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# Inhibition by somatostatin of amylase secretion induced by calcium and cyclic AMP in rat pancreatic acini

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It has recently been shown that somatostatin inhibits amylase secretion from isolated pancreatic acini by reducing cyclic AMP (cAMP) production [Matsushita, Okabayashi, Hasegawa, Koide, Kido, Okutani, Sugimoto and Kasuga (1993) Gastroenterology **104**, 1146–1152]. To date, however, little is known as to the other mechanism(s) by which somatostatin inhibits amylase secretion in exocrine pancreas. To investigate the action of somatostatin independent of cAMP generation, we examined the effect of somatostatin in isolated rat pancreatic acini stimulated by 1 $\mu$M calcium ionophore A23187 and 1 mM 8-bromo-cyclic AMP (8Br-cAMP). Somatostatin inhibited amylase secretion evoked by a combination of A23187 and 8Br-cAMP in a dose-dependent manner. The maximum inhibition was obtained by $10^{-7}$ M somatostatin, and at this concentration somatostatin inhibited the effect of A23187 and 8Br-cAMP by approximately 30%. In electrically permeabilized acini, an elevation of free calcium concentration resulted in an increase in amylase secretion and cAMP enhanced the secretion evoked by calcium. cAMP shifted the dose–response curve for calcium-induced secretion leftwards and elevated the peak value of secretion. Somatostatin inhibited the effect of cAMP on calcium-induced amylase secretion by shifting the dose–response curve to the right. To determine the involvement of a G-protein(s), we examined the effect of somatostatin in acini pretreated with pertussis toxin. Pretreatment of acini with pertussis toxin completely blocked somatostatin-inhibition of amylase-secretion evoked by A23187 and 8Br-cAMP. These results indicate that somatostatin decreases amylase secretion induced by cAMP and calcium by reducing the calcium sensitivity of exocytosis. A pertussis toxin-sensitive G-protein is also involved in this step.

## INTRODUCTION

Somatostatin, a hypothalamic peptide isolated as an inhibitor of pituitary growth hormone release, also inhibits the secretion of many other hormones, exocrine enzymes and neurotransmitters [1]. In exocrine pancreas, studies *in vivo* have shown that somatostatin inhibits cholecystokinin (CCK) and/or secretin-stimulated exocrine secretion [2–4]. Studies *in vitro* have also revealed inhibitory action of somatostatin on amylase release [5,6] and it is only recently that somatostatin has been shown to inhibit amylase secretion from isolated pancreatic acini [6]. Those studies indicated that the inhibitory effect was elicited by reducing cyclic AMP (cAMP) production stimulated by vasoactive intestinal peptide (VIP) or secretin [5–7]. However, in excitable cells, for example in growth hormone-secreting pituitary cells, other inhibitory mechanisms of somatostatin are involved. These include a hyperpolarizing effect resulting from an increase in potassium conductance [8–10] and an inhibition of voltage-dependent calcium current leading to a decrease in cytosolic free calcium concentration [11,12]. By contrast, in pancreatic acini, little is known about the mechanism by which somatostatin inhibits amylase secretion besides reduction of cAMP generation. Since pancreatic acinar cells are non-excitable, voltage-dependent mechanisms described above are unlikely. Yet, a previous study indicated that somatostatin completely suppressed secretin-induced amylase secretion in the presence of partial reduction of secretin-induced cAMP production [6]. The result suggests that other inhibitory mechanism(s) of somatostatin besides reduction of cAMP generation may be involved. Somatostatin receptors have been characterized in pancreatic acinar cells [13] and have recently been purified and cloned [14–17]. These data demonstrate that somatostatin acts via cell-surface membrane receptors which are coupled to pertussis toxin (PTX)-sensitive GTP-binding proteins.

The present study was therefore conducted to determine if a mechanism independent of cAMP generation is involved in the inhibitory effect of somatostatin on exocrine pancreas. To this end, we first used A23187 and 8-bromo-cAMP (8Br-cAMP) as secretagogues, which bypass receptor- and G-protein-mediated systems [18] and examined the effect of somatostatin. Experiments are then expanded using electro-permeabilized acini to gain an insight into the intracellular events influenced by somatostatin. Additionally, we determined the role of a pertussis toxin-sensitive G-protein in the action of somatostatin. Our results indicate that somatostatin inhibits exocytosis of amylase by reducing calcium sensitivity of the exocytotic machinery and that a pertussis toxin-sensitive G-protein is also involved in this regulation.

