Arachidonic Acid Metabolites Alter G Protein–mediated Signal Transduction in Heart

Effects on Muscarinic K⁺ Channels

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ABSTRACT The muscarinic acetylcholine receptor (mAChR)–stimulated, inwardly rectifying K⁺ current (Ir₄ₐᵥ) was examined in single bullfrog atrial cells using the whole-cell patch clamp technique. Ir₄ₐᵥ was activated either by bath addition of 1 µM ACh or via activation of the G protein, Gₐ, with guanosine-γ-thiotriphosphate (GTPγS). Arachidonic acid (AA) modulated Ir₄ₐᵥ under both conditions. AA decreased mAChR-stimulated Ir₄ₐᵥ and increased the rate of decay from the peak current (desensitization). In addition, AA affected GTPγS-activated Ir₄ₐᵥ by modulation of Gₐ. The effects of AA and its metabolites on Gₐ were assessed by examining their effects on both the basal rate of Gₐ activation by GTPγS, and the mAChR-mediated increase in activation rate produced by nanomolar ACh. AA increased the basal rate of GTPγS-mediated Ir₄ₐᵥ activation, but reduced the ACh-induced augmentation of this rate. All of the effects of AA on GTPγS-mediated Ir₄ₐᵥ activation were produced by metabolites. A lipoxygenase inhibitor, nordihydroguaiaretic acid (NDGA), decreased the basal and ACh-enhanced rate of Ir₄ₐᵥ activation in both the presence and absence of exogenous AA. In contrast, indomethacin (INDO), a cyclooxygenase inhibitor, increased the basal rate of Ir₄ₐᵥ activation by GTPγS in both the presence and absence of exogenous AA, and reversed the effects of AA on the ACh-augmented basal rate. AA metabolites produced via lipoxygenase and cyclooxygenase pathways thus have opposing effects on the signal transduction pathway from mAChR to Ir₄ₐᵥ. We directly tested a lipoxygenase pathway metabolite, LTB₄, on GTPγS-mediated Ir₄ₐᵥ activation and found that it not only overcame the inhibitory effects of NDGA, but also increased both the basal and ACh-augmented rate of Ir₄ₐᵥ activation. From these data, we propose that AA metabolites modulate the function of Gₐ by altering its kinetic properties.

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Acetylcholine (ACh) binding to muscarinic acetylcholine receptors (mAChR) on atrial cells activates an inwardly rectifying potassium current, $I_{K(ACh)}$ (Garnier et al., 1978; Simmons and Hartzell, 1987). Although it is clear that a GTP-binding protein ($G_{\alpha}$) mediates the coupling between mAChR and the ion channel (Breitwieser and Szabo, 1985; Pfaffinger et al., 1985), the exact nature of the coupling remains elusive.

Activation of $I_{K(ACh)}$ by $G_{\alpha}$ has been suggested to involve the direct interaction of $G_{\alpha}$ with both mAChR and the ion channel. Initial experiments (Logothetis et al., 1987; Yatani et al., 1987) demonstrated that isolated, preactivated G proteins (or activated subunits) were capable of inducing $I_{K(ACh)}$ in excised patches of atrial cell membranes. Cytoplasmic second messengers were apparently not involved, since the channels were not activated in on-cell patches when ACh was applied to the cell, but were activated when ACh was included in the patch pipette during on-cell recording (Soejima and Noma, 1984).

The ability to apply isolated G protein subunits to the cytoplasmic face of excised membrane patches has produced some interesting insights into G protein–mediated signal transduction. Both isolated $\alpha$ (for review, see Brown and Birnbaumer, 1988) and $\beta\gamma$ (for review, see Neer and Clapham, 1988) subunits have been shown to produce $I_{K(ACh)}$-like currents. The mode of action of the isolated subunits was thought to be the same: insertion of either the $\alpha$ or $\beta\gamma$ subunits into the patch membrane, and direct interaction with the $I_{K(ACh)}$ channel protein.

Although the initial reports of $\beta\gamma$-mediated activation of $I_{K(ACh)}$ assumed a direct interaction of the subunits with the $I_{K(ACh)}$ channel (Logothetis et al., 1987), it now seems clear that the $\beta\gamma$ subunits are mediating at least some of their effects via activation of phospholipase A$_2$ (PLA$_2$). G proteins are involved in the activation of PLA$_2$ (for review, see Burch, 1989), and it has been suggested that in some systems, activation is mediated by $\beta\gamma$ subunits (Jelksena and Axelrod, 1987). Antibodies to PLA$_2$ block the ability of $\beta\gamma$ subunits to activate $I_{K(ACh)}$ in excised patches (Kim et al., 1989), and bath application of arachidonic acid (AA), blockers of AA metabolism, and 5-lipoxygenase metabolites are all capable of altering $I_{K(ACh)}$ single channel activity (Kim et al., 1989; Kurachi et al., 1989). Pertussis toxin did not block the ability of AA metabolites to modulate $I_{K(ACh)}$ (Kurachi et al., 1989), nor were inhibitors of AA metabolism able to block the GTP$\gamma$S-mediated activation of $I_{K(ACh)}$, suggesting that $\alpha$ subunits mediate $I_{K(ACh)}$ activation in the absence of the $\beta\gamma$ pathway. Taken together, the results of experiments with exogenously applied G protein subunits suggested that $I_{K(ACh)}$ may be modulated by complex interactions between the $\alpha$ subunit and products of $\beta\gamma$-activated metabolic pathways.

To dissect out the effects of AA and its metabolites on each of the components of the normal activation cascade, we used a kinetic approach which can directly assess the functional state of $G_{\alpha}$ (Breitwieser and Szabo, 1988). The key feature of the method is the use of high concentrations of hydrolysis-resistant GTP analogues at fixed ratios with GTP (e.g., for GTP$\gamma$S/GTP > 20/1), which results in the irreversible activation of every $G_{\alpha}$ molecule that releases its bound GDP. This process is directly reflected in the appearance of $I_{K(ACh)}$. Under these special conditions, the
rate of $I_{K_{ACh}}$ activation is likely to correspond to the rate-limiting step in the receptor-independent activation of $G_\alpha$, namely, GDP release (Breitwieser and Szabo, 1988; Szabo and Otero, 1989). The kinetic approach proved to be a remarkably reproducible and sensitive assay of the state of $G_\alpha$, and amplified the effects of AA and its metabolites. Our results suggest that AA can modulate both the rate of $mAChR$-independent $G_\alpha$ activation and the ability of ACh to increase this rate. AA metabolites thus set the “tone” of the $mAChR-G_\alpha-I_{K_{ACh}}$ pathway. A preliminary account of these results has appeared (Scherer and Breitwieser, 1990).

