UC Irvine
UC Irvine Previously Published Works

Title

G protein control of potassium channel activity in a mast cell line.

Permalink

Journal

ISSN

Authors

McCloskey, MA
Cahalan, MD

Publication Date

DOI

Copyright Information

Peer reviewed

G Protein Control of Potassium Channel Activity in a Mast Cell Line

MICHAEL A. MCCLOSKEY and MICHAEL D. CAHALAN

From the Department of Physiology and Biophysics, University of California, Irvine, California 92717

ABSTRACT   Using the patch-clamp technique, we studied regulation of potassium channels by G protein activators in the histamine-secreting rat basophilic leukemia (RBL-2H3) cell line. These cells normally express inward rectifier K^+ channels, with a macroscopic whole-cell conductance in normal Ringer ranging from 1 to 16 nS/cell. This conductance is stabilized by including ATP or GTP in the pipette solution. Intracellular dialysis with any of three different activators of G proteins (GTPγS, GppNHp, or AlF_4^-) completely inhibited the inward rectifier K^+ conductance with a half-time for decline averaging ~300 s after "break-in" to achieve whole-cell recording. In addition, with a half-time averaging ~200 s, G protein activators induced the appearance of a novel time-independent outwardly rectifying K^+ conductance, which reached a maximum of 1–14 nS. The induced K^+ channels are distinct from inward rectifier channels, having a smaller single-channel conductance of ~8 pS in symmetrical 160 mM K^+, and being more sensitive to block by quinidine, but less sensitive to block by Ba^{2+}. The induced K^+ channels were also highly permeable to Rb^+ but not to Na^+ or Cs^+. The current was not activated by the second messengers Ca^{2+}, inositol 1,4,5-trisphosphate, inositol 1,3,4,5-tetrakisphosphate, or by cyclic AMP-dependent phosphorylation. Pretreatment of cells with pertussis toxin (0.1 μg/ml for 12–16 h) prevented this current's induction both by guanine nucleotides and aluminum fluoride, but had no effect on the decrease in inward rectifier conductance. Since GTPγS is known to stimulate secretion from patch-clamped rat peritoneal mast cells, it is conceivable that K^+ channels become inserted into the plasma membrane from secretory granules. However, total membrane capacitance remained nearly constant during appearance of the K^+ channels, suggesting that secretion induced by GTPγS was minimal. Furthermore, pertussis toxin had no effect on secretion triggered by antigen, and triggering of secretion before electrical recording failed to induce the outward K^+ current. Finally, GTPγS activated the K^+ channel in excised inside-out patches of membrane. We conclude that two different GTP-binding proteins differentially regulate two subsets of K^+ channels, causing the inward rectifier to close and a novel K^+ channel to open when activated.

INTRODUCTION

GTP-binding (G) proteins have been implicated in the regulation of ion channels in several types of electrically excitable cells. Neurotransmitter inhibition of voltage-

gated Ca^{2+} channels in dorsal root ganglion neurons (Holz et al., 1986), neuroblastoma x glioma hybrid cells (Hescheler et al., 1987), and a clonal pituitary cell line (Lewis et al., 1986) appears to be mediated by G proteins. Stimulation of cardiac and skeletal muscle Ca^{2+} channels by β-adrenergic agonists may involve direct channel activation by G_s, the stimulatory protein of adenylate cyclase (Yatani et al., 1987b), whereas stimulation of voltage-gated Ca^{2+} channels in GH_3 pituitary cells by leuteinizing hormone releasing hormone (LHRH) occurs via a pertussis toxin substrate, presumably a G_i species or G_o (Rosenthal et al., 1988). Similarly, receptor-mediated stimulation of K^+ channels in cardiac atria (Breitwieser and Szabo, 1985; Pfaffinger et al., 1985; Kurachi et al., 1986; Logothetis et al., 1987; Yatani et al., 1987a) and certain neurons (Andrade et al., 1986; North et al., 1987; Sasaki and Sato, 1987; VanDongen et al., 1988) appears to involve one or more G proteins. Inhibition of K^+ current by activation of G proteins has been reported for inward rectifier K^+ channels in nucleus basalis neurons (Nakajima et al., 1988) and for M current in frog sympathetic ganglion cells (Pfaffinger, 1988). So far, however, there is little or no evidence to suggest that a similar mechanism may control ion channel activity in cells excitable by means other than membrane depolarization.

Mast cells and basophils secrete histamine and other allergic mediators in response to binding of polyvalent antigen to IgE present in high affinity cell-surface Fc_ϵ receptors (Metzger et al., 1986). The rat basophilic leukemia (RBL-2H3) cell line, a homologue of mucosal mast cells (Seldin et al., 1985), has proven especially useful in biochemical and physiological studies of IgE-mediated secretion. In these cells, aggregation of Fc_ϵ receptors depolarizes the plasma membrane, provided that Ca^{2+} or Na^+ ions are present in the extracellular medium; in the absence of Ca^{2+}, cross linkage of IgE receptors causes the uptake of extracellular sodium (Kanner and Metzger, 1984). Although depolarization per se neither triggers secretion nor opens voltage-gated Ca^{2+} channels (Kanner and Metzger, 1983; Mohr and Fewtrell, 1987), antigenic stimulation causes an increase in the concentration of cytosolic Ca^{2+} (Beaven et al., 1984) and there is evidence that this is due to Ca^{2+} influx across the plasma membrane as well as Ca^{2+} release from intracellular stores (Beaven et al., 1984; Fewtrell and Sherman, 1987). There is also evidence that antigen binding to RBL cells causes a delayed efflux of cellular K^+, as well as a partial repolarization of the plasma membrane (Labrecque et al., 1988, 1989).

The present work is part of a larger effort to examine the molecular basis of the ionic permeability changes that occur upon antigenic stimulation of RBL cells. Previous patch-clamp studies have failed to detect IgE-operated ion channels in either rat peritoneal mast cells (Lindau and Fernandez, 1986a) or RBL-2H3 cells (Lindau and Fernandez, 1986b). However, these experiments with RBL cells were all conducted using the conventional (fast) whole-cell recording mode, in which cytoplasmic molecules rapidly diffuse into the patch pipette. We speculated that the loss of some essential cytoplasmic constituent(s) might have contributed to the previous failure to observe channel activation in RBL cells. This, combined with the observation that G_s or another cholera toxin substrate modulates transmembrane signaling via the IgE receptor (McCloskey, 1988; Narasimhan et al., 1988), led us to explore the possible effects of GTP analogues on the electrical properties of RBL cells.

We found that dialysis of cells with various G protein activators caused the time-dependent appearance of an outward K$^+$ conductance, while accelerating decay of the inward K$^+$ rectifier channels present in these cells (Lindau and Fernandez, 1986*b*). The channel responsible for the induced outward current has a different voltage dependence, a smaller unitary conductance and different pharmacology than the inward rectifier. Our evidence suggests that these new K$^+$ channels are activated by a G protein, although the commonly known soluble second messengers do not appear to be involved. This G protein appears to be a substrate for ADP-ribosylation by pertussis toxin, suggesting that it may be related to G$_i$ or G$_o$. The receptors that normally regulate either K$^+$ channel remain to be identified.

This work was presented in preliminary form at the 1988 meeting of the Society of General Physiologists (McCloskey and Cahalan, 1988).

MATERIALS AND METHODS

Chemicals

Guanosine 5'-(3-*O*-thio)triphosphate, tetralithium salt, (GTPγS) was purchased from Boehringer-Manheim (Indianapolis, IN). Quinidine hydrochloride as well as the sodium salts of 5'-guanylylimidodiphosphate (GppNHp) and adenosine-5'-triphosphate (ATP) were obtained from Sigma Chemical Co. (St. Louis, MO). Inositol 1,4,5-triphosphate (IP$_3$, K$^+$ salt) also was purchased from Sigma, and inositol 1,3,4,5-tetrakisphosphate (IP$_4$, K$^+$ salt) was obtained from Calbiochem-Behring Corp. (La Jolla, CA). Pertussis and cholera toxins were purchased from List Biological Laboratories (Campbell, CA) and stored in phosphate-buffered saline at 4°C. Ascites fluid containing the mouse monoclonal antidinitrophenyl (DNP) IgE from the H1-ε-DNP-26.82 hybridoma (Liu et al., 1980) was supplied by Dr. Fu-tong Liu (Medical Biology Institute, La Jolla, CA). Trinitrophenylated-bovine serum albumin (TNP-BSA) was prepared by treatment of BSA with a 15-fold molar excess of trinitrobenzenesulfonic acid in borate-buffered saline (pH 9.0) for 5 h at 24°C. The yellow protein was separated from unreacted reagent on a small column of polyacrylamide P-4 gel and then dialyzed against borate-buffered saline (pH 8.0).

Cell Culture

The secreting rat basophilic leukemia subline (RBL-IV-2H3) was obtained from Dr. Reuben Siraganian of the NIH. The RBL-1 line was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown as monolayers in Eagle's minimum essential medium (Earle's salts) supplemented with 20% fetal bovine serum as previously described (Barsumian et al., 1981). Fetal bovine serum was obtained from any of the following vendors: Gibco (Grand Island, NY), HyClone (Logan, UT), Armour Pharmaceuticals (Kankakee, IL), and Whittaker Bioproducts (Walkersville, MD). Every 4 d the cells were eluted by trypsinization and seeded in new 75-cm^2 flasks at a density of 2 × 10^6 per flask. For electrical recording the cells (5 × 10^4) were plated 12–24 h before use on 12-mm-diam round glass coverslips (#1 thickness). In some experiments, cells were sensitized with anti-DNP IgE by diluting ascites fluid 1,000-fold into the culture medium at the time of seeding.

