The K^+ channel inward rectifier subunits form a channel similar to neuronal G protein-gated K^+ channel

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Abstract **G protein-activated inwardly rectifying K^+ channel subunits GIRK1 (Kir 3.1), GIRK2 (Kir 3.2), and CIR (Kir 3.4) were expressed individually or in combination in *Xenopus* oocytes and CHO cells. GIRK1 coexpressed with CIR or GIRK2, produced currents up to 10-fold larger than any of the subunits expressed alone. No such clear synergistic effects were observed upon coexpression of CIR/GIRK2 under the same conditions. Coexpression of G protein $\beta\gamma$ ($G_{\beta 1\gamma 2}$) increased the current through GIRK1/GIRK2 and GIRK2 channels. $G_{\beta\gamma}$ subunits purified from bovine brain, increased channel activity 50–1000-fold in patches from cells expressing GIRK1/GIRK2 or GIRK2 alone. The single GIRK1/GIRK2 channels resembled previously described neuronal G protein-gated K^+ channels. In contrast, single GIRK2 channels were short-lived and unlike any previously described neuronal K^+ channel. We propose that some neuronal G protein-activated inward rectifier K^+ channels may be formed by a GIRK1/GIRK2 heteromultimer and that $G_{\beta\gamma}$ activation may involve both subunits.**

Key words: K^+ channels; G proteins; Inward rectifier K^+ channels

1. Introduction

G protein-gated inwardly rectifying K^+ channels [1] regulate heart rate and modulate slow synaptic activity in central neurons [2]. The native G protein-gated inwardly rectifying K^+ channel in cardiac cells, I_{KACh} [3,4], resembles a current in neonatal rat locus coeruleus [5,6]. Agonist-induced increases of both cardiac and neuronal G protein-linked K^+ current are pertussis toxin (PTX)-sensitive, sharply inwardly rectify, and have similar single channel properties. Neuronal and cardiac single channels have 32–35 pS conductance in symmetrical 140 mM K^+ with mean open times of ~1 ms [4,7,8]. Somatostatin, μ-opioid, α^2-adrenergic, m2 muscarinic, and a number of other receptors activate neuronal inward rectifying current through a PTX-sensitive G protein [5,7,9]. As in cardiac atria, acetylcholine (ACh) has been shown to activate m2 receptors in the rat nucleus parabrachialis, causing an increase in K^+ conductance and hyperpolarizing these cells [10].

The inward rectifier K^+ channels may be divided into 5 families (Kir 1–5) based on homology [11]. The G protein-linked inward rectifier class of ion channels (Kir 3.0) consists of clones called GIRK1 (Kir 3.1), GIRK2 (Kir 3.2), GIRK3 (Kir 3.3), and CIR or rcKATP (Kir 3.4). The cardiac I_{KACh} channel is composed of two recently cloned members of the inward rectifier K^+ channel family, the GIRK1 (Kir 3.1) and CIR (Kir 3.4) subunits [12]. When coexpressed in oocytes, these clones give rise to a channel that is activated by $G_{\beta\gamma}$ and has characteristics of native cardiac I_{KACh}. GIRK1 and GIRK2 have been isolated from brain libraries [13], and in situ hybridization of mouse brain with ^{35}S-labeled oligonucleotide probes demonstrates an extensive colocalization of message for these two clones in many brain regions, including hippocampus, pons, and cerebellar granular layer [14].

Sequence homology mapping showed a relatively high degree of sequence identity between CIR from heart and GIRK2 from brain (74%). Reasoning that GIRK2 may substitute for CIR in a functional K^+ channel multimer, we coexpressed these clones in *Xenopus* oocytes and Chinese Hamster Ovary (CHO) cells (see also [15,16]). Here we show that coexpressed GIRK1 and GIRK2 form functional heteromultimers with properties similar to native G protein-coupled inward rectifiers in brain [7,8] and cardiac cells [4]. We also demonstrate a 16-fold increase in GIRK2 current when coexpressed with recombinant $G_{\beta\gamma}$ subunits. Together with existing evidence proposed for $G_{\beta\gamma}$ activation of GIRK1, this suggests an important role for both GIRK1 and GIRK2 in $G_{\beta\gamma}$ activation of this multimeric channel in vivo.

2. Materials and methods

2.1. Xenopus *oocyte recording*

Stage V and VI *Xenopus* oocytes were defolliculated by collagenase treatment and injected with 2 ng of CIR, GIRK2, or GIRK1 mRNA and 1 ng of m2 receptor mRNA and/or 2.5 ng of $G_{\beta 1}$ mRNA and 1.3 ng of $G_{\gamma 2}$. mRNA was transcribed in vitro from cDNA clones (mCAP; Stratagene, Megascript, Ambion). After 72 h, currents were recorded using two-electrode voltage clamp (NPI Turbotec, Darmstadt) and filtered at 1 kHz (8-pole Bessel filter). The bath solution contained (in mM): KCl 96, NaCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, and Na-HEPES 5 (pH 7.4).

Patch clamp experiments were performed using an Axopatch 200A patch clamp amplifier, and currents were filtered at 5 kHz (8-pole Bessel filter), and sampled at 20 kHz. The pipette and bath solutions contained (in mM): KCl 140, $MgCl_2$ 2, HEPES 10, EGTA 5, pH 7.2. $GdCl_3$ (10 μM) was added to the pipette solution to inhibit abundant stretch-activated channels. For mean open time and conductance determinations, the filter cutoff was set to 5 kHz.