METHODS

Solutions

HEPES-buffered Ringer solution contained (in millimolar): NaCl, 90; KCl, 2.5; MgCl$_2$, 5; CaCl$_2$, 2.5; and HEPES, 20; pH 7.4. For the electrophysiological experiments, 5 μM TTX and 0.5 mM CdCl$_2$ were added to block Na$^+$ and Ca$^{2+}$ currents, respectively. Other additions to the superfusing solution are noted in the figures. Stock solutions of AA (20 mM, in ethanol) were stored in aliquots under nitrogen at −20°C. Fresh aliquots were used daily. The sulfidopeptide leukotriene C$_4$ (LTC$_4$) was purchased in ethanol and stored at −80°C until the day of the experiment. Aliquots were diluted into HEPES-buffered Ringer and used within 4 h. Stock solutions of indomethacin (INDO) in ethanol and nordihydroguaiaretic acid (NDGA) in dimethylsulfoxide were made fresh daily. Neither solvent (maximal concentration, 3.0%) had any effect on GTPγS- or ACh-stimulated $I_{K_{ACh}}$ (data not shown). The intracellular solution contained (in millimolar): K$^+$ aspartate, 80; KCl, 30; HEPES, 5; K$^+$ EGTA, 1; GTP, 0.05; MgATP, 5; pH 7.4. When present, GTPγS was added to the intracellular solution as the Li salt.

All salts were reagent grade. All chemicals and nucleotides were obtained from Sigma Chemical Co. (St. Louis, MO) except HEPES (Ultrol) and AA (Calbiochem Corp., San Diego, CA), and LTC$_4$ (Cayman, Ann Arbor, MI).

Cell Dissociation

Isolated atrial cells from the bullfrog *Rana catesbeiana* were obtained as described by Fischmeister and Hartzell (1986) with minor modifications. Briefly, the heart was removed, rinsed in HEPES-buffered Ringer, and then perfused with 25 ml Ca$^{2+}$-free Ringer solution (in millimolar): NaCl, 88.4; KCl, 2.5; MgCl$_2$, 1.8; NaHCO$_3$, 23.8; NaH$_2$PO$_4$, 0.6; and Na$^+$ pyruvate, 2.5; fatty acid-free bovine serum albumin, 1 mg/ml; and MEM vitamins and essential amino acids, 1 μl/ml, equilibrated with 5% CO$_2$ at 28°C. The heart was then perfused (at 28°C) with 25 ml of a recirculating dissociation solution (Ca$^{2+}$-free Ringer solution with 5 mM creatine, 50 U/ml penicillin, and 50 μg/ml streptomycin) containing 10 mg trypsin (bovine pancreas; Boehringer Mannheim Biochemicals, Indianapolis, IN) and 62.5 μg collagenase B (*Clostridium histolyticum*, lot 144702; Boehringer Mannheim Biochemicals). After 60–90 min the heart was placed in dissociation medium without enzymes and the atrium dissected. Cells were released by gentle agitation of the atrium in dissociation medium for 30–45 min. The myocytes were then diluted twofold with storage solution (Ca$^{2+}$-free Ringer solution with 0.9 mM CaCl$_2$; 5 mM glucose; 1 μl/ml MEM vitamins and essential amino acids; 50 U/ml penicillin; and 50 μg/ml streptomycin). Cells remained viable for 6–8 h after isolation.
**Electrophysiology**

Currents were measured by the whole-cell patch clamp technique (Hamill et al., 1981) as previously described (Breitwieser and Szabo, 1985, 1988). Patch electrodes were fabricated from square bore glass (Glass Co. of America, Millville, NJ) on a Flaming Brown micropipette puller (Sutter Instrument Co., San Rafael, CA) and were used without firepolishing. Junction potentials were not corrected. Voltage pulses were generated with a computer-loaded Arbitrary Waveform generator (model 75; Wavetek, San Diego, CA). The protocol for monitoring $I_{K_ACh}$ used sequential 250-ms steps to $-90$, $-125$, and $-5$ mV from the holding potential of $-85$ mV (with return to the holding potential between steps). Steady-state current–voltage relationships were determined by assessing the average current at the end of 250-ms voltage steps. Pulse protocols were applied to the cell continuously from the moment of patch rupture. A 5-mV hyperpolarizing step from the holding potential was included in all pulse protocols to allow continuous monitoring of the pipette series resistance, which was not electronically compensated. Currents were measured with a patch clamp amplifier (EPC-7; List, Darmstadt-Eberstadt, FRG) and monitored with an oscilloscope (model 310; Nicolet Instrument Corp., Madison, WI). Signals were converted to digital signal via a PCM-1 instrumentation adaptor (Medical Systems Corp., Greenvale, NY) and stored on VCR tape (VHS, model AG-1230; Panasonic, Secaucus, NJ). Rates were estimated by recording the analogue signal on either an Astromed (model Z100; Astromed, West Warwick, RI) or Gould recorder (model 220; Gould Inc., Cleveland, OH). All experiments were performed at room temperature (22–24°C).

**RESULTS**

**AA Alters Both the Magnitude and Time Course of $I_{K_ACh}$ Activation**

ACh induces an inwardly rectifying K⁺ current in isolated bullfrog atrial myocytes, as illustrated by the steady-state current–voltage relationship shown in Fig. 1 A. To assess whether AA could activate whole-cell $I_{K_ACh}$ to a level comparable to that obtained with ACh, we superfused bullfrog atrial cells with 20 μM AA. AA produced minimal activation of $I_{K_ACh}$ (Fig. 1 B): in this particular experiment, there was a 3-pA increase in outward current at $-5$ mV.

Also illustrated in Fig. 1 B is the response of the myocyte to ACh in the continued presence of AA. After a 2-min exposure to 20 μM AA, stimulation with 1 μM ACh elicited a smaller current than that seen in response to ACh in the absence of AA (compare Fig. 1, A and B). Since AA also altered the kinetics of $I_{K_ACh}$ decay after ACh application (see below), the mean ACh-induced $I_{K_ACh}$ in the presence of AA was assessed 30 s after the peak response and was $37.8 \pm 4.0$ pA (SEM; $n = 17$) at $-5$ mV. This is less than half of the steady-state $I_{K_ACh}$ observed in the absence of AA ($88.1 \pm 8.1$ pA; $n = 32$).

AA modified the kinetics of decay of the current induced by ACh. Illustrated in Fig. 2 A is the control time course of decay of $I_{K_ACh}$ at $-5$ mV in response to 1 μM ACh. Application of ACh induced a peak $I_{K_ACh}$ which decreased to $57 \pm 5\%$ ($n = 4$) of peak within 1 min. There was, in addition to the decrease in magnitude of the peak current in the presence of 20 μM AA, a more marked time-dependent decline in $I_{K_ACh}$ (Fig. 2 B). In this representative experiment, the peak $I_{K_ACh}$ in the presence of AA was lower than control, and $I_{K_ACh}$ decayed to <20% of peak current within 1
min. $I_{K[ACh]}$ after 1 min of 1 μM ACh in the presence of 20 μM AA was only 30 ± 6% of the corresponding peak $I_{K[ACh]}$ also measured in the presence of AA ($n = 4$).

