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Bombesin Stimulates Gastrin Secretion in the Rat without Increasing Serum Calcitonin (40699)[1, 2]

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Gastrin and its synthetic analog, pentagastrin, are potent calcitonin (CT) secretagogues in several mammalian species, including man (1–5). However, in the laboratory rat we have not found either gastrin or pentagastrin to be a potent CT secretagogue (6–8). This conclusion was based both on *in vivo* studies where large doses of gastrin or pentagastrin were given i.v. (6) and on *in vitro* studies where thyroid glands were incubated in medium containing various concentrations of gastrin or pentagastrin (7). Similar independent *in vivo* studies in the rat by Garel and associates (9, 10) have led them to this conclusion, also.

The present study was designed to see whether or not endogenous secretion of gastrin in the rat would act as a CT secretagogue. Since the structure of rat gastrin has not been elucidated, it was possible that some unique unrecognized feature of homologous rat gastrin might endow it with CT secretory activity in the rat. To provoke gastrin release, we employed the amphibian tetradecapeptide, bombesin, which has been shown to be a potent and highly efficacious secretagogue in the dog, cat, and human (11–14). Our results show that bombesin stimulates gastrin release in the rat as well but that the increased gastrin does not, in turn, provoke demonstrable increases in blood levels of CT.

Materials and methods. Animals. Male Sprague–Dawley rats, 5–8 weeks old and weighing 120–200 g, were obtained from ARS/Sprague–Dawley (Madison, Wis.). Following their receipt, the rats were maintained on Wayne Laboratory Blox (Granville Milling Co., Creedmore, N.C.) until use. The rats were fasted for 18–26 hr just before use.

Procedures. Separate groups of rats were lightly anesthetized with ether and injected i.v. (tail vein) with synthetic bombesin (Bachem, Inc., Torrance, Calif.). In one experiment synthetic cyclic ovine somatostatin (SRIF, Beckman Co., Palo Alto, Calif.) also was injected i.v., and it was given 1 min before bombesin. Both peptides were dissolved in 0.15 *M* NaCl to give the desired dose in 0.5 ml, and this volume was injected. Control rats received 0.5 ml of 0.15 *M* NaCl. At the desired time intervals after injection, blood samples were drawn under ether anesthesia by cardiac puncture using a 20-gauge, 1-in. needle. Samples were placed on ice and allowed to clot. Serum was obtained by centrifugation within 1 hr of blood collection. Each serum sample was subdivided, one portion being analyzed freshly for calcium and inorganic phosphorus and the other being stored frozen at −20° until subjected to radioimmunoassays.

Analytical procedures. Calcium and inorganic phosphorus in serum were determined using an AutoAnalyzer II (Technicon, Tarrytown, N.Y.). The procedures are modifications of previously described analytical procedures (15–17). CT in serum was measured using a radioimmunoassay for rat CT which has been described in detail previously (7, 8). The homologous assay utilizes a chicken antiserum to rat CT (final dilution 1:10,000) and purified native rat CT for iodination and as unlabeled reference standard. Gastrin in serum was measured using a radioimmunoassay described earlier in detail (18, 19). A guinea pig antiserum to porcine gastrin (final dilution 1:100,000) was used, and synthetic human G-17 (ICI, England) was employed for iodination and as unlabeled reference standard.

Statistical analyses. For serum calcium and phosphorus, the data were subjected to anal-

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ysis of variance, and standard errors (SEM) were calculated from the residual error term of the appropriate analysis (20). The significance of differences between mean values was evaluated using either the F test or the multiple comparisons test of Schéffe (20). Radioimmunoassay values were subjected to nonlinear regression analysis (21) on an IBM 1130 computer. For experiments where assay values in most groups exceeded the lower limit of assay detectability, mean values ± SEM were calculated; where most values were below limits of detectability, individual values are presented. For hormone values, the significance of differences between groups was determined using the nonparametric rank sum test of Wilcoxon for unpaired measurements (20).

