Somatostatin-induced paradoxical increase in intracellular Ca^{2+} concentration and insulin release in the presence of arginine vasopressin in clonal HIT-T15 β -cells

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Somatostatin, a hormone that signals via G_i/G_o , usually inhibits increases in intracellular calcium concentration ($[\text{Ca}^{2+}]_i$) and insulin release from β -cells. We have found that in the presence of arginine vasopressin (AVP), which signals via G_q , somatostatin increased $[\text{Ca}^{2+}]_i$, leading to insulin release in HIT-T15 cells. The increase in $[\text{Ca}^{2+}]_i$ by somatostatin was observed even after 60 min of AVP treatment. Somatostatin alone failed to increase $[\text{Ca}^{2+}]_i$ and insulin release. Somatostatin induced changes in $[\text{Ca}^{2+}]_i$ in a biphasic pattern, characterized by a sharp and transient increase followed by a rapid decline to sub-basal levels. Pretreatment with pertussis toxin, which inactivates G_i/G_o , abolished the effects of somatostatin. U-73122, an inhibitor of phospholipase C, antagonized the somatostatin-induced increase in $[\text{Ca}^{2+}]_i$. In Ca^{2+} -free medium, somatostatin still increased

$[\text{Ca}^{2+}]_i$. Depletion of intracellular Ca^{2+} stores with thapsigargin, a microsomal Ca^{2+} -ATPase inhibitor, abolished somatostatin's effect. In the presence of bradykinin, another G_q -coupled receptor agonist, somatostatin also increased $[\text{Ca}^{2+}]_i$, but not in the presence of isoproterenol (a G_s -coupled receptor agonist) or medetomidine (a G_i/G_o -coupled receptor agonist). Our findings suggest that somatostatin signals through G_i/G_o , and involves phospholipase C and Ca^{2+} release from the endoplasmic reticulum. The increase in $[\text{Ca}^{2+}]_i$ by somatostatin leads to insulin release. This cross-talk is specific to G_q and G_i/G_o , and is not limited to the AVP and somatostatin receptors.

Key words: Ca^{2+} release, cross-talk, G_i/G_o , G_q , phospholipase C.

INTRODUCTION

The hormone insulin is synthesized and released from pancreatic β -cells, and is the most important regulator of elevated blood glucose levels. The mechanism that regulates insulin release is rather complex from an endocrinal point of view. Although an increase in blood glucose levels is one of the most potent stimulators for insulin release, several hormones and neurotransmitters act directly or indirectly in the pancreas to stimulate or inhibit its release. Inhibition of insulin release by somatostatin has been well characterized in studies utilizing the whole pancreas, pancreatic islets and several β -cell lines [1–4]. Arginine vasopressin (AVP), a hormone normally found in the posterior pituitary gland, is also present in the pancreas [5]. Previously we demonstrated AVP's ability to stimulate the release of glucagon and insulin from the rat pancreas, and in the α -cell line InR1G9 and β -cell line RINm5F [6,7].

Somatostatin was initially isolated and identified as an inhibitor of growth-hormone release from anterior pituitary cells [8]. In the pancreas, somatostatin is synthesized and released from δ -cells, and inhibits both endocrine and exocrine secretions [9,10]. The inhibitory effects of somatostatin on insulin release have been associated with decreases in cAMP and intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) through the inhibition of Ca^{2+} influx via L-type voltage-dependent Ca^{2+} channels [4] and the opening of ATP-sensitive K^+ channels [11]. High-resolution measurements of cell capacitance have also suggested that inhibition of insulin release by somatostatin is associated with activation of the Ca^{2+} -dependent protein phosphatase calcineurin, and in-

