Gs and Gq/11 Couple Vasoactive Intestinal Peptide and Cholinergic Stimulation to Lacrimal Secretion

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**Purpose.** The intent of this study was to determine the physiological role of selected G proteins in receptor-mediated protein release by lacrimal acini.

**Methods.** The role of G proteins in lacrimal secretion was determined in tissues obtained from the lacrimal glands of adult male New Zealand White rabbits. Pertussis toxin treatment of primary acinar cultures and permeabilization of cultured acini with streptolysin-O and insertion of GDPβS or antibodies against the α subunit of Gs or Gq/11 were used to determine the role of G proteins in vasoactive intestinal peptide (VIP) and carbachol-stimulated lacrimal secretion. Gs and Gq/11 were identified in lacrimal membranes obtained from freshly isolated lacrimal gland fragments, freshly isolated acini, and cultured acini by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting.

**Results.** Permeabilization by streptolysin-O and introduction of guanosine thiodiphosphate into cultured acini blocked stimulation of protein released by either 100 nM VIP or 100 μM carbachol by approximately 50%. Exposure of cultured acini to 100 ng/ml pertussis toxin for 36 to 48 hours did not affect stimulated release by either agonist, indicating that the guanosine triphosphate-dependent actions of VIP and carbachol are mediated through pertussis toxin-insensitive G proteins. Pertussis toxin-insensitive G proteins in lacrimal membranes obtained from freshly isolated glands, freshly isolated acini, and cultured acini were identified with polyclonal antibodies to the α subunits of Gs and Gq/11. Immunoblotting of lacrimal membranes with anti-Gs α antiserum showed two immunoreactive bands at 44 and 47 kDa. Anti-Gq/11 α antiserum detected a single band at 46 kDa in similar membrane preparations. Anti-Gs α antiserum reduced the secretory response to VIP by 64% and to carbachol by 37%. Introduction of anti-Gq/11 α antiserum reduced the response to carbachol by 70%; however, the response to VIP was unchanged. Simultaneous introduction of both antisera caused no further reduction of VIP-stimulated release than did anti-Gs α antiserum alone. However, simultaneous introduction of both anti-Gs α and anti-Gq/11 α antisera resulted in complete inhibition of the effects of carbachol on protein release by cultured acini.

**Conclusions.** These results show that VIP receptor activation of lacrimal protein release is mediated through Gs, whereas cholinergic stimulation involves both Gs and Gq/11. From the authors' results, the authors conclude that Gs links VIP receptor activation to adenylyl cyclase and cyclic adenosine 3′-5′ monophosphate production and the ultimate release of protein by acinar cells and that Gq/11 links muscarinic receptor activation to phospholipase C and IP3 and diacylglycerol accumulation, which also leads to protein release. Furthermore, it is hypothesized that Gs has an additional role in the regulation of vesicular traffic and exocytosis.


In exocrine tissues such as lacrimal gland, protein secretion involves activation of surface receptors by neurotransmitters and neuropeptides and mediation by transmembrane signaling and second-messenger generation. In the cyclic adenosine 3′-5′ monophosphate (cAMP) pathway, receptor-mediated activation of adenylyl cyclase results in a rise in intracellular cAMP. Cyclic AMP-dependent protein kinases alter the phosphorylation state of specific proteins in the basolateral membrane as well as proteins associated with secretory release. Control of lacrimal protein secretion by way of cAMP involves stimulation by noradrenaline, the adrenergic agonist isoproterenol.
and vasoactive intestinal peptide (VIP), and inhibition by met-enkephalin.\textsuperscript{5-8}

In lacrimal gland, adenylyl cyclase activity is enhanced by NaF, a direct activator of heterotrimeric G proteins, and by guanosine triphosphate (GTP\gamma S).\textsuperscript{9,10} The VIP stimulation of the enzyme also is dependent on GTP.\textsuperscript{11} Specific lacrimal membrane proteins have been identified by adenosine diphosphate ribosylation and with peptide-directed antiserum as G\textsubscript{a} subunits.\textsuperscript{5,12} G\textsubscript{a} specifically links seven transmembrane-spanning receptors to activation of adenylyl cyclase.\textsuperscript{13,14} Anti-G\textsubscript{a} antiserum blocks VIP stimulation of adenylyl cyclase activity in lacrimal membrane preparations\textsuperscript{12}; therefore, it is likely that G\textsubscript{a} couples VIP receptors to cAMP-mediated stimulation of secretion of lacrimal proteins. However, the definitive role of G\textsubscript{a} in secretion has not been determined.

