**Gβγ Transduces [Ca^{2+}]_{i} Oscillations and Gαq a Sustained Response during Stimulation of Pancreatic Acinar Cells with [Ca^{2+}]_{i}-mobilizing Agonists**

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A central unresolved question in agonist-evoked [Ca^{2+}]_{i} signaling is the pathway by which [Ca^{2+}]_{i} oscillations and a sustained response are transduced. We show here that activation of Gβγ signal [Ca^{2+}]_{i} oscillations and activation of Gαq signal a sustained response during stimulation by a number of Ca^{2+}-mobilizing agonists. Thus, infusion of purified Gβγ into pancreatic acinar cells through a patch pipette evokes [Ca^{2+}]_{i} oscillations by Ca^{2+} release from internal stores, which were inhibited by two independent scavengers of Gβγ, the β-adrenergic receptor kinase fragment, and a mutated Gα_{q1203A}. These proteins, as well as an inhibitory antibody against Gα_{q121}, prevent [Ca^{2+}]_{i} oscillations and the sustained response when applied before cell stimulation, possibly by preventing the dissociation of Gα into its subunits. After cell stimulation and dissociation of Gα into Gβγ and Gαq, scavenging Gβγ stabilized the sustained response and inhibited reassociation of the subunits on termination of cell stimulation with antagonist, whereas scavenging Gαq inhibited the sustained response and uncovered the Gβγ-dependent oscillations. These findings provide a general mechanism by which Ca^{2+}-mobilizing agonists can control the type of [Ca^{2+}]_{i} signal to be transduced to the cell interior.

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Biochemical and molecular evidence demonstrated that receptors of Ca^{2+}-mobilizing agonists are coupled to the Gα_{q11} family of G proteins that activate phospholipase Cβ (PLCβ) to generate IP_{3} in the cytosol (Berridge, 1993; Hepler and Gilman, 1992). Ca^{2+} mobilization from the endoplasmic reticulum by IP_{3}, initiates a sequence of Ca^{2+} transporting events culminating in either a repetitive change in [Ca^{2+}]_{i}, in the form of [Ca^{2+}]_{i} oscillations or in a single, large transient increase in [Ca^{2+}]_{i}, the so-called sustained response (Berridge, 1993; Muallem, 1992). A central unresolved issue in Ca^{2+} signaling is how the agonists determine the type of the [Ca^{2+}]_{i} signal to be transduced.

A defining characteristic of [Ca^{2+}]_{i} oscillations is that they are evoked at low concentrations of agonists, which causes a small or no change in IP_{3} levels. The sustained response requires intense stimulation and a large increase in IP_{3} levels (Berridge, 1993; Muallem, 1992). In vitro, PLCβ can be activated well by Gα_{q11} (Bernstein et al., 1992; Hepler et al., 1993; Smrcka et al., 1991; Taylor et al., 1991; Wu et al., 1992) and Gβγ (Blank et al., 1992; Boyer et al., 1992; Camps et al., 1992; Park et al., 1993; Smrcka and Sternweis, 1993). However, in intact cells, receptors coupled to Gα, which are believed to initiate Ca^{2+} signaling through Gβγ (Clapham and Neer, 1993), are less efficient than receptors coupled to Gα_{11} in activating PLCβ (Peralta et al., 1988; Ashkenazi et al., 1989; Conklin et al., 1993; Liu et al., 1995; Wu et al., 1993). This raised the possibility that the intensity of PLCβ stimulation, and thus the type of the [Ca^{2+}]_{i} signal transduced, is determined by whether PLCβ is activated by Gβγ or Gα_{q11}.

Another potential mechanism by which Gβγ and/or Gαq can regulate Ca^{2+} signaling is by affecting the different Ca^{2+}-transporting pathways regulating [Ca^{2+}]_{i} (Muallem, 1992), in particular the IP_{3}-activated Ca^{2+} channel (IPACC). It has been shown that this channel is regulated by several kinases and phosphatases (Cameron et al., 1995; Zhang, et al., 1993), the activity of which may be regulated by G proteins. In addition, we showed recently that G proteins regulate two distinct steps in Ca^{2+} signaling. Stimulation of all cellular G proteins by high concentrations of GTPγS activates PLC to generate IP_{3}. Selective stimulation of G proteins with low concentration of GTPγS or AlF_{4} regulates the apparent affinity of the IPACC for IP_{3} to allow robust agonist-mediated Ca^{2+} release at IP_{3} levels present in resting cells (Xu et al., 1996b). The latter effect is independent of PLC activation or IP_{3} metabolism (Xu et al., 1996b) and may be mediated by the Gβγ released from the G proteins affected by the low concentration of GTPγS.

To begin to understand how G proteins regulate Ca^{2+} signaling, in the present study we determined the type of [Ca^{2+}]_{i} signal transduced by activation of Gα_{q11} or Gβγ by various Ca^{2+}-mobilizing agonists in pancreatic acinar cells. Infusion through a patch pipette of purified Gβγ, the Gβγ binding protein βARK fragment, the Gβγ scavenger Gα_{q1203A}, and a Gα_{q11} inhibitory antibody, before or after cell stimulation, showed that agonist-dependent activation of Gβγ leads to Ca^{2+} oscillations, whereas activation of Gα_{q11} results in a sustained response. Hence, agonists use selective activation of Gβγ or Gα_{q11} to specify the pattern of the Ca^{2+} signal to be transduced.

