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Effects of enterally- and parenterally-administered bombesin on intestinal luminal tryptic activity and protein in the suckling rat

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Summary. Because of the presence of bombesin-like immunoreactivity in milk, we investigated if enteral administration of bombesin affects the intestinal luminal content of trypsin and protein in 12–14-day-old rats. Bombesin (40 μg/kg), given either orogastrically or subcutaneously, produced a significant elevation in the intestinal content of trypsin activity. Thus, enterally-administered bombesin can produce acute biologic effects in suckling rats.
Key words. Bombesin; milk peptides; pancreas; trypsin; suckling rat.

Bombesin is a tetradecapeptide first isolated from the skin of the frog, *Bombina bombina*[1]. Subsequently, structurally and functionally homologous peptides have been isolated and characterized in a number of mammalian species[2]. Bombesin and bombesin-related peptides have been shown to be potent pancreatic secretagogues, both in vivo[3] and in vitro[4], and specific bombesin receptors have been demonstrated on the pancreatic acinar cell[5]. Substances with bombesin-like immunoreactivity and biologic activity[6–9] have recently been demonstrated in mild. Since other substances contained in milk have been shown to exert biologic activity in suckling animals when given orally[10], it seemed reasonable to hypothesize that the bombesin-like peptides found in milk might also be capable of eliciting biologic effects on the suckling animal.

We therefore conducted experiments seeking to determine if bombesin, administered orogastrically, exerted effects on pancreatic exocrine secretions of suckling rats. Specifically, changes in the intestinal luminal content of trypsin and protein were evaluated. A preliminary report of these findings has been presented[11].

Methods. Sprague Dawley rats raised in our own colony were used throughout these experiments. Animals were exposed to an alternating cycle of light and darkness for 12-h periods. The date of birth was set as day 0, and litter size was corrected to 10 on day 2 of life in order to standardize growth and development. The mothers and their pups had free access to water and standard chow (Lab-Blox, Allied Mills, Chicago, IL). The pups were allowed to suckle normally until day 12–14 when they were fasted 12 h and then studied. They were kept in cages with half of the floor placed on a heating pad to enable them to maintain their own normal body temperature[12]. Experiments were conducted within litters; rats in each litter were divided into three groups of equal body weight. The first group, designated 'control' received 0.1 ml of 0.9 N NaCl with 0.2% bovine serum albumin (Sigma, St. Louis, MO), designated 'SAL/BSA' by s.c. injection as well as orogastrically through a 3.5 French catheter. The second group was designated 'OG' since rats in this group received 0.1 ml of a solution containing 40 μg/kg of bombesin tetradecapeptide (Bachem, Torrance, CA) dissolved in SAL/BSA orogastrically through a 3.5 French catheter, and 0.1 ml of SAL/BSA by s.c. injection. The third group was designated 'SQ' since rats in this group received 0.1 ml of a solution containing 40 μg/kg of bombesin tetradecapeptide dissolved in SAL/BSA by s.c. injection and 0.1 ml of SAL/BSA orogastrically through a 3.5 French catheter.

The goal of our study was to determine if OG bombesin could influence the luminal contents of trypsin or protein in an absolute sense. We therefore chose a dose of bombesin that has been shown to produce maximal increases of pancreatic protein, trypsin, and DNA contents in the pancreas of suckling rats [13], and maximal increases in serum gastrin concentration and antral gastrin content in adult rats [14]. All experiments were begun between 08.00 and 09.00 h. In one set of experiments, rats were killed by decapitation 15 or 30 min after bombesin administration. In another series of experiments, rats were killed by decapitation 1 h after injection. The small intestine was removed and divided into three segments of equal length, each of which was then flushed with ice-cold phosphate buffered saline at a volume of 1 ml/10 g b. wt followed by an equal volume of air. Each sample was then centrifuged for 20 min at 2000 × g and the supernatant removed and frozen at −20 °C. All samples of flush were assayed for content of protein and trypsin activity within 48 h of collection. Protein was measured by the method of Lowry [15] using bovine serum albumin as the standard. Trypsin activity was measured by the release of p-nitroaniline from the substrate benzoyl-DL-arginine p-nitroanilide (Sigma, St. Louis, MO) as described by Erlanger [16]. Units were expressed as nanomoles p-nitroaniline produced per min. All chemicals were of reagent grade. Values for the luminal flush content of protein and trypsin activity were determined for each of the three intestinal segments. These values for each animal were then added to yield the protein and trypsin activity per total intestine.

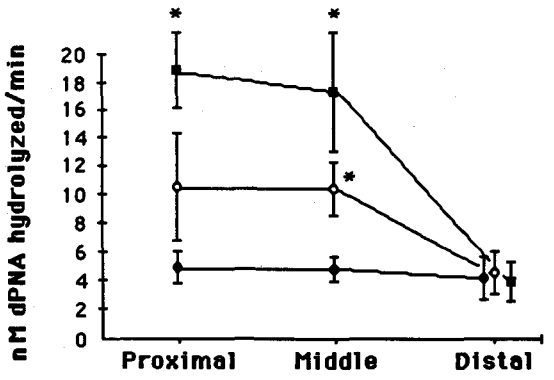
Data were expressed as the mean ±SE and evaluated statistically by analysis of variance and unpaired t-test when the F-test showed significant group differences [17]. Differences were considered significant when $p < 0.05$.