The inhibitory effects of AA on $I_{K[ACh]}$ induced by ACh may be due to either (a) a direct block of the open $I_{K[ACh]}$ channel by AA or a metabolite, or (b) an effect of AA on mAChR and/or $G_{i}$. The next section uses GTPγS, a hydrolysis-resistant GTP analogue, to examine whether AA can block the $I_{K[ACh]}$ channel directly.

**AA Does Not Block Open $I_{K[ACh]}$**

Including 1 mM GTPγS in the electrode solution induces a slow, mAChR-independent activation of $I_{K[ACh]}$, which reaches a maximum, steady-state $I_{K[ACh]}$ in ~3 min (Breitwieser and Szabo, 1988). Addition of 1 μM ACh does not induce additional current once the steady-state $I_{K[ACh]}$ has been reached. Furthermore, unlike $I_{K[ACh]}$ induced by ACh in the presence of GTP, the current generated in the presence of GTPγS does not decay after removal of ACh. A representative time course showing the slow, GTPγS-mediated generation of $I_{K[ACh]}$ and parameters used to calculate the basal activation rate are illustrated in Fig. 3. The basal rate of GTPγS-mediated $I_{K[ACh]}$ activation is calculated by dividing the absolute rate (picoamperes per minute) of current increase during the linear, initial rate portion of $I_{K[ACh]}$ activation by the final steady-state current, $I_{K[ACh]}$ (picoamperes).

Also illustrated in Fig. 3 is the lack of effect of 20 μM AA on $I_{K[ACh]}$ once GTPγS-dependent activation of $G_{i}$, and hence $I_{K[ACh]}$, has occurred. This suggests that the inhibition by AA of $I_{K[ACh]}$ activated by ACh in the presence of GTP
FIGURE 2. Effect of AA on the time course of $I_{K(ACh)}$ decay in response to ACh. $I_{K(ACh)}$ was monitored by measuring the current at the end of 250-ms voltage pulses to $-5$ mV, from a holding potential of $-85$ mV. Plots are aligned to allow comparison of the time course after ACh application. Data are plotted from the time of patch rupture. The pipette contained control intracellular solution. A, Time course of $I_{K(ACh)}$ decay after exposure to 1 μM ACh. B, Time course of $I_{K(ACh)}$ decay after exposure to 1 μM ACh in the presence of 20 μM AA. The cell was superfused sequentially with Ringer solution containing first 10 and then 20 μM AA. $I_{K(ACh)}$ was then elicited by switching to a Ringer solution containing both 20 μM AA and 1 μM ACh.

(Fig. 2 B) is not due to a direct block of the open $I_{K(ACh)}$ channel by AA, and further implies that AA must produce its effect by modulating mAChR, $G_{b}$, and/or their interactions.

AA and Its Metabolites Modify the Rate of GTPγS-mediated $I_{K(ACh)}$ Activation

The physiological effects of AA can be complex since AA is rapidly metabolized via two major routes: the cyclooxygenase and lipoxygenase pathways. Blocking either pathway results in both reduced production of metabolites via the blocked pathway
and enhanced production of metabolites via the unblocked pathway. We therefore examined the effects on $I_{K(ACh)}$ activation rates of blocking either the cyclooxygenase (with INDO) or lipoxygenase (with NDGA) pathways. We summarize our conclusions in Fig. 4 to aid in an examination of the data. As illustrated, cyclooxygenase and lipoxygenase pathway metabolites have opposing effects on $I_{K(ACh)}$ activation. The rate of $I_{K(ACh)}$ activation is the result of a balance between the inhibitory actions of cyclooxygenase metabolites and the stimulatory effects of lipoxygenase metabolites, including LTC₄. Thus, blocking production of lipoxygenase metabolites with NDGA inhibits $I_{K(ACh)}$ activation, while blocking production of cyclooxygenase metabolites with INDO potentiates $I_{K(ACh)}$ activation. In this section, the rate of $I_{K(ACh)}$ activation

![Figure 3](https://example.com/figure3)

**FIGURE 3.** Effect of AA on $I_{K(ACh)}$ activated by GTPγS. 1 mM GTPγS (20/1 ratio with GTP) was added to the control intracellular solution. Generation of $I_{K(ACh)}$ by GTPγS was monitored from the moment of patch rupture by periodic 250-ms voltage steps from the holding potential (−85 mV) to −5 mV. Maximal $I_{K(ACh)}$ was assessed by switching to a Ringer solution containing 1 μM ACh, which resulted in a peak current that decayed to a steady-state level ($I_{K(ACh)\text{ss}}$). The rate of activation of $I_{K(ACh)}$ was calculated from the slope of the change in current with time (picoamperes per minute) normalized to $I_{K(ACh)\text{ss}}$ (picoamperes). For this cell, the basal activation rate was 0.22 min⁻¹. After $I_{K(ACh)\text{ss}}$ was reached, ACh was washed from the bath to test for complete irreversibility of $I_{K(ACh)}$ and then the superfusing solution was switched to a Ringer solution containing 20 μM AA, as indicated in the figure.

in the presence of GTPγS is used to isolate the Gₓ−$I_{K(ACh)}$ interaction and to examine the effect of AA and its metabolites on the rate of GDP release by Gₓ.

The rate of $I_{K(ACh)}$ activation induced by hydrolysis-resistant guanine nucleotides (GXP) depends only on the ratio of GXP/GTP for GXP concentrations >0.1 mM. At high ratios (≥20/1 for GTPγS) the maximal basal $I_{K(ACh)}$ activation rate has been shown to be equivalent to the rate of release of GDP from Gₓ (Breitwieser and Szabo, 1988; Szabo and Otero, 1989), and is of the order of 0.3 min⁻¹. The results of the previous section demonstrate that AA does not alter $I_{K(ACh)}$ once Gₓ has been fully activated by GTPγS, implying that the channel itself is not blocked by AA. Thus, it is possible to use the rate of $I_{K(ACh)}$ activation to assess whether AA or its
metabolites can affect the activation of $G_k$ by GTP$_\gamma$S, or the interaction of
$G_k$-GTP$_\gamma$S with the channel. The tabulated data are illustrated in Fig. 5. With a ratio
of 20/1 of GTP$_\gamma$S/GTP (1 mM GTP$_\gamma$S/50 $\mu$M GTP), the $I_{K_{ACH}}$ activation rate was
0.28 $\pm$ 0.02 min$^{-1}$ (SEM; $n = 18$), as has been shown previously (Breitwieser and
Szabo, 1988). In the presence of 20 $\mu$M AA this rate was significantly increased to
0.47 $\pm$ 0.03 min$^{-1}$ ($n = 24$). Similar results were obtained in the presence of 10 $\mu$M
atropine (control, 0.27 $\pm$ 0.03, $n = 3$; 20 $\mu$M AA, 0.44 $\pm$ 0.05, $n = 5$), implying that
AA does not produce its effect by binding to the agonist site of mACHR. While an
effect of AA on unoccupied mACHR cannot be ruled out, it is more likely that the
ability of AA to increase the basal rate of $I_{K_{ACH}}$ activation by GTP$_\gamma$S may be ascribed
to either an effect of AA (or its metabolites) on the release of GDP from $G_k$ or to an
enhancement of the ability of activated $G_k$ to interact with the channel.