Results. Results shown in Fig. 1 are representative of those obtained in several identical experiments conducted. Figure 1A shows that bombesin, given in doses of 10 or 50 μg i.v. for 5 or 30 min, produced no change in serum calcium compared to control rats given saline i.v. for 5 min. In this experiment no change in serum phosphorus was observed either, although a small but significant hypophosphatemia occurred after bombesin in some of the experiments not shown. Figure 1B shows the values for serum CT and gastrin in the same rats. Both doses of bombesin produced a significant increase of approximately twofold in gastrin within 5 min, and by 30 min even greater increases were apparent. In spite of the marked hypergastrinemia induced by bombesin, serum CT remained undetectable in all four groups given bombesin, and this was confirmed in other experiments not shown.

To ensure that a possible bombesin-induced increase in serum CT was not inadvertently missed, both dose–response and time-course studies were conducted using bombesin (Table I and Fig. 2). Figure 2A and Table I show that no substantial effects of bombesin on serum calcium or phosphorus were observed. The results in Fig. 2B show that large and significant increases in serum gastrin occurred between 5 min and 2 hr after bombesin at a dose of 50 μg/rat. Results shown in Table I illustrate that the doses of bombesin used in most of the studies (10 and 50 μg/rat) were appropriate, since a dose higher than 50 μg/rat did not lead to a further increase in serum gastrin 30 min after injection. Both Fig. 2B and Table I illustrate that,

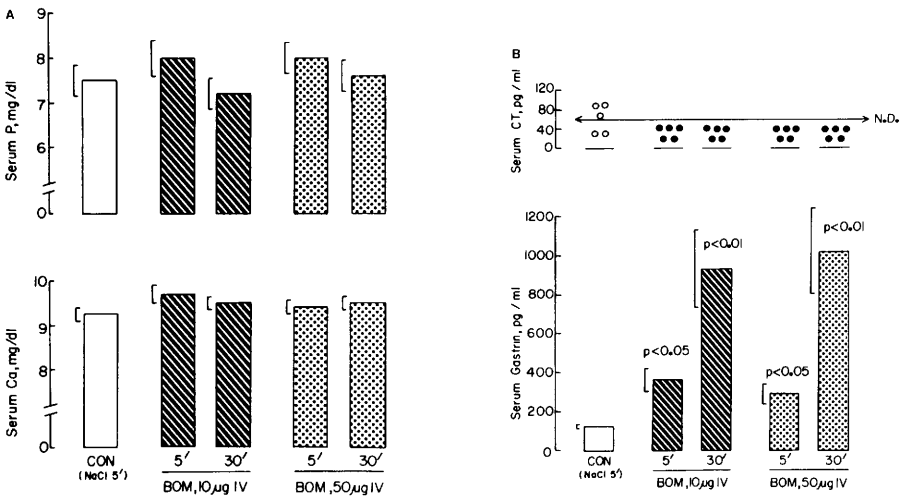

FIG. 1. (A) Concentrations of calcium and inorganic phosphorus in serum of 7-week-old rats injected with bombesin (BOM) either 5 min (5') or 30 min (30') earlier. The height of each bar represents the mean value for a separate group of five rats, and the bracket shows the SEM. The control rats (CON) received saline i.v. 5 min before blood collection. All rats had been fasted for 21 hr. (B) Concentrations of gastrin and CT in the same rats shown in (A). Bars and brackets in lower graph represent mean values ± SEM. Upper graph shows individual values for each rat; horizontal arrow represents lower limit of detectability for the immunoassay (N.D. = not detectable). P values refer to comparison of mean values of rats given BOM to CON rats.

TABLE I. SERUM CALCIUM, GASTRIN, AND CT 30 MIN AFTER INJECTION OF VARIOUS DOSES OF BOMBESIN[a]

Dose of bombesin (μg)	No. rats	Serum Concentrations: Calcium (mg/100 ml)	Gastrin (pg/ml)	CT (pg/ml)
0 (Control)	6	9.53 ± 0.12	50 ± 10	ND[b]
0.016	5	9.41 ± 0.12	30 ± 13	ND
0.08	6	9.55 ± 0.12	40 ± 11	ND
0.4	5	9.36 ± 0.12	38 ± 12	ND
2.0	6	9.20 ± 0.12	100 ± 43	ND
10.0	6	9.35 ± 0.12	152 ± 59*	ND
50.0	6	9.48 ± 0.12	269 ± 82**	ND
100.0	6	9.53 ± 0.12	204 ± 58*	ND

[a] Each value presented represents the mean ±SEM for a separate group of rats. Differs from control group: * $P < 0.05$, ** $P < 0.01$. Control rats received 0.5 ml 0.15 *M* NaCl i.v.

