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Bombesin stimulates insulin secretion by a pancreatic islet cell line

(gastrin-releasing peptide/somatostatin/glucagon/HIT-T15 cells)

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ABSTRACT **The amphibian tetradecapeptide, bombesin (BBS) has been shown to stimulate insulin secretion both *in vivo* and by pancreatic islet cells *in vitro*. To determine whether BBS can act directly on pancreatic β cells, we examined its effects on insulin secretion by HIT-T15 cells (HIT cells), a clonal islet cell line. Addition of 100 nM BBS to HIT cells stimulated insulin release 25-fold within 30 sec. The rapid stimulatory effect of BBS on insulin release was short-lived: the secretory rate returned to basal levels after 90 min of BBS treatment. The decrease in the rate of insulin release in the continued presence of BBS was due not to depletion of intracellular insulin stores but to specific desensitization to this peptide. Stimulation of insulin secretion by BBS was dose dependent with an ED_{50} value (0.51 ± 0.15 nM) similar to the concentration of BBS-like immunoreactive material in rat plasma. Five BBS analogs, including porcine gastrin-releasing peptide, were as powerful as BBS in stimulating insulin release. The relative potencies of the analogs tested indicated that the COOH-terminal octapeptide sequence in BBS was sufficient for stimulation of release. In contrast, 14 peptides structurally unrelated to BBS did not alter insulin secretion. BBS action was synergistic with that of glucagon; insulin secretion in the presence of maximal concentrations of both peptides was greater than the additive effects of the two peptides added individually. Somatostatin inhibited BBS-stimulated release by 69 ± 1% with an ID_{50} value of 3.2 ± 0.3 nM. These results show that BBS stimulation of insulin secretion by a clonal pancreatic cell line closely parallels its effects *in vivo* and support the hypothesis that BBS stimulates insulin secretion by a direct effect on the pancreatic β cell. The clonal HIT cell line provides a homogeneous cell preparation amenable for studies on the biochemical mechanisms of BBS action in the endocrine pancreas.**

Bombesin (BBS), a tetradecapeptide originally isolated from the skin of the frog *Bombina bombina* (1), is one of a group of amphibian peptides with homologous counterparts in mammalian brain and gut (2–8). BBS modulates many behavioral, metabolic, and endocrine processes in mammals when introduced into the central nervous system or the blood stream (4, 6, 9). These processes include regulation of body temperature, food and water intake, blood glucose levels, and the release of pituitary, pancreatic, and gut hormones. Therefore, endogenous BBS-like peptides have been proposed to play a role in the maintenance of metabolic homeostasis (4).

In this study, we have focused on the effect of BBS on hormone secretion by the endocrine pancreas. Intravenous infusion of BBS in humans and dogs causes an increase in the plasma concentrations of both insulin and glucagon and lowers blood glucose levels (10–14). *In vitro*, BBS stimulates the secretion of insulin and glucagon by perfused dog pancreas (15, 16), whereas it has been shown to either stimulate (17) or inhibit insulin release by isolated rat pancreas (18). Because pancreatic islet cells consist of a mixture of hormone-secreting cell types, these studies cannot distinguish between a direct or indirect effect of BBS on pancreatic β cells. To elucidate the nature of any direct effect of BBS on pancreatic β cells, we examined its actions in a clonal insulin-secreting cell line. HIT-T15 (HIT) cells were derived from simian virus 40-transformed hamster pancreatic islets and retain many characteristics of normal differentiated β cells (19). HIT cells synthesize and process proinsulin, contain secretory granules, and respond appropriately to a variety of compounds known to modulate insulin release in normal islet cells (19). Our results indicate that HIT cells provide a useful *in vitro* model system for investigating the action of BBS in the β cell of the pancreas. A preliminary report of some of these experiments has been presented.†

MATERIALS AND METHODS

Materials. Synthetic BBS, [Tyr^4]BBS, ranatensin, litorin, porcine gastrin-releasing peptide (GRP), GRP(14-27), cholecystokinin octapeptide, oxytocin, vasopressin, growth-hormone releasing factor(1-44), secretin, and vasoactive intestinal peptide were purchased from Peninsula Laboratories (San Carlos, CA). Somatostatin, calcitonin, substance P, and neurotensin were purchased from Beckman. [Leu]Enkephalin was obtained from Bachem Fine Chemicals (Torrance, CA). Thyrotropin-releasing hormone was provided by Abbott (North Chicago, IL). Culture medium, horse serum, and fetal calf serum were purchased from GIBCO, and plastic culture dishes were from Falcon. IgGsorb (*Staphylococcus aureus* protein A) was purchased from Enzyme Center (Boston). Glucagon, melatonin, phenylmethylsulfonyl fluoride, and iodoacetamide were purchased from Sigma. Monocomponent porcine insulin (lot no. 615-07J-256) and HIT cells were generous gifts from Eli Lilly.

