We report a novel signaling pathway linking M₂ muscarinic receptors to metabotropic ion channels. Stimulation of heterologously expressed M₂ receptors, but not other G₁/G₂-associated receptors (M₁ or α₂), activates a calcium- and voltage-independent chloride current in Xenopus oocytes. We show that the stimulatory pathway linking M₂ receptors to these chloride channels consists of Gβγ stimulation of phosphoinositide 3-kinase γ (PI-3Kγ), formation of phosphatidylinositol 3,4,5-trisphosphate (PIP₃), and activation of atypical protein kinase C (PKC). The chloride current is activated in the absence of M₂ receptor stimulation by the injection of PIP₃, and PIP₃ current activation is blocked by a pseudosubstrate inhibitory peptide of atypical PKC but not other PKCs. Moreover, the current is activated by injection of recombinant PKCε at concentrations as low as 1 nM. M₂ receptor-current coupling was disrupted by inhibition of PI-3K and by injection of βγ binding peptides, but it was not affected by expression of dominant negative p85α RNA. We also show that this pathway mediates M₂ receptor coupling to metabolotropic nonselective cation channels in mammalian smooth muscle cells, thus demonstrating the broad relevance of this signaling cascade in neurotransmitter signaling.

M₂ muscarinic receptors mediate numerous cellular functions including presynaptic regulation of transmitter release by neurons in the brain and autonomic nervous system and postsynaptic control of the heart, smooth muscle, and secretory cells. Stimulation of M₂ receptors in heart cells opens inward rectifier K⁺ channels through a direct interaction between released G protein βγ subunits and channel proteins (1–6), but the signaling pathways linking M₂ receptors to ion channels in nerve, smooth muscle, and secretory cells are poorly understood. Hormone-stimulated phosphoinositide 3-kinase (PI-3K) plays an important role in cell growth, adhesion, and survival and in actin assembly (7). The identification of the Gβγ-stimulated PI-3Kγ (8–10) extends the potential range of processes mediated by PI-3Kγ to G protein-coupled receptors, although specific physiological processes mediated by PI-3Kγ have not been identified. One potential target of lipid second messengers generated by PI-3K are atypical protein kinase C enzymes (aPKCs), which lack a diacylglycerol binding site and are activated in vitro by phosphatidylinositol phosphates (11–14). Here we show that stimulation of heterologously expressed M₂ receptors, but not other G₁/G₂-linked receptors, opens endogenous metabotropic chloride channels in Xenopus oocytes by activation of PI-3Kγ, generation of PIP₃, and stimulation of aPKC. We also show that this signaling pathway mediates physiological coupling between M₂ receptors and nonselective cation channels in mammalian smooth muscle cells.

EXPERIMENTAL PROCEDURES

Xenopus Oocyte Procedures—Surgical removal of oocytes was performed in the laboratory of Dr. Peter Drain in accordance with a protocol approved by the University of Pennsylvania Animal Care and Use Committee. Oocyte defolliculation, injection, and dual-electrode voltage clamp were as described previously (15). Currents were amplified (OC-725C, Warner Instruments, Hamden, CT), filtered at 200 Hz (~3 dB; 8-pole Bessel filter, model 902, Frequency Devices, Haverhill, MA), digitized at 1 kHz (TL125, Axon Instruments, Foster City, CA), and monitored and simultaneously stored on disk (Axotape, Axon Instruments). All currents shown were leak-subtracted using identical voltage paradigms before exposure to mACH. Pipettes with resistances between 0.5 and 1 megaohm were filled with 3 M KCl. Extracellular bath solution was (concentrations in mM): 115 NaCl, 2.8 KCl, 1 MgCl₂, and 10 Hepes. Intracellular injection of all substances consisted of 50-nanoliter volumes, and the indicated concentrations assume a 20-fold dilution in the oocyte cytosol (1 μl volume). Injections were made 10 min before oocyte stimulation. Antibodies directed against specific Gα subunits were injected at the obtained concentration (1:20 final titer). Solution changes were made by washing the bath with at least 25× bath volume (1 ml).