2.2. Mammalian cell recordings

For CHO cell recordings, GIRK1 and GIRK2 were subcloned into pcDNA1 downstream from the CMV promoter and GIRK2 cDNA (10 μg), and/or GIRK1 cDNA (10 μg) with 5 μg of the L3T4 (CD4 cDNA) construct were transfected via calcium phosphate precipitation [12]. Fluorescently-labelled CD4-expressing CHO cells were assayed for cur-

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Abbreviations: CHO, Chinese hamster ovary; GIRK1 (Kir 3.1), GIRK2 (Kir 3.2) and CIR (rcKATP, Kir 3.4), G protein-gated inwardly rectifying potassium channel clones; ROMK1 (Kir 1.1) and IRK1 (Kir 2.1), inward rectifying potassium channel clones; E_K, potassium equilibrium potential; ACh, acetylcholine; G protein, GTP binding protein; $G_{\beta\gamma}$, G protein β and γ subunits; G_α, G protein α subunit.

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rents. The pipette and bath solutions were identical (in mM): KCl 140, $MgCl_2$ 2, HEPES 10, EGTA 5 (pH 7.2). Currents were recorded using an Axopatch 200A patch clamp amplifier, filtered at 5 kHz (8-pole Bessel filter), and sampled at 50 kHz. Mean open time values were obtained from inside-out patches held at −70 mV. Open time histograms were truncated to remove data binned at times < 0.2 ms.

2.3. $G_{\beta\gamma}$ preparation

G protein heterotrimers were isolated from bovine brain cholate extracts using the protocol as described by [12].

3. Results

3.1. Electrophysiological characteristics of GIRK1/GIRK2 current

G protein-activated inwardly rectifying K^+ channel clones GIRK1, GIRK2, and CIR were expressed individually or in combination in *Xenopus laevis* oocytes. The muscarinic m2 receptor clone was coinjected with each group, and current responses after agonist stimulation (ACh 5 μM) in high $[K^+]_o$ (96 mM) were compared (Fig. 1). Expression of single inward rectifier clones yielded relatively small currents at −100 mV, an average of −0.27 ± 0.02 μA for control (n = 14), −0.74 ± 0.1 μA for GIRK1 (n = 12), −0.59 ± 0.1 μA for CIR (n = 12), and −0.65 ± 0.1 μA for GIRK2 (n = 11; Fig. 1A–D). However, when GIRK1 was coexpressed with CIR or GIRK2, up to 10-fold larger currents were recorded (Fig. 1E and F). The GIRK1/CIR (Fig. 1E) and GIRK1/GIRK2 (Fig. 1F) combinations yielded −8.35 ± 0.7 μA (n = 13) and −6.72 ± 0.2 μA (n = 35) peak currents, respectively, upon voltage clamp steps to −100 mV. No such clear synergistic effects were observed upon coexpression of CIR/GIRK2 under the same conditions (average peak current −0.7 ± 0.1 μA; n = 12), suggesting that these two subunits do not synergize.

The GIRK1/GIRK2 I–V relation was characteristic of inward rectifiers while the I–V relation of control oocytes displayed relatively linear leak on this scale (Fig. 1A,G). GIRK1/GIRK2 current activated with two time courses upon hyperpolarization from the holding potential at −10 mV to −100 mV; one instantaneous phase followed by a slower, time-dependent phase (Fig. 1F). The time-dependent component of GIRK1/GIRK2 (τ = 17 ms) was slightly faster than that observed for the time-dependent activation of GIRK1/CIR (τ = 49 ms; Fig. 1E). A small voltage-dependent K^+ current with time-dependent inactivation present in most oocyte batches prevented an accurate estimate of the time constant of activation of GIRK1, CIR or GIRK2 alone (Fig. 1B–D). We attempted to express CIR with Kir 1.1 (ROMK1) or Kir 2.1 (IRK1), but large currents consistent with only ROMK1 or only IRK1 were observed. To date we have not found heteromultimer formation between subfamilies.

We tested the hypothesis that the GIRK1/GIRK2 current has properties similar to those of native neuronal G protein-coupled inward rectifier currents. We coinjected GIRK1, GIRK2 and m2 receptor mRNA into oocytes and measured current properties under voltage clamp conditions. Oocytes coinjected with the GIRK1/GIRK2 combination expressed significant inwardly rectifying current even without agonist stimulation (basal current; Fig. 2A). Current amplitudes averaging −1.1 ± 0.12 μA (n = 20) at −100 mV were increased an average

