Pancreatic changes in somatostatin content and receptor/effector system after intrapancreatic injection of 5,7-dihydroxytryptamine

G Muñoz-Acedo, I Alvaro-Alonso and E Arilla

Unidad de Neuroendocrinologia Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad de Alcalá, Alcalá de Henares 28871, Madrid, Spain

(Requests for offprints should be addressed to E Arilla)

Abstract

To date, it is unknown whether intrapancreatic serotonergic nerves can influence pancreatic somatostatin (SS) content and the SS receptor/effector system in the exocrine pancreas. In this study, the intrapancreatic serotonergic nerves were chemically ablated by injecting a specific serotonin (5-HT) neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), into the substance of the gland. Three days after the injection, the 5-HT-like immunoreactive levels in the pancreas were reduced by more than 85% whereas somatostatin-like immunoreactive levels had increased (86%). The number of SS receptors in the pancreatic acinar cell membranes of the 5,7-DHT-treated rats was also increased (72%). No significant differences were seen in basal or forskolin-stimulated adenylate cyclase (AC) enzyme activities in the control and the 5,7-DHT-treated groups. In spite of the increase in the number of SS receptors in the pancreatic acinar cell membranes of 5,7-DHT-treated rats, SS caused a significantly lower inhibition of AC activity in these membranes. This finding is related to the observed decrease of a 41 kD pertussis toxin-sensitive substrate, presumably the α_i subunit of the guanine nucleotide inhibitory protein, in pancreatic acinar cell membranes 3 days after intrapancreatic 5,7-DHT administration when compared with the corresponding controls. The functions of pancreatic serotonergic nerves seem to be associated with enteropancreatic communication. These data together with the present results suggest that pancreatic SS content and the SS receptor/effector system in the exocrine pancreas may be regulated by enteropancreatic serotonergic nerve fibers and may participate in enteropancreatic reflexes.

Journal of Endocrinology (1995) **145,** 227–234

Introduction

Several studies have shown anatomical and functional interconnections between the serotonergic and somatostatinergic systems in the rat brain (Tanaka & Tsujimoto 1981, Kakigi & Maeda 1992). However, the possible interconnection of these two systems at the pancreatic level is less well known. The pancreas receives a serotonergic innervation which may be derived exclusively from the myenteric plexus of the stomach and duodenum (Kirchgessner & Gershon 1990). Most serotonergic immunoreactive axons in the pancreas terminate in ganglia although some fibers are also observed near acini, ducts, vessels, and islets of Langerhans cells (Kirchgessner & Gershon 1990). Recently pancreatic serotonergic receptors (5-HT_{1A} and 5-HT_{1P}) were found along nerve fiber bundles located in the stroma between acini, and over single cells scattered in the pancreatic parenchyma (Kirchgessner *et al.* 1992, 1993). The density of 5-HT_{1P} binding sites was higher than that of 5-HT_{1A} binding sites in the rat pancreas (Kirchgessner *et al.* 1993). On the other hand, somatostatin (SS) in the pancreas is localized in the (so-called) D cells in the islets of Langerhans (Arimura *et al.* 1975). Normal rat pancreatic acinar cell membranes possess specific SS receptors which have been characterized previously (Srikant & Patel 1986, Viguerie *et al.* 1988). These receptors are probably coupled to the adenylate cyclase (AC) enzyme system via a guanine nucleotide inhibitory protein (Gi) (Sakamoto *et al.* 1987). Guanine nucleotides inhibit SS binding to its receptors in pancreatic acinar cell membranes (Sakamoto *et al.* 1987). These receptors are accessible to circulating SS as well as to high local concentrations of islet SS. Serotonin (5-HT) and SS have been described as inhibiting insulin (Feldman *et al.* 1972, Alberti *et al.* 1973) and exocrine pancreatic secretion on a functional level (Mori *et al.* 1979, Susini *et al.* 1980, Matsushita *et al.* 1993). To date, whether intrapancreatic serotonergic nerves influence pancreatic SS content and the SS mechanism of action in the exocrine pancreas is not known. The experiments in this paper investigate the effects of chemical ablation of intrapancreatic serotonergic nerves by 5,7-dihydroxytryptamine (5,7-DHT) on the SS receptor/effector system in pancreatic acinar membranes and on total pancreatic somatostatin-like immunoreactive (SSLI) content.

