Pertussis Toxin Blocks Both Cyclic AMP-mediated and Cyclic AMP-independent Actions of Somatostatin

EVIDENCE FOR COUPLING OF Ni TO DECREASES IN INTRACELLULAR FREE CALCIUM*

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The neuropeptide somatostatin inhibits hormone release from GH4C1 pituitary cells via two mechanisms: inhibition of stimulated adenylate cyclase and a cAMP-independent process. To determine whether both mechanisms involve the guanyl nucleotide-binding protein Nix, we used pertussis toxin, which ADP-ribosylates Nix and thereby blocks its function.

Pertussis toxin treatment of GH4C1 cells blocked somatostatin inhibition of both vasoactive intestinal peptide (VIP)-stimulated cAMP accumulation and prolactin secretion. In membranes prepared from toxin-treated cells, somatostatin inhibition of VIP-stimulated adenylate cyclase activity was reduced and [125I-Tyr]somatostatin binding was decreased more than 95%. In contrast, pertussis toxin did not affect the biological actions or the membrane binding of thyrotropin-releasing hormone. These results indicate that ADP-ribosylated Nix cannot interact with occupied somatostatin receptors and that somatostatin inhibits VIP-stimulated adenylate cyclase via Nix.

To investigate somatostatin's cAMP-independent mechanism, we used depolarizing concentrations of K+ to stimulate prolactin release without altering intracellular cAMP levels. Measurement of Quin-2 fluorescence showed that 11 mM K+ increased intracellular [Ca2+] within 5 s. Somatostatin caused an immediate, but transient, decrease in both basal and K+-elevated [Ca2+]. Consistent with these findings, somatostatin inhibited K+-stimulated prolactin release, also without affecting intracellular cAMP concentrations. Pertussis toxin blocked the somatostatin-induced reduction of [Ca2+]. Furthermore, the toxin antagonized somatostatin inhibition of K+-stimulated VIP-stimulated prolactin secretion with the same potency (ED50 = 0.3 ng/ml).

These results indicate that pertussis toxin acts at a common site to prevent somatostatin inhibition of both Ca2+ and cAMP-stimulated hormone release. Thus, Ni appears to be required for somatostatin to decrease both cAMP production and [Ca2+] and to inhibit the actions of secretagogues using either of these intracellular messengers.

The hypothalamic peptide somatostatin is a physiological regulator of growth hormone release and also inhibits the secretion of many other hormones and neurotransmitters by specific target cells of the pancreas, gut, and nervous system (1). The mechanisms by which somatostatin elicits these inhibitory effects are still poorly understood. The GH4C1 rat pituitary cell strain has been a particularly useful model system for studying the biochemical events involved in somatostatin inhibition of hormone release (2, 3). In these cells, we have shown that somatostatin inhibits growth hormone and prolactin secretion by at least two different mechanisms: one mediated by changes in cAMP levels and the other independent of changes in cAMP concentrations (4, 5).

Two lines of evidence indicate that the cAMP-mediated effects of somatostatin result from the interaction of the somatostatin receptor with membrane adenylate cyclase. First, somatostatin inhibits VIP-stimulated adenylate cyclase activity in GH4C1 cell membranes in a manner which parallels its effect on cAMP levels in cells (6). Second, the phosphodiesterase inhibitor IBMX does not reduce somatostatin inhibition of VIP-stimulated cAMP production in intact cells (4).

