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Bombesin induces a reduction of somatostatin inhibition of adenylyl cyclase activity, G_i function, and somatostatin receptors in rat exocrine pancreas

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Abstract

To analyze the effect of bombesin on the somatostatin (SS) mechanism of action in the exocrine pancreas, male Wistar rats (250–270 g) were injected intraperitoneally with bombesin (10 μg/kg) three times daily at 8-h intervals for 7 or 14 days. Bombesin attenuated the ability of SS to inhibit forskolin-stimulated adenylyl cyclase activity in pancreatic acinar membranes. However, it did not decrease the ability of forskolin to stimulate the adenylyl cyclase catalytic subunit. The ability of 5′-guanylylimidodiphosphate [Gpp(NH)p] (a nonhydrolyzable GTP analog) to inhibit forskolin-stimulated adenylyl cyclase activity was diminished in pancreatic acinar cell membranes from bombesin-treated rats. Bombesin administration did not affect the ADP-ribosylation of a 41-kDa G protein catalyzed by pertussis toxin. The maximal SS binding capacity of pancreatic acinar membranes from bombesin-treated rats was decreased when compared with controls at the two time periods studied. The bombesin/gastrin-releasing peptide antagonist [D-Tpi^6,Leu$^{13}\psi$(CH$_2$NH)Leu^{14}]bombesin (6–14) (RC-3095) (10 μg/kg ip), injected three times daily at 8-h intervals for 7 or 14 days, had a similar effect to that of bombesin on the SS mechanism of action. The combined administration of bombesin and its antagonist RC-3095 had a greater effect on the SS receptor–effector system than when administered separately. The present study indicates that the pancreatic SS receptor–effector system may be regulated by bombesin in vivo.

Keywords: Bombesin; RC-3095; Proglumide; Somatostatin receptor; G_i protein; Adenylyl cyclase

1. Introduction

Bombesin, a tetradecapeptide originally isolated from amphibian skin [2], belongs to a family of structurally related peptides recently characterized in amphibians as well as in mammals [52]. It is a neural peptide present in enteric and intrapancreatic nerves [18,35]. Bombesin is known to play a role as a neurotransmitter in the exocrine pancreas [19,25]. Receptors for bombesin and related peptides have been functionally and structurally identified in several cell systems [24,50], including the exocrine pancreas [24]. A great deal of interest has been generated by the demonstration that bombesin-like peptides are potent mitogens in different cell types [8,41,52], including pancreatic acinar cells [29,31]. In the latter tissue, bombesin has long been known to be a powerful stimulant of secretion [11] and of electrical activity [23]. Thus, the effects of exogenously administered bombesin in vivo could be the result of a direct action on the pancreas. This does not discard the possibility that the effects of bombesin on the exocrine pancreas are also mediated through the modulation of other hormones that act on the pancreatic acinar cells. In this regard it has recently been shown that pretreatment of pancreatic acini with bombesin reduces subsequent binding of labeled somatostatin (SS) to acinar membranes [44]. The tetradecapeptide SS has been shown to exert a negative control on bombesin receptor-stimulated phosphatidylinositol turnover [32]. In addition, SS and bombesin have contrary effects on exocrine pancreatic secretion [3,21]. However, to date, the effect of bombesin on the postreceptor SS mechanism of

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action is unknown. In pancreatic acinar cells, SS receptors [12,42,46] are coupled to the adenylate cyclase (AC) enzyme system via the guanine nucleotide binding inhibitory protein G_i [43]. The extent of G_i modification correlates with the ability of SS to inhibit adenosine 3′,5′-cyclic monophosphate (cAMP) formation.