Results. Total small intestinal flush content of protein and trypsin activity was measured at 15, 30, and 60 min after treatment, and the results are shown in the table. There were not significant differences in protein content between groups at 15 min. The protein content of the intestinal flush was significantly elevated in the SQ group compared to the OG group at 30 min, and compared to both other groups at 60 min. Values for the control and OG groups did not differ at any time point studied.

Effect of bombesin (40 μg/kg) administered orogastrically (OG) or subcutaneously (SQ) on the content of protein and trypsin activity in the intestinal luminal flush. Values from the proximal, middle and distal segments were summed to yield results per whole intestine, and are shown as the mean ±SE of these summed values.

Group	Protein (μg/intestine)			Trypsin (units/intestine)		
	15 min	30 min	60 min	15 min	30 min	60 min
Control	620.73 ±17.7 (5)	541.9 ±63.6 (8)	435.6 ±54.8 (11)	12.4 ±2.2 (5)	13.9 ±1.8 (8)	16.7 ±1.2 (11)
OG	632.9 ±36.0 (7)	535.4 ±37.1 (11)	361.2 ±34.7 (12)	16.8 ±1.5 (7)	25.2[a] ±3.5 (11)	18.9 ±1.0 (12)
SQ	774.4 ±77.9 (6)	705.1[b] ±49.1 (9)	640.2[a,b] ±30.3 (11)	38.6[a,b] ±6.2 (6)	39.1[a,b] ±5.5 (9)	79.2[a,b] ±4.4 (11)

[a] $p < 0.05$ compared to control; [b] $p < 0.05$ compared to OG. Number of rats in each group is shown in parentheses.



Effect of subcutaneously (SQ) or orogastrically (OG) administered bombesin on the content of trypsin activity in the flush from proximal, middle, and distal intestinal segments at 15, 30 and 60 min after bombesin administration.
dPNA = d-para-nitroaniline. ■, Control; ○, OG; ◆, SQ. Values represent mean ±SEM of individual values. (*) = $p < 0.05$ when compared to control. Number of rats in each group are the same as shown in the table.

Fifteen min after bombesin administration, trypsin activity was significantly increased in the SQ group. At 30 min, trypsin activity was significantly increased in both the OG and SQ group, while at 1 h after treatment, trypsin activity was significantly increased in the SQ group. Results were similar when expressed as specific activity, i.e., units/mg protein (data not shown).

The control values for both protein and trypsin activity at the 15- and 30-min time periods did not differ significantly. Since the experiments using the 60-min time period were performed separately, no statistical comparisons were performed. Nonetheless, values for protein content did seem to fall with increasing time, although such a trend was not observed for trypsin activity.

Since both OG and SQ bombesin produced significant elevations of tryptic activity at 30 min, this time point was chosen for a more detailed analysis of the effects of the two routes of peptide delivery. The figure shows the mean ±SE values for content of trypsin activity in the luminal flush from the proximal, middle, and distal segments 30 min after bombesin administration. Differences between experimental groups in each time period were evaluated by analysis of variance and F-test for simple effects; differences between individual segments were evaluated using one-way analysis of variance and unpaired t-test when the F value indicated significant differences [17]. The pattern of changes in the content of trypsin activity in the intestinal flush of each segment observed in the OG and SQ groups were qualitatively similar. Values for the proximal and middle segments were significantly higher than those for the distal segments in both the SQ and OG groups, while no such gradient occurred in the control group. Trypsin activity in the proximal and middle segments was highest in the SQ group, did not

differ significantly from corresponding values in the OG group, but were significantly greater than control. Trypsin activity in these two segments in the OG group was more than double the corresponding control values, but only values for the middle segment achieved statistical sigificance ($p = 0.0169$) because of the large variation in values for the proximal segment in the OG group. Values for trypsin activity in the distal segment for all experimental groups did not differ.

Discussion. We found that orogastric administration of bombesin to suckling rats is associated with significant increases in the content of trypsin activity in the intestinal luminal flush. To our knowledge, this is the first time that enterally-administered bombesin has been shown to produce acute biologic effects in the suckling rat, and that these effects are qualitatively similar to those seen with subcutaneously-administered bombesin.

