Alternate Coupling of Receptors to \( G_s \) and \( G_i \) in Pancreatic and Submandibular Gland Cells* \\

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Many \( G_s \)-coupled receptors can activate both cAMP and Ca\(^{2+} \) signaling pathways. Three mechanisms for dual activation have been proposed. One is receptor coupling to both \( G_s \) and \( G_{15} \) (a \( G_q \) class heterotrimeric G protein) to initiate independent signaling cascades that elevate intracellular levels of cAMP and Ca\(^{2+} \), respectively. The other two mechanisms involve cAMP-dependent protein kinase-mediated activation of phospholipase Cβ either directly or by switching receptor coupling from \( G_s \) to \( G_i \). These mechanisms were primarily inferred from studies with transfected cell lines. In native cells we found that two \( G_s \)-coupled receptors (the vasoactive intestinal peptide and \( \beta \)-adrenergic receptors) in pancreatic acinar and submandibular gland duct cells, respectively, evoke a Ca\(^{2+} \) signal by a mechanism involving both \( G_s \) and \( G_i \). This inference was based on the inhibitory action of antibodies specific for \( G_{a_s} \), \( G_a_i \), and phosphatidylinositol 4,5-bisphosphate, pertussis toxin, RGS4, a fragment of \( \beta \)-adrenergic receptor kinase and inhibitors of cAMP-dependent protein kinase. By contrast, Ca\(^{2+} \) signaling evoked by \( G_s \)-coupled receptor agonists was not blocked by \( G_i \) class-specific antibodies and was unaffected in \( G_{a_i} \)–/– knockout mice. We conclude that sequential activation of \( G_s \) and \( G_i \) mediated by cAMP-dependent protein kinase, may represent a general mechanism in native cells for dual stimulation of signaling pathways by \( G_s \)-coupled receptors.

A family of heterotrimeric guanine nucleotide-binding proteins (G proteins) transduces a variety of signals across the plasma membrane by sequential interactions with receptor and effector proteins (e.g. second messenger-generating enzymes and ion channels). These interactions result from guanine nucleotide-driven conformational changes in G protein α subunits (1). Agonist-bound receptors catalyze the exchange of GDP for GTP on the α subunits of their cognate G proteins to promote dissociation of α from a high affinity complex of βγ subunits. Dissociated subunits are competent to modulate the activity of effectors. GTP hydrolysis ultimately returns Ga to the GDP-bound state, thus allowing reformation of inactive heterotrimeric. Sixteen distinct genes encode G protein α subunits in mammals. The family is commonly divided into four classes based on amino acid sequence identity and function: \( G_s \), \( G_i \), \( G_q \), and \( G_{15} \). Members of a newly identified family of regulators of G protein signaling (RGS proteins) have been shown to stimulate the GTPase activity of \( G_s \) and \( G_i \) class α subunits, thus attenuating signaling (2).

One of the more thoroughly characterized examples of G protein-mediated signal transduction is carried out by the hormone-sensitive adenyl cyclase system. Relevant receptors communicate with homologous G proteins, one of which (\( G_s \)) activates adenyl cyclase while others (\( G_i \)) inhibit the enzyme (1). The second messenger (cAMP) mediates diverse cellular responses, primarily by activating cAMP-dependent protein kinase (PKA). In the case of Ca\(^{2+} \)-mobilizing agonists, G protein activation is followed by stimulation of phospholipase Cβ (PLCβ) to generate IP\(_3\) in the cytosol, which initiates the [Ca\(^{2+} \)]\(_i\) signal by release of Ca\(^{2+} \) from internal stores (1, 3). PLCβ can be activated by each of the four \( G_s \) class α subunits or by G\(G_y\) subunits released from \( G_i \) class proteins (4). Only \( G_s \)-mediated PLCβ activation is inhibited by pertussis toxin (4). In this study we sought to learn the mechanism by which \( G_s \)-coupled receptors evoke Ca\(^{2+} \) signaling.