  Printed in Great Britain
0022–0795/95/0145–0227 $08.00/0

Materials and Methods

Materials

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, Bucks, UK); 5,7-DHT, bacitracin, phenyl-methylsulphonylfluoride (PMSF), guanosine triphosphate (GTP), 5′-guanylylimidodiphosphate (Gpp-(NH)p); 3-isobutyl-1-methylxanthine (IBMX) and bovine serum albumin (BSA) were purchased from Sigma (St Louis, MO, USA). The rabbit antibody used in the radioimmunoassay technique was purchased from the Radiochemical Centre (Amersham). This antiserum was raised in rabbits against SS-14 conjugated to BSA and is specific for SS, but since SS-14 constitutes the C-terminal portion 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 the disulfide bond with 0·1% mercaptoethanol boiling water bath for 5 min does 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 residue is 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.

Experimental animals

The specific 5-HT neurotoxin, 5,7-DHT (Azmitia & Marovitz 1980), was dissolved to a final concentration of 5×10^{-4} M in Krebs Ringer buffer, pH 7·4, supplemented with ascorbic acid (10^{-3} M) as an antioxidant and the monoamine oxidase inhibitor, pargyline (10^{-4} M) (Jansson *et al.* 1985). Male Wistar rats weighing 225–250 g were anaesthetized with ether and the pancreas was exposed through a midline laparatomy. The pancreas was infiltrated completely with 2 ml 5,7-DHT solution injected into the substance of the gland through a 25 gauge needle as previously described (Jansson *et al.* 1985). The abdominal wound was closed. Control rats received an identical surgical procedure and infiltration of the pancreas with the buffer alone. The rats were decapitated 3 days after the infiltration. The pancreas was removed and trimmed free of fat, connective tissue and lymph nodes.

Measurement of 5-HT

Analysis by HPLC was performed as described earlier (Hansson & Rosengren 1978). Briefly, the pancreas was homogenized with an internal standard in 2 ml 0·4 M perchloric acid and after centrifugation the supernatant was further processed. α-Methyl-5-HT (150 ng) was used as internal standard. A model 6000A Waters high pressure pump equipped with a Rheodyne sample valve injector (Model 7125) and an electrochemical detector (Bioanalytical Systems, Model LC 10) were used. The detector potential was set at +0·75 V vs an Ag/AgCl reference electrode. 5-HT was adsorbed on a weak cation-exchange resin at pH 6·0 (Amberlite XE-64, 20 × 4 mm). After washing, the column was eluted in 0·25 ml fractions with 1·2 M HCl. The different fractions were analyzed by HPLC using a reversed phase column (5 μM Nucleosil C_{18}, 250 × 4·6 mm). The mobile phase consisted of aqueous methanol (17%) with 40 g methanesulphonic acid and 2·9 g phosphoric acid at pH 2·5. The method yields a 78% recovery.

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 collagenase/ml in an oxygenated Krebs-Ringer medium as described by Amsterdam *et al.* (1978). After thorough washing by sedimentation, acini were transferred to 0·3 M sucrose and homogenized at 4 °C by use of a Potter homogenizer following the method described by Meldolesi *et al.* (1971). 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 pelleted and stored at −70 °C.

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 (Colas *et al.* 1992). Tyr^{11}-SS was radioiodinated by chloramine-T iodination according to the method of Greenwood *et al.* (1963). 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 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 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. After incubation at 20 °C for 90 min, bound and free ligand were separated by centrifugation at 11 000 ***g*** for 4 min 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.

Evaluation of radiolabelled peptide degradation

To determine the extent of tracer degradation during incubation, the ability of preincubated peptide to bind to fresh pancreatic acinar membranes was measured. 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 had been obtained in control experiments performed in the absence of pancreatic acinar cell membranes during the preincubation period. Pancreatic acinar cell membranes from control and 5,7-DHT-treated rats showed a similar peptide degradation capacity and the values varied by no more than 9% in all the experimental groups.

Adenylate cyclase assay

Activity of AC was measured as previously reported (Houslay *et al.* 1976) with minor modifications. Briefly, rat pancreatic acinar membranes (0·12 mg/ml) were incubated with 1·5 mM ATP, 5 mM $MgSO_4$, 1 μM Gpp(NH)p and ATP-regenerating system (7·5 mg/ml creatine phosphate and 1 mg/ml creatine kinase), 1 mM IBMX, 0·1 mM PMSF, 1 mg/ml bacitracin, 1 mM EDTA, and test substances (10^{-11}–10^{-6} M SS or 10^{-4} M forskolin (FK)) in 0·1 ml 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 cooling, 0·2 ml alumina slurry (0·75 g/ml in triethanolamine–HCl buffer, pH 7·4) was added and the suspension centrifuged. The supernatant was taken for assay of cAMP by the method of Gilman (1970).