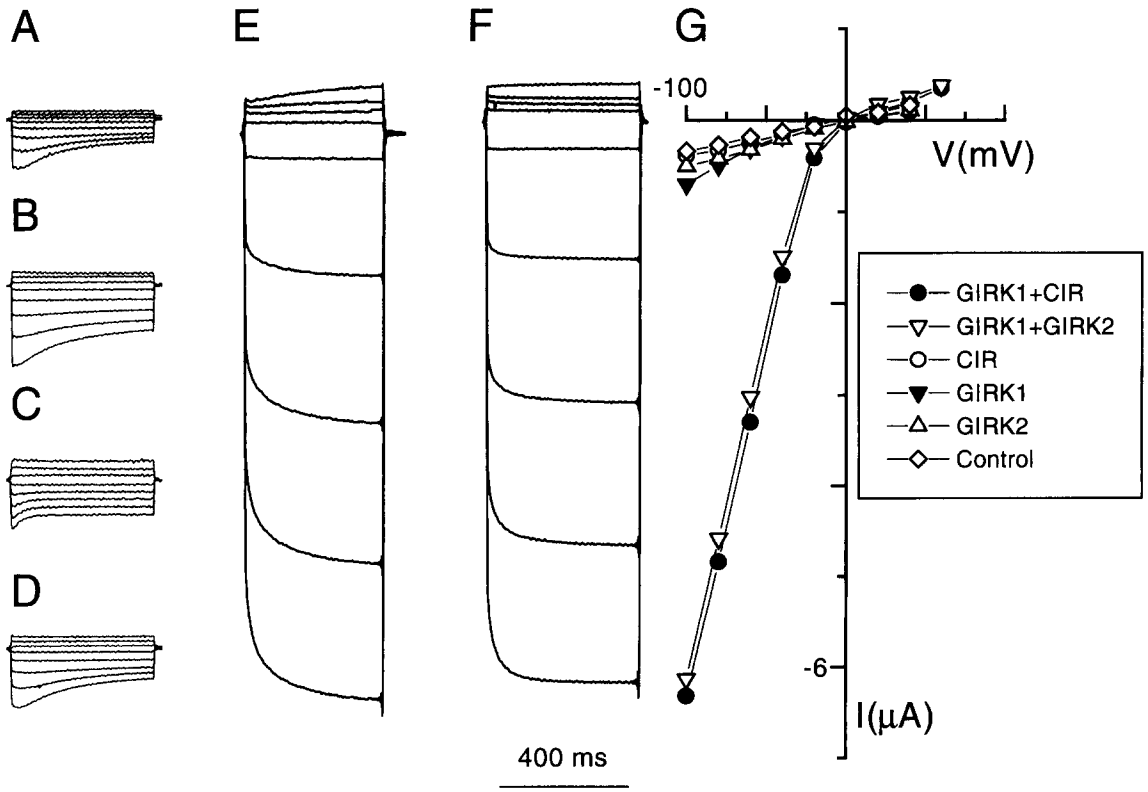

Fig. 1. Coexpression of inward rectifier clones with muscarinic M2 acetylcholine receptor. Representative current tracings for control (A), GIRK1 (B), CIR (C), GIRK2 (D), GIRK1/CIR (E), and GIRK1/GIRK2 (F). All recordings were performed in high $[K^+]_o$ (96 mM) and after agonist stimulation (ACh 5 μM). V_H = −10 mV. Voltage steps from −100 to +40 mV (A–D) or +60 mV (E,F) were made in 20 mV increments. The current scale in I–V relation (G) is common for all traces (A–F).

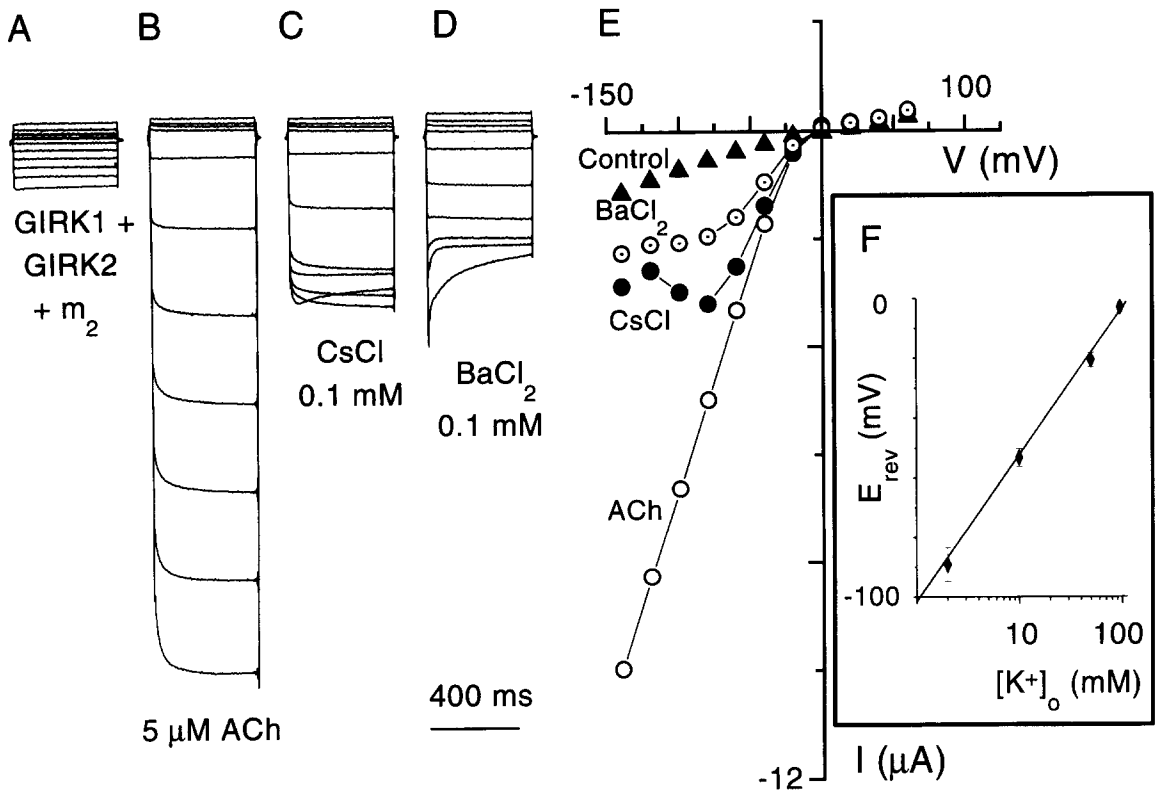

Fig. 2. Pharmacological characterization of the GIRK1/GIRK2 current. (A) GIRK1/GIRK2 coexpressed with M2 receptor were recorded in 96 mM $[K^+]_o$. After 5 μM ACh stimulation (B), oocytes were exposed to 0.1 mM CsCl (C), or 0.1 mM $BaCl_2$ (D). $V_H = -10$ mV. The current scale in the *I–V* relation (E) is common for all traces (A–E). (F) Reversal potentials were plotted as a function of $[K^+]_o$ and fit by a straight line with slope of 51 mV/decade.