Several \( G_s \)-coupled receptors can activate dual signaling cascades. For example, increases in both cAMP and [Ca\(^{2+} \)]\(_i\) have been observed by histamine acting on \( H_2 \) receptors in parietal cells (5), parathyroid hormone acting on osteoblasts (6), and isoprenaline acting on cardiac myocytes (7) or salivary gland cells (8, 9). In contrast to the simple paradigm that each receptor molecule can activate a single class of G protein (10), activation of more than one signaling cascade could be due to coupling of one receptor type to two classes of G proteins. This model is supported by experiments in heterologous expression systems. Overexpression of histaminergic \( H_2 \) (11), parathyroid hormone (12), luteinizing hormone (13), P2Y11 (14), vasopressin V2, dopamine D1A, and adenosine A2A (15) receptors resulted in stimulation of adenyl cyclase and PLCβ. The \( \beta \)-adrenergic receptor (which is considered to be a classical \( G_s \)-coupled receptor) and the vasopressin V2, dopamine D1A, and adenosine A2A can functionally interact with the \( G_s \) family member, \( G_{15} \), when both proteins are overexpressed in COS cells (15, 16).

An alternate mechanism for stimulation of Ca\(^{2+} \) signaling by...
**Fig. 1.** Signaling pathways tested in this study. Double slashes through arrows in the pathway signify inhibition by the indicated agents. A positive control for Ca\(^{2+}\) release was tested by carbamyl stimulation of G\(_s\)-coupled muscarinic m3 receptors (R\(_m\)), which stimulate PLC\(\beta\) via G\(_s\) class \(\alpha\) subunits. Ca\(^{2+}\) signaling in this work is followed by measuring the activity of Ca\(^{2+}\)-activated Cl\(^-\) current, \(g_{Cl}\). G\(_s\)-coupled receptors (R\(_s\)) such as the VIP or \(\beta\)-adrenergic receptor might activate the G\(_s\) class heterotrimeric G protein, G\(_{s,\alpha}\). A wide variety of G\(_s\)-coupled receptors can couple to G\(_{i,15}\) activate PLC\(\beta\) to produce IP\(_3\), and release Ca\(^{2+}\)\(\text{from intracellular stores (15, 16). This potential pathway would be absent from G}_{i,15}\) knockout mice. b, R\(_s\) activation of G\(_s\) and stimulation of adenylyl cyclase (AC) to increase production of cAMP, activates PKA which could activate PLC\(\beta\). c, agonist stimulation of R\(_i\) typically activates G\(_i\) and stimulates AC to increase production of cAMP (inhibited by carboxyl-terminal G\(_a\) antibodies). PKA activity is required (directly or indirectly) for generation of R\(_s\), thereby eliciting a switch (17) or an augmentation to account for activation of G\(_i\) by G\(_s\)-coupled receptor agonists. G\(_i\)-released by activation of G\(_s\) could stimulate PLC\(\beta\) activity, produce IP\(_3\), and release Ca\(^{2+}\) from intracellular stores. PTX and antibodies (Abs) to the carboxyl terminus of G\(_i\) inhibit receptor activation of G\(_i\). PIP\(_2\) antibodies prevent PLC\(\beta\) hydrolysis of PIP\(_2\) and production of IP\(_3\). The Ca\(^{2+}\) ionophore, A23187, bypasses the need for IP\(_3\) production needed for Ca\(^{2+}\) release from intracellular stores.

G\(_s\)-coupled receptors is activation of PLC\(\beta\) by PKA. In several cell types, increasing cellular cAMP with forskolin (5, 8, 9) or membrane permeable cAMP analogues (5) increased [Ca\(^{2+}\)\(\text{intracellular stores. PTX and antibodies (Abs) to the carboxyl terminus of G}_i\) inhibit receptor activation of G\(_i\). PIP\(_2\) antibodies prevent PLC\(\beta\) hydrolysis of PIP\(_2\) and production of IP\(_3\). The Ca\(^{2+}\) ionophore, A23187, bypasses the need for IP\(_3\) production needed for Ca\(^{2+}\) release from intracellular stores.