[b] Not detectable (all values < 30 pg/ml).

despite the increases in serum gastrin produced by bombesin, the hypergastrinemia was not accompanied by demonstrable elevations in serum CT. In fact, serum CT remained low or undetectable in both control and bombesin-treated rats. These low or undetectable levels are characteristic for fasted rats of this age (6).

In one experiment (Fig. 3) bombesin and somatostatin (SRIF) were given both alone and together. No effects on serum calcium or phosphorus were observed (Fig. 3A). Figure 3B shows that bombesin alone produced a marked increase in serum gastrin. SRIF alone did not alter serum gastrin levels, but when SRIF was given with bombesin it significantly reduced, but did not eliminate, the bombesin-induced hypergastrinemia. In this particular experiment almost all of the rats had detectable levels of serum CT (Fig. 3B, top graph), the values ranging from ≈ 100 to 200 pg/ml. However, no significant changes in serum CT were observed in any of the experimental groups receiving SRIF or bombesin.

Discussion. In several mammals, including man, the gastrointestinal hormones, gastrin and cholecystokinin, clearly are CT secretagogues (1–5). In the pig, we even have shown that gastrin is an effective stimulator of CT release at blood levels of gastrin which occur normally after a meal (8). Surprisingly, however, in the rat gastrin is weak or ineffective in provoking CT release as judged by both *in vivo* or *in vitro* experiments where thyroid glands were exposed to high doses of pentagastrin and human or porcine heptadecapeptide gastrin (6–8). Since the biological activity of gastrin resides in the C-terminal tetrapeptide amide, a region highly conserved in the mammalian gastrins which have been se-

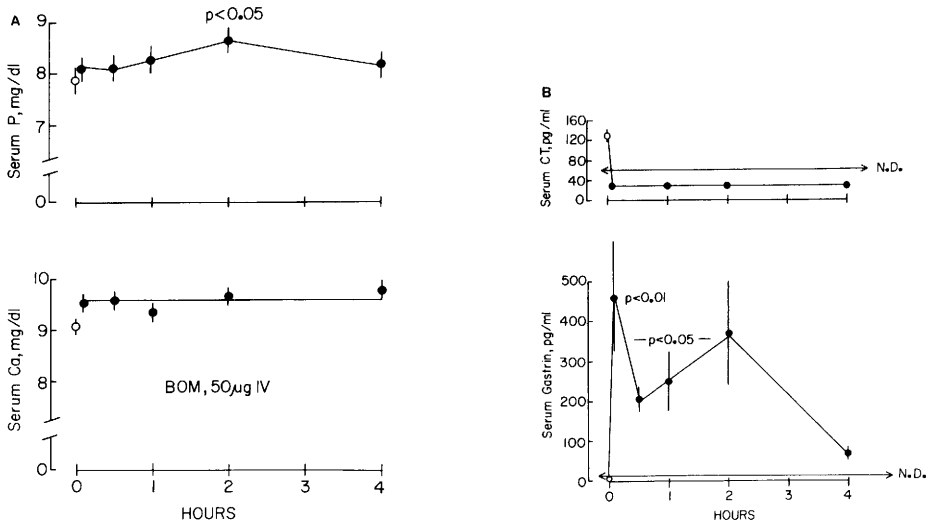

FIG. 2. (A) Concentrations of calcium and inorganic phosphorus in serum of 6-week-old rats injected with a single dose of bombesin (BOM) and bled at times ranging from 5 min to 4 hr later (●). Control rats not given bombesin (○) received an injection of saline and were bled 5 min later. Each point represents the mean value for a separate group of five rats, and vertical lines shown the SEM. (B) Concentrations of gastrin and CT in the same rats shown in (A). See Fig. 1B legend for additional details.