Preparation of cRNAs—cRNA was prepared using the mMessage mMachine kit (Ambion). Plasmid DNAs were linearized with appropriate restriction enzymes, and cRNAs were synthesized using the appropriate RNA polymerase. The integrity of the cRNAs was tested on ethidium bromide-stained agarose gels, and concentrations were estimated by spectrophotometry. The pBR5 construct (16) in PGEX was obtained from Dr. M. Kasuga and subcloned in pBlueScript KS+ (Stratagene). M₂, M₃, and M₄ clones were kindly provided by Dr. E. Peralta and Dr. T. Morelli.

Patch-clamp and Myocyte Dispersion—Equine tracheal tissue was obtained in accordance with protocols approved by the University of Pennsylvania Animal Care and Use Committee. Cell isolation, whole cell recording, and agonist application were as described previously (17). Seals were formed with 3–5-megohm pipettes, and cells were dialyzed with the following (concentrations in mM): 130 CsCl, 1.2 MgCl₂, 1 MgATP, 0.1 EGTA, and 10 Hepes, pH 7.3. The bath solution was (concentrations in mM): 125 NaCl, 5 KCl, 1 MgSO₄, 1.8 CaCl₂, 10 glucose, and 10 Hepes, pH 7.4. Cells were allowed to adhere to a glass coverslip, and recordings in relaxed cells were made at room temperature. Following break-in, cells were dialyzed for 5 min before activation of currents by application of the muscarinic agonist using a puffer pipette.

Chemicals—Chelerythrine, GF109203X, G6976, and cPKC pseudosubstrate peptide were obtained from Calbiochem and PIP₃ and

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‡ To whom correspondence should be addressed: Dept. of Animal Biology, University of Pennsylvania, 3800 Spruce St., Philadelphia, PA 19104-6046. Tel.: 215-898-2389; Fax: 215-573-6810; E-mail: mlk@vet.upenn.edu.

§ The abbreviations and trivial names used are: PI-3K, phosphoinositide 3-kinase; PIP₃, phosphatidylinositol 3,4,5-trisphosphate; PKC, protein kinase C; aPKC, atypical PKC; cPKC, conventional PKC; mACH, methacholine; GF109203X, bisindolylmaleimide I.
**RESULTS AND DISCUSSION**

Heterologous expression of M₂ receptors in *Xenopus* oocytes indicated that receptor stimulation activates a novel metabotropic chloride current. The muscarinic agonist methacholine (mACH) activated only a sustained inward current in oocytes injected with M₃ receptor cRNA and recorded in calcium-free conditions, whereas a transient inward current was observed in oocytes expressing G₁₂/₁₃-coupled M₂ receptors (Fig. 1A). The transient current was shown to be the ubiquitous endogenous calcium-activated chloride current, as it was blocked by chelating intracellular calcium or by inhibiting calcium release with heparin, whereas activation of the M₂ current could be obtained repeatedly in calcium-free solutions and was not affected by intracellular calcium chelation or by blockade of calcium release. These currents were identified in early original experiments characterizing muscarinic acetylcholine receptor subtypes in *Xenopus* oocytes (18), although the sustained current was not isolated and was reported to be cation selective. Ion substitution experiments clearly identified the M₂ current as anion selective, with a selectivity sequence of I⁻ > Cl⁻ > isethionate, whereas substitution of more than 90% of the sodium for Tris had no effect on current reversal potential (Fig. 1, B and C). The chloride current activated following M₂ receptor binding was voltage-independent, and no measurable current was available in the absence of M₂ stimulation (no shift in background current observed with anion substitution), indicating that receptor binding is required for channel opening.