Also illustrated in Fig. 5 are the effects of INDO and NDGA on the basal rate of
$I_{K_{ACH}}$ activation under control conditions, and in the presence of either 20 $\mu$M AA
or 10 $\mu$M LTC$_4$. INDO (10 $\mu$M) increased the rate of $I_{K_{ACH}}$ activation in both the
absence and presence of exogenously applied AA. In contrast to the effect of INDO,
NDGA (10 $\mu$M) reduced the rate of $I_{K_{ACH}}$ activation by $\sim$30%. NDGA also blocked
the increase in the basal rate determined in the presence of 20 $\mu$M AA. It should be
pointed out that NDGA reduced the magnitude of $I_{K_{ACH}}$ elicited by either ACh or
GTP$_\gamma$S (data not shown). However, since the rate of GTP$_\gamma$S-mediational $I_{K_{ACH}}$
activation was always normalized to the final ACh-stimulated $I_{K_{ACH}}$ in the same cell
(as illustrated by the control activation rate in Fig. 3), the rates are directly
comparable with those measured in the absence of NDGA.

These data suggest that normal cellular AA metabolism must contribute to the
ability of $G_k$ to release GDP and become activated. This is suggested by both the
effects of NDGA and INDO on basal $I_{K_{ACH}}$ activation rates in the absence of
exogenous AA, and by the fact that the effects of the inhibitors are the same in the
presence of exogenous AA. Furthermore, the data imply that metabolites produced
by both the cyclooxygenase and lipoxigenase pathways modulate $G_k$: lipoxigenase
metabolites appear to enhance the rate of $G_k$ activation, while cyclooxygenase
metabolites appear to inhibit it.

To confirm this hypothesis, we examined the effects of a 5-lipoxygenase metabo-
lite, LTC$_4$, on the rate of $I_{K_{ACH}}$ activation by GTP$_\gamma$S, in the presence and absence of
either NDGA or INDO. LTC$_4$ was chosen for two reasons: (a) it had been shown to
produce $I_{K_{ACH}}$ single channel activity (Kim et al., 1989; Kurachi et al., 1989) and (b)
it is the sulfidopeptide leukotriene that gives rise to a variety of further metabolites,
allowing isolation of one branch of the 5-lipoxygenase metabolite cascade. 10 μM LTC₄ in the absence of ACh had only a minor effect on whole-cell $I_{K_{ACh}}$ in the presence of GTP (<2 pA at -5 mV), but it greatly accelerated the basal rate of GTPγS-mediated $I_{K_{ACh}}$ activation, as illustrated in Fig. 5. Significantly, NDGA did not block the stimulatory effect of LTC₄. INDO on the other hand acted synergistically with LTC₄, producing a rate of $I_{K_{ACh}}$ activation 10-fold higher than that seen under control conditions. These results are consistent with products of AA metabolism mediating opposing effects on the rate of GTPγS-mediated $I_{K_{ACh}}$ activation, and hence on the rate of GDP release from $G_{k}$.

**Figure 5.** Effect of AA, metabolites, and blockers on the basal rate of $I_{K_{ACh}}$ activation. The basal rate of $I_{K_{ACh}}$ activation was measured as illustrated in Fig. 3, using an intracellular solution containing 1 mM GTPγS. Immediately upon patch rupture the superfusing solution was changed to a Ringer solution containing the indicated mixes of control, 20 μM AA, or 10 μM LTC₄, plus the indicated inhibitors (either 10 μM NDGA or 10 μM INDO). Bars indicate the mean ± SEM of four or more observations. The bar indicating the mean rate of activation in the presence of LTC₄ + INDO (3.6 ± 0.18 min⁻¹) was truncated for clarity. The rate of $I_{K_{ACh}}$ activation in the presence of either 20 μM AA or 10 μM LTC₄ was significantly greater than the control basal rate ($P < 0.01$). The blockers, NDGA or INDO, significantly altered the corresponding rate in the absence of blocker ($P < 0.01$), except for the effect of NDGA in the presence of LTC₄ (which was not significantly different from the rate in the presence of LTC₄ alone). The rate in the presence of AA + NDGA was not significantly different from the rate in the presence of NDGA alone.

AA and Its Metabolites Modulate the mAChR-induced Increase in the Rate of GTPγS-mediated $I_{K_{ACh}}$ Activation

Nanomolar concentrations of ACh, which do not activate appreciable $I_{K_{ACh}}$ in the absence of GTPγS, lead to a marked acceleration of the rate of GTPγS-mediated $I_{K_{ACh}}$ activation (Breitwieser and Szabo, 1988). This is consistent with the notion that mAChR activation enhances the rate of GDP release from $G_{k}$, and reflects the mAChR–$G_{k}$ interaction. Fig. 6 illustrates the action of 5 nM ACh on the rate of activation of $I_{K_{ACh}}$ at high GTPγS/GTP (20/1). The current was sampled at -5 mV from a holding potential of -85 mV starting immediately after breaking into the cell. GTPγS (1 mM) in the pipette quickly began to diffuse into the cell and $I_{K_{ACh}}$ began to activate at the mAChR-independent rate. The superfusing solution was then switched to one containing 5 mM ACh, causing an abrupt increase in the rate of $I_{K_{ACh}}$ activation. $I_{K_{ACh}}$ was then assessed by switching to a Ringer solution...
containing 1 μM ACh. In this cell, 5 nM ACh had already induced the maximal $I_{K_{ACh}}$ and 1 μM ACh thus had no further effect. Both the basal and the ACh-augmented rates were normalized to this maximal $I_{K_{ACh}}$. In this particular cell the rates were 0.23 and 5.07 min⁻¹, respectively. We examined the effects of AA and its metabolites on the ACh-induced increase in the rate of activation of $I_{K_{ACh}}$: AA, metabolites, and/or blockers were present from the moment of patch rupture, and various ACh concentrations were applied as described above.