### EXPERIMENTAL PROCEDURES

**Reagents**—Affinity purified B087, C260, and C267 polyclonal antibodies specific for G\(_{s,\alpha}\) and G\(_{i,\alpha}\) (G\(_{s,12}\)), G\(_{s,\alpha}\), and G\(_{i,\alpha}\) (G\(_{i,12}\)) and G\(_{s,\alpha}\), respectively (19), and anti-G\(_{s}\) IgG (20, 21) were prepared as described. Monoclonal antibody against PIP\(_2\) was purchased from Prescibe Diagnostics. Pertussis toxin (PTX) (from List Biological Laboratories) was reconstituted into distilled H\(_2\)O and diluted into a pipette solution containing 0.5 mM dithiobister. A glutathione-tagged fragment of \(\beta\)-adrenergic receptor kinase (\(\bar{\beta}\)ARK1) was kindly provided by Dr. Robert Lefkowitz (Duke University, Durham, NC). His-tagged RGS4 was expressed in Escherichia coli and purified as described (22). Stock solutions of all antibodies, the \(\bar{\beta}\)ARK1 fragment, and RGS4 were dialyzed against a solution containing 100 mM KCl and 10 mM HEPES (pH 7.2 with NaOH) and stored at \(-20^\circ\)C until dilution into the pipette solution. H89 and Rp-8-CPT-cAMP-S were obtained from Biomole and BioLog, respectively. The pipette solution contained (in mM): 150 KCl, 10 HEPES (pH 7.2 with NaOH), 2 MgCl\(_2\), 1 ATP, and 0.1 EGTA. The standard bath solution A contained (in mM): 150 NaCl, 5 KCl, 1 MgCl\(_2\), 1 CaCl\(_2\), 10 HEPES (pH 7.2 with NaOH), and 10 glucose. When this solution was supplemented with 10 mM pyruvate, 1 mg/ml bovine serum albumin, and 0.02% soybean trypsin inhibitor, it was abbreviated PSA. Alternate Receptor Coupling

**Alternate Receptor Coupling**

The Ca\(^{2+}\)\(\text{ionophore, A23187, bypasses the need for IP}_3\) production needed for Ca\(^{2+}\) release from intracellular stores.

**Current Recording**—The Ca\(^{2+}\)\(\text{ionophore, A23187, bypasses the need for IP}_3\) production needed for Ca\(^{2+}\) release from intracellular stores.
used as a positive control. Three mechanisms were tested: (a) activation of $G_{o15}$ by $R_s$, (b) direct activation of PLC$eta$ by PKA, and (c) switching or augmentation of coupling specificity of $R_s$ from $G_{o}$ to $G_i$. We tested these mechanisms using two $G_s$-coupled receptors which evoke different types of Ca$^{2+}$ signals: pancreatic acinar cells stimulated with VIP and SMG duct cells stimulated with Iso. Ca$^{2+}$ signaling was followed by measuring the activity of the Ca$^{2+}$-activated Cl$^-$/channel current in each cell type. Previous work showed that pancreatic acinar and SMG cells express the Ca$^{2+}$-activated Cl$^-$/channel (21, 25, 27) and this current faithfully reflects changes in [Ca$^{2+}$]i (21, 27).

Fig. 2a shows that stimulation of pancreatic acinar cells with a saturating concentration of VIP-induced [Ca$^{2+}$], oscillations which lasted for the duration of cell stimulation, as previously reported (28). Maximal stimulation of the $G_s$-coupled muscarinic m3 receptor with 1 mM carbachol in the same cells resulted in a typical biphasic response of a spike and a plateau. This response was highly reproducible in mouse pancreatic acinar cells; similar responses were observed in 15/15 cells from 13 mice. Fig. 2b shows that stimulation of SMG duct cells with the $\beta$-adrenergic agonist Iso caused a sustained increase in the Ca$^{2+}$-activated Cl$^-$/channel current with no apparent oscillations. Following removal of Iso, stimulation with carbachol caused a large biphasic response. The Cl$^-$/current responses are similar in shape and time course to the previously reported changes in [Ca$^{2+}$], caused by these agonists in SMG cells (8, 9). Among cells which responded to carbachol, prior stimulation with Iso elicited a response similar to that in Fig. 2b in 19/25 SMG duct cells from 17 mice.