**EXPERIMENTAL PROCEDURES**

Solutions and Reagents—The standard bath solution (solution A) contained in (mM) 140 NaCl; 5 KCl; 1 MgCl_{2}; 1 CaCl_{2}; 10 glucose, and 10 HEPES (pH 7.4 with NaOH). The pipette (intracellular) solution contained in (mM) 140 KCl; 1 MgCl_{2}; 0.2 EGTA; 5 Na_{2}ATP, and 10 HEPES (pH 7.3 with KOH). The Gβγ subunit (a generous gift from Dr. P. Sternweis, University of Texas Southwestern Medical Center, Dallas) was purified from bovine brain and stored at −80 °C as described before (Sternweis and Robishaw, 1984). For use in the present experiments batches of Gβγ were dialyzed with a solution containing 140 mM KCl 30 mM HEPES (pH 7.2 with NaOH), and 0.2% sodium cholate. Reduction in the sodium cholate concentration was required to obtain
stable seals. The βARK fragment and a GST peptide (a generous gift from Drs. B. Stoessel and R. Lefkowitz, Duke University, Durham) were dialyzed with a solution containing 140 mM KCl and 10 mM HEPES (pH 7.2 with NaOH) and concentrated to about 1 mM using centricon filters. Control experiments showed that the GST peptide had no effect on [Ca\(^{2+}\)] signaling by Gβγ or the agonists. Recombinant mutated Gα\(_{i1G203A}\) (a generous gift from Drs. A. Raw, J. R. Hepler, and A. Gilman, University of Texas Southwestern Medical Center, Dallas) was prepared using a procedure described before (Lee et al., 1992) and stored at −80°C in a solution containing 1 mM EDTA and 50 mM HEPES (pH 7.6 with KOH). The Gα\(_{i1G203A}\) antibodies (a generous gift from Dr. P. Sternweis) were raised against the C-terminal portion of Gα\(_i\), as described (Gutowski et al., 1991). The IgG fraction was purified and gel-filtrated through a G-50 Sephadex column in a solution containing 120 mM KCl and 20 mM HEPES (pH 7.4 with KOH). Preimmune serum was obtained from the animals that were used to obtain the anti Gα\(_{i1G203A}\) antibodies. The antibodies and serum were stored at −80°C until use. In control experiments the preimmune serum had no effect on I\(_{Cl^−}\) oscillations as illustrated in Fig. 1, 30 nM of the patch pipette solution to obtain the desired final concentrations.

Isolation of Pancreatic Acinar Cells—Single rat pancreatic acinar cells were prepared by a standard technique (Mualem et al., 1988a). In brief, the pancreas of a 75–100-g rat was removed, minced, and incubated in an 0.025% trypsin, 0.02% EDTA solution (Sigma) for 5 min at 37°C. The tissue was washed, and single cells were liberated by a 7-min incubation at 37°C in solution A containing 0.02% soybean trypsin inhibitor and 160 units/ml pure collagenase (CLSP, Worthington). The cells were washed and kept on ice until use.

Whole Cell Current Recording—The tight seal whole cell current recording of the patch clamp technique was used (Hamill et al., 1981) for measurement of I\(_{Cl^−}\). Numerous studies by O. H. Petersen group and others (Petersen, 1992) have shown that in pancreatic acini this I\(_{Cl^−}\) faithfully follows changes in [Ca\(^{2+}\)], measured with fluorescent dyes and can be used to monitor changes in [Ca\(^{2+}\)] in whole cell voltage-clamped acinar cells. The experiments were carried out at room temperature using the standard bath and pipette solutions. The patch pipettes had resistance of 3–5 MΩ. Seals of better than 5 GΩ (range 6–10) were produced on the cell membrane, and the whole cell configuration was established by gentle suction or voltage pulses of 0.5 V for 0.3–1 ms. Formation of the whole cell configuration was followed by an increase in capacitance and noise. The patch clamp output (Axopatch-1B, Axon Instruments) was filtered at 20 Hz. Recording was performed with pClamp6 and a Digi-Dta 1200 interface (Axon Instruments). In most experiments acinar cells were voltage-clamped at a holding potential of −30 mV, and depolarizing voltage jumps of 100-ms duration at a frequency of 1 Hz to a membrane potential of +10 mV were repetitively applied throughout the experiment to measure the leak current. In some experiments the cells were voltage-clamped at −30 mV, and leak current was measured only at the beginning and the end of experiments. In all experiments shown the Cl− and cation equilibrium potential was about 0 mV. All the traces shown were at a holding potential of −30 mV and were corrected for the leak currents.