In the present study, we investigated the effects of bombesin and a new short chain pseudononapeptide bombesin/gastrin-releasing peptide (GRP) antagonist, [D-Tpi^6,Leu$^{13}\psi$(CH$_2$NH)Leu^{14}]bombesin (6–14) (RC-3095), on SS inhibition of forskolin (FK)-stimulated AC activity, the overall catalytic activity of AC by means of stimulation of the AC catalytic subunit with FK, specific [^{125}I-Tyr^{11}]somatostatin ([^{125}I-Tyr^{11}]SS) binding and its inhibition by the stable GTP analog 5′-guanylylimidodiphosphate [Gpp(NH)p] [43] and pertussis toxin (PTX)-sensitive inhibitory GTP binding proteins (G_i proteins) in rat pancreatic acinar membranes. In addition, the effects of these compounds on pancreatic somatostatin-like-immunoreactive content (SSLI) was examined. Because bombesin increases cholecystokinin (CCK) and gastrin release, it is possible that these hormones may mediate the effects of bombesin on the SS receptor-effector system. The pretreatment with proglumide (PG), a gastrin/CCK receptor antagonist [39], was used to evaluate whether the effects of bombesin on the pancreatic somatostatinergic system involved the activation of gastrin/CCK receptors.

We present evidence that the ability of Gpp(NH)p to inhibit FK-stimulated AC activity is diminished in pancreatic acinar cell membranes from bombesin-treated rats in addition to an already reported decrease in SS receptor levels in rat pancreatic acinar membranes. Likewise, evidence that bombesin and its antagonist RC-3095 have similar effects on the SS receptor–effector system in rat pancreatic acinar membranes is also introduced.

2. Methods

2.1. Experimental animals

Male Wistar rats (250–270 g) were injected intraperitoneally three times daily at 8-h intervals during 7 or 14 days [9,50] with the following agents: bombesin alone (10 μg/kg), PG alone (20 mg/kg), RC-3095 alone (10 μg/kg), bombesin combined with PG, and bombesin combined with RC-3095 and saline in a volume (250:l) equal to that of the other compounds. Drug doses were selected according to the effective doses reported in previous studies [10,30]. Rats were decapitated 18 to 20 h after the last injection. The pancreas was removed and trimmed free of fat, connective tissue, and lymph nodes.

2.2. Chemicals

Synthetic Tyr^{11}-SS was purchased from Universal Biologicals Ltd (Cambridge, UK); carrier-free ^{125}I-Na (IMS 300, 100 mCi/ml) was obtained from the Radiochemical Center (Amersham, UK); bombesin, PG, FK, bacitracin, phenylmethylsulfonyl fluoride, guanosine triphosphate (GTP), Gpp(NH)p; 3-isobutyl-1-methylxanthine, PTX, and bovine serum albumin (BSA) were purchased from Sigma (St Louis, MO, USA). Bombesin receptor antagonist [D-Tpi^6, Leu$^{13}\psi$(CH$_2$NH)Leu^{14}]bombesin (6–14) or RC-3095, originally synthesized by Radulovic et al. [40] and Cai et al. [6], was provided by Asta Pharma (Frankfurt/M, Germany). Tpi [2,3,4,9,-tetrahydro-1H-pyrido(3,4-b)indol-3-carboxylic acid] is a conformationally constrained analog of Trp and is more hydrophobic than Trp. The rabbit antibody used in the radioimmunoassay technique was purchased from the Radiochemical Center (Amersham, UK). This antiserum was raised in rabbits against SS-14 conjugated to BSA and is specific for SS-14. Because SS-14 constitutes the C-terminal portions of both SS-25 and SS-28, the antiserum does not distinguish between these three forms. All other reagents were of the highest purity commercially available.

2.3. Preparation of rat pancreatic acinar membranes

Dispersed pancreatic acini were obtained from male Wistar rats after enzymatic degradation of the organ with 0.2 U of collagenase/ml in an oxygenated Krebs–Ringer medium as described by Amsterdam et al. [1]. After thorough washing by sedimentation, acini were homogenized in 0.3 M sucrose at 4°C by use of a Potter homogenizer following the method of Meldolesi et al. [34]. After sedimentation at 1500 *g* for 12 min, the homogenized membranes were resuspended in 1.56 M sucrose. This suspension was overlaid with 0.3 M sucrose and centrifuged at 105 000 *g* for 150 min. The plasma membrane-enriched fraction collected from the interphase was diluted with distilled water and centrifuged at 15 000 *g* for 30 min. The supernant was discarded and the pellet was resuspended in 50 mM Tris-HCl, pH 7.4, 0.01 mg/ml bacitracin, and 0.2 mM $CaCl_2$ and stored at −70°C. An aliquot was taken for protein determination by the method of Lowry et al. [33].