$\beta$-Adrenergic, vasopressin V2, dopamine D1A, and adenosine A2A receptors overexpressed in COS cells can couple to $G_{o15}$, but not other members of the $G_s$ class, and stimulate PLC$\beta$ activity (15, 16). This would suggest that $G_{o15}$ has the unique ability to couple to receptors which are usually coupled to $G_s$. Currently, there are no good biochemical tools to specifically evaluate $G_{o15}$ function in native cells. Genetics provide an alternative approach. We measured the effect of VIP and Iso on Ca$^{2+}$ signaling in cells prepared from mutant $G_{o15}$ (-/-) mice to rule out the possibility that $G_{o15}$ contributes to Ca$^{2+}$ signaling by $G_s$-coupled receptors in SMG and pancreatic acinar cells. Fig. 2c shows that VIP- and carbachol-induced Ca$^{2+}$ signaling was completely normal in pancreatic acini for $G_{o15}$ (-/-) mice. The same results were obtained in six out of six experiments with acini from six mice. Fig. 2d shows that Iso- and carbachol-induced Ca$^{2+}$ signaling was normal in SMG duct cells from $G_{o15}$ (-/-) mice. Similar results were obtained in four out of six experiments with SMG ducts prepared from the six mice that were used to study the response of pancreatic acinar cells. These findings exclude coupling to $G_{o15}$ as obligatory for activation of Ca$^{2+}$ signaling by the $G_s$-coupled receptors. Coupling of $R_s$ to other members of the $G_s$ class is also excluded by experiments with antibodies described below.

Experiments with RGS4 supplied our first evidence that activation of Ca$^{2+}$ signaling by VIP and Iso involves more than activation of $G_s$. RGS4 accelerates GTP hydrolysis by $G_s$ and $G_i$ class $\alpha$ subunits but not $G_{o}$ (29, 30). In Fig. 3, infusion of 100 pm RGS4 through a patch pipette into pancreatic acinar (Fig. 3a) or SMG duct (Fig. 3b) cells completely inhibited the Ca$^{2+}$ response to VIP and Iso, respectively. The control shows that the response to subsequent stimulation with carbachol was markedly reduced, as we reported recently (23). Measurement of cAMP production in streptolysin O-permeabilized cells showed that inhibition of Ca$^{2+}$ signaling by RGS4 was not due to inhibition of cAMP production by the $G_s$-coupled receptors (not shown). The results with RGS4 exclude model b of Fig. 1a as the mechanism by which $R_s$ evokes a Ca$^{2+}$ signal.

In the next set of experiments we systematically tested the model for PKA-dependent G$\gamma$ switching (or augmentation) of receptor specificity shown in Fig. 1c (17, 18). We first tested if stimulation of $G_o$ is obligatory for launching a Ca$^{2+}$ signal by the VIP and Iso receptors. This was achieved by introducing antibodies specific for $G_o$ into the cells through a patch pipette. Antibodies to the carboxyl terminus of $G_o$ were used because they have been reported to block receptor-mediated activation of adenylyl cyclase (31). Fig. 4 shows that the antibodies specific for $G_o$ inhibited Ca$^{2+}$ signaling induced by VIP stimulation of pancreatic acinar cells and the Ca$^{2+}$ signal stimulated by Iso acting on SMG duct cells without affecting the oscillations or the biphasic response evoked by stimulation of the $G_s$-coupled m3 receptor with carbachol. Similar findings were observed in 4 additional acinar and 3 additional duct cells. As discussed below, infusion of $G_o$ specific antibodies did not effect VIP- or Iso-evoked Ca$^{2+}$ signaling. Therefore, $G_o$ stimulation was essential for launching a Ca$^{2+}$ signal by the two classical $G_s$-coupled receptors.