volves activation of G-proteins but not inhibition of voltage-dependent Ca^{2+} currents or adenylate cyclase activity [12]. Somatostatin receptors are coupled to the pertussis toxin (PTX)-sensitive family of G-proteins (G_i/G_o) [13]. Binding of somatostatin to its receptor leads to conformational changes in G-proteins, where α and $\beta\gamma$ subunits assume their active form and dissociate from the receptor. Both PTX-sensitive and -insensitive G-proteins have the ability to transduce signals between receptors and phospholipase C (PLC) [14]. Overall, α and $\beta\gamma$ subunits from different G_i/G_o -coupled receptors have been shown to activate various target effectors [15,16]. The α subunit can inhibit adenylate cyclase and Ca^{2+} influx via voltage-dependent Ca^{2+} channels, increase K^+ efflux via ATP-sensitive K^+ channels and activate cGMP phosphodiesterase, whereas the $\beta\gamma$ subunit can activate several enzymes such as PLC- β , G-protein-coupled receptor kinase, phospholipase A_2 and phosphoinositide 3-kinase [17–20].

An increasing number of cross-talk mechanisms have emerged between several G-protein-coupled receptors, which involve all levels of the signalling cascade. Cross-talk between G_q and G_i/G_o has been described in human neuroblastoma SH-SY5Y and COS cells [21–23]. In HIT-T15 cells, AVP binds V_{1b} receptors [24], which couple to G_q , thus activating PLC- β through the α_q subunit with subsequent hydrolysis of $\text{PtdIns}(4,5)P_2$ to $\text{Ins}(1,4,5)P_3$ and diacylglycerol (DAG) [25]. DAG activates protein kinase C, whereas $\text{Ins}(1,4,5)P_3$ promotes Ca^{2+} release from the endoplasmic reticulum (ER), leading to an increase in $[\text{Ca}^{2+}]_i$ [7,26]. In SH-SY5Y cells, somatostatin and neuropeptide Y increase $[\text{Ca}^{2+}]_i$ after pretreatment with carbachol, a G_q -coupled

Abbreviations used: AVP, arginine vasopressin; $[\text{Ca}^{2+}]_i$, intracellular Ca^{2+} concentration; PTX, pertussis toxin; PLC, phospholipase C; DAG, diacylglycerol; ER, endoplasmic reticulum; TG, thapsigargin; BK, bradykinin; fura 2/AM, fura 2 acetoxymethyl ester.

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receptor agonist [22]. Somatostatin and neuropeptide Y alone fail to increase $[Ca^{2+}]_i$. Stimulation of PLC- β by the $\beta\gamma$ dimer of different G-proteins has been shown to occur in the presence of α_q [27]. Studies in COS-1 cells provide further support for the $\beta\gamma$ dimer mediation of the cross-talk between the G_q - and G_i/G_o -coupled receptors [23]. Enhancement of G_q -dependent signals by G_i/G_o -coupled receptors in COS-7 cells requires an activated PLC- β and is mediated by the $\beta\gamma$ dimer [21]. In addition, activation of G_i/G_o -coupled receptors has been shown to enhance the inositol phosphate signals generated by G_q -coupled receptors [28,29]. Although there are reports of cross-talk where an increase in inositol phosphate mass formation and $[Ca^{2+}]_i$ by G_i/G_o has occurred after activation of G_q -coupled receptors, our findings represent the first example in an endocrine cell line, and the first example of cross-talk between somatostatin and AVP receptors. The objective of the present study was to provide insight into the mechanism by which somatostatin increases $[Ca^{2+}]_i$ and insulin release in the presence of AVP in β -clonal HIT-T15 cells.

EXPERIMENTAL

Materials

All reagents were purchased from Sigma (St. Louis, MO, U.S.A.), except fura 2 acetoxymethyl ester (fura 2/AM), which was from Molecular Probes (Eugene, OR, U.S.A.), U-73122 {1-[6-[[17 β -3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1H-pyrrole-2,5-dione} and U-73343 {1-[6-[[17 β -3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-2,5-pyrrolidine-dione} were from Biomol Research Laboratory (Plymouth Meeting, PA, U.S.A.).

Cell culture

HIT-T15 cells (simian virus 40-transformed Syrian hamster islets) were maintained in RPMI 1640 with 10% fetal bovine serum and aerated with 5% CO_2 /95% air at 37 °C. All experiments were performed using cells from passages 80–90.