**RESULTS**

**Gβγ Evokes [Ca\(^{2+}\)] Oscillations—Oscillations of [Ca\(^{2+}\)] were followed by measurement of the current mediated by the Ca\(^{2+}\)-activated Cl− channels (I\(_{Cl^−}\) –Ca\(^{2+}\)) (Petersen et al., 1992; Thorn et al., 1993). Infusion of Gβγ through the patch pipette, by including 200 nM Gβγ purified from bovine brain in the pipette solution, evoked two types of [Ca\(^{2+}\)] oscillations as illustrated in Fig. 1, a and b. In 27/43 (63%) cells rapid oscillations in I\(_{Cl^−}\) –Ca\(^{2+}\) with an amplitude of 115 ± 43 pA and a return of the current to near baseline were observed (Fig. 1a). In 16/43 (37%) of the cells the I\(_{Cl^−}\) –Ca\(^{2+}\) developed more slowly, each transient lasted for a longer period of time, and the frequency of the oscillations was low. In these cells I\(_{Cl^−}\) –Ca\(^{2+}\) averaged 164 ± 30 pA.**

Preliminary experiments suggest that the pattern of the oscillations was not a function of the Gβγ concentrations. In 3/8 experiments with 80 nM Gβγ a response similar to that in Fig. 1b and in 3/6 experiments with 700 nM Gβγ a response similar to that in Fig. 1a were observed. For this analysis only cells responding to both Gβγ infusion and agonist stimulation were included. In an additional eight experiments the cells responded to agonist, but the Gβγ-induced oscillations were less than twice the noise. In all experiments in which Gβγ induced clear oscillations (n = 57), subsequent stimulation with high agonist concentration always further increased I\(_{Cl^−}\) –Ca\(^{2+}\) to produce a sustained, nonoscillatory response (Fig. 1a). This indicates that Gβγ could mobilize part but not all of the Ca\(^{2+}\) stored in the IP\(_3\)-mobilizable pool.

Among the noted characteristics of the Gβγ-induced oscillations is the relatively rapid activation of I\(_{Cl^−}\) –Ca\(^{2+}\) usually within 30 s of establishing the whole cell configuration. Subsequently, the amplitude of the oscillations continued to increase and reached its maximal value after about 120–150 s of cell dialysis (Fig. 1, a and b). This was significantly faster than expected from theoretical considerations (see below). The high concentration of Gβγ used and its hydrophobicity may account for the rapid onset of Ca\(^{2+}\) oscillation by Gβγ.

The specificity of the Gβγ effect is documented in Fig. 1, c–f. Infusion of as high as 700 nM boiled Gβγ had no effect on I\(_{Cl^−}\) –Ca\(^{2+}\) (n = 4) (Fig. 1c). 47.4 μM of the jARK fragment, which binds Gβγ and inhibits its activity (Koch et al., 1993; Koch et al., 1994), completely inhibited the effect of 200 nM Gβγ (n = 6) (Fig. 1d). The recombinant mutant Gα, α\(_{i1G203A}\), avidly binds Gβγ and subsequently fails to undergo a GTP- or GTPγS-induced conformational change necessary for dissociation from Gβγ\(^{2+}\) similar to previous findings made with the equivalent Gα\(_i\) (Lee et al., 1992) and Gα (Slepak et al., 1993) mutants. Thus, Gα\(_{i1G203A}\) effectively sequestrs and inhibits the effect of Gβγ. At 200 nM, α\(_{i1G203A}\) partially inhibited oscillations evoked by 200 nM Gβγ (Fig. 1e), whereas 400 nM α\(_{i1G203A}\) completely inhibited the effect of 200 nM Gβγ in all experiments tested (n = 5) (Fig. 1f).

Fig. 2 shows that Gβγ evoked I\(_{Cl^−}\) –Ca\(^{2+}\) oscillations by Ca\(^{2+}\) release from the IP\(_3\)-mobilizable intracellular pool. Thus, removal of extracellular Ca\(^{2+}\) did not prevent the oscillations (n = 4) (Fig. 2a); buffering the cytosol with 1,2-bis-(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid to prevent changes in [Ca\(^{2+}\)], prevented Gβγ-dependent oscillations and greatly attenuated the effect of 10 μM carbachol (n = 3) (Fig. 2b). Finally, including 1.5 mg/ml heparin, a selective inhibitor of IPACC (Ghosh et al., 1988; Zhao et al., 1990), in the pipette solution inhibited the effect of Gβγ and that of the agonist (Fig. 2c).
Similar results were obtained in five additional experiments using between 1.5 and 2.5 mg/ml heparin.

Inhibition of Gβγ Inhibits Agonist-evoked Ca2+ Oscillations—The overall results in Figs. 1 and 2 show that Gβγ specifically evokes Ca2+ oscillations when infused into pancreatic acinar cells. To determine the role of Gβγ in agonist-mediated Ca2+ oscillations, we tested the effects of α11G203A and the βARK fragment on agonist-induced ICl(Ca2+). Figs. 3 and 4 show that both α11G203A and the βARK fragment inhibited the oscillations induced by three different agonists, which bind to separate receptors in acinar cells (Williams and Yule, 1993). The results in Figs. 3 and 4 were separated for clarity but are described together to highlight the finding that inhibition of Gβγ inhibited oscillations evoked by all major Ca2+-mobilizing agonists and the different patterns of Ca2+ oscillations evoked by each of the agonists. Oscillations of ICl(Ca2+) were initiated with a low and a sustained response with high agonist concentrations (Figs. 3a, 4a, 4c, and 4e). With all agonists the oscillatory current was between 80 and 200 pA, whereas maximal concentration of the various agonists caused a single large transient increase in ICl(Ca2+) of between 500 and 1000 pA (note the different scales for the oscillatory and sustained ICl(Ca2+) in both figures). Preliminary studies showed that maximal effects with all inhibitory proteins were achieved within 200–250 s of dialysis. Considering the size of these proteins, all in the range of 40 kDa, this is the time expected from theoretical consideration of diffusion of substances in solutions (see below). This indicates minimal binding of the proteins used to the glass pipette and minimal hindrance for diffusion of molecules from pipette to cells. However, to ensure complete equilibration of proteins between pipette solution and the cell interior, 400 s of dialysis with the various peptides was allowed before cell stimulation was initiated.