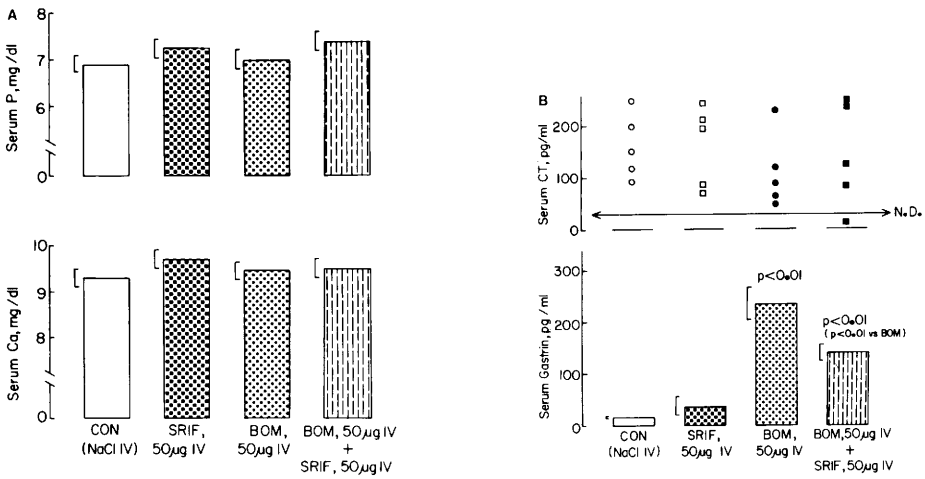

FIG. 3. (A) Concentrations of calcium and inorganic phosphorus in serum of 7-week-old rats injected with somatostatin (SRIF) or bombesin (BOM) alone or in combination. Rats were bled 30 min after injection; control rats (CON) received saline i.v. 30 min before blood collection. All rats had been fasted for 20 hr. See Fig. 1A legend for additional details. (B) Concentrations of gastrin and CT in the same rats shown in (A). See Fig. 1B legend for additional details.

quenced (22), it seemed unlikely that failure to elicit a CT response in the rat could be attributed to use of heterologous gastrins. However, since the structures of gastrin moieties in the rat, including G-17, are not known, it was possible that some unappreciated special feature of the rat gastrin molecule is required for CT-secretory activity in this species. Therefore, in the present study we attempted to provoke secretion of endogenous rat gastrin in large amounts to see whether or not the hypergastrinemia would lead to increased secretion of CT. For a gastrin secretagogue we chose bombesin, an amphibian tetradecapeptide which has been shown to be effective in a number of mammalian species (11–14).

Our findings clearly show that bombesin is effective in promoting gastrin secretion in the rat (Figs. 1B, 2B, and 3B and Table I) as has been shown with other mammals. The effect on gastrin in the rat is dose dependent (Table I), rapid in onset (Figs. 1B and 2B), and lasts for several hours after a single i.v. injection (Fig. 2B). Furthermore, the increase in gastrin after bombesin can be partially inhibited by somatostatin (Fig. 3B), which itself did not influence basal serum gastrin in these experiments. It is of interest (i) that somatostatin did not inhibit basal gastrin in the rat, as it does in a variety of other mammalian species, including the pig (19), and (ii) that unlike the case in man (23) and the pig (24), somatostatin did not inhibit CT secretion in the rat (Fig. 3B).

Despite the fact that bombesin produced a marked hypergastrinemia in the rat, in none of the experiments did we find an increase in serum CT. This was true both for experiments where basal serum CT was close to or below the lower limit of assay detectability (Fig. 1B and Table I) and those where it was clearly measurable (Figs. 2B and 3B). In none of the experiments did we observe any consistent effects of bombesin on serum calcium or phosphorus. Our findings in the rat are in marked contrast to those in the pig where bombesin-induced hypergastrinemia rapidly is followed by six- to eightfold increases in CT secretion (25). The failure to observed CT secretion in the rat during hypergastrinemia confirms our previous impression that gastrin is a poor CT secretagogue in this species. We conclude that some other unidentified gastrointestinal factor or event must be involved in the CT response observed in the rat following feeding or administration of calcium-free solutions (8, 10, 26, 27).

Summary. Bombesin was administered to young fasted rats, and blood levels of immunoreactive gastrin and calcitonin (CT) were measured. Doses of bombesin up to 100 μg/

rat i.v. did not affect serum calcium or phosphorus. Five minutes after bombesin, 10 or 50 μg/rat, serum gastrin increased 1- to 2-fold and, by 30 min, it was elevated 5- to 10-fold. Time-course and dose–response studies showed that doses of 10–100 μg/rat increased gastrin and that gastrin rose within 5 min after 50 μg/rat and remained elevated for at least 2 hr. Despite bombesin-induced hypergastrinemia, CT remained low or undetectable (<50–100 pg/ml) at all times. In one study, somatostatin (50 μg/rat i.v.), given together with bombesin, inhibited the rise in serum gastrin by 50%, but serum CT, which was detectable, was unaffected by either bombesin or somatostatin. We conclude that bombesin stimulates gastrin release in the rat, but the elevated gastrin does not promote CT secretion. The results support our earlier findings which indicate that gastrin is weak or ineffective in the rat as a CT secretagogue.

