The effect of pentagastrin on the somatostatin receptor/effect system in rat pancreatic acinar membranes

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Abstract

An intraperitoneal (i.p.) injection of pentagastrin (250 μg/kg, three times daily) for 1 week increased somatostatin like-immunoreactivity (SSLI) content in the pancreas and the number of somatostatin (SS) receptors in pancreatic acinar membranes without influencing their apparent affinity as compared with control animals. No significant differences were seen in basal or forskolin (FK)-stimulated adenylate cyclase (AC) enzyme activities in the control and pentagastrin-treated rats. In spite of the increase in the number of SS receptors, SS caused a significantly lower inhibition in AC activity in these membranes. This finding is related to the fact that the stable GTP analogue, 5'-guanylylimidodiphosphate (Gpp[NH]p) was a much less potent inhibitor of binding in the pancreatic acinar cell membranes from pentagastrin-treated animals than in those from controls. In addition the ability of Gpp(NH)p to inhibit FK-stimulated AC activity was also decreased in pancreatic acinar cell membranes from pentagastrin-treated rats. Pretreatment with proglumide, (20 mg/kg i.p.) a gastrin/cholecystokinin (CCK) receptor antagonist, prevented the pentagastrin-induced changes in SS level and binding as well as the inhibitory effect of SS on AC activity in pancreatic acinar cell membranes. Proglumide alone had no observable effect on the somatostatinergic system. These data suggest a SS receptor/G protein uncoupling as a result of binding of pentagastrin to gastrin receptors present in pancreatic acinar cell membranes.

Keywords: Pentagastrin; Somatostatin receptor; Adenylate cyclase; Pancreatic acinar membrane; Rat

1. Introduction

Somatostatin (SS), originally discovered as a hypothalamic inhibitor of pituitary hormone secretion [1], is a 14 amino acid peptide that is found throughout the brain as well as in peripheral tissues and that exerts a wide spectrum of biological actions [2,3]. High-affinity SS receptors have been demonstrated in various tissues [2,3]. Recent studies have shown the existence of SS receptors on pancreatic acinar cells [4,5], indicating that SS acts directly on acinar cells. These receptors are probably coupled to the
adenylate cyclase (AC) enzyme system via a guanine nucleotide inhibitory protein, Gi [6]; guanine nucleotides inhibit SS binding to its receptor [6]. Specific binding of SS to receptors in the exocrine pancreas is regulated not only by SS [4] but also by heterogeneous pancreatic peptides such as cholecystokinin (CCK) and carbachol, agents that act by mobilizing intracellular Ca^{2+} to provoke enzyme secretion in the pancreas [7]. It is also well established that pancreatic secretagogues such as vasoactive intestinal peptide (VIP) or secretin, which act via an increase in intracellular cyclic AMP (cAMP) levels, decrease the maximum binding capacity of SS [8]. However, it is still unknown whether gastrin binding to its pancreatic acinar cell membrane receptors [9–11] could regulate the binding of SS to its receptors in these membranes. On the other hand, gastrin and SS exert opposite effects on the growth of the exocrine pancreas [12–15].

The aims of the present study were to investigate the effect of pentagastrin on SS receptors and SS-inhibited AC activity in rat pancreatic acinar cell membranes. Pretreatment with proglumide, an antagonist of receptors of the gastrin/CCK peptide family [9,12], was used in order to evaluate whether the effects of pentagastrin on the pancreatic somatostatinergic system involved activation of gastrin/CCK receptors. Experiments were also performed to measure the inhibition of specific [125I]-Tyr^{1-SS} binding by the stable GTP analogue, 5'-guanylylimidodiphosphate (Gpp[NH]p), in order to determine the integrity of SS receptor binding site-G protein interactions. In addition, somatostatin-like immunoreactivity (SSLI) in the pancreas of pentagastrin-treated animals and controls was also studied.

2. Materials and methods

2.1. Chemicals

Pentagastrin was kindly donated by ICI-Farma (Spain). Synthetic Tyr^{1-SS} was purchased from Universal Biologicals Ltd. (Cambridge, UK); carrier-free Na^{125I} (IMS 30, 100 mCi/ml) was purchased from the Radiochemical Center (Amersham, UK); forskolin, bacitracin, phenylmethylsulfonylfluoride (PMSF), guanosine triphosphate (GTP), 5'-guanylylimidodiphosphate (Gpp[NH]p), 3-isobutyl-1-methylxantine (IBMX) and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). The specific activity of the purified labelled peptide was about 600 Ci/mmol. The rabbit antibody used in the radioimmunoassay technique was purchased from the Radiochemical Centre (Amersham, UK). This antiserum was raised in rabbits against SS-14 conjugated to BSA and is specific for SS. Since SS-14 constitutes the C-terminal portions of both SS-25 and SS-28, the antiserum does, not distinguish between these three forms. The binding of SS-14 to this antibody does not depend on an intact disulfide bond in the molecule, since breaking of the disulfide bond by reaction with 0.1% mercaptoethanol (boiling water bath, 5 min) did not change peptide immunoreactivity. Cross-reactivity with other peptides was less than 0.5%. Cross-reaction with several SS analogues demonstrated that neither the N-terminal glycine nor the C-terminal cysteine residues are required for antibody binding, suggesting that the antigen site is directed towards the central part of the molecule containing the tryptophan residue. All other reagents were of the highest purity commercially available.

2.2. Experimental animals

Male Wistar rats weighing 200–250 g received, three times daily and for 1 week, intraperitoneal injections of saline or pentagastrin (Peptavlon) (250 μg/kg) [13] alone and in combination with proglumide (20 mg/kg). This dose of pentagastrin causes maximal stimulation of DNA synthesis in the rat [12]. Rats were killed 8 h after the last injection. The pancreas was removed and trimmed free of fat, connective tissues and lymph nodes.
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 units of collagenase/ml in an oxygenated Krebs-Ringer medium as described by Amsterdam et al. [16]. After thorough washing by sedimentation, acini were transferred to 0.3 M sucrose. In 0.3 M sucrose the acini were homogenized at 4°C by use of a Potter homogenizer following the Meldolesi et al. method [17]. 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 90 min. The plasma-membrane-enriched fraction collected from the interphase was pelleted and stored at -70°C.

2.4. Binding of [125I]-Tyr11-SS

Binding of [125I]-Tyr11-SS was assayed on pancreatic acinar membranes from rats by a modified method [18]. Tyr11-SS was radiiodinated by chloramine-T iodination according to the method of Greenwood [19]. Separation of iodinated SS from unincorporated iodide was carried out on a Sephadex G-25 (fine) column equilibrated and eluted with 0.1 M acetic acid in BSA (0.1%, w/v). The specific activity of the radioligand was 600 Ci/mmol.

Binding of [125I]-Tyr11-SS to pancreatic acinar membranes was carried out in a total volume of 250 μl in 50 mM Tris-HCl buffer (pH 7.4) containing 0.5 mM MgCl2, 3 mM NaCl, 0.2 mM CaCl2, 0.2% (w/v) 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 [125I]-Tyr11-SS in the absence or presence of 0.01~10 nM unlabelled 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 scintillation counter. Non-specific 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 non-specific membrane-associated radioactivity. The effects of Gpp[NH]p on [125I]-Tyr11-SS binding were determined after addition of a range of Gpp[NH]p concentrations (10^{-11}~10^{-4} M) in the binding assay buffer.

2.5. Evaluation of radiolabelled 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. Briefly, [125I]-Tyr11-SS (35 pM) was incubated with pancreatic acinar membranes (75 μg 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 radiolabelled peptide which specifically bound during the second incubation was measured and expressed as a percentage of the binding that has been obtained in control experiments performed in the absence of pancreatic acinar membranes during the preincubation period.

2.6. Adenylate cyclase assay

AC activity was measured as previously reported [20] with minor modifications. Briefly, rat pancreatic acinar membranes (0.12 mg protein/ml) were incubated with 1.5 mM ATP, 5 mM MgSO4, 1 μM ATP and ATP-regenerating system (7.5 mg/ml creatine phosphate and 1 mg/ml creatine kinase), 1 mM 3-isobutyl-1-methylxantine, 0.1 mM PMSF, 1 mg/ml bacitracin, 1 mM EDTA, and tested substances (10^{-9} M SS or 10^{-5} M FK) in 0.1 ml of 0.025 M triethanolamine/HCl buffer (pH 7.4). After 30 min incubation at 30°C, the reaction was stopped by heating the mixture for 3 min. After refrigeration, 0.2 ml of an alumina slurry (0.75 g/ml in triethanolamine/HCl buffer, pH 7.4) was added and the suspension centrifuged. The supernatant was
harvested for the assay of cyclic AMP by the method of Gilman [21].

2.7. Tissue extraction and SS radioimmunoassay

For SSLI measurement, the pancreases were rapidly homogenized in 1 ml 2 M acetic acid using a Brinkman polytron (setting 5, 30 s). The extracts were boiled for 5 min in an ice-chilled water-bath, and aliquots (100 μl) were removed for protein determination [22]. 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 assayed. The SS concentration was determined in tissue extracts by a modified radioimmunoassay method [23], with a sensitivity limit of 10 pg/ml.

Incubation tubes prepared in duplicate contained 100 μl samples of tissue extracts or standard solutions of 0–500 pg cyclic SS-14 diluted in phosphate buffer (0.05 M, pH 7.2 containing 0.3% BSA, 0.01 M EDTA), 200 μl appropriately diluted anti-SS serum, 100 μl freshly prepared [125I]-Tyr11-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 dextran-coated charcoal (dextran T-70, 0.2%, w/v), Pharmacia, Uppsala, Sweden; charcoal: Norit A, 2% (w/v), Serva, Feinbiochemica, Heidelberg, Germany). Serial dilution curves for the samples were parallel with the standard curve. The intra and inter-assay variation coefficients were 6.0 and 8.8% respectively.

2.8. Data analysis

The computer program LIGAND [24] was used to analyze the binding data. The use of this program allowed us to select those receptor models which best fit given sets of binding data. The same program was also used to present data in the form of Scatchard plots and to compute values for receptor affinity (Kd) and density (Bmax) that best fit the sets of binding data for each rat. The paired t test was applied to means derived from the best fit of data points for each individual animal.

3. Results

Pentagastrin administration increased the SSLI content in the pancreas as compared with the control group (Table 1). Preliminary experiments confirmed that specific binding of [125I]-Tyr11-SS to pancreatic acinar cell membranes changed linearly with protein concentration and was time-dependent in all experimental groups. An apparent equilibrium was observed between 60 and 120 min at 20°C (data not shown). All subsequent binding experiments were therefore conducted at 20°C for 90 min.

Daily i.p. injections of pentagastrin for 7 days were associated with an increase in SS binding (Fig. 1), which was the result of the increase in the

<table>
<thead>
<tr>
<th>Groups</th>
<th>SS receptor</th>
<th>SS LI</th>
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<tr>
<td></td>
<td>Bmax</td>
<td>Kd</td>
</tr>
<tr>
<td>Controls</td>
<td>625 ±112</td>
<td>0.075 ±0.011</td>
</tr>
<tr>
<td>Peptavlon</td>
<td>1131 ±47**</td>
<td>0.078 ±0.003</td>
</tr>
<tr>
<td>Proglumide</td>
<td>675 ±85</td>
<td>0.073 ±0.005</td>
</tr>
<tr>
<td>Pre plus peptavlon</td>
<td>668 ±93</td>
<td>0.074 ±0.007</td>
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</table>

Table 1
Effect of peptavlon and proglumide on somatostatin-like immunoreactive (SSLI) concentration in rat pancreas and on the equilibrium parameters for somatostatin (SS) binding to rat pancreatic acinar membranes

Binding parameters were calculated from Scatchard plots by linear regression. Units for SSLI are ng SS per mg protein, units for Kd are nM and units for Bmax are fmol of SS bound per mg of protein. The results are represented as the means ± S.E.M. of five separate experiments. Statistical comparison versus control: *P<0.05, **P<0.01.
growth of the maximal number of binding sites as
revealed by Scatchard plots of the binding data
(Fig. 1 and Table 1). Pentagastrin administration
was not associated with any appreciable change in
the \( K_d \) of SS binding to pancreatic acinar
membranes. Pancreatic acinar membranes from
control and pentagastrin-treated rats showed a similar
peptide degradation capacity and the values varied by
no more than 11% in all the experimental groups.

No significant differences were seen for either
basal or FK-stimulated AC activities between the
control and pentagastrin groups. The capacity of SS
(10^{-9} M) to inhibit basal and FK stimulated AC
activity in control and pentagastrin treated rats is
shown in Table 2. It is to be noted that SS did not
modify basal AC activity. The effect of SS on FK-
stimulated AC activity was markedly decreased
in pancreatic acinar membranes from pentagastrin-
treated rats as compared to control animals
(Table 2).

The modulation of \([^{125}I]\)-Tyr^{11}-SS binding by
guanine nucleotides was subsequently studied in
control and pentagastrin treated rats. After 7 days of
pentagastrin administration Gpp[NH]p inhibition
curves had shifted to the right. Gpp[NH]p was a
much less potent inhibitor of binding in the mem-
branes from pentagastrin treated animals than in
those from controls (Fig. 2).

Further experiments explored the effect of pentag-
astatin on GTP proteins in rat pancreatic acinar mem-
branes by determining the ability of low Gpp(NH)p

<p>| Table 2 |
|-----------------|-----------------|-----------------|-----------------|
| Effect of somatostatin (SS) ((10^{-5} \text{ M})) and forskolin (FK) ((10^{-5} \text{ M})) on adenylate cyclase (AC) activity (pmol cAMP/min/mg protein) in pancreatic acinar membranes of control, pentavaxion, proglumide + pentavaxion and proglumide-treated rats |</p>
<table>
<thead>
<tr>
<th>Control</th>
<th>Peptavaxion</th>
<th>Proglumide + peptavaxion</th>
<th>Proglumide</th>
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</thead>
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<tr>
<td>Basal activity</td>
<td>16.4 ± 2.5</td>
<td>15.2 ± 1.3</td>
<td>16.1 ± 1.5</td>
</tr>
<tr>
<td>Basal activity + 10^{-9} M SS</td>
<td>13.3 ± 1.3</td>
<td>12.6 ± 0.8</td>
<td>14.1 ± 1.2</td>
</tr>
<tr>
<td>+ 10^{-5} M FK</td>
<td>23.5 ± 0.9</td>
<td>22.1 ± 1.2</td>
<td>22.3 ± 1.0</td>
</tr>
<tr>
<td>Fold FK stimulation over basal</td>
<td>1.4 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>10^{-3} M FK + 10^{-9} M SS</td>
<td>14.6 ± 0.4</td>
<td>17.5 ± 0.3</td>
<td>13.9 ± 0.4</td>
</tr>
<tr>
<td>% SS inhibition of FK stimulation</td>
<td>37.9 ± 3.8</td>
<td>20.8 ± 2.2**</td>
<td>37.6 ± 4.4</td>
</tr>
</tbody>
</table>

Experiments were performed as described in Materials and methods. Values represent the mean ± S.E.M. of the determinations performed. Statistical comparison versus control: **P < 0.01.
concentrations to inhibit forskolin-stimulated AC activity. Using this assay, membranes derived from control rats yielded a characteristic biphasic response curve (Fig. 3). Gpp(NH)p concentrations between 0.01–1 nM decreased AC activity ($P < 0.05$) due to $G_s$ 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 was markedly decreased in pancreatic acinar membranes from pentagastrin-treated rats (Fig. 3).

Pretreatment with proglumide (20 mg/kg, i.p.) prevented the pentagastrin-induced changes in SSSI level, SS binding as well as the inhibitory effect of SS on AC activity in pancreatic acinar cell membranes while proglumide alone had no observable effect (Tables 1 and 2).

4. Discussion

In rats, a daily intraperitoneal injection of pentagastrin for 7 days increases the mean pancreatic weight as well as the SSSI content and the number of specific SS receptors in pancreatic acinar cell membranes. The ability of Gpp[NH]p to inhibit SS binding to its receptors was decreased in pancreatic acinar cell membranes from pentagastrin-treated rats. SS significantly lowered the inhibition of AC activity in these membranes.

The SSSI levels as well as the binding parameters of SS receptors in the control rats were similar to those previously reported by others [4,5,25]. SS did not modify basal AC activity and was a partial antagonist of FK stimulated pancreatic AC activity in agreement with other authors [25,26].

The molecular mechanism by which pentagastrin modifies the somatostatinergic system in the...
pancreas is unknown. However, the gastrin receptors seem to mediate the action of pentagastrin, since the changes induced by pentagastrin on the somatostatinergic system were prevented by the gastrin/CCK receptor antagonist proglumide. In addition, proglumide alone had no demonstrable effect on these parameters.

Recently, two types of receptors for the gastrin/CCK peptide family have been identified in pancreatic acinar cells [9–11], the CCK₆ receptors, that have a much higher affinity for CCK than for gastrin [10], and the gastrin receptors (CCK₅ or GR), that have approximately the same affinity for CCK and gastrin [10]. The CCK-A receptor [27] and the GR [28] have each been cloned. With one exception, all CCK receptor antagonists described to date interact only with receptors that interact with the CCK/gastrin family of peptides.

The mechanism by which pentagastrin in vivo raises an increase in SSLI levels is unknown. However, several studies have shown that gastrin stimulates pancreatic SS secretion [29] and that agents that stimulate SS secretion may also regulate SS biosynthesis [30].

Since growth factor-induced cell division is paralleled by heteroregulation of other cell surface receptors [31] it is possible that SS receptors are targets for heterologous regulation by growth factors. Thus, the increase of SS receptors could be secondary to the mitogenic signal of gastrin [12,14]. It is well known that cell surface receptor regulation by heterologous ligands is an important mechanism in the physiological response to a given factor.

Compared with controls, the ability of SS to inhibit AC is decreased in pancreatic acinar membranes from pentagastrin-treated animals. However, this did not appear to be due to any defect in the catalytic unit of the AC itself. Indeed, similar levels of activities were noted in membranes from both control and pentagastrin-treated animals when this enzyme was stimulated directly by the diterpene FK. The present results support the hypothesis that the cAMP pathway is not involved in the inhibitory effect of SS on pancreatic growth caused by pentagastrin. The increase in SS receptors in the pancreatic acinar membranes following pentagastrin administration was not accompanied by a corresponding increase in SS-inhibited AC activity. This may be because the coupling between the SS receptors and the catalytic subunit of AC, mediated via Gi, was affected. Interestingly, the inhibitory effect of Gpp(NH)p on SS receptor binding was markedly decreased in pancreatic acinar membranes from pentagastrin treated rats. In addition the inhibitory effect of Gpp(NH)p on FK-stimulated AC was markedly decreased in pancreatic acinar membranes from pentagastrin-treated rats. These findings suggest that there is an abnormality at the Gi level in these membranes that would explain the decreased inhibition of AC by SS after pentagastrin administration.

The mechanism by which Gi is altered after pentagastrin administration is not clear. It is possible that a covalent modification of the a-subunit such as a change in phosphorylation might cause this alteration of Gi. In this regard, the activation of protein kinase C may be involved in the mechanism of action of gastrin [31,32]. Another possibility that could be considered is the sequestration of Gi. It has recently been shown that growth factor-induced cell division is paralleled by a translocation of Gi from the plasma membrane to the nucleus, which leads to a loss of Gi activity in the plasma membrane [33]. A possibility that must be checked is that there was a translocation in the exocrine pancreas after pentagastrin administration simultaneous to a strong cellular proliferation.

Alternatively, it is possible that new SS receptors may represent a subpopulation of SS receptors that are either coupled to an alternative signal transduction mechanism [18]. This possibility is consistent with the fact that molecular cloning has revealed the presence of five structurally related SS receptors [34].

These results suggest that SS receptors might be regulated by gastrin receptors, and that there is an abnormality in the integrity of the SS receptor
binding site--G protein interaction, that would explain the decreased inhibition of AC by SS after pentagastrin administration.

Since gastrin increases proliferation of pancreatic acinar cells [12,14] and SS appears to be an inhibitor of this proliferation [13,15], it is tempting to speculate that the rise in the number of SS receptors found in the present study may constitute a protective mechanism to shield the pancreas from hyperplasia caused by overstimulation.

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