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Potassium Channels in Cardiac Cells Activated by Arachidonic Acid and Phospholipids

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Two types of potassium-selective channels activated by intracellular arachidonic acid or phosphatidylcholine have been found in neonatal rat atrial cells. In inside-out patches, arachidonic acid and phosphatidylcholine each opened outwardly rectifying potassium-selective channels with conductances of 160 picosiemens ($I_{K\cdot AA}$) and 68 picosiemens ($I_{K\cdot PC}$), respectively. These potassium channels were not sensitive to internally applied adenosine triphosphate (ATP), magnesium, or calcium. Lowering the intracellular *p*H from 7.2 to 6.8 or 6.4 reversibly increased $I_{K\cdot AA}$ channel activity three- or tenfold, respectively. A number of fatty acid derivatives were tested for their ability to activate $I_{K\cdot AA}$. These potassium-selective channels may help explain the increase in potassium conductance observed in ischemic cells and raise the possibility that fatty acid derivatives act as second messengers.

MANY K^+ CHANNELS ARE MODULATED by intracellular second messengers. Most regulatory mechanisms described to date have involved soluble second messengers such as adenosine 3′,5′-monophosphate (cAMP). More recently, membrane-permeant lipophilic mediators, specifically arachidonic acid or its metabolites, have been shown to mediate signal transduction in neuronal and cardiac cells (*1, 2*). In cardiac cells, inwardly rectifying K^+ channels are controlled by intracellular Na^+ ($I_{K\cdot Na}$