Less is known about the mechanism by which somatostatin inhibits hormone secretion independently of changes in intracellular cAMP levels (5). However, the recent observation that somatostatin reduces [Ca2+], in GH cells (7) suggests that changes in cellular Ca2+ metabolism may be involved. [125I-Tyr]somatostatin binding to pituitary membranes is modulated by guanylnucleotides, indicating that the somatostatin receptor is likely to regulate adenylate cyclase via a guanylnucleotide-binding protein. Such a regulatory component (Ni) has recently been identified and shown to couple the enzyme to other inhibitory receptors (9, 10). A 41,000-Da subunit of this protein is ADP-ribosylated by pertussis toxin, a protein produced by Bordetella pertussis (9, 10). Following pertussis toxin treatment of cells, receptor-mediated inhibition of adenylate cyclase is blocked and the specific

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1 The abbreviations used are: VIP, vasoactive intestinal peptide; 8-Br-cAMP, 8-bromoadenosine 3':5'-monophosphate; [Ca2+]i, the intracellular free calcium ion concentration; F-101h, Ham's F-10 medium supplemented with 5 mg/ml lactalbumin hydrolysate; Gpp(NH)p, guanyl-5'-yl imidodiphosphate; HBS, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes)-buffered salt solution containing 20 mM Hepes, 115 mM NaCl, 4.6 mM KCl, 1 mM CaCl2, 10 mM D-Glucose (pH 7.2); HBS/BSA, HBS supplemented with 0.1% bovine serum albumin; IBMX, 3-isobutyl-1-methylxanthine; Ni, the inhibitory guanyl nucleotide-binding regulatory subunit of adenylate cyclase; PdIms, 4.5-Pd, 1-(3-isobutylphosphatidyl)-o-myoinositol 4,5-bisphosphate; TRH, thyrotropin-releasing hormone; Met-TRH, [3-methylhistidine]thyrotropin-releasing hormone; EGTa, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid.
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binding of inhibitory agonists to membrane preparations is decreased (11–14). Both of these actions of pertussis toxin are believed to result from the inability of ADP-ribosylated N\textsubscript{i} to poorly couple inhibitory receptors to adenylate cyclase.

Since pertussis toxin treatment of GH cell membranes results in the ADP-ribosylation of a single 41,000-Da protein (15), we have used this toxin to address the role of N\textsubscript{i} in somatostatin action. First, we examined whether N\textsubscript{i} was the guanylyl nucleotide-binding protein which coupled the somatostatin receptor to adenylate cyclase. Second, we investigated the role of N\textsubscript{i} in the CAMP-independent actions of somatostatin, including its effects on [Ca\textsuperscript{2+}]. Our results indicate that both the CAMP-dependent and CAMP-independent actions of somatostatin require a functional N\textsubscript{i}. Preliminary reports of some of these data have been presented.\textsuperscript{2}

EXPERIMENTAL PROCEDURES

Materials

Pertussis toxin was the kind gift of Dr. Erik Hewlett, University of Virginia, or was purchased from List Biologicals (Campbell, CA). VIP, somatostatin, and bombesin were obtained from Bachem (Torrance, CA). TRH was obtained from Beckman Instruments. [\textsuperscript{125}I]-\textsuperscript{Tyr\textsubscript{1}}-Somatostatin-1-14 was purchased from Peninsula Laboratories (Belmont, CA). Somatostatin receptor antagonist (1000 \textsuperscript{g} for 10 min at 4 \textdegreeC) and the cAMP concentration in the medium was measured by radioimmunoassay (20). Under the conditions of these experiments, changes in extracellular cAMP concentrations parallel effects on intracellular cAMP levels (4).

The effect of drugs on intracellular CAMP concentrations was determined in HBS/BSA without IBMX. Cells were preincubated for two periods of 30 min in HBS/BSA, and then fresh HBS/BSA containing chemicals or hormones was added. At the indicated time, the medium was aspirated and the cells were extracted with 1 ml of ice-cold 95% ethanol, 0.1% Triton X-100. The extracts were dried in a Speed-Vac concentrator, resuspended in 50 mM acetate buffer (pH 6.2), and centrifuged to remove precipitated protein. Hormones were added to the cuvette from −200 µl stock solutions. Tracings were digitized on a bidigitizer, normalized by converting the fluorescence readings to percentage saturation of the Quin-2, and plotted on a laser printer.