To examine the linkage between M₂ receptors and the novel chloride current, we used antibodies, specific peptides, dominant negative constructs, and enzyme inhibitors to disrupt receptor-effector coupling. M₂ receptor-chloride current coupling was blocked by preinjection of antibodies directed against Go₁ or Go₉, but not Go₉, proteins (Fig. 2, A and B). Anti-Gα₁/Gα₉ and anti-Gα₉ antibodies blocked 83% (n = 6) and 52% (n = 6) of the current, respectively. Whereas M₂ signaling was clearly coupled by G/Gα proteins, the M₂ current was not activated by heterologously expressed adrenergic α₁C receptors or muscarinic M₄ receptors, which coupled weakly to intracellular calcium release and the associated calcium-activated chloride current (Fig. 2C). These receptors also preferentially associate with Gα₁/Gα₉ proteins (19, 20), indicating that the signaling pathway leading to activation of the M₂ chloride current discriminates between receptors signaling through pertussis toxin-sensitive G proteins. Moreover, whereas both M₂ and M₄ receptors are capable of activating inward rectifying K⁺ channels through βγ proteins (21), the stimulatory pathway linking M₂ receptors and chloride channels effectively distinguishes between these closely related receptors.

Protein kinase C (PKC) molecules that are activated by diacylglycerol and calcium following stimulation of phospholipase C by G protein-coupled receptors have been implicated in M₂ signaling (22–25). M₂ coupling to the novel chloride current was disrupted by exposure of oocytes to the nonselective protein kinase C inhibitor chelerythrine. However, GF109203X and Go₆976 (not shown), more selective inhibitors of several conventional and novel PKC isoforms, had no effect on current activation (Fig. 3, A and B). None of the protein kinase C inhibitors affected M₃ coupling to phospholipase C, and conversely, M₃ receptor coupling was not affected by phospholipase C inhibition (Fig. 3A). PKCs that are activated by phosphatidylinositol 4,5-diphosphate, PIP₂, and cissafftyl acids have been implicated as effectors in mitogenic, apoptotic, and contractile signaling (11–13, 26, 27), although involvement of aPKCs in ion channel signaling has not been reported. We examined the role of protein kinase C subtypes in M₂ receptor coupling using peptides that selectively bind and inhibit conventional PKCs or aPKCs. Preinjection of aPKC pseudosubstrate inhibitory peptides (28), but not cPKC inhibitory peptides (29), inhibited the M₂ chloride current coupling, suggesting that aPKC activation is necessary for M₂-chloride

**Fig. 1.** M₂ receptors activate an endogenous chloride current in *Xenopus* oocytes. A, application of 50 μM mACH to oocytes injected with M₂ or M₃ cRNA elicited markedly different responses. The M₂ current response was slowly activating, sustained during muscarinic stimulation, and could be activated repeatedly in nominally Ca²⁺-free solution (inset shows second response in same oocyte). Neither calcium chelation by incubation for 4 h with 50 μM 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) nor block of intracellular calcium release by preinjection of heparin (10 mg/ml) altered the M₂ current. Conversely, stimulation of M₄-expressing oocytes activated the transient, calcium-activated chloride current, which could not be repeatedly activated in Ca²⁺-free solution and was blocked by BAPTA incubation and heparin injection. B, the M₂ current is anion-selective. Cation substitution (equimolar Tris-Cl (TrisCl)) substituted for NaCl in the bath solution did not alter the current-voltage relationship of the M₂ current, but anion substitution (sodium isethionate and NaCl for NaCl in the bath solution) markedly altered the magnitude and reversal potential of the current, as predicted for an anion-selective current. Currents shown are from voltage clamp steps to between −90 and 60 mV in 10-mV increments (Vᵣ = −60 mV), imposed during activation of the M₂ current. Current families were obtained before and after changing bath solution from the control to the test solution. Figure shows control currents for Tris-Cl experiment only. Changes in background current were negligible in test solutions. Each experiment shown was performed in at least 5 oocytes. C, current-voltage relationships for the experiments shown in B, indicating a relative anion permeability sequence of I⁻ > Cl⁻ > isethionate and no shift with cation substitution.
channel coupling.