We first examined the effects of LTC₄ on ACh-augmented $I_{K_{ACh}}$ activation, since its effects on the basal rate of $I_{K_{ACh}}$ activation were purely stimulatory. Confirming a previous study (Breitwieser and Szabo, 1988), ACh over the range from 1 to 10 nM produced an enhancement of the rate of GTPγS-mediated $I_{K_{ACh}}$ activation. LTC₄, which stimulated the basal rate of $I_{K_{ACh}}$ activation by GTPγS, also enhanced the ACh-mediated increase in this rate (Fig. 7). This effect of LTC₄ was not altered by the presence of 10 μM NDGA, although NDGA by itself virtually prevented the activation of $I_{K_{ACh}}$, even in the presence of 10 nM ACh. The ability of LTC₄ to overcome the block by NDGA suggests that NDGA is specifically inhibiting metabolism via lipoxygenases (and preventing production of a required activating metabolite), rather than interacting nonspecifically with either mAChR or the channel.

The effects of exogenously applied AA are more complex than those of LTC₄, since both stimulatory and inhibitory metabolites are produced, as suggested by the results in Fig. 5. To simplify an examination of the data, the effects of NDGA and INDO on the ACh-augmented rate of $I_{K_{ACh}}$ activation in the presence of exogenous AA are displayed separately.
20 μM AA (Fig. 8A) produced a rightward shift in the activation curve as compared with the control (in contrast to its stimulatory effect on mAChR-independent \( I_{[K^{+}ACh]} \) activation rates in Fig. 5). 10 μM NDGA, as discussed above, virtually blocked the enhancement of \( I_{[K^{+}ACh]} \) activation rate produced by ACh, and AA was not able to overcome this block. These results suggest that inhibitory metabolites (cyclooxygenase pathway) have a greater effect on the ACh-mediated rate of \( I_{[K^{+}ACh]} \) activation than on the mAChR-independent basal rates.

10 μM INDO (Fig. 8B), by blocking utilization of exogenously applied AA via the cyclooxygenase pathway, produced an ACh-augmented rate of \( I_{[K^{+}ACh]} \) activation which was not significantly different from control. Neither INDO + AA nor INDO alone was able to shift the ACh-mediated activation curve to the level of \( \text{LTC}_4 \), suggesting that the normal ACh-mediated augmentation of the \( I_{[K^{+}ACh]} \) activation rate (in the absence of any exogenous lipid metabolites or blockers) is limited by cellular production of stimulatory metabolites, such as \( \text{LTC}_4 \). The ACh-induced augmentation of the \( I_{[K^{+}ACh]} \) activation rate in the absence of exogenous AA metabolites represents the maximal rate at which \( \text{LTC}_4 \) or its further metabolites can be produced. Only when exogenous \( \text{LTC}_4 \) is added to the superfusing solution do the activation curves shift to the left of control, implying that cellular metabolism, under these conditions, is rate limiting. The results further suggest that significant levels of the inhibitory metabolite(s) are not produced unless exogenous AA is provided. An excess of AA saturates the lipoxygenase pathways and results in the production of

![Figure 7](https://example.com/figure7.png)
metabolites via the cyclooxygenase pathway, which apparently has a lower affinity for AA than the lipoxygenase pathway.

**DISCUSSION**

In this paper we present evidence that AA metabolites modulate the ACh-stimulated K⁺ channel of bullfrog atrial cells by altering the function of the signal-transducing, GTP-binding protein, G₆₅, which couples mAChR to the ion channel. AA (20 μM) is able to elicit a modest activation of \( I_{K(ACh)} \) under whole-cell recording conditions (2–4 pA, compared with 88.1 pA for 1 μM ACh), although the major effect of AA metabolites appears to be in modulating the cellular response to ACh. ACh, in the presence of AA, elicits a smaller peak \( I_{K(ACh)} \) and the current decays to significantly lower steady-state levels, suggesting that AA metabolites may modulate \( I_{K(ACh)} \) desensitization.

**Metabolism of AA Is Required**

Modulation of ion channel function by AA can proceed via either a direct mechanism (Kim and Clapham, 1989; Ordway et al., 1989; Vacher et al., 1989) or through
the production of metabolites (Piomelli et al., 1987; Kim et al., 1989; Kurachi et al., 1989). To assess the contribution of each of these possibilities to the effects of AA on $I_{K[ACa]}$ activation, we (a) examined the effects of the metabolic inhibitors NDGA and INDO on the response to AA, and (b) tested whether AA metabolites could overcome the blocking effects of the inhibitors.

Virtually all of the effects of AA on $I_{K[ACa]}$ activation are mediated by AA metabolites. Both inhibitory and stimulatory metabolites are produced (as discussed below; see also Fig. 4). The effect of AA on the basal activation rate was blocked by NDGA to a level not statistically different from that found in the presence of NDGA alone ($0.24 \text{ min}^{-1} [\text{AA + NDGA}]$ vs. $0.21 \text{ min}^{-1} [\text{NDGA alone}]$). If the block of the basal rate of activation of $I_{K[ACa]}$ by NDGA results in a residual rate that is due to direct effects of AA, then this effect is saturated by cellular AA levels, since addition of 20 μM exogenous AA did not significantly increase this rate, suggesting that all of the effects of AA are due to metabolites.

**Lipoxygenase Metabolites Increase the Rate of $I_{K[ACa]}$ Activation**

NDGA blocks lipoxygenases, and when added to the bath in the presence or absence of exogenous AA, decreases both the basal and ACh-augmented rate of $I_{K[ACa]}$ activation. NDGA reduced the amplitude of $I_{K[ACa]}$ activated via either ACh (+ GTP) (Scherer and Breitwieser, unpublished observations) or GTPγS (see Fig. 9 A). All of the effects of NDGA are reversed by the 5-lipoxygenase product, LTC4. These results strongly suggest that products of the 5-lipoxygenase pathway, specifically sulfidopeptide leukotrienes, enhance the rate of $G_k$ activation. The specific metabolites of NDGA-blockable pathways that modulate $G_k$ are not known since NDGA blocks not only 5-lipoxygenase, but also 12-lipoxygenase and epoxygenases (Needleman et al., 1986; Capdevila et al., 1988). Although we find that LTC4 reverses the effect of NDGA, other leukotrienes or metabolites may be equally effective, since LTC4 breaks down readily to LTD4, LTE4, etc.