Cell Culture. The establishment and properties of the HIT cell line have been described (19). After several months of propagation in our laboratory, the cells became more flattened and more tightly adherent to culture dishes. The flattened cells continued to secrete insulin and their responses to glucagon and somatostatin were similar to that described by Santerre *et al.* (19). The new morphology has been retained during continuous passage of the cells for 2 years. Because increased adherence to the substratum was experimentally advantageous, HIT cells with the flattened morphology were used in all experiments.

Experiments were carried out using replicate 35-mm dishes inoculated with 1.5 ml from a single donor suspension. Cultures were grown in Ham's F12 medium supplemented with 15% horse serum/2.5% fetal calf serum at 37°C in humidified 5% CO_2/95% air. The culture medium was changed

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Abbreviations: BBS, bombesin; GRP, gastrin-releasing peptide.

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every 3–4 days and experiments were carried out 1 day after the medium was changed. Cells were maintained in culture for 3–5 changes of medium before use, and the number of cells per plate was determined at the time of each experiment using a hemocytometer.

Insulin Radioimmunoassay. Radiolabeled insulin (specific activity, 150–250 Ci/g; 1 Ci = 37 GBq) was prepared by Chloramine T iodination of monocomponent porcine insulin (20). Antiserum against monocomponent porcine insulin was produced in guinea pigs (Hartley strain) according to published procedures (21, 22) An antiserum designated 792 and shown to be specific for insulin was used at a final concentration of 1:160,000. Free antigen was separated from antibody–antigen complex by precipitation with either 20% polyethylene glycol (M_r, 8000)/20% horse serum (23) or IgGsorb (*Staphylococcus aureus* protein A) (24). The limit of assay sensitivity was 15 pg of insulin per 0.1 ml of sample buffer. HIT cell (hamster) insulin and porcine insulin gave parallel displacement curves for inhibition of iodinated antigen binding. The intra-assay variability was ±5% and inter-assay variation was ±27%.

Measurement of Insulin Secretion. To determine the effect of BBS and other neuropeptides on insulin secretion, replicate dishes were washed twice with a Hepes-buffered salt solution containing 118 mM NaCl/4.6 mM KCl/0.5 mM $CaCl_2$/1 mM $MgCl_2$/10 mM glucose/5 mM Hepes/0.1% $NaHCO_3$/0.1% bovine serum albumin, pH 7.2. Subsequently, 1 ml of Hepes-buffered salt solution, preequilibrated at 37°C in 5% CO_2/95% air and containing the appropriate concentration of peptide, was added. The cultures were then incubated for the desired time at 37°C in humidified 5% CO_2/95% air. For the short time course experiment shown in Fig. 1*A*, cells were washed with buffer then incubated in Hepes-buffered salt solution without $NaHCO_3$ (pH 7.2) at 37°C in ambient atmosphere. At the end of each incubation, the buffers were collected, floating cells were removed by centrifugation, and the supernatants were stored at −20°C for the subsequent determination of insulin concentration by RIA.

To determine the extent of insulin degradation by HIT cells, we measured the recovery of exogenous [^{125}I]insulin and nonradiolabeled insulin from the incubation buffers by precipitation with 10% trichloroacetic acid and by RIA, respectively. After incubation with HIT cells for 60 min at 37°C, [^{125}I]insulin (0.15–6 ng/ml) was degraded only 21 ± 2% and nonradiolabeled insulin (100 ng/ml) was degraded only 25 ± 12%. Furthermore, the addition of 100 nM BBS did not alter the stability of either radiolabeled or nonradioactive insulin (data not shown). Therefore, the 5- to 25-fold increase in insulin accumulation induced by BBS cannot be due to inhibition of insulin degradation in the medium.