## MATERIALS AND METHODS

### Reagents

Drugs and chemicals were obtained from the following sources. BSA (Fraction V) from Miles Scientific (Naperville, LA, U.S.A.), soybean trypsin inhibitor (SBTI) and collagenase (type CLSPA) from Worthington Biochemicals (Freehold, NJ, U.S.A.), $NAD^+$ and thymidine from Wako Pure Chemical Industries (Osaka, Japan), somatostatin 14 (SS-14) and cholecystokinin octapeptide

Abbreviations used: cAMP, cyclic AMP; 8Br-cyclic AMP, 8-bromo-cAMP; CCK, cholecystokinin; SBTI, soybean trypsin inhibitor; IBMX, 3-isobutyl-1-methyl-xanthine; HR, Hepes-buffered Ringer's solution; PTX, pertussis toxin; VIP, vasoactive intestinal peptide; SS-14, somatostatin 14.

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(CCK-8) from Peptide Institute (Osaka, Japan), A23187, 3-isobutyl-1-methyl-xanthine (IBMX), 8Br-cAMP, cAMP, magnesium salt of ATP (Mg-ATP), ADP-ribose and quinacrine from Sigma Chemical (St. Louis, MI, U.S.A.), pertussis toxin (PTX) from Seikagaku Kogyo (Tokyo, Japan) and [$\alpha$-$^{32}$P]NAD$^+$ from New England Nuclear (Boston, MA, U.S.A.). Other reagents were from commercial sources.

### Preparation of isolated pancreas acini

Pancreatic acini were isolated from pancreas of male Sprague–Dawley rats weighing 200–250 g by the collagenase digestion method described by Hootman et al. [19] with slight modification as described by Habara and Kanno [20]. Acini were suspended in Hepes-buffered Ringer's solution (HR) containing 10 mM Hepes/NaOH (pH 7.4), 118 mM NaCl, 4.7 mM KCl, 1 mM $Na_2HPO_4$, 1.13 mM $MgCl_2$, 5.5 mM D-glucose, 2.5 mM $CaCl_2$, Eagle's minimal essential medium amino acid supplement, 2 mM L-glutamine, 0.2% BSA, and 0.01% SBTI. After a 30 min recovery incubation, the following experiments were performed.

### Membrane permeabilization

After isolation, acini were suspended in the permeabilization buffer containing 20 mM Pipes (pH 7.0), 130 mM KCl, 1.2 mM $KH_2PO_4$, 2 mM $MgSO_4$, 15 mM D-glucose, 0.2% BSA, 0.01% SBTI, and were then exposed to an intensive electric field (ten exposures to a field strength of 2 kV/cm from 3 $\mu$F capacitor; time constant 100 $\mu$s). Note that more than 95% of the acinar cells were stained with Trypan Blue after the permeabilization. Immediately after the permeabilization, the incubation was started by adding the acini to the permeabilization buffer supplemented with 1 mM Mg-ATP, 0.1 mM free magnesium, 1 mM EGTA and various concentrations of free $Ca^{2+}$ and cAMP. Different free $Ca^{2+}$ concentrations were obtained by altering the $Ca^{2+}$/EGTA ratio. Free $Ca^{2+}$ concentrations were calculated as described by Waisman et al. [21]. When cAMP was added, 0.1 mM IBMX was included.

### Measurement of amylase release

Intact isolated acini were suspended in HR and incubated in a shaking water-bath at 30 °C with various secretagogues in the presence or absence of SS-14. Amylase activity was assayed in triplicate by the method of Bernfeld [22]. In experiments using permeabilized acini, amylase activity was measured by a modified Bernfeld method as described by O'Sullivan and Jamieson [23] using Mops-buffered solution instead of phosphate buffer in which the EGTA buffer used slightly inhibited amylase activity. Amylase release was expressed as a percentage of total amylase activity initially present in acini.

### Preparation of pertussis toxin-treated pancreatic acini

To obtain pertussis toxin-treated acini, we injected 50 $\mu$g of the toxin or its vehicle (0.1 M sodium phosphate buffer, pH 7.5) into the peritoneal cavity of an intact rat as described by Matozaki et al. [24]. Seventy-two hours after the toxin injection, pancreatic acini were isolated as described above.

