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Desensitization of Inositol 1,4,5-Trisphosphate/Ca^{2+}-induced Cl^- Currents by Prolonged Activation of G Proteins in *Xenopus* Oocytes*

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Expression of G protein α subunits of the G_q family with various G protein-coupled receptors induces activation of an inositol 1,4,5-trisphosphate (IP_3)/Ca^{2+}-mediated Cl^- conductance in *Xenopus* oocytes. Our present data show that two members of this family, the human $G\alpha_{16}$ subunit and the murine homologue $G\alpha_{15}$, can induce both activation and inhibition of these agonist-induced currents. Although extremely low amounts (10–50 pg) of injected $G\alpha_{16}$ subunit cRNA cause modest (~2-fold) enhancement of ligand-induced Cl^- currents in oocytes co-injected with thyrotropin-releasing hormone (TRH) receptor cRNA 48 h postinjection, larger $G\alpha_{16}$ and $G\alpha_{15}$ cRNA injections cause >10-fold inhibition of TRH or 5HT2c receptor responses. The inhibition is analyzed in this study. The inhibited currents are recovered if various $G\beta\gamma$ subunit combinations are also expressed with the $G\alpha$ subunits. The constitutively active mutant, $G\alpha_{16}$Q212L, also causes a strong attenuation of the ligand-induced Cl^- currents, but this inhibition is not recovered by co-expression of $G\beta\gamma$ subunits. These results indicate that the free $G\alpha$ subunit is responsible for the inhibitory signal. Although expression of TRH receptor alone produces maximum responses approximately 48 h after injection, co-expression of TRH receptor with $G\alpha_{16}$ results in enhanced responses 6–12 h postinjection, followed by complete attenuation at 36 h. Furthermore, injection of $G\alpha_{16}$ cRNA alone at comparable levels gives rise to spontaneous Cl^- currents within 6–12 h postinjection, suggesting that the early spontaneous activation underlies the later suppression. Expression of other G protein α subunits of the G_q family, at cRNA levels considerably higher than effective for $G\alpha_{16}$, produces both analogous spontaneous Cl^- currents and, later, inhibition of ligand-induced Cl^- currents. Experiments with direct injection of IP_3 and of Ca^{2+} suggest that this inhibition is consistent with the down-regulation of IP_3 receptors. These data indicate that both enhancement and inhibition of signaling through G protein-coupled receptors can be mediated by the expression level and/or activity of an individual G protein.

Many hormones, neurotransmitters, and growth factors act via G protein-coupled receptors (GPCRs)[1] in a wide range of transduction processes. The phosphoinositide cascade, which controls calcium-stimulated processes such as secretion, chemotaxis, and fertilization, is regulated by G proteins. Phosphoinositide phospholipase C (PLC) catalyzes the hydrolysis of phosphatidyl 4,5-bisphosphate to generate two second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. Diacylglycerol in turn activates protein kinase C; IP_3 binds to an intracellular IP_3 receptor/channel to release Ca^{2+} stores (Ref. 1 and references therein). In mammals, several families of PLC isoforms have been described (2, 3). The PLC β isoform is regulated by G proteins. All four β isoforms of PLC are activated via the pertussis toxin-insensitive $G\alpha_q$ family of G proteins: $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, $G\alpha_{15}$, and $G\alpha_{16}$, although isoforms appear to vary with respect to potency for activation (4–6). Two isoforms, PLC β2 and PLC β3, can also be activated by $G\beta\gamma$ (6–9). It also has been shown that the $G\alpha$ and $G\beta\gamma$ subunits bind to different regions of the PLC β enzyme (10, 11).

The complexity of the molecular components and the functional interaction among them enable many variations in the control and timing of particular signaling pathways. Such specificity of signaling may be important in determining the cell's response to an extracellular signal. The pattern of expression of receptors, G proteins, and effectors may also play an important role in this selectivity process. For instance, the $G\alpha_{16}$ and $G\alpha_{15}$ subunits show a restricted pattern of expression (12, 13); conversely, they have a promiscuous behavior with respect to receptor coupling (14, 15). Regulatory processes such as short and long term desensitization may also determine the efficiency of signal transduction in a cell.

In *Xenopus* oocytes, activation of PLC by ligand-activated GPCRs produces the opening of a Cl^- channel via Ca^{2+} release from intracellular stores; this system has been extensively used for the study of GPCRs. Many receptors couple to an endogenous pertussis toxin-sensitive G protein when expressed in oocytes (16–18); this pathway seems to involve G_o. It is still unclear whether the $\beta\gamma$-dependent activation of PLC β accounts for the pertussis toxin-sensitive regulation of PLC. Recent data suggest that the $G\beta\gamma$ dimer regulates the activity of PLC in oocytes (19). Other receptors, *e.g.* the thyrotropin-releasing hormone (TRH) receptor, activate PLC via pertussis toxin-insensitive G proteins in oocytes (20, 21). Antisense experiments show that these G proteins are members of the G_q family. Additionally, the expression level of G proteins influences the selectivity of receptor/G protein coupling (21). Promiscuity of receptor/G protein interactions appears with increas-

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[1] The abbreviations used are: GPCR, G protein-coupled receptor; PLC, phospholipase C; TRH, thyrotropin-releasing hormone.

ing levels of G protein expression, indicating that the level of expression of G proteins and receptors are important for modulating transmembrane signaling.

Regulation of the PLC cascade in oocytes is an important step in oocyte maturation and fertilization. For example, it has been shown that G proteins of the G_q family increase in expression level as the oocyte matures and acquires the capacity to respond to IP_3 (22). Although the IP_3 receptors are localized mostly in the cytoplasm of the oocyte, they are found mostly in the cortical region of the mature egg (23). In the egg, an increase in Ca^{2+} concentration occurs at fertilization and propagates Ca^{2+} waves that are required for the prevention of polyspermy and for the induction of cell cycle changes (24). In this study, we present further evidence for regulation of the pathway leading from the activation of G proteins to the activation of Cl^- channels. We use this signaling cascade to examine the role of G proteins of the G_q family to GPCR stimulation. We show that overstimulation of PLC, by means of expression of Gα subunits of the G_q family, produces signal inhibition and that this effect is the result of a decreased physiological response to IP_3 activation. We suggest that this effect is part of a desensitization process controlling the stimulation of the PLC pathway and that both enhancement and inhibition of signaling through GPCRs can be mediated by the level of G protein expression and/or activity.