Protein secretion in lacrimal gland also is regulated by agonists that activate phosphatidylinositol turnover and mobilize the intracellular calcium pool. M\textsubscript{3} muscarinic receptor activation results in an increase in phosphatidylinositol 4,5 bisphosphate (PIP\textsubscript{2})-specific phospholipase C (PLC) activity and rapid production of 1,4,5-inositol trisphosphate (1,4,5-IP\textsubscript{3}).\textsuperscript{15-17} The IP\textsubscript{3} mobilizes Ca\textsuperscript{2+} from nonmitochondrial stores, and the increase in intracellular Ca\textsuperscript{2+} and formation of a Ca\textsuperscript{2+}-calmodulin complex results in alteration of the activation of Ca\textsuperscript{2+}-calmodulin-dependent protein kinases and phosphorylation of specific membrane proteins.\textsuperscript{3} Diacylglycerol, also a product of the PLC reaction, activates protein kinase C by way of an alteration in the affinity of the kinase for Ca\textsuperscript{2+} and subsequent translocation to the membrane. The activation of protein kinase C leads to enhanced exocytosis.\textsuperscript{18}

The GTP-dependent receptor activation of IP\textsubscript{3} generation may be coupled to PLC by way of pertussis toxin-sensitive G\textsubscript{i} and G\textsubscript{q}, or by way of pertussis toxin-insensitive proteins of the G\textsubscript{i} family.\textsuperscript{19} In parotid gland, cholinergic stimulation of PIP\textsubscript{2} hydrolysis is dependent on GTP\gamma S and is the result of M\textsubscript{3} receptor regulation of PLC.\textsuperscript{20} The activation of PLC in this issue is blocked by antiserum directed against sequences common to the \alpha subunits of G\textsubscript{i} and G\textsubscript{q}.\textsuperscript{20} It is presumed that PLC activation and subsequent secretion of protein in lacrimal gland also is dependent on coupling to a muscarinic receptor by a G protein. However, this has not been tested.

Antibodies to peptides corresponding to amino acid sequences of the G\textsubscript{a} subunits allow specific identification of and discrimination among G\textsubscript{a} subunits.\textsuperscript{21} Furthermore, because the antisera uncouple receptors from \alpha-subunit regulation of effectors, they also are useful as probes of the functional interactions of G proteins with receptors and effectors that are central to the regulation of cell functions.\textsuperscript{14,22} The current study was conducted to test directly the hypothesis that specific heterotrimeric G proteins identified with selected polyclonal antibodies couple VIP and cholinergic receptor activation to protein secretion. We first assessed the GTP dependence of VIP and cholinergic stimulation of secretion and characterized this dependence as to its toxin sensitivity. Based on these experiments, the presence of the G proteins, thought to be important for lacrimal secretion, was confirmed using immunoblotting. Permeabilization of cultured acini and insertion of antibodies directed against the \alpha subunits of G\textsubscript{i} and G\textsubscript{q/11} were used to determine the physiological role of these G proteins in cholinergic and VIP regulation of protein secretion. Our data provide direct evidence for a role for G\textsubscript{i} in VIP stimulation of secretion and for G\textsubscript{q/11} and G\textsubscript{i} in cholinergic stimulation of secretion. The data from this study in conjunction with our previous work, and the work of others, show that G\textsubscript{i} couples VIP to cAMP-dependent secretion, and G\textsubscript{q/11} couples M\textsubscript{3} receptors to PLC, which also leads to an increase in lacrimal protein release. In addition, a non-cell-surface receptor–effector role for G\textsubscript{i} in cholinergic and in VIP stimulation of protein release is proposed.