2.4. Binding of [^{125}I-Tyr^{11}]SS

Binding of [^{125}I-Tyr^{11}]SS was performed on rat pancreatic acinar membranes by a modification of the method of Colás et al. [7]. Tyr^{11}-SS was radioiodinated by the chloramine-T method as described by Greenwood et al. [15]. Separation of iodinated SS from free iodine was performed on a Sephadex G-25 (fine) column using 0.1 M acetic acid with BSA (0.1%, wt/vol). The specific activity of the radioligand was 600 Ci/mmol. Binding of [^{125}I-Tyr^{11}]SS to pancreatic acinar membranes was performed in a total volume of 250 μl in 50 mM Tris-HCl buffer (pH 7.4) containing 0.5 mM $MgCl_2$, 3 mM NaCl, 0.2 mM $CaCl_2$, 0.2% (wt/vol) BSA, 0.5 mg/ml bacitracin, and 0.3 mg/ml soybean trypsin inhibitor (binding buffer). Plasma membranes (75 μg of protein/ml) were incubated for 90 min at 20°C with 35 pM

[^{125}I-Tyr^{11}]SS in the absence or presence of 0.01 to 10 nM unlabeled SS. Bound and free ligand were separated by centrifugation at 11 000 *g* for 4 min at 4°C in a microcentrifuge. Radioactivity in the pellet was measured with a gamma counter. Nonspecific binding was estimated as membrane-associated radioactivity in the presence of 1 μM SS and specific binding was calculated as the difference between total and nonspecific membrane-associated radioactivity. The effects of Gpp(NH)p on [^{125}I-Tyr^{11}]SS binding were determined after addition of various Gpp(NH)p concentrations (10^{-11}–10^{-4} M) to the binding assay buffer.

2.5. Evaluation of radiolabeled peptide degradation

To determine the extent of tracer degradation during incubation, we measured the ability of preincubated peptide to bind to fresh pancreatic acinar membranes after an initial preincubation of the peptide. In brief, [^{125}I-Tyr^{11}]SS (35 pM) was incubated with pancreatic acinar membranes (75 μg of protein/ml) for 90 min at 20°C. After this preincubation, aliquots of the medium were added to fresh pancreatic acinar membranes and incubated for an additional 90 min at 20°C. The fraction of the added radiolabeled peptide that was specifically bound during the second incubation was measured and expressed as a percentage of the binding obtained in control experiments performed in the absence of pancreatic acinar membranes during the preincubation period.

2.6. Adenylyl cyclase assay

AC activity was measured as previously reported by Houslay [20] with minor modifications. In brief, rat pancreatic acinar membranes (0.12 mg of protein/ml) were incubated with 1.5 mM ATP, 5 mM $MgSO_4$, 1 μM GTP and an ATP-regenerating system (7.5 mg/ml creatine phosphate and 1 mg/ml creatine kinase), 1 mM 3-isobutyl-1-methylxanthine, 0.1 mM phenylmethylsulfonyl fluoride, 1 mg/ml bacitracin, 1 mM EDTA, and tested substances (10^{-9} M SS-14 or 10^{-5} M FK) in 0.1 ml of 0.025 M triethanolamine/HCl buffer (pH 7.4). After a 30-min incubation at 30°C, the reaction was stopped by heating the mixture for 3 min. After cooling, 0.2 ml of an alumina slurry (0.75 g/ml in triethanolamine/HCl buffer, pH 7.4) was added and the suspension was centrifuged. The supernatant was taken for assay of adenosine 3′,5′-cyclic monophosphate (cAMP) by the Gilman method [14].