Secretion Assays

The effectiveness of various degranulation stimuli was tested by measuring the release of the secretory granule enzyme *N*-acetyl-β-D-glucosaminidase. Cells were seeded in 24-well plates at

a density of 0.5–1.0 × 10^5 per well (1 ml of medium). 12–24 h later the monolayers were rinsed three times with 1 ml of HEPES-buffered saline (HBS) (in millimolar: 135 NaCl/5 KCl/0.5 MgCl$_2$/1.0 CaCl$_2$/5.6 glucose/10 HEPES, pH 7.4/0.05% bovine serum albumin). The appropriate stimulus was added in 0.8 ml HBS and the plates covered with Parafilm and floated on a 37°C water bath for periods ranging from 5 to 60 min. An aliquot of the supernatant was removed for assay, the remainder of the supernatant was discarded, and the cells were lysed for 45 min with 1 ml of 0.15% Brij-35 in HBS. An aliquot of the lysate was collected and assayed together with the supernatant using a colorimetric assay with the enzyme substrate p-nitrophenyl β-D-N-acetylglucosamine. Release of enzyme from digitonin-permeabilized cells was measured as follows. RBL monolayers were rinsed three times with standard K aspartate pipette solution (see below) containing 10^{-9} M free calcium. Solutions of differing free calcium concentrations were prepared as described by Miller and Smith (1984). Cells were then permeabilized by the addition of 20 μg/ml digitonin (Calbiochem-Behring Corp., La Jolla, CA) in the same solution for 5 min. The solution was replaced with one of several test solutions containing free calcium buffered at 10^{-9} to 10^{-5} M, with or without 100 μM GTPγS, and the cells were incubated for another 20 min at room temperature. Control cells that had been sensitized with anti-DNP IgE were triggered with 20 ng/ml of TNP-BSA at 37°C. Enzyme activity in the supernatant and cell layer was quantitated as above.

Electrical Recording

To record from the cells, the cover glass was rinsed with mammalian Ringer solution (in millimolar: 160 NaCl, 4.5 KCl, 2 CaCl$_2$, 1 MgCl$_2$, 5 HEPES, titrated to pH 7.4 with NaOH) and placed in a recording chamber (~300 μl volume) made of a cover glass with a plastic rim (Thomas Scientific, Philadelphia, PA). Patch-clamp recording was performed in the whole-cell and excised outside-out and inside-out patch configurations (Hamill et al., 1981). Microelectrodes were fashioned from Accu-fill 90 Micropets (Becton, Dickinson & Co., Parsippany, NJ), coated with Sylgard (Dow Corning Corp., Midland, MI), and fire-polished to resistances of 2–5 MΩ. All records were collected at ambient room temperature (22–26°C), although before recording in some experiments the cells were triggered at 37°C either with antigen or ionomycin.

Mammalian Ringer solution was the standard extracellular buffer; sometimes K$^+$, Rb$^+$, or Cs$^+$ ions were substituted for Na$^+$. The polyvalent cations Ba^{2+}, Sr^{2+}, Zn^{2+}, Cd^{2+}, and La^{3+} were added to mammalian Ringer in some experiments. Several different pipette solutions were used; the control solution contained (in millimolar): 160 K aspartate, 1.1 EGTA, 0.1 CaCl$_2$, 2.0 MgCl$_2$, 10 HEPES titrated to pH 7.2 with KOH. In separate experiments we added to this basal solution the following components: 100 μM GTPγS, 100 μM GppNHp, 5 mM KF plus 100 μM AlCl$_3$, 0.3 mM GTP, 0.3 mM GTP plus 0.5 mM ATP, 0.5 mM ATP, 0.5 mM ATP plus 100 μM 3',5'-cyclic AMP, 20 μM IP$_3$, 20 μM IP$_3$ plus 20 μM IP$_4$. Pipette solutions were introduced into the cytoplasm by dialysis after patch rupture, referred to as "break in," to achieve whole-cell recording. Solutions are referred to in the figure legends in "double slash" form, as, for example, Ringer//K aspartate + GTPγS.

Experiments were performed on-line using a List EPC-7 voltage-clamp (Medical Systems Corp., Great Neck, NY) interfaced to a Scientific Microsystems 11/73 computer. The current trace was filtered at 1–2 kHz (8-pole Bessel filter; Frequency Devices, Inc., Haverhill, MA), digitized, and stored in data files for subsequent off-line analysis. The membrane potential was held at −80 mV; in a few experiments, holding the potential at −40 or −25 mV did not affect the results. Voltage ramps (0.64 mV/ms) were the most common type of stimulus used to rapidly assess the two types of K$^+$ current described in this paper. Current-voltage relations during the voltage ramp provide a valid representation of the voltage dependence of both channel types described here, because the inward rectifier K$^+$ channel opens and closes

very rapidly, and the GTPγS-induced K^+ channel does not exhibit time-dependent kinetic transitions during voltage pulses. At various times during each experiment a series of 32 depolarizing pulses 10 mV in amplitude were delivered from the holding potential while sampling the unfiltered current at 50 kHz. Membrane capacitance, C_m, was determined by integrating the capacitive current transients; series resistance, R_s, was estimated by fitting a single exponential function to the fall of the capacitive current, assumed to discharge with a time constant, $\tau = C_m \cdot R_s$. Values for C_m ranged from 7 to 20 pF, while R_s values ranged from 4 to 25 MΩ. All potentials are corrected for pipette liquid junction potentials. Further information on the patch-clamp methods used can be found elsewhere (Cahalan et al., 1985).

RESULTS

The predominant conductance in RBL cells, as described previously by Lindau and Fernandez (1986*b*), is mediated by inwardly rectifying (IR) K^+ channels. When irreversible G protein activators gain access to the cytoplasm during whole-cell recording, current carried through IR channels decreases within a few minutes of dialysis and, usually with an overlapping time course, current through a second type of previously inactive outwardly rectifying (OR) K^+ channel is induced. Because each of these two different K^+ conductances has a distinct voltage dependence, currents carried through either K^+ channel during a voltage ramp stimulus can readily be distinguished. This section will first consider the IR conductance and its regulation by ATP and by irreversible G protein activators. Then, properties of the novel K^+ channel induced by G protein activators will be described, along with evidence showing that these two channel types are independent.

Regulation of Inward Rectifier by Nucleotides

In whole-cell recording from RBL-2H3 cells, with no added nucleotides in the pipette solution, current through IR channels can be easily visualized in I-V curves, as shown in Fig. 1. At the most negative potentials, inward current is carried by K^+ ions present in Ringer solution (4.5 mM), while between -80 and about -30 mV, outward current passes through the IR channels. No other conductances are evident from the I-V relation; above 0 mV the whole-cell input resistance is $\sim$10 GΩ. The properties of IR channels in RBL cells are similar to those in several other cell types, such as cardiac myocytes (Trube and Hescheler, 1984) and some invertebrate eggs (Hagiwara and Yoshii, 1979); the IR conductance increases dramatically and shifts to more positive potentials when extracellular $[K^+]$ is raised (data not shown). Fig. 1 *A* shows that prolonged whole-cell recording with control K aspartate pipette solution results in the eventual decline of current through IR channels. In this cell, the conductance was stable for $\sim$12 min and then began to decline. In 18 experiments of this type, without nucleotides in the pipette solution, after 10–20 min of whole-cell recording the IR conductance decayed partially in 6 cells and completely in 12 cells. To quantify changes of the IR conductance, we performed a least-squares fit of current between -90 and -100 mV to determine the IR slope conductance, $G_{K(IR)}$ (Fig. 1 *B*). From plots of $G_{K(IR)}$ at various times after break-in we extracted average values of the initial whole-cell K^+ conductance and the half-time, $t_{1/2}$, for its decay, summarized in Table I.

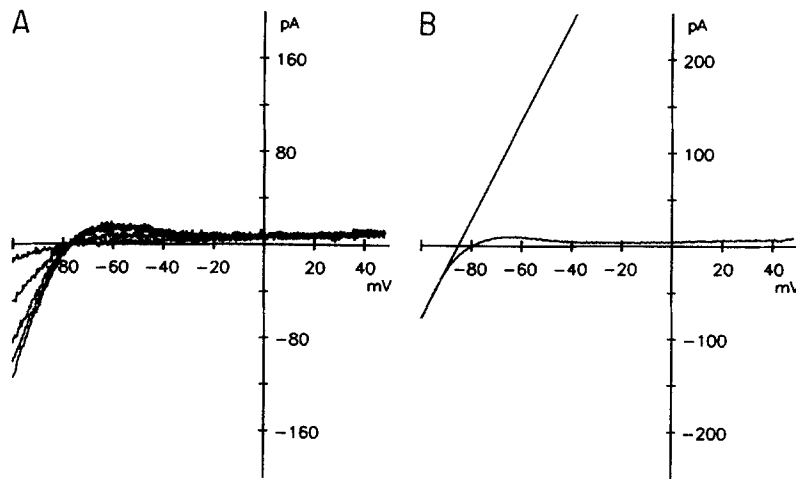Washout of the IR conductance can be greatly slowed or prevented by including

FIGURE 1.   Changes of IR K^+ conductance after break-in. Solutions: Ringer//standard K aspartate. (A) Whole-cell current-voltage curves illustrating washout of IR conductance after prolonged dialysis without nucleotides. Ramp voltage stimuli (0.64 V/s) of −100 to +50 mV were applied from a holding potential of −80 mV once every 10 s. The illustrated I-V curves were recorded between 730 and 980 s after break-in. In this cell the IR K^+ currents were stable up to 720 s, and then decayed. (B) Linear least-squares fit with a slope conductance of 10.5 nS in the interval −100 to −90 mV illustrating the method of determining $G_{K(IR)}$.