**METHODS**

**Materials**

- Dulbecco's modified Eagle's medium (DMEM)–Ham's nutrient mixture F\textsubscript{12} (1:1), insulin-transferrin-selenium, gentamicin, epidermal growth factor, soybean trypsin inhibitor, aprotinin, dexamethasone, carbamylcholine chloride (carbachol), atropine sulfate, and N-2-hydroxyethylpiperazine-N'-2 ethanesulfonic acid (Hepes) were from Sigma Chemical (St. Louis, MO).
- Certified fetal bovine serum (FBS), collagenase, and streptolysin-O (SLO) were from GIBCO (Grand Isle, NY).
- Hylauronidase was from Worthington Biomedicals (Freehold, NJ), and DNase I was from Boehringer Mannheim Biochemicals (Indianapolis, IN).
- Vasoactive intestinal peptide (VIP) was from Peninsula Laboratories (Belmont, CA).
- Nonimmune rabbit serum was from Calbiochem–Novabiochem (La Jolla, CA).
- The C-terminal decapetides of G\textsubscript{qa} and G\textsubscript{q/11} were synthesized by Core Laboratories of the LSU Medical Center.

Matrigel and dispase were obtained from Collaborative Biomedical Products (Bedford, MA). Surfase siliconizing fluid was from Pierce (Rockford, IL). G\textsubscript{qa} antiserum (RM/1) and G\textsubscript{q/11} antiserum were obtained from Calbiochem–Novabiochem and from DuPont–New England Nuclear (Boston, MA). Recombinant G\textsubscript{qa} and G\textsubscript{q/11} subunits were from Calbiochem–Novabiochem.
Precast sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gels, prestained SDS–PAGE molecular weight standards, polynylini- dene difluoride membranes, alkaline-phosphatase conjugated antirabbit immunoglobulin G, 5-bromo-4-chloro-3-indoyl phosphate, and nitroblue tetrazolium were from Bio–Rad (Hercules, CA). All other reagents and supplies were from standard sources.

Isolation and Culture of Lacrimal Acini

All experimental procedures were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Adult male New Zealand White rabbits were sedated with an intramuscular injection of 30 mg/kg ketamine and 3 mg/kg xylazine and killed with an intracardiac injection of 70 mg/kg sodium pentobarbital. Isolation of acini was accomplished by a modification of the procedures for single-cell isolations with the omission of ethylenediamine-tetraacetic acid (EDTA) chelation in a calcium and magnesium-free medium.10 Isolated lacrimal gland fragments, freshly isolated acini, and cultured acini were homogenized with a Tekmar Tissumizer at a setting of 45 (model TK-10 power control, Tekmar, Cincinnati, OH) for 15 minutes on ice. All subsequent isolation procedures were conducted at 4°C. The supernatants from two 1000 g, 10-minute centrifugations were combined and centrifuged for 20 minutes at 40,000 g. The resulting pellet was washed three times at 40,000 g for 20 minutes. The pellet membranes were resuspended in the isolation medium, aliquoted, frozen in liquid nitrogen, and stored at −70°C. Membrane protein concentration was determined by the method of Lowry et al.27

Preparation of Membranes

Membranes were prepared from freshly isolated lacrimal gland fragments, freshly isolated acini, and cultured acini. Lacrimal glands from several rabbits were pooled, minced, and portioned into three aliquots. Membranes were prepared directly from gland fragments or from acini isolated as described above. Remaining acini were cultured for 3 days, and membranes were prepared from the cultured acini. Lacrimal gland fragments or freshly isolated acini were placed in ice-cold isolation medium containing 5% sorbitol, 0.5 mM EDTA, 5 mM histidine–imidazole buffer, pH 7.5, 9 μg/ml aprotonin, 3 mM DTT, and 0.2 mM phenylmethylsulfonyl fluoride. For preparation of membranes from cultured acini, the medium was removed from six-well plates and acini were incubated with 1.5 ml dispase at 37°C for 2 hours. The cells were collected, washed three times by centrifugation, and resuspended in the membrane isolation medium. Tissues (gland fragments, freshly isolated acini, or cultured acini) were homogenized with a Tekmar Tissumizer at a setting of 45 (model TK-10 power control, Tekmar, Cincinnati, OH) for 15 minutes on ice. All subsequent isolation procedures were conducted at 4°C. The supernatants from two 1000 g, 10-minute centrifugations were combined and centrifuged for 20 minutes at 40,000 g. The resulting pellet was washed three times at 40,000 g for 20 minutes. The pellet membranes were resuspended in the isolation medium, aliquoted, frozen in liquid nitrogen, and stored at −70°C. Membrane protein concentration was determined by the method of Lowry et al.27

