We have investigated the effect of α1-adrenergic agonist phenylephrine (PE) on acetylcholine-activated K+ currents (I_kach). I_kach was recorded in mouse atrial myocytes using the patch clamp technique. I_kach was activated by 10 μM ACh and the current decreased by 44.27 ± 2.38% (n = 12) during 4 min due to ACh-induced desensitization. When PE was applied with ACh, the extent of desensitization was markedly increased to 69.34 ± 2.22% (n = 9), indicating the presence of PE-induced desensitization. I_kach was fully recovered from desensitization after a 6-min washout. PE-induced desensitization of I_kach was not affected by protein kinase C inhibitor, calphostin C, but abolished by phospholipase C (PLC) inhibitor, neomycin. When phosphatidylinositol 4,5-bisphosphate (PIP2) replenishment was blocked by wortmannin (an inhibitor of phosphatidylinositol 3-kinase and phophatidylinositol 4-kinase), depletion of PIP2. This result may imply that PIP2 is a final regulator of the K_ACh channel in cardiac myocytes. The underlying mechanism of PIP2 action was investigated in detail for GIRK, and it was shown that the activation of GIRK channels by Gβγ depends on the presence of PIP2 (4, 6, 10–12). This result may imply that PIP2 is a final regulator molecule for GIRK channel activity and that Gβγ exerts its effect by strengthening the interaction of the channel with PIP2.

The K_ACh channel in cardiac myocytes is believed to be heterotetrameric complex formed by GIRK1 and GIRK4. Slowing of heart rate by vagal stimulation is known to be mediated by the activation of K_ACh channels, which leads to hyperpolarization of membrane potentials in pacemaker cells and in atrial myocytes (13–17). It is also well known that the effect of vagal stimulation fades gradually (vagal escape), due to desensitization of K_ACh currents (18–22). Molecular mechanisms of K_ACh activation and desensitization have been subjects of intense researches for more than a decade. However, it is not yet clear whether the recent view on the mechanism of GIRK channel regulation is valid for native K_ACh channels. It has been generally believed that direct binding of Gβγ to the channel results in activation (23, 24), but the role of PIP2 in this process is still controversial. In rat atrial myocytes, exogenously applied PIP2 and other phospholipids were reported to block agonist-mediated K_ACh channel activation (25).

The aim of the present study is to investigate the role of PIP2 in normal signaling pathway for the regulation of native K_ACh channels. Considering that PIP2 content in the membrane can be controlled by the PLC-linked receptor (26, 27), we used mouse atrial myocytes that possess both PLC-linked receptors, such as α1-adrenergic receptor and K_ACh channels. The results of the present study show that the α1-adrenergic agonist accelerates desensitization of the K_ACh channel, possibly through the depletion of the PIP2 pool in plasma membrane, supporting the hypothesis that PIP2 is acting as a signal molecule in the regulation of K_ACh channels through α1-adrenergic pathway.

**EXPERIMENTAL PROCEDURES**

**Cell Isolation**—The isolation of single atrial myocytes from mice was performed as described by Harrison et al. (28) with minor modifications. Mice were killed by cervical dislocation, and the heart was quickly removed. The heart was cannulated by a 24-gauge needle and then retrogradely perfused via the aorta on a Langendorff apparatus. During coronary perfusion all perfusates were maintained at 37 °C and equilibrated with 100% O2. Initially the heart was perfused with normal Tyrode solution for 2–3 min to clear the blood. The heart was then perfused with Ca2+-free solution for 2 min. Finally the heart was perfused with enzyme solution for 14–16 min. Enzyme solution contains 0.14 mg ml−1 collagenase (Sigma Type 5) in Ca2+-free solution. After perfusion with enzyme solution, the atria were separated from the ventricles and chopped into small pieces. Single cells were dissociated in high K+, low Cl− storage medium from these small pieces using blunt-tip glass pipette. Cells were stored at 4 °C until use.
Materials and Solutions—Normal Tyrode solution contained (mM): 140 NaCl, 5.4 KCl, 0.5 MgCl\(_2\), 1.8 CaCl\(_2\), 10 glucose, 5 HEPES, titrated to pH 7.4 with NaOH. Ca\(^{2+}\)-free solution contained (mM): 140 NaCl, 5.4 KCl, 0.5 MgCl\(_2\), 10 glucose, 5 HEPES, titrated to pH 7.4 with NaOH. The high K\(^+\), low Cl\(^-\) storage medium contained (mM): 70 KOH, 40 KCl, 50 L-glutamic acid, 20 taurine, 20 KH\(_2\)PO\(_4\), 3 MgCl\(_2\), 10 glucose, 5 HEPES, 0.5 EGTA. Pipette solution contained (mM): 140 KCl, 10 HEPES, 1 MgCl\(_2\), 5 EGTA, titrated to pH 7.2 with KOH. ACh chloride (Sigma), phenylephrine (Sigma), and neomycin (Biomol) were dissolved in deionized water to make a stock solution and stored at \(-20^\circ\) C. On the day of experiments one aliquot was thawed and used. Calphostin C (Biomol) and wortmannin (WMN; Biomol) were first dissolved in dimethyl sulfoxide (MeSO) as a stock solution and then used at the final concentration in the solution. All experiments were performed at 35 \(\pm\) 1 \(^\circ\) C in the presence of ACh, 10 \(\mu\)M glibenclamide was applied to inhibit the ATP-sensitive K\(^+\) channel. Cells were superfused with solution by gravity at \(-5\) ml/min. Approximately 30 s were required to change completely the bath contents.