Measurement of Intracellular Insulin. For determination of intracellular insulin levels, HIT cells were extracted into 5 M acetic acid containing bovine serum albumin (5 mg/ml)/phenylmethylsulfonyl fluoride (0.3 mg/ml)/iodoacetamide (0.3 mg/ml). Samples were snap-frozen and thawed 3 times and then extracted at 4°C for 16–20 hr. Extracts were centrifuged and the supernatants were lyophilized. The lyophilized samples were dissolved in a buffer of 10 mM NaH_2PO_4/1 mM Na_2EDTA/0.1% Triton X-100/phenylmethylsulfonyl fluoride (0.3 mg/ml)/iodoacetamide (0.3 mg/ml), pH 7.6. Insulin concentrations in the extracts were determined by RIA.

RESULTS

The Effect of BBS on Insulin Secretion. The data in Fig. 1 show the effect of 100 nM BBS on insulin secretion by HIT cells. The stimulatory effect of BBS was very rapid; within 30 sec BBS caused a 25-fold increase in insulin release (Fig. 1*A*). Thereafter, the secretory rate decreased and returned to basal levels by 90 min (Fig. 1*B*). The lack of a prolonged

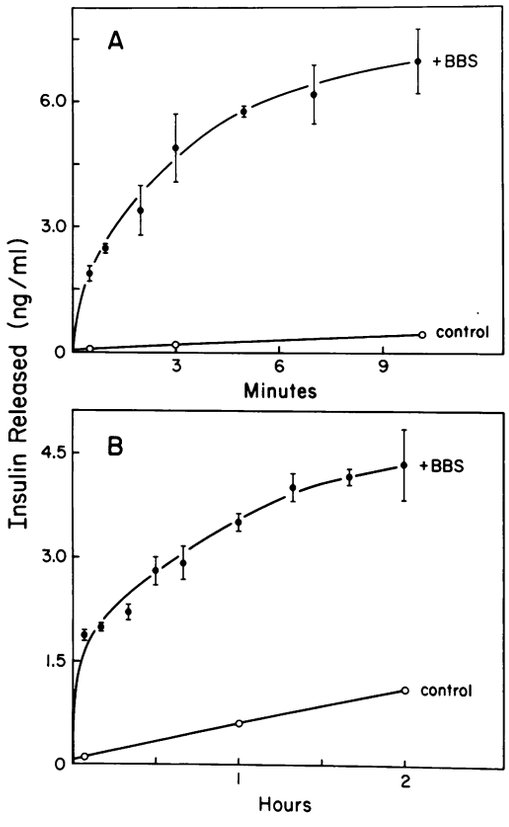

FIG. 1. Time course for BBS stimulation of insulin secretion. HIT cells were incubated at either 37°C in the ambient atmosphere in Hepes-buffered salt solution without bicarbonate (*A*) or at 37°C in 5% CO_2/95% air in Hepes-buffered salt solution with bicarbonate (*B*). Incubations were carried out in the absence (○) or presence (●) of 100 nM BBS for the times shown. The concentration of insulin released into the buffer was measured by RIA. Each point represents the mean value for triplicate (*A*) or quadruplicate (*B*) dishes, and the brackets show the SEM. Cell number was 3.7 ± 0.1 × 10^6 cells per dish (*A*) and 5.1 ± 0.4 × 10^6 cells per dish (*B*).

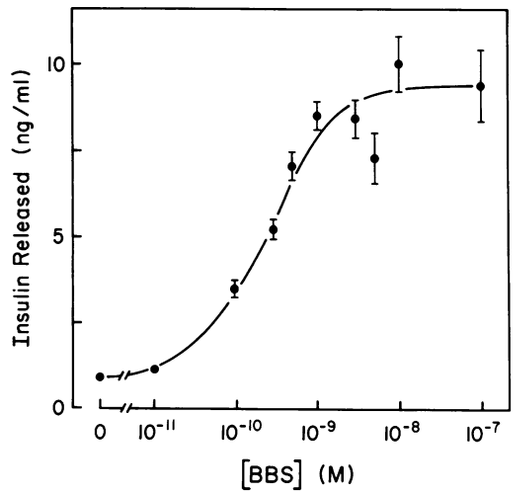

FIG. 2. Concentration dependence for BBS stimulation of insulin secretion. HIT cells (3.3 ± 0.2 × 10^6 cells per dish) were incubated at 37°C in 1.0 ml of Hepes-buffered salt solution containing the indicated concentration of BBS. After 60 min, the buffers were collected and the released insulin was measured by RIA. Each point represents the mean value of quadruplicate dishes and the bars show the SEM.