If PKA-dependent phosphorylation were involved, then inhibition of PKA activity should block Ca$^{2+}$, but not Ca$^{2+}$, signaling (Fig. 1c). The $R_s$ in both cell types met this criterion.
as shown in Fig. 5. In control experiments, Ca^{2+} oscillations were initiated by stimulation of pancreatic acinar cells with VIP. After termination of VIP stimulation by removing the agonist, very similar oscillations were initiated by stimulating the same cells with low concentrations of carbachol, which acts through the Gq-coupled muscarinic receptor. Finally, the cell was stimulated with a supermaximal concentration of carbachol (Fig. 5). Similar results were obtained in 14 cells. In four separate experiments, the VIP response was completely abolished when pancreatic acinar cells were treated with 10 μM H89, a selective and potent inhibitor of PKA (32), whereas the ability of a low concentration of carbachol to induce oscillations or of a supermaximal concentration to induce a biphasic response was unaltered (Fig. 5b). Similarly, treatment of SMG duct cells with 10 μM H89 abolished Iso-dependent [Ca^{2+}] increase, without affecting the carbachol-dependent response (Fig. 5d). Inhibition of the response to Iso was observed in all 6 SMG duct cells treated with H89. The requirement for PKA stimulation was further verified by testing the effect of the potent and selective inhibitor of PKA, Rp-8-CPT-cAMP-S. Infusing the cells with 10 μM Rp-8-CPT-cAMP-S through the pipette abolished the response to VIP (n = 7) and Iso (n = 5) in all cells tested (Fig. 5c, e and f). Again, control experiments in the same cells showed that all forms of Gα-dependent responses were unaffected by inhibition of PKA with Rp-8-CPT-cAMP-S. These inhibitory effects of the two PKA inhibitors argued against the possibilities that unregulated VIP or β-adrenergic receptors are coupled directly to Gq (33) or that Gα directly modulates Ca^{2+} channels (34) in these systems.

To directly address a role for Gα in Ca^{2+} signaling by VIP and Iso we measured the effect of infusing the cells with PTX or antibodies specific for certain members of the Gα subclass of α subunits. Preliminary studies showed that concentrations of PTX below 20 ng/ml in the pipette solution did not consistently inhibit VIP-induced signaling. At concentrations above 50 ng/ml, PTX rapidly caused a large, time-dependent, nonselective increase in membrane conductance, as if PTX caused cell permeabilization. We therefore limited our testing to the effect of 20 ng/ml PTX on Ca^{2+} signaling in pancreatic acinar cells. Fig. 6 shows that treatment with PTX inhibited VIP but not carbachol-dependent Ca^{2+} signaling. Similar results were obtained in four experiments. In 13 additional experiments, PTX-treated acinar cells lysed before the experimental protocol could be completed. We were unable to find a concentration of PTX that inhibited the Iso response in SMG duct cell without causing cell lysis.

Antibodies generated against peptides representing the carboxyl termini of Gα1 and Gα4 subunits inhibit receptor-initiated activation of these G proteins (20, 21, 35). The results obtained by infusing antibodies into pancreatic acinar cells are illustrated in Fig. 7. Two types of polyclonal antibodies against Gα were used, one recognizing Gα5 and Gα12 or one specific for Gα4 and Gα13 (19). Fig. 7a shows that infusing 17.5 μg/ml antibodies specific for Gα12 and Gα4 had no effect on Ca^{2+} signaling induced by Gα1 or Gα2-coupled receptors. Similar results were obtained in four experiments. However, these antibodies were not without effect, as seen for SMG cells (described below). Fig. 7b shows that infusing pancreatic acinar cells with 9 μg/ml Gα1,12-
specific antibodies completely inhibited the response to VIP without affecting the response to carbachol. Similar results were observed in six cells. An important control is shown in Fig. 7c. In contrast to the effect of Gq-specific antibodies, infusing the cells with Gqi1,11 antibodies (at sufficient concentration to abolish the oscillation and largely inhibit the sustained response to carbachol) had no effect on the ability of VIP to induce oscillations. In seven experiments with cells infused with 80 µg/ml anti-Gq, IgG the response to VIP remained normal, while the response to the low concentration of carbachol was abolished and the response to supermaximal concentration of carbachol was inhibited by 83 ± 7%.

Activation of Gq by Iso is further suggested by the results for SMG duct cells shown in Fig. 8. In six cells infused with Gq1,11 reactive IgG, the response to supermaximal concentrations of carbachol was reduced by 91 ± 6% while the response to Iso was not affected (Fig. 8b). Unlike the findings in pancreatic acinar cells stimulated with VIP, both Gq antibody preparations effectively inhibited the response to Iso in SMG duct cells. Gq1,12-specific antibodies, at a concentration of 9 µg/ml, completely inhibited the Ca2+ response to Iso (Fig. 8c). Infusion of only 3.5 µg/ml Go13,0 antibodies completely inhibited the response to Iso in two cells and partially (63 ± 14%) in three cells (Fig. 8d). At a concentration of 7 µg/ml the anti-Go13,0 completely inhibited the response to Iso in five cells (Fig. 8e).

The findings in Figs. 7 and 8 provide strong evidence that activation of Ca2+ signaling by Gq-coupled receptors is independent of members of the Gq class. The inhibitory Goq antibodies used in the present work recognizes the predominant Gq class a subunits expressed in these cells, Goq1, Goq11, and Goq2,4 (22). Furthermore, these antibodies were shown to inhibit Ca2+ signaling evoked by several Gq-coupled receptors in pancreatic (21) and other cell types (36, 37). At a concentration inhibiting the oscillatory and the biphasic response to cholinergic stimulation, the antibodies had no apparent effect on the response to either VIP or Iso. This data supports the conclusion that inhibition of VIP- and Iso-induced Ca2+ signaling by RGS4 was due to acceleration of GTPase activity of a Gq class a subunit(s).

The use of PTX and Goq antibodies indicates that receptor-mediated activation of Gq was required for activation of Ca2+ signaling by VIP or Iso. It is notable that both Goq antibody preparations inhibited Iso-stimulated Ca2+ signaling in SMG duct cells whereas only the Gq1,12-specific preparation was effective for inhibiting VIP-stimulated signaling in the pancreatic acinar cells. This minor difference between the two systems may be attributed to cell type-specific expression patterns of Goq isoforms or the degree of Goq selectivity exhibited by putative PKA-phosphorylated VIP and β-adrenergic receptors. It is puzzling that the β-adrenergic Ca2+ response is inhibited completely by either Goq antibody preparation. If the β-adrenergic receptor couples to all members of the Gq class, then each antibody preparation would be expected to only partially inhibit and a mixture of the antibodies to completely inhibit
signaling by these receptors. The complete inhibition of signaling by either antibody preparation suggests that partial inhibition of IP$_3$ production by stimulation of the β-adrenergic receptor had reduced IP$_3$ below a threshold level needed to trigger Ca$^{2+}$ release. This interpretation is supported by previous work showing that Iso released Ca$^{2+}$ from the IP$_3$ mobilizable Ca$^{2+}$ pool (9) without causing a detectable increase in global IP$_3$ concentration (8).

In the G$_s$/G$_i$ switching model, activated receptor, phosphorylated by PKA, couples to G$_i$, (18). This predicts that Gβγ released from G$_i$ could activate PLCβ. Thus, inhibition of Gβγ or PLCβ activity is expected to inhibit the effect of the G$_s$-coupled receptors on [Ca$^{2+}$]. To test these predictions, we measured the effect of the Gβγ scavenging protein βARK1 (21, 38) and of the inhibitory PIP$_2$ antibody (39, 40) on VIP-dependent Ca$^{2+}$ signaling. Fig. 9a shows that infusing 5 μM βARK1 into pancreatic acinar cells completely inhibited the response to VIP. As we (21) and others (37) reported earlier, βARK1 also inhibited the response to stimulation of the G$_q$-coupled muscarinic receptor. Inhibition by βARK1 was upstream of the Ca$^{2+}$ increase because elevation of [Ca$^{2+}$], with A23187 strongly activated the Cl$^-$ current. Results similar to those in Fig. 9a, including the positive control with A23187, were obtained in five experiments. Fig. 9b shows that cytoplasmic PIP$_2$ antibodies completely inhibited the response to VIP and reduced the response to carbachol by $88 \pm 11\%$ ($n = 7$). These experiments indicate that both VIP and carbachol stimulate PLCβ to cause the hydrolysis of PIP$_2$.

In summary, our examination of the [Ca$^{2+}$] increase triggered by G$_i$-coupled receptors supports a model for switching or augmentation of receptor coupling to extend to G$_i$ in native cells freshly isolated from tissue. We conclude that the pathway involves activation of G$_i$ and PKA, receptor stimulation of G$_i$, and activation of PLCβ by Gβγ (derived from G$_i$). We acknowledge that the PKA substrate(s) responsible for activation of G$_i$ are not known but, as suggested by the switching model (17, 18), they could be the same receptors that were initially coupled only to G$_s$. We use caution, however, in referring to the Ca$^{2+}$ pathway (Fig. 1c), as a receptor switching model. PKA-dependent phosphorylation of the VIP or β-adrenergic receptors could allow G$_i$ to replace G$_s$ but the data are also consistent with broadening of receptor coupling to G$_i$ plus G$_s$. One mode for augmentation of receptor coupling can be envisioned if it is assumed that most β-adrenergic or VIP receptors are productively coupled to G$_s$ but a smaller subpopulation are poised to couple to G$_i$. Effective G$_i$ coupling would occur only when the receptors are phosphorylated by PKA. Because expression of a mutant (phosphorylation negative) β-adrenergic receptor prevented PKA-dependent activation of G$_i$ in HEK 293 cells (17), it is unlikely that phosphorylation of proteins downstream of the VIP or β-adrenergic receptors are responsible for activation of G$_i$ in pancreatic acinar or submandibular gland cells. An alternative to the assumption that mutant receptor is unable to couple to G$_i$ (17) is that the mutant cannot regulate its interaction with an RGS protein that may ordinarily suppress G$_i$ activation stimulated by the β-adrenergic receptor. A role for regulation of RGS protein function by receptor phosphorylation is attractive, not only because RGS proteins exhibit selectivity among receptor signaling complexes (23, 41, 42), but also because phosphorylation is not necessary for purified β-adrenergic receptors to activate G$_i$ in vitro (33). Additional experimental tools are needed to distinguish between these and other potential mechanisms. Independent of the mode of coupling it is clear that in pancreatic acinar and submandibular cells G$_i$-coupled receptors activate Ca$^{2+}$ signaling by coupling to G$_i$ and this coupling requires activation of G$_s$.

An equally important conclusion is that VIP and β-adrenergic receptor regulation of Ca$^{2+}$ release is completely independent of G$_s$ class proteins. The observation that PKA-dependent switching/augmentation in receptor/G protein coupling occurs in two different native cell types via two different receptors (that generate different types of Ca$^{2+}$ signals) suggests a generalization of the mechanism by which G$_i$-coupled receptors generate a second signal to activate a distinct signaling cascade.

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**Fig. 9. Inhibition of VIP-induced Ca$^{2+}$ signaling by βARK1 and PIP$_2$ antibodies.** Pancreatic acinar cells were dialyzed with pipette solutions containing 5 μM recombinant βARK1 fragment (a) or PIP$_2$ antibodies (b) prior to stimulation with 10 mM VIP and 1 mM carbachol (Car). As indicated, [Ca$^{2+}$], was increased by the addition of A23187 to the cell in a. The number of similar observations is given in the text.
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