0.3 mM GTP or 0.5 mM ATP in the pipette solution (Fig. 2). In 17 experiments similar to that illustrated in the top curve of Fig. 2, lasting over 20 min each, complete decay was never observed; partial washout occurred in 11 cells, and in 6 cells no decay occurred. It is likely, therefore, that IR channels are maintained in the

TABLE I

Decline of Inward Rectifier Conductance during Whole-Cell Recording*

Pipette additive	$G_{K(IR)}$	$t_{1/2}$
	nS	s
None	4.48 ± 1.45	580 ± 306
(n = 12)		
GTPγS	5.28 ± 2.33	282 ± 119
(n = 67)		P < 0.001
GTPγS + Na₂ATP	5.35 ± 2.67	386 ± 139
(n = 9)		
AlF₄⁻	4.83 ± 1.15	359 ± 67
(n = 7)		P ≤ 0.10
GppNHp	9.34 ± 2.61	186 ± 41
(n = 4)		P < 0.05

*Three different activators of G proteins significantly accelerate decay of the inward K^+ rectifier in RBL cells. The slope conductance, $G_{K(IR)}$, was measured between −90 and −100 mV. Activators were added to standard K aspartate pipette solution as described in the Methods section. Probabilities refer to significance of difference between decay rates with additives or with standard K aspartate alone.

open state by cytoplasmic ATP (or GTP), which is lost during dialysis of the cell contents. It is possible that GTP sustains the IR channels by providing a substrate for conversion to ATP. A similar dependence on ATP was demonstrated previously for IR channels in excised patches from cardiac myocytes (Trube and Hescheler, 1984). Although intracellular Na^+ is required for maintenance of the IR conductance in starfish eggs (Hagiwara and Yoshii, 1979), inclusion of 15–40 mM Na aspartate in the pipette solution did not promote survival of the IR conductance in RBL cells (data not shown).

The IR conductance never washed out completely in cells dialyzed with solutions containing 0.5 mM ATP; however, inclusion of the G protein activator, GTPγS, in the pipette solution resulted in a rapid decline in IR conductance (Fig. 2, Table I). Furthermore, the rate of decline of the IR conductance in cells dialyzed without ATP was increased by GTPγS, GppNHp, or AlF_4^-, suggesting that a G protein can
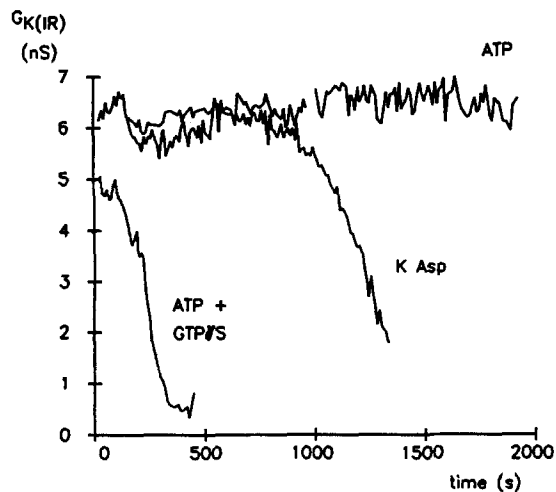

FIGURE 2. Dual control of IR K^+ conductance by ATP and guanine nucleotides. The slope conductance of the IR K^+ channel, calculated as described in Fig. 1 *B*, is plotted as a function of time after break-in. The plots show data from three individual experiments representative of all data collected for the three different pipette solutions indicated. Note that the IR conductance decays in control cells (K aspartate), that the addition of 0.5 mM ATP prevents this washout, and that even in the presence of 0.5 mM ATP, 100 μM GTPγS induces rapid loss of the IR conductance.

regulate the IR channels. Whether this involvement is direct or indirect is unclear, although no decline in IR conductance was caused by cAMP, IP_3, or IP_4. Loss of the IR conductance was useful for our present purposes because it allowed us to study another K^+ current without using pharmacological probes to block the IR channels (see below).

Induction of Outward K^+ Current by G Protein Activators

When GTPγS or another G protein activator was included in the pipette solution, a novel type of K^+ channel opened during whole-cell dialysis. The new current, visualized by ramp stimuli during its induction in Fig. 3 *A*, rectifies outwardly, in contrast to the IR current, as seen by the flattening slope of the *I–V* curve at hyperpolarized potentials. As indicated below, in ion-substitution experiments the outward current

is K^+ selective. Because of its outward rectification, K^+ selectivity, and induction by G protein activators, we refer to the channel as the OR K^+ channel, and to its conductance as $G_{K(GTP)}$. To quantify the rate of its induction by G protein activators, we measured the outward slope conductance, $G_{K(GTP)}$, between $+20$ and $+40$ mV, a region of membrane potential in which IR channels are closed (cf. Figs. 1 B and 3 B). We then plotted $G_{K(GTP)}$ as a function of time after break-in to the whole-cell mode and determined the average values for the time to half-maximal induction, $t_{1/2}$, and the maximal conductance achieved under different experimental conditions. As indicated in Table II, dialysis with a second nonhydrolyzable GTP analogue, GppNHp, as well as another G protein activator, AlF_4^-, also induced the outward conductance.
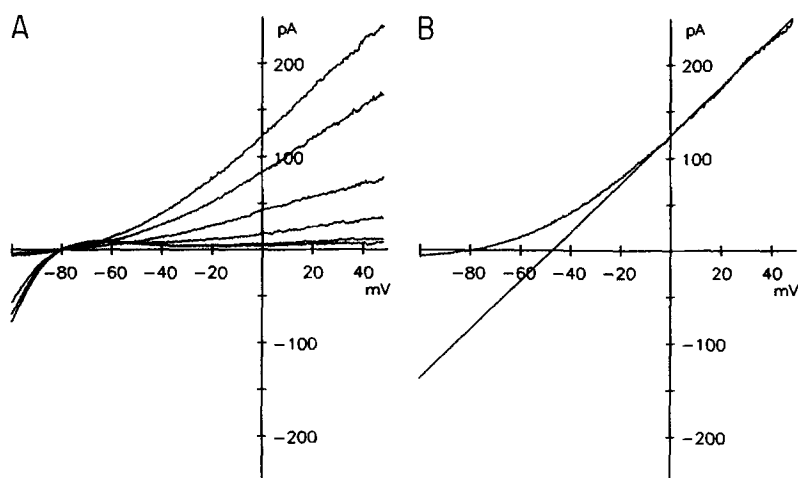

FIGURE 3.   Induction of outward K^+ current by GTPγS. Solutions: Ringer//K aspartate + GTPγS. (A) Ramp currents at 50-s intervals, starting 50 s after break-in with K aspartate pipette solution containing 100 μM GTPγS. Note the decay of IR conductance and development of a large outward current at potentials more positive than ~ -60 to -70 mV. (B) Method for analysis of the induced outward K^+ conductance, $G_{K(GTP)}$. A least-squares fit (slope conductance, $G_{K(GTP)} = 5.2$ nS, for the illustrated record) was performed between $+20$ and $+40$ mV for successive ramps collected every 10 s after break-in.

Given that the IR conductance often decays while the OR conductance appears, one may ask if there is a causal relationship between the two events. Fig. 4 illustrates the relative kinetics of $G_{K(GTP)}$ induction and $G_{K(IR)}$ decay in two different cells, which demonstrates the lack of consistent correlation between these two events. In some cells, as in Fig. 4 A, $G_{K(IR)}$ decayed almost completely before induction of the new K^+ channel, whereas in others, as in Fig. 4 B, complete induction of $G_{K(GTP)}$ occurred before the decline of $G_{K(IR)}$. On average, $G_{K(IR)}$ decayed more slowly than the new conductance appeared, the mean ($\pm$SD) half-times for induction of $G_{K(GTP)}$ and washout of $G_{K(IR)}$ being 219 ± 71 and 309 ± 116 s, respectively (Tables I and II). These results suggest that the IR channel is not a precursor of the OR channel, since $G_{K(IR)}$

TABLE II

*Induction of $G_{K(GTP)}$ by G Protein Activators**

Pipette additive	$G_{K(GTP)}$	$t_{1/2}$
	nS	*s*
GTPγS	2.58 ± 1.59	212 ± 73
(n = 58)		
GppNHp	1.83 ± 0.62	157 ± 14
(n = 3)		
AlF$_4^-$	2.00 ± 1.15	140 ± 56
(n = 7)		

*Three irreversible activators of G proteins induce the appearance of OR K$^+$ conductance in RBL cells. The maximal slope conductance (between +20 and +40 mV) and the half-time for induction are indicated for each compound. Activators were added to standard K aspartate pipette solution (see Methods).

did not consistently decay before, or simultaneously with, the appearance of $G_{K(GTP)}$.