Measurement of Adenylate Cyclase Activity—GH\textsubscript{4}C\textsubscript{1} cell membranes were prepared as previously described (6), except that the buffer contained the following: 10 mM Tris-Cl buffer (pH 7.4), 5 mM MgCl\textsubscript{2}, 0.5 mM dithiothreitol, and 0.01% Brij 35. Membranes were incubated with 0.1 µM [\textsuperscript{35}S]GTP\textsubscript{γS} (specific activity: 5000 Ci/mmol) in the presence of 100 nM unlabeled GTP, 30 nM [\textsuperscript{3H}]-cAMP (specific activity: 5000 Ci/mmol) and varying concentrations of somatostatin. Membranes were incubated for 10 min at 30 \textdegreeC with 30 nM somatostatin and then washed twice with fresh HBS/BSA at 4 \textdegreeC. Membranes were then frozen and stored at −70 \textdegreeC until used.

RESULTS

Effect of Pertussis Toxin on Somatostatin Receptor Function in Membranes—Two lines of evidence have indicated that the


\textsuperscript{3} When K\textsuperscript{+} was used as a secretagogue, the indicated concentration is the final, not the added, K\textsuperscript{+} concentration. Since in Quin-2 experiments the sodium ion concentration could not be decreased to compensate for the added K\textsuperscript{+}, this adjustment was also omitted in most hormone secretion and cAMP experiments. However, control experiments have shown that the additional sodium has no effect on secretory responses.

Prolactin radioimmunoassay was performed as previously described (5). Rat prolactin (NIADDKD-rPRL-1-5) was radiolabeled by the chloramine-T method (18). The prolactin standard was NIADDKD-rPRL-4-38. Antibody-bound antigen was precipitated by the addition of inactivated Staphylococcus aureus [\textsuperscript{125}I]-Somatostatin for 90 min at 37 \textdegreeC in 50 mM Tris, 2 mM EDTA, 7 mM MgCl\textsubscript{2}, 150 mM NaCl, 3 mM benzamidine, 5 µg/ml leupeptin, 7 µg/ml pepstatin, 5 µg/ml soybean trypsin inhibitor, and 5 µg/ml lima bean trypsin inhibitor. Adenylate cyclase activity was determined by the method of Salomon et al. (Ref. 22; as modified in Ref. 6).

Ligand Binding—\textsuperscript{[\textsuperscript{35}S]Somatostatin (2200 Ci/mmol) binding to GH\textsubscript{4}C\textsubscript{1} cells was determined during a 60-min incubation at 37 \textdegreeC in F-10 after as previously described (23). Nonsaturable binding was determined in the presence of 100 nM unlabeled somatostatin and was subtracted from total binding.
somatostatin receptor is coupled to adenylate cyclase in GHL C cell membranes. First, somatostatin inhibits stimulated adenylate cyclase activity (6). Second, guanyl nucleotides decrease the binding affinity of somatostatin receptors in membrane preparations (6). Therefore, we examined the effect of pertussis toxin treatment on these two indicators of somatostatin receptor function.

In membranes prepared from control cells, VIP stimulated adenylate cyclase activity 3.6-fold and somatostatin inhibited this stimulation by 15% (Table I). In membranes from pertussis toxin-treated cells, VIP caused a similar stimulation of adenylate cyclase activity (4.5-fold), but somatostatin only inhibited this stimulation by 3%. Thus, somatostatin inhibition of stimulated adenylate cyclase activity was blocked by pertussis toxin treatment.

The data in Fig. 1 show the effects of pertussis toxin treatment on the membrane binding of [125I]-Ty r-binding to membranes. Although TRH has been shown to stimulate PtdIns-4,5-P hydrolysis (24–26), its receptor also appears to be coupled to a guanyl nucleotide-binding protein (27). In control membranes, Gpp(NH)p, a nonhydrolyzable analog of GTP, reduced [125I]-Ty r-binding by 78% (Fig. 1, left panel) and [3H]Me-TRH binding by 63% (Fig. 1, right panel). These observations confirm published results (6, 27). In membranes prepared from pertussis toxin-treated cells, [125I]-Ty r-somatostatin binding in the absence of Gpp(NH)p was reduced by more than 95%, whereas [3H]Me-TRH binding was unaffected. As in control membranes, Gpp(NH)p reduced [3H]Me-TRH binding by 65% in pertussis toxin-treated membranes. In contrast, this guanyl nucleotide had no detectable effect on the low level of saturable [125I]-Ty r-somatostatin binding measured in pertussis toxin-treated membranes.