We confirmed the role of aPKC in receptor-effector coupling by direct injection of aPKC into the cytosol (Fig. 4). Injection of recombinant PKCζ activated the chloride current in a concentration-dependent fashion, with currents observed at final concentrations of PKCζ as low as 1 nM. Moreover, injection of phosphatidylinositol 3,4,5-trisphosphate (PIP3), which stimulates PKCζ in vitro (12), activated the current. Current activation was specific to PIP3 injection. Phosphatidylglycerol, phosphatidic acid, phosphatidylethanolamine, arachidonic acid, and heated aqueous medium failed to induce any current (data not shown). PIP3 and PKCζ currents were indistinguishable from that observed following M2 receptor stimulation in several respects. First, PIP3 and PKCζ activated an anion selective current with a permeability sequence of I– > Cl– > isethionate and a current-voltage relationship identical to the M2 current. Second, the current was sustained in the absence of extracellular calcium following activation by either agent and was insensitive to calcium chelation. Third, currents activated by M2 receptor stimulation and by PIP3 or PKCζ injection were not additive. Following activation of the current by 50 μM mACH, little or no further current was elicited by the injection of PIP3 or PKCζ (n = 6), and PIP3 or PKCζ current activation abrogated subsequent mACH current (n = 5) (Fig. 4A). Finally, the aPKC pseudosubstrate inhibitor blocked both the M2 and PIP3-induced chloride currents (Fig. 4, A and B). PIP3 has been shown to stimulate novel PKCζ-ε and PKCζ-η (30) as well as atypical PKCζ activity, and the block of PIP3 currents by the aPKC pseudosubstrate peptide indicates the specific nature of PKCs mediating current activation. Moreover, neither diacylglycerol analogues nor phorbol esters activated the current (although 1-oleoyl-2-acetyl-sn-glycerol weakly activated the calcium-activated chloride current), and M2 receptor-channel current coupling was not affected by prior exposure to these agents (Fig. 4A). Thus pharmacologic and peptide inhibitors that are selective for aPKCs blocked activation of the M2 chloride current, and lipid activators of aPKCs (but not known activators of conventional and novel PKCs) and PKCζ evoked the current in the absence of M2 receptor stimulation, indicating that stimulation of aPKC is both necessary and sufficient to open M2-coupled chloride channels.