EXPERIMENTAL PROCEDURES

In Vitro Synthesis of RNA—In vitro transcription of sense RNA was carried out as described previously (25) with a few modifications. Recombinant plasmids containing cDNA inserts were linearized by digestion with appropriate restriction enzymes. The transcription of linearized templates was performed in 7.6 mM Tris-HCl, pH 7.6, 6 mM $MgCl_2$, 0.6 mM NaCl, and 10 mM dithiothreitol containing 0.5 mM each ATP, CTP, and UTP, 0.1 mM GTP, 0.5 mM 5′-(7-methyl)-GTP, and 180 units of the respective polymerase in a total volume of 250 μl. The reaction mixture was incubated for 150 min at 37 °C. The DNA template was subsequently removed by treatment with 5 units of RNase-free DNase I for 15 min at 37 °C. Free nucleotides were removed using a Sephadex G50 column. The mRNA was phenol-chloroform-extracted and recovered by ethanol precipitation. The RNA was dissolved in RNase-free water at the corresponding concentration (see figure legends), divided into aliquots, and stored at −70 °C until used.

Oocyte Expression and Electrophysiology—These procedures are described in detail elsewhere (26). Briefly, oocytes were defolliculated and maintained at 18 °C in incubation medium containing ND96 (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.4), 1.8 mM $CaCl_2$, 50 μg/ml gentamycin, and 5% horse serum (27). Whole cell currents were measured at room temperature using either a Dagan 8500 amplifier or an Axon Instruments GeneClamp in a standard two-microelectrode voltage clamp configuration. Current was measured on-line by oscilloscope and chart recorder. Electrodes were filled with 3 M KCl and had a resistance of 1–2 megaohms. During experiments, the oocytes were clamped at −80 mV and superfused continuously in ND96 medium; all drugs were applied in this solution. Injections of IP_3 and Ca^{2+} were performed using a pressure injector and a small bore (~2-μm) injection pipette.

Materials—TRH was from Peninsula Laboratories (Belmont, CA), and 5HT was from Research Biochemicals Inc. (Natick, MA). All other drugs and reagents were from Sigma.

RESULTS

Enhancement and Inhibition of Responses by G Protein Subunits—Superfusion of TRH onto oocytes voltage-clamped at negative holding potentials (−80 mV) and expressing TRH receptor produced an inward current characteristic of the PLC- and IP_3-mediated Ca^{2+}-activated Cl^- conductance (Fig. 1*A*). We have shown previously (21) that the response increases monotonically with the receptor cRNA concentration under the conditions of these experiments. In addition, co-injection of cRNA for some G protein α subunits (Gα$_q$, Gα$_{11}$, Gα$_{14}$, Gα$_o$a, and Gα$_o$b in the range of 1–5 ng of cRNA) as well as for receptor enhances agonist-induced currents (maximum 4–6-fold increase) over the values for receptor injections alone (21).

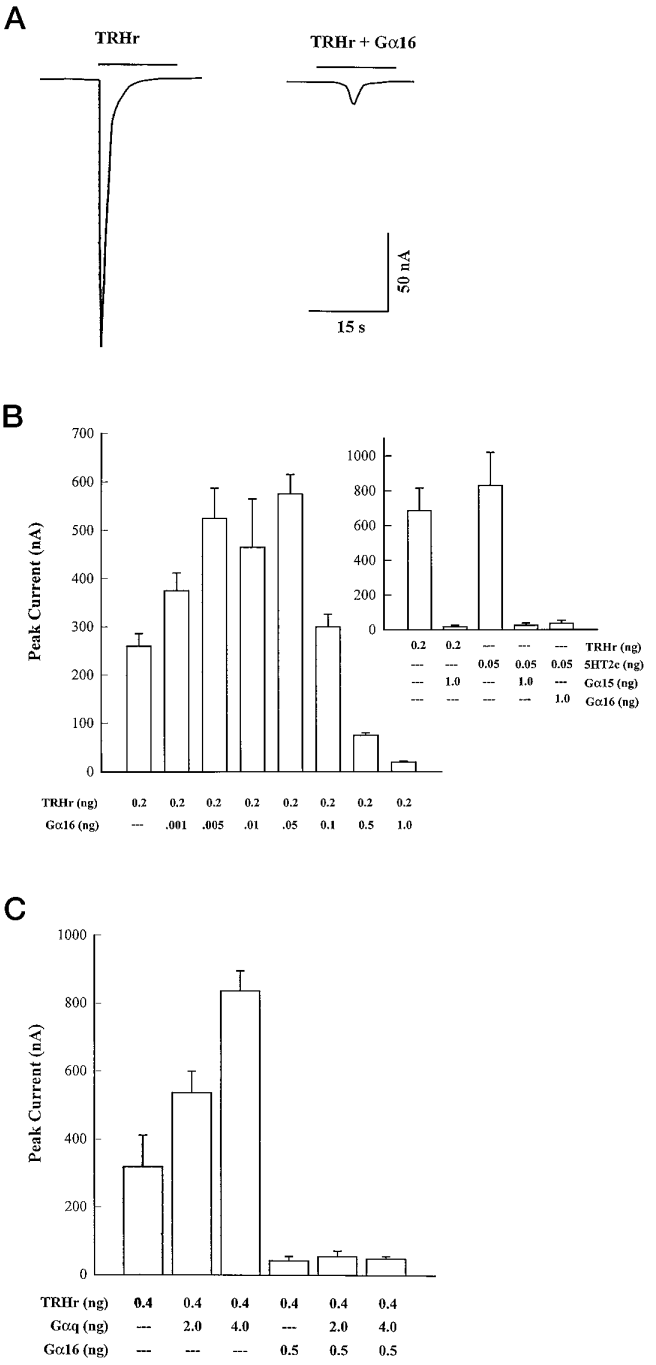

FIG. 1. **Agonist-induced Cl$^-$ currents in oocytes are inhibited by co-expression of Gα$_{16}$.** *A*, recordings from individual oocytes injected 48 h earlier with 0.2 ng of TRH receptor cRNA alone or in combination with 1 ng of Gα$_{16}$ cRNA. The *solid bars above* each *trace* represent the application of 10 nM TRH for 20 s, used for all data on TRH responses. *B*, biphasic relationship between the amount of injected Gα$_{16}$ cRNA and the magnitude of inward Cl$^-$ current elicited. The TRH responses are enhanced for Gα$_{16}$ cRNA ≤0.05 ng and inhibited at higher concentrations. Amounts of injected cRNA (ng) for each condition are shown *below* the *abscissa*. Comparable inhibitions were found substituting Gα$_{15}$ cRNA and/or 5HT2c receptor cRNA (*inset*). For the 5HT2c injected oocytes, currents were elicited with a 20-s application of 1 nM 5HT. Data are the mean ± S.E. for 7–12 oocytes/condition recorded 48 h postinjection. *C*, increased TRH-induced Cl$^-$ currents due to Gα$_q$ are inhibited by Gα$_{16}$ co-expression. Amounts of injected cRNA (ng) for each condition are shown *below* the *abscissa*. Data are the mean ± S.E. for 5–8 oocytes/condition recorded 48 h postinjection.