of 6.7-fold by application of 5 μM ACh ($n = 20$; Fig. 2B). In order to further characterize this current, we measured current–voltage relations after applying different concentrations of Cs^+ (1 μM–1 mM) and Ba^{2+} (0.1 μM–1 mM) in the bath. The current was blocked by either Cs^+ (0.1 mM) or Ba^{2+} (0.1 mM; Fig. 2C,D), in a voltage-dependent fashion similar to the block of a native neuronal K^+ channel [17]. The EC_{50} for either $BaCl_2$ or CsCl inhibition of GIRK1/GIRK2 was ~0.1 mM at −100 mV. GIRK1/GIRK2 current was K^+-selective; the slope of the GIRK1/GIRK2 reversal potential plotted as a function of $[K^+]_o$ was 51 mV/decade (Fig. 2F).

3.2. *GIRK1/GIRK2 single channels are activated by G protein βγ subunits*

Since cardiac I_{KACh} is activated by $G_{\beta\gamma}$, not G_α [4,18,19], we tested $G_{\beta\gamma}$ modulation of GIRK1/GIRK2. Macropatches excised from oocytes expressing GIRK1/GIRK2 contained large numbers of active channels (basal activation). Transmembrane voltage was held at −80 mV during the experiment with brief steps to 0 and +80 mV to test for rectification. Basal activity of the channel was observed in the cell-attached and inside-out modes (Fig. 3A, first expanded trace). $G_{\beta\gamma}$ subunits (20 nM) purified from bovine brain [12] applied to the cytoplasmic side of the membrane activated channels in 7 of 7 patches (Fig. 3A). $G_{\beta\gamma}$ increased channel activity (Np_o) 50- to 200-fold, depending on the level of basal activity, comparable to the $G_{\beta\gamma}$ activation of cardiac recombinant I_{KACh} (GIRK1/CIR; [12]). Controls in which $G_{\beta\gamma}$ was preincubated with G_α-GDP (30 min, 30°C), prevented $G_{\beta\gamma}$ activation of the channel, as expected since G_α-GDP/$G_{\beta\gamma}$ heterotrimers are inactive [18,20]. This experiment rules out nonspecific activation of the channel by contaminants or detergents. Macropatches excised from H_2O- or m2 receptor mRNA-injected oocytes did not respond to $G_{\beta\gamma}$ application.

GIRK1/GIRK2 was also expressed in mammalian cells using the Chinese Hamster Ovary (CHO) cell line. $G_{\beta\gamma}$ applied to the cytoplasmic face of excised patches activated GIRK1/GIRK2 channels. The $G_{\beta\gamma}$-activated channels had a conductance of 32 pS and mean open time 0.9 ms at −70 mV, similar to the cardiac, neuronal, and oocyte GIRK1/GIRK2 channels. $G_{\beta\gamma}$ increased GIRK1/GIRK2 channel activity (Np_o) up to 1000-fold above basal levels (Fig. 3B).

3.3. *The single channel properties of GIRK1/GIRK2 channels*

The single channel properties of GIRK1/GIRK2 channels expressed in oocytes and activated by $G_{\beta\gamma}$ or GTPγS closely mimic those of neuronal and cardiac G protein-gated inward rectifier K^+ channels (Fig. 4A). Single channel conductance in symmetrical 140 mM K^+ (−100 to 0 mV) was 37 pS (Fig. 4C) and the mean open time measured at −80 mV was 1.4 ms (Fig. 4B). The single channel *I–V* sharply rectified in the inward direction in the presence of 2 mM bath Mg^{2+} (Fig. 4C). These properties are comparable to the measured single channel properties of neuronal G protein-gated K^+ channels [7,8].

Intracellular cations, such as Mg^{2+} and polyamines, have been proposed to contribute to the mechanism of inward rectification [4,21,22,23]. Rectification of GIRK1/GIRK2 inward