Pertussis toxin-catalyzed ADP ribosylation

The pertussis-toxin (PTX)-catalyzed ADP ribosylation was carried out as previously reported (Bokoch *et al.* 1983). After PTX activation, membranes (1·6 mg protein/ml) were incubated with the 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, 2 μM [^{32}P]NAD^+ (30 Ci/mmol) and an ATP-regeneration system. After 30 min at 30 °C, the reaction was stopped by addition of 1 ml ice-cold 100 mM Tris–HCl buffer (pH 8·0), 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% SDS (SDS-sample buffer). After heating for 30 min at 60 °C, the suspension was centrifuged for 10 min at 100 000 ***g*** and aliquots of the supernatant were submitted to SDS-PAGE using the procedure of Laemmli (1970) as previously described (Laburthe *et al.* 1984). The gels were run, fixed, dried and exposed to Dupont films (cronex 4) for 1–7 days at −80 °C using an intensifying screen.

Tissue extraction and SS radioimmunoassay

For SSLI measurement, the pancreata 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 and aliquots (100 μl) were removed for protein determination (Lowry *et al.* 1951). 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 (Patel & Reichlin 1978) with a sensitivity limit of 10 pg/ml. Incubation tubes prepared in duplicate contained 100 μl samples of unknown 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 c.p.m. (equivalent to 5–10 pg) and enough buffer to give 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 interassay coefficients of variation were 6·0 and 8·8% respectively.

Data analysis

The LIGAND computer program (Munson & Rodbard 1980) was used to analyze the binding data. The use of this program made it possible to select the models of receptors which best fit a given set of binding data. The same program was also used to present data in the form of Scatchard plots (Scatchard 1949) and to compute the values for receptor affinity (K_d) and density (B_{max}) that best fit the sets of binding data for each rat. Statistical comparisons of all the data were analyzed by ANOVA and the Newman-Keuls *t*-test. Means among groups were considered significantly different when the *P* value was less than 0·05. Each individual experiment was performed in duplicate.

TABLE 1. Effect of 5,7-dihydroxytryptamine (5,7-DHT) on somatostatin-like immunoreactive (SSLI) concentration, on the levels of serotonin (5-HT) in rat pancreas and on the equilibrium parameters for somatostatin (SS) binding to rat pancreatic acinar membranes

	SS receptor			
	B_{max}	K_d	SSLI	5-HT
Groups				
Control	922 ± 39·53	0·12 ± 0·01	5·8 ± 1·1	0·20 ± 0·01
5,7-DHT	1587 ± 134·39**	0·15 ± 0·01	10·8 ± 0·9**	0·03 ± 0·002***

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, units for B_{max} are fmol of SS bound per mg of protein and units for 5-HT levels are µmol per kg of pancreatic wet weight. The results are represented as the means ± S.E.M. of five separate experiments. Statistical comparison versus control: **P<0·01; ***P<0·001.