Surprisingly, when cRNA for another member of the G_q family, Gα$_{16}$, was co-injected with the TRH receptor cRNA, a

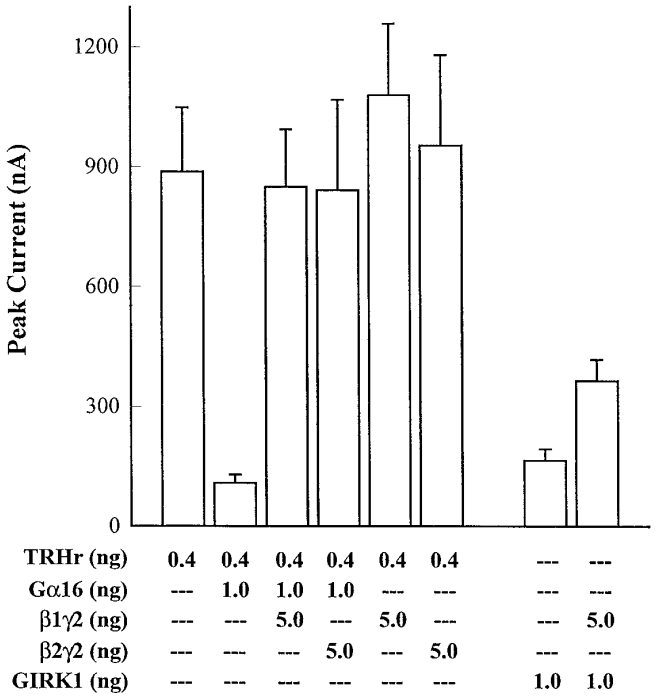

FIG. 2. **Inhibition of agonist-induced Cl^- currents by $G\alpha_{16}$ is eliminated by co-expression of various G protein β and γ subunits.** Amounts of injected cRNA (ng) for each condition are shown *below* the *abscissa*. Functional Gβ and Gγ subunit expression was confirmed by co-expression with 1 ng of GIRK1 cRNA and measurement of currents in the presence of saline containing high K^+ (96 mM KCl, 1 mM NaCl; see Ref. 30). Data are the mean ± S.E. for 6–8 oocytes/condition recorded 48 h postinjection.

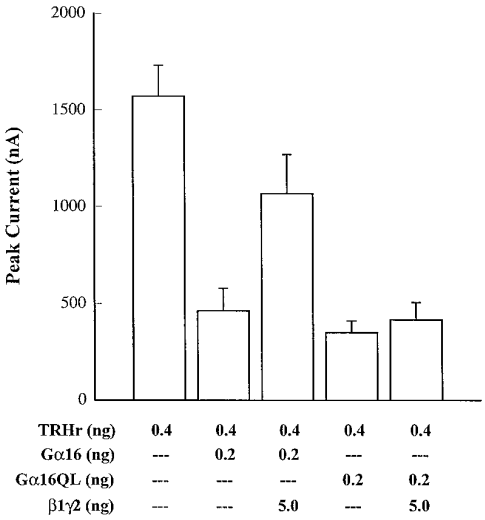

FIG. 3. **Expression of constitutively active $G\alpha_{16}$ inhibits agonist-induced Cl^- currents that are not recovered by co-expression of Gβ and Gγ subunits.** Amounts of injected cRNA (ng) for each condition are shown *below* the *abscissa*. Data are the mean ± S.E. for 6–8 oocytes/condition recorded 48 h postinjection.

strong inhibition of the TRH-induced peak inward current was observed 48 h postinjection (Fig. 1*A*). The inhibition depended on the amount of cRNA injected (Fig. 1*B*), reaching >90% at 1 ng of $G\alpha_{16}$ cRNA, and occurred at cRNA amounts 10 times lower than the injection amounts at which other G protein α subunits produced detectable increases in ligand-induced Cl^- currents (*e.g.* see Fig. 1*C*). This suggested that $G\alpha_{16}$ was more potent than other members of the $G\alpha_q$ subfamily. High potency is also suggested by the fact that even smaller quantities of $G\alpha_{16}$ cRNA (0.001–0.05 ng) actually increased TRH-induced currents (Fig. 1*B*). However, this paper focuses on the novel inhibition produced by $G\alpha_{16}$ cRNA levels at injected levels ≥0.5 ng. Each of several cRNA preparations yielded such inhibition. As was seen with oocytes expressing $G\alpha_{16}$ and TRH receptor, $G\alpha_{16}$ also inhibited peak inward currents in oocytes injected with the serotonin 5HT2c receptor and perfused with 5HT (Fig. 1*B*, *inset*). $G\alpha_{16}$ is the human homologue of murine $G\alpha_{15}$, another member of the G_q family of G protein α subunits. The murine homologue $G\alpha_{15}$ inhibited TRH or 5HT responses similarly to $G\alpha_{16}$ (Fig. 1*B*, *inset*).

We have examined other aspects of the inhibition of the signaling pathway by these two G protein α subunits. We studied first the effect of co-injecting $G\alpha_q$ cRNA (which leads to enhancement of TRH-induced Cl^- currents) with $G\alpha_{16}$ cRNA. In oocytes injected with 8 times less $G\alpha_{16}$ cRNA than $G\alpha_q$ (at $G\alpha_q$ levels that by themselves increased the TRH-induced Cl^- current 2–3-fold), the >5-fold inhibition of Cl^- currents by $G\alpha_{16}$ was the dominant effect (Fig. 1*C*).

$G\alpha_{16}$ and $G\alpha_{15}$ are expressed in hematopoietic lineages of human and mouse, respectively (12, 13). They activate the PLC β2 expressed in these lineages and also other PLC β isoforms (10, 28, 29). We considered the possibility that the inhibition observed in oocytes could be due to the inability of $G\alpha_{16}$ or $G\alpha_{15}$ to activate the corresponding PLC β isoform, termed PLC Xβ, present in *Xenopus* oocytes. However, the enhancement observed at low injected $G\alpha_{16}$ concentrations argues against this possibility. Also, we have recently shown that $G\alpha_{16}$ and $G\alpha_{15}$ can activate PLC Xβ[2] by co-expressing *Xenopus* phospholipase PLC Xβ with various Gα subunits in transiently transfected COS-7 cells. Therefore, it seems unlikely that the inhibitory effect we observed when expressing $G\alpha_{15}$ or $G\alpha_{16}$ in oocytes was due to the inability of these subunits to activate downstream effectors.

