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The effect of pentagastrin on the somatostatin receptor/effector 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 inibit 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

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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 the activation of gastrin/CCK receptors. Experiments were also performed to measure the inhibition of specific [^{125}I]-Tyr^{11}-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^{11}-SS was purchased from Universal Biologicals Ltd. (Cambridge, UK); carrier-free Na^{125}I (IMS 30, 100 mCi/ml) was purchased from the Radiochemical Center (Amersham, UK); forskolin, bacitracin, phenylmethylsulphonylfluoride (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 [^{125}I]-Tyr^{11}-SS

Binding of [^{125}I]-Tyr^{11}-SS was assayed on pancreatic acinar membranes from rats by a modified method [18]. Tyr^{11}-SS was radioiodinated by chloramine-T iodination according to the method of Greenwood [19]. Separation of iodinated SS from unincorporated iodine 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 [^{125}I]-Tyr^{11}-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 $MgCl_2$, 3 mM NaCl, 0.2 mM $CaCl_2$, 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 [^{125}I]-Tyr^{11}-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 [^{125}I]-Tyr^{11}-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, [^{125}I]-Tyr^{11}-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 $MgSO_4$, 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 assay. 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 [^{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 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 (K_d) and density (B_{max}) 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 [^{125}I]-Tyr^{11}-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), wich was the result of the increase in the

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

Groups	SS receptor		SSLI
	B_{max}	K_d	
Controls	625 ± 112	0.075 ± 0.011	4.84 ± 1.12
Peptavlon	1131 ± 47**	0.078 ± 0.003	6.15 ± 0.62*
Proglumide + peptavlon	675 ± 85	0.073 ± 0.005	4.76 ± 0.92
Proglumide	668 ± 93	0.074 ± 0.007	4.79 ± 0.87

Binding parameters were calculated from Scatchard plots by linear regression. Units for SSLI are ng SS per mg protein, units for K_d are nM and units for B_{max} 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: *$P<0.05$, **$P<0.01$.

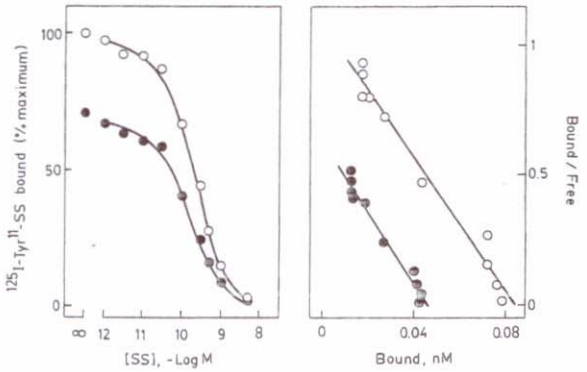

Fig. 1. (Left panel) Competitive inhibition of [^{125}I]-Tyr^{11}-somatostatin ([^{125}I]-Tyr^{11}-SS, 35 pM) binding to pancreatic acinar membranes by unlabelled somatostatin (SS). The membranes (75 μg protein/ml) were incubated for 90 min at 20°C in the presence of 35 PM [^{125}I]-Tyr^{11}-SS and increasing concentrations of native peptide. Points correspond to values for control (●) and peptavlon-treated rats (○). Each point is the mean of six separate experiments, each performed in duplicate. The results express the value of a pool of the control groups, since the B_{max} and the K_d values of the control groups were not affected. For the sake of clarity S.E.M. values are not represented but were always below 10% of the mean values. (Right panel) Scatchard analysis of the same data.

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 membranes from pentagastrin treated animals than in those from controls (Fig. 2).