$[Ca^{2+}]_i$ measurement in cell suspension

Cells (5×10^6) were grown in 75 cm² culture flasks for 5 days until 80–90% confluency had been reached. Thereafter, the cells were harvested by treatment with trypsin/EDTA and prepared for experiments. Measurement of $[Ca^{2+}]_i$ was accomplished by loading 20×10^6 cells with 2 μ M fura 2/AM for 30 min at 37 °C in Krebs/Ringer bicarbonate buffer (KRB) containing 136 mM NaCl, 4.8 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM Hepes, 4 mM glucose and 0.1% BSA, pH 7.4. The loaded cells were centrifuged at 300 g for 2 min and resuspended at a density of 2×10^6 cells/ml with KRB. The 340/380 nm fluorescence ratios were monitored using an SLM-8000 spectrofluorometer (SLM Instruments, Urbana, IL, U.S.A.). The $[Ca^{2+}]_i$ was calibrated as described previously [4]. Experiments in Ca^{2+} -free medium were created by centrifugation at 300 g for 60 s followed by resuspension of the cells in Ca^{2+} -free KRB containing 10 μ M EGTA. Depletion of ER Ca^{2+} was accomplished by pretreatment with thapsigargin (TG), a microsomal Ca^{2+} -ATPase inhibitor [30], for 30 min prior to somatostatin. The involvement of G_i/G_o in the somatostatin-induced increase in $[Ca^{2+}]_i$ was examined by pretreatment with PTX (100 ng/ml) for 24 h. All experiments were repeated four times.

$[Ca^{2+}]_i$ measurement in single cells

Cells (1000–2000) were grown for 2–3 days on a 22 mm² glass coverslip inside a 35 mm culture dish under similar conditions as

described above. Thereafter, the culture medium was discarded and the cells loaded with fura 2/AM for 30 min at 37 °C. Each coverslip was mounted inside a custom-made perfusion chamber, where it was possible to control the exposure of cells to different treatments. Measurement of $[Ca^{2+}]_i$ from single cells was accomplished by mounting the perfusion chamber on the stage of an inverted fluorescence microscope (Carl Zeiss, Thornwood, NY, U.S.A.). The fluorescence images were obtained (excitation at 340 and 380 nm; emission at 510 ± 20 nm), the background subtracted and the images divided on a pixel-by-pixel basis to generate spatially resolved maps of the $[Ca^{2+}]_i$. The emitted signals were digitalized, recorded and processed using the Attofluor Digital Fluorescence Imaging System (Atto Instruments, Rockville, MD, U.S.A.). The $[Ca^{2+}]_i$ was calculated as described previously [31]. Calibration was performed *in situ* according to the procedure provided by Attofluor, using fura 2 penta K⁺ salt as a standard.

Measurement of insulin release under perfusion conditions

The perfusion system used in this study was as described previously [32] with some modifications. HIT-T15 cells were grown in 10 mm round glass coverslips inside a 35 mm culture dish for 3–4 days until confluency ($400\text{--}700 \times 10^3$ cells). Each coverslip was then removed from the culture dish and mounted inside a 0.7 ml perfusion chamber (Millipore Swinnox Filter Holders, Waters, Milford, MA, U.S.A.) with cells facing inside the chamber. Initially, the cells were perfused for a 20 min equilibration period at 37 °C with modified KRB containing 136 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4 , 1.2 mM $MgSO_4$, 5 mM $NaHCO_3$, 10 mM Hepes, 4 mM glucose and 0.1% BSA, pH 7.4. The flow rate was adjusted to 0.5 ml/min prior to the experiments and samples collected at 30 s intervals. Each coverslip was exposed to one of four treatments: KRB-treated control, 1 nM AVP, 100 nM somatostatin and 1 nM AVP followed by 100 nM somatostatin. At the end of each experiment, cells were treated with 20 mM KCl to test their ability to release insulin. Once the experiments had been completed, the glass coverslips were removed from the perfusion chambers and the number of cells quantified. Insulin concentration from effluent samples was measured by RIA. Each treatment group was replicated four times.

