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# Interference with the Serum Gastrin Response to Feeding after Surgical Intervention with the Rat Thyroparathyroid Complex[1] (39472)

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Previous work by us and by others has established that gastrin is a potent thyrocalcitonin secretagogue in a number of mammalian species, including man (1-4). Conversely, in man and other mammals some work has suggested that increased blood levels of thyrocalcitonin, in turn, can act to suppress a number of gastrointestinal functions, including secretion of gastrin (5-9).

Recently, we reported large increases in both serum gastrin and serum thyrocalcitonin shortly after the onset of feeding in rats adapted to a fixed schedule of daily feeding (10). Whether the increased secretion of thyrocalcitonin postprandially occurred in response to the increased level of circulating gastrin or to some other gastrointestinal factor or event remains to be clarified (10, 11). In the present study we used both rats adapted to a feeding schedule (10) and unadapted rats. The purpose of the experiments was to explore whether or not a decrease in blood thyrocalcitonin after thyroidectomy might lead to an increased blood level of gastrin indicating that thyrocalcitonin normally acts to suppress gastrin release. Surprisingly, the results showed that surgical intervention of any kind with the thyroparathyroid complex resulted in a suppression of the gastrin response to feeding.

*Materials and methods. Animals.* Male Holtzman rats 40-50 days old and weighing 150-200 g were adapted to a fixed schedule of daily feeding for 2 weeks or more as described previously (10). Food (Purina Laboratory Chow) was made available to each individually housed rat for 12 hr and then withheld for 12 hr. The time of onset of feeding and of food removal were kept the same (± 5 min) for each day. In order to ascertain possible effects of the light-dark period (12 hr each), half of the rats were fed during the dark period and half during the light period. However, since no differences were found between rats fed during the dark and those fed during the light, results from appropriate groups were combined for presentation. As described previously (10), food consumption by rats during the first 2 hr of feeding was monitored to ensure that no significant differences occurred between different groups being compared. Rats not adapted to a feeding schedule were simply fed Purina Laboratory Chow *ad lib.* except for the 24 hr period before the experiment when they were fasted; after a 24 hr fast, food was presented and the rats allowed to feed for the desired time interval. All rats were allowed water *ad lib.*

*Surgical procedures.* All surgery was performed under ether anesthesia. In order to allow time for resumption of normal feeding patterns, rats were not used for experiments until at least 2 days after surgery. Thyroparathyroidectomy, thyroidectomy, and parathyroidectomy all were achieved by blunt dissection. Thyroidectomy was performed on rats which had previously been subjected to autotransplantation of the parathyroid glands, and rats were supplemented with thyroxine as previously described (12). Parathyroid autotransplantation was achieved by surgically removing both glands and placing them either on or within the thyroid tissue or laterally away from the thryoid in the sternohyoid muscle. Sucess of the transplantation procedure was determined for each rat 10-14 days after surgery by plasma calcium analysis as described previously (12). To interrupt vagal innervation to the stomach and still permit

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gastric emptying, truncal vagotomy plus pyloroplasty were performed through a single midventral incision. Both trunks of the vagus nerve were transected at the distal end of the esophagus. A 3 mm longitudinal incision then was made at the pylorus through the mucosa, and the incision was closed with 6-0 nylon sutures. Control rats were subjected to pyloroplasty only.

*Serum analyses.* Radioistopes ($^{45}Ca$ and $^{32}P$, 100 $\mu$Ci each) were injected 1 week before blood samples were obtained. Blood was drawn from each rat by cardiac puncture and allowed to clot. Serum was obtained by centrifugation within 1 hr of blood collection. Analyses of calcium (13), phosphorus (14), $^{45}Ca$, and $^{32}P$ (10, 15) were performed on freshly obtained aliquots of serum; the remaining serum sample from each rat was stored at −20°C for subsequent radioimmunoassay.

The procedures used for the radioimmunoassay for serum gastrin have been described previously in detail (16, 17). Equilibrium assays were conducted using a guinea pig antiserum to porcine gastrin (final dilution = 1:100,000), and synthetic 1-17 human gastrin (ICI, England) was used as unlabeled reference standard. Thus the values we report for gastrin in rat serum are in terms of pg-equivalents of synthetic human gastrin. Bound and free labeled gastrin were separated using amberlite resin (16, 17). The lower limit of detectability of the assay as conducted was 20 pg/ml, and intra- and interassay variations, as reported previously (10), did not exceed 15 and 20%, respectively.