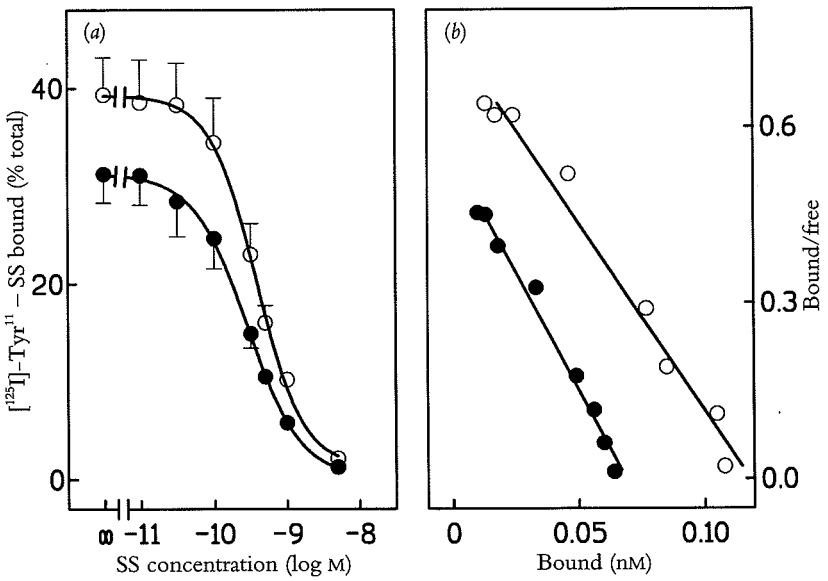

FIGURE 1. (*a*) Competitive inhibition of specific [^{125}I]-Tyr^{11}-somatostatin ([^{125}I]-Tyr^{11}-SS, 35 pM) binding to pancreatic acinar membranes by unlabelled somatostatin (SS). The membranes (75 µg protein/ml) were incubated for 90 min at 20 °C in the presence of 35 pM [^{125}I]-Tyr^{11}-SS and increasing concentrations of native peptide. Points correspond to values for animals in the control (●) and 5,7-dihydroxytryptamine (5,7-DHT)-treated (○) groups. (*b*) Scatchard analysis of the same data.

Results

Intrapancreatic injection of 5,7-DHT *in vivo* resulted in an approximately 85% depletion of 5-HT in the pancreas at 3 days after the injection whereas the SSLI content was increased (86%) 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. The binding of [^{125}I]-Tyr^{11}-SS to its specific receptors in pancreatic acinar cell membranes of rats treated with 5,7-DHT was significantly increased (Table 1 and Fig. 1). Scatchard analysis indicated that the number of SS receptors (B_{max}) in this tissue was markedly increased after lesions with 5,7-DHT, but that receptor affinity (K_d) was not altered (Table 1).

SS receptors were negatively coupled to the AC system in pancreatic acinar cell membranes from control and 5,7-DHT treated rats (Fig. 2). SS inhibited basal and FK-stimulated AC activity in the control and 5,7-DHT-treated groups. However, the effect of SS on basal and

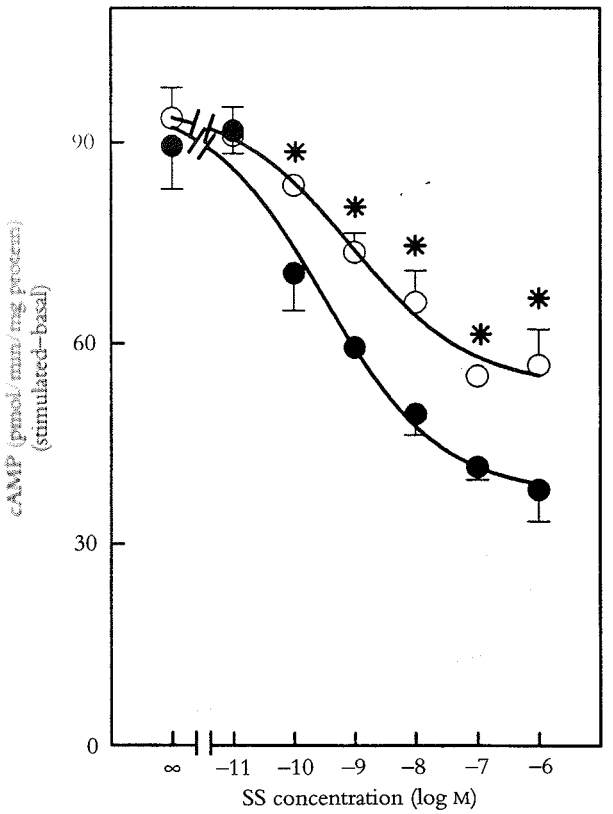

FIGURE 2. Ability of somatostatin (SS) to inhibit adenylate cyclase activity in rat pancreatic acinar membranes from the control (●) and 5,7-dihydroxytryptamine (5,7-DHT)-treated (○) groups. Basal levels of cAMP were subtracted from the corresponding stimulated values. Data are expressed as the mean ± S.E.M. of six duplicate experiments. Statistical comparison versus control: $*P<0.05$.

FK-stimulated AC activity was markedly diminished in pancreatic acinar membranes from 5,7-DHT-treated rats as compared with the control animals (11 ± 1% vs 28 ± 4% and 12 ± 1% vs 25 ± 2% respectively). This decrease was related to a reduced efficacy of SS in inhibiting AC, although there was no change in the potency of the neuropeptide (Fig. 2).

To test if the changes observed after injecting 5,7-DHT were related to a modification in AC activity or to an alteration in the coupling of the enzyme to regulatory proteins (Gi), we measured the response of AC to the diterpene FK, which is assumed to act directly upon the catalytic subunit of AC, and then examined the α_i subunits of Gi using PTX-catalyzed ADP ribosylation of membranes from control and 5,7-DHT-treated rats. Neither the potency nor the efficacy of FK to stimulate AC activity were significantly altered by the intrapancreatic injection of 5,7-DHT. In addition, no significant differences were seen for basal AC activities between the control and the 5,7-DHT-treated groups.