### ADP-ribosylation of pancreatic acinar membrane proteins by pertussis toxin

Pancreatic acinar cell membranes were prepared from control and pertussis toxin-treated acini as described by Ponnappa et al. [25]. Briefly, isolated acini were homogenized with a Dounce tissue grinder in 0.3 M sucrose buffered with Hepes/NaOH, pH 7.4, containing 1 mM benzamidine. The homogenate was initially centrifuged at 110 *g* for a few seconds. After the supernatant had been centrifuged at 1000 *g* for 10 min, the membrane pellet was resuspended in 50 mM Tris/HCl buffer, pH 7.5. A 5 $\mu$l aliquot of membrane suspension was incubated with 5 mCi/ml [$\alpha$-$^{32}$P]NAD and 10 $\mu$g/ml of preactivated pertussis toxin at 30 °C for 30 min in 50 mM Tris/HCl buffer, pH 7.4, containing 1 mM Na-EDTA, 10 mM thymidine, 1 mM ADP-ribose, 15 mM isonicotic acid hydrazide and 2.5 $\mu$M NAD [26]. After cessation of the reaction by adding Laemmli's sample buffer, radiolabelled membranes were analysed by electrophoresis in 12.5% polyacrylamide gels. Autoradiograms were obtained from the dried gel after exposure to Kodak X-Omat AR film at −70 °C.

## RESULTS

### Amylase secretion from intact acini

In an attempt to determine whether somatostatin inhibits amylase secretion by a mechanism independent of cAMP generation, we examined the effect of SS-14 on cAMP-induced amylase secretion. To this end, we first characterized the effect of 8Br-cAMP on amylase secretion in the presence and absence of a calcium ionophore A23187. Table 1 depicts the dose–response relationship for 8Br-cAMP-induced amylase secretion from intact acini in the presence and absence of A23187. 8Br-cAMP by itself exerted little effect on amylase release, but in the presence of $10^{-6}$ M A23187, amylase release was markedly stimulated at 8Br-cAMP concentrations of $10^{-4}$ M or higher. At a concentration of $10^{-3}$ M, 8Br-cAMP caused more than 30% release of amylase. We therefore used 1 mM 8Br-cAMP in the subsequent experiments. Table 2 demonstrates the dose–response relationship for A23187-stimulated amylase release in the presence and absence of 1 mM 8Br-cAMP. In the absence of 8Br-cAMP, A23187 evoked a dose-dependent increase in amylase release at concentrations above $10^{-8}$ M. At $10^{-6}$ M, A23187 caused approximately 14% release of amylase. The effect of A23187 was markedly enhanced by the addition of 8Br-cAMP. Effects of A23187 and 8Br-cAMP were synergistic at an A23187 concentration of $10^{-7}$ M–$10^{-6}$ M. Based on these results, we employed a combination of 1 mM 8Br-cAMP and 1 $\mu$M A23187 to stimulate amylase release from intact acini.

### Inhibitory effect of somatostatin on amylase release from intact acini

As shown in Table 3, 100 nM SS-14 significantly inhibited amylase release stimulated by 8Br-cAMP and A23187. Note that

**Table 1 Effect of 8Br-cAMP on amylase secretion in pancreatic acini**

Acini were incubated for 60 min with various concentrations of 8Br-cAMP in the presence and absence of 1 $\mu$M A23187. Values are the means ± S.E.M. for three experiments. n.d., not determined.

| [8Br-cAMP] (M) | Amylase secretion (%) | |
|---|---|---|
| | None | A23187 |
| 0 | 1.1 ± 0.12 | n.d. |
| $10^{-6}$ | 1.5 ± 0.61 | 11.6 ± 1.32 |
| $10^{-5}$ | 2.4 ± 0.12 | 14.9 ± 2.11 |
| $10^{-4}$ | 2.4 ± 0.64 | 21.1 ± 0.82 |
| $10^{-3}$ | 3.1 ± 0.20 | 32.0 ± 0.68 |

**Table 2 Dose–response relationship for A23187-induced amylase secretion**

Acini were incubated for 60 min with various concentrations of A23187 in the presence and absence of 1 mM 8Br-cAMP. Values are the means ± S.E.M. for three experiments. n.d., not determined.