Further experiments explored the effect of pentagastrin on G_i proteins in rat pancreatic acinar membranes by determining the ability of low Gpp(NH)p

Table 2

Effect of somatostatin (SS) (10^{-9} M) and forskolin (FK) (10^{-5} M) on adenylate cyclase (AC) activity (pmol cAMP/min/mg protein) in pancreatic acinar membranes of control, peptavlon, proglumide + peptavlon and proglumide-treated rats

	Control	Peptavlon	Proglumide + peptavlon	Proglumide
Basal activity	16.4 ± 2.5	15.2 ± 1.3	16.1 ± 1.5	16.7 ± 1.9
Basal activity + 10^{-9} M SS	13.3 ± 1.3	12.6 ± 0.8	14.1 ± 1.2	13.8 ± 1.1
+ 10^{-5} M FK	23.5 ± 0.9	22.1 ± 1.2	22.3 ± 1.0	23.8 ± 1.5
Fold FK stimulation over basal	1.4 ± 0.2	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.4
10^{-5} M FK + 10^{-9} M SS	14.6 ± 0.4	17.5 ± 0.3	13.9 ± 0.4	15.2 ± 0.7
% SS inhibition of FK stimulation	37.9 ± 3.8	20.8 ± 2.2**	37.6 ± 4.4	36.1 ± 5.2

Experiments were performed as described in Materials and methods. Values represent the mean ± S.E.M. of the determinations performed. Statistical comparison versus control: $^{**}P < 0.01$.

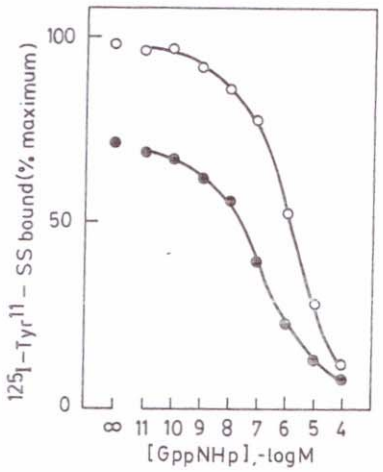

Fig. 2. Dose-effect curves for 5′-guanylylimidodiphosphate (Gpp[NH]p) on the specific binding of [^{125}I]-Tyr^{11}-somatostatin ([^{125}I]-Tyr^{11}-SS 35 pM) to rat pancreatic acinar membranes from control (●) and peptavlon-treated rats (○). The results express the values of a pool of the control groups, since differences among them were not found. Each point is the mean of six separate experiments, each performed in duplicate. For the sake of clarity, S.E.M. values are not represented but were always below 9% of the mean values.

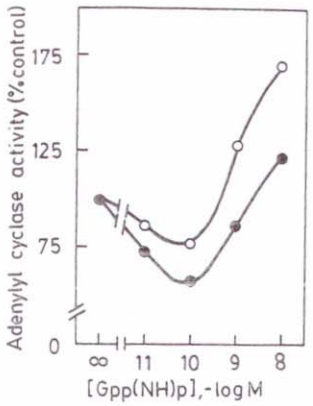

Fig. 3. Dose-effect curves for 5′-guanylylimidodiphosphate (Gpp[NH]p) on forskolin-stimulated adnylyl cyclase (AC) activity in rat pancreatic acinar membranes from control (●) and pentagastrin-treated (○) rats. The enzyme activity was measured in the presence of 100 nM forskolin and the indicated concentration of Gpp[NH]p. Date are expressed as a percentage of forskolin-stimulated activity (100%) in the absence of Gpp(NH)p. The results are given as the mean ± S.E.M. of three determinations performed in duplicate. For the sake of clarity, S.E.M. are not represented but were always below 10% of the mean values.

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_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 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 SSLI 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 SSLI 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 SSLI levels as well as the binding parameters of SS receptors in the control rats were similar to those previously reported by others [4,5,23]. 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 gastric/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 CCK_A receptors, that have a much higher affinity for CCK than for gastrin [10], and the gastrin receptors (CCK_C 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 wich pentagastrin in vivo cuases 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 paralelled 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 G_i level in these membranes that would explain the decreased inhibition of AC by SS after pentagastrin administration.