**FIG. 2.** Activation of the metabotropic chloride current is not common to all Gq/11-linked receptors. A and B, injection of subtype-specific antibodies directed against Gq/11 proteins inhibited muscarinic receptor-chloride current coupling with no effect on the M2 current, whereas anti-G12 antibodies had no effect on the M2 current, but blocked M3 calcium release and the attendant current. C, stimulation of heterologously expressed inhibitory G protein-coupled M2 and α2C receptors resulted in weak stimulation of calcium release (relative to M2 receptors) but did not activate the calcium-insensitive M2 current. Activation was specific to PIP3 injection. Phosphatidylinositol 4,5-bisphosphate, phosphatidic acid, phosphatidylcholine, linolenic acid, phosphatidylinositol 3,4,5-trisphosphate (PIP3), which stimulates PKCζ, activated the chloride current in a concentration-dependent fashion, with currents observed at final concentrations of PKCζ as low as 1 nM. Moreover, injection of phosphatidylinositol 3,4,5-trisphosphate (PIP3), which stimulates PKCζ in vitro (12), activated the current. Current activation was specific to PIP3 injection. Phosphatidylglycerol, phosphatidic acid, phosphatidylethanolamine, arachidonic acid, and heated aqueous medium failed to induce any current (data not shown). PIP3 and PKCζ currents were indistinguishable from that observed following M2 receptor stimulation in several respects. First, PIP3 and PKCζ activated an anion selective current with a permeability sequence of I– > Cl– > isethionate and a current-voltage relationship identical to the M2 current. Second, the current was sustained in the absence of extracellular calcium following activation by either agent and was insensitive to calcium chelation. Third, currents activated by M2 receptor stimulation and by PIP3 or PKCζ injection were not additive. Following activation of the current by 50 μM mACH, little or no further current was elicited by the injection of PIP3 or PKCζ (n = 6), and PIP3 or PKCζ current activation abrogated subsequent mACH current (n = 5) (Fig. 4A). Finally, the aPKC pseudosubstrate inhibitor blocked both the M2 and PIP3-induced chloride currents (Fig. 4, A and B). PIP3 has been shown to stimulate novel PKCζ-ε and PKCζ-η (30) as well as atypical PKCζ activity, and the block of PIP3 currents by the aPKC pseudosubstrate peptide indicates the specific nature of PKCs mediating current activation. Moreover, neither diacylglycerol analogues nor phorbol esters activated the current (although 1-oleoyl-2-acetyl-sn-glycerol weakly activated the calcium-activated chloride current), and M2 receptor-channel current coupling was not affected by prior exposure to these agents (Fig. 4A). Thus pharmacologic and peptide inhibitors that are selective for aPKCs blocked activation of the M2 chloride current, and lipid activators of aPKCs (but not known activators of conventional and novel PKCs) and PKCζ evoked the current in the absence of M2 receptor stimulation, indicating that stimulation of aPKC is both necessary and sufficient to open M2-coupled chloride channels.
Hormone-stimulated PI-3K activity results in the formation of PIP3, which has been implicated as a second messenger in a wide variety of cellular processes such as glucose transport, actin rearrangement, chemotaxis, and apoptosis (see Ref. 7). Activation of chloride channels by PIP3 suggested the involvement of PI-3K in the stimulatory pathway linking M2 receptor stimulation to chloride channel opening. Consistent with such a signaling cascade, the covalent PI-3K inhibitor wortmannin blocked the M2-stimulated current (Fig. 5), with no effect on M3 receptor activation, sustained chloride current (left). If injected after the chloride current was induced by mACH (50 μM), PIP3 had no effect (center). The current was blocked by preinjection of the aPKC inhibitory peptide (inhib pep, right). Middle, PKCε injection induced a chloride current with similar slow activation kinetics in a concentration-dependent fashion (left and center). The current was not available following muscarinic stimulation (right). Bottom, neither OAG (10 μM) nor phorbol 12-myristate 13-acetate (PMA, 5 μM) activated the chloride current (although OAG weakly stimulated the calcium-activated chloride current), and their application had no effect on stimulation of the chloride current by mACH. Symbol (V) indicates point of injection. B, average currents evoked in oocytes from the same batches. *, indicates significantly different from PIP3 injection alone.

Fig. 4. The M2 chloride current is activated by PIP3 and atypical PKC. A, top, injection (V) of PIP3 (10 μM) induced a slowly activating, sustained chloride current (left). If injected after the chloride current was induced by mACH (50 μM), PIP3 had no effect (center). The current was blocked by preinjection of the aPKC inhibitory peptide (inhib pep, right). Middle, PKCε injection induced a chloride current with similar slow activation kinetics in a concentration-dependent fashion (left and center). The current was not available following muscarinic stimulation (right). Bottom, neither OAG (10 μM) nor phorbol 12-myristate 13-acetate (PMA, 5 μM) activated the chloride current (although OAG weakly stimulated the calcium-activated chloride current), and their application had no effect on stimulation of the chloride current by mACH. Symbol (V) indicates point of injection. B, average currents evoked in oocytes from the same batches. *, indicates significantly different from PIP3 injection alone.