| | Amylase secretion (%) | |
|---|---|---|
| [A23187] (M) | None | 8Br-cAMP |
| 0 | 2.2 ± 0.32 | n.d. |
| $10^{-9}$ | 3.9 ± 0.42 | 5.4 ± 0.37 |
| $10^{-8}$ | 4.5 ± 0.80 | 9.9 ± 1.36 |
| $10^{-7}$ | 6.2 ± 0.12 | 12.7 ± 0.18 |
| $10^{-6}$ | 14.0 ± 0.12 | 30.1 ± 2.17 |

**Table 3 Effect of somatostatin on 8Br-cAMP- and A23187-mediated amylase secretion**

Pancreatic acini were incubated for 60 min with 1 mM 8Br-cAMP and/or 1 μM A23187 in the presence and absence of 100 nM SS-14. Values are the means ± S.E.M. for three experiments. Statistical analysis was done by Students *t*-test. $^*P < 0.05$ versus without SS-14.

| Secretagogues | Amylase secretion (%) |
|---|---|
| None | 3.3 ± 0.21 |
| 8Br-cAMP | 6.1 ± 0.6 |
| 8Br-cAMP + SS-14 | 5.5 ± 0.7 |
| A23187 | 12.5 ± 0.1 |
| A23187 + SS-14 | 11.3 ± 0.3 |
| 8Br-cAMP + A23187 | 30.1 ± 1.0 |
| (8Br-cAMP + A23187) + SS-14 | 20.1 ± 1.8* |

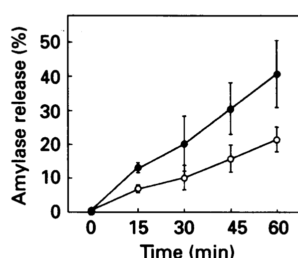


**Figure 1 Time course of effect of somatostatin on amylase secretion**

Acini were incubated for the indicated times with 1 μM A23187 and 1 mM 8Br-cAMP in the presence (○) and absence (●) of 100 nM SS-14. Values are the means ± S.E.M. for three experiments. Amylase release in unstimulated acini was 3.8 ± 0.5%/60 min.

SS-14 did not inhibit amylase release stimulated by either 8Br-cAMP or A23187 alone (Table 3). Figure 1 depicts the time course of amylase secretion induced by 8Br-cAMP and A23187 in the presence and absence of SS-14. The inhibition was observed even at 15 min and was remarkable at 60 min. Then dose-dependency of the inhibitory effect of SS-14 was investigated and the results are documented in Figure 2. SS-14 inhibited amylase secretion in a dose-dependent manner and the maximal inhibition was observed with 100 nM SS-14. The half maximal inhibition was obtained with 8 nM SS-14.

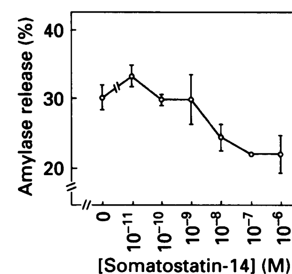


**Figure 2 Dose–response relationship for the inhibitory effect of somatostatin**

Acini were incubated for 60 min with 1 μM A23187, 1 mM 8Br-cAMP and various concentrations of SS-14. Values are the means ± S.E.M. for three experiments. Amylase release in unstimulated acini was 3.7 ± 0.15%.

**Table 4 Effect of quinacrine on inhibitory action of somatostatin**

Acini were incubated with 10 μM carbachol or a combination of 1 μM A23187 and 1 mM 8Br-cAMP in the presence and absence of 100 μM quinacrine. SS-14 (100 nM) was included in some experiments. Values are the means ± S.E.M. for three experiments. n.d., not determined.

| | Amylase secretion (%) | |
|---|---|---|
| Addition | None | Quinacrine |
| None | 1.9 ± 0.40 | n.d. |
| Carbachol | 22.0 ± 1.35 | 2.7 ± 0.20 |
| (A23187 + 8Br-cAMP) | 30.1 ± 1.14 | 32.0 ± 0.78 |
| (A23187 + 8Br-cAMP) + SS-14 | 22.3 ± 1.75 | 24.1 ± 0.18 |

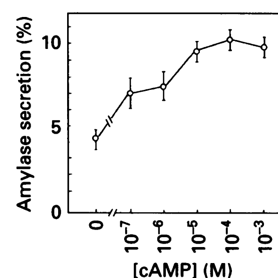


**Figure 3 Dose–response relationship for cAMP-induced amylase secretion in permeabilized acini**

Permeabilized acini were incubated for 30 min with 10 μM calcium and various concentrations of cAMP. Values are the means ± S.E.M. for three experiments. Amylase release in unstimulated acini was 2.1 ± 0.23%.