*Results.* Figure 1 summarizes results obtained for serum gastrin in rats adapted to a fixed schedule of daily feeding. Previously we reported the serum gastrin changes in these rats during the period extending from 5 hr before feeding to 2 hr after the onset of feeding (10). Also reported were changes in calcium, phosphate, $^{45}Ca$, $^{32}P$, and thyrocalcitonin (10). The results in Fig. 1 extend our findings to cover the gastrin changes over a 24 hr period. Serum gastrin levels were measurable at all time periods studied, reaching a nadir 1 hr before feeding (~150 pg/ml), rising rapidly more than fivefold 1-2 hr after the onset of feeding and remaining at levels >500 pg/ml for most of the daily

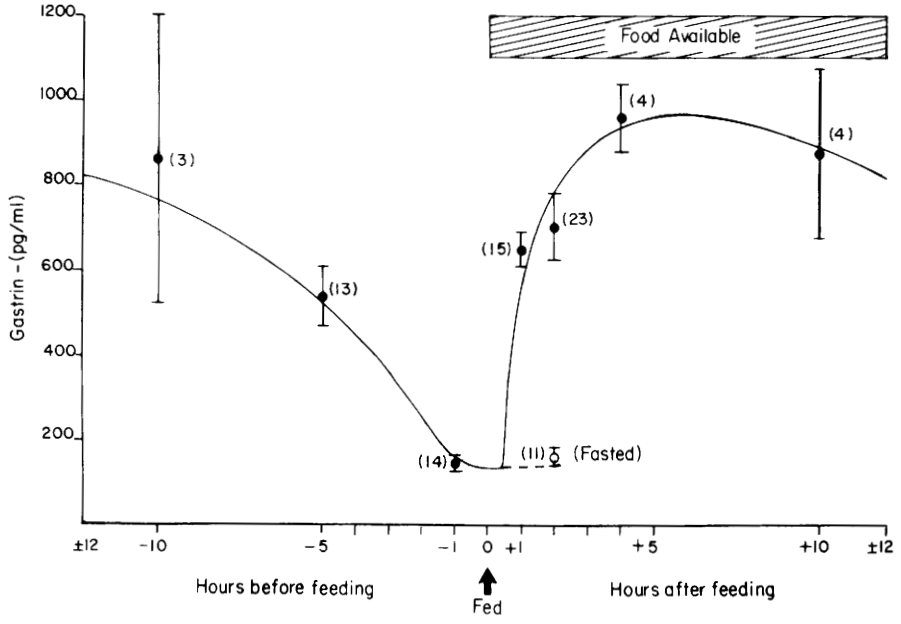


FIG. 1. Cyclic change in serum gastrin over 24 hr in intact rats adapted to a fixed schedule of daily feeding. Time is expressed in relation to the onset of feeding (= 0 time). Each point represents the mean value for a group of rats whose number is shown in parentheses; vertical lines show the SE. Open circle and dashed line represent a group of rats whose food was withheld at 0 time. Light-dark schedules were adjusted so that some rats were fed during light and others fed during dark (see Methods).

period before the next scheduled feeding. Serum gastrin remained low 2 hours after the scheduled feeding period if food was withheld. The postprandial increase in gastrin was not associated with an increase in blood calcium, since this is when daily serum calcium is lowest in adapted rats (10). Furthermore, as we have noted previously (10), serum gastrin levels were unusually high in these "adapted" rats. Even the lowest value obtained (~150 pg/ml) was higher than values obtained 1 hr after feeding in most nonadapted rats (Table I). The results shown in Table I are representative of our findings in several experiments with nonadapted rats, namely that serum gastrin was undetectable (<20 pg/ml) in rats fasted for 20 hr or longer and that subsequent feeding raised serum gastrin to only about 100–150 pg/ml 1–2 hr later.

The results shown in Table II illustrate that the pattern of gastrin response in rats adapted to the regular feeding schedule was not abolished by disruption of the normal vagal influence on gastrin release. As reported previously by others (18), we found that vagotomized rats exhibit an elevated serum level of gastrin before and after feeding compared to control rats which, in this experiment, had been subjected to pyloroplasty alone. The rats with pyloroplasty alone exhibited serum gastrin levels which were not different from unoperated rats (see Fig. 1) at the time periods studied.