SDS–PAGE and Immunoblotting

The G protein subunits were identified by SDS–PAGE and Western blotting using commercially available antisera developed against C-terminal peptide sequences of the α subunits of G, and Gq.12 Membranes were centrifuged at 13,800 g for 15 minutes, and the pelleted proteins were solubilized by incubation on ice for 60 minutes in freshly prepared 20 mM Tris, 1 mM EDTA, 1 mM DTT, 100 mM sodium chloride, and 0.9% Na cholate, pH 8.28 The suspensions were centrifuged at 13,800 g for 3 minutes. Protein concentration of the solubilized samples was determined with the Bio–Rad (Hercules, CA) protein reagent. The proteins were resolved by SDS–PAGE on 1-mm slab 12.5% minigels at 200 V29 and transblotted on polyvinylidene difluoride membranes at 100 V for 2 hours in a Bio–Rad transblot apparatus.30 Membranes were blocked for 1 hour with blotto and incubated for 18 hours at room temperature with the primary antiserum diluted 1:1000. Control blots were prepared by incubation in blotto without primary antiserum. Membranes were washed three times and incubated with
alkaline phosphatase-conjugated goat antirabbit immunoglobulin G (1:1500) for 2 hours. Immunoactive proteins were visualized with 0.15 mg/ml of the alkaline phosphatase substrate, 5-bromo-4-chloro-3-indolyl phosphate, and 0.33 mg/ml nitroblue tetrazolium in 100 mM Tris and 0.5 mM magnesium chloride, pH 9.5. The reaction was terminated by washing in deionized water and the blots were air dried.

**Protein Secretion by Cultured Acini**

Cultured acini were washed three times with a K+- Krebs–Ringer–Hepes buffer (permeabilization buffer) containing 145 mM potassium chloride, 2 mM magnesium chloride, 1.2 mM KH2PO4, 10 μM calcium chloride, 10 mM glucose, 10 mM Heps, 0.2% BSA, and 0.1% soybean trypsin inhibitor, pH 7.3. Acini were permeabilized by exposure to 0.4 U/ml SLO in the permeabilization buffer for 30 minutes at 37°C alone or in combination with GDPβS, antibodies or nonimmune rabbit serum. Control experiments were conducted in which antibodies were coincubated with the C-terminal decapeptides of Gsa or Gs/qa (1 μM) for 30 minutes at 37°C before introduction into the cells. The acini then were washed three times with a balanced salt solution containing 116 mM sodium chloride, 5.4 mM potassium chloride, 0.81 mM magnesium sulfate, 1.01 mM disodium acid phosphate, 1.8 mM calcium chloride, and 10 mM Heps, pH 7.4, and equilibrated for 30 minutes at 37°C with 5% CO2. The medium then was replaced and the acini were equilibrated for a 20-minute period at 37°C with 5% CO2. After a final 20-minute period of incubation with fresh medium supplemented with the appropriate agonist, antagonists, or vehicle, the medium was removed and assayed for total protein.27 For determination of the effect of pertussis toxin on protein secretion, the culture medium was supplemented with 100 ng/ml or 1 mg/ml pertussis toxin on day 2, and the secretory response was measured on day 4 with omission of the permeabilization protocol. In all secretion experiments, at least three experiments were performed with duplicate wells for each condition.

**Statistical Analysis**

Data are expressed as the means ± standard errors. Statistical significance of P < 0.05 was determined by Student’s t-tests for unpaired data.

**RESULTS**

The effect of permeabilization on secretion was determined by the measurement of the release of protein by cultured acini that were exposed to vehicle or SLO.
TABLE 2. Release of Secretory Protein by Nonpermeabilized Acini: Effect of Pertussis Toxin

<table>
<thead>
<tr>
<th></th>
<th>No PTX (mg protein/ml)</th>
<th>100 ng/ml PTX (mg protein/ml)</th>
<th>1 mg/ml PTX (mg protein/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.056 ± 0.01</td>
<td>0.065 ± 0.02</td>
<td>0.065 ± 0.01</td>
</tr>
<tr>
<td>VIP</td>
<td>0.445 ± 0.05</td>
<td>0.466 ± 0.05</td>
<td>0.422 ± 0.02</td>
</tr>
<tr>
<td>Carbachol</td>
<td>0.497 ± 0.03</td>
<td>0.500 ± 0.03</td>
<td>0.536 ± 0.02</td>
</tr>
<tr>
<td>Carbachol ± DALA</td>
<td>0.140 ± 0.02</td>
<td>0.298 ± 0.01*</td>
<td>0.496 ± 0.03*</td>
</tr>
</tbody>
</table>

PTX = pertussis toxin, VIP = vasoactive intestinal polypeptide; DALA = D-ala²-enkephalinamide. Values are the mean ± SE of three experiments. Doses were 100 nM VIP, 100 μM carbachol, and 10 μM DALA. * Statistically significant difference when compared with values determined in the absence of PTX.

Pertussis toxin-insensitive G proteins include Gα and Gq/11. Identification of the specific nonpertussis toxin-sensitive G proteins that may be linked to regulation of secretion by VIP or carbachol was accomplished by examination of immunoblots of membrane preparations of lacrimal tissues incubated with commercially available antibodies directed against the α subunits of these two G proteins. Examination of lacrimal membranes obtained from cultured acini probed with anti-Gαs antiserum (Fig. 1) showed the presence of two immunoreactive bands at 44 kDa and 47 kDa. Bands at identical kilodaltons were present in the membranes prepared from gland fragments and from freshly isolated acini. Immunoblotting of membrane proteins with antibody directed against a C-terminal peptide common to both Gαs and Gq/11 (Fig. 1) detected a single immunoreactive band at 46 kDa in membranes prepared from fragments, freshly isolated acini, and cultured acini. Specificity of the antisera was assessed with recombinant Gαs and Gq/11 subunits (Fig. 2). Immunoreactivity of the antisera was restricted to the expected antigen in each case, with no detectable cross-reactivity. To determine the origin of the band that was present in blots reacted with either antisera, blots containing all three tissue preparations were incubated in the absence of primary antiserum (Fig. 3). The upper band, which also was present in Figure 1, was most prominent in membranes prepared from whole lacrimal gland fragments. This band appears to be caused by nonspecific immunoreactivity of constituents of the fragment and freshly isolated acinar preparations with the secondary antibody.

Because antisera against the C-terminus of α subunits uncouple receptors from regulation of effectors, we tested the effect of insertion of Gαs and Gq/11 antibodies into permeabilized acini on the release of protein in response to VIP or carbachol.

FIGURE 1. G protein α subunits in membranes of lacrimal tissues. Membrane proteins (25 μg per lane) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes, and immunoblotted with a 1:1000 dilution of anti-Gαs (A) or anti-Gq/11α (B) antisera as described in the Methods section. Lane 1 = 5 μl molecular weight standards. Lane 2 = membranes prepared from whole gland fragments. Lane 3 = membranes prepared from freshly isolated acini. Lane 4 = membranes prepared from cultured acini.
In nonpermeabilized acini, the increase in protein release in the presence of 100 nM VIP or 100 µM carbachol was significantly greater than unstimulated secretion (0.409 ± 0.03 and 0.416 ± 0.01 mg/ml secreted protein, respectively, as compared with 0.046 ± 0.004 mg/ml secreted protein). Secretion in response to either agonist was not altered by exposure of the nonpermeabilized cells to anti-Gsa, or anti-Gq/11 antisera (Fig. 4). However, anti-Gsa and anti-Gq/11 antisera resulted in significant alterations in receptor-mediated activation of secretion after their introduction into permeabilized acini. Introduction of anti-Gsa antiserum into permeabilized acini (Fig. 5) resulted in a 64% reduction in the secretory response to VIP from 0.474 ± 0.04 mg protein/ml to 0.170 ± 0.02 mg protein/ml. The release of protein in response to carbachol also was reduced significantly by the antiserum. Anti-Gq/11 antiserum blocked the response to carbachol by 37%, from 0.543 ± 0.05 mg protein/ml to 0.348 ± 0.04 mg protein/ml.

In similar experiments (Fig. 6), anti-Gq/11 antiserum introduced into permeabilized acini had no effect on the response to 100 nM VIP. However, in the cells treated with SLO, anti-Gq/11 antiserum specifically blocked the response to 100 µM carbachol. The release of protein in response to carbachol, 0.501 ± 0.06 mg protein/ml, was reduced 70% to 0.153 ± 0.02 mg/ml protein by the presence of the antiserum to the a subunit of Gq/11.

To determine whether Gs and Gq/11 mediate cho-
linergic receptor activation of secretion through the same or different intracellular pathways, acini were permeabilized to introduce anti-Gs or anti-Gq/11 antisera alone or in combination (Fig. 7). Under unstimulated conditions, protein release was 0.048 ± 0.004 mg protein/ml. The addition of carbachol resulted in the release of 0.624 ± 0.06 mg protein/ml. As in previous experiments, both antisera blocked cholinergic stimulation of secretion with an approximate reduction of 45% by anti-Gs antisera and of 71% by anti-Gq/11 antisera. In the presence of both antisera, cholinergic stimulation of secretion was blocked completely, resulting in release of protein that was not different from release under unstimulated conditions.

In separate cultures, similar experiments were conducted to determine the effect of the simultaneous introduction of anti-Gs and anti-Gq/11 antisera on the release of protein in response to peptidergic receptor activation by VIP (Fig. 8). Release of protein by permeabilized acini in the presence of 100 nM VIP was 0.421 ± 0.06 mg protein/ml, which represented a significant increase when compared with the release of protein under unstimulated conditions. As in previous experiments, anti-Gs antisera reduced significantly the stimulatory effect of VIP to 0.195 ± 0.02 mg/ml, whereas anti-Gq/11 antisera had no effect on the stimulation of secretion by VIP. The combination of antisera resulted in an inhibition of VIP stimulation of secretion that was equivalent to the effect of anti-Gs antisera alone.

To establish specificity of the antibodies at the concentrations used in the permeabilization experiments, antibodies were coincubated with 1 μM peptide for 60 minutes before permeabilization (Table 3). The effect of each antisera was blocked com-
agonists that increase second messengers (cAMP, IP3, DAG) lead to protein release, it has been presumed that G proteins are linked to secretion in intact cells. In this study, introduction of polyclonal antibodies to peptide sequences of the α subunits of selected G proteins into cultured acini was used to determine directly that the receptor-mediated physiological response of secretion is G protein dependent. We have shown that VIP and cholinergic receptor activation is linked to secretion of lacrimal protein by at least two G proteins. The VIP stimulation of secretion involves Gαs, whereas both Gαs and Gq/11 are involved in the muscarinic activation of secretion.

Exposure of acini to antibody raised against the carboxyl terminal decapptide of Gs resulted in a significant reduction in the VIP-stimulated release of protein. Because the C-terminus of Gs is the site of interaction with receptors,13,14 these results suggest that the antiserum binds to the α subunit of Gs and blocks the VIP–receptor–Gsα interaction that would otherwise result in the cAMP-dependent increase in protein release. These results directly confirm recent evidence for a role for Gs in VIP-stimulated secretion obtained by indirect methods in which the same antibody blocked VIP stimulation of adenylyl cyclase in lacrimal membrane preparations.12

In contrast to VIP induction of secretory protein release, cholinergic stimulation of lacrimal secretion is mediated through the pertussis toxin-insensitive G protein detected by anti-Gq/11α antiserum. Immunoblotting showed that a protein that reacts with antiserum directed against a peptide sequence common to the α subunits of Gαs and Gαq is present in lacrimal membranes. This Gαq/11α subunit apparently is involved directly in mediation of cholinergic stimulation of secretion, because anti-Gq/11α antiserum blocked cholinergic stimulation of protein release. The mechanism by which the introduction of anti-Gq/11α antiserum into permeabilized cells blocked cholinergic stimulation of secretion in lacrimal acini most likely is inactivation of Gq/11 coupling of muscarinic receptors to PLC. The PLC pathway, central to lacrimal secretion, is well defined in this gland and involves M3 receptor activation, increased PLC activity, and a rise in the production of diacylglycerol and inositol phosphates, including 1,4,5-inositol triphosphate.16,18 Coupling to PLC by Gq/11 is common to seven transmembrane domain receptors in a variety of tissues,19 and mediation of muscarinic receptor activation by Gq/11 has been shown in parotid gland in which M3 receptor activation of PLC and PIP2 hydrolysis is blocked by exposure of membranes to Gq/11α-peptide-directed antiserum.20 Reversal of atropine-sensitive20 carbachol-induced secretion by insertion of anti-Gq/11α antiserum suggests that muscarinic activation of secretion involves Gq/11.

**TABLE 3. Release of Secretory Protein by Permeabilized Acini: Specificity of Antiserum**

<table>
<thead>
<tr>
<th>Serum</th>
<th>VIP (mg/ml)</th>
<th>Carbachol (mg/ml)</th>
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<tbody>
<tr>
<td>Nonimmune serum</td>
<td>0.418 ± 0.01</td>
<td>0.495 ± 0.03</td>
</tr>
<tr>
<td>Anti-Gas</td>
<td>0.164 ± 0.01*</td>
<td>0.377 ± 0.004*</td>
</tr>
<tr>
<td>Anti-Gas + Gαs peptide</td>
<td>0.404 ± 0.01</td>
<td>0.469 ± 0.02</td>
</tr>
<tr>
<td>Anti-Gαs + Gq/11α peptide</td>
<td>0.186 ± 0.01*</td>
<td>0.365 ± 0.02*</td>
</tr>
<tr>
<td>Anti-Gq/11α</td>
<td>0.454 ± 0.02</td>
<td>0.206 ± 0.01*</td>
</tr>
<tr>
<td>Anti-Gq/11α + Gαs peptide</td>
<td>0.418 ± 0.01</td>
<td>0.218 ± 0.01*</td>
</tr>
<tr>
<td>Anti-Gq/11α + Gq/11α peptide</td>
<td>0.402 ± 0.02</td>
<td>0.454 ± 0.02</td>
</tr>
</tbody>
</table>

VIP = vasoactive intestinal peptide.
Nonimmune serum or antiserum (1:100 dilution) was incubated with or without 1 μM peptide for 60 minutes at 37°C before introduction into acini. Release of protein was measured in response to 100 nM VIP or 100 μM carbachol. Values are the mean ± SE of three experiments.

* Statistically significant difference when compared with nonimmune serum.
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...motion. If the mechanism by which Gs affects cholinergic stimulation is through regulation of trafficking independent of PLC activation, it is likely that Gs has the same function in the release of VIP-induced protein release in addition to linking the receptor to adenyl cyclase. This hypothesis remains to be tested.

To conclude, in this study, we have shown that VIP and cholinergic stimulation of secretion by lacrimal acini are G protein dependent, because GDP/S, which inactivates G proteins, blocked both VIP and carbachol stimulation of secretion. We have shown that the G proteins involved are not pertussis toxin-sensitive, because the toxin did not block stimulation of secretion. The pertussis toxin-insensitive G protein α subunits of Gs and Gq/n were identified in lacrimal membranes by immunoblotting using freshly isolated and cultured tissues. Stimulation of secretion by cultured lacrimal acini by VIP required Gs, but not Gq/n. Cholinergic induction of secretion required both G proteins. It is proposed that Gs links VIP receptor activation to the effector adenyl cyclase and that Gq/n links M3 muscarinic receptor activation to PLC. Additionally, it is suggested that Gs may have a receptor-second-messenger independent role in the regulation of vesicular traffic and exocytosis.

Key Words

cell permeabilization, G protein, lacrimal gland, muscarinic activation, secretion, vasoactive intestinal peptide

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