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1. Care, A. D., Bruce, J. B., Boelkins, J., Kenny, A. D., Conaway, H., and Anast, C. S., Endocrinology **89,** 262 (1971).
2. Cooper, C. W., Schwesinger, W. H., Mahgoub, A. M., and Ontjes, D. A., Science **172,** 1238 (1971).
3. Cooper, C. W., Schwesinger, W. H., Ontjes, D. A., Mahgoub, A. M., and Munson, P. L., Endocrinology **91,** 1079 (1972).
4. Hennessy, J. F., Wells, S. A., Jr., Ontjes, D. A., and Cooper, C. W., J. Clin. Endocrinol. Metabol. **39,** 487 (1974).
5. Heath, H., III, and Sizemore, G. W., J. Clin. Invest. **60,** 1135 (1977).
6. Cooper, C. W., Obie, J. F., and Hsu, W. H., Proc. Soc. Exp. Biol. Med. **151,** 183 (1976).
7. Cooper, C. W., Ramp, W. K., Becker, D. I., and Onties, D. O., Endocrinology **101,** 304 (1977).
8. Cooper, C. W., Bolman, R. M., III, Linehan, W. M., and Wells, S. A., Jr., Recent Progr. Horm. Res. **34,** 259 (1978).
9. Garel, J.-M., and Jullienne, A., J. Endocrinol. **75,** 373 (1977).
10. Garel, J.-M., and Besnard, P., Endocrinology **104,** 1617 (1979).
11. Bertaccini, G., Erspamer, V., Melchiorri, P., and Sopranzi, N., Brit. J. Pharmacol. **52,** 219 (1974).
12. Erspamer, V., and Melchiorri, P., *in* "Gastrointestinal Hormones" (J. C. Thompson, ed.), p. 375, Univ. of Texas Press, Austin (1975).
13. Llanos, O. L., Villar, H. V., Konturek, S. J., Rayford, P. L., and Thompson, J. C., Ann. Surg. **186,** 614 (1977).
14. Melchiorri, P., *in* "Gut Hormones" (S. R. Bloom, ed.), p. 534. Churchill Livingstone, New York (1978).
15. Kessler, G., and Wolfman, M., Clin. Chem. **10,** 686 (1964).
16. Kraml, M., Clin. Chem. Acta **13,** 422 (1966).
17. Gitelman, H. J., Anal. Biochem. **18,** 521 (1967).
18. Hsu, W. H., and Cooper, C. W., Proc. Soc. Exp. Biol. Med. **154,** 401 (1977).
19. Bolman, R. M. III, Cooper, C. W., and Wells, S. A., Jr., Endocrinology **103,** 259 (1978).
20. Snedecor, G. W., and Cochran, W. G., "Statistical Methods," 6th ed., pp. 59, 130, and 265, Iowa State Univ. Press, Ames (1967).
21. Burger, H. G., Lee, V. W. K., and Rennie, G. C., J. Lab. Clin. Med. **80,** 302 (1972).
22. Grossman, M. I., Nature (London) **228,** 1147 (1970).
23. Gordin, A., Lamberg, B. A., Pelkonen, R., and Almqvist S., Clin. Endocrinol. **8,** 289 (1978).
24. Linehan, W. M., Cooper, C. W., Bolman, R. M., III, and Wells, S. A., Jr., Endocrinology **104,** 1602 (1979).
25. Cooper, C. W., Obie, J. F., and Crowell, J. A., Jr., Fed. Proc. **38,** 850 (1979).
26. Talmage, R. V., Doppelt, S. H., and Cooper, C. W., Proc. Soc. Exp. Biol. Med. **149,** 855 (1975).
27. Cooper, C. W., and Obie, J. F., Proc. Soc. Exp. Biol. Med. **157,** 374 (1978).

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