Figure 2 summarizes the serum gastrin changes in rats adapted to the daily feeding schedule and subjected to surgical intervention with the thyroparathyroid complex. At the time periods examined just before and after feeding, thyroparathyroidectomized, parathyroidectomized, and thyroidectomized (with autotransplanted parathyroids) rats all showed marked reductions in fasting gastrin levels and a severely impaired gastrin response to feeding. Rats with intact thyroid glands and autotransplanted parathyroid glands exhibited a delayed rise (at 1 hr) and less marked (at 2 and 6 hr) suppression of serum gastrin. In addition to a reduced response to feeding, these groups of rats also appeared to have a delayed response, since unlike in intact control rats no significant rise in serum gastrin was observed at 1 hr after the onset of feeding. In a few rats (Fig. 2—sham) the parathyroid glands were excised and then replaced either in the normal location on the surface of the thyroid or within the thyroid tissue. These rats (seven at each time period), adapted to the fixed feeding schedule, showed serum gastrin levels of 671 ± 124 pg/ml 1 hour after feeding, and 839 ± 287 pg/ml 2 hr after feeding. These preliminary findings suggest but do not establish that a close approximation of the parathyroid and thyroid glands is required to prevent the reduced gastrin response observed after surgical intervention with the thyroparathyroid complex.

In order to determine whether the re-

TABLE I. SERUM GASTRIN IN FASTED AND FED RATS NOT ADAPTED TO A FIXED SCHEDULE OF DAILY FEEDING.

| Treatment | Serum gastrin pg/ml |
|---|---|
| 1. Fasted 24 hr (4) | N.D.[a] |
| 2. Fasted 24 hr + fed 1 hr (9) | 89 ± 23 |

[a] N.D. = Not detectable (value for each rat was <20 pg/ml). Fed rats were offered Purina Laboratory Chow for 1 hr after a 24 hr fasting period. Each rat consumed 4–6 g. Serum gastrin was measurable in each rat in group 2. Serum Ca (mg/dl) for each rat was between 10.0 and 10.5. Numbers in parentheses represent the number of rats in each group.

TABLE II. SERUM GASTRIN IN VAGOTOMIZED RATS ADAPTED TO A FIXED SCHEDULE OF DAILY FEEDING.

| Treatment | Serum gastrin pg/ml | | | |
|---|---|---|---|---|
| | −5 Hr | −1 Hr | +1 Hr | +2 Hr |
| 1. Pyloroplasty | 401 ± 111 (4) | N.T.[a] | 558 ± 94 (6) | 561 ± 106 (4) |
| 2. Pyloroplasty + Vagotomy | 1108 ± 318 (4) | 297 ± 52 (4) | N.T. | 1619 ± 274 (5) |

[a] N.T. = Not tested. Times shown are expressed relative to the time of onset of feeding (0 hr) when rats were offered Purina Laboratory Chow. Each rat consumed 4–6 g. Numbers in parentheses represent the number of rats in each group. Daily plasma calcium was within the range for normal rats at each hour tested.

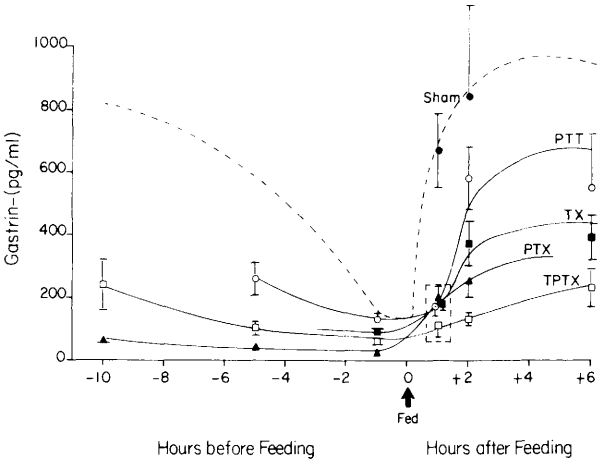


FIG. 2. Cyclic changes in serum gastrin in rats adapted to a fixed schedule of daily feeding and subjected to surgical intervention with the thyroparathyroid complex. Surgical procedures employed were "Sham" (parathyroids removed and replaced in thyroid), PTT (parathyroids autotransplanted in neck muscle), TX (thyroidectomy with parathyroids autotransplanted to neck muscle), PTX (parathyroidectomy), and TPTX (thyroparathyroidectomy). Box at +1 hr emphasizes the reduced serum gastrin in all groups except "Sham" with parathyroids in normal location. Numbers of animals at −1 hr to +2 hr = 9–23; all other points represent 3–12 animals. Dashed line represents curve for normal rats shown in Fig. 1. See Fig. 1 legend for additional details. Plasma calcium levels for all rats with functional parathyroid glands were >10 mg/dl at −5 hr; for rats without parathyroids plasma calcium levels were between 7 and 8 mg/dl.