### Effect of inhibitor of phospholipase $A_2$ on inhibitory action of somatostatin

In other cell systems, the inhibitory action of somatostatin is mediated by a metabolite of arachidonic acid [27]. To assess the involvement of a metabolite of arachidonic acid, we examined the effect of quinacrine, an inhibitor of phospholipase $A_2$ action, on the inhibitory action of somatostatin. This compound was shown to attenuate the action of carbachol and CCK on amylase secretion in exocrine pancreas [28]. As shown in Table 4, 100 μM

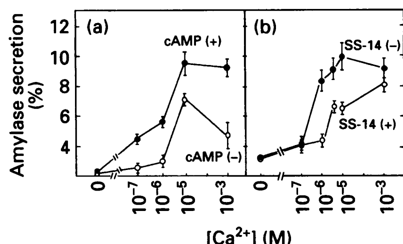


**Figure 4 Effect of cAMP and somatostatin on calcium-induced amylase secretion**

(**a**) Permeabilized acini were incubated for 30 min with various concentrations of calcium in the presence (●) and absence (○) of 100 $\mu$M cAMP. (**b**) Permeabilized acini were incubated with various concentrations of calcium and 100 $\mu$M cAMP in the presence (○) and absence (●) of 100 nM SS-14. Values are the means ± S.E.M. for three experiments.

**Table 6 Effect of somatostatin on amylase secretion induced by calcium and cAMP in permeabilized acini obtained from PTX-treated and untreated rats**

Acini were obtained from rats treated with PTX or saline. Acini were permeabilized as described in the Materials and methods section. Permeabilized acini were incubated for 30 min in medium containing $10^{-5}$ M calcium and 100 $\mu$M cAMP in the presence of various concentrations of SS-14. Values are the means ± S.E.M. for three experiments.

| SS-14 concentration (M) | Amylase secretion (%) | |
|---|---|---|
| | PTX-treated | PTX-untreated |
| 0 | 9.7 ± 0.28 | 9.9 ± 1.31 |
| $10^{-8}$ | 10.1 ± 1.31 | 8.6 ± 0.42 |
| $10^{-7}$ | 8.4 ± 0.32 | 3.9 ± 0.65 |
| $10^{-6}$ | 9.2 ± 2.00 | 2.9 ± 1.31 |

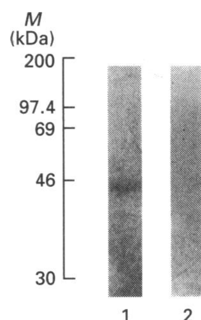


**Figure 5 ADP-ribosylation catalysed by PTX in pancreatic acini**

Membranes obtained from naive (lane 1) and PTX-treated acini were incubated with [$^{32}$P]NAD$^+$ and activated PTX.

**Table 5 Effect of somatostatin on amylase secretion induced by A23187 and 8Br-cAMP in PTX-treated and untreated acini**

PTX-treated and untreated acini were incubated for 60 min with 1 $\mu$M A23187 and 1 mM 8Br-cAMP in the presence of various concentrations of SS-14. Values are the means ± S.E.M. for three experiments.

| SS-14 concentration (M) | Amylase secretion (%) | |
|---|---|---|
| | PTX-treated | PTX-untreated |
| 0 | 30.9 ± 0.41 | 30.1 ± 2.04 |
| $10^{-8}$ | 30.1 ± 1.31 | 27.0 ± 3.71 |
| $10^{-7}$ | 30.2 ± 1.56 | 20.1 ± 1.84 |
| $10^{-6}$ | 31.1 ± 0.90 | 19.4 ± 2.46 |

quinacrine blocked the effect of carbachol. In contrast, amylase secretion induced by A23187 and 8Br-cAMP was not affected by the compound, suggesting that quinacrine affects receptor-mediated early events. SS-14 attenuated amylase secretion induced by A23187 plus 8Br-cAMP, even in the presence of 100 $\mu$M quinacrine.