Dialysis with 150 nm α11G203A largely inhibited the oscillations (Fig. 3b) and reduced the sustained response by all agonists by about 84 ± 16% (n = 11). At 450 nm, α11G203A completely inhibited agonist-evoked Ca2+ oscillations (Fig. 4d) and better than 90% (n = 6) of the sustained response. The results in Figs. 3c, 4b, and 4f show that the βARK fragment was as effective as α11G203A in inhibiting the different patterns of ICl(Ca2+) oscillations and the sustained response induced by carbachol (Fig. 3c), bombesin (Fig. 4b), and CCK (Fig. 4f). The βARK fragment also inhibited oscillations evoked by the partial CCK agonist, CCK-J 180 (not shown). This partial agonist binds to state A of the CCK receptor to exclusively induce [Ca2+]i oscillations (Matozaki et al., 1990; Loessberg et al., 1991).

Gγ11 Is Not Required for Agonist-evoked [Ca2+]i Oscillations—Diffusion of charged and uncharged molecules from a patch pipette into cells depends on several properties of the molecules, pipettes, and the cells. Pusch and Neher (1988) have
affected the time of dialysis needed to achieve maximal inhibition was achieved faster. Two examples for the inhibition of maximal inhibition. At higher concentrations, maximal inhibition was shown in Fig. 5. Fig. 5  

shown that for most molecules equilibration time between pipette solution and cells follows a single exponential time course and is approximated by Equations 1 and 2:

\[ \tau = (0.6 \pm 0.17) \cdot R_A \cdot M^{1/3} \] \hspace{1cm} (Eq. 1)

\[ \tau = V/N_0 \] \hspace{1cm} (Eq. 2)

where \( \tau \) is the time constant in seconds, \( R_A \) is the access resistance in M\( \Omega \), and \( M \) is the molecular mass of the diffusing molecule in daltons. Equation 2 is a volume scaling formula used to relate the time constant of the cell of interest (\( \tau \)) to that of chromatin cells (\( \tau_i \)), for which the constant 0.6 ± 0.17 was obtained. The proteins used in the present studies, G\( \beta \gamma \), G\( \alpha_q \), and the pARK fragment all have a molecular mass of around 40 kDa, and the IgG anti G\( \alpha_q \), has a molecular mass around 150 kDa. The access resistance in our experiments averaged 9.9 ± 1.1 M\( \Omega \), and the dimensions of acinar cells are close to those of chromatin cells (15–17 \( \mu \)m). Thus, it can be calculated that the 40-kDa proteins should equilibrate within about 204 s and the IgG within 316 s.

As indicated above, preliminary experiments indeed showed that between 200 and 250 s of dialysis were required for obtaining maximal inhibition with the proteins of 40 kDa. Because of the design of the experiments in Figs. 6 and 7, we performed the most extensive studies with the IgG anti G\( \alpha_q \). In 28 experiments with 40–240 \( \mu \)g/ml anti-G\( \alpha_q \) in the pipette solution, maximal inhibition was obtained when the antibody was above 80 \( \mu \)g/ml. The concentration of the antibody only affected the time of dialysis needed to achieve maximal inhibition. With 80 \( \mu \)g/ml, 7–10 min of dialysis were required for maximal inhibition. At higher concentrations, maximal inhibition was achieved faster. Two examples for the inhibition of agonist-evoked Ca\( ^{2+} \) signaling by 200 \( \mu \)g/ml IgG anti-G\( \alpha_q \) are shown in Fig. 5. Fig. 5a shows the protocol used to evaluate the inhibition and that nonimmune serum had no effect on Ca\( ^{2+} \) signaling. At a designated time after establishing the whole cell configuration and starting the cytosolic dialysis, the cell was stimulated with maximal concentration of carbachol. Shortly afterwards the cell was inhibited with atropine to terminate the stimulation and force Ca\( ^{2+} \) back into the internal stores for rapid and maximal reloading of the stores (Muallem et al. 1988b; Muallem et al. 1988c). After continuous dialysis with the pipette solution for an additional 400–500 s, the cells were stimulated with high concentration of bombesin (BS) to evaluate the additional inhibition gained by the continuous dialysis. Fig. 5, b and c, shows that about 120 s after establishing the whole cell configuration, 200 \( \mu \)g/ml anti-G\( \alpha_q \) inhibited the carbachol response by more than 80% (82 ± 7%, n = 10). Dialysis for an additional 400 s with the antibody increased the inhibition by only about 10% to 91 ± 6%. To show that the antibody did not affect the I\( _{Cl} \) (Ca\( ^{2+} \)) itself or the electrical properties of the cells, the I\( _{Cl} \) (Ca\( ^{2+} \)) was activated by increasing [Ca\( ^{2+} \)], with ionomycin. The relatively rapid and extensive inhibition of the agonist-induced Ca\( ^{2+} \) signaling by the high concentration of the antibody allowed us to determine their effect before and after cell stimulation as depicted in Figs. 6 and 7.