Using RBL-2H3 cells, induction of $G_{K(GTP)}$ was normally robust, resulting in a conductance of ~2 nS. However, the parental RBL cell line, RBL-1, exhibited much lower $G_{K(GTP)}$ values.
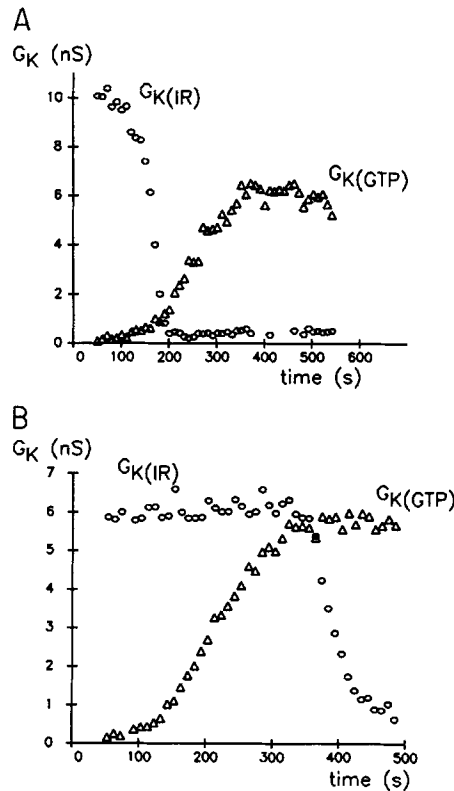

FIGURE 4. Time courses of $G_{K(GTP)}$ induction and $G_{K(IR)}$ decay in two cells. Solutions: Ringer//K aspartate + GTPγS. (A) $G_{K(IR)}$ (O) washed out before $G_{K(GTP)}$ (△) had fully evolved. (B) $G_{K(GTP)}$ appeared before the decline in $G_{K(IR)}$, resulting in simultaneous expression of both channel types between about 100 and 400 s. The lack of correlation from cell to cell between the decay of $G_{K(IR)}$ and the appearance of $G_{K(GTP)}$ suggests that the IR channel is not converted into the OR K$^+$ channel. A similar plot for a cell in which $G_{K(GTP)}$ induction and $G_{K(IR)}$ decay overlapped is shown in Fig. 11.

Open Channel Properties

Although the current induced by G protein activators rectifies outwardly during ramp stimulation, no voltage-dependent relaxations of current could be observed in response to step depolarizations, as shown in Fig. 5. Thus, curvature in the *I-V* relation is due either to extremely fast gating events or to properties of the open channel. The ion-substitution experiment shown in Fig. 6 *A* indicates that the induced channel is permeable to both K$^+$ and Rb$^+$, with a permeability ratio, P_{Rb}/P_K, of 0.67 calculated from the shift in reversal potential. In Rb$^+$ Ringer, current magnitudes are smaller than expected from the permeability ratio, and several inflections of current can be observed, indicating that Rb$^+$ is a more reluctant current carrier than K$^+$. The *I-V* relation in K$^+$ Ringer exhibits a decrease in conductance at hyperpolarized potentials, shown to -200 mV in Fig. 6 *B*. This decreased conductance is not due to ionic block by external Ca^{2+}, Mg^{2+}, or Na$^+$ ions, as removal of all three did not appreciably change the *I-V* shape.
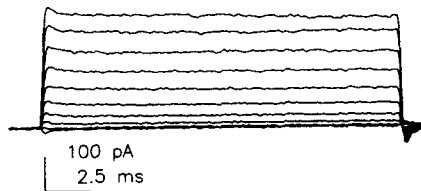

FIGURE 5. Currents through the OR K$^+$ channel during a series of step depolarizations. Solutions: Ringer//K aspartate + GTPγS. After $G_{K(GTP)}$ was induced by GTPγS and stabilized, the membrane voltage was rapidly stepped for 200 ms from the holding potential of -80 mV to potentials ranging from -100 to $+40$ mV in 20-mV increments. Except for residual uncompensated capacity current, current after the voltage step appears instantaneously, within the time resolution of the voltage clamps, and does not inactivate.

100 pA

2.5 ms

The channel is not measurably permeable to Na$^+$ or Cs$^+$. Instead, Cs$^+$ ions block both OR and IR channels in a voltage-dependent manner, as shown in Fig. 7. We analyzed the steepness of Cs$^+$ block by forming ratios of current in the presence and absence of Cs$^+$, and then fitting with a Boltzmann relation, as described in the legend of Fig. 7. Cs$^+$ block of the IR channels is steeper than expected for movement of a single monovalent cation within the electric field of the membrane. In two determinations, the steepness factor, *k*, averaged 13 mV, corresponding roughly to two charges moving across the membrane. The strong voltage dependence for Cs$^+$ block of IR channels can be attributed to multiple binding sites for Cs$^+$ within the channel (Hille and Schwarz, 1978). The voltage dependence for Cs$^+$ block of the OR channels is less steep and shifted to more negative potentials than that for the IR channels.

The K$^+$ current through the induced channel is quite noisy at hyperpolarized potentials when the cell is bathed in K$^+$ Ringer (Fig. 7). The origin of this noise can

be traced to behavior of the single-channel currents. Fig. 8 *A* shows single-channel events in an outside-out patch of membrane excised from a cell that had been dialyzed for 5 min with GTPγS to induce $G_{K(GTP)}$. The channel alternates between closed and open states; when open, the channel appears to flicker rapidly. The rapid fluctuations and transitions from open to closed states may account for the noise in the macroscopic *I–V* curve (Fig. 7). Such flicker is not present in the much larger single-channel currents through the IR channel (Fig. 8 *B*).
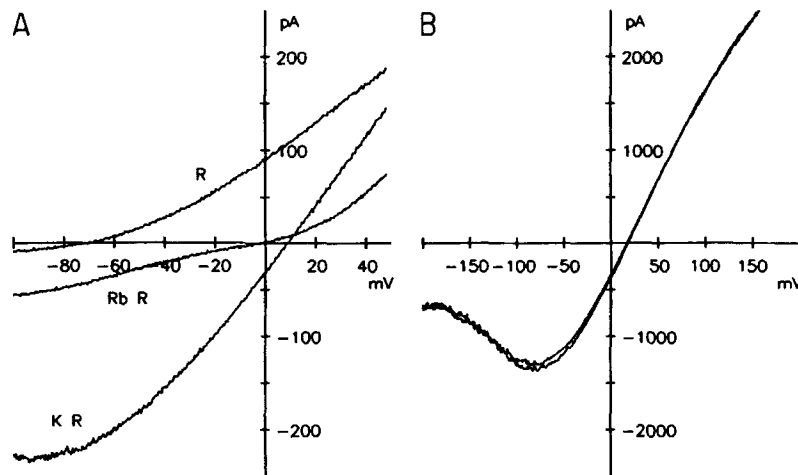

FIGURE 6. (*A*) Ionic selectivity of the GTPγS-induced channel. Whole cell *I–V* curves recorded with three different external solutions bathing the cell. Ramp currents for three to five consecutive traces in each solution were averaged. Prior to the ionic substitutions, $G_{K(GTP)}$ was maximally induced by dialysis with GTPγS, and $G_{K(IR)}$ had decayed completely. The solutions were: *R*, normal mammalian Ringer (4.5 mM K⁺); *KR*, potassium Ringer (160 mM K⁺); *RbR*, rubidium Ringer (160 mM Rb⁺). Upon replacement of Na⁺ with K⁺, the reversal potential shifts to ~0 mV as predicted for a K⁺-permeable channel. The junction potential between Ringer and K Ringer of ~5 mV has not been corrected. The channel is also permeable to Rb⁺; from the 10-mV difference in reversal potential between KR and RbR we calculate a permeability ratio P_{Rb}/P_K of 0.67. (*B*) Voltage dependence of the GTPγS-induced channel is independent of external Na⁺ or divalent ions. *I–V* curves are similar in K Ringer and 160 mM KCl without Na⁺, Ca^{2+}, or Mg^{2+}.