Data analysis

Traces from $[Ca^{2+}]_i$ measurements are representative of four experiments. Results from the insulin release under perfusion conditions are presented as mean $\pm$ S.E.M., and were analysed using ANOVA to determine the effect of treatment. Fisher's least-significant-difference test was used to determine the difference between means for which the ANOVA indicated a significant *F* ratio ($P < 0.05$).

RESULTS

Somatostatin-induced increase in $[Ca^{2+}]_i$ in the presence of AVP

In the presence of 1 nM AVP, 100 nM somatostatin induced a sharp and transient increase in $[Ca^{2+}]_i$, followed by a rapid decline to the sub-basal level. To determine if this effect was due to hormone treatment, we replaced AVP and somatostatin with KRB. Somatostatin failed to increase $[Ca^{2+}]_i$ in KRB and no further increases in $[Ca^{2+}]_i$ were observed when KRB was added in the presence of AVP. Treatment with somatostatin prior to AVP did not induce a further increase in $[Ca^{2+}]_i$ by AVP compared with AVP alone. The increase in $[Ca^{2+}]_i$ by somatostatin in the

Figure 1 Somatostatin-induced increase in $[Ca^{2+}]_i$ in the presence of AVP

Upper panel: the effect of somatostatin (SS) was confirmed by replacing 1 nM AVP with working buffer (KRB). No increase in $[Ca^{2+}]_i$ was observed after 100 nM somatostatin treatment (left-hand panel). When KRB was replaced for somatostatin, only the typical increase in $[Ca^{2+}]_i$ by AVP was observed (middle panel). Lower panel: in the presence of AVP, somatostatin increased $[Ca^{2+}]_i$ (left-hand panel). The increase in $[Ca^{2+}]_i$ by somatostatin was observed even after 60 min of AVP treatment (right-hand panel). Traces are representative of four experiments.

Figure 2 Dose responses to AVP and somatostatin

Upper panel: the response to 100 nM somatostatin (SS) gradually increased with a decrease in the AVP concentration from 100 nM to 100 pM, where a near-maximal response was obtained in the presence of 1 nM AVP. Lower panel: a concentration-dependent response to 100 pM–1 μ M somatostatin was also observed in the presence of 1 nM AVP, where a near-maximal response was obtained with 100 nM somatostatin. Traces are representative of four experiments.

presence of AVP was still apparent even after 60 min of treatment (Figure 1). The response to 100 nM somatostatin gradually increased with a decrease in the AVP concentration from 100 nM to 100 pM. In the presence of 1 nM AVP, 100 nM somatostatin caused a near-maximal increase in $[Ca^{2+}]_i$. A concentration-dependent response to 100 pM–1 μ M somatostatin was also observed in the presence of 1 nM AVP, where near-maximal response was obtained with 100 nM somatostatin (Figure 2). For this reason, we chose 1 nM AVP followed by 100 nM somato-

Figure 3 Effect of AVP washout on somatostatin-induced increase in $[Ca^{2+}]_i$

Upper panel: in the presence of 1 nM AVP, 100 nM somatostatin (SS) increased $[Ca^{2+}]_i$ in single cells during Ca^{2+} imaging analysis ($n = 10$ cells). Lower panel: temporary pre-exposure to AVP, followed by a 5 min washout period, abolished the increase in $[Ca^{2+}]_i$ by somatostatin ($n = 9$ cells). Traces are representative of four experiments.

statin to characterize the mechanism underlying somatostatin-induced increase in $[Ca^{2+}]_i$ and insulin release in HIT-T15 cells.

By measuring $[Ca^{2+}]_i$ in single cells through imaging analysis, we determined whether temporary pre-exposure to AVP or its presence was required for the somatostatin-induced increase in $[Ca^{2+}]_i$. In the presence of AVP somatostatin increased $[Ca^{2+}]_i$, whereas temporary pre-exposure to AVP followed by a 5 min washout period abolished the increase in $[Ca^{2+}]_i$ by somatostatin (Figure 3).