The mechanism by which G_i is altered after pentagastrin administration is not clear. It is possible that a covalent modification of the α-subunit such as a change in phosphorylation might cause this alteration of G_i. 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 G_i. It has recently been shown that growth factor-induced cell division is parallelled 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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References

[1] Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J. and Guillemin, R., Hypothalamic peptide that inhibits the secretion of immunoreactive pituitary growth hormone, Science, 179 (1973) 77–79.

[2] Epelbaum, J., Somatostatin in the central nervous system: physiology and pathological modification, Prog. Neurobiol., 27 (1983) 63–100.

[3] Reichlin, S., Somatostatin, N. Engl. J. Med., 309 (1983) 1495–1556.

[4] Srikant, C.B. and Patel, Y.C., Somatostatin receptor in rat pancreatic acinar cells: pharmacological and strucutral characterization and demonstration of downregulation in streptozotocin diabetes, J. Biol. Chem., 261 (1986) 7690–7696.

[5] Knuhtsen, S., Esteve, J.P., Cambillau, C., Colás, B., Susini, C. and Vaysse, N., Solubilization and characterization of active somatostatin receptors from rat pancreas, J. Biol. Chem., 265 (1990) 1129–1133.

[6] Sakamoto, C., Matozaki, T., Nagao, M. and Baba, S., Coupling of guanine nucleotide inhibitory protein to somatostatin receptors on pancreatic acinar membranes, Am. J. Physiol., 253 (1987) G308–G314.

[7] Sakamoto, C., Matozaki, T., Nagao, M., Nishisaki, H. and Baba, S., Pancreatic secretagogues regulate somatostatin binding to its receptors on rat pancreatic acinar membranes, Pancreas, 3 (1988) 18–24.

[8] Matozaki, T., Sakamoto, C., Nagao, M. and Baba, S., Pancreatic secretagogues regulate somatostatin binding to acinar cell membranes via two-functional distinct pathways, Horm. Metabol. Res., 20 (1988) 141–144.

[9] Vinayek, R. and Gardner, J.D., Receptor identification, Methods Enzymol., 191 (1990) 609–639.

[10] Presti, M. and Gardner, J.D., Receptor antagonists for gastrointestinal peptides, Am. J. Physiol., 264 (1993) G399–G406.

[11] Laburthe, M., Kitabgi, P., Couvineau, A. and Amironoff, B., Peptide receptors and signal transduction in the digestive tract, Handbook of Experimental Pharmacology, Gastrointestinal Regulatory Peptides. In D.R. Brown (Ed.), Springer-Verlag, New York, Vol. 106, 1993, pp. 133–178.

[12] Johnson, L.R. and Guthrie, P.D., Mucosal DNA synthesis: a short term index of the tropic action of gastrin, Gastroenterology, 67 (1974) 453–459.

[13] Morisset, J., Somatostatin: a potential antigrowth factor for the exocrine pancreas, Regul. Pept., 10 (1984) 11–22.

[14] Hajri, A., Aprahamian, M. and Damge, C., Effect of prolonged adminstration of long-acting somatostatin on caerulein, CCK-8 and GRP induced pancreatic growth in the rat, Regul. Pept., 32 (1991) 227–237.

[15] Rünzi, M., Müller, M.K. and Goebell, H. Endogenous somatostatin possibly controls pancreatic growth: further evidence for feed back regulation, Regul. Pept., 47 (1993) 65–72.

[16] Amsterdam, A., Salomon, T.E. and Jamieson, J.D., Sequential dissociation of the exocrine pancreas into lobules, acini and individual cells, Methods Cell. Biol., 20 (1978) 361–378.

[17] Meldolesi, J., Jamieson, J.D. and Palade, G.E., Composition of cellular membranes in the pancreas of the guinea pig, J. Cell. Biol., 49 (1971) 109–129.

[18] Colás, B., Cambillau, C., Buscall, L., Zeggari, M., Esteve, J.P., Lautre, V., Thomas, F., Vaysse, N. and Susini, C., Stimulation of a membrane tyrosine phosphatase activity by somatostatin analogues in rat pancreatic acinar cells, Eur. J. Biochem., 207 (1992) 1017–1024.