Voltage Clamp Recording and Analysis—Membrane currents were recorded in nystatin-perforated patch configuration using an Axopatch-1C amplifier (Axon Instruments). Nystatin forms voltage-insensitive ion pores in the membrane patch that are somewhat selective for cations over anions but are impermeant to Ca\(^{2+}\) and other multivalent ions or molecules >0.9 nm in diameter (29). This method, therefore, minimizes dialysis of intracellular constituents with the internal pipette solution. Nystatin was dissolved in MeSO at a concentration of 50 mg/ml and then added to the internal pipette solution to yield a final nystatin concentration of 200 \(\mu\)g/ml. The patch pipettes were pulled from borosilicate capillaries (Clark Electromedical Instruments, Pangbourne, United Kingdom) using a Narishige puller (FP-83; Narishige, Tokyo, Japan). We used patch pipettes with a resistance of 2–3 megohms when filled with the above pipette solutions. The electrical signals were displayed during the experiments on an oscilloscope (Tektronix, TDS 210) and a chart recorder (Gould). Data were digitized with pClamp software 5.7.1 (Axon Instruments) at a sampling rate of 1–2 kHz and filtered at 5 kHz. Voltage clamp and data acquisitions were performed by a digital interface (Digitida 1200, Axon Instruments) coupled to an IBM-compatible computer using pClamp software 5.7.1 (Axon Instruments) at a sampling rate of 1–2 kHz and filtered at 5 kHz.

Statistics and Presentation of Data—The results in the text and in the figures are presented as means \(\pm\) S.E., n = number of cells tested. Statistical analyses were performed using the Student’s t test. The difference between two groups was considered to be significant when p < 0.01 and not significant when p > 0.05.

RESULTS

Activation and Desensitization of \(I_{KACCH}\)—Acetylcholine-activated K\(^+\) current (\(I_{KACCH}\)) was activated by adding 10 \(\mu\)M acetylcholine (ACh) to the bath solution, while the cell was voltage-clamped at the holding potential of \(-40\) mV (Fig. 1A). Upon the application of ACh, a rapid increase in outward current was observed. Despite the continuous presence of ACh, the activation of \(I_{KACCH}\) was not sustained at its peak, but the amplitude of \(I_{KACCH}\) decreased slowly. We regarded this decrease as a result of ACh-induced desensitization of \(I_{KACCH}\). The ACh-induced desensitization was recovered after washout of ACh, so that the amplitude of \(I_{KACCH}\) at the second exposure to ACh in a 6-min interval was similar to that at the first exposure. In subsequent experiments, such a paired application of ACh was used for investigating the effect of various signal molecules on regulation of \(K_{ACCH}\) channel, regarding the \(I_{KACCH}\) at the first response as the control.