Fig. 5. M2 receptor-chloride coupling occurs through Gβγ-stimulated PI-3Kγ. A, mACH (50 μM) induced similar currents in oocytes expressing M2 receptors alone or M2 receptors and a dominant negative P85 construct (ΔP85), whereas injection of a peptide encoding a region of adenylyl cyclase 2 that binds Gβγ (QEHA peptide, 500 μM) or transducin α (5 μM) markedly inhibited the M2 current. In the experiment shown, the ΔP85 construct was injected at a 3.6-fold higher concentration than the M2 cRNA (1.8 μg/μl ΔP85 and 0.5 μg/μl M2). B, mean data from experiments described in A. Wort, wortmannin. C, concentration-dependent inhibition of the M2 current by the QEHA peptide relative to the current evoked by mACH alone in paired experiments.
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rent activation requires the release of intracellular Ca2+, although such release is not itself sufficient for channel opening without simultaneous M2 receptor engagement (37). Not surprisingly, dialysis of tracheal myocytes with PI3P did not result in the activation of Icat (data not shown). Taken together, however, these data indicate that the M2-PI3Kγ-aPKC coupling pathway likely underlies physiological postsynaptic muscarinic signaling in smooth muscle.

In summary, we demonstrate a novel signaling pathway linking M2 receptors to metabotropic ion channels. Receptor binding results in the stimulation of the Gγz-regulated PI-3Kγ, formation of PI3P, and activation of aPKC. This signaling pathway leads to the opening of a novel, second messenger-activated chloride current in Xenopus oocytes and mediates activation of nonselective cation channels in smooth muscle cells. These findings define a novel signaling cascade linking G protein-coupled receptors to membrane ion channels and provide further insight into the intricate role of lipid second messengers in receptor signaling.