[19] Grenwood, F.C., Hunter, W.M. and Glover, J.S., The preparation of [^{131}I]-labeled human growth hormone of high specific radioactivity, Biochem. J., 89 (1963) 114–123.

[20] Houslay, M.D., Metcalfe, J.C., Warren, G.P., Hesketh, T.R. and Smith, G.A., The glucagon receptor of rat liver plasma membrane can couple to adenylate cyclase without activating it, Biochim. Biophys. Acta, 436 (1976) 489–494.

[21] Gilman, A.G., A protein binding assay for adenosine 3′,

5′-cyclic monophosphate, Proc. Natl. Acad. Sci. USA, 67 (1970), 305–312.

[22] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the Folin phenol reagent, J. Biol. Chem., 193 (1951) 265–275.

[23] Patel, Y.C. and Reichlin, S., Somatostatin in hypothalamus, extrahypothalamic brain and peripheral tissues of the rat, Endocrinology, 102 (1978) 523–531.

[24] Munson, P.J. and Rodbard, D., LIGAND: A versatile computerized approach for characterization of ligand binding systems, Anal. Biochem., 1207 (1980) 220–239.

[25] Susini, C., Vaysse, N., Esteve, J.P., Pradayrol, L., Slassi, C. and Ribet, A., Biology of Normal and Cancerous Exocrine Pancreatic Cells. In A. Ribet, L. Pradayrol and C. Susini (Eds.). Elsevier, North Holland, New York, 1980, pp. 119–126.

[26] Heisler, S., Forskolin potentiates calcium-dependent amylase secretion from rat pancreatic acinar membranes, Can. J. Physiol. Pharmacol., 61 (1983) 1168–1176.

[27] Wank, S.A., Harkins, R., Jensen, R.T., Shapira, H., De Weerth, A. and Slattery, T., Purification, molecular cloning and functional expression of the cholecystokinin receptor from rat pancreas, Proc. Natl. Acad. Sci. USA, 89 (1992) 3125–3129.

[28] A.S., Lee, Y.M., Mc Bride, E.W., Miller, L.J., Lu, M., Lin, H.Y., Kolakowski, L.F. and Beinborn, M., Expression cloning and characterization of the, canine parietal cell gastrin receptor, Proc. Natl. Acad. Sci. USA, 89 (1992) 3605–3609.

[29] Ipp, E., Dobbs, R.E., Harris, V., Arimura, A., Vale, W. and Unger, R.H., The effects of gastrin, gastric inhibitory polypeptide, secretin and the octapeptide of cholecystokinin upon immunoreactive somatostatin release by the perfused canine pancreas, J. Clin. Invest., 60 (1977) 1216–1219.

[30] Goodman, R.H., Mandel, G., Sevariano, K.A. and Montminy, M., Somatostatin: Basic and Clinical Status. In S. Reichlin (Ed.), Plenum Press, New York, 1987, pp. 21–32.

[31] Bold, R.J., Ishizuka, J., Townsend, C.M. and Thompson, J.C., Biomolecular advances in gastrointestinal hormones, Arch. Surg., 128 (1993) 1268–1273.

[32] Bastie, M.J., Delvaux, M., Dufresne, M., Saunier-Blache, J.S., Vaysse, N. and Ribet, A., Distinct activation of Na^+-H^+ exchange by gastrin and CCK peptide in acini from guinea pig, 254 (1988) G25–G32.

[33] Crouch, M.F., Belford, D.A., Milburn, P.J. and Hendry, I.A., Pertussis toxin inhibits EGF, phorbol ester- and insulin-stimulated DNA synthesis in Balb/c 3T3 cells: evidence for postreceptor activation of G_{ix}, Biochem. Biophys. Res. Commun., 167 (1990), 1369–1376.

[34] Bell, G.I. and Reisine T., Molecular biology of somatostatin receptors, Trends. Neurol. Sci., 16 (1993) 34–38.