2.7. Tissue extraction and SS radioimmunoassay

For measurement of somatostatin-like-immunoreactive content, the pancreata were rapidly homogenized in 1 ml of 2 M acetic acid, using a Brinkman Polytron (setting 5, 30 s). The extracts were boiled for 5 minutes and aliquots (100 μl) were removed for protein determination as described by Lowry et al. [33]. The homogenates were subsequently centrifuged at 15 000 *g* for 15 min at 4°C and the supernatant was neutralized with 2 M NaOH. The extracts were stored at −70°C until assay. The SS concentration in the tissue extracts was determined by a modified radioimmunoassay method [38], with a sensitivity limit of 10 pg/ml. Incubation tubes prepared in duplicate contained 100-μl samples of tissue extracts or standard solutions of 0 to 500 pg SS-14 diluted in phosphate buffer (0.05 M, pH 7.2, containing 0.3% BSA, 0.01 M EDTA), 200 μl of diluted anti-SS serum, 100 μl of freshly prepared [^{125}I-Tyr^{11}]SS diluted in buffer to give 6000 cpm (equivalent to 5–10 pg), in a final volume of 0.8 ml. All reagents as well as the assay tubes were kept chilled on ice before their incubation for 48 h at 4°C. Bound hormone was separated from free hormone by the addition of 1 ml of dextran-coated charcoal (dextran T-70, 0.2% wt/vol, Pharmacia, Uppsala, Sweden; charcoal: Norit A, 2% wt/vol Serva, Feinbiochemica, Heidelberg, Germany). Serial dilution curves for the samples were parallel with the standard curve. The intraassay and interassay variation coefficients were 6.0 and 8.8%, respectively.

2.8. Pertussis toxin-catalyzed ADP-ribosylation

PTX-catalyzed ADP-ribosylation was performed as previously reported [4]. After PTX activation, membranes (0.8 mg of protein/ml) were incubated with PTX (16 μg/ml) in 100 mM Tris-HCl buffer (pH 8.0) containing 10 mM thymidine, 1 mM ATP, 100 μM GTP, 2.5 mM $MgCl_2$, 1 mM EDTA, and 2 μM [^{32}P]NAD^+ (30 Ci/mmol) and an ATP-regenerating system. After 30 min at 30°C, the reaction was stopped by addition of 1 ml of ice-cold 100 mM Tris-HCl buffer (pH 8.0) and the proteins were sedimented by centrifugation for 10 min at 30 000 *g* and solubilized with 0.1 ml 60 mM Tris-HCl buffer (pH 6.8) containing 10% glycerol, 0.001% bromophenol blue, and 3% sodium dodecyl sulfate (SDS) (SDS sample buffer). After heating for 30 minutes at 60°C, the suspension was centrifuged for 10 min at 100 000 *g* and aliquots of the supernatant were submitted to SDS–polyacrylamide gel electrophoresis, using the procedure of Laemmli [28] as previously described [27]. The gels were run, fixed, dried, and exposed to Dupont films (cronex 4) for 1 to 7 days at −80°C, using an intensifying screen.

2.9. Statistical analysis

The computer program LIGAND [36] was used to analyze the binding data. Statistical comparisons of all the data were performed with one-way analysis of variance and the Student–Newman–Keuls test. Means among groups were considered significantly different when P was <0.05. Each individual experiment was performed in duplicate.

Table 1
Effect of somatostatin (SS) (10^{-9} M) and forskolin (FK) (10^{-5} M) on adenylyl cyclase (AC) activity (pmol of cAMP/min/mg of protein) in pancreatic acrinar membranes of rats treated for 7 or 14 days with saline, bombesin, RC-3095, or bombesin combined with proglumide or with RC-3095

	Controls	Bombesin	Proglumide + Bombesin	RC-3095 + Bombesin	RC-3095
7 days					
Basal activity	14.7 ± 1.3	15.1 ± 1.3	13.9 ± 1.3	15.3 ± 2.1	14.3 ± 1.1
$+10^{-9}$ M of SS	12.6 ± 0.8	14.3 ± 1.5	13.1 ± 1.5	13.5 ± 0.9	12.9 ± 1.2
10^{-5} M FK	23.6 ± 1.7	24.3 ± 1.8	24.2 ± 2.0	25.1 ± 2.0	24.7 ± 1.2
10^{-5} M FK + 10^{-9} M	16.3 ± 1.0	20.5 ± 1.2	20.1 ± 1.1	22.1 ± 1.5	22.6 ± 1.3
%SS inhibition of FK stimulation	30.9 ± 1.7	15.6 ± 1.4†	16.9 ± 1.1†	11.9 ± 0.9†	8.5 ± 0.5†
14 days					
Basal activity	15.1 ± 0.8	16.1 ± 1.7	14.2 ± 1.6	16.2 ± 1.9	15.2 ± 1.5
$+10^{-9}$ M of SS	13.0 ± 1.0	14.2 ± 1.1	12.9 ± 1.3	14.5 ± 1.4	13.7 ± 1.1
10^{-5} M FK	24.8 ± 1.2	25.3 ± 2.0	24.8 ± 1.8	25.9 ± 1.1	25.5 ± 1.5
10^{-5} M FK 10^{-9} M SS	17.3 ± 1.4	21.7 ± 1.0	21.1 ± 1.5	20.9 ± 1.4	22.3 ± 1.2
%SS inhibition of FK stimulation	30.3 ± 1.7	14.2 ± 1.1†	15.0 ± 1.7†	19.3 ± 1.3*	12.6 ± 1.4†