To determine the role of G\( \alpha_q \) in different aspects of agonist-evoked [Ca\( ^{2+} \)] oscillations, we first tested the effect of the G\( \alpha_q \) antibody on Ca\( ^{2+} \) oscillations. Fig. 6a shows the control experiment in which I\( _{Cl} \) (Ca\( ^{2+} \)) oscillations persisted for the duration of cell stimulation with 0.5 \( \mu \)M carbachol. Stimulation of the same cells with 10 \( \mu \)M carbachol produced the usual sustained response. In this set of experiments, dialyzing the cells for 250–400 s with 200 \( \mu \)g/ml of the G\( \alpha_q \) antibodies prior to the first cell stimulation completely inhibited the os-
obtained with the oscillatory agonist CCK-J 180. A 400-s dialysis of the antibodies completely inhibited CCK-J 180-evoked \([\text{Ca}^2+]\) oscillations (Fig. 6d), but when the cells were stimulated 30 s after establishing the whole cell configuration the oscillations continued uninterrupted (although with progressively reduced amplitude) for at least 12 min. The combined experiments indicate that once the \(G_{\alpha_q}\) subunits were dissociated by agonist stimulation to induce oscillations, binding of the released \(G_{\alpha_q}\) did not completely inhibit the oscillations.

\(G_{\alpha_q}\) Is Required for the Sustained Response and \(G_{\beta\gamma}\) for \([\text{Ca}^2+]\) Oscillations—Inhibition of agonist-dependent \(\text{Ca}^2+\) signaling by both binding of \(G_{\beta\gamma}\) (Figs. 3 and 4) and \(G_{\alpha_q}\) (Figs. 5 and 6) before cell stimulation can be because the inhibitory proteins \((\text{GqG12/11}, \text{ARK fragment}, G_{\alpha_q}\) antibody) prevented the agonist-dependent dissociation of the \(G_{\alpha_q}\) to its subunits or because a favored and rapid binding to the respective subunits after their release and inhibition of their activity. To distinguish between these possibilities we performed the experiment shown in Fig. 7. The diagram at the top of Fig. 7 depicts the rationale of these experiments. Immediately after establishing the whole cell configuration, the cells were stimulated with a high concentration of carbachol to completely dissociate the \(G_{\alpha_q}\) coupled to the cholinergic receptors into \(G_{\beta\gamma}\) and \(G_{\alpha_q}\). Including the \(\text{ARK fragment}\) in the pipette allowed the slow infusion of \(G_{\beta\gamma}\) into the cytosol and the scavenging of the free \(G_{\beta\gamma}\). All the remaining signals should be mediated by \(G_{\alpha_q}\). Fig. 7a shows that this resulted in stabilization, rather than inhibition, of the sustained response. Another consequence of \(G_{\beta\gamma}\) binding by the \(\text{ARK fragment}\) is that the effect of the antagonist should be inhibited due to the inhibition of the reassociation between \(G_{\beta\gamma}\) and \(G_{\alpha_q}\). Comparing Fig. 7a and b shows that the \(\text{ARK fragment}\) increased the duration of atropine-dependent termination of cell stimulation from about 17 ± 4 to 314 ± 56 s \((n = 4)\). Demonstrating that \(G_{\beta\gamma}\) was indeed bound to the \(\text{ARK fragment}\) allows us to conclude that \(G_{\alpha_q}\), on its own can transduce and support the sustained response.

The reciprocal experiment is shown in Fig. 7c. In this experiment the patch pipette included the \(G_{\alpha_q}\) antibodies. After dissociations of the \(G_{\alpha_q}\) subunits by high carbachol concentration, scavenging \(G_{\alpha_q}\) by the antibodies inhibited the sustained response and slowly uncovered \([\text{Ca}^2+]\) oscillations, which lasted for at least 5 min \((n = 6)\). That the oscillations in Fig. 7c were dependent on \(G_{\beta\gamma}\) is shown in Fig. 7d. Including both \(G_{\alpha_q}\) antibodies and the \(\text{ARK fragment}\) in the pipette now completely inhibited all forms of \([\text{Ca}^2+]\), with return of the \([\text{Ca}^2+]\), to base line with no subsequent oscillations \((n = 4)\). The complete inhibition of \(\text{Ca}^2+\) oscillations is further shown by the inability of a second stimulation to change \([\text{Ca}^2+]\). Therefore the experiments in Fig. 7, c and d, further support the conclusion that the sustained response is mediated by \(G_{\alpha_q}\) and also shows that \(\text{Ca}^2+\) oscillations can be transduced by \(G_{\beta\gamma}\).