Table 1. The effect of different neuropeptides on insulin release by HIT cells

Peptide added	Insulin secreted, ng per dish	
	Control	With BBS
None	0.50 ± 0.06	5.06 ± 0.50
Secretin	0.42 ± 0.05	5.33 ± 0.30
Vasoactive intestinal peptide	0.50 ± 0.04	5.68 ± 0.32
Thyrotropin-releasing hormone	0.51 ± 0.03	5.27 ± 0.63
Substance P	0.44 ± 0.05	5.29 ± 0.53
Neurotensin	0.45 ± 0.03	5.08 ± 0.51

HIT cells ($3.9 \pm 0.2 \times 10^6$ cells per dish) were incubated with the peptides shown. The final concentration of all peptides was 100 nM. After 60 min, the buffers were removed and the concentration of insulin was determined by RIA. Values represent mean ± SEM of triplicate dishes.

stimulatory effect of BBS on secretion could have been due to depletion of intracellular insulin stores. However, during a 60-min incubation at 37°C, 100 nM BBS caused the release of only 1.04 ± 0.07% (mean ± range; $n = 2$) of intracellular insulin. Furthermore, there was no detectable difference in the insulin content of untreated and BBS-treated cells (data not shown). Therefore, stimulation of insulin release by BBS does not deplete intracellular stores. The explanation for the transient nature of the response to BBS is discussed below.

The concentration dependence for the stimulation of insulin release by BBS is shown in Fig. 2. In this experiment, a half-maximal response was elicited by 0.3 nM BBS. In four experiments, the ED_{50} value for BBS was 0.51 ± 0.15 nM (mean ± SEM). The concentration of extractable BBS-like immunoreactivity in rat plasma has been reported to be about 0.2 nM (3). Therefore, BBS stimulates insulin secretion in a dose-dependent manner with an ED_{50} in the physiological concentration range.

The Effect of Other Neuropeptides on Insulin Secretion. To determine the specificity of BBS action, we tested the ability of other peptides to regulate insulin secretion in HIT cells. The results in Table 1 show that five neuropeptides structurally unrelated to BBS did not alter basal insulin release nor did they modulate the stimulatory action of BBS. In independent experiments, oxytocin, pentagastrin, epidermal growth factor, [Leu]enkephalin, vasopressin, growth hormone-releasing factor, cholecystokinin octapeptide, melatonin, and calcitonin at concentrations of 100 nM had no effect on insulin secretion. Therefore, the ability of BBS to stimulate insulin release by HIT cells is not shared by all brain–gut peptides.

To analyze the structural requirements for biological activity in BBS-like peptides, we tested five BBS analogs shown in Table 2. [Tyr^4]BBS, which has a tyrosine instead of a leucine at position four, is the synthetic analog used for radiolabeling in receptor-binding studies (25, 26). Litorin and ranatensin, peptides originally isolated from amphibian skin (6), both contain the last eight COOH-terminal amino acids of BBS with a penultimate leucine for phenylalanine substitution. GRP, a mammalian BBS analog isolated from porcine gut, has the same COOH-terminal decapeptide sequence as BBS except for a histidine for glutamine substitution (2). GRP differs from BBS in its NH_2-terminal 17 amino acids. GRP(14-27) is the COOH-terminal tetradecapeptide fragment of GRP.

Table 3. Potency of BBS analogs to stimulate insulin secretion

Peptide	ED_{50}, nM
BBS	0.51 ± 0.15
[Tyr^4]BBS	0.46 ± 0.17
Ranatensin	1.11 ± 0.08
Litorin	0.63 ± 0.05
GRP	2.06 ± 0.61
GRP(14–27)	0.78 ± 0.30

The concentration of peptide required to produce half-maximal stimulation of hormone release (ED_{50}) was determined in experiments such as that shown in Fig. 2. Statistical analysis using Duncan's multiple range test showed that the ED_{50} value for GRP was significantly different from that for the other analogs [$P < 0.01$ compared to BBS, litorin, and [Tyr^4]BBS; $P < 0.05$ compared to ranatensin and GRP(14-27)]. The ED_{50} values for the analogs other than GRP did not differ significantly from that for BBS or each other ($P > 0.99$). Values represent mean ± SEM of four experiments for BBS or mean ± range of two experiments for BBS analogs.