Experiments were performed as described in Methods. Data are ± SEM values of five separate experiments, each performed in duplicate cAMP, adenosine 3′, 5′-cyclic nonophosphate.

Statistical comparison versus control: * $P < 0.01$; † $P < 0.001$.

3. Results

The effect of SS-14 on FK-stimulated AC activity was markedly decreased in pancreatic acinar membranes from bombesin-treated rats compared with control animals after 7 and 14 days of bombesin administration (Table 1). It should be noted that SS did not modify basal AC activity. To test if the observed changes were related to modifications in the expression of AC, we measured the response of AC to the diterpene FK, which acts directly on the catalytic subunit of AC. No significant differences were seen for either basal or FK-stimulated AC activities between the control and bombesin groups (Table 1).

The G_i proteins were evaluated by PTX labeling. Pancreatic acinar cell membranes from control and bombesin-treated rats were incubated with PTX and [^{32}P]NAD^+ and subsequently analyzed by SDS–polyacrylamide gel electrophoresis and autoradiography (data not shown). This technique labeled a 41-kDa band. The labeling intensity of this band was similar in the membranes of all the groups studied.

Additional experiments were performed to explore the effect of bombesin on the functionality of G_i proteins in rat pancreatic acinar membranes. These included determination of the ability of low Gpp(NH)p concentrations to inhibit FK-stimulated AC activity. Use of this assay showed that membranes derived from control rats yielded a characteristic biphasic response curve (Fig. 1). Gpp(NH)p concentrations between 0.01 and 1 nM decreased AC activity ($P < 0.05$) because of G_i activation, whereas higher nucleotide concentrations (10 nM) resulted in stimulation ($P < 0.01$) of both AC and G_s activities. The inhibitory effect of Gpp (NH)p on FK-stimulated AC activity, however, was markedly decreased in pancreatic acinar membranes from bombesin-treated rats (Fig. 1). These findings provide strong evidence for a G_i functional abnormality in the bombesin-treated rats.

Experiments were also performed to measure the inhibition of specific [^{125}I-Tyr^{11}]SS binding by the stable GTP analog Gpp(NH)p (Fig. 2). These were performed to determine whether the reduced SS-mediated AC activity observed in bombesin-treated rats could also be attributed to alterations in the integrity of the SS receptor binding site–G protein interaction. The IC_{50} of Gpp(NH)p is approximately the same in the bombesin-treated and non–bombesin-treated groups, suggesting no difference in potency.

Stoichiometric experiments with [^{125}I-Tyr^{11}]SS and increasing concentrations of the unlabeled peptide (Fig. 3) showed that in the animals treated with bombesin for 7 and 14 days, the maximal binding of [^{125}I-Tyr^{11}]SS to rat pancreatic acinar membranes was significantly lower than in

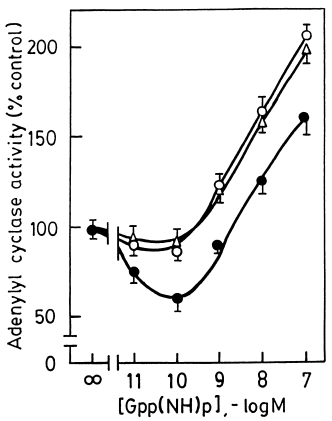

Fig. 1. Dose–effect curves for 5′guanylylimidodiphosphate [Gpp(NH)p] on forskolin (FK)-stimulated adenylyl cyclase (AC) activity in rat pancreatic acinar membranes from control (●) and rats treated with bombesin for 7 days (○) or 14 days (△). The enzyme activity was measured in the presence of 100 nM FK and the indicated concentrations of Gpp(NH)p. Data are expressed as percentages of FK-stimulated activity (100%) in the absence of Gpp(NH)p. Each point is the mean of three separate experiments, each performed in duplicate.