**DISCUSSION**

Agonists coupled to \(G\) proteins transduce their information by catalyzing the GTP/GDP exchange reaction of the \(\alpha\) subunits to dissociate the \(G_{\alpha}\) from the \(G_{\beta\gamma}\) subunits (Gilman, 1987). In the case of \([\text{Ca}^2+]\)-mobilizing agonists, activation of the \(G\) proteins is followed by activation of PLC to generate IP3 in the cytosol, which initiates the \([\text{Ca}^2+]\) signal by \(\text{Ca}^2+\) release from internal stores (Berridge, 1993; Mualllem, 1992). The agonist can signal two types of \([\text{Ca}^2+]\), repetitive change in \([\text{Ca}^2+]\), in the form of \([\text{Ca}^2+]\), oscillations seen with low agonist concentration or a single large transient change in \([\text{Ca}^2+]\), as a result of intense stimulation (Berridge, 1993; Mualllem, 1992). How the two signals are transduced is not known. The present studies suggest that activation of \(G_{\beta\gamma}\) is required and may be
inhibitory proteins prior to cell stimulation ensured maximal oscillations. After completion of the present studies, it was shown that both types of proteins can bind to the undissociated Gq and inhibit signaling by preventing the dissociation of Gq into its subunits. Hence, addition of the antibodies did not prevent the oscillations, while almost eliminating the sustained response. The ability to mediate the agonist-evoked [Ca2+]i oscillations since scavenging the Gq subsequent to cell stimulation did not prevent the oscillations, while almost eliminating the sustained response (Fig. 6, c and e). We interpret these experiments as follows. Stimulation of the cells before the Gq/11 antibodies entered the cytosol resulted in sufficient dissociation of Gβγ and Gαi to start the oscillations. As the antibodies penetrated the cytosol they bound all free and remaining undissociated Gq. This had two effects. Binding of the antibodies to Gq prevents further dissociation of the subunits to inhibit activation of the sustained response. Binding of the free Gαi by the antibodies did not prevent the oscillations since they were largely mediated by Gβγ. The partial reduction in the amplitude of the oscillations observed in the first few min of cell stimulation with carbachol (Fig. 6c) and after longer stimulation with CCK-J 180 (Fig. 6e) indicates that Gαi contributes to, but is not essential for, agonist-evoked [Ca2+]i oscillations. The possible contribution of Gαi to [Ca2+]i oscillations is discussed below.

Infusion of Gβγ and Gαi binding proteins for a long period before cell stimulation inhibited the oscillatory and the sustained response. After completion of the present studies, it was reported in Xenopus oocytes that injection of α subunits, the βARK fragment, or the Gq/11, antibody 5 min before cell stimulation inhibited the acetylcholine-induced sustained response (Stehno-Bittel et al., 1995). Based on the finding that injection of Gβγ but not Gαi caused Ca2+ release from internal stores and the complete inhibition of acetylcholine-induced Ca2+ release by the Gβγ binding proteins, it was concluded that Gβγ transduces the muscarinic signal for Ca2+ release by activation of all required PLC, whereas the Gαi only determines specificity of the receptor-G protein coupling (Stehno-Bittel et al., 1995). Although we also could activate [Ca2+]i release from internal stores of pancreatic acini with Gβγ (Fig. 1) but not with a wild type or constitutively active Gαq or Gq/12/13 (not shown), our results do not support such an interpretation (Fig. 7). The inability of injected or infused Gαi in oocytes and pancreatic acini, respectively, to induce Ca2+ release may be because the α subunit could not access the relevant PLC; it was inactivated as it entered the cytosol or due to other technical reasons. The fact that in pancreatic acini as well as in Xenopus oocytes both the Gβγ and Gα binding proteins inhibited Ca2+ signaling suggests that both types of proteins can bind to the undissociated Gq and inhibit signaling by preventing the dissociation of the Gq into its subunits. Hence, addition of the inhibitory proteins prior to cell stimulation ensured maximal inhibition of all forms of Ca2+ signaling. The inhibitory proteins can probably also bind to the free Gq subunits and inhibit their action. In this case exposure of the Gq subunits to the inhibitory proteins after cell stimulation revealed a clear specificity in mediating different forms of the [Ca2+], signal. Thus, after subunit dissociation, inhibition of Gβγ by the βARK fragment did not inhibit the Ca2+ signal as would be expected if Gβγ mediated all PLC activation. On the contrary, binding of Gβγ stabilized the sustained response and markedly attenuated antagonist-dependent inactivation, probably by interfering with the rebinding of Gβγ to Gαi (Fig. 7, a and b). Binding of Gαi by the antibodies after cell stimulation inhibited the sustained response (Fig. 7c) but not [Ca2+]i oscillations (Fig. 7c), which required free Gβγ (Fig. 7d). The combined evidence strongly supports a role for Gαi in transducing and maintaining the sustained response.