These results indicate that pertussis toxin treatment specifically blocked the interaction of the somatostatin receptor with a guanyl nucleotide-binding protein, thereby disrupting the functional coupling of this receptor to adenylate cyclase. In the same membrane preparations, the apparent interaction of the TRH receptor with a guanyl nucleotide-binding protein was unaffected.

Effect of Pertussis Toxin on the CAMP-mediated Actions of Somatostatin in Cells—Since pertussis toxin treatment blocked somatostatin inhibition of adenylate cyclase, we examined the toxin's effects on those cellular actions of somatostatin which appear to result from this inhibition (3–6). In control cells, VIP stimulated cAMP accumulation 11-fold, and somatostatin inhibited the VIP stimulation by 52% (Fig. 2, lower panel). In pertussis toxin-treated cells, VIP stimulation of cAMP accumulation was unchanged, but somatostatin no longer inhibited this stimulation. The effect of pertussis toxin treatment on prolactin release paralleled the effects on cAMP accumulation (Fig. 2, upper panel): toxin pretreatment did not alter VIP stimulation, but blocked somatostatin inhibition.

### Table I

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Adenylate cyclase activity*</th>
<th>Control</th>
<th>Toxin-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenylate cyclase activity*</td>
<td>Control</td>
<td>Toxin-treated</td>
</tr>
<tr>
<td></td>
<td>nmoI/min/mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.51 ± 0.013 (3)</td>
<td>0.33 ± 0.010 (4)</td>
<td></td>
</tr>
<tr>
<td>Somatostatin</td>
<td>0.48 ± 0.012 (4)</td>
<td>0.30 ± 0.007 (4)</td>
<td></td>
</tr>
<tr>
<td>VIP</td>
<td>1.85 ± 0.021 (4)</td>
<td>1.47 ± 0.046 (4)</td>
<td></td>
</tr>
<tr>
<td>VIP + somatostatin</td>
<td>1.68 ± 0.035 (4)</td>
<td>1.44 ± 0.040 (3)</td>
<td></td>
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</tbody>
</table>

*Mean ± S.E.; the number of observations is shown in parentheses.
In intact cells, pertussis toxin blockade of somatostatin action was not simply due to inhibition of somatostatin binding. The saturable binding of [125I-Tyr]somatostatin to GH3C1 cells was unaffected by pertussis toxin treatment, whereas somatostatin inhibition of hormone secretion was abolished (Table II). The apparently different effects of pertussis toxin treatment on [125I-Tyr]somatostatin binding to cells and membranes (Fig. 1 and Table II) probably result from the different forms of the receptor measured under the two conditions. In membranes incubated in the absence of guanyl nucleotides, [125I-Tyr]somatostatin binding is primarily a measure of the high affinity state of the somatostatin receptor which exists in the absence of GTP (6). In contrast, [125I-Tyr]somatostatin binding to intact cells probably reflects binding to the low affinity form of the somatostatin receptor induced by GTP.

In summary, pertussis toxin has parallel effects on somatostatin inhibition of VIP-stimulated adenylate cyclase activity, cAMP accumulation, and prolactin secretion. This indicates that somatostatin inhibition of stimulated CAMP levels and prolactin secretion results from its inhibition of adenylate cyclase via Ni.