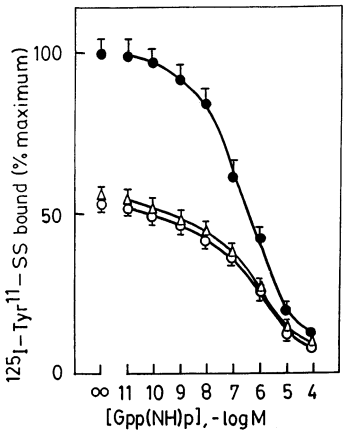

Fig. 2. Dose-effect curves for 5′-guanylylimidodiphosphate [Gpp(NH)p] on the specific binding of [^{125}I-Tyr^{11}]somatostatin ([^{125}I-Tyr^{11}]SS) (35 pM) to rat pancreatic acinar membranes from control rats (●) and rats treated with bombesin (7 days (○) and 14 days (△)). The results express the values of a pool of the control groups, because no differences among them were found. Each point is the mean of six separate experiments, each performed in duplicate. The calculated EC_{50} values were 0.43, 1.7, and 1 μM, respectively.

controls. Scatchard plots of the stoichiometric binding data were linear and essentially parallel (Fig. 3). Interpretation with the LIGAND computer program [36] resulted in the best fit for a model with one SS receptor. Pancreatic acinar membranes from bombesin-treated rats exhibited significant decreases in the maximum SS binding capacity at the two time periods studied (Table 2). The corresponding K_d values, however, were unchanged after bombesin administration.

The percentage of labeled SS degraded by pancreatic acinar membranes from each experimental group during the binding experiments was similar in both treated and untreated animals (Table 3).

Table 2
Characteristics of [^{125}I-Tyr^{11}]somatostatin ([^{125}I-Tyr^{11}]SS) binding to pancreatic acinar membranes from rats treated for 7 and 14 days with saline, bombesin alone or combined with proglumide, and RC-3095 alone or combined with bombesin

Groups	Days	
	7	14
Saline		
B_{max} (fmol/mg of protein)	523 ± 37	520 ± 25
K_d (nM)	0.07 ± 0.01	0.07 ± 0.02
Bombesin		
B_{max} (fmol/mg of protein)	281 ± 71*	288 ± 54*
K_d (nM)	0.09 ± 0.03	0.07 ± 0.01
Proglumide + bombesin		
B_{max} (fmol/mg of protein)	285 ± 27*	279 ± 32*
K_d (nM)	0.08 ± 0.01	0.07 ± 0.01
RC-3095 + bombesin		
B_{max} (fmol/mg of protein)	157 ± 14†	153 ± 21‡
K_d (nM)	0.09 ± 0.01	0.07 ± 0.01
RC-3095		
B_{max} (fmol/mg of protein)	209 ± 23†	205 ± 31†
K_d (nM)	0.08 ± 0.02	0.08 ± 0.03

Binding parameters were calculated from Scatchard plots by linear regression to determine the total number of binding sites (B_{max}, fentomoles of SS bound per milligram of protein) and their equilibrium dissociation constant (K_d, nM). Data are mean ± SEM values of the combined data from five rats in each group. Determinations were made in duplicate for each experiment. The results express the value of a pool of the control groups, because the B_{max} and K_d values of the control groups were not affected by the vehicle.

Statistical comparison versus controls: $^{*}P < 0.05$; $^{\dagger}P < 0.01$; $^{\ddagger}P < 0.001$.

Because bombesin increases CCK and gastrin release, these hormones could mediate the effects of bombesin on the SS receptor–effector system. The pretreatment with the gastrin/CCK receptor antagonist PG was used to evaluate

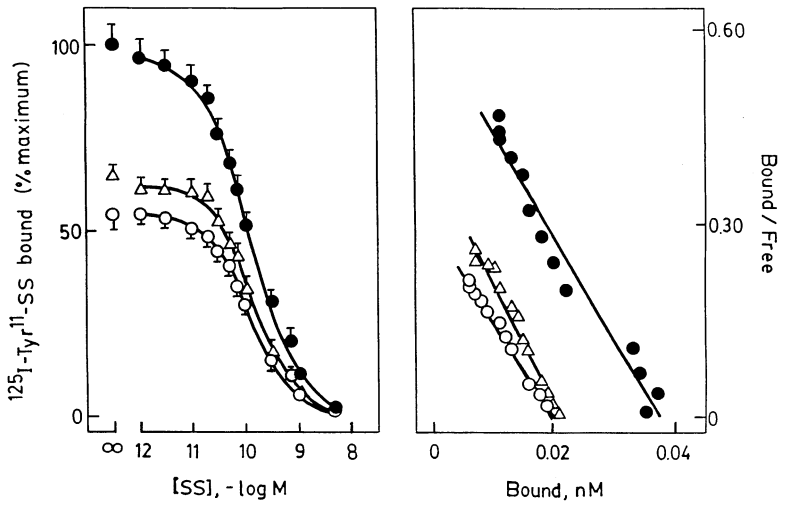

Fig. 3. Left: Competitive inhibition of [^{125}I-Tyr^{11}]somatostatin ([^{125}I-Tyr^{11}]SS) (35 pM) binding to pancreatic acinar membranes by unlabeled somatostatin (SS). The membranes (75 μg of protein/ml) were incubated for 90 min at 20°C in the presence of 35 pM [^{125}I-Tyr^{11}]SS and increasing concentrations of native peptide. Points correspond to values for control rats (●) and rats treated with bombesin [7 days (○) and 14 days (△)]. Each point is the mean of five separate experiments, each performed in duplicate. The results express the value of a pool of the control groups, because the B_{max} and the K_d values of the control groups were similar. Right: Scatchard analysis of the same data.

Table 3
Degradation of the tracer [^{125}I-Tyr^{11}]somatostatin ([^{125}I-Tyr^{11}]SS) after incubation with pancreatic acinar cell membranes from each experimental group

Groups	% of Degraded tracer
Controls	10.1
Bombesin	10.9
Bombesin + proglumide	9.2
RC-3095 + bombesin	10.7
RC-3095	9.4

whether the effects of bombesin on the pancreatic somatostatinergic system involved the activation of gastrin/CCK receptors. The combination of PG with bombesin failed to affect the bombesin-induced changes in the SS receptor–effector system (Tables 1–3). PG did not affect SS content or the SS receptor–effector system (data not shown).

The bombesin antagonist RC-3095 had similar effects on the number of SS receptors and on SS-mediated inhibition of FK-stimulated AC activity as bombesin. Bombesin administration in combination with its antagonist RC-3095 induced a greater SS-mediated inhibition of FK-stimulated AC activity as well as a greater decrease in the binding capacity of the SS receptors (Tables 1 and 2). Bombesin administration did not affect amount of somatostatin-like-immunoreactive content in the rat pancreas at the two time periods studied (Table 4).

4. Discussion

In this study, we have shown that the ability of Gpp(NH)p to inhibit FK-stimulated AC activity was diminished in pancreatic acinar cell membranes from bombesin-treated rats in addition to an already reported decrease in the number of SS receptors and that both bombesin and its antagonist RC-3095 have similar effects on the SS receptor–effector system in rat pancreatic acinar membranes.

Recently, it has been reported that the adenosine 3′,5′-cyclic monophosphate (cAMP) pathway is involved in the inhibitory effect of SS on pancreatic exocrine secretion [48].

Table 4
Pancreatic somatostatin-like immunoreactivity (SSLI) levels in rats treated for 7 or 14 days with saline, bombesin, or bombesin combined with proglumide

Groups	SSLI (ng of SS-14/mg of protein)	
	7	14
Saline	4.20 ± 0.72	4.27 ± 0.61
Bombesin	4.34 ± 0.62	5.24 ± 0.73
Proglumide + bombesin	4.43 ± 0.57	4.32 ± 0.25

The SSLI levels are expressed as nanograms of SS per milligram of protein. Data are mean ± SEM values of the combined data from five rats in each group. Determinations were made in duplicate for each experiment.

Therefore, in the present study we examined the effect of SS on AC activity in pancreatic acinar cell membranes from control and bombesin-treated rats. Our results confirm the observations of other authors showing that SS inhibits FK-stimulated AC activity but does not modify rat pancreatic basal AC activity [17]. SS was a partial antagonist of FK-stimulated AC activity in pancreatic acinar membranes, in agreement with Heisler [17]. In this respect, it is well established that the inhibition of FK-stimulated AC activity by SS in pancreatic acinar cell membranes and other cell types involves three protein components found in the plasma membrane [12,42,43,47], ie, the SS receptor, the AC catalytic unit, and the GTP binding protein G_i, which couples the SS receptor to the catalytic unit. Therefore, impairment of any of these three components by bombesin could attenuate the effect of SS. This study did not reveal any defect in the AC catalytic unit because in membranes from control or bombesin-treated animals, similar levels of activity were noted after direct stimulation of this enzyme by the diterpene FK.

Although bombesin did not vary the PTX-catalyzed ADP-ribosylation of G_i proteins, nor the ability of Gpp(NH)p to inhibit binding of ^{125}I-Tyr^{11}]-SS, the inhibitory effect of Gpp(NH)p on FK-stimulated AC was markedly decreased in pancreatic acinar membranes from bombesin-treated rats. This, together with the decrease in the number of SS receptors, would explain, at least in part, the attenuated inhibition of FK-stimulated AC activity.

Although no changes in pancreatic SS content were detected by radioimmunoassay, it has been shown that bombesin stimulates pancreatic SS release [16]. In this case, an increased SS release or turnover might then lead to downregulation and sensitization of the SS receptor–effector system.

Because bombesin is a potent secretagogue of pancreatic enzyme secretion [21], the decrease of the number of SS receptors observed in bombesin-treated rats is consistent with the view that SS may function as an inhibitor of pancreatic exocrine secretion [3]. In this regard, other pancreatic secretagogues such as vasoactive intestinal peptide, secretin, CCK, and carbachol have been shown to decrease the maximum binding capacity of SS in pancreatic acinar membranes [44].

The parameters of the SS receptors in the control rats were similar to those previously reported by others [12,42, 47,48]. Although five SS receptor subtypes have been cloned [22], the exocrine pancreas appears to express only receptor subtype 2 (SSTR2) [5]. It is tempting to speculate that the decrease in the SS receptor density in bombesin-treated rats could result, at least in part, from downregulation of the SSTR2 subtype.

The results of this study show that both bombesin and its antagonist RC-3095 have similar effects on the SS receptor–effector system. It is conceivable that molecules with different properties might produce similar effects through a commonly activated system. It has been shown that chronic administration of bombesin or GRP will result in a down-

regulation of bombesin/GRP receptors similar to that produced by the antagonists of these peptides, such as RC-3095 [49]. This may explain why bombesin and RC-3095 have similar effects. In this context, it is also well established that treatment with bombesin and its antagonist RC-3095 decreases the binding capacity of epidermal growth factor (EGF) receptors in pancreatic cancers [49]. The greatest reduction in the concentration of EGF receptors and tumor growth inhibition were observed after treatment with RC-3095 plus GRP [49]. Recently, Vidal et al. [51] have shown that EGF increases the SS receptor density as well as the biological response to SS in pancreatic tumor AR42J cells. Therefore, it is possible that the decrease in functional EGF receptors induced by bombesin or its antagonist RC-3095 may be the common mechanism of action of both peptides on the SS receptor–effector system.

The physiological role of the decrease in the SS receptor–effector system induced by bombesin in the exocrine pancreas cannot be stated at this time. However, bombesin-like immunoreactivity was identified by immunocytochemistry within intrapancreatic nerves, both in the exocrine portion of the gland and within the islets [26]. Specific bombesin receptors have been functionally and structurally identified in rat, mouse, guinea pig, and human pancreatic acinar membranes [13,24,45]. Several findings support that bombesin-like peptides are important in the mediation of the pancreatic response to vagal stimulation in the anesthetized rat model [37]. Thus, that bombesin, an activator of pancreatic enzyme secretion [21], inhibits the SS receptor–effector system, which is considered to be an inhibitor of this secretion [3], suggests that the regulation of SS binding by bombesin could be physiologically important in permitting the pancreas to secrete more enzymes after stimulation of the organ.

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