such paired bones do not vary by more than 5% in initial ^{45}Ca content (8). This was confirmed by the present results. One hour after removal of the left tibia and after saline administration, the right tibia contained 75 ± 6% of ^{45}Ca content of the control left tibia, i.e., about 25 ± 4% of ^{45}Ca has been released into the ECF within 1 hr. On the other hand, gastrin administration resulted in a suppression of the release of ^{45}Ca. Since the distribution of ^{45}Ca injected 17 hr earlier should be in the vicinity of the bone fluid compartment (BFC) (9) which was referred to by Parfitt as the rapidly exchangeable calcium (10), our data on ^{45}Ca content only represent the distribution of calcium between the BFC and ECF and not the total calcium content of bone. Although the present study shows neither the site nor the mechanism of gastrin, it is likely that the suppressive action of gastrin is through the retention within this rapidly exchangeable calcium pool of calcium which should have otherwise been released into the ECF.

The *in vitro* experimental results confirmed those obtained from the *in vivo* investigation. The suppressive action of gastrin on calcium efflux from bone became apparent within the first hour and continued for at least 5 hr. This prolonged effect of gastrin was probably due to the lack of the usual continual excretion and inactivation of gastrin. The *in vitro* experiments, in addition, confirm the direct and independent action of gastrin. The observation that gastrin was ineffective in suppressing the release of ^{45}Ca from heat-inactivated bone suggests that the viability of cells is important for the movement of calcium out of the bone compartments. The cellular basis of the influx and efflux of bone calcium and the control of plasma calcium were hypothesized by Talmage and Grubb (11). Our results on the action of gastrin seem to fit into this model in that gastrin has no effect on the movement of calcium from the plasma. This flux occurs passively along the concentration gradient between the bone compartments and the plasma which has a higher concentration of calcium ions than the bone fluid compartment. The suppressive action of gastrin is likely to be on the active efflux of calcium from bone fluid compartment, leading to a drop in the plasma calcium concentration.

In conclusion, the gastrin-induced hypocalcemia occurs independently of CT. The mechanism of the gastrin-mediated hypocalcemia was strongly suggested by the *in vivo* and *in vitro* studies to be due to a suppression of the efflux of calcium from bone. However, the details of the mechanism of action of gastrin remain to be clarified.

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