In addition, we investigated if the rapid decline in $[Ca^{2+}]_i$ to sub-basal levels after the increase by somatostatin was due to closure of voltage-dependent Ca^{2+} channels, thereby inhibiting the Ca^{2+} influx, or through efflux from the cytosol via plasma-membrane Ca^{2+} -ATPases. Pretreatment with Bay K8644, which opens voltage-dependent Ca^{2+} channels, or experiments in Ca^{2+} -free medium failed to alter the decline in $[Ca^{2+}]_i$. Pretreatment with Ca^{2+} -ATPase inhibitors, such as sodium orthovanadate, eosin Y, lanthanum chloride, Ruthenium Red or nickel sulphate, also failed to alter the sharp decrease in $[Ca^{2+}]_i$ (results not shown).

Somatostatin-induced insulin release

We measured insulin release under perfusion conditions to determine if the increase in $[Ca^{2+}]_i$ by somatostatin also resulted in insulin exocytosis. Perfusion with KRB or somatostatin alone did not increase insulin release. As for Ca^{2+} -measurement experiments, in the presence of AVP somatostatin induced a significant increase in insulin release, which was also characterized by a sharp peak followed by a rapid decline to sub-basal levels. The stimulatory effect by somatostatin lasted between 2 and 3 min. Treatment with AVP alone induced a gradual increase in insulin release with a lower amplitude and longer duration (Figure 4).

Figure 4 Effect of somatostatin on insulin release in the presence of AVP

Upper panel: in control experiments, perfusion with KRB (○) or 100 nM somatostatin (SS) alone (●) did not increase insulin release. Lower panel: in the presence of 1 nM AVP, somatostatin (◆) increased insulin release, which was characterized by a sharp peak, followed by a rapid decline to sub-basal levels. Treatment with AVP alone (◇) induced a gradual increase in insulin release with a lower amplitude and longer duration. Treatment with 20 mM KCl at the end of the perfusion experiments stimulated insulin release. Experiments represent means $\pm$ S.E.M. ($n = 4$), * $P < 0.05$ between the AVP- and AVP + somatostatin-treated groups at the times indicated.

PTX-sensitive protein(s) mediate the somatostatin effects

Since somatostatin receptors belong to the PTX-sensitive G_i/G_o family of G-proteins, we hypothesized that G_i/G_o also mediated the increase in $[Ca^{2+}]_i$ by somatostatin. Pretreatment of HIT-T15 cells with PTX abolished the increase in $[Ca^{2+}]_i$ by 100 nM somatostatin, whereas it did not alter the increase in $[Ca^{2+}]_i$ by 1 nM AVP, which signals via G_q (Figure 5).

Involvement of the PLC pathway

We examined whether the PLC pathway was also involved in somatostatin signalling by using U-73122, a specific PLC inhibitor [33]. Pretreatment with U-73122 (1–4 μ M) for 100 s before 100 nM somatostatin but after 1 nM AVP inhibited the increase in $[Ca^{2+}]_i$ by somatostatin in a concentration-dependent manner (results not shown). U-73122 (4 μ M) abolished the

Figure 5 Effect of PTX on somatostatin-induced increase in $[Ca^{2+}]_i$

Pretreatment with 100 ng/ml PTX for 24 h abolished the increase in $[Ca^{2+}]_i$ by 100 nM somatostatin (SS). PTX pretreatment had no effect on the response to AVP, which signals via G_q . Traces are representative of four experiments.

Figure 6 Effect of U-73122 and U-73343 on somatostatin-induced increase in $[Ca^{2+}]_i$

Pretreatment with 4 μ M U-73343 (left-hand panel), an inactive analogue of U-73122, failed to inhibit the increase in $[Ca^{2+}]_i$ by 100 nM somatostatin (SS). Pretreatment with U-73122 (4 μ M), a specific PLC inhibitor, after 40 s of 1 nM AVP and 100 s prior to somatostatin abolished the increase in $[Ca^{2+}]_i$ by somatostatin (middle panel). Pretreatment with U-73122 for 100 s prior to AVP (right-hand panel) abolished the responses to AVP and subsequently to somatostatin. Traces are representative of four experiments.