Maximal concentrations of every BBS analog tested stimulated insulin release to the same extent as did 100 nM BBS (data not shown). The results in Table 3 show that the four short peptides were equipotent with BBS. However, BBS was 4-fold more potent than GRP, the NH_2-terminal extended analog (Table 3). The relative potencies of these peptides indicate that the COOH-terminal octapeptide sequence in BBS is sufficient for stimulation of insulin secretion.

The Effect of Somatostatin and Glucagon on BBS-Stimulated Secretion. Both *in vivo* and in pancreatic islets *in vitro*, glucagon stimulates insulin secretion (27), whereas somatostatin inhibits it (28). Because HIT cells respond in the expected manner to these two peptides (19), we investigated the effect of glucagon and somatostatin on the stimulatory action of BBS.

A maximal concentration of BBS (100 nM) increased insulin release 10-fold, whereas, in the same experiment, glucagon (1 μM) caused a 6-fold increase (Table 4). The simultaneous addition of glucagon and BBS at maximal concentrations resulted in a 40-fold stimulation of insulin secretion (Table 4). In three independent experiments, the effect of glucagon plus BBS was 1.7 ± 0.3-fold greater than the additive effect of the two peptides alone. Therefore, BBS and glucagon act synergistically to stimulate insulin release.

Although 100 nM somatostatin had no effect on basal insulin secretion, it inhibited the stimulatory effect of BBS by 71% (Table 4). In four experiments, this concentration of somatostatin inhibited BBS-stimulted insulin release by 69 ± 1% (mean ± SEM). Fig. 3 shows that the inhibitory effect of somatostatin was dose dependent: the ID_{50} for somatostatin in two experiments was 3.2 ± 0.3 nM (mean ± range).

We have characterized specific, high-affinity receptors for BBS in HIT cells and have shown that the binding of [^{125}I-Tyr^4]BBS is not affected by either somatostatin or glucagon (unpublished observations). Therefore, the modulatory ef-

Table 2. Amino acid sequences of BBS analogs*

BBS		pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-MetNH_2
[Tyr^4]BBS		pGlu-Gln-Arg-Tyr-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-MetNH_2
Ranatensin		pGlu - - - - - - - - - Val-Pro-Gln-Trp-Ala-Val-Gly-His-Phe-MetNH_2
Litorin		pGlu - - - - - - - - - - - - - - - Gln-Trp-Ala-Val-Gly-His-Phe-MetNH_2
GRP	Ala . . .	Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-MetNH_2
GRP(14–27)		Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-MetNH_2

*Summarized from refs. 2 and 6. pGlu denotes pyroglutamic acid. The NH_2-terminal amino acids 2–13 in GRP are symbolized by . . . . Amino acids differing from BBS sequence are underlined. The hyphens indicate amino acid deletions.

Table 4. The effect of glucagon and somatostatin on the stimulatory action of BBS

Peptide added	Insulin secreted, ng per dish	
	Control	With BBS
None	0.50 ± 0.06	5.06 ± 0.50
Glucagon (1 μM)	2.92 ± 0.15	17.5 ± 1.3
Somatostatin (100 nM)	0.48 ± 0.03	1.48 ± 0.10

HIT cells (3.9 ± 0.2 × 10^6 cells per dish) were incubated with the peptides shown in the absence or presence of 100 nM BBS. After 60 min, the buffers were removed and the concentration of insulin was determined by RIA. Values represent mean ± SEM of triplicate dishes.

fects of somatostatin and glucagon on BBS action must be mediated by independent receptors.