## Inhibitory effect of somatostatin on amylase release from permeabilized acini

To further characterize the inhibitory effect of SS-14, we measured amylase secretion in permeabilized acini. In this system, we could measure amylase secretion in the presence of varying concentrations of free calcium. Figure 3 demonstrates the dose–response relationship for the effect of cAMP on amylase in the presence of 10 $\mu$M calcium. cAMP augmented amylase release in a dose-dependent manner and the maximal release was obtained with $10^{-5}$ M cAMP. To provide enough concentration of intracellular cAMP, 100 $\mu$M cAMP was employed to examine the effect of SS-14 in the following experiments. As shown in Figure 4a, calcium-induced secretion was biphasic and the maximal amylase release was detected at 10 $\mu$M calcium. cAMP shifted the dose–response curve for calcium-induced amylase secretion to the left and stimulation of amylase release was observed at 100 nM calcium. cAMP also elevated the peak value. As shown in Figure 4b, SS-14 inhibited amylase secretion induced by calcium and cAMP. SS-14 reduced secretion by shifting the dose-response curve for calcium sensitivity to the right. At $10^{-5}$ M calcium, SS-14 inhibited cAMP-induced amylase release approximately 40%.

## Effect of pertussis toxin on somatostatin-mediated inhibition of amylase secretion

To determine whether a GTP-binding protein(s) was involved in SS-14-mediated inhibition, we pretreated pancreatic acini with PTX. The extent of ADP-ribosylation of G-protein in acinar cell membranes after PTX treatment is shown in Figure 5. In control membranes, activated pertussis toxin selectively incorporated [$^{32}$P]NAD$^+$ into a 41 kDa protein (lane 1). After the treatment of pancreatic acini with PTX, ADP-ribosylation *in vitro* catalysed by PTX (lane 2) was not detected on the autoradiogram. Using PTX-treated and intact acini, we examined the effect of PTX on SS-14-induced inhibition of amylase secretion (Table 5). Amylase secretion stimulated by A23187 and 8Br-cAMP from toxin-pretreated and untreated acini was identical. In naive acini, SS-14 inhibited amylase secretion induced by A23187 and 8Br-cAMP. In toxin-pretreated acini, however, the inhibitory effect was completely abolished (Table 5).

Finally, we examined the effect of somatostatin in permeabilized acini obtained from PTX-treated rats. As depicted in Table 6, somatostatin did not affect amylase secretion induced by

calcium and cAMP in permeabilized acini obtained from PTX-treated rats.

## DISCUSSION

Reduction of cAMP production by inhibiting adenylate cyclase activity has been the only mechanism known so far by which somatostatin inhibits amylase release from exocrine pancreas [6,29]. In the present work, we demonstrate for the first time that somatostatin inhibits amylase release by a mechanism distal to the production of cAMP. Exocrine pancreatic secretion is regulated by hormonal and neuronal interaction. To observe receptor-mediated action of somatostatin, we used isolated pancreatic acini which exclude a neuronal mechanism [30]. In exocrine pancreas, there are two types of receptor-mediated secretagogues. One is $Ca^{2+}$-mobilizing agonists such as CCK, carbachol and bombesin [31,32]. The other is cAMP-producing agonists such as secretin and VIP [33]. Interaction of the two types of agonists is complicated. For example, CCK is shown to regulate somatostatin-binding to its receptor [34,35] and secretin also has the potential to mobilize intracellular $Ca^{2+}$ [36]. Since A23187 and 8Br-cAMP bypass receptor-mediated second messenger generation, we can assess the effect of SS-14 on events distal to the second messenger generation.

The present results demonstrate that somatostatin attenuates exocytosis induced by an elevation of free calcium concentration. As shown in Table 2 and Figure 5, calcium-induced exocytosis is greatly potentiated by cAMP. This potentiation is due to both sensitization of exocytotic machinery to calcium and increasing the maximal velocity of exocytotic reactions. Indeed, somatostatin attenuates exocytosis by inhibiting cAMP action. In this regard, Ullrich et al. [37] reported that somatostatin attenuated calcium-induced exocytosis of insulin in permeabilized insulinoma cells. Our results extend their data by showing that somatostatin reduces the calcium sensitivity of exocytosis. It is presumed that cAMP phosphorylates via A-kinase a protein involved in exocytotic machinery and thereby enhances calcium-induced exocytosis. Somatostatin may affect the function of the putative protein. Recent studies have provided evidence that G-proteins are involved in the regulation of exocytosis [38]. For example, exocytosis of histamine is modulated by $G_{i3}$ in mast cells [39]. The present results, however, do not address whether or not PTX-sensitive G-protein modulated by somatostatin directly regulates exocytosis in pancreatic acini.