Characteristics of \(I_{KACCH}\) activation and desensitization were further investigated from the current-voltage (I-V) curves. I-V curves were obtained from the current response induced by voltage ramps between \(+60\) and \(-120\) mV (at a speed of \(\pm\) 0.6 V s\(^{-1}\)) from the holding potential of \(-40\) mV. The ramps were applied before ACh application (a), at peak (b), 4 min in ACh (c), and washout of ACh (d), as indicated in Fig. 1A. Corresponding I-V curves were plotted in Fig. 1B; the reversal potentials were shifted slightly to negative potentials toward \(K^+\) equilibrium potential, and the shape of I-V curves was changed by ACh. The degree of inward rectification was less strong in the presence of ACh (b and c) compared with that in control (a). A strong inward rectification in “a” is considered to be typical for inward rectifying K\(^+\) currents, IRK (30). The I-V curves for net \(I_{KACCH}\) were obtained by subtracting the control curve (a) from the I-V curves in the presence of ACh, as shown in Fig. 1C: “b-a” represents \(I_{KACCH}\) at peak (\(I_{KACCH}\), peak), and “c-a” represents \(I_{KACCH}\) at 4 min in ACh (\(I_{KACCH}\), 4 min). The shape of inward rectification and the reversal potential of two curves were not different, indicating that the decrease in current amplitude during exposure to ACh occurs uniformly over the voltage range tested.

Desensitization of \(I_{KACCH}\) Is Accelerated by \(\alpha_1\)-Adrenergic Agonist—In Fig. 2A, 100 \(\mu\)M phenylephrine (PE) was applied together at the second exposure to ACh. From the continuous recording of current trace at \(-40\) mV, it was noticed that the process of desensitization was markedly accelerated by PE, resulting in a greater reduction of \(I_{KACCH}\) after the same period (Fig. 2A). But, the amplitude of \(I_{KACCH}\) at peak was not signifi-
We then tested the effect of PLC inhibitor, neomycin. When neomycin (500 μM) was pretreated before the application of PE at the second exposure to ACh, the increase in desensitization by PE was no longer observed (Fig. 3C). This finding suggests that PE-induced desensitization of \(I_{K\text{ACH}}\) is antagonized by neomycin. The I-V curve for desensitized current in the presence of neomycin and PE (b'-c') was significantly smaller than that in the presence of PE (b-a). The extent of desensitization in the presence of PE and neomycin was 48.79 ± 3.95% (n = 9), showing a significant difference from the extent of desensitization in the presence of PE (69.34 ± 2.22%), but not different from the control (44.27 ± 2.38%). Neomycin itself did not significantly affect the activation of \(I_{K\text{ACH}}\) and ACh-induced desensitization (\(I_{K\text{ACH, peak}}\): 759.20 ± 93.43 pA; desensitization: 48.65 ± 3.76%, n = 7).

The extent of desensitization obtained in various conditions was summarized in Fig. 3E. Based on these results, it is suggested that PE-induced desensitization of \(I_{K\text{ACH}}\) is the result of the activation of PLC, but not through the activation of PKC.

### Effect of the Depletion of PIP\(_2\) Pool by Wortmannin on \(I_{K\text{ACH}}\)

We postulated that PLC involvement is related with PIP\(_2\), and this possibility was tested by using WMN (an inhibitor of PI 3-kinase and PI 4-kinase). It was reported that WMN inhibits the replenishment of PIP\(_2\) after the depletion of PIP\(_2\) by the receptor-mediated activation of PLC (27). Therefore, we examined whether the inhibition of PIP\(_2\) replenishment by WMN affects PE-induced desensitization of \(K_{\text{ACH}}\) current and its recovery. In Fig. 4A, we first applied ACh and PE simultaneously to induce the increased desensitization of \(I_{K\text{ACH}}\) and confirmed the full recovery of \(I_{K\text{ACH}}\) from desensitization after a 6-min washout with normal Tyrode solution. Then the same series of experiment was performed in the presence of 100 μM WMN (Fig. 4B). PE-induced desensitization of \(I_{K\text{ACH}}\) was greatly accelerated by WMN, and \(I_{K\text{ACH, 4 min}}\) became very smaller. The extent of desensitization in the presence of WMN was 97.25 ± 7.63% (n = 6), and this value was significantly greater than 64.22 ± 5.70% (n = 7) in the absence of WMN. In contrast, WMN alone without PE did not significantly affect the activation and desensitization of \(I_{K\text{ACH}}\) (\(I_{K\text{ACH, peak}}\): 603.14 ± 80.63 pA; desensitization: 45.84 ± 4.07%, n = 6, data not shown). These results suggest that blockade of PIP\(_2\) synthesis by WMN facilitated PE-induced desensitization, but not ACh-induced desensitization.