These results prompted us to seek other evidence for the hypothesis that $G\alpha_{16}$ or $G\alpha_{15}$ subunits in oocytes activate the PLC Xβ and that the prolonged activation by free Gα subunits induces Cl^- current inhibition. We co-injected G$\beta\gamma$ subunits with $G\alpha_{16}$ in presence of the TRH receptor (Fig. 2). Functional activity of the G$\beta\gamma$ dimer was verified by co-expressing these subunits with the inward rectifying atrial potassium channel (GIRK1), which is activated by free G$\beta\gamma$ subunits (31–33). Co-injection of G$\beta\gamma$ with TRH receptor and $G\alpha_{16}$ prevented most of the inhibition produced by co-injection of TRH receptor and $G\alpha_{16}$ alone. Subunit combinations consisting of $G\beta_1\gamma_2$ and $G\beta_2\gamma_2$ were each capable of recovering these currents. We believe that this effect occurs because G$\beta\gamma$ sequesters free Gα and therefore prevents the action of free Gα subunits on downstream effectors. We also considered the possibility that G$\beta\gamma$ alone had an effect on the activity of PLC Xβ independent of the Gα subunit, but we observed no significant change in ligand-induced Cl^- currents when TRH receptor was co-injected with G (in the absence of injected Gα subunit cRNA) (Fig. 2). This result argued against a direct effect of G$\beta\gamma$ on ligand-induced PLC activation under our conditions. We further addressed this question by injecting the constitutively active mutant of $G\alpha_{16}$, $G\alpha_{16}$Q212L, which does not bind to G$\beta\gamma$. The $G\alpha_{16}$Q212L mutation produced the same inhibitory effect on TRH-induced Cl^- currents as did wild-type $G\alpha_{16}$, but this effect could not be reversed by the presence of G$\beta\gamma$ (Fig. 3).

The data presented thus far are consistent with the hypothesis that free $G\alpha_{16}$ subunits produce the inhibitory effect. More specifically, $G\alpha_{16}$ would inhibit via the sequelae of overstimulation of the PLC β pathway.

Spontaneous Responses with G Protein Subunits—Further

[2] Ma, H., Magnusson, R. P., Iyengar, R., and Aragay, M., submitted for publication.