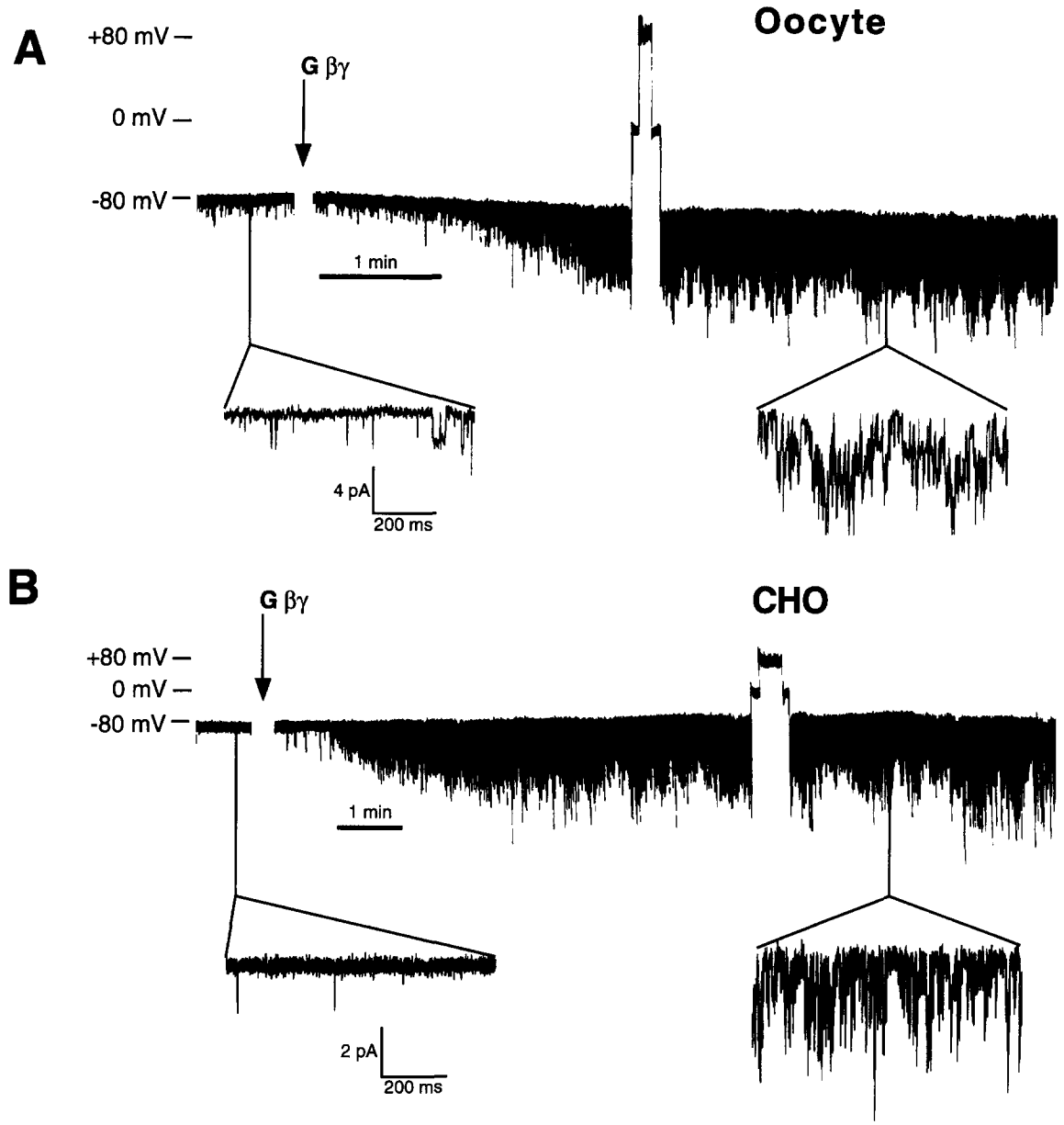

Fig. 3. $G_{\beta\gamma}$ activation of GIRK1/GIRK2 single channels. An excised macropatch from a coinjected oocyte was held at −80 mV and perfused with solution containing 20 nM $G_{\beta\gamma}$ (A). The current steps are in response to voltage steps to 0 and +80 mV. (B) Same experiment from excised patch from CHO cell cotransfected with GIRK1/GIRK2 clones.

rectifier channels was measured in macropatches excised from oocytes and activated with purified $G_{\beta\gamma}$. The excised macropatch was held at −80 mV and short potential steps to 0 and +80 mV were applied while different cytoplasmic solutions were perfused into the bath (Fig. 4D). Single channels were not observed in the outward direction in the presence of 2 mM bath Mg^{2+} (Fig. 4D, left). After perfusion with Mg^{2+}-free solution (5 mM EDTA), outward channels were observed when the potential was stepped to +80 mV. The outward component was reversibly blocked by spermine: outward currents were again observed after washout with Mg^{2+}-free solution (Fig. 4D). Application of 1 μM spermine to the cytoplasmatic side of the patch blocked outward current after Mg^{2+} removal, consistent with previous data for IRK1 [21].

3.4. *GIRK2 current is strongly augmented when coinjected with G protein βγ subunit mRNA*

Purified bovine brain $G_{\beta\gamma}$ activates CIR channels and GIRK1/CIR when applied to patches containing channels expressed in CHO oocytes and Sf9 cells [12]. To test the ability of $G_{\beta\gamma}$ to activate GIRK2 alone, GIRK2 inward rectifier subunits were expressed with recombinant $G_{\beta\gamma 2}$ in *Xenopus* oocytes. For comparison, oocytes coinjected with the M2 receptor and GIRK2 were tested after application of 5 μM ACh, yielding an average peak current at −100 mV of −0.65 ± 0.1 μA, $n = 11$ (Fig. 5A). When coexpressed with $G_{\beta 1\gamma 2}$, much larger GIRK2 currents were measured in the basal state (Fig. 5B); peak current averaged −10.37 ± 0.6 μA, $n = 12$, at −100 mV, 16-fold higher than agonist-activated (m2, ACh) GIRK2