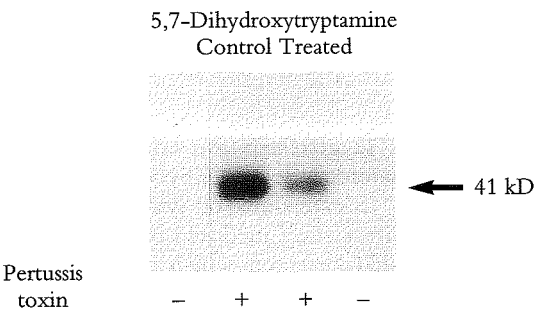

FIGURE 3. Autoradiograph of [^{32}P]ADP-ribosylated pancreatic acinar membrane proteins. Pancreatic acinar membranes from control and 5,7-dihydroxytryptamine (5,7-DHT)-treated rats were incubated in the presence of [^{32}P]NAD^+, with and without pertussis toxin. This experiment is representative of six others.

The study of PTX-catalyzed ADP-ribosylation showed a decrease of a 41 kD PTX substrate, presumably the α_i subunit of Gi, in pancreatic acinar membranes from 5,7-DHT treated rats (Fig. 3).

Discussion

The aim of the present study was to evaluate the role of intrapancreatic serotonergic nerves on the SS receptor/effector system and SS content in the rat pancreas. The levels of 5-HT in the pancreas of control rats were similar to those previously reported (Lundquist *et al.* 1989). The injection of 5,7-DHT severely decreased the level of 5-HT (85%) in the rat pancreas 3 days after its injection in agreement with previously published values (Jansson *et al.* 1985). Thus, 5-HT axon innervation in the pancreas was nearly abolished by 5,7-DHT.

The SSLI content in pancreata from control rats was similar to that previously reported by others (Patel & Reichlin 1978) and the SS receptors from rat pancreatic acinar membrane consist of a single class of high affinity sites (Srikant & Patel 1986, Viguerie *et al.* 1988). The binding affinity of SS receptors in this tissue was higher than that previously observed in rat brain, pituitary and adrenal cortex using the same radioligand (Srikant & Patel 1986). Thus, the SS receptors in the exocrine pancreas appear to contain the highest affinity for SS so far identified in the rat. The binding capacity of the SS receptors was comparable to that reported previously (Srikant & Patel 1986, Viguerie *et al.* 1988). Although the number of SS-receptor subtypes (SSTRs) cloned is growing (Bell & Reisine 1993), the rat pancreas appears to express only the subtype SSTR2 (Bruno *et al.* 1993).

Intrapancreatic serotonergic nerves are present not only within the islets but also between the exocrine acini

(Koevary *et al.* 1983, Kirchgessner & Gershon 1990). Pancreatic 5-HT_{1A} and 5-HT_{1P} receptors were found along nerve fiber bundles that were located in the stroma between acini, and over single cells scattered in the pancreatic parenchyma (Kirchgessner *et al.* 1992, 1993). These locations suggest that the effect of ablation of intrapancreatic serotonergic nerves by 5,7-DHT on the SS content and its receptor/effector system could be due either to an indirect effect mediated by the nerve terminals carrying 5-HT receptors which release other transmitters, or a direct effect that would be mediated by the already described 5-HT receptors in the acini and islets of Langerhans.

The ablation of 5-HT nerve fibers within the pancreas increased pancreatic SSLI content as compared with the control group but the reason for this increase is still unclear. Whether this increase in SSLI reflects a decreased release, increased biosynthesis or decreased degradation of SSLI cannot be answered at this time. In agreement with the present results, a widespread increase in rat hippocampal and cortical SSLI levels has been reported after intracerebroventricular injection of 5,7-DHT (Kakigi & Maeda 1992, Kondo *et al.* 1993).

It is important to emphasize that our data indicate that the number of SS receptors, as assessed by agonist binding studies, increased after ablation of intrapancreatic 5-HT nerves whereas a 41 kD PTX substrate, presumably the α_i subunit of Gi, decreased. Thus this study indicates that 5-HT axons exert a strong influence on SS receptors and on some post-SS-receptor mechanisms.