Figure 7 Effects of a Ca^{2+} -free environment and TG on somatostatin-induced increase in $[Ca^{2+}]_i$

Upper panel: in Ca^{2+} -free medium, 100 nM somatostatin (SS) maintained its ability to increase $[Ca^{2+}]_i$ in the presence of 1 nM AVP. Lower panel: pretreatment with TG, which depletes ER Ca^{2+} stores, abolished the increase in $[Ca^{2+}]_i$ by somatostatin. Traces are representative of four experiments.

increase in $[Ca^{2+}]_i$ by somatostatin. Pretreatment with 4 μ M U-73343, an inactive analogue of U-73122, failed to inhibit the increase in $[Ca^{2+}]_i$ by somatostatin. When U-73122 was given prior to AVP, the responses to AVP and subsequently to somatostatin were abolished (Figure 6).

ER as the main source for Ca^{2+} release

Activation of the PLC pathway often leads to the formation of $Ins(1,4,5)P_3$, which increases Ca^{2+} release from the ER by binding to $Ins(1,4,5)P_3$ receptors [34]. We hypothesized that ER was the primary source of Ca^{2+} for the sharp and transient increase in $[Ca^{2+}]_i$ by somatostatin. Pretreatment with 1 μ M TG after 1 nM AVP abolished the increase in $[Ca^{2+}]_i$ by 100 nM somatostatin. In Ca^{2+} -free medium, somatostatin maintained its ability to increase $[Ca^{2+}]_i$ (Figure 7).

Cross-talk between G_q and G_i/G_o

The increase in $[Ca^{2+}]_i$ and insulin release by somatostatin in the presence of AVP may involve cross-talk between G_q and G_i/G_o .

Figure 8 Cross-talk between G_q and G_i/G_o

Upper panel: in the presence of BK, another G_q -coupled receptor agonist, somatostatin (SS) also increased $[Ca^{2+}]_i$. Lower panel: in the presence of isoproterenol, a G_s -coupled receptor agonist (left-hand panel) or medetomidine, a G_i/G_o -coupled receptor agonist (middle panel), somatostatin failed to increase $[Ca^{2+}]_i$. In the presence of AVP, medetomidine increased $[Ca^{2+}]_i$ (right-hand panel). Traces are representative of four experiments.

We examined whether in the presence of bradykinin (BK), another G_q -coupled receptor agonist [35], and also in the presence of isoproterenol or medetomidine (G_s - and G_i/G_o -coupled receptor agonists, respectively) [36,37], somatostatin would maintain its ability to increase $[Ca^{2+}]_i$. In the presence of 1 nM–1 μ M BK, 100 nM somatostatin increased $[Ca^{2+}]_i$. As for AVP, the response to somatostatin gradually increased with a decrease in the BK concentration from 1 μ M to 10 nM. In the presence of 10 μ M isoproterenol, which induced a small increase in $[Ca^{2+}]_i$ or 10 μ M medetomidine, somatostatin failed to increase $[Ca^{2+}]_i$. In the presence of 1 nM AVP, 10 μ M medetomidine also increased $[Ca^{2+}]_i$ (Figure 8).

DISCUSSION

The present study demonstrated that in the presence of AVP somatostatin increased $[Ca^{2+}]_i$, which was characterized by a sharp and transient peak followed by a rapid decline to the sub-basal level. Experiments with TG, which depletes Ca^{2+} stores from the ER, suggested that Ca^{2+} released from this organelle is mainly responsible for the sharp and transient increase in $[Ca^{2+}]_i$ by somatostatin. Pretreatment with Bay K8644 or Ca^{2+} -ATPase inhibitors or experiments in Ca^{2+} -free medium failed to alter the rapid decline in $[Ca^{2+}]_i$ to sub-basal levels. Further investigation will be needed to determine the mechanism responsible for this sharp decline in $[Ca^{2+}]_i$.