**Cyclooxygenase Metabolites Decrease the Rate of $I_{K[ACa]}$ Activation**

INDO, a cyclooxygenase inhibitor, consistently increased both the basal rate of $I_{K[ACa]}$ activation in either the absence or presence of exogenously applied AA, and reversed the inhibitory effect of AA on the ACh-mediated augmentation of the basal activation rate. These results are consistent with the notion that a metabolite of the cyclooxygenase pathway slows the rate of $G_k$ activation. A complication is that diversion of AA into the lipoxygenase pathways may occur upon cyclooxygenase blockade (Aehringhaus et al., 1984), thus producing more of the stimulatory metabolites. Two of our results rule out diversion of AA into the stimulatory lipoxygenase pathway as the sole explanation for the effects of INDO and argue for the production of an inhibitory metabolite: first, INDO reverses the inhibition of the ACh-augmented $I_{K[ACa]}$ activation rate mediated by exogenously applied AA; and second, INDO greatly potentiates the effect of exogenously applied LTC4. The cyclooxygenase metabolite(s) that inhibit $I_{K[ACa]}$ activation are unknown since prostaglandins, thromboxanes, and 12-hydroxy-heptadecatrienoic acid are all produced via this pathway. Experiments to identify the cyclooxygenase pathway metabolites that inhibit $G_k$ activation are underway.
Figure 9. Effect of NDGA on GTPγS-activated $I_{K_{ACh}}$. $I_{K_{ACh}}$ was activated by 1 mM GTPγS in the intracellular solution; maximal $I_{K_{ACh}}$ was obtained by a brief pulse of 1 μM ACh, which was then washed from the bath. Illustrated is the effect of switching to a Ringer solution containing NDGA plus various other effectors on the GTPγS-activated current. A, 10 μM NDGA; B, 10 μM NDGA plus 1 μM ACh; C, 10 μM LTC4 plus 10 μM NDGA.

Mechanism of Action of AA Metabolites

Our data would suggest that both lipoygenase and cyclooxygenase products of AA modulate $I_{K_{ACh}}$ through $G_{\kappa}$. Three configurations of a receptor-G protein-effector signal-transducing pathway could account for our data, as diagrammed below, with LTR representing a leukotriene receptor, LT representing an unspecified leukotriene, and PGH$_2$X representing one or more of the metabolites produced from prostaglandin H$_2$ (PGH$_2$) via the cyclooxygenase pathway, which negatively modulate...
the effects of LTR. Model 1 assumes mAChR and LTR converge at \( G_k \), with both receptors capable of increasing the rate of GDP release from \( G_k \). Model 2 differs from model 1 in that mAChR and LTR interact with distinct G proteins, both of which are capable of activating \( I_{K[ACH]} \), and thus the two pathways converge at the level of the channel. Model 3 states that metabolites of AA alter the kinetic properties of \( G_k \) and affect its interactions with mAChR and/or the ion channel.

Models 1 and 2 assume that the relevant LTR is coupled to a G protein. Although \(^{3}H\)LTD\textsubscript{4} binding in lung is modulated by guanine nucleotides, binding of LTC\textsubscript{4} is not, suggesting that not all leukotriene receptors are coupled to effectors via G proteins (Hogaboom et al., 1983). If the relevant LTR is coupled to a G protein, it cannot be \( G_k \), since the effect of leukotrienes on single-channel \( I_{K[ACH]} \) activation was not blocked by pertussis toxin (PTX) treatment (Kurachi et al., 1989), which interferes with the interaction of receptors with \( G_k \) (Pfaffinger et al., 1985). Model 1 is thus ruled out. Model 2 is still plausible if it is assumed that \( G_k2 \) is not sensitive to PTX, and hence is not uncoupled from LTR by PTX treatment.

Agonists for G protein–coupled receptors act to enhance GDP release from inactive G proteins. Once the G protein becomes activated with GTP\textsubscript{γS}, removal of agonist (e.g., ACh) is without effect (Breitwieser and Szabo, 1985, 1988). Similarly,
removal of lipoxygenase products by addition of NDGA should be without effect if the leukotrienes are acting only by increasing the rate of GDP release from G\textsubscript{\alpha} via a classical receptor–G protein interaction. Fig. 9 illustrates the test of these predictions. In all three panels, \( I_{[K(AC)]} \) was maximally activated by GTP\textsubscript{yS}. NDGA was able to block the GTP\textsubscript{yS}-activated \( I_{[K(AC)]} \) (Fig. 9 A). Addition of ACh with NDGA did not overcome the effect of NDGA (Fig. 9 B). LTC\(_4\), however, if added at the same time as NDGA, was able to prevent the inhibition of the GTP\textsubscript{yS}-activated \( I_{[K(AC)]} \) (Fig. 9 C), and indeed, resulted in a slight increase in the level of GTP\textsubscript{yS}-activated current. These results further rule out models 1 and 2, since in both cases one would expect uncoupling of all of the receptors from their respective G proteins once the G proteins had been activated by GTP\textsubscript{yS}.

Model 3 is the most parsimonious explanation of our data. We suggest that both lipoxygenase and cyclooxygenase metabolites directly modulate the functional state of G\textsubscript{\alpha}. These metabolites produce their effects on both the inactive, GDP-bound form of G\textsubscript{\alpha} and on \( \alpha\textsubscript{\gamma}-\text{GTP}\textsubscript{\gammaS} \), suggesting that the \( \alpha \) subunit is modified. Modulation of GTP\textsubscript{yS}-activated \( I_{[K(AC)]} \) by AA metabolites requires that either (a) the channel-associated \( \alpha\textsubscript{\gamma}-\text{GTP}\textsubscript{\gammaS} \) can still be modulated by AA metabolites or (b) there is a finite amount of dissociation of \( \alpha\textsubscript{\gamma}-\text{GTP}\textsubscript{\gammaS} \) from the ion channel during steady-state current activation. The ability of LTC\(_4\) to increase steady-state, GTP\textsubscript{yS}-activated \( I_{[K(AC)]} \) (Fig. 9 C) suggests it is able to promote \( \alpha\textsubscript{\gamma}-\text{GTP}\textsubscript{\gammaS} \) – channel association:

\[
\alpha\textsubscript{\gamma}-\text{GTP}\textsubscript{\gammaS} + \text{channel} \xrightleftharpoons{} \text{channel} \cdot \alpha\textsubscript{\gamma}-\text{GTP}\textsubscript{\gammaS},
\]

implying that both unassociated \( \alpha\textsubscript{\gamma}-\text{GTP}\textsubscript{\gammaS} \) and ion channels exist under steady-state conditions. LTC\(_4\) could thus increase the steady-state, GTP\textsubscript{yS}-activated \( I_{[K(AC)]} \) by shifting the equilibrium in favor of channel \( \cdot \alpha\textsubscript{\gamma}-\text{GTP}\textsubscript{\gammaS} \). This has implications for the physiological activation of \( I_{[K(AC)]} \) by ACh. As illustrated in Fig. 2 A, peak \( I_{[K(AC)]} \) desensitizes to the steady-state current within the first 60 s after ACh application, implying that not all of the available ion channels are active during the steady state. Thus, both the net ability of G\textsubscript{\alpha} to activate \( I_{[K(AC)]} \) and desensitization may, in part, be the result of alterations in the balance of inhibitory and stimulatory AA metabolites with time after mAChR activation.

It is necessary to rule out direct modifications of either the ion channel or mAChR by AA metabolites. Several lines of evidence suggest that the ion channel is not directly blocked or modulated by AA metabolites. Fig. 3 suggests that the GTP\textsubscript{yS}-activated current is not directly modulated by AA, and the results of Fig. 9, A and C suggest that NDGA does not produce its effects by directly blocking the ion channel, since LTC\(_4\) is able to overcome the blocking effects.