A possible explanation for transduction of [Ca2+]i oscillation by Gβγ and a sustained response by Gαi may lie in their specificity in activating selective PLC isoforms and thus the extent to which they increase IP3 levels. In vitro experiments and transfection of selective isoforms showed that Gβγ stimulates PLCβ3 and PLCβ2 better than PLCβ1, whereas Gαi stimulates PLCβ1 better than PLCβ2 and PLCβ3 (Camp et al., 1992; Katz et al., 1992; Smrcka and Sternweis, 1993). [Ca2+]i oscillations in many cells, including pancreatic acini (Muallem, 1992), are associated with a low, if any, increase in IP3 and the periodic activation and inactivation of the Ca2+ transporting pathways governing the Ca2+ signal (Muallem, 1992; Zhang et al., 1992). On the other hand intense stimulation is associated with a large increase in IP3 levels (Berridge, 1993; Muallem, 1992) and a persistent activation of all Ca2+ transporters for the duration of cell stimulation (Muallem et al., 1988b; Muallem et al., 1988c; Muallem, 1992). It is therefore possible that stimulation with a low concentration of agonist liberates sufficient Gβγ to activate PLCβ3 and/or PLCβ2 and causes a small and localized increase in IP3 to evoke Ca2+ oscillations. This can account for the stimulation of Ca2+ oscillations by agonists that activate Gq/Gi in pancreatic acinar cells (Kase et al., 1991) and duct cells (Zhao et al., 1994) and in salivary duct cells (Xu et al., 1996a). In salivary ducts we showed that isoproterenol, a Gq/Gi-coupled agonist, releases Ca2+ from the IP3 pool by a modest activation of PLC (Xu et al., 1996a).

A more attractive mechanism by which Gβγ can transduce Ca2+ oscillations and Gαi the sustained response is that Gβγ modifies the activity of the IP3-activated Ca2+ channel (IPACC), whereas Gαi stimulates PLC to generate IP3. In a recent study we showed that treatment of pancreatic acinar cells with very low concentrations of GTPγS or AIF3 markedly increased the apparent affinity of the IPACC to its ligand IP3 to sensitize and allow agonist-mediated Ca2+ release at the low IP3 concentrations present in resting cells (Xu et al., 1996b). These effects were independent of PLC activation or generation of IP3. It is therefore possible that infusion of Gβγ through the patch pipette sensitized the IPACC, similar to the effect of GTPγS, to initiate [Ca2+]i oscillations at the IP3 concentration present in resting cells. The need for IP3 in Gβγ-induced oscillations is shown by their inhibition by heparin, an inhibitor of the IPACC. The fact that in acinar cells Gβγ at high concentrations never depleted all the IP3-mobilizable Ca2+ pools (Fig. 1) would suggest that stimulation of PLC may not play a major role in Gβγ-evoked [Ca2+]i oscillations. Finally, the partial reduction in the amplitude of the oscillations by the Gαi antibody when added after cell stimulation (Fig. 6, c and e) can be interpreted to suggest that at low agonist concentrations some Gαi and Gβγ were liberated to start the oscillations. Gαi generated some IP3 whereas Gβγ increased the affinity of the IPACC for IP3 to facilitate Ca2+ release and recruit more of the internal pool in the face of the quantal properties of Ca2+ release (Muallem et al., 1989; Tortorici et al., 1994). Inhibition of Gαi by the antibody after initiation of cell stimulation inhibited the stimulated production of IP3. This reduced IP3 levels to
those present in resting cells, but the free Gβγ allowed continuation of the oscillations. Similarly, on intense stimulation (Fig. 7, c and d) binding of all the Gαq inhibited production of IP3 to inhibit the sustained response but allowed the oscillations due to the free Gβγ. Hence, Gβγ may induce (Fig. 1) or transduce (Figs. 3–7) Ca2+ oscillations by regulating the activity of the IPACC. It is interesting that the forms of Gβγ(CTa) oscillations induced by Gβγ are reminiscent of those induced by the apical pool of acinar cells by infusion of IP3 (Thorn et al., 1993). The site of the Gβγ-induced [Ca2+]i oscillations and the suggested direct regulation of IPACC by Gβγ are now being tested in our laboratory.

In summary, through the application to the cytosol of specific proteins that bind Gβγ or Gαq, before and after cell stimulation, we were able to show that Gβγ and Gαq may transduce different patterns of agonist-dependent Ca2+ signaling. Activation of Gαq is required for transduction of a sustained response, probably by mediating most of the agonist-dependent activation of PLC and generation of IP3. Transduction of [Ca2+]i oscillations requires Gβγ and appears to be common and shared by different agonists coupled to Gαq.