In excitable cells, somatostatin receptor is known to be coupled to PTX-sensitive G-proteins and elicits inhibitory effects via both cAMP-dependent and -independent pathways [40–42]. As to pancreatic acinar cells, it has only been reported that SS-14 inhibits adenylate cyclase in isolated pancreatic acini [6]. In a pancreatic acinar cell line, AR-42J cells, somatostatin action was blocked by PTX [29]. In the present study, we documented direct evidence that somatostatin inhibits exocytosis of amylase via a PTX-sensitive G-protein. In rat pancreatic acini, there are multiple PTX-sensitive G-proteins. These include $G_{i1}$, $G_{i2}$, $G_{i3}$ and $G_0$ [43]. Recent studies have shown that the signal transduction of the somatostatin receptor system is more complex than was thought. For example, somatostatin receptor expressed in AR42J cells could couple to multiple G-proteins [14]. Moreover, multiple somatostatin receptor subtypes have been cloned in various organs [15–17] and receptor subtype-specific coupling to multiple G-proteins has been demonstrated [44]. In addition, somatostatin alters, via multiple G-proteins, various effector systems including adenylate cyclase [5–7], potassium and calcium channels [8–12], phospholipase $A_2$ [27,45] and protein phosphatase [46]. Among these effector systems, involvement of phospholipase $A_2$ is unlikely since quinacrine has no effect on the inhibitory action of somatostatin. In any case, further studies are needed to elucidate whether cAMP-dependent and -independent inhibitory effects of somatostatin on exocrine pancreas are mediated by the same receptor with different G-proteins or by different receptors with different G-proteins.

In conclusion, we have described a yet undescribed inhibitory mechanism of somatostatin in exocrine pancreas. This mechanism operates distal to the cAMP generation modulating the sensitivity of exocytotic machinery to cytosolic free calcium via a PTX-sensitive G-protein.

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## REFERENCES


1 Reichlin, S. (1983) N. Engl. J. Med. **309**, 1495–1501
2 Boden, G., Sivitz, M. C., Owen, O. E., Essa-Koumar, N. and Landor, J. H. (1975) Science **190**, 163–165
3 Konturek, S. J., Tasler, J., Obtulowicz, W., Coy, D. H. and Schally, A. V. (1976) J. Clin. Invest. **58**, 1–6
4 Gyr, K., Beglinger, C., Kohler, E., Trautzl, U., Keller, U. and Bloom, S. R. (1987) J. Clin. Invest. **79**, 1595–1600
5 Singh, P., Asada, I., Owlia, A., Collins, T. J. and Thompson, J. C. (1988) Am. J. Physiol. **254**, G217–G223
6 Matsushita, K., Okabayashi, Y., Hasegawa, H., Koide, M., Kido, Y., Okutani, T., Sugimoto, Y. and Kasuga, M. (1993) Gastroenterology **104**, 1146–1152
7 Esteve, J. P., Vaysse, N., Susini, C., Kunsch, J. M., Fourmy, D., Pradayrol, L., Wunsch, E., Moroder, L. and Ribet, A. (1983) Am. J. Physiol. **245**, G208–G216
8 Yamashita, N., Shibuya, N. and Ogata, E. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 6198–6202
9 Koch, B. D. and Schonbrunn, A. (1988) J. Biol. Chem. **263**, 226–234
10 Moore, S. T., Madamba, S. G., Joels, M. and Siggins, G. R. (1988) Science **239**, 278–280
11 Koch, B. D., Blalock, J. B. and Schonbrum, A. (1988) J. Biol. Chem. **263**, 216–225
12 Lewis, D. L., Weight, F. F. and Luini, A. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 9035–9039
13 Garry, D. J., Garry, M. G., Williams, J. A., Mahoney, W. C. and Sorenson, R. L. (1989) Am. J. Physiol. **256**, G897–G904
14 Brown, P. J. and Schonbrunn, A. (1993) J. Biol. Chem. **268**, 6668–6676
15 Yamada, Y., Post, S. R., Wang, K., Tager, H. S., Bell, G. I. and Seino, S. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 251–255
16 Kluxen, F., Bruns, C. and Lubbert, H. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 4618–4622
17 Rohrer, L., Raulf, F., Bruns, C., Buettner, R., Hofstaedter, F. and Schule, R. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 4196–4200
18 Williams, J. A. and Lee, M. (1974) Biochem. Biophys. Res. Commun. **60**, 542–548
19 Hootman, S. R., Brown, M. E., Williams, J. A. and Logsdon, C. D. (1986) Am. J. Physiol. **251**, G75–G83
20 Habara, Y. and Kanno, T. (1991) Cell. Calcium **12**, 533–542
21 Waisman, D. H., Gimble, J. H., Goodmann, D. B. P. and Rasmussen, H. (1981) J. Biol. Chem. **256**, 409–414
22 Bernfeld, P. (1955) Methods. Enzymol. **1**, 149–158
23 O'Sullivan, A. J. and Jamieson, J. D. (1992) Biochem. J. **285**, 597–601
24 Matozaki, T., Sakamoto, C., Nagao, M., Nishizaki, H. and Baba, S. (1988) Am. J. Physiol. **255**, E652–E659
25 Ponnappa, B. C., Dormer, R. L. and Williams, J. A. (1981) Am. J. Physiol. **240**, G122–G129
26 Nishimoto, I., Hata, Y., Ogata, E. and Kojima, I. (1987) J. Biol. Chem. **262**, 12120–12126
27 Schweitzer, P., Madamba, S. and Siggins, G. R. (1990) Nature (London) **346**, 464–467
28 Grosfils, K., Gomez, F. and Dehaye, J. P. (1992) Biochem. Biophys. Res. Commun. **184**, 408–413
29 Viguerie, N., Tahiri-Jouti, N., Esteve, J. P., Clerc, P., Logsdon, C., Svoboda, M., Susini, C., Vaysee, N. and Ribet, A. (1988) Am. J. Physiol. **255**, G113–G120
30 Mulvihill, S. J., Bunnett, N. W., Goto, Y. and Debas, H. T. (1990) Metabolism **39**, 143–148