Effect of Pertussis Toxin on CAMP-independent Actions of Somatostatin—Somatostatin inhibition of basal hormone secretion in F-10lh occurs without a concomitant reduction of basal CAMP concentrations (5). Furthermore, this inhibition is not blunted when cellular CAMP concentrations are pharmacologically raised to levels many times those required to maximally stimulate hormone release (5). To determine whether Ni might also be involved in this CAMP-independent action of somatostatin, we first examined the effect of pertussis toxin treatment on somatostatin inhibition of basal hormone secretion in F-10lh. The results in Table II demonstrate that somatostatin inhibition of basal hormone secretion was totally blocked in pertussis toxin-treated cells.

To further characterize the effect of pertussis toxin treatment on the CAMP-independent actions of somatostatin, we used four secretagogues which do not regulate adenylate cyclase activity (Fig. 3). 8-Br-cAMP directly stimulates CAMP-dependent protein kinases and therefore elicits CAMP-inducible effects at a site distal to the activation of adenylate cyclase. High K+ concentrations are believed to stimulate hormone release by depolarizing the plasma membrane, opening voltage-sensitive Ca2+ channels, and increasing [Ca2+]. (28). TRH and bombesin do not reproducibly affect CAMP levels in [Ca2+]i-Although the biochemical basis of the CAMP-independent mechanism of somatostatin action is unknown, several studies have suggested that regulation of [Ca2+]i might

![Fig. 3. Effect of pertussis toxin treatment on somatostatin inhibition of secretagogue-stimulated prolactin release. Cells were pretreated without (upper panels) or with (lower panels) 50 ng/ml pertussis toxin. Prolactin release was then determined in HBS/BSA, either in the absence (a) or presence (b) of 100 nM somatostatin and the secretagogues shown. The concentrations of secretagogues used were: VIP, 100 nM; 8-Br-cAMP, 5 mM; K+, 11 mM; TRH, 1 µM; bombesin, 100 nM. Each bar represents the mean ± S.E. (n = 6).](http://example.com/fig3)

**TABLE II**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Peptide added</th>
<th>Prolactin released*</th>
<th>[125I-Tyr]Somatostatin bound*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control</td>
<td>128 ± 2.7</td>
<td>10,750 ± 150</td>
</tr>
<tr>
<td></td>
<td>Somatostatin</td>
<td>98 ± 2.7</td>
<td>1,670 ± 90</td>
</tr>
<tr>
<td>Pertussis toxin</td>
<td>Control</td>
<td>119 ± 2.0</td>
<td>11,730 ± 610</td>
</tr>
<tr>
<td></td>
<td>Somatostatin</td>
<td>114 ± 3.3</td>
<td>1,720 ± 90</td>
</tr>
</tbody>
</table>

*Mean ± S.E. (n = 8).
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be involved (7, 30, 31). We therefore used the fluorescent chelating dye Quin-2 to determine the effect of somatostatin on basal and K+-elevated [Ca²⁺], with and without pertussis toxin treatment (Fig. 5). In control GH₃C₁ cells, somatostatin caused a transient drop in resting [Ca²⁺] (Fig. 5A). [Ca²⁺]i reached a minimum approximately 30 s after the addition of somatostatin, and then slowly returned to the original resting level. Pretreatment of the cells with pertussis toxin resulted in an almost complete block of the somatostatin reduction in [Ca²⁺]i (Fig. 5B). Table III summarizes the results of a number of such experiments. In control cells, somatostatin reduced [Ca²⁺] below resting level, whereas in pertussis toxin-treated cells, somatostatin no longer had a significant effect on basal [Ca²⁺].

We next explored the effect of somatostatin on K⁺-stimulated [Ca²⁺] (Fig. 5, C and D). Addition of 11 mM K⁺ caused a very rapid rise in [Ca²⁺], which decayed to a nadir before rising to a relatively sustained plateau level. Addition of 100 nm somatostatin during the plateau phase caused a transient drop in [Ca²⁺], in control cells (Fig. 5C). However, in pertussis toxin-treated cells, somatostatin had no significant effect on K⁺-stimulated [Ca²⁺], (Fig. 5D and Table II). The effect of somatostatin on [Ca²⁺] is likely to be receptor-mediated, since 100 nm dicarboxymethyl somatostatin caused a much smaller decrease in [Ca²⁺], than the same concentration of somatostatin (Fig. 5, B and C). Dicarboxymethyl somatostatin, like other reduced analogs (32), is at least 100-fold less potent than somatostatin at inhibiting hormone secretion and adenylylate cyclase activity (data not shown). Thus, somatostatin reduces both basal and potassium-elevated [Ca²⁺], and these effects are blocked by pertussis toxin treatment. Therefore somatostatin’s effects on [Ca²⁺], appear to be mediated via Ni.