Our results rule out the mAChR as the major locus of AA metabolite action. While AA decreases the binding of the muscarinic antagonist \([\text{H}]\text{quinuclidinylbenzilate} \) to cardiac (Rauch et al., 1989) or synaptic membranes (Aronstam et al., 1977), it has also been reported to increase binding of the muscarinic agonist carbamylcholine (Baron and Kloog, 1984). Our results exclude an effect of AA or its metabolites at the agonist-binding site of mAChR binding, since AA has the same effect(s) on the rate of \( I_{[K(AC)]} \) activation in the absence or presence of atropine (10 \( \mu \text{M} \)). In addition, the effects of AA metabolites and blockers on the ACh-augmented
rate of $I_{K(ACh)}$ activation can be predicted from their effects on the basal rate of $G_k$ activation, suggesting that mAChR is interacting normally with an altered $G_k$. Finally, AA metabolites affect the GTPγS-activated $I_{K(ACh)}$ (Fig. 9) under conditions in which mAChR is uncoupled from $G_k$. Thus, although we cannot at this time rule out a minor effect of AA and/or its metabolites on mAChR, our data would suggest that the primary site of action for AA and its metabolites is at the level of $G_k$.

It is of interest to speculate about the ways in which $G_k$ could be modified by AA and its metabolites. There have been reports of phosphorylation of $G_i$ in response to protein kinase C activation (Katada et al., 1985). In addition, there is evidence that hydroperoxy acids decrease the activity of the calcium-calmodulin protein kinase II (Piomelli et al., 1989). Therefore, it is possible that a tonic level of phosphorylation of $G_k$ is modulated by AA and its metabolites. It is also possible that some other type of dynamic covalent modification of $G_k$ occurs in response to the balance of stimulatory and inhibitory metabolites of AA that are present in the membrane. Myristoylation (Buss et al., 1987; James and Olson, 1989a), palmitoylation (James and Olson, 1989b) and farnesylation (Casey et al., 1989; Hancock et al., 1989) have all been described as regulated modifications of eukaryotic proteins, including ras (Willumsen et al., 1984; Magee et al., 1987). Such lipid-mediated covalent modifications may be the reason that cloned $G_i$ subtypes are much less potent at promoting activation of $I_{K(ACh)}$ than biochemically purified, native G proteins (Yatani et al., 1988). Alternately, some type of noncovalent interaction of AA metabolites or further products with $G_k$ may occur.

Comparison with Excised Patch Results

Our data confirm and extend the results of Kim et al. (1989) and Kurachi et al. (1989): both groups have reported that AA and various lipoxygenase metabolites are capable of stimulating single-channel $I_{K(ACh)}$, although not to the same extent as GTPγS. Our results confirm the submaximal stimulation of $I_{K(ACh)}$ by AA at the whole-cell level: 20 μM AA induced only 2–4 pA of $I_{K(ACh)}$, compared with 88.1 pA inducible by ACh. The inability of AA to maximally activate whole-cell $I_{K(ACh)}$ in the absence of ACh, the attenuation of ACh-induced $I_{K(ACh)}$ by AA (Fig. 2), and the influence of AA and metabolites on the rate of $G_k$ activation (e.g., Fig. 5) suggest that AA and metabolites play a modulatory role in $I_{K(ACh)}$ activation. Both Kurachi et al. (1989) and Kim et al. (1989) have demonstrated stimulatory effects of lipoxygenase metabolites. Kurachi et al. (1989) concluded that these metabolites either enhance GDP/GTP exchange of $G_k$ in a receptor-independent manner, or that they couple directly to the channel via membrane receptors interacting with PTX-insensitive G proteins. Our results demonstrate that lipoxygenase metabolites increase the GDP release rate from $G_k$ and thus modulate $I_{K(ACh)}$. Our data also provide an explanation for the observation of Kurachi et al. (1989) of the slight stimulatory effect of INDO on single-channel $I_{K(ACh)}$. Since cyclooxygenases are present in endoplasmic reticulum (Needleman et al., 1986), one might expect a greater contribution of inhibitory metabolites in the whole-cell recording configuration. Our whole-cell data define a distinct inhibitory role for cyclooxygenase products in modulating $I_{K(ACh)}$ and suggest that $I_{K(ACh)}$ is dynamically regulated by the balance of stimulatory and inhibitory AA metabolites.
Physiological Implications

We have shown that metabolites of AA specifically modulate $I_{K(AC3)}$ and suggest that they act by altering the kinetic properties of $G_k$. Stimulation of mACHR results in activation of $I_{K(AC3)}$, inhibition of adenylate cyclase, and stimulation of phosphatidylinositol metabolism. All of these effects are mediated by G proteins and thus may be subject to modulation by AA. Physiological responses to ACh such as alterations in heart rate, contractility, and conduction velocity may also be affected by AA.

The results we have obtained in the presence of exogenously applied AA may be more relevant to pathological states, in which large amounts of AA and metabolites are produced. For example, exogenously generated leukotrienes have been shown to alter cardiac performance. Leukotrienes (LTC₄ and LTD₄) are produced in isolated heart preparations in response to antigenic stimulation (Aehringhaus et al., 1983; Levi et al., 1985; Yacoob and Piper, 1988) or during myocardial infarction (Evers et al., 1985). Although all of the leukotrienes produced during pathophysiological states may not be produced by the myocytes, leukotrienes do impinge directly on cardiac muscle, probably through specific receptors (Hogaboom et al., 1985), resulting in a negative inotropic effect (Hattori and Levi, 1984; Levi et al., 1985; Bjornsson et al., 1987). Our data would suggest that part of the mechanism by which leukotrienes reduce myocardial contractility may be modulation of $I_{K(AC3)}$, and possibly other second messenger pathways.

There also appears to be a defined role for AA metabolism under nonpathological states. The effects of NDGA and INDO on $I_{K(AC3)}$ activation rates in the absence of exogenous AA suggest that cellular metabolism of AA contributes to the normal functioning of $G_k$ and of the signal transduction pathway, which ultimately activates $I_{K(AC3)}$. Since prostaglandins are produced by isolated hearts in response to muscarinic receptor stimulation (Jaiswal and Malik, 1988), it is likely that modulation of $I_{K(AC3)}$ by AA metabolites occurs as a normal consequence of mAChR stimulation. Indeed, lipoxygenase products of AA metabolism may be necessary for full expression of $I_{K(AC3)}$, since $I_{K(AC3)}$ is greatly attenuated by NDGA. Finally, AA release resulting from activation of phospholipase A₂ or phospholipase C plus diacylglycerol lipase (Burch, 1989) via other receptor systems may modulate $I_{K(AC3)}$ indirectly by altering $G_k$ responsiveness to mAChR stimulation.