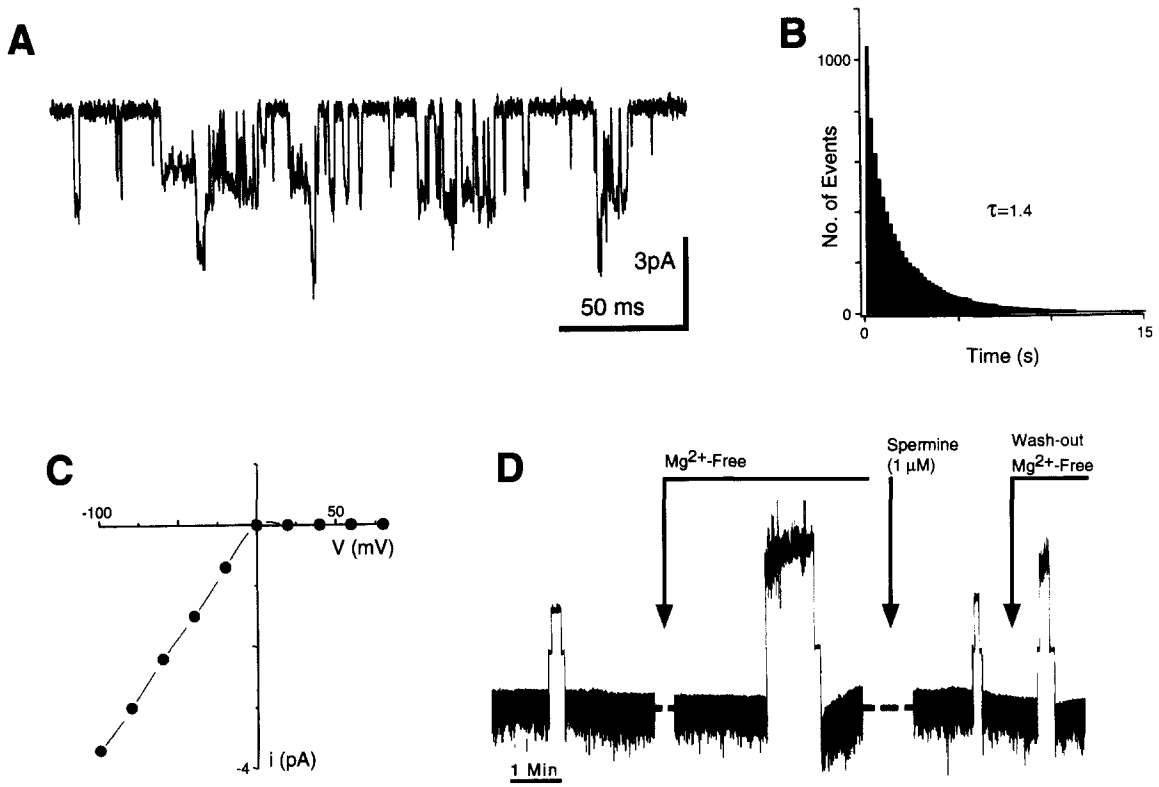

Fig. 4. Single channel properties of GIRK1/GIRK2. (A) Inside-out patch from an oocyte coexpressing GIRK1/GIRK2 activated by $G_{\beta\gamma}$ subunits. V_H = −80 mV. (B) Open time duration histogram was fit by a single exponential time constant of 1.4 ms. (C) Current–voltage relation of single channel amplitudes reveals a 37 pS conductance. (D) Excised macropatch preactivated with $G_{\beta\gamma}$ was held at −80 mV with intermittent steps to +80 mV.

(Fig. 5C,D). Interestingly, in oocytes expressing GIRK2, $G_{\beta 1\gamma 2}$, and m2, subsequent ACh application did not result in larger currents (not shown), indicating excess $G_{\beta\gamma}$ subunits had fully activated the available channels. Additionally the GIRK1/GIRK2 combination could also be further augmented when coexpressed with the $G_{\beta 1\gamma 2}$; the average current at −100 mV was −17.7 ± 1.2 μA (n = 11) compared to −6.72 ± 0.2 μA (n = 35) for agonist stimulated current (see Fig. 1F). In contrast GIRK1 currents activated either by agonist stimulation (m2, ACh) or more directly by $G_{\beta 1\gamma 2}$ coexpression, were comparable in magnitude (−0.74 ± 0.1 μA, n = 12, and −0.79 ± 0.1 μA, n = 10), and an order of magnitude smaller than GIRK2 currents with $G_{\beta 1\gamma 2}$ coexpression. In conclusion, $G_{\beta\gamma}$ strongly activated GIRK2.

To directly assay the activation of GIRK2 with $G_{\beta\gamma}$, excised macropatches of GIRK2 expressing oocytes were exposed to 20 nM purified bovine brain $G_{\beta\gamma}$. As shown in Fig. 5E, $G_{\beta\gamma}$ potently activated GIRK2 channels. Thus, GIRK1 and GIRK2 channels might both be activated by $G_{\beta\gamma}$. As a corollary, $G_{\beta\gamma}$ directly binds both GIRK1 and CIR subunits of I_{KACh} as determined by direct biochemical analysis of purified subunits and native channels [24].

Representative traces of GIRK2 recorded from oocyte patches are shown in Fig. 5F. GIRK2 channels exhibited the same properties as CIR channels expressed in oocytes, Sf9 and CHO cells: channels were short-lived and of variable conductance as previously described [12]. Single channel recordings of GIRK2 (alone) or CIR (alone) were difficult to analyze due to their short open times (see [12]). Open time constants were less than 0.1 ms and conductance varied between 20 and 40 pS. Similar channels have not been reported in native cells and are clearly different than inward rectifiers reported in neurons.