duced gastrin response to feeding in the parathyroid transplanted rats conditioned to a feeding schedule (Fig. 2) was somehow unique, rats *not* adapted to this feeding schedule also were studied to examine the effect of thyroparathyroid surgery on serum gastrin 2 hr after feeding (Table III). Rats subjected only to parathyroid autotransplantation showed an increase in blood gastrin similar to that seen in nonadapted intact rats (see Table I). However, rats which in addition had been thyroidectomized for 48 hr showed no detectable increase in serum gastrin after feeding.

In order to explore further the apparent 1 hr delay in the serum gastrin response to feeding in the adapted rats subjected to surgery (Fig. 2), the study summarized in Fig. 3 was conducted. Changes in plasma calcium, phosphate, $^{45}Ca$, and $^{32}P$ as well as gastrin were evaluated in intact and parathyroid transplanted rats from 1 hr before feeding to 2 hr after feeding. In this experiment both parathyroid glands were excised and transplanted laterally in the neck. Except for serum gastrin, values are shown as percent change from the plasma concentration observed 1 hr before feeding. Plasma calcium is not shown in Fig. 3 because by 1 hr before feeding, as previously reported (10), plasma calcium had already fallen in both groups and did not change further at either +1 hr or +2 hr. Serum gastrin levels in the rats with parathyroids transplanted away from the thyroid did not differ significantly from those in intact rats 2 hr after feeding. However, at +1 hr the transplanted rats exhibited a significantly lower serum gastrin

TABLE III. SERUM GASTRIN IN THYROIDECTOMIZED AND THYROID INTACT RATS WITH AUTOTRANSPLANTED PARATHYROID GLANDS AND NOT ADAPTED TO A FIXED SCHEDULE OF DAILY FEEDING.

| *Treatment* | *Serum gastrin pg/ml* |
|---|---|
| 1. Parathyroid transplanted | |
| a. Fasted 24 hr | N.D.[a] |
| b. Fasted 24 hr + fed 2 hr | 88 ± 22 |
| 2. Parathyroid transplanted + thyroidectomized | |
| a. Fasted 24 hr | N.D. |
| b. Fasted 24 hr + fed 2 hr | N.D. |

[a] N.D. = Not detectable (value for each rat was <20 pg/ml). Fed rats were offered Purina Laboratory Chow for 2 hr after a 24 hr fasting period. Each rat consumed 5–10 g. Plasma Ca values for each group averaged between 10.1 and 10.3 mg/dl (five rats/group).

than intact rats. Likewise, the transplanted rats showed either a delayed or reduced change at +1 or +2 hr in serum phosphate, $^{32}P$, and $^{45}Ca$ (Fig. 3).

*Discussion.* Several recent studies have suggested that in man and certain other species high levels of blood thyrocalcitonin may act to suppress gastrin secretion and a variety of other gastrointestinal functions (5-9). However, these studies generally have employed large doses of administered calcitonins (8, 9). Because of this, no strong evidence exists to support the idea that physiological levels of circulating thyrocalcitonin influence secretion of gastrin.

This study was originally designed to determine whether or not removal of endogenous thyrocalcitonin by thyroidectomy might lead to an elevation in blood gastrin. We relied heavily on studies in rats adapted to a specific feeding schedule (Fig. 1), because these rats exhibit postprandial increases in gastrin and fasting serum gastrin levels which are much greater than those in nonadapted rats (Tables I and III) and which become even more pronounced after truncal vagotomy (Table II). Unexpectedly, we found that thyroidectomy produced a delayed and reduced increase in serum gastrin instead of an increase (Fig. 2) and that this occurred also in rats that had not been adapted to a daily feeding schedule (Table III). Further studies revealed that any surgical intervention which disrupted the normally close apposition of the thyroid and parathyroid glands reduced the basal serum gastrin and delayed the usual increase in gastrin after feeding (Fig. 2). The reasons for the reduced and/or delayed rise in gastrin are unclear at present. A low blood calcium in PTX and TPTX rats might be involved since gastrin release is known to be influenced by serum calcium (1, 19, 20). Alternatively, parathyroid hormone may have some direct effect on gastrin secretion (21-23). However, a reduced serum gastrin was observed also in thyroidectomized rats with parathyroid transplants having a normal serum calcium and thyroxine supplement (Fig. 2); presumably these rats were deficient in thyrocalcitonin. Even rats with both parathyroid and thyroid glands intact but with the glands separated anatomically showed a reduced and delayed rise in gastrin