BBS-Induced Desensitization. The observation that BBS had a transient effect on insulin release (see Fig. 1) without depleting intracellular insulin stores suggested that HIT cells desensitize to the stimulatory effect of BBS. To test this hypothesis, cells were incubated for 2 hr in the presence or absence of 100 nM BBS and then challenged in fresh buffer with three different secretagogues. During the first incubation, 100 nM BBS caused a 10-fold increase in insulin secretion (Fig. 4*A*). However, cells exposed to BBS in the first incubation did not respond to subsequent treatment with BBS (Fig. 4*B*). In contrast, cells preincubated with BBS did respond to glucagon and a high concentration of K^+ with increased insulin release (Fig. 4*B*). These data show that BBS induces specific desensitization in HIT cells.

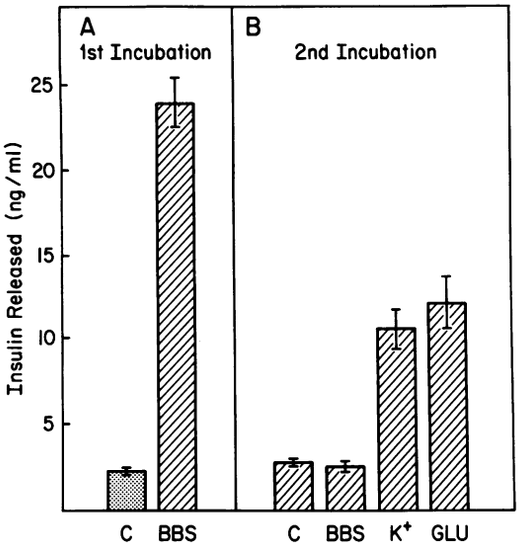

FIG. 4. BBS-induced desensitization of HIT cells. HIT cells (6.0 ± 0.4 × 10^6 cells per dish) were incubated in 1.0 ml of Hepes-buffered salt solution at 37°C for 2 hr in the absence (C) or presence of 100 nM BBS. The amount of insulin released during this incubation was measured by RIA and is shown in *A*. Replicate dishes that had been incubated with 100 nM BBS for 2 hr were subsequently incubated again in fresh buffer containing either no secretagogue (C), 100 nM BBS, 50 mM KCl (K^+), or 1 μM glucagon (GLU). After 90 min, the buffers were removed and secreted insulin was measured by RIA (*B*). Each bar represents the mean ± SEM of triplicate dishes.

DISCUSSION

BBS is a potent stimulator of insulin secretion when infused intravenously into either humans or experimental animals (10–14). In addition, BBS-like peptides are present in peptidergic neurons of the mammalian pancreas, and electrical stimulation of the splanchnic nerve has been shown to increase BBS levels in peripheral plasma to concentrations sufficient to stimulate insulin release (29, 30). These results strongly suggest that BBS-like peptides play an important role in the physiological control of insulin secretion.

The data presented here show that BBS directly stimulates insulin release by a clonal line of hamster pancreatic cells. The characteristics of the insulin response to BBS in HIT cells are remarkably similar to what is observed both *in vivo* and in pancreatic islets *in vitro*. BBS is effective in HIT cells at the same low concentrations that stimulate insulin secretion in perfused dog pancreas (15, 16) and increase plasma insulin levels in dogs and humans (10, 14). In all systems, the response to BBS is rapid and transient. Infusion of BBS in dogs and humans produced a maximal stimulation of insulin secretion within 5 to 15 min followed by a decrease in insulin levels during continued infusion of the peptide (11–14). Similarly, in perfused isolated canine pancreas, stimulation of insulin release was maximal after 2 min and decreased thereafter (15, 16). In HIT cells, a 25-fold stimulation of insulin release occurred 30 sec after the addition of BBS and the secretory rate subsequently decreased to the basal rate. As *in vivo* (13), somatostatin inhibited the stimulatory effect of BBS in HIT cells. Finally, a mammalian BBS-like peptide, GRP, increased insulin release both when infused intravenously into dogs (14) and when added to HIT cells. These observations suggest that the effects of BBS-like peptides on insulin secretion *in vivo* are due to direct action on β cells.