The mechanism by which axonal 5-HT influences SS receptors has not been determined although it may involve an effect by a 5-HT axonal component (either 5-HT itself or a cotransmitter) on SS receptor regulatory mechanisms at the level of cell membrane events, such as increased insertion or decreased degradation of existing receptors, or on earlier events such as the synthesis of new SS receptors.

The observations of other authors showing that FK stimulated rat pancreatic AC activity (Dehaye *et al.* 1985) are confirmed here. SS was a partial antagonist of FK-stimulated pancreatic AC activity in agreement with Heisler (1983). The efficacy of SS to inhibit AC is diminished in pancreatic acinar membranes from 5,7-DHT-treated animals as compared with controls. However, there did not appear to be any defect in the catalytic unit of AC itself since similar levels of activities were noted in membranes from either control or 5,7-DHT-treated rats when this enzyme was stimulated directly by the diterpene FK. However, the decreased inhibitory effect of SS on AC activity may possibly be related to the observed decrease of the 41 kD PTX substrate, presumably the α_i subunit of Gi, in pancreatic acinar cell membranes. Although the present results do not discard the possibility that the intrapancreatic injection of 5,7-DHT may exert effects directly on pancreatic tissue, other authors (Alonso & Soubrie 1991) do not find a direct effect of 5,7-DHT on G protein levels in the brain. Since the exposure of intact cells to AC inhibitory agonists can apparently decrease the level of Giα proteins (Jones & Bylund 1988), the increase in the SS level and its receptors would be compatible with a decrease in the level of Giα proteins. Reithmann *et al.* (1990) have proposed that the down-regulation of the level of membrane Giα proteins is a rather general cellular response to long term AC inhibition that serves as an intracellular negative feedback control against prolonged receptor activation. Whether a loss of Gi protein after ablation of 5-HT intrapancreatic nerves represents a quantitative decrease in the level of this protein or a qualitative change resulting from a transcriptional or posttranslational modification will require further investigation.

An inconsistency between changes in receptors and signal transduction is not unprecedented. For example, chronic exposure of primary cultures of mouse cortical neurons to the muscarinic antagonist atropine has been shown to increase the density of muscarinic receptors while decreasing carbachol-stimulated phosphoinositide hydrolysis (Smith *et al.* 1989). Alternatively, the lack of correlation between the magnitude of SS inhibition of AC and the density of [^{125}I]-Tyr^{11}-SS receptors may suggest the coupling of SS receptors to multiple signal transduction mechanisms or may be related to factors such as multiple and spare receptor sites. Of relevance to this observation is a recent report demonstrating no apparent match between the level of SS receptor sites and the magnitude of SS inhibition of AC activity in the frontal cortex of a group of Alzheimer's disease patients (Bergström *et al.* 1991).

It is probable that all of the serotonergic axons in the pancreas are of enteric origin since no serotonergic nerve cell bodies have been observed in pancreatic ganglia (Kirchgessner & Gershon 1990). Therefore, the functions of pancreatic serotonergic nerves are likely to be associated with enteropancreatic communication. These findings together with the present results suggest that pancreatic SS content and the SS receptor/effector system in the exocrine pancreas may be regulated by enteropancreatic serotonergic nerve fibers and thus may participate in enteropancreatic reflexes.

Besides the well-known rise in pancreatic SS content in diabetes (Patel & Weir 1976) there is evidence that suggests a role for serotonergic innervation in the pathogenesis of abnormal islet function in humans with type II diabetes (de Leiva *et al.* 1978). The fact that serotonergic neurons may control the SS receptor/effector system could be of interest in the understanding of the pathophysiology of type II diabetes.

Acknowledgements

The authors thank C F Warren from the Alcalá de Henares University Institution of Education Sciences and L Puebla from the Department of Biochemistry of Alcalá

de Henares University for their linguistic assistance and A Martin for invaluable technical assistance. This study was supported by a Grant from the Dirección General de Investigación Científica y Técnica of Spain.

References

Alberti KGMM, Christensen NJ, Christensen SE, Pange-Hansen A, Iversen J, Lundbaek K, Seyer-Hansen K & Orskow H 1973 Inhibition of insulin secretion by somatostatin. *Lancet* **2** 1299–1301.

Alonso R & Soubrie P 1991 Effects of serotonergic denervation on the density and plasticity of brain muscarinic receptors in the rat. *Synapse* **8** 30–37.

Amsterdam A, Salomon TE & Jamieson JD 1978 Sequential dissociation of the exocrine pancreas into lobules, acini and individual cells. *Methods in Cellular Biology* **20** 361–378.