31 Williams, J. A. (1980) Am. J. Physiol. **238**, G269–G279
32 Ochs, D. L., Korenbrot, J. I. and Williams, J. A. (1983) Biochem. Biophys. Res. Commun. **117**, 122–128
33 Gardner, J. D. and Jensen, R. T. (1981) in Physiology of the Gastrointestinal Tract (Johnses, L. R., ed.), pp. 831–871, Raven Press, New York
34 Sakamoto, C., Goldfine, I. D. and Williams, J. A. (1984) J. Biol. Chem. **259**, 9623–9627
35 Esteve, J. P., Susini, C., Vaysse, N., Antoniotti, H., Wunsch, E., Berthon, G. and Ribet, A. (1984) Am. J. Physiol. **247**, G62–G69
36 Trimble, E. R., Bruzzone, R., Biden, T. J. and Farese, R. V. (1986) Biochem. J. **239**, 257–261
37 Ullrich, S., Prentki, M. and Wollheim, C. B. (1991) Biochem. J. **270**, 273–276
38 Burgoyne, R. D. and Morgan, A. (1993) Biochem. J. **293**, 305–316

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39 Aridor, M., Rajmilevich, G., Beaven, M. A. and Sagi-Eisenberg, R. (1993) Science **262**, 1569–1572
40 Jakobs, K. H., Aktories, K. and Schultz, G. (1983) Nature (London) **303**, 177–178
41 Koch, B. D., Dorflinger, L. J. and Schonbrunn, A. (1985) J. Biol. Chem. **260**, 13138–13145
42 Yajima, Y., Akita, Y. and Saito, T. (1986) J. Biol. Chem. **261**, 2684–2689
43 Schnefel, S., Profrock, A., Hinsch, K.-D. and Schulz, I. (1990) Biochem. J. **269**, 483–488
44 Yamada, Y., Reisine, T., Law, S. F., Ihara, Y., Kubota, A., Kagimoto, S., Seino, M., Seino, Y., Bell, G. I. and Seino, S. (1992) Mol. Endocrinol. **6**, 2136–2143
45 Schweitzer, P., Madamba, S., Champagnat, J. and Siggins, G. R. (1993) J. Neurosci. **13**, 2033–2049
46 Pan, M. G., Florio, T. and Stork, J. S. (1992) Science **256**, 1215–1217