4. Discussion

We have presented evidence indicating that GIRK1 and GIRK2 form functional heteromultimers when expressed in *Xenopus* oocytes and CHO cells. The relatively small currents observed for expression of either GIRK1 or GIRK2 alone were synergistically increased when GIRK1 and GIRK2 were expressed together. This result is similar to the coexpression in oocytes of GIRK1 and CIR, two subunits that were defined biochemically to comprise the I_{KACh} channel in cardiac atria [12]. Furthermore, our results suggest that the inwardly rectifying K^+ channels described in neurons may be heteromultimers of GIRK1 and GIRK2. Coexpression of GIRK1 and GIRK2 in oocytes and CHO cells produce channels that have macroscopic and single channel characteristics similar to those described in central neurons [7,8]. In contrast, the single channel properties of either GIRK2 or CIR expressed alone are clearly different than any of the native inward rectifiers so far described. Additionally, GIRK1 does not appear to form functional homomeric channels in oocytes [25] or mammalian cell lines, and probably does not form homomeric channels in vivo [25]. The lack of a synergistic effect when GIRK2 and CIR are coexpressed suggests that the coassembly of these subunits is unlikely in oocytes, CHO cells and perhaps in vivo. Finally, our hypothesis is supported by the fact that GIRK1 and GIRK2

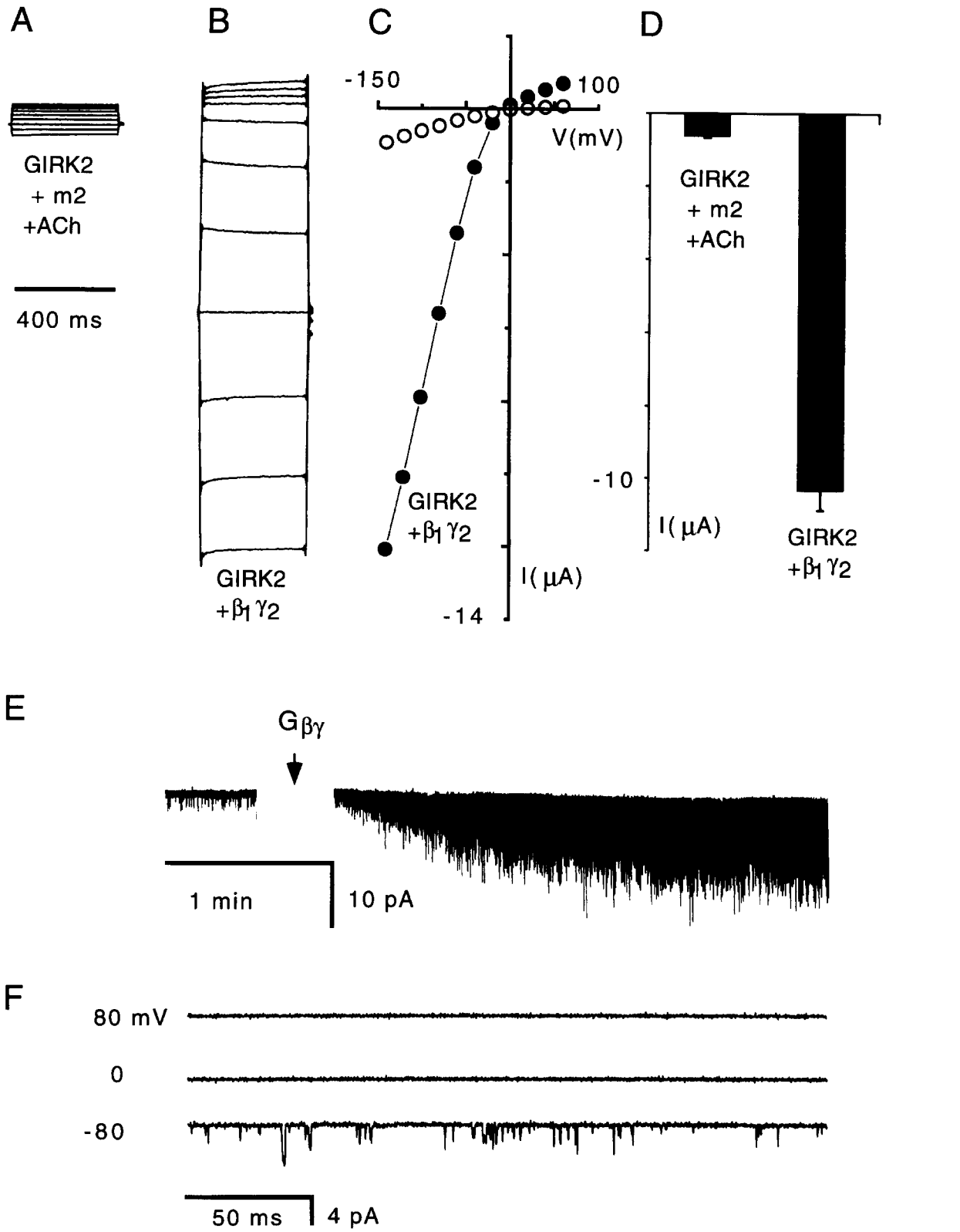

Fig. 5. $G_{\beta\gamma}$ activates GIRK2. (A) GIRK2 coexpressed in oocytes with the m2 muscarinic receptor yielded relatively small currents when exposed to 5 μM ACh. (B) Coexpression with $G_{\beta 1\gamma 2}$ dramatically increased currents to levels 16-fold above m2 + GIRK2 alone; V_H = −80 mV. (C) I–V relation for currents shown in (A) and (B). (D) Comparison of averaged currents at −100 mV. Current scale for (C) is the same for (A)–(D). (E) 20 nM bovine brain $G_{\beta\gamma}$ activated GIRK2 in macropatches from *Xenopus* oocytes. V_H = −80 mV. (F) Single Kir channels from cell-attached patch in an oocyte expressing GIRK2 and m2 receptor.

were shown to colocalize by in situ hybridization in a variety of brain regions [14]. Native neuronal channels with distinct functional characteristics may consist of other combinations of inward rectifier subunits since GIRK3 and CIR are also present in brain [12–14] and since other subunits remain to be identified. A definitive determination of the subunit composition of native inward rectifying K channels in various neurons awaits coimmunoprecipitation data from these specific cell types, a difficult task due to brain microheterogeneity.