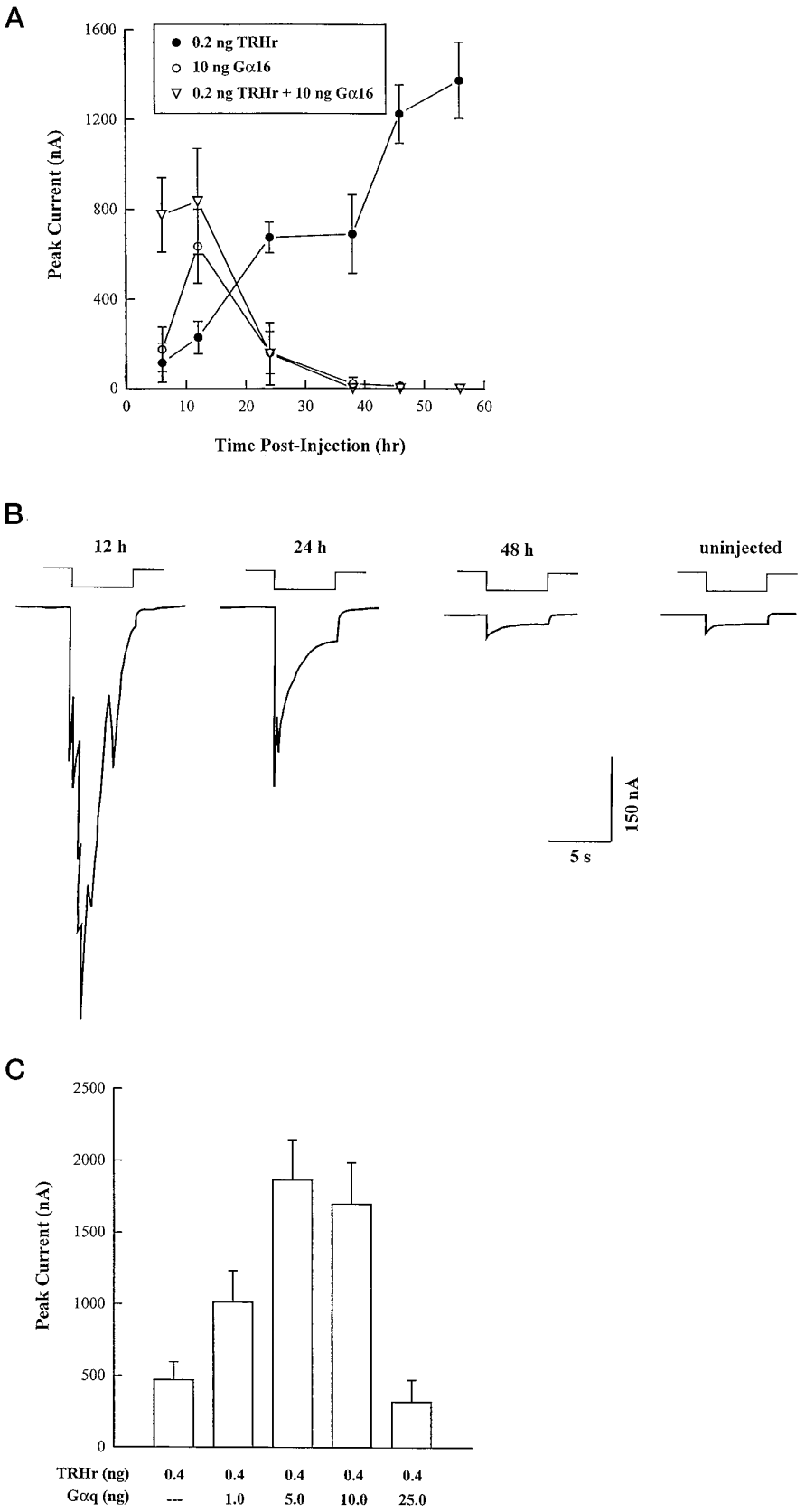

FIG. 4. **Time course of inhibition and presence of spontaneous currents for coexpression of $G\alpha_{16}$ alone.** *A*, oocytes were injected with cRNA encoding the TRH receptor (*filled circles*), $G\alpha_{16}$ (*open circles*), or both (*open triangles*). Measurement of Cl^- currents for each group of oocytes was performed at various times following cRNA injection (*abscissa*). For the two oocyte groups expressing TRH receptor, currents were elicited with at a holding potential of −80 mV. The holding current prior to TRH application was less than −200 nA in all oocytes. For the oocytes injected with $G\alpha_{16}$ cRNA alone, the oocyte was clamped at the Cl^- equilibrium potential (approximately −25 mV) and then jumped to −80 mV for 5 s. Data are the mean ± S.E. for 5–7 oocytes/condition/time point; some oocytes in each group were measured at more than one time point. *B*, recordings from individual oocytes injected with 10 ng of $G\alpha_{16}$ cRNA at various time points postinjection. The *solid bars above* each *trace* represent a voltage jump from the Cl^- equilibrium potential to −80 mV. For comparison, the response of a representative uninjected oocyte is included. *C*, increasing the concentration of injected Gaq cRNA inhibits agonist-induced Cl^- currents. Amounts of injected cRNA (ng) for each condition are shown *below* the *abscissa*. Data are the mean ± S.E. for 4–6 oocytes/condition recorded 48 h postinjection.

evidence for an activity of free Gα was obtained from examining the effect of expressing $G\alpha_{16}$ alone or with TRH receptor at various times following cRNA injection. The results of these time course experiments are shown in Fig. 4*A*. Injection of cRNA for TRH receptor produces a monotonic increase in Cl^- currents from 6 to 50 h postinjection. This response is most likely due to increasing amounts of receptor with time after injection. At 6 h postinjection, co-injection of TRH receptor and $G\alpha_{16}$ cRNA produced a ligand-induced peak inward current 4 times larger than in oocytes expressing TRH receptor alone. Contrary to the responses of oocytes expressing TRH receptor alone, oocytes with both TRH receptor and $G\alpha_{16}$ showed a

TABLE I
Comparison of spontaneous oscillations in oocytes following injection of $G\alpha_{16}$ or $G\alpha_q$ cRNA

Oocytes were injected with either $G\alpha_{16}$ (0.05 or 0.5 ng; see Fig. 1*B*) or $G\alpha_q$ (5 or 25 ng; see Fig. 4*C*). Spontaneous currents were measured (holding potential, −80 mV) in recordings lasting for 5 min at 12, 24, 36, and 48 h postinjection.

Subunit	RNA amount	Time postinjection	Spontaneous events[a]		Peak spontaneous current	
			Cell 1	Cell 2	Cell 1	Cell 2
	ng	*h*			*nA*	*nA*
$G\alpha_{16}$	0.05	12	0	0	0	0
		24	0	1:2	0	~2
		36	2:13	4:10	~3	~5
		48	2:7	2:6	~2	~3
	0.5	12	6:18	14:15	72	63
		24	13:10	3:9	~8	49
		36	0	7:11	0	~2
		48	0	0	0	0
$G\alpha_q$	5.0	12	0	0	0	0
		24	0	0	0	0
		36	0	2:6	0	~3
		48	1:8	0	~6	0
	25.0	12	1:12	8:14	40	22
		24	5:6	8:12	27	31
		36	1:15	0	~8	0
		48	1:8	0	~5	0

[a] Spontaneous events are shown as the ratio of the number of events to duration (in seconds).

decrease in peak inward currents after 12 h and complete attenuation after 36 h.

In oocytes injected with $G\alpha_{16}$ alone, we also observed an important new phenomenon: spontaneous oscillatory Cl^- currents. These spontaneous responses with $G\alpha_{16}$ also provide further evidence that the $G\alpha_{16}$ subunit alone can activate PLC Xβ. In general, injection of larger cRNA amounts resulted in larger peak spontaneous currents, larger numbers of spontaneous events, and a longer response duration at earlier time points as compared with oocytes injected with smaller amounts of cRNA. For 10-ng injections, the spontaneous signals were maximal (~600 nA) at 12 h and decreased afterwards (Fig. 4, *A* and *B*). For 0.5-ng injections, the spontaneous currents also peaked in amplitude at 12 h and vanished by 48 h but had considerably smaller amplitudes (Table I). For 0.05-ng injections, the spontaneous currents occurred only after ≥24 h, persisted until at least 48 h, and were only a few nA in amplitude (Table I). Fig. 4*A* shows that the peak and subsequent decline in the spontaneous responses for oocytes expressing $G\alpha_{16}$ alone has a time course that matches the peak and decline of TRH responses for oocytes expressing both TRH receptor and $G\alpha_{16}$, as if the spontaneous responses are first activated and later inhibited by the same mechanism that first enhances and later inhibits the receptor responses. In the most straightforward hypothesis, inhibition of the signaling pathway is the eventual result of spontaneous activation induced by $G\alpha_{16}$.

Because we hypothesize that $G\alpha_{16}$ differs from other G proteins, for instance $G\alpha_q$, primarily in the quantitative potency of coupling, we have sought and found spontaneous oscillations induced by the latter G protein as well (Table I). These responses occur at injection levels (5 and 25 ng) greater than those previously studied (21).

Inhibition at High $G\alpha_q$ Levels—The hypothesis that $G\alpha_{16}$ differs only quantitatively from other G proteins, because of intrinsic activity, also led us to seek an inhibitory effect of other G proteins. An inhibitory effect analogous to that produced by $G\alpha_{16}$ could indeed be observed with other G protein α subunits that activate PLC, again when injected at higher cRNA concentrations than previously tested (21). In Fig. 4*C* we present the results for co-injection of increasing quantities of $G\alpha_q$ cRNA with TRH receptor cRNA. Similar results were observed with $G\alpha_{11}$ co-expression and with other receptors (data not shown).

Localization of the Inhibitory Effect at IP_3 Receptors—We next sought to determine where along the pathway the inhibitory signal was produced. Mammalian PLC is the target of phosphorylation by various kinases (34, 35). Some of these modifications inactivate the enzyme. The IP_3 receptor can also be phosphorylated by various kinases, although the effects on the activity are not clear (see Ref. 36). We tested the hypothesis that activation of PLC, which leads to generation of diacylglycerol and the concomitant activation of protein kinase C, would in turn lead to the inactivation of a key messenger of this signaling cascade. Oocytes injected with 1 ng of $G\alpha_{16}$ were treated either chronically (from 1 h postinjection to the time of assay) or acutely (1 h prior to assay) with protein kinase C inhibitors (either staurosporine or bisindolylmaleimide). Little change in spontaneous Cl^- currents induced by $G\alpha_{16}$ expression was observed with any of the conditions tested (data not shown). We also co-injected excess PLC Xβ cRNA to determine whether it is the rate-limiting step in $G\alpha_{16}$ inhibition. Co-injection of PLC Xβ produced a 2-fold increase in the ligand-induced Cl^- currents but did not prevent the inhibition produced by the presence of $G\alpha_{16}$ (data not shown).