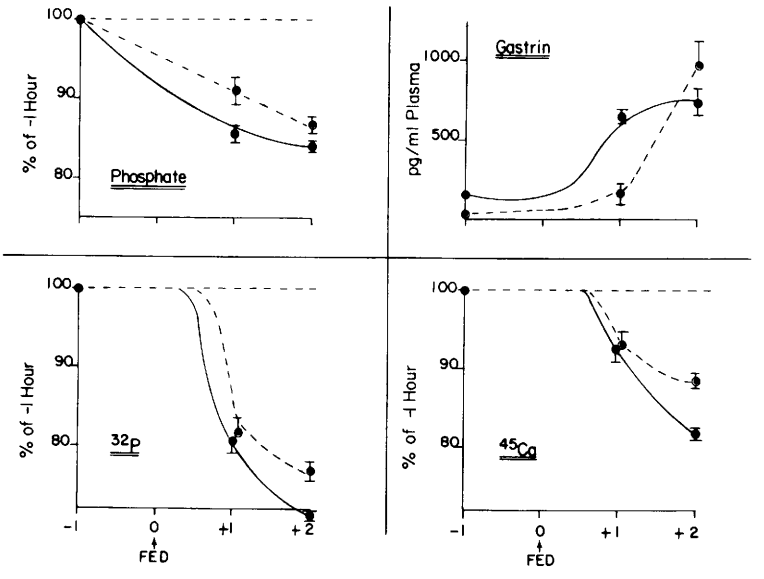


FIG. 3. Changes in serum gastrin, phosphate, $^{32}P$, and $^{45}Ca$ in intact rats (—●—) and parathyroid autotransplanted (PTT) rats (--●--) adapted to a fixed schedule of daily feeding. Parathyroid glands were autotransplanted laterally into neck muscles. Except for gastrin, the results are expressed as relative change of each group from the value observed 1 hr before feeding. Plasma calcium and phosphate values at the −1 hr period were not statistically different for the two groups. See Fig. 1 legend for additional details.

after eating (Fig. 2, PTT vs Control). In similarly treated rats, delayed or reduced changes in phosphate, $^{32}P$, and $^{45}Ca$ also were observed (Fig. 3). Interestingly, rats with their own parathyroids autotransplanted back within or on the surface of the thyroid showed a normal rise in blood gastrin after feeding. The results show that the thyroid and parathyroid glands are required for normal gastrin secretory responses after feeding and suggest that the normally close anatomical apposition of these two glands may be of some special significance.

*Summary.* Rats adapted for 2–3 weeks to a fixed schedule of daily feeding exhibited high serum gastrin levels and large increases in serum gastrin after feeding. Serum gastrin was >500 pg/ml throughout most of the 24 hr daily cycle and fell to ~150 pg/ml 1 hr before the scheduled time of feeding; by 1–2 hr after feeding serum gastrin rose again to >500 pg/ml. This postprandial increase in gastrin was not observed if food was withheld and it was seen also after truncal vagotomy which itself raised serum gastrin. In contrast, in nonadapted rats serum gastrin was unmeasurable (<20 pg/ml) after a 24 hr fast and rose only to ~100 pg/ml 1–2 hr after feeding. In nonadapted rats, thyroidectomy prevented the 2 hr rise in serum gastrin after feeding. In adapted rats parathyroidectomy, thyroparathyroidectomy, and thyroidectomy with parathyroid autotransplantation, all reduced serum gastrin before and after feeding indicating that any surgical intervention with the thyroparathyroid complex interfered with gastrin release. Rats with autotransplanted parathyroid glands and intact thyroid glands showed a delayed increase in serum gastrin after feeding, as well as a delay in the usual fall in plasma phosphate, $^{45}Ca$, and $^{32}P$, but if the parathyroids were replaced within the thyroid tissue, this delay did not occur.

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