In addition to sequence similarity, another unifying feature of GIRK1, GIRK2 and CIR is regulation by $G_{\beta\gamma}$. We observed potent stimulation of single channel current when purified $G_{\beta\gamma}$ was added to excised patches containing GIRK2, as shown previously for patches containing CIR [12]. Furthermore, whole cell currents were augmented ~16-fold when GIRK2 was coexpressed with recombinant $G_{\beta 1\gamma 2}$. Activation of basal GIRK1 [19,26], or GIRK1/GIRK2 [15,16] currents in oocytes by coexpressed $G_{\beta\gamma}$ was demonstrated earlier. Interestingly, we find that $G_{\beta\gamma}$-stimulated GIRK2 currents are also an order of magnitude larger than the maximum GIRK1 currents obtained by either receptor or $G_{\beta\gamma}$-stimulation. There are two potential explanations for this result. First, GIRK2 may be more potently activated by $G_{\beta\gamma}$ than GIRK1. Alternatively GIRK1 functional expression may be limited in oocytes. Of course, when any subunit is expressed alone in oocytes or in other expression systems, the possibility remains that the native system may contribute unknown subunits. Indeed, we have suggested that GIRK1 expression in oocytes was limited by the availability of a CIR oocyte homolog. The fact that GIRK1 does not express in mammalian cells at all unless CIR is also expressed, and the presence of an oocyte CIR-like homolog on Northern blot analysis of noninjected oocytes, supports the latter hypothesis [24]. The next task for understanding $G_{\beta\gamma}$ activation of G protein-activated heteromultimers will be to determine whether $G_{\beta\gamma}$ binds to common regions of GIRK1, GIRK2, and CIR individually, or to a domain unique to the heteromultimeric protein.

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References

[1] Clapham, D.E. (1994) Annu. Rev. Neurosci. 17, 441–464.
[2] Nicoll, R.A. (1988) Science 241, 545–551.
[3] Sakmann, B., Noma, A. and Trautwein, W. (1983) Nature 303, 250–253.
[4] Logothetis, D.E., Kurachi, Y., Galper, J., Neer, E.J. and Clapham, D.E. (1987) Nature 325, 321–326.
[5] Inoue, M., Nakajima, S., and Nakajima, Y. (1988) J. Physiol. 407, 177–198.
[6] Velimirovic, B.M., Koyano, K., Nakajima, S. and Nakajima, Y. (1995) Proc. Natl. Acad. Sci. USA 92, 1590–1594.
[7] Miyake, M., MacDonald, J.C. and North, R.A. (1989) Proc. Natl. Acad. Sci. USA 86, 3419–3422.
[8] Grigg, J.J., Kozasa, T., Nakajima, Y. and Nakajima, S. (1995) J. Neurophys. (in press).
[9] Nicoll, R.A., Malenka, R.C. and Kauer, J.A. (1990) Physiol. Rev. 70, 513–565.
[10] Egan, T. and North, R.A. (1986) Nature 319, 405–407.
[11] Doupnik, C.A. and Lester, H.A. (1995) Curr. Opin. Neurobiol. 5, 268–277.
[12] Krapivinsky, G., Gordon, E.A., Wickman, K., Velimirovic, B., Krapivinsky, L. and Clapham, D.E. (1995) Nature 374, 135–141.
[13] Lesage, F., Duprat, F., Fink, M., Guillemare, E., Coppola, T., Lazdunski, M. and Hugnot, J.-P. (1994) FEBS Lett. 353, 37–42.
[14] Kobayashi, T., Ikeda, K., Ichikawa, T., Abe, S., Tagashi, S. and Kumanishi, T. (1995) Biochem. Biophys. Res. Commun. 208, 1166–1173.
[15] Kofuji, P., Davidson, N. and Lester, H.A. (1995) Proc. Natl. Acad. Sci. USA 92, 6542–6546.
[16] Duprat, F., Lesage, F., Guillemare, E., Fink, M., Hugnot, J-P., Bigay, J., Lazdunski, M., Romey, G., and Barhanin, J. (1995) Biochem. Biophys. Res. Commun. 212, 657–663.
[17] Yamaguchi, K., Nakajima, Y., Nakajima, S. and Stanfield, P.R. (1990) J. Physiol. 426, 499–520.
[18] Wickman, K., Iniguez-Lluhi, J., Davenport, P., Taussig, R.A., Krapivinsky, G.B., Linder, M.E., Gilman, A. and Clapham, D.E. (1994) Nature 368, 255–257.
[19] 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 370, 143–146.
[20] Logothetis, D.E., Kim, D., Northup, J.K., Neer, E.J. and Clapham, D.E. (1988) Proc. Natl. Acad. Sci. USA 85, 5814–5818.
[21] Lopatin, A.N., Makhina, E.N. and Nichols, C.G. (1994) Nature 372, 366–369.
[22] Kubo, Y., Reuveny, E., Slesinger, P.A., Jan, Y.N. and Jan, L.Y. (1993) Nature 364, 802–806.
[23] Yamada, M., and Kurachi, Y. (1995) J. Biol. Chem. 270, 9289–9294.
[24] Krapivinsky, G., Krapivinsky, L., Wickman, K. and Clapham, D.E. (1995) J. Biol. Chem. 270, 29059–29062.
[25] Hedin, K.E., Lim, N.F. and Clapham, D.E. (1995) (in press).
[26] Lim, N.F., Dascal, N., Labarca, C., Davidson, N. and Lester, H.A. (1995) J. Gen. Physiol. 105, 421–439.