Concentration Dependence for Pertussis Toxin Inhibition of Somatostatin Action—The results in Fig. 3 show that pertussis toxin treatment prevented somatostatin inhibition of both VIP-stimulated and K⁺-stimulated hormone secretion. Since these secretagogues are believed to stimulate prolactin release

**Table III**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Decrease in [Ca²⁺], by somatostatin*</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Basal</td>
<td>20.7 ± 2.60 (3)</td>
</tr>
<tr>
<td>Pertussis toxin</td>
<td>11 mM K⁺</td>
<td>3.7 ± 2.03 (3)</td>
</tr>
</tbody>
</table>

*Mean ± S.E.; the number of observations is shown in parentheses.

**Fig. 5.** Effect of pertussis toxin treatment on somatostatin regulation of [Ca²⁺]. Cells were preincubated with either medium alone (top panels) or with pertussis toxin (lower panels: 10 ng/ml in left panel; 100 ng/ml in right panel). Changes in [Ca²⁺], were determined in suspended cells by monitoring the fluorescence of intracellular Quin-2. Arrows mark the addition of 100 nm somatostatin (SRIF), 100 nm dicarboxymethyl somatostatin (CMSRIF), or vehicle. Neither 11 mM K⁺ nor somatostatin had any effect on the intrinsic fluorescence of GH₃C₁ cells (data not shown). The left two panels show the effects of somatostatin on basal [Ca²⁺], and the right two panels show the effects of somatostatin on [Ca²⁺], stimulated by 11 mM K⁺.
by a cAMP-mediated and a Ca\textsuperscript{2+}-mediated mechanism, respectively, this result suggested that Ni was involved in both the cAMP-dependent and cAMP-independent actions of somatostatin. Although pertussis toxin has been shown to ADP-ribosylate only a single 41,000-Da protein in GH cell membranes (15), it remained possible that the toxin had multiple targets in intact cells. To address this possibility, we determined the concentration dependence for pertussis toxin blockage of somatostatin inhibition of both VIP-stimulated (Fig. 6, left panel) and K\textsuperscript{+}-stimulated (Fig. 6, right panel) prolactin secretion. Both effects of somatostatin were completely blocked by pertussis toxin with the same ED_{50} (0.3 ng/ml), indicating that the toxin acts at a single, common site to prevent somatostatin inhibition of VIP-stimulated and K\textsuperscript{+}-stimulated prolactin secretion.

**DISCUSSION**

We have previously shown that somatostatin inhibits hormone secretion from GH\textsubscript{4}C\textsubscript{1} cells by at least two mechanisms: inhibition of stimulated adenylate cyclase and a second cAMP-independent process (3-6). The results presented here demonstrate that pertussis toxin blocks somatostatin inhibition of VIP-stimulated adenylate cyclase activity, cAMP accumulation, and prolactin secretion. Since pertussis toxin ADP-ribosylates only a 41,000-Da polypeptide, presumably N\textsubscript{i}, in GH cell membranes (15), these results indicate that N\textsubscript{i} mediates somatostatin inhibition of VIP-stimulated prolactin secretion by coupling the somatostatin receptor to adenylate cyclase. However, pertussis toxin treatment also blocked the cAMP-independent inhibition of hormone secretion by somatostatin. Furthermore, pertussis toxin prevented the reduction of basal and K\textsuperscript{+}-elevated [Ca\textsuperscript{2+}], by somatostatin, effects which were also produced without any changes in intracellular cAMP levels. These results indicate that N\textsubscript{i} not only mediates somatostatin inhibition of adenylate cyclase, but also the cAMP-independent components of somatostatin action, including the reduction in [Ca\textsuperscript{2+}]. To our knowledge, this is the first evidence for the coupling of N\textsubscript{i} to inhibition of [Ca\textsuperscript{2+}].