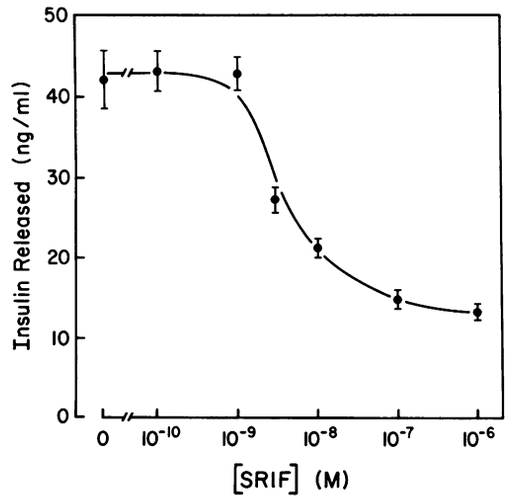

FIG. 3. Concentration dependence for somatostatin inhibition of BBS-stimulated insulin release. HIT cells (3.4 ± 0.4 × 10^6 cells per dish) were incubated at 37°C in 1.0 ml of Hepes-buffered salt solution containing 100 nM BBS and the indicated concentration of somatostatin (SRIF). After 60 min, the buffers were collected and the released insulin was measured by RIA. Insulin secretion in the absence of any peptide was 5.1 ± 0.7 ng/ml. Each point gives the mean ± SEM of triplicate dishes.

To determine the structural requirements for BBS action, we compared the potencies of several BBS analogs to stimulate insulin secretion by HIT cells. Our data indicate that the COOH-terminal octapeptide sequence in BBS is sufficient for potent stimulation. Of additional interest is the observation that porcine GRP was less potent than the amphibian peptide BBS. Similar results have been observed in other systems. For example, when injected intracisternally into rats, BBS is more potent than GRP in producing hypothermia and hyperglycemia (31) and in causing inhibition of gastric acid secretion and stimulation of gastrin release (32). Furthermore, BBS has been shown to bind to receptors in a rat pituitary cell line with a 3-fold higher apparent affinity than does GRP (26). The greater potency of the amphibian peptide BBS than its mammalian analog probably reflects

differential sensitivity of certain target cells to the various molecular forms of endogenous BBS-like peptides. The presence of several forms of BBS-like substances in mammals was recognized in early studies of the distribution of immunoreactive BBS-like peptides (3, 6–8). Recently, three biologically active BBS-like peptides have been purified from canine intestine (5). These peptides share a common COOH-terminal sequence that has substantial homology with both porcine GRP and amphibian BBS. The observation that BBS and GRP(14-27) are more potent than GRP in some target cells (refs. 31 and 32; Table 3) indicates that the COOH-terminal fragments of endogenous BBS-like peptides are physiologically more potent than their NH_2-terminal extended counterparts. However, diverse target tissues do show differential sensitivity to various BBS-like peptides (4, 6, 7). Molecular heterogeneity and differential cellular responsiveness to active forms have also been reported for the neuropeptides somatostatin, cholecystokinin, and gastrin (28, 33, 34). Further studies are clearly warranted to determine the relative importance of the different forms of mammalian BBS in the various biological actions of this peptide.

Both *in vivo* and in perfused pancreas the stimulatory effect of BBS on insulin secretion is transient (11–16). Similarly, in HIT cells the time course for BBS-stimulated insulin secretion shows a return to the basal secretory rate by 90 min in the continued presence of the peptide. This decrease in secretory rate is not due to a significant decrement in intracellular insulin, but rather to specific desensitization of the cellular response to BBS. Desensitization to BBS has also been observed in dispersed pancreatic acinar cells (35, 36). Since BBS-induced desensitization and resensitization in acinar cells occur in the absence of protein synthesis, BBS receptors must be able to cycle between active and inactive states. However, the mechanism by which BBS desensitization occurs remains to be determined.

An interesting result obtained in our study was the synergistic effect of BBS and glucagon on insulin secretion. Glucagon elicits its biological effects by increasing intracellular cAMP levels in target cells (37). The intracellular mechanism of BBS action has been most thoroughly investigated in the exocrine pancreas where its effects appear not to be mediated by cAMP (38–40). In pancreatic exocrine cells BBS has been shown to modulate membrane potential, calcium efflux, cGMP accumulation, and phosphatidylinositol turnover (38–40). The relative importance of these biochemical changes in initiating the biological actions of BBS remains to be clarified. Our results indicate distinct and synergistic mechanisms for BBS and glucagon action. HIT cells provide a homogeneous well-defined system for determining the mechanism by which BBS stimulates insulin secretion both alone and in conjunction with other regulatory agents.

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