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REFERENCES

Aehringhaus, U., A. Dembinska-Kiec, and B. A. Peskar. 1984. Effects of exogenous prostaglandins on the release of leukotriene C₄-like immunoreactivity and on coronary flow in indomethacin-treated anaphylactic guinea-pig hearts. Naunyn-Schmiedeberg's Archives of Pharmacology. 326:368–374.
Jaiswal, N., and K. U. Malik. 1988. Prostaglandin synthesis elicited by cholinergic stimuli is mediated by activation of M₂ muscarinic receptors in rabbit heart. The Journal of Pharmacology and Experimental Therapeutics. 245:59–66.

James, G., and E. N. Olson. 1989a. Myristoylation, phosphorylation, and subcellular distribution of the 80-kDa protein kinase C substrate in BC₃H₁ myocytes. Journal of Biological Chemistry. 264:20928–20933.

James, G., and E. N. Olson. 1989b. Identification of a novel fatty acylated protein that partitions between the plasma membrane and cytosol and is deacylated in response to serum and growth factor stimulation. Journal of Biological Chemistry. 264:20998–21006.

Jelsema, C. L., and J. Axelrod. 1987. Stimulation of phospholipase A₂ activity in bovine rod outer segments by the βγ subunits of transducin and its inhibition by the α subunit. Proceedings of the National Academy of Sciences USA. 84:3623–3627.

Katada, T., A. G. Gilman, Y. Watanabe, S. Bauer, and K. H. Jakobs. 1985. Protein kinase C phosphorylates the inhibitory guanine-nucleotide-binding regulatory component and apparently suppresses its function in hormonal inhibition of adenylate cyclase. European Journal of Biochemistry. 151:431–437.

Kim, D., and D. E. Clapham. 1989. Potassium channels in cardiac cells activated by arachidonic acid and phospholipids. Science. 244:1174–1176.

Kim, D., D. L. Lewis, L. Grazidei, E. J. Neer, D. Bar-Sagi, and D. E. Clapham. 1989. G-protein βγ-subunits activate the cardiac muscarinic K⁺-channel via phospholipase A₂. Nature. 337:557–560.

Kurachi, Y., H. Ito, T. Sugimoto, T. Shimizu, I. Miki, and M. Ui. 1989. Arachidonic acid metabolites as intracellular modulators of the G protein-gated cardiac K⁺ channel. Nature. 337:555–557.

Levi, R., Y. Hattori, J. A. Burke, Z.-G. Guo, U. Hachfeld del Balzo, W. A. Scott, and G. A. Rouzer. 1985. Leukotriene C₄ is released from the anaphylactic heart: a case for its direct negative inotropic effect. In Prostaglandins, Leukotrienes, and Lipoxins: Biochemistry, Mechanism of Action, and Clinical Applications. J. M. Bailey, editor. Plenum Publishing Corp., New York. 275–288.

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.

Magee, A. I., L. Gutierrez, I. A. McKay, C. J. Marshall, and A. Hall. 1987. Dynamic fatty acylation of p21ras. The EMBO Journal. 6:3353–3357.

Needleman, P., J. Turk, B. A. Jakusch, A. R. Morrison, and J. B. Lefkowith. 1986. Arachidonic acid metabolism. Annual Review of Biochemistry. 55:69–102.

Neer, E. J., and D. E. Clapham. 1988. Roles of G protein subunits in transmembrane signalling. Nature. 333:129–134.

Ordway, R. W., J. V. Walsh, Jr., and J. J. Singer. 1989. Arachidonic acid and other fatty acids directly activate potassium channels in smooth muscle cells. Science. 244:1176–1179.

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.

Piomelli, D., A. Volterra, N. Dale, S. A. Siegelbaum, E. R. Kandel, J. H. Schwartz, and F. Belardetti. 1987. Lipoxynegenase metabolites of arachidonic acid as second messengers for presynaptic inhibition of Aplysia sensory cells. Nature. 328:38–43.

Piomelli, D., J. K. T. Wang, T. S. Sihra, A. C. Nairn, A. J. Czernik, and P. Greengard. 1989. Inhibition of Ca²⁺/calmodulin-dependent protein kinase II by arachidonic acid and its metabolites. Proceedings of the National Academy of Sciences USA. 86:8550–8554.
Rauch, B., R. A. Colvin, and F. C. Messineo. 1989. Inhibition of [3H]quinucilidinyl benzylate binding to cardiac muscarinic receptor by long chain fatty acids can be attenuated by ligand occupation of the receptor. *Journal of Molecular and Cellular Cardiology.* 21:495–506.

Scherer, R. W., and G. E. Breitwieser. 1990. Arachidonic acid and LTC4 modulate muscarinic potassium channel activation in heart. *Biophysical Journal.* 57:313a. (Abstr.)

Simmons, M. A., and H. C. Hartzell. 1987. A quantitative analysis of the acetylcholine-activated potassium current in single cells from frog atrium. *Pflügers Arch.* 409:454–461.

Scherer, R. W., and G. E. Breitwieser. 1990. Arachidonic acid and LTC4 modulate muscarinic potassium channel activation in heart. *Biophysical Journal.* 57:313a. (Abstr.)

Simmons, M. A., and H. C. Hartzell. 1987. A quantitative analysis of the acetylcholine-activated potassium current in single cells from frog atrium. *Pflügers Arch.* 409:454–461.

Soejima, M., and A. Noma. 1984. Mode of regulation of the ACh-sensitive K-channel by the muscarinic receptor in rabbit atrial cells. *Pflügers Arch.* 400:424–431.

Szabo, G., and A. S. Otero. 1989. Muscarinic activation of potassium channels in cardiac myocytes: kinetic aspects of G protein function in vivo. *Trends in Pharmacological Sciences.* 10 (Suppl):46–49.

Vacher, P., J. McKenzie, and B. Duly. 1989. Arachidonic acid affects membrane ionic conductances of GH3 pituitary cells. *American Journal of Physiology (Endocrinology and Metabolism).* 257:E203–E211.

Willumsen, B. M., A. Christensen, N. L. Hubbert, A. G. Papageorge, and D. R. Lowy. 1984. The p21 ras C-terminus is required for transformation and membrane association. *Nature.* 310:583–586.

Yacoob, H. B., and P. J. Piper. 1988. Inhibition of leukotriene release in anaphylactic guinea-pig hearts by a 5-lipoxygenase inhibitor, CGS 8515. *British Journal of Pharmacology.* 95:1322–1328.

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

Yatani, A., R. Mattera, J. Codina, R. Graf, K. Okabe, E. Parell, R. Iyengar, A. M. Brown, and L. Birnbaumer. 1988. The G protein-gated atrial K+ channel is stimulated by three distinct Gqα-subunits. *Nature.* 336:680–682.