In parallel with our results in GH\textsubscript{4}C\textsubscript{1} pituitary cells, pertussis toxin treatment has been shown to reduce somatostatin inhibition of stimulated adenylate cyclase, cAMP accumulation, or hormone secretion in other systems. In S49 lymphoma cell membranes, pertussis toxin treatment blocks somatostatin inhibition of forskolin-stimulated adenylate cyclase activity (18). Pertussis toxin treatment also blocks somatostatin inhibition of stimulated cAMP accumulation and hormone secretion from several types of endocrine cells: pancreatic \(\beta\)-cells (33), pituitary somatotrophs (34), and AtT20/D16 pituitary tumor cells (35). The general observation that pertussis toxin blocks somatostatin inhibition of hormone secretion, in addition to stimulated adenylate cyclase activity and cAMP accumulation, has been taken as evidence that somatostatin elicits its inhibitory effects on hormone secretion by regulating adenylate cyclase.

Two lines of evidence support the hypothesis that somatostatin can inhibit hormone secretion from GH\textsubscript{4}C\textsubscript{1} cells by a cAMP-independent mechanism. First, somatostatin can inhibit hormone secretion under a variety of conditions in which there are no changes in intracellular cAMP concentrations. These include inhibition of basal hormone secretion (4, 5), inhibition of the stimulatory effect of 11 mM K\textsuperscript{+}, and inhibition of the stimulatory effects of TRH and bombesin (36). Second, somatostatin can inhibit secretion stimulated by 8-Br-cAMP, which raises intracellular cAMP levels without affecting adenylate cyclase, and by forskolin, which produces supramaximal intracellular cAMP levels even in the presence of somatostatin (5). The observation that pertussis toxin blocked a cAMP-independent effect of somatostatin with the same potency as it blocked its cAMP-mediated action suggested that both mechanisms required the same pertussis toxin-sensitive site, namely N\textsubscript{i}. Thus, our results provide a new function for N\textsubscript{i} in addition to mediating inhibition of adenylate cyclase.

Somatostatin has been shown to decrease basal [Ca\textsuperscript{2+}], in GH cells (7), and this reduction in [Ca\textsuperscript{2+}], provides a possible mechanism for its cAMP-independent action. Consistent with this possibility, we have observed that somatostatin decreases [Ca\textsuperscript{2+}], in the presence of a maximal concentration of the cAMP analog 8-(4-chlorophenylthio)-cAMP. The result that pertussis toxin prevented both somatostatin reduction of [Ca\textsuperscript{2+}], and its inhibition of K\textsuperscript{+}-stimulated secretion suggests that N\textsubscript{i} may activate the cAMP-independent mechanism of somatostatin action by triggering decreases in [Ca\textsuperscript{2+}]. The biochemical mechanism by which N\textsubscript{i} may reduce [Ca\textsuperscript{2+}], clearly provides an interesting problem for future research.

Examination of guanyl nucleotide effects on \textsuperscript{125}I-Tyr\textsuperscript{1} somatostatin binding to GH\textsubscript{4}C\textsubscript{1} cell membrane preparations (6) indicated that the somatostatin receptor interacts with a guanyl nucleotide-binding protein by a process similar to that