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REFERENCES

Ashkenazi, A., Peralta, E. G., Winslow, J. W., Ramachandran, J., and Capon, D. J. (1989) Cell 56, 487–493
Berger, S., Blank, J. L., Smrcka, A. V., Higashijima, T., Sternweis, P. C., Exton, J. H., and Roz, E. M. (1992) J. Biol. Chem. 267, 8081–8088
Berridge, M. (1993) Nature 361, 315–325
Blank, J. L., Brattain, K. A., and Exton, J. H. (1992) J. Biol. Chem. 267, 23069–23075
Boyer, J. L., Waldo, G. L., and Harden, T. K. (1992) J. Biol. Chem. 267, 25451–25456
Cameron, A. M., Stein, J. P., Roskams, A. J., Ali, S. M., Ronnett, G. V., and Snyder, S. H. (1995) Cell 83, 463–472
Campi, M., Carozzi, A., Schnabel, P., Scheer, A., Parker, P. J., and Gierschik, P. (1992) Nature 360, 684–686
Clapham, D. E., and Nee, E. J. (1993) Nature 365, 403–406
Conklin, B. R., Farfel, Z., Lustig, K. D., Julius, D., and Bourne, H. R. (1993) Nature 363, 274–276
Dhish, T. K., Eis, P. S., Mullaney, J. M., Ebert, C. L., and Gill, D. L. (1988) J. Biol. Chem. 263, 11075–11079
Gilman, A. G. (1987) Annu. Rev. Biochem. 56, 615–649
Gutowski, S., Smrcka, A., Nowak, L., Wu, D., Simon, M., and Sternweis, P. C. (1991) J. Biol. Chem. 266, 20519–20524
Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981) Pflügers Arch. 391, 85–100
Hepler, J. R., and Gilman, A. G. (1992) Trends Biochem. Sci. 17, 383–387
Hepler, J. R., Koza, T., Smrcka, A. V., Simon, M. I., Rhee, S. G., Sternweis, P. C., and Gilman, A. G. (1993) J. Biol. Chem. 268, 14367–14375
Kase, H., Waki, M., and Petersen, O. H. (1991) Pflügers Arch. 419, 668–670
Katz, A., Wu, D., and Simon, M. I. (1992) Nature 360, 686–689
Koch, W. J., Inglesi, J., Stone, W. C., and Lefkowitz, R. J. (1993) J. Biol. Chem. 268, 8526–8520
Koch, W. J., Hawes, B. E., Inglesi, J., Luttrell, L. M., Lefkowitz, R. J. (1994) J. Biol. Chem. 269, 6193–6197
Lee, E. Taussig, R., and Gilman, A. G. (1992) J. Biol. Chem. 267, 1212–1218
Liu, J., Conklin, B. R., Blin, N., Yun, J., and Wess, J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 11642–11646
Loessberg, P. A., Zhao, H., and Muallem, S. (1991) J. Biol. Chem. 266, 1363–1366
Motozaki, T., Gokke, B., Tsunoda, Y., Rodriguez, M., Martinez, J., and Williams, J. A. (1990) J. Biol. Chem. 265, 6247–6254
Muallem, S. (1992) in Advances in Second Messenger and Phosphoprotein Research (Putney, J. W., Jr., ed) pp. 351–368, Raven Press, New York
Muallem, S., Pandol, S., and Beeker, T. G. (1988a) Biochem. J. 255, 301–307
Muallem, S., Schoeffield, M., Fimmel, C. J., and Pandol, S. F. (1988b) Am. J. Physiol. 255, G221–G228
Muallem, S., Schoeffield, M., Fimmel, C. J., and Pandol, S. F. (1988c) Am. J. Physiol. 255, G229–G235
Park, D., Jhon, D. Y., Lee, C. W., Lee, K. H., and Rhee, S. G. (1993) J. Biol. Chem. 268, 1414–1423
Smrcka, A. V., and Sternweis, P. C. (1993) J. Biol. Chem. 268, 9667–9674
Smrcka, A. V., and Sternweis, P. C. (1991) Science 251, 804–807
Stein-Behlott, L., Krapivinsky, G., Krapivinsky, L., Perez-Terzic, C., and Clapham, D. E. (1995) J. Biol. Chem. 270, 3068–3074
Stehno-Bittel, L., Krapivinsky, G., Krapivinsky, L., Perez-Terzic, C., and Clapham, D. E. (1995) J. Biol. Chem. 270, 3068–3074
Sternweis, P. C., and Robishaw, J. D. (1984) J. Biol. Chem. 259, 13806–13813
Taylor, S. J., Chae, H. Z., Rhee, S. G., and Exton, J. H. (1993) Nature 360, 516–518
Thorn, P., Lawrie, A. M., Smith, P. M., Gallacher, D. V., and Petersen, O. H. (1993) Cell 74, 661–668
Tortoriol, G., Zhang, B.-X., Xu, X., and Muallem, S. (1994) J. Biol. Chem. 269, 29621–29626
Williams, J. A., and Yule, D. I. (1993) in The Pancreas, Biology, Pathobiology, and Disease (Go, V. W., ed) pp. 167–189, Raven Press, New York
Wu, D. O., Lee, C. H., Rhee, S. G., and Simon, M. I. (1992) J. Biol. Chem. 267, 1811–1817
Wu, D., LaRosa, G. J., Simon, M. I. (1993) Science 263, 101–103
Xu, X., Diao, J., Zhao, H., and Muallem, S. (1996a) J. Biol. Chem. 271, 1414–1423
Zhao, X. Zeng, W., and Muallem, S. (1996b) J. Biol. Chem. 271, 11737–11744
Zhang, B.-X., Zhao, H., Loessberg, P. A., and Muallem, S. (1992) J. Biol. Chem. 267, 25393–25393
Zhang, B.-X., Zhao, H., and Muallem, S. (1993) J. Biol. Chem. 268, 10997–11001
Zhao, H., Khademazad, M., and Muallem, S. (1990) J. Biol. Chem. 265, 14282–14287
Zhao, H., Star, R. A., and Muallem, S. (1994) J. Gen. Physiol. 104, 57–85