Arimura A, Haruko S, Dupont A, Nishi N & Schally AV 1975 Somatostatin: abundance of immunoreactive hormone in rat stomach and pancreas. *Science* **189** 1007–1009.

Azmitia EC & Marovitz WF 1980 *In vitro* hippocampal uptake of tritiated serotonin (^{3}H-5HT); a morphological, biochemical and pharmacological approach to specificity. *Journal of Histochemistry and Cytochemistry* **28** 636–644.

Bell GI & Reisine T 1993 Molecular biology of somatostatin receptor. *Trends in Neurological Science* **16** 34–38.

Bergström L, Garlind A, Nilsson L, Alafuzoff I, Fowler CJ, Winblad B & Cowburn RF 1991 Regional distribution of somatostatin receptor binding and modulation of adenylyl cyclase activity in Alzheimer's disease brain. *Journal of Neurological Science* **105** 225–233.

Bokoch GM, Katada T, Northup JK, Hewlett EL & Hilman AG 1983 Identification of the predominant substrate for ADP-ribosylation by islet activating protein. *Journal of Biological Chemistry* **258** 2072–2075.

Bruno JF, Xu Y, Song J & Berelowitz M 1993 Tissue distribution of somatostatin receptor subtype messenger ribonucleic acid in the rat. *Endocrinology* **133** 2561–2567.

Colas B, Cambillau C, Buscail L, Zeggari M, Esteve JP, Lautre V, Thomas F, Vaysse N & Susini C 1992 Stimulation of a membrane tyrosine phosphatase activity by somatostatin analogues in rat pancreatic acinar cells. *European Journal of Biochemistry* **207** 1017–1024.

Dehaye JP, Guillard M, Poloczek P, Stievenart M, Winand J & Christophe J 1985 Effect of forskolin on adenylate cyclase activity and amylase secretion in the rat exocrine pancreas. *Journal of Cyclic Nucleotide Protein Phosphorylation Research* **10** 269–280.

Feldman JM, Quickel KE & Lebowitz HE 1972 Potentiation of insulin secretion *in vitro* by 5-HT antagonists. *Diabetes* **21** 779–788.

Gilman AG 1970 A protein binding assay for adenosine 3′,5′-cyclic monophosphate. *Proceedings of the National Academy of Sciences of the USA* **67** 305–312.

Greenwood FC, Hunter WM & Glover JS 1963 The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochemical Journal* **89** 114–123.

Hansson C & Rosengren E 1978 Quantitative analysis of 5-hydroxytryptamine in biological material by high performance liquid chromatography and electrochemical detection. *Analytical Letters* **11** 901–912.

Heisler S 1983 Forskolin potentiates calcium-dependent amylase secretion from rat pancreatic acinar membranes. *Canadian Journal of Physiology and Pharmacology* **61** 1168–1176.

Houslay MD, Metcalfe JC, Warren GP, Hesketh TR & Smith GA 1976 The glucagon receptor of rat liver plasma membrane can couple to adenylate cyclase without activating it. *Biochimica and Biophysica Acta* **436** 489–494.

Jansson L, Leung PE & McEvoy RC 1985 Ablation of serotonergic nerves in the rat pancreas. Lack of effects on hormone secretion and intrinsic blood flow. *Acta Endocrinologica* **110** 515–521.

Jones SB & Bylund DB 1988 Characterization and possible mechanisms of α_2-adrenergic receptor-mediated sensitization of forskolin-stimulated cyclic AMP production in HT 29 cells. *Journal of Biological Chemistry* **263** 14236–14244.

Kakigi T & Maeda K 1992 Effect of serotonergic agents on regional concentrations of somatostatin- and neuropeptide Y-like immunoreactivities in rat brain. *Brain Research* **599** 45–50.

Kirchgessner AL & Gershon MD 1990 Innervation of the pancreas by neurons in the gut. *Journal of Neuroscience* **10** 1626–1642.

Kirchgessner AL, Liu MT, Tamir H & Gershon MD 1992 Identification and localization of 5-HT_{1P} receptors in the guinea pig pancreas. *American Journal of Physiology* **262** G553–G566.

Kirchgessner AL, Liu MT, Howard MJ & Gershon MD 1993 Detection of the 5-HT_{1A} receptor mRNA in the rat bowel and pancreas: Comparison with 5-HT_{1P} receptors. *Journal of Comparative Neurology* **327** 233–250.

Koevary SB, Azmitia EC & McEvoy RC 1983 Rat pancreatic serotonergic nerves: morphologic, pharmacologic and physiologic studies. *Brain Research* **265** 328–332.