### PLC, but Not PKC, Is Involved in the Increased Desensitization by PE

To elucidate the mechanisms for PE-induced desensitization, we blocked each step of the signal transduction pathway related with PE. When PKC inhibitor, calphostin C (2.5 μM), was pretreated before the application of PE at the second exposure to ACh, acceleration of \(I_{K\text{ACH}}\) desensitization by PE was still observed (Fig. 3A). To focus on the change in desensitization, I-V curves for desensitized current were plotted in Fig. 3B. It was noticed that desensitized current in the presence of PE and calphostin C (b'-c') was almost completely overlapped by that in the presence of PE only (b-c). The extent of desensitization in the presence of PE and calphostin C was 79.20 ± 4.46% (n = 4), indicating no significant difference from that in the presence of PE only (69.34 ± 2.22%). Furthermore, phorbol 12-myristate 13-acetate (100 nM), a specific PKC activator, did not mimic the effect of PE on the desensitization of \(I_{K\text{ACH}}\) (n = 5, data not shown). The amplitude of \(I_{K\text{ACH}}\) at peak was affected neither by calphostin C nor by phorbol 12-myristate 13-acetate.

The recovery from PE-induced desensitization was also affected by WMN (Fig. 4B). In the continuous presence of WMN,
the amplitude of $I_{K_{ACH}, \text{peak}}$ measured at the second exposure to ACh was only $19.65 \pm 2.61\%$ ($n = 6$) of the preceding level (Fig. 4D), indicating that the recovery from desensitization was significantly inhibited by WMN. WMN also inhibited the recovery from ACh-induced desensitization, but to a lesser extent: $I_{K_{ACH}, \text{peak}}$ measured at the second exposure to ACh in the absence of PE was $70.92 \pm 9.18\%$ of preceding level ($n = 6$, data not shown). The degree of inhibition is comparable with the magnitude reduction of basal PIP$_2$ levels in unstimulated cells by WMN in this period time (27), suggesting that the incomplete recovery of ACh-induced desensitization was the result of basal reduction of PIP$_2$ level by WMN.

Facilitation of PE effect by WMN was further confirmed in Fig. 4C, where PIP$_2$ pool in the membrane was depleted before the first exposure to ACh by pretreating WMN and PE for 3 min. The amplitude of $I_{K_{ACH}, \text{peak}}$ was only $178.33 \pm 85.36\%$ ($n = 3$), and this value was significantly smaller than $I_{K_{ACH}, \text{peak}}$ in control ($775.90 \pm 90.29\%$, $n = 12$). Above results imply that depletion of PIP$_2$ pool by PE causes a decrease in $I_{K_{ACH}}$ and that the increase in desensitization by PE can be explained by the same mechanism.

**Effect of PE on IRK**—As well as $K_{ACH}$ channel, IRK is known to be regulated by PIP$_2$ (4, 6). We examined whether IRK is affected by the various substances used in the present study. IRK was determined from the I-V curve, as described previously in Fig. 1B. The amplitude was measured at $-120$ mV, where IRK is large. IRK was not affected either by PE (100 $\mu$M) or by WMN (100 $\mu$M). However, the addition of PE in the presence of WMN decreased IRK by $31.41 \pm 1.93\%$. These results suggest that the change of PIP$_2$ level induced by normal signal molecule such as PE may not contribute to IRK regulation, although IRK is inhibited when PIP$_2$ level is reduced further down by inhibiting its replenishment.

**DISCUSSION**

The main question addressed in the present study is whether PIP$_2$ is acting as a signal molecule for the regulation of native $K_{ACH}$ channels. The results obtained can be summarized as follows: 1) PE, $\alpha_1$-adrenergic agonist, accelerates desensitization of the $I_{K_{ACH}}$; 2) PE-induced desensitization was inhibited by PLC inhibitor, neomycin, but not by PKC inhibitor, calphostin C; 3) when wortmannin, an inhibitor PI 3-kinase and PI 4-kinase was applied with PE, desensitization of $I_{K_{ACH}}$ was further accelerated, and the recovery from desensitization was inhibited. From these results it was suggested that $K_{ACH}$ channels are regulated by $\alpha_1$-adrenergic agonist through the depletion of the PIP$_2$ pool in plasma membrane.