IP_3 binds to a specific receptor on intracellular membranes and is a key control point in the regulation of phospholipase-activating pathways. To study the role of the IP_3 receptor in $G\alpha_{16}$ inhibition, we injected IP_3 directly into the oocyte, bypassing G protein induction of PLC. These experiments were performed with oocytes expressing either $G\alpha_{16}$ alone or $G\alpha_{16}$ plus TRH receptor. A rapid induction of Cl^- currents was observed upon injection of 100 pmol of IP_3 in all oocytes. Fig. 5*A* summarizes the data and shows representative traces. However, oocytes injected with $G\alpha_{16}$ with or without receptor showed a marked reduction in IP_3-induced Cl^- currents (to ~30% of control).

We next studied the effect of activating the Cl^- channel directly by injecting Ca^{2+} into the oocyte. Injection of 50 pmol of $CaCl_2$ produced comparable responses in control oocytes and in oocytes injected with $G\alpha_{16}$ (Fig. 5*B*). These results indicate that the Cl^- channels themselves were not affected by the prolonged stimulation of the PLC β pathway and suggest that the reduction of the peak currents observed in oocytes expressing $G\alpha_{16}$, $G\alpha_{15}$, or other $G\alpha$ subunits in excess is a consequence of physiological alterations in IP_3 receptor responses.

DISCUSSION

Activation of GPCRs expressed in *Xenopus* oocytes generates a well characterized second messenger cascade that leads to opening of Ca^{2+}-activated Cl^- channels. However, regulation of the sensitivity in this cascade is only partially understood. In the present study, we show that the expression of the human $G\alpha_{16}$, or the murine homologue $G\alpha_{15}$, produces inhibition of peak inward Cl^- currents activated via GPCRs when measured more than 24 h postinjection. This inhibitory effect is likely the result of a desensitization process that blocks the release of Ca^{2+} from internal stores, due to the prolonged activation of PLC β by free $G\alpha_{16}$ subunits. As evidence for such a mechanism, we show that (i) expression of $G\alpha_{16}$ or $G\alpha_{15}$ inhibits the currents induced by ligands for each of two co-expressed receptors; (ii) co-expression of $G\beta\gamma$ with $G\alpha_{16}$ subunits restores this current; (iii) the presence of the activated mutant of $G\alpha_{16}$, $G\alpha_{16}$Q212L, results in a similar block of the ligand-induced Cl^- conductance, but co-injection of $G\beta\gamma$ does not restore the signal; (iv) oocytes injected with $G\alpha_{16}$ alone display a spontaneous Cl^- conductance at 6–12 h postinjection, after which peak currents decrease; and (v) in oocytes injected with $G\alpha_{16}$ and TRH receptor cRNA, agonist produces larger than normal currents at

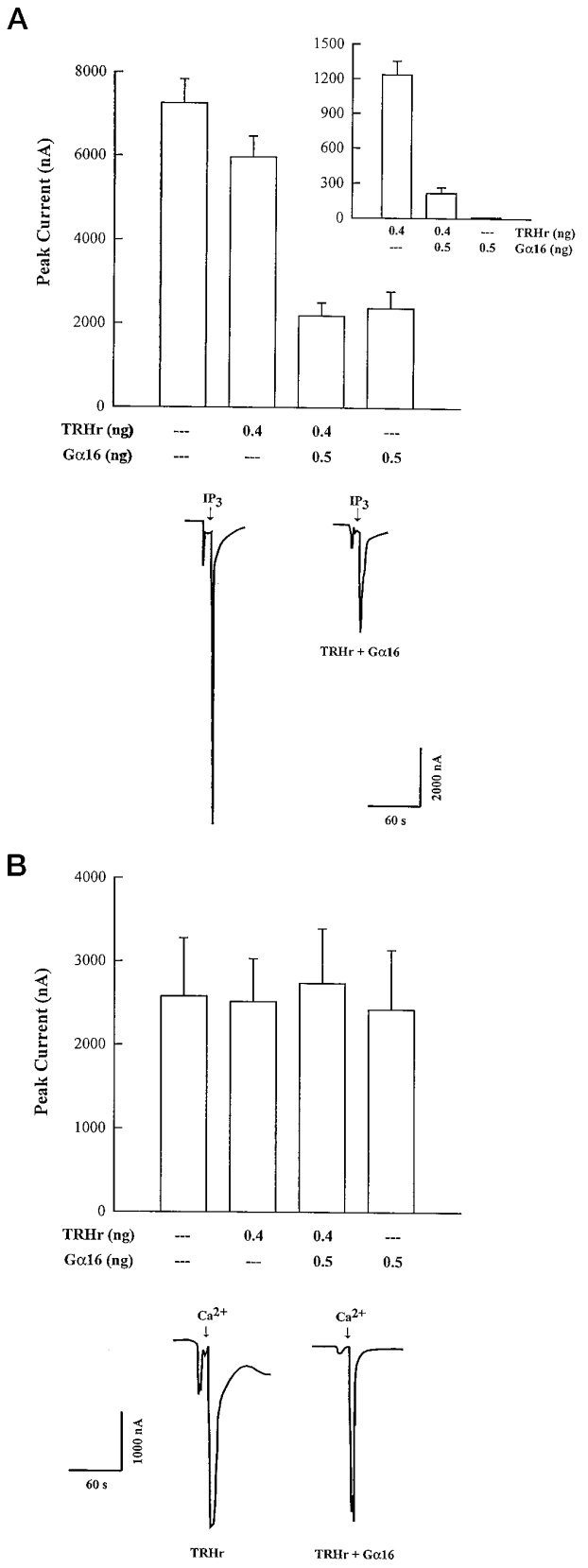

FIG. 5. **Cl^- currents induced by IP_3 injection are smaller in oocytes expressing $G\alpha_{16}$, but currents induced by Ca^{2+} injection are normal.** *A*, voltage clamp currents were measured at a holding potential of −80 mV during injection of 25 nl of H_2O containing 100 pmol of IP_3. Amounts of injected cRNA (ng) for each condition are shown *below* the *abscissa*. For comparison, currents elicited with a 20-s application of 10 nM TRH are shown in the *inset*. Data are the mean ± S.E. for 6–8 oocytes/condition recorded 48 h postinjection. *B*, injection of Ca^{2+} eliminates the $G\alpha_{16}$-mediated inhibition of Cl^- currents. Voltage-clamp currents were measured at a holding potential of −80 mV during injection of 25 nl of H_2O containing 100 pmol of IP_3. Amounts of injected cRNA (ng) for each condition are shown *below* the *abscissa*. Data are the mean ± S.E. for 4 oocytes/condition recorded 48 h postinjection. Representative *traces below* each *graph* are for oocytes expressing TRH receptor alone or in combination with $G\alpha_{16}$. The initial inward deflection prior to IP_3 or Ca^{2+} injection is a mechanical artifact due to insertion of the injection pipette.

early times postinjection (6–12 h) but a reduced response at later times. These effects suggest that the signaling cascade initiated through a single GPCR can be both enhanced and inhibited depending upon the level of G protein expression and/or activity.