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REFERENCES

1. Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J., and Clapham, D. E. (1987) Nature 325, 321–326
2. Wickman, K. D., Iniguez-Lluhi, J. A., Davenport, P. A., Tassios, R., Krapivinsky, G. B., Linder, M. E., Gilman, A. G., and Clapham, D. E. (1994) Nature 368, 255–257
3. Reuveny, E., Slesinger, P. A., Inglese, J., Morales, J. M., Iniguez-Lluhi, J. A., Lefkowitz, R. J., Bourne, H. R., Jan, Y. N., and Jan, L. Y. (1994) Nature 369, 143–146
4. Huang, C. L., Slesinger, P. A., Casey, P. J., Jan, Y. N., and Jan, L. Y. (1995) Neuron 15, 1135–1141
5. Slesinger, P. A., Reuveny, E., Jan, Y. N., and Jan, L. Y. (1995) Neuron 15, 1145–1156
6. Kunkel, M. T., and Peralta, E. G. (1995) Cell 83, 443–449
7. Toker, A., and Cantley, L. C. (1997) Nature 387, 673–676
8. Stephens, L., Smrcka, A., Cooke, F. T., Jackson, T. R., Sternweis, P. C., and Hawkins, P. T. (1994) Cell 77, 83–89
9. Thomason, P. A., James, S. K., Casey, P. J., and Downes, C. P. (1994) J. Biol. Chem. 269, 16525–16528
10. Stoyanov, B., Volinio, S., Hanck, T., Rubio, I., Loubtchenkov, M., Malek, D., Stoyanova, S., Vanhaesebroeck, B., Dhand, R., and Nurnberg, B. (1995) Science 269, 690–693
11. Ono, Y., Fujii, T., Ogita, K., Kikkawa, U., Ishiguro, K., and Nishizuka, Y. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 1103–1107
12. Nakashiba, N., Brewer, K. A., and Exton, J. H. (1993) J. Biol. Chem. 268, 13–16
13. Lifshitz, M., and Cantley, L. C. (1994) J. Biol. Chem. 269, 729–737
14. Nishizuka, Y. (1995) Science 269, 673–676
15. Toker, A., and Cantley, L. C. (1997) Science 267, 623–625
16. Hawkins, R. J., Bourne, H. R., Jan, Y. N., and Jan, L. Y. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7415–7419
17. Wang, Y.-X., and Kotlikoff, M. I. (1998) J. Biol. Chem. 273, 14920–14924
18. Hara, K., Yonezawa, K., Sakaue, H., Ando, A., Kotani, K., Kitamura, T., Kitamura, Y., Ueda, H., Stephens, L., and Jackson, T. R. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 14918–14923
19. Fukuda, K., Kubo, T., Akiba, I., Maeda, A., Mishina, M., and Numa, S. (1987) Nature 327, 623–625
20. Couphy, I., Duzic, E., and Lanier, S. (1992) J. Biol. Chem. 267, 9852–9857
21. Felder, C. C. (1995) J. Biol. Chem. 270, 4619–4625
22. Gasbrot, A. P., Riccardi, D. W., Wu, L., Hebert, S. C., and Galper, J. B. (1996) J. Biol. Chem. 271, 6388–6402
23. Morita, S., and Kobayashi, H. (1988) Neurosci. Lett. 86, 201–206
24. Marshall, J. C. (1995) Science 269, 16525–16528
25. Krapivinsky, G. B., Linder, M. E., Gilman, A. G., and Clapham, D. E. (1994) J. Biol. Chem. 271, 729–737
26. Toker, A., and Cantley, L. C. (1994) J. Biol. Chem. 269, 1019–1026
27. Lozano, J., Berra, E., Muniz, M. M., Diaz-Meco, M. T., Dominguez, S., and Moscat, J. (1994) J. Biol. Chem. 269, 19280–19289
28. Guilly, P., Gong, M. C., Somlyo, A. V., and Somlyo, A. P. (1997) J. Physiol. (London) 500, 95–109
29. Dominguez, I., Diaz-Meco, M. T., Municio, M. M., Berra, E., Garcia, de Herreros, A., Cornet, M. E., Sanz, L., and Moscat, J. (1992) Mol. Cell. Biol. 12, 3767–3773
30. House, C., and Kemp, B. E. (1987) Science 238, 1726–1728
31. Toker, A., Meyer, M., Reddy, K. K., Felck, J. R., Aneja, R., Aneja, S., Parra, A., Burns, D. J., Balis, L. A., and Cantley, L. C. (1994) J. Biol. Chem. 269, 32358–32367
32. Stephens, L. R., Eguinoa, A., Erdjument-Bromage, H., Lai, M., Cooke, F., Caudo, J., Smrcka, A. S., Theelen, M., Cadwallader, K., Temple, G., and Hawkins, P. T. (1997) Cell 89, 105–114
33. Tang, X., and Downes, C. P. (1997) J. Biol. Chem. 272, 14193–14199
34. Leopold, D., Hanck, T., Enner, T., Maier, U., Wetakier, R., and Nurnberg, B. A.
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(1998) J. Biol. Chem. 273, 7024–7029
34. Chen, J., DeVivo, M., Dingus, J., Harry, A., Li, J., Sui, J., Carty, D. J., Blank, J. L., Exton, J. H., Stoffel, R. H., Inglese, J., Lefkowitz, R. J., Logothetis, D. E., Hildebrandt, J. D., and Iyengar, R. (1995) Science 268, 1166–1169
35. Benham, C. D., Bolton, T. B., and Lang, R. J. (1985) Nature 316, 345–347
36. Byrne, N. G., and Large, W. A. (1987) Br. J. Pharmacol. 92, 371–379
37. Wang, Y. X., Fleischmann, B. K., and Kotlikoff, M. I. (1997) Am. J. Physiol. 273, C500–C508
38. Large, W. A., and Wang, Q. (1996) Am. J. Physiol. 271, C435–C454
39. Inoue, R., and Isenberg, G. (1990) J. Physiol. (London) 424, 73–92
40. Pacaud, P., and Bolton, T. B. (1991) J. Physiol. (London) 444, 477–499
41. Martiny-Baron, G., Kazanietz, M. G., Mischak, H., Blumberg, P. M., Kochs, G., Hug, H., Marne, D., and Schachtele, C. (1993) J. Biol. Chem. 268, 9184–9197