One unexpected finding was the ability of somatostatin to increase $[Ca^{2+}]_i$ even after 60 min of AVP treatment. In HIT-T15 cells, the V_{1b} receptor is coupled to G_q , which upon activation stimulates PLC- β through its α_q subunit [24]. The hydrolysis of $PtdIns(4,5)P_2$ by PLC- β results in $Ins(1,4,5)P_3$ and DAG formation, where DAG activates protein kinase C, whereas $Ins(1,4,5)P_3$ promotes Ca^{2+} release from the ER [7,26,34]. Phosphorylation of AVP receptors and PLC- β by protein kinase C after activation of the AVP receptor occurs during desensitization [38–41]. This event accounts in part for the reduction in the responsiveness of cells to further stimulation by AVP. In our system, desensitization of AVP receptors and PLC- β may not be apparent during AVP stimulation, since we demonstrated that the presence of AVP is

required for somatostatin to increase $[Ca^{2+}]_i$ and this increase could be observed even after 60 min of AVP treatment.

An increase in $[Ca^{2+}]_i$ usually triggers exocytosis [42]. During perfusion experiments, somatostatin alone did not increase insulin release, but potentiated the AVP-induced insulin release. These results were consistent with data on $[Ca^{2+}]_i$, which suggested that the increase in insulin release was mediated by the increase in $[Ca^{2+}]_i$.

Five distinct somatostatin receptor subtypes have been characterized and designated as sstr1–5, where two splice variants of sstr2 exist, sstr2a and sstr2b [43,44]. All five receptor subtypes are coupled to G_i/G_o . Pretreatment with PTX abolished the increase in $[Ca^{2+}]_i$ by somatostatin, suggesting that G_i/G_o also mediated the somatostatin effects. PTX pretreatment did not alter the increase in $[Ca^{2+}]_i$ by AVP, which signals via G_q and belongs to the PTX-insensitive family of G-proteins. The somatostatin receptor subtype that mediates the increase in $[Ca^{2+}]_i$ and insulin release remains to be characterized.

Activation of PLC- β frequently leads to an increase in $Ins(1,4,5)P_3$ mass formation and $[Ca^{2+}]_i$, where the involvement of G_i/G_o subunits (mainly the $\beta\gamma$ dimer) in these increases has been reported by other laboratories [17,21,23]. In our study, the increase in $[Ca^{2+}]_i$ by somatostatin was inhibited by U-73122, a specific PLC inhibitor, but not by U-73343, an inactive analogue of U-73122. These findings suggest that the effects of somatostatin are mediated through the PLC pathway. Pretreatment with U-73122 prior to AVP abolished the responses to AVP and subsequently to somatostatin, which suggest that activation of PLC- β by AVP is essential for somatostatin to increase $[Ca^{2+}]_i$. To date, reports addressing the stimulatory effects of G_i/G_o -coupled receptor agonists on the PLC- β pathway have been made through measurement of $Ins(1,4,5)P_3$ mass formation or using inhibitors/antibodies against PLC [18,21,23], which does not provide evidence to preclude the events occurring prior to activation of PLC- β , such as an increase in the formation of $PtdIns(4,5)P_2$.

We have demonstrated that the cross-talk is specific to G_q and G_i/G_o , although not limited to the AVP and somatostatin receptors, since in the presence of BK somatostatin increased $[Ca^{2+}]_i$. After AVP, treatment with medetomidine, an α_2 -adrenoceptor agonist that stimulates G_i/G_o , also increased $[Ca^{2+}]_i$. When isoproterenol, a β -adrenoceptor agonist that stimulates G_s , or medetomidine were given prior to somatostatin, they failed to increase $[Ca^{2+}]_i$.

In summary, our findings suggest that in the presence of AVP somatostatin increased $[Ca^{2+}]_i$ and insulin release from HIT-T15 cells through G_i/G_o , which involves the PLC pathway and Ca^{2+} release from the ER. The cross-talk mechanism is specific to G_q and G_i/G_o , although it may not be limited to the G-protein system coupled to the AVP or somatostatin receptors. Further work on primary β -cells and islets will be necessary to determine the physiological significance of the present findings.

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