The prolonged activation of PLC β by free $G\alpha_{15}$ or $G\alpha_{16}$ that results in signal inhibition is likely not a peculiarity of $G\alpha_{16}$ or $G\alpha_{15}$ subunits. Low quantities of other Gα subunit cRNAs enhance transmitter responses (21), but when higher quantities of other Gα subunit cRNAs are injected there is an inhibitory effect analogous to that seen with smaller quantities of $G\alpha_{16}$, in agreement with previous observations injecting large amounts of wild-type $G\alpha_q$ (37). We suggest that any prolonged stimulation of the PLC pathway in oocytes will result in signal down-regulation.

It is at first surprising that the rather small spontaneous currents with 0.5 ng of $G\alpha_{16}$ cRNA (60–70-nA peak, at 12 h after injection) represent activation sufficient to desensitize most of the release mechanism. We point out, however, that the spontaneous activation is present for many hours, whereas most experiments on oocytes utilize a single application of agonist, lasting just seconds. Furthermore, the IP_3 receptors that mediate the spontaneous currents have presumably been desensitized by the same process that desensitizes the responses to exogenously applied agonists. We do not know whether Ca^{2+} itself is the desensitizing factor, but previous studies show that neither activation nor desensitization of the Cl^- channel response is simply related to the concentration of intracellular Ca^{2+} (38, 39). The barely detectable spontaneous responses to 0.05 ng of $G\alpha_{16}$ (Table I) were evidently not associated with activation sufficient to desensitize the signaling pathway (Fig. 1*B*).

One important question concerns the mechanism by which the wild-type $G\alpha_{16}$ or $G\alpha_{15}$ subunit produces this dramatic effect even at relatively low quantities of cRNA injected, while other subunits need to be constitutively active or injected at higher quantities. These two subunits have the peculiarity that they couple to a broad range of GPCRs (14). One possibility could be that these G proteins in the oocyte are being activated by endogenous receptors. On the other hand, a lower affinity for endogenous Gβγ subunits or a higher efficiency in activating endogenous PLC β isoforms may account for the unusual effects of $G\alpha_{16}$ or $G\alpha_{15}$ on the Cl^- current response. It is interesting to note that injection of 1–5 ng of the other Gα subunit cRNAs produces an increase in peak current when co-injected with TRH receptor (21). Above this amount, all Gα subunits of the G_q family induce desensitization. Presumably, above a threshold at which excess Gα subunits can no longer interact with Gβγ and couple to the receptor, the free Gα subunits induce activation of PLC and induce signal desensitization. Because we have no direct measurements of Gα subunit levels, it remains possible that the unusual effects of $G\alpha_{16}$ or $G\alpha_{15}$ occur simply because unusually large amounts of these Gα subunits are produced in the oocyte.

This study shows that $G\alpha_{16}$ and $G\alpha_{15}$ subunits join the list of pertussis toxin-insensitive G protein subunits that can activate an effector in oocytes, leading to Ca^{2+} mobilization (21). cDNAs

for other pertussis toxin-insensitive G proteins, including $G\alpha_q$ and $G\alpha_{11}$, have been cloned from *Xenopus* (37, 40). Using antibodies against the C-terminal sequence of mouse G_q it has been shown that oocytes express several forms of the G_q family of α subunits (22). There has, however, been some controversy concerning the ability of G proteins of the $G\alpha_q$ family to activate the PLC cascade in the oocytes. Our earlier study showed that responses are enhanced by injection of cRNA for $G\alpha_q$ or $G\alpha_{11}$ (21), but in other reports $G\alpha_q$ or $G\alpha_{11}$ injected into the oocyte has reduced Cl^- currents (19, 41). The results presented here support the idea that the $G\alpha$ subunit of all members of the $G\alpha_q$ family can couple to GPCRs and activate the oocyte's IP_3/Ca^{2+}-induced Cl^- conductance. Under certain conditions, this coupling can eventually produce an inhibitory signal that we suggest may be due to the overexpression of G protein α subunits and, therefore, to overstimulation of phospholipase Cβ.

IP_3 is a key second messenger for the mobilization of Ca^{2+} from internal stores. An IP_3 receptor isoform has been cloned from oocytes (23). Previous experiments had shown that injection of IP_3 into oocytes renders them unresponsive to subsequent stimulation by either receptor activation or IP_3 injection (42). Recently, Honda *et al.* (43) showed that the loss of IP_3 sensitivity due to down-regulation of the IP_3 receptor is the main reason for the $G\alpha_q$-induced desensitization of the platelet-activating factor receptor response. The results presented here demonstrate that the presence of wild type $G\alpha_{16}$ and $G\alpha_{15}$ is enough to produce a similar desensitization effect and extend the observation to the other members of the G_q family. Taken together, these results suggest that the regulation of the IP_3 receptor plays a pivotal role in the propagation of agonist-mediated Ca^{2+} transients. This study provides a model to study the mechanism of a more physiological effect like heterologous desensitization, where the activation of several receptors may trigger similar down-regulation responses. The precise mechanism of down-regulation of the IP_3 receptor remains unclear.

In mammals three different isoforms of IP_3 receptors have been identified. There is accumulating evidence that individual isoforms have distinct tissue and subcellular localization, pharmacological properties, and regulation by calcium and phosphorylation (see Ref. 36). For example, the type I IP_3 receptor is down-regulated in carbachol-stimulated human neuroblastoma cells (44, 45). It will be interesting to ascertain how persistent activation of PLC by different G protein α subunits or $\beta\gamma$ subunits affects the regulation of agonist-mediated Ca^{2+} transients in other cellular environments.