Classical signal molecules produced by the activation of PLC-linked receptors are IP$_3$ and diacylglycerol. The role of IP$_3$ on ion channels has not been reported in cardiac myocytes, but diacylglycerol may possibly contribute to the regulation of ion channels via PKC. Desensitization of $K_{ACH}$ channel was known to be caused by phosphorylation of m2 muscarinic receptor (31–34), so it is possible that PKC is involved in the increased desensitization of $K_{ACH}$ channel by PE. We tested this possibility, but calphostin C, a PKC-specific inhibitor did not inhibit PE action (desensitization of 79.20 $\pm$ 4.46%, Fig. 3, A and E). Furthermore, the effect of PE was not mimicked by direct pharmacological activation of PKC with phorbol 12-myristate 13-acetate. These results support the idea that the mechanism for PE-induced desensitization was the depletion of PIP$_2$ rather than the production of PIP$_2$ metabolites that may inhibit the $K_{ACH}$ channel. Although we did not carry out the biochemical measurements of PIP$_2$ concentration in the present experiment, the decrease in PIP$_2$ concentration in the plasma membrane by PLC-linked receptor has been reported previously in
Chinese hamster ovary cells and human neuroblastoma cells (26, 27). The involvement of PIP$_2$ in the PE effect was further supported by the finding that the PE-induced desensitization became irreversible when the replenishment of PIP$_2$ was blocked by WMN (Fig. 4B). Furthermore when the PIP$_2$ pool was depleted by preincubation of PE and WMN, activation of the K$_{ACh}$ current was reduced (Fig. 4C).

IRK channels, on the other hand, showed a different response. In the case of IRK, PE did not affect the channel activity, although PE with WMN affected the channel activity. These data suggest that interaction of IRK with PIP$_2$ is stronger than that of K$_{ACh}$ channel, as suggested previously (4, 6, 35), and that the effect of PIP$_2$ depletion on IRK occurs at much lower concentration. Another possibility that should be tested in future studies is a co-localization of a specific PLC-linked receptor and a specific ion channel. In this view, the PIP$_2$ pool, which is regulated by PE, may not be uniformly distributed over the whole membrane, but localized closely with K$_{ACh}$ channels.

It has previously been reported by other studies that several PLC-linked receptor can inhibit K$_{ACh}$ channel. Braun et al. (36) reported that the selective α$_1$-adrenergic agonist methoxamine reduced both the IK$_1$ and K$_{ACh}$ current in rabbit atrial myocytes. Yamaguchi et al. (37) reported that endothelin-1 and endothelin-3 inhibited K$_{ACh}$ current in guinea pig atrial myocytes. Their observation is similar to the effect of PE presented in this paper, but they failed to identify the mechanism of the α$_1$-adrenergic agonist or endothelin-induced inhibition. They only demonstrated that these effects were not mediated by PKC or IP$_3$. But it now seems to be very likely that PIP$_2$ is involved in those effects. Recently, channel expression studies have demonstrated that PLC-linked receptors inhibit GIRK1/GIRK4 channels (38) or K$_{ATP}$ channels (39), and these effects were mediated by depletion of the PIP$_2$ pool in membrane.

The functional consequence of accelerated desensitization of I$_{K_ACh}$ by α$_1$-adrenergic receptor may be an early cessation of parasympathetic effect in the continuous presence of ACh. This discovery may be of particular importance, since it provides a novel pathway for sympathetic-parasympathetic interaction. Interaction between sympathetic and parasympathetic system was recognized early in various experimental conditions, and this interaction is also considered to be of clinical importance. However, the precise signal transduction pathways involved in this interaction are not fully understood, except the inhibition of adenylate cyclase by acetylcholine as a mechanism of parasympathetic antagonism to sympathetic stimulation. To our knowledge, the pathway presented in the present study seems to be the first report of a reciprocal pathway through which sympathetic stimulation can antagonize parasympathetic activity.

In conclusion, α$_1$-adrenergic agonist accelerates the desensitization of K$_{ACh}$ channel through the regulation of PIP$_2$ pool, suggesting that the receptor mediated regulation of the PIP$_2$ pool may play an important role in the control of cellular function through the modulation of ion channels.