Naturally-occurring compounds found in milk have been shown to produce biologic effects when administered orally[18]. Thus far, the only peptide which has been shown to have effects on the exocrine pancreas when given enterally has been EGF[19]. Recently, Lehy[20] has presented preliminary data which show a stimulation of several indices of pancreatic and intestinal proliferation by bombesin administered enterally over several days. The results of this latter study of chronic oral administration of bombesin and our own study of the acute effects of enteral bombesin therefore demonstrate that bombesin, as well as EGF, affects the growth of the exocrine pancreas and/or its secretions when administered enterally. They also suggest that the bombesin-like peptides present in milk may produce both acute and chronic effects on the developing pancreas. Based on previously reported concentrations of bombesin-like immunoreactivity in milk[6-8], however, the doses of bombesin used in the study of Lehy[20] and our own are probably much higher than those to which the suckling rat is normally exposed. Utilization of more physiological doses of bombesin will require direct measurement of bombesin-like immunoreactivity in rat milk. It also remains to be determined whether these effects are produced directly by bombesin, by degradative products of bombesin, or indirectly by the stimulated release of other hormones[21]. It will also be necessary to study the effects of bombesin's mammalian counterparts, the gastrin-releasing peptides, as part of the evaluation of the physiologic significance of the effect described here.

A likely explanation for the increase in intestinal luminal content of trypsin activity seen with enteral administration of bombesin is stimulation of pancreatic enzyme secretion. Certainly, the pattern of response with OG bombesin was similar to that seen with SQ bombesin (fig.), a known stimulant of pancreatic exocrine secretion[3-5]. Chronic parenteral administration of cholecystokinin (CCK) has also been shown to increase the trypsin content of the intestinal luminal fluid, albeit after chronic parenteral administration[22]. This effect was also presumed to be produced by chronic stimulation of pancreatic enzyme secretion. Other, less likely mechanisms include the inhibition of trypsin absorption[23,24] or decreased luminal trypsin degradation secondary to increased bile secretion[25].

Regardless of the exact mechanism of the effect of OG bombesin observed in this study, however, the point to be emphasized is that we have clearly demonstrated that enteral bombesin is biologically active in the suckling rat. Moreover, this biological effect (increased luminal tryptic activity) may have nutritional significance in terms of altering the degree of intraluminal protein digestion occurring in the intestine at this age. This study, as well as the preliminary data of Lehy[20] would support further study into the exact nature, mechanism, and importance of the actions of enterally-delivered bombesin-like peptides in developmental physiology.

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Luffolide, a novel anti-inflammatory terpene from the sponge *Luffariella* sp.

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Summary. Luffolide (**4**) is a minor metabolite of the sponge *Luffariella* sp. from Palau. The structure of luffolide was determined by single crystal X-ray analysis. Luffolide is relatively unstable and undergoes a complex cyclization reaction to give the hexacyclic products **5** and **6**. Luffolide (**4**) has some of the anti-inflammatory properties of manoalide (**1**): this may help to define the chemical reaction between manoalide (**1**) and phospholipase A_2.
Key words. Luffolide; *Luffariella* sp.; X-ray structure determination; anti-inflammatory; phospholipase A_2.

Sponges of the genus *Luffariella* have provided a series of sesterterpenes that are potent anti-inflammatory agents. Both manoalide (**1**) and seco-manoalide (**2**), isolated from the sponge *Luffariella variabilis*[3], are irreversible inhibitors of the enzyme phospholipase A_2 (PLA_2)[4] while luffariellolide (**3**) is a partially reversible PLA_2 inhibitor that was isolated as a major metabolite of a Palauan species of *Luffariella*[5]. The same specimen of *Luffariella* sp. also contained a minor polycyclic sesterterpene, luffolide (**4**), that also inhibits hydrolysis of phosphatidylcholine by bee venom PLA_2.

The hexane-soluble material from a methanol extract of *Luffariella* sp. was chromatographed on silica gel, using solvents of increasing polarity from hexane to ethyl acetate, to obtain two antimicrobial fractions containing luffariellolide (**3**, 15.4% dry weight) and luffolide (**4**, 0.0037% dry weight). Quite unexpectedly, luffolide (**4**) crystallized from $CDCl_3$ in an NMR tube to produce colorless rods, mp. 123 °C, and a crystal was reserved for an X-ray study. Crystals formed in space group $P2_1$ with $a = 8.317(2)$, $b = 20.238(7)$, $c = 10.259(2)$ Å, and $\beta = 111.30(1)°$ with an asymmetric unit of $C_{28}H_{40}O_6 \cdot CHCl_3$. All diffraction maxima with $2\theta < 114°$ were collected on a computer-controlled four-circle diffractometer using 1° ω-scans and CuK$\bar{\alpha}$ radiation (1.54178 Å). Of the 2257 symmetry unique reflections, 1931 (94%) were judged observed ($|F_o| \geqq 3\sigma(F_o)$) after correcting for background, Lorentz, and polarization effects[6]. The structure was solved routinely using direct methods and was refined, using anisotropic nonhydrogens and fixed isotropic hydrogens, to a conventional crystallographic residual of 0.083 for the observed reflections. Additional crystallographic details have been deposited at the Cambridge Crystallographic Data Centre. The figure is a computer-generated perspective drawing of the final X-ray model of luffolide (**4**). Hydrogens have been omitted from the drawing, and the absolute configuration was not established by the X-ray experiment. The