*B. D. Koch and A. Schonbrunn, unpublished observations.*
originally proposed for the β-adrenergic receptor (57). Namely, guanyl nucleotides appear to destabilize a high affinity ternary complex of the somatostatin receptor, bound hormone, and a guanyl nucleotide-binding protein, thereby producing a decrease in [125I-Tyr']somatostatin binding. [125I-Tyr']Somatostatin binding to membrane preparations primarily reflects binding to this high affinity ternary complex (5); thus, the observed decrease in membrane binding and loss of guanyl nucleotide sensitivity upon pertussis toxin treatment are consistent with inactivation of Nᵢ. However, it is not clear why pertussis toxin treatment reduced [125I-Tyr']somatostatin binding to membrane preparations more than did maximal concentrations of Gpp(NH)p. Perhaps ADP-ribosylation completely prevents Nᵢ interaction with the somatostatin receptor, whereas the binding of Gpp(NH)p to Nᵢ only reduces its affinity for the receptor. Unfortunately, the binding of [125I-Tyr']somatostatin to pertussis toxin-treated membranes was too low to permit experimental examination of this hypothesis. Nevertheless, the ability of pertussis toxin to reduce [125I-Tyr']somatostatin binding to GH₄C₁ cells strongly suggests that the somatostatin receptor interacts with Nᵢ, and that this interaction is disrupted by pertussis toxin treatment.

Unexpectedly, we found that although [125I-Tyr']somatostatin binding to membrane preparations was reduced by pertussis toxin treatment, [123I-Tyr']somatostatin binding to cells was unaffected. The presence of GTP in cells, but its absence in the membrane incubations, may be responsible for this difference. In contrast to membrane binding, [125I-Tyr']somatostatin binding to intact cells is likely to reflect binding to the low affinity form of the somatostatin receptor induced by GTP. Therefore, the different effects of pertussis toxin on [125I-Tyr']somatostatin binding to cells and membranes may be due to the different forms of the receptor measured in the two assays.

The action of pertussis toxin to ADP-ribosylate a 41,000-Da polypeptide component of the inhibitory guanyl nucleotide-binding subunit of adenylate cyclase, and thereby reduce hormonal inhibition of the enzyme, has been demonstrated in many target cells (11-14). Furthermore, pertussis toxin treatment has been shown to decrease the affinity of specific membrane receptors for a number of neurotransmitters which cause inhibition of adenylate cyclase (14). Thus, pertussis toxin treatment appears to generally disrupt both Nᵢ-dependent inhibition of adenylate cyclase activity and the interaction of inhibitory receptors with Nᵢ. Recently, pertussis toxin has also been shown to block the action of a stimulatory agent, the chemicotactic peptide fMet-Leu-Phe (38-41). This effect of pertussis toxin also correlates with ADP-ribosylation of a 41,000-Da polypeptide (38-40). Since fMet-Leu-Phe has been shown to increase PtdIns-4,5-P₂ hydrolysis and [Ca²⁺], in neutrophils (41, 42), the ability of pertussis toxin to block its action via receptors which are coupled to a pertussis toxin substrate, Nᵢ, does not play such a role in GH₄C₁ cells: pertussis toxin treatment did not affect the guanyl nucleotide-sensitive binding of TRH to GH₄C₁ cell membranes, nor did it reduce TRH stimulation of hormone secretion. Since TRH also stimulates PtdIns-4,5-P₂ hydrolysis and increases [Ca²⁺] in GH cells (21, 24-26), our results suggest that not all secretagogues which utilize these intracellular messengers act via receptors which are coupled to a pertussis toxin substrate.

We have previously demonstrated that a series of somatostatin analogs show the same potency for inhibition of VIP-stimulated cAMP accumulation and inhibition of basal prolactin secretion in GH₄C₁ cells (43). These results indicated that the cAMP-mediated and cAMP-independent actions of somatostatin were triggered by the same receptor. The results presented here provide evidence that both somatostatin inhibition of adenylate cyclase and its cAMP-independent inhibitory actions require a pertussis toxin substrate, Nᵢ. Furthermore, the ability of pertussis toxin to block somatostatin reduction of [Ca²⁺], suggests a possible biochemical mechanism for somatostatin’s cAMP-independent actions and demonstrates a new function for Nᵢ.

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REFERENCES

Role of Ni in Somatostatin Action