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REFERENCES

1. Berridge, M. J. (1993) *Nature* **361,** 315–325
2. Rhee, S. G., and Choi, K. D. (1992) *J. Biol. Chem.* **267,** 12393–12396
3. Exton, J. H. (1996) *Annu. Rev. Toxicol. Pharmacol.* **36,** 481–509
4. Taylor, S. J., Chae, H. Z., Rhee, S. G., and Exton, J. H. (1991) *Nature* **350,** 516–518
5. Lee, C. H., Park, D., Wu, D., Rhee, S. G., and Simon, M. I. (1992) *J. Biol. Chem.* **267,** 16044–16047
6. Smrcka, A. V., and Sternweis, P. C. (1993) *J. Biol. Chem.* **268,** 9667–9674
7. Camps, M., Carozzi, A., Schnabel, P., Scheer, A., Parker, P. J., and Gierschik, P. (1992) *Nature* **360,** 684–686
8. Katz, A., Wu, D. Q., and Simon, M. I. (1992) *Nature* **360,** 686–689
9. Park, D., Jhon, D.-Y., Lee, C.-W., Lee, K.-H., and Rhee, S. G. (1993) **268,** 4573–4576
10. Wu, D. Q., Katz, A., and Simon, M. I. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 5297–5301
11. Kuang, Y., Wu, Y., Smrcka, A., Jiang, H., and Wu, D. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 2964–2968
12. Wilkie, T., Scherle, P. A., Strathmann, M. P., Slepak, V. Z., and Simon, M. I. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 10049–10053
13. Amatruda, T. T., III, Steele, D. A., Slepak, V. Z., and Simon, M. I. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 5587–5591
14. Offermanns, S., and Simon, M. I. (1995) *J. Biol. Chem.* **270,** 15175–15180
15. Zhu, X., and Birnbaumer, L. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 2827–2831
16. Blitzer, R. D., Omri, G., Devivo, M., Carty, D. J., Premont, R. T., Codina, J., Birnbaumer, L., Cotecchia, S., Caron, M. G., Lefkowitz, R. J., Landau, E. M., and Iyengar, R. (1993) *J. Biol. Chem.* **268,** 7532–7537
17. Sakuta, H., Sekiguchi, M., Okamoto, K., and Sakai, Y. (1991) *Eur. J. Pharmacol.* **208,** 31–39
18. Moriarty, T. M., Gillo, B., Sealfon, S., Roberts, J. L., Blitzer, R. D., and Landau, E. M. (1988) *Mol. Brain Res.* **4,** 75–79
19. Stehno-Bittel, L., Krapivinsky, G., Krapivinsky, L., Perez-Terzic, C., and Clapham, D. E. (1995) *J. Biol. Chem.* **270,** 30068–30074
20. Moriarty, T. M., Sealfon, S. C., Carty, D. J., Roberts, J. L., Iyengar, R., and Landau, E. M. (1989) *J. Biol. Chem.* **264,** 13524–13530
21. Quick, M. W., Simon, M. I., Davidson, N., Lester, H. A., and Aragay, A. M. (1994) *J. Biol. Chem.* **269,** 30164–30172
22. Gallo, C. J., Jones, T. L., Aragay, A. M., and Jaffe, L. A. (1996) *Dev. Biol.* **177,** 300–308
23. Kume, S., Muto, A., Aruga, J., Nakagawa, T., Michikawa, T., Furuichi, T., Nakade, S., Okano, H., and Mikoshiba, K. (1993) *Cell* **73,** 555–570
24. Jaffe, L. F. (1983) *Dev. Biol.* **99,** 265–276
25. Guastella, J., Nelson, N., Nelson, H., Czyzyk, L., Keynan, S., Miedel, M., Davidson, N., Lester, H. A., and Kanner, B. I. (1990) *Science* **249,** 1303–1305
26. Quick, M. W., and Lester, H. A. (1994) in *Methods in Neuroscience: Ion Channels of Excitable Cells* (Conn, P. M., ed) pp. 261–279, Academic Press, Inc., San Diego
27. Quick, M. W., Naeve, J., Davidson, N., and Lester, H. A. (1992) *Biotechniques* **13,** 357–362
28. Amatruda, T. T., III, Gerard, N. P., Gerard, C., and Simon, M. I. (1993) *J. Biol. Chem.* **268,** 10139–10144
29. Wu, D. Q., Katz, A., Lee, C.-H., and Simon, M. I. (1992) *J. Biol. Chem.* **267,** 25798–25802
30. Dascal, N., Lim, N. F., Schreibmayer, W., Wang, W., Davidson, N., and Lester, H. A. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 6596–6600
31. Wickman, K. D., Iñiguez-Lluhi, J. A., Davenport, P. A, Taussig, R., Krapivinsky, G. B., Linder, M. E., Gilman, A. G., and Clapham, D. E. (1994) *Nature* **368,** 255–257
32. Lim, N. F., Dascal, N., Labarca, C., Davidson, N., and Lester, H. A. (1995) *J. Gen. Physiol.* **105,** 421–439
33. Kofuji, P., Davidson, N., and Lester, H. A. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 6542–6546
34. Ryu, S. H., Kim, U.-H., Wahl, M. I., Brown, A. B., Carpenter, G., Huang, K.-P., and Rhee, S. G. (1990) *J. Biol. Chem.* **265,** 17941–17945
35. Liu, M., and Simon, M. I. (1996) *Nature* **382,** 83–87
36. Joseph, S. K. (1996) *Cell. Signal.* **8,** 1–7
37. Guttridge, K. L., Smith, L. D., and Miledi, R. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 1297–1301
38. Parker, I., and Yao, Y. (1994) *Cell Calcium* **15,** 276–288
39. Ilyin, V., and Parker, I. (1994) *J. Physiol.* (*Lond.*) **477,** 503–509
40. Shapira, H., Way, J., Lipinsky, D., Oron, Y., and Battey, J. F. (1994) *FEBS Lett.* **348,** 89–92
41. Lipinsky, D., Gershengorn, M. C., and Oron, Y. (1992) *FEBS Lett.* **307,** 237–240
42. Sugiyama, H., Ito, I., and Hirono, C. (1987) *Nature* **325,** 531–533
43. Honda, Z., Takano, T., Hirose, N., Suzuki, T., Muto, A., Kume, S., Mikoshiba, K., Itoh, K., and Shimizu, T. (1995) *J. Biol. Chem.* **270,** 4840–4844
44. Wojcikiewicz, R. J. H., Nakade, S., Mikoshiba, K., and Nahorski, S. R. (1992) *J. Neurochem.* **59,** 393–386
45. Wojcikiewicz, R. J. H., Furuichi, T., Nakade, S., Mikoshiba, K., and Nahorski, S. R. (1994) *J. Biol. Chem.* **269,** 7963–7969