Unitary Conductance of K⁺ Channels

We estimated the average conductance of single open channels by applying voltage ramps to outside-out patches to obtain *I–V* curves for single open channels. Examples of single OR channels from two different patches are shown in Fig. 9, *A* and *B*, while Fig. 9 *C* illustrates single-channel ramp currents for the IR channel. The average conductance of the IR channel in symmetrical 160 mM K⁺ was 26 pS, whereas that of the induced OR channel was ~8 pS.
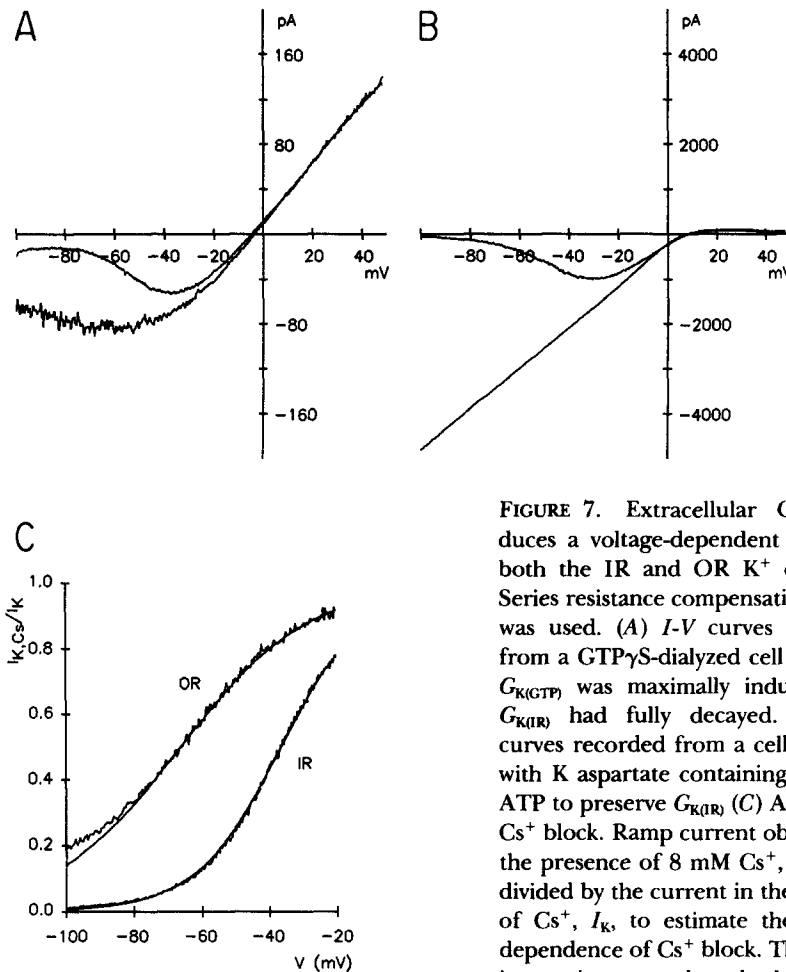
FIGURE 7. Extracellular Cs$^+$ produces a voltage-dependent block of both the IR and OR K$^+$ channels. Series resistance compensation (60%) was used. (A) I-V curves recorded from a GTPγS-dialyzed cell in which $G_{K(GTP)}$ was maximally induced and $G_{K(IR)}$ had fully decayed. (B) I-V curves recorded from a cell dialyzed with K aspartate containing 0.5 mM ATP to preserve $G_{K(IR)}$ (C) Analysis of Cs$^+$ block. Ramp current obtained in the presence of 8 mM Cs$^+$, $I_{K,Cs}$, was divided by the current in the absence of Cs$^+$, I_K, to estimate the voltage dependence of Cs$^+$ block. The resulting ratios are plotted along with smooth curves fit (by eye) to a Boltzmann equation,

$$I_{K,Cs}/I_K = 1/\{1 + \exp[(E_h - E)/k]\},$$

where E_h is the voltage at which half the channels are blocked, and k is the steepness of block (millivolts per e-fold change). Note that Cs$^+$ block of the induced OR channels occurs at more negative membrane potentials and with less steepness than Cs$^+$ block of the IR channels. The parameters for the Boltzmann fits were: for the OR channels, $E_h = -66$ mV and $k = 19$ mV; for the IR channels, $E_h = -37$ mV and $k = 13$ mV. Similar determinations on two other cells agreed well: for the OR channels, $E_h = -63$ mV and $k = 21$ mV; for the IR channels, $E_h = -36$ mV and $k = 13$ mV. A systematic deviation from the simple Boltzmann relation occurred at very negative membrane potentials for the OR channel. This could originate from residual unsubtracted "leakage" current, or from relief of block if Cs$^+$ is sparingly permeant.

Effects of Channel Blockers on K^+ Currents

Pharmacological experiments support the distinction between the IR channels and the induced OR channels. Quinidine, which has been shown to block antigen-induced K^+ efflux (Labrecque et al., 1988), also blocks $G_{K(GTP)}$ by more than 50% at 50 μM (data not shown). In contrast, the IR conductance is not affected by 100 μM quinidine, even after prolonged incubation (10 min) of the cells with the drug. Of a variety of multivalent cations tested, including Zn^{2+}, Cd^{2+}, and La^{3+}, only extracellular Sr^{2+} and Ba^{2+} affected $G_{K(GTP)}$. Strontium produces a voltage-dependent block of $G_{K(GTP)}$ at hyperpolarized potentials (data not shown), qualitatively similar to that caused by Cs^+ (Fig. 7). As indicated in Fig. 10, Ba^{2+} blocks $G_{K(GTP)}$ with much lower potency than it blocks $G_{K(IR)}$. 100 μM Ba^{2+} was sufficient to block $G_{K(IR)}$ completely, whereas 20 mM Ba^{2+} did not completely inhibit $G_{K(GTP)}$. Thus, two different pharma-
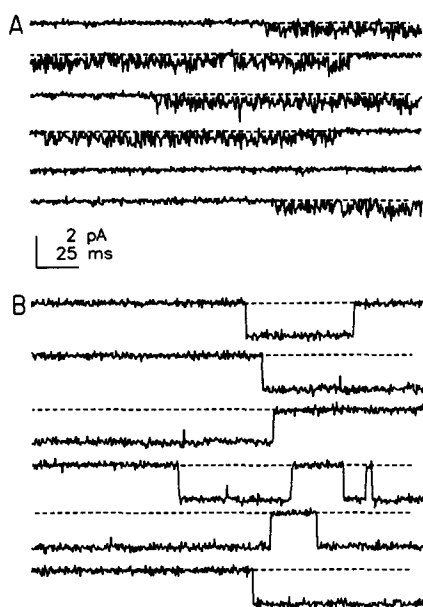

FIGURE 8. Two types of single K^+ channels in outside-out patches. Continuous recordings of current at -80 mV. Levels at which channels are closed are indicated by dashed lines. (A) Steady-state currents in a patch with a single GTPγS-induced channel. Note the marked "flicker" when the channel is open. Solutions: K Ringer//K aspartate + GTPγS. (B) Currents through a single IR channel. Solutions: K Ringer//K aspartate + ATP.

cological probes—quinidine and Ba^{2+}—discriminate between the IR and the OR K^+ conductances. Tetraethylammonium blocked both channels with similar potency; half-block was observed at 10–15 mM (data not shown).

Effects of Pertussis and Cholera Toxins on K^+ Conductance

Pertussis toxin is a bacterial toxin that catalyzes ADP-ribosylation of G_i, G_o, and related proteins, thereby inhibiting their function (Ui, 1984). Preincubation of intact cells with pertussis toxin (100–400 ng/ml) for 12–16 h strongly inhibited induction of the outward current by GTPγS (Table III) and also by AlF_4^- (not shown). Measurement of the half-time was not always possible because of the very small value of $G_{K(GTP)}$ in many pertussis toxin–treated cells. In contrast, pertussis toxin had no significant effect on the decay rate of the inward rectifier in the pres-

ence of GTPγS, further suggesting that the two K$^+$ currents are mediated by distinct molecular species of channel. These results also provide further evidence that OR channel activation occurs via a pertussis toxin–sensitive G protein.

Cholera toxin from *Vibrio cholera* catalyzes ADP-ribosylation of G$_s$, the stimulatory GTP-binding protein of adenylate cyclase (Cassel and Pfeuffer, 1978). This modification inhibits the GTPase activity of G$_s$ and enhances its coupling to adenylate cyclase (reviewed by Gill, 1977). G$_s$ also appears to have other cAMP-independent functions, including regulation of Mg^{2+} uptake in S49 lymphoma cells (Maguire and
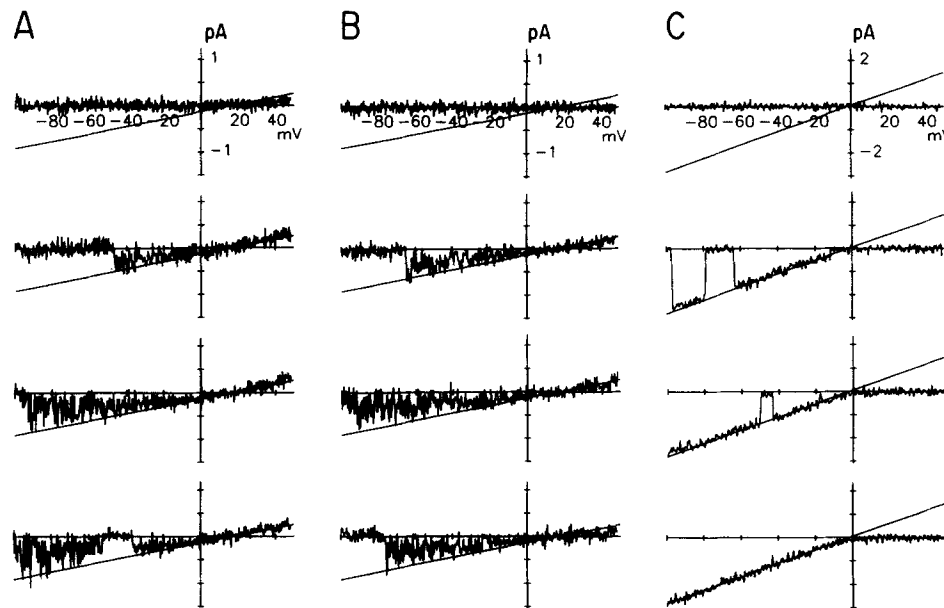

FIGURE 9.   Single channel *I-V* curves obtained during voltage ramps to the indicated membrane potentials. All records have been corrected for leak currents by subtraction of average sweeps with no channel openings. (*A* and *B*) For two separate cells, OR K$^+$ channels were first induced by GTPγS, and then outside-out patches were excised. A line corresponding to a single-channel conductance of 8 pS has been drawn for each sweep. (*C*) IR K$^+$ channel in an outside-out patch excised from a cell dialyzed with a pipette solution containing standard K aspartate without nucleotides. Records were digitally filtered at 500 Hz. A line representing a single-channel conductance of 26 pS was determined by fitting records between −100 and −50 mV.