**REFERENCES**

1. Hilgemann, D. W., and Ball, R. (1996) Science 273, 956–959
2. Lupu, V. D., Kanzacheeva, E., Krishna, U. M., Falck, J. R., and Bezprozvanny, I. (1998) J. Biol. Chem. 273, 14067–14070
3. Zhananzarov, A. B., and Ache, B. W. (1999) J. Neurosci. 19, 2929–2937
4. Huang, C. L., Feng, S., and Hilgemann, D. W. (1998) Nature 391, 803–806
5. Liu, H.-H., Zhou, S.-S., and Huang, C.-L. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 5820–5825
6. Zhang, H., He, C., Yan, X., Misrahi, T., and Logothetis, D. E. (1999) Nat. Cell Biol. 1, 183–188
7. Shyng, S. L., and Nichols, C. G. (1988) Science 242, 1138–1141
8. Baukrowitz, T., Schulte, U., Oliver, D., Herlitze, S., Krauter, T., Tucker, S. J., Ruppersberg, J. P., and Fakler, B. (1998) Science 292, 1144–1144
9. Fan, Z., and Makrides, J. C. (1997) J. Pharmacol. Exp. Ther. 283, 538–540
10. Sui, J. L., Petit, Jacques, J., and Logothetis, D. E. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 1307–1312
11. Ho, I. H. M., and Murrell-L现实ado, R. (1999) J. Physiol. (Lond.) 520, 645–651
12. Logothetis, D. E., and Zhang, H. (1999) J. Physiol. (Lond.) 520, 630
13. Sakmann, B., Noma, A., and Trautwein, W. (1983) Nature 303, 250–253
14. Breitwieser, G. E., and Szabo, G. (1985) Nature 317, 538–540
15. Pfaffinger, P. J., Martin, J. M., Hunter, D. D., Nathanison, N. M., and Hille, B. (1985) Nature 317, 536–538
16. Carmeliet, E., and Mubagw, K. (1986) J. Physiol. (Lond.) 371, 239–255
17. Kurachi, Y., Nakajima, T., and Sugimoto, T. (1986) Am. J. Physiol. 251, H1780–H1789
18. Martin, P., Levy, M. N., and Matsuda, Y. (1982) J. Physiol. (Lond.) 243, H219–H225
19. Martin, P. (1982) J. Physiol. (Lond.) 245, H584–H591
20. Boyett, M. R., and Roberts, A. (1987) J. Physiol. (Lond.) 393, 171–194
21. Boyett, M. R., Kirby, M. S., Orchard, C. H., and Roberts, A. (1988) J. Physiol. (Lond.) 404, 613–635
22. Honjo, H., Kodama, I., Zang, W. J., and Boyett, M. R. (1992) Am. J. Physiol. 263, H1779–H1789
23. Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J., and Clapham, D. E. (1987) Nature 325, 321–326
24. Krapivinsky, G., Krapivinsky, L., Wickman, K., and Clapham, D. E. (1995) J. Biol. Chem. 270, 29059–29062
25. Kim, D., and Bang, H. (1998) J. Physiol. (Lond.) 517, 59–74
26. Jenkinson, S., Nahorski, S. R., and Challiss, R. A. (1994) Mol. Pharmacol. 46, 1138–1148
27. Williams, G. B., Nahorski, S. R., and Challiss, R. A. (1998) J. Biol. Chem. 273, 5037–5046
28. Harrison, S. M., McCull, E., and Boyett, M. R. (1992) J. Physiol. (Lond.) 449, 517–550
29. Korn, S. J., and Horn, R. (1989) J. Gen. Physiol. 94, 789–812
30. Simonous, M. A., and Hartzell, H. C. (1987) Pfluegers Arch. 409, 454–461
31. Shui, Z., Boyett, M. R., Zang, W.-J., Haga, T., and Kameyama, K. (1995) J. Physiol. (Lond.) 487, 359–366
32. Richardson, R. M., and Hosey, M. M. (1992) J. Biol. Chem. 267, 22249–22255
33. Kwatra, M. M., Leung, E., Maan, A. C., McMahon, K. K., Ptasienski, J., Green, R. D., and Hosey, M. M. (1987) *J. Biol. Chem.* **262**, 16314–16321
34. Vorobiov, D., Levin, G., Lotan, I., and Dascal, N. (1998) *Pfluegers Arch.* **436**, 56–68
35. Rohacs, T., Chen, J., Prestwich, G. D., and Logothetis, D. E. (1999) *J. Biol. Chem.* **274**, 36065–36072
36. Braun, A. P., Fedida, D., and Giles, W. R. (1992) *Pfluegers Arch.* **421**, 431–439
37. Yamaguchi, H., Sakamoto, N., Watanabe, Y., Saito, T., Masuda, Y., and Nakaya, H. (1997) *Am. J. Physiol.* **273**, H1745–H1753
38. Kohrinsky, E., Mirshahi, T., and Logothetis, D. E. (1999) *Biophys. J.* **76**, A411
39. Xie, L., Horie, M., and Takano, M. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 15292–15297