Kondo Y, Ogawa N, Asanuma M, Hirata H, Tanaka K, Kawada Y & Mori A 1993 Regional changes in neuropeptide levels after 5,7-dihydroxytryptamine-induced serotonin depletion in the rat brain. *Journal of Neural Transmission* **92** 151–157.

Laburthe M, Breant B & Rouyer-Fessard C 1984 Molecular identification of receptors for vasoactive intestinal peptide in rat intestinal epithelium by covalent cross-linking. Evidence for two classes of binding sites with different structural and functional properties. *European Journal of Biochemistry* **139** 181–187.

Laemmli UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227** 680–685.

de Leiva A, Tanenberg RJ, Andersson B, Greenberg B, Senske B & Goetz FC 1978 Serotonergic activation and inhibition: effects on carbohydrate tolerance and plasma insulin and glucagon. *Metabolism* **27** 511–520.

Lowry OH, Rosebrough AL, Farr L & Randall RJ 1951 Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193** 265–275.

Lundquist I, Ahren B, Hansson C & Hakanson R 1989 Monoamines in pancreatic islets of guinea pig, hamster, rat and mouse determined by high performance liquid chromatography. *Pancreas* **4** 662–667.

Matsushita K, Okabayashi Y, Hasegawa H, Koide M, Kido Y, Okutani T, Sugimoto Y & Kasuga M 1993 *In vitro* inhibitory effect of somatostatin on secretion action in exocrine pancreas of rats. *Gastroenterology* **104** 1146–1152.

Meldolesi J, Jamieson JD & Palade GE 1971 Composition of cellular membranes in the pancreas of the guinea pig. *Journal of Cell Biology* **49** 109–129.

Mori J, Satoh H, Satoh Y & Honda F 1979 Amines on the rat exocrine pancreas: (3) effects of amines on pancreatic secretion. *Japanese Journal of Pharmacology* **29** 923–933.

Munson PJ & Rodbard D 1980 LIGAND: a versatile computerized approach for characterization of ligand binding systems. *Analytical Biochemistry* **107** 220–239.

Patel YC & Weir GC 1976 Increased somatostatin content of islets from streptozotocin-diabetic rats. *Clinical Endocrinology* **5** 191–194.

Patel YC & Reichlin S 1978 Somatostatin in hypothalamus, extrahypothalamic brain and peripheral tissues of the rat. *Endocrinology* **102** 523–531.

Reithmann C, Gierschik P, Werdan K & Jacobs KH 1990 Hormonal regulation of Giα level and adenylyl cyclase responsiveness. *British Journal of Clinical Pharmacology* **30** 118S–120S.

Sakamoto C, Matozaki T, Nagao M & Baba S 1987 Coupling of guanine nucleotide inhibitory protein to somatostatin receptors on pancreatic acinar membranes. *American Journal of Physiology* **253** G308–G314.

Scatchard G 1949 The attractions of proteins for small molecules and ions. *Annals of the New York Academy of Sciences* **51** 669–671.

Smith CJ, Court JA, Keith AB & Perry EK 1989 Increases in muscarinic-stimulated hydrolysis of inositol phospholipids in rat hippocampus following cholinergic deafferentation are not paralleled by alterations in cholinergic receptor density. *Brain Research* **485** 317–324.

Srikant CB & Patel YC 1986 Somatostatin receptors on rat pancreatic acinar cells. Pharmacological and structural characterization and demonstration of down-regulation in streptozotocin diabetes. *Journal of Biological Chemistry* **261** 7690–7696.

Susini C, Vaysse N, Esteve JP, Pradayrol L, Slassi C & Ribet A 1980 Action of somatostatin 28 and somatostatin 14 on amylase release and cyclic AMP content in rat pancreatic acini. In *Biology of Normal and Cancerous Exocrine Pancreatic Cells*, pp 119–126. Eds A Ribet, L Pradayrol & C Susini. New York: Elsevier North Holland.

Tanaka S & Tsujimoto A 1981 Somatostatin facilitates the serotonin release from rat cerebral cortex, hippocampus and hypothalamus slices. *Brain Research* **208** 219–222.

Viguerie N, Tahiri-Jouti N, Esteve JP, Clerc P, Logsdon C, Svosoba M, Susini C, Vaysse N & Ribet A 1988 Functional somatostatin receptors on a pancreatic acinar cell line. *American Journal of Physiology* **255** G113–G120.

Received 4 August 1994
Accepted 7 December 1994