Erdos, 1980) and activation of Ca^{2+} channels in cardiac myocytes (Yatani et al., 1987*b*). As shown in Table III, under conditions in which endogenous G$_s$ is modified by cholera toxin, pretreatment with toxin had no significant effect on the decay of G$_{K(IR)}$ or the development of G$_{K(GTP)}$. This suggests that G$_s$ does not mediate either the decay of the IR conductance or the evolution of the OR conductance.

Is K$^+$ Channel Induction a Consequence of Exocytosis?

Exocytosis causes an increase in cell surface area as secretory vesicles fuse with the plasma membrane. To determine whether a significant increase in RBL membrane
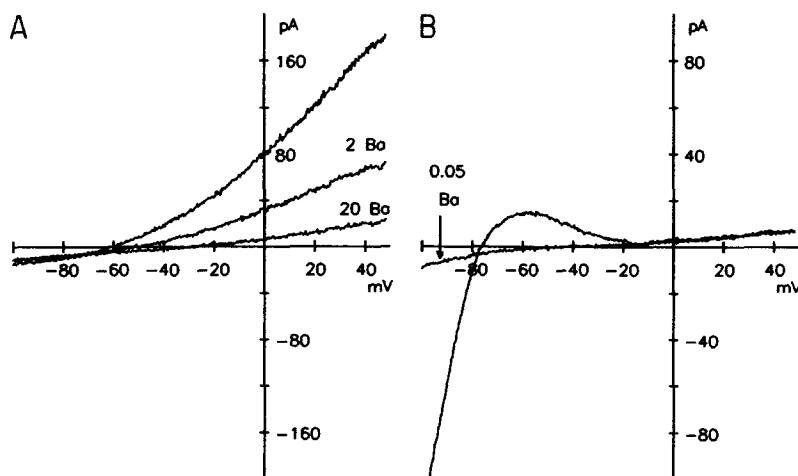
FIGURE 10.   Differential effects of extracellular barium on IR and OR K⁺ channel conductance. 20 mM Ba^{2+} produces incomplete block of OR K⁺ channel (*A*), whereas 50 μM Ba^{2+} yields nearly complete inhibition of the IR K⁺ channel (*B*). Inhibition was completely reversible for both conductances.

area occurred in response to GTPγS, we measured total cell capacitance during the course of typical channel induction experiments. As shown in Fig. 11, little or no change in capacitance was observed. The same procedure in rat peritoneal mast cells results in degranulation accompanied by a two- to threefold increase in membrane capacitance (see e.g., Neher, 1988). Thus, it appears that GTPγS did not induce substantial exocytosis in RBL cells.

Our standard pipette solution with 100 μM GTPγS was also ineffective in triggering secretion when applied to digitonin-permeabilized RBL cells. Although a small amount of β-hexosaminidase (<10% of the total cellular content) was released as $[Ca^{2+}]$ was raised from 10^{-7} to 10^{-6} M, GTPγS did not elicit >2% further release, and at the $[Ca^{2+}]$ used in our patch-clamp experiments, no release above basal was

TABLE III

Inhibition of $G_{K(GTP)}$ by Pertussis Toxin

	OR K⁺ channel		IR K⁺ channel	
Pretreatment	$G_{K(GTP)}$	$t_{1/2}$	$G_{K(IR)}$	$t_{1/2}$
	nS	s	nS	s
None	2.58 ± 1.59	212 ± 73	5.28 ± 2.33	282 ± 119
	n – 58		n – 67	
Pertussis toxin	0.47 ± 0.43	ND	4.26 ± 1.80	282 ± 80
	n – 12ᶻ		n – 11	
Cholera toxin	2.92 ± 1.59	168 ± 64	3.86 ± 1.72	234 ± 79
	n – 5		n – 6	

*Pertussis toxin selectively blocks development of the OR K⁺ channel in RBL cells. $G_{K(GTP)}$ was induced and $G_{K(IR)}$ was inhibited upon intracellular dialysis with standard K aspartate pipette solution containing 100 μM GTPγS.
ᶻ$P < 0.001$ relative to control cells.

obtained in the presence or absence of GTPγS. This finding supports the conclusion from capacitance measurements that GTPγS causes little or no secretion from RBL cells under the conditions of the patch-clamp recording.

Triggering the cells to produce substantial degranulation before electrical recording did not generate the outward K^+ current. Intact cells were stimulated to secrete either with 400 nM ionomycin or antigen at 37°C for periods of 5–30 min. Recording then was performed with 100 μM GTPγS in the pipette solution. No $G_{K(GTP)}$ was present in these cells just after break-in, even when parallel secretion assays indicated that the cell populations had released >50% of the granule enzyme β-hexosaminidase. With dialysis of GTPγS, these cells developed $G_{K(GTP)}$ normally.
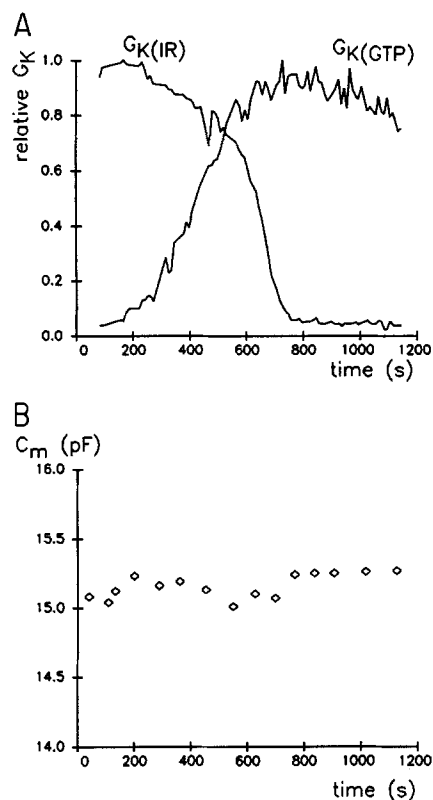

FIGURE 11. Capacitance and conductance measurements on cells during dialysis with 100 μM GTPγS. (A) Normalized conductance values are plotted as a function of time after break-in. The maximum conductances were 10.2 nS for $G_{K(IR)}$ and 6.1 nS for $G_{K(GTP)}$. (B) Cell membrane capacitance, determined from integration of leak pulses taken at different times during the run. Note that capacitance remains stable while $G_{K(IR)}$ declines and $G_{K(GTP)}$ develops. This suggests that changes in the area of the plasma membrane during dialysis are minimal, and that membrane insertion is not required for GTPγS-induced appearance of the OR K^+ channel in the plasma membrane.

Finally, in five of eight experiments we found that excision of an inside-out patch into a bath solution containing GTPγS resulted in activation of the OR K^+ channel (Fig. 12). This observation strengthens the idea that secretion per se is an unlikely origin of the outward K^+ conductance.

Do Second Messengers Activate $G_{K(GTP)}$?

We tested the possible involvement of four different second messengers in activation of the OR K^+ channel. Inclusion of 100 μM cAMP plus 0.5 mM ATP in the pipette solution did not give rise to the outward K^+ conductance (n = 5), nor was $G_{K(GTP)}$ present upon break-in to cells pretreated for 1 h with 1 mM dibutyryl cyclic

AMP, with or without 0.5 mM ATP in the pipette solution ($n = 3$). Likewise, cAMP plus ATP did not cause a decline of $G_{K(IR)}$. Thus, cAMP is unlikely to mediate either the appearance of the outward K^+ conductance or the diminution of the IR conductance by G protein activators.

The possible involvement of Ca^{2+} was checked in two ways. First, we dialyzed cells with a strongly Ca-buffered pipette solution (11 mM EGTA) containing very low free Ca^{2+} (10^{-9} M); this concentration of EGTA is known to block the GTPγS or IP_3-induced rise in cytosolic Ca^{2+} in rat peritoneal mast cells (Neher, 1988). Even with this buffer, however, 100 μM GTPγS caused the appearance of $G_{K(GTP)}$ averaging 3.5 ± 1.3 nS with a half-time for induction of 180 ± 4 s ($n = 3$). Second, we dialyzed cells with K aspartate containing Ca^{2+} buffered at the relatively high value of 10^{-6} M in the absence of GTPγS. Induction of $G_{K(GTP)}$ did not occur even over a 15-min period ($n = 3$). Thus, an increase in intracellular Ca^{2+} probably does not mediate the induction of $G_{K(GTP)}$ by guanine nucleotides. Furthermore, dialysis with K aspartate containing 20 μM IP_3 alone ($n = 7$) or in combination with 20 μM IP_4 ($n = 6$) neither induced the outward K current nor inhibited the IR conductance.
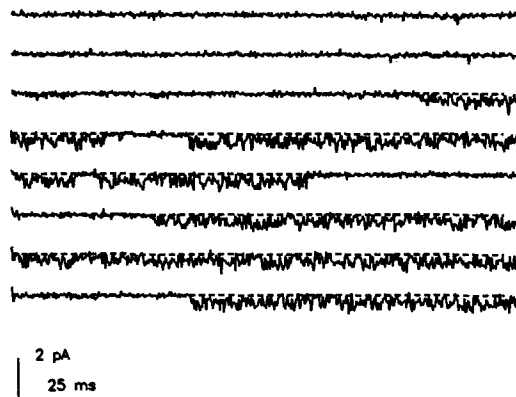

Figure 12. Activation of a single OR K^+ channel in an inside-out patch excised into GTPγS solution. The dashed line indicates the level at which the channel is closed. Cells were bathed in a solution consisting of (in millimolar): 140 KCl, 2 $MgCl_2$, 1 EGTA, 0.5 ATP, 5 HEPES, pH 7.3, with 100 μM GTPγS. The pipette contained K Ringer solution. Holding potential, −80 mV. Patch excision into solution without GTPγS did not result in channel activity.

Does the IgE Receptor Regulate K^+ Channels?

We tested in two ways the possible role of the IgE receptor in the regulation of the OR and IR K^+ channels. First, IgE-sensitized cells were treated for 5–30 min at 37°C with antigen (20–100 ng of TNP-BSA or DNP-IgG per ml HBS). The cells were then placed on the microscope stage at room temperature, using Ringer containing the same concentration of antigen as a bathing solution. The whole-cell mode was achieved using pipettes filled with K aspartate containing 100 μM GTPγS. As noted above, this pretreatment with antigen ($n = 7$) did not cause the appearance of $G_{K(GTP)}$ at time zero; moreover, it did not affect significantly the half-time for $G_{K(GTP)}$ induction (225 ± 114 s) or the maximal value of $G_{K(GTP)}$ (4.5 ± 2.7 nS). Similarly, the maximal IR K^+ conductance (4.1 ± 1.9 nS, $n = 9$) and the half-time for its decay (240 ± 106 s, $n = 8$) were unaltered by this treatment.

Second, we formed the whole-cell configuration using 0.3 mM GTP plus 0.5 mM ATP in the pipette solution, using cells not previously treated with antigen. Within 20 s to 3 min of break-in, antigen (50–100 ng TNP-BSA or DNP-IgG per ml Ringer)

was added to the bath ($n = 8$). This procedure neither induced the appearance of $G_{K(GTP)}$ nor caused the washout of $G_{K(IR)}$. Although these findings suggest that neither the IR nor the OR K^+ channel is linked via a G protein to the IgE receptor, further studies are necessary to confirm this conclusion (see below).

DISCUSSION

The only readily discernible conductance present in unstimulated RBL cells is mediated by IR K^+ channels (Lindau and Fernandez, 1986*b*). In our experiments we found that intracellular application of any of three different activators of G proteins (GTPγS, GppNHp, or AlF_4^-) inhibited the IR K^+ conductance, and also induced the appearance of a novel OR K^+ current. There is no precursor-product relation between these two conductances, as the time course for changes in the two conductances did not correlate in different experiments (Fig. 4). The channels that mediate these two conductances are distinct entities, having different unitary conductances (Fig. 8), different open channel rectification (Figs. 1 and 6), and markedly different sensitivities to inhibition by Ba^{2+} (Fig. 10) and quinidine. Both channels are susceptible to block by external Cs^+ ions, but display different voltage dependencies (Fig. 7). The differential effects of pertussis toxin on regulation of the two conductances (Table III) suggest that separate GTP-binding proteins mediate these two processes.

Regulation of the Inward Rectifier

In our experiments the IR K^+ channel in RBL cells appears to be regulated by two different mechanisms. First, cellular ATP or GTP stabilizes $G_{K(IR)}$, as previously demonstrated for ATP in cardiac myocytes (Trube and Hescheler, 1984). In a previous patch-clamp study of RBL cells, Lindau and Fernandez (1986*b*) did not observe washout of the IR conductance, probably because their pipette solutions contained ATP. Second, activation of a pertussis toxin–insensitive G protein appears to inhibit the channel by a route that does not involve cAMP, elevated intracellular $[Ca^{2+}]$, or hydrolysis of inositol phospholipids. Nakajima et al. (1988) recently reported that activation of a pertussis toxin–insensitive G protein inhibits IR K^+ channels in nucleus basalis neurons, and Kurtz and Penner (1989) found that activation of a G protein coupled to angiotensin II blocks the IR K^+ channel in renal juxtaglomerular cells. In RBL cells, the identities of the G protein and putative receptor coupling to either K^+ channel remain unknown. However, the G proteins that regulate the IR and OR K^+ channels appear to differ, based on their differential susceptibility to pertussis toxin in the absence of receptor stimulation.

Mechanism of Induction of Outward K^+ Conductance

We consider three main hypotheses to explain how G protein activators might work to induce the outward K^+ conductance. First, the OR K^+ channel might originate in the membrane of the secretory granule, and become inserted into the plasma membrane during guanine nucleotide-induced secretion. Second, G protein activation may stimulate formation of a second messenger, e.g., cAMP, which then indirectly gates the channel. The third hypothesis is that an activated G protein directly gates the channel.

Dialysis of rat peritoneal mast cells with GTPγS is known to cause cellular degranulation, as measured by a twofold or greater increase in membrane capacitance or area (Fernandez et al., 1984). However, little or no increase in membrane area of RBL cells was observed during the course of channel induction by GTPγS (Fig. 11). Our results with digitonin-permeabilized cells also suggest that GTPγS-induced secretion is very limited, if it occurs at all. The question then becomes whether a very small amount of exocytosis might account for the increase in whole-cell K$^+$ conductance. The increase in capacitance, if any, is <2%, on the order of 0.2 pF in the cell illustrated in Fig. 11. We estimate that at full expression there are an average of ~600–700 conducting OR channels in the plasma membrane. The probability of a channel's being open is not known with certainty; preliminary estimates ranged from 0.1 to 0.3. Taking the open probability as 0.2, there are ~3,000–3,500 channels per cell after full induction with GTPγS. Estimating the cell surface area from the average whole-cell capacitance of 11.6 ± 3.6 pF (mean ± SD, $n = 248$), and assuming a specific membrane capacitance of 1 μF/cm^2, the average lateral density of the induced K$^+$ channel at full expression in the plasma membrane is ~2.6–3.0 μm^{-2}. Based on an estimated upper limit of 0.2 pF for the increase in capacitance during dialysis with GTPγS, if the channels originate from secretory granule membranes, they would have to be present in the granule membrane at a channel density of 150–175 μm^{-2}. Although this estimate is within the range for lateral densities of known channel proteins (Hille, 1984), nevertheless, it seems unlikely that a miniscule amount of degranulation could explain the outward K$^+$ conductance, because elicitation of marked secretion prior to break-in does not lead to the appearance of $G_{K(GTP)}$. The differential effects of pertussis toxin on channel induction and IgE-mediated secretion also argue against a role for membrane insertion in generating the outward K current.

Second messenger production resulting from G protein stimulation might regulate K$^+$ channels. However, dialysis of cells with solutions containing cyclic AMP, IP$_3$, IP$_4$, or elevated [Ca^{2+}] did not mimic the GTPγS effect. Further evidence against a role for cAMP is that pertussis toxin blocks induction by GTPγS even though the stimulatory protein of adenylate cyclase, G$_s$, is not a substrate for this toxin. A similar argument pertains to the finding that cholera toxin, which does modify G$_s$ in RBL cells (McCloskey, 1988), did not affect the development of $G_{K(GTP)}$. The fact that we could activate the OR K$^+$ channel by bath application of GTPγS to excised inside-out patches of membrane supports the third hypothesis, namely, this channel is activated directly by a GTP-binding protein or by local generation of second messengers.

Possible Coupling of the Fc$_\epsilon$ Receptor to the K$^+$ Channel

Labrecque et al. (1988, 1989) have shown that Fc$_\epsilon$ receptor aggregation is followed by a delayed efflux of Rb$^+$ from, and repolarization of, RBL-2H3 cells, and they hypothesize that a calcium-activated K$^+$ channel mediates this efflux. Quinidine produces 50% block of this Rb$^+$ efflux and repolarization at a concentration that also partially blocks $G_{K(GTP)}$, supporting the speculation that the OR K$^+$ channel may mediate IgE receptor-triggered K$^+$ efflux. However, we were unable to activate the OR channel in RBL cells during dialysis with pipette solutions containing calcium

buffered at 10^{-6} M. Nor could we activate $G_{K(GTP)}$ by aggregation of the IgE receptors on cells dialyzed with pipette solutions containing GTP. Although this suggests that the IgE receptor is not coupled directly to the OR K^+ channel via a G protein, this failure might be explained by the rapid loss, during whole-cell dialysis, of a cytosolic factor required for coupling. Signal transduction via the IgE receptor in RBL cells also is known to be very temperature dependent (see e.g., Woldemussie et al., 1986); because all of our electrical recording was performed at room temperature, we may not have detected coupling of the IgE receptor to channel induction.

Significance of Findings

The list of ion channels known to be activated by G proteins continues to grow; to our knowledge this is one of the first reports of such a coupling mechanism in non-excitable cells. The K^+ channel induced by G protein activation appears not to have been described before. K^+ channels in RBL cells may provide an excellent system for investigating the details of the molecular interaction between the G protein and the ion channel.

REFERENCES

Andrade, R., R. C. Malenka, and R. A. Nicoll. 1986. A G protein couples serotonin and GABA$_B$ receptors to the same channels in hippocampus. *Science.* 234:1261–1265.

Barsumian, E. L., C. Isersky, M. B. Petrino, and R. P. Siraganian. 1981. IgE-induced histamine release from rat basophilic leukemia cell lines: isolation of releasing and nonreleasing clones. *European Journal of Immunology.* 11:317–323.

Beaven, M. A., J. Rogers, J. P. Moore, T. R. Hesketh, G. A. Smith, and J. C. Metcalfe. 1984. The mechanism of the calcium signal and correlation with histamine release in 2H3 cells. *Journal of Biological Chemistry.* 259:7129–7136.

Breitwieser, G. E., and G. Szabo. 1985. Uncoupling of cardiac muscarinic and α-adrenergic receptors from ion channels by a guanine nucleotide analogue. *Nature.* 317:538–540.

Cahalan, M. D., K. G. Chandy, T. E. DeCoursey, and S. Gupta. 1985. A voltage-gated potassium channel in human T lymphocytes. *Journal of Physiology.* 358:197–237.

Cassel, D., and T. Pfeuffer. 1978. Mechanism of cholera toxin action: covalent modification of the guanyl nucleotide-binding protein of the adenylate cyclase system. *Proceedings of the National Academy of Sciences.* 75:2669–2673.

Fernandez, J. M., E. Neher, and B. D. Gomperts. 1984. Capacitance measurements reveal stepwise fusion events in degranulating mast cells. *Nature.* 312:453–455.

Fewtrell, C., and E. Sherman. 1987. IgE receptor-activated calcium permeability pathway in rat

basophilic leukemia cells: measurement of the unidirectional influx of calcium using quin2-buffered cells. *Biochemistry.* 26:6995–7003.

Gill, D. M. 1977. Mechanism of action of cholera toxin. *Advances in Cyclic Nucleotide Research.* 8:85–118.

Hagiwara, S., and M. Yoshii. 1979. Effects of internal potassium and sodium on the anomalous rectification of the starfish egg as examined by internal perfusion. *Journal of Physiology.* 8:85–118.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv.* 391:85–100.

Hescheler, J., W. Rosenthal, W. Trautwein, and G. Schultz. 1987. The GTP-binding protein, Gₒ, regulates neuronal calcium channels. *Nature.* 325:445–447.

Hille, B. 1984. Ionic Channels of Excitable Membranes. Sinauer Associates Inc., Sunderland, MA. 225 pp.

Hille, B., and W. Schwarz. 1978. Potassium channels as multi-ion single-file pores. *Journal of General Physiology.* 72:409–442.

Holz IV, G. G., S. G. Range, and K. Dunlap. 1986. GTP-binding proteins mediate transmitter inhibition of voltage-dependent calcium channels. *Nature.* 319:670–672.

Kanner, B. I., and H. Metzger. 1983. Crosslinking of the receptors for immunoglobulin E depolarizes the plasma membrane of rat basophilic leukemia cells. *Proceedings of the National Academy of Sciences.* 80:5744–5748.

Kanner, B. I., and H. Metzger. 1984. Initial characterization of the calcium channel activated by the cross-linking of the receptors for immunoglobulin E. *Journal of Biological Chemistry.* 259:10188–10193.

Kurachi, Y., T. Nakajima, and T. Sugimoto. 1986. On the mechanism of activation of muscarinic K⁺ channels by adenosine in isolated atrial cells: involvement of GTP-binding proteins. *Pflügers Archiv.* 407:264–274.

Kurtz, A., and R. Penner. 1989. Angiotensin II induces oscillations of intracellular calcium and blocks anomalous inward rectifying potassium current in mouse renal juxtaglomerular cells. *Proceedings of the National Academy of Sciences.* 86:3423–3427.

Labrecque, G. F., D. Holowka, and B. Baird. 1988. Characterization of an increased K permeability associated with the stimulation of the receptor for immunoglobulin E (IgE) on rat basophilic leukemia (RBL) cells. *Biophysical Journal.* 53:348a. (Abstr.).

Labrecque, G. F., D. Holowka, and B. Baird. 1989. Antigen-triggered membrane potential changes in IgE-sensitized rat basophilic leukemia cells: evidence for a repolarizing response that is important in the stimulation of cellular degranulations. *Journal of Immunology.* 142:236–243.

Lewis, D. L., F. F. Weight, and A. Luini. 1986. A guanine nucleotide-binding protein mediates the inhibition of voltage-dependent calcium current by somatostatin in a pituitary cell line. *Proceedings of the National Academy of Sciences.* 83:9035–9039.

Lindau, M., and J. M. Fernandez. 1986a. IgE-mediated degranulation of mast cells does not require opening of ion channels. *Nature.* 319:150–153.

Lindau, M., and J. M. Fernandez. 1986b. A patch-clamp study of histamine-secreting cells. *Journal of General Physiology.* 88:349–368.

Liu, F.-T., J. W. Bohn, E. L. Ferry, H. Yamamoto, C. A. Molinaro, L. A. Sherman, N. R. Klinman, and D. H. Katz. 1980. Monoclonal dinitrophenyl-specific murine IgE antibody: preparation, isolation, and characterization. *Journal of Immunology.* 124:2728–2737.

Logothetis, D. E., Y. Kurachi, J. Galper, E. J. Neer, and D. E. Clapham. 1987. The βγ subunits of GTP-binding proteins activate the muscarinic K channel in heart. *Nature.* 325:321–326.

Maguire, M. E., and J. J. Erdos. 1980. Inhibition of magnesium uptake by β-adrenergic agonists and prostaglandin E1 is not mediated by cyclic AMP. *Journal of Biological Chemistry.* 255:1030–1035.

McCloskey, M. A. 1988. Cholera toxin potentiates IgE-coupled inositol phospholipid hydrolysis and mediator secretion by RBL-2H3 cells. *Proceedings of the National Academy of Sciences.* 85:7260–7264.

McCloskey, M. A., and M. D. Cahalan. 1988. Activation of a K⁺ channel in RBL-2H3 cells by a pertussis toxin-sensitive GTP-binding protein. *Journal of General Physiology.* 92:53a. (Abstr.)

Metzger, H., G. Alcaraz, R. Hohmam, J.-P. Kinet, V. Pribluda, and R. Quarto. 1986. The receptor with high affinity for immunoglobulin E. *Annual Review of Immunology.* 4:419–470.

Miller, D. J., and G. L. Smith. 1984. EGTA purity and the buffering of calcium ions in physiological solutions. *American Journal of Physiology.* 245:C160–C166.

Mohr, F. C., and C. Fewtrell. 1987. IgE receptor-mediated depolarization of rat basophilic leukemia cells measured with the fluorescent probe bis-oxonol. *Journal of Immunology.* 138:1564–1570.

Nakajima, Y., S. Nakajima, and M. Inoue. 1988. Pertussis toxin-insensitive G protein mediates substance P-induced inhibition of potassium channels in brain neurons. *Proceedings of the National Academy of Sciences.* 85:3643–3647.

Narasimhan, V., D. Holowka, C. Fewtrell, and B. Baird. 1988. Cholera toxin increases the rate of antigen-stimulated calcium influx in rat basophilic leukemia cells. *Journal of Biological Chemistry.* 263:19626–19632.

Neher, E. 1988. The influence of intracellular calcium concentration on degranulation of dialysed mast cells from rat peritoneum. *Journal of Physiology.* 395:193–214.

North, R. A., A. Williams, A. Suprenant, and M. J. Christie. 1987. μ and δ receptors both belong to a family of receptors which couple to a potassium conductance. *Proceedings of the National Academy of Sciences.* 84:5487–5491.

Pfaffinger, P. J. 1988. Muscarine and t-LHRH suppress M-current by activating an IAP-insensitive G-protein. *Journal of Neuroscience.* 8:3343–3352.

Pfaffinger, P. J., J. M. Martin, D. D. Hunter, N. M. Nathanson, and B. Hille. 1985. GTP-binding proteins couple cardiac muscarinic receptors to a K channel. *Nature.* 317:536–538.

Rosenthal, W., J. Hescheler, K.-D. Hinsch, K. Spicher, W. Trautwein, and G. Schultz. 1988. Cyclic AMP-independent, dual regulation of voltage-dependent Ca²⁺ currents by LHRH and somatostatin in a pituitary cell line. *EMBO Journal.* 7:1627–1633.

Sasaki, K., and M. Sato. 1987. A single GTP-binding protein regulates K⁺-channels coupled with dopamine, histamine and acetylcholine receptors. *Nature.* 325:259–262.

Seldin, D. C., S. Adelman, K. F. Austen, R. L. Stevens, A. Hein, J. P. Caulfield, and R. G. Woodbury. 1985. Homology of the rat basophilic leukemia cell and the rat mucosal mast cell. *Proceedings of the National Academy of Sciences.* 82:3871–3875.

Trube, G., and J. Hescheler. 1984. Inward-rectifying channels in isolated patches of the heart cell membrane: ATP-dependence and comparison with cell-attached patches. *Pflügers Archiv.* 401:178–184.

Ui, M. 1984. Islet-activating protein, pertussis toxin: a probe for functions of the inhibitory guanine nucleotide regulatory component of adenylate cyclase. *Trends in Physiological Sciences.* 277–279.

VanDongen, A. M. J., J. Codina, J. Olate, R. Mattera, R. Joho, L. Birnbaumer, and A. M. Brown. 1988. Newly identified brain potassium channels gated by the guanine nucleotide binding protein Gₒ. *Science.* 242:1433–1437.

Woldemussie, E., K. Maeyama, and M. A. Beaven. 1986. Loss of secretory response of rat baso-

philic leukemia (2H3) cells at 40°C is associated with reversible suppression of inositol phospholipid breakdown and calcium signals. *Journal of Immunology.* 137:1674–1680.

Yatani, A., J. Codina, A. M. Brown, and L. Birnbaumer. 1987*a*. Direct activation of mammalian atrial muscarinic potassium channels by GTP regulatory protein G_K. *Science.* 235:207–211.

Yatani, A., J. Codina, Y. Imoto, J. P. Reeves, L. Birnbaumer, and A. M. Brown. 1987*b*. A G protein directly regulates mammalian cardiac calcium channels. *Science.* 238:1288–1292.