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Effect of Bombesin and Gastrin-Releasing Peptide on the Release of Gastric Inhibitory Polypeptide and Insulin in Rats[1] (42377)

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Abstract. The objective of this study was to determine whether bombesin- or gastrin-releasing peptide-induced release of insulin occurs before or after the release of gastric inhibitory polypeptide (GIP) in rats. The present results demonstrate that GIP release occurs before insulin release and suggest that bombesin-like peptides and GIP interact to stimulate insulin secretion.

Bombesin is a 14-amino acid peptide which was originally isolated from frog skin (1, 2). A larger variant of bombesin, called gastrin-releasing peptide (GRP-27), has been isolated and characterized from porcine nonantral stomach (3, 4). Bombesin-like immunoreactivity is found widespread in the gastrointestinal tract and pancreas (5–9), suggesting that bombesin-like peptides play a role in gut and pancreatic function. Bombesin and GRP have been shown to increase circulating levels of insulin and gastric inhibitory polypeptide (GIP) as well as other gut peptides in a number of species (10–20). In fact, McDonald and coworkers (12) have reported that GIP release occurs after insulin release which suggests that GIP does not contribute to the initial insulinotropic action of bombesin or GRP. Whether bombesin and GRP stimulate GIP release before or after insulin release in rats is not known. The objective of this paper, therefore, was to determine whether bombesin and GRP cause GIP release before or after insulin release.

Materials and Methods. *Animals.* Male rats (220–225 g) were purchased from Timco (Houston, Tex.) and maintained in an air-conditioned (24 ± 2°C) and light-regulated (lights on, 0500–1700 hr) animal quarters for at least 7 days before the following experiments were done. All rats had free access to rat chow and water unless described otherwise.

Radioimmunoassays (RIAs): Insulin. A double-antibody RIA procedure was used to measure serum levels of insulin as described previously (19).

GIP. Serum GIP levels were measured using a double-antibody RIA method according to the published procedure of Kuzio and coworkers (21). The GIP antiserum was purchased from Quadra Logic, (Vancouver, British Columbia). The cross-reactivity of this GIP antiserum with glucagon, secretin, and VIP is <0.01%. In addition, it does not recognize bombesin or GRP.

Experimental design: Experiment 1. GRP (10 μg in 0.2 ml) was given intravenously (iv) under light ether anesthesia to rats which had been fasted for 24 hr (glucose concentration = 81 ± 4 mg/dl). Six rats were sacrificed before (0 min), and 5 and 10 min after intravenous administration of GRP. Control rats were given vehicle iv and sacrificed at identical times. Trunk blood was collected and serum was separated and stored at −20°C until it was analyzed for serum GIP and insulin levels.

Experimental design: Experiment 2. Bombesin (29 μg in 0.5 ml) was administered intraperitoneally (ip) to rats fed *ad libitum* (glucose concentration = 155 ± 8 mg/dl). Rats were sacrificed before (0 min) and at 5, 10, 15, 30, and 60 min after administration of bombesin. Trunk blood was collected and serum was analyzed for GIP and insulin levels as described above. Control rats were given vehicle iv and sacrificed at the identical times. GRP and bombesin were suspended in a vehicle consisting of 0.1% bovine serum albumin (BSA), 0.9% NaCl, and 0.05 *M* acetic acid. GRP and bombesin were purchased from Peninsula Laboratories (San Carlos, Calif.).

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Statistics. Concentrations of insulin and GIP are expressed as means ± SEM. Differences were evaluated by an analysis of variance (ANOVA) and the Newman-Keuls test (22), and differences with a P value of <0.05 were considered significant.

Results. *Experiment 1.* Serum insulin levels were elevated significantly 10 min after intravenous administration of GRP. Intravenous administration of vehicle alone did not alter circulating levels of insulin (Fig. 1). By contrast, serum GIP levels were significantly ($P < 0.05$) elevated at 5 and 10 min after intravenous administration of GRP, whereas intravenous administration of vehicle alone failed to alter circulating levels of GIP.

Experiment 2. In *ad libitum* fed rats, serum GIP levels were significantly elevated 5, 10, 15, 30, and 60 minutes after administration of bombesin (Fig. 2). Although administration of vehicle alone resulted in a significant elevation of serum GIP levels at 5, 10, and 15 min, the vehicle-induced elevations in serum GIP levels were significantly less when compared to rats given bombesin. Serum insulin levels were increased significantly 15 min after administration of bombesin (Fig. 3).

Discussion. The results of the present study show that in fasted rats, intraveneous administration of GRP, and in fed rats, intraperitoneal administration of bombesin result in a release of GIP before the release of insulin. This finding is important in the sense that bombesin- or GRP-induced release of GIP probably contributes to the insulinotropic action of bombesin-like peptides, and suggests that the elevation in circulating insulin levels after bombesin or GRP administration *in vivo* is a result of their combined stimulatory actions. Bombesin- or GRP-induced insulin release *in vivo* is probably not due to bombesin- or GRP-induced GIP release alone, since our laboratory has shown earlier that GRP and bombesin can stimulate insulin release directly from isolated rat islets *in vitro* (23).

The present findings contradict the observations of McDonald and colleagues (12), who demonstrated that administration of GRP to dogs stimulates release of insulin before serum GIP levels rise. Although speculative, the disparities in the results between these two studies

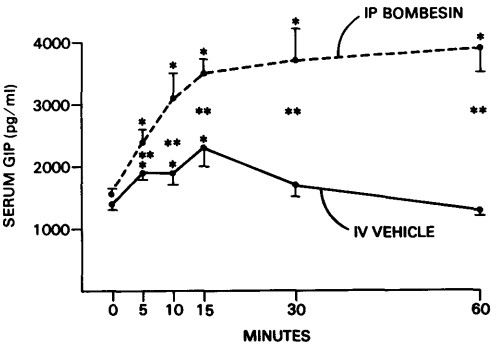

FIG. 2. Serum GIP levels ($\bar{X}$ ± SEM) in fed rats rose significantly in response to bombesin (29 μg) given intraperitoneally. Control rats were given vehicle alone. Six rats were sacrificed at each time point. *$P < 0.05$ vs basal GIP level; **$P < 0.05$ vs GIP response to IV vehicle.

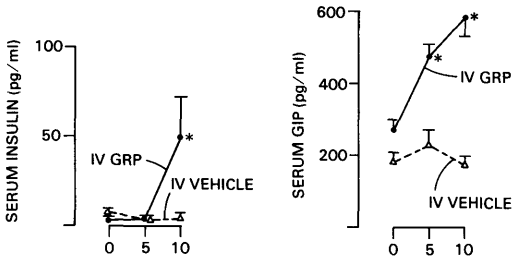

FIG. 1. Serum insulin and GIP levels ($\bar{X}$ ± SEM) in fasted rats rose significantly in response to GRP (10 μg) given intravenously (iv). Control rats were given vehicle iv alone. Six rats were sacrificed at each time point. *$P < 0.05$ vs basal or vehicle.

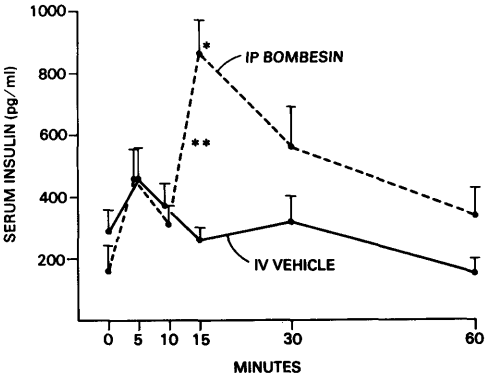

FIG. 3. Serum insulin levels ($\bar{X}$ ± SEM) in fed rats rose significantly in response to bombesin (29 μg) given intraperitoneally. Control rats were given vehicle alone. Six rats were sacrificed at each time point. *$P < 0.05$ vs basal insulin level; **$P < 0.05$ vs response to vehicle alone.

may be attributed to species differences or to differences in experimental design. In the present study, we used rats and administered bombesin or GRP as a single bolus, whereas McDonald and co-workers administered GRP as a constant intravenous infusion to dogs.

We suggest that bombesin-like peptides might play a physiologic role in the regulation of meal-stimulated insulin secretion. Bombesin-like peptides can apparently exert their insulinotropic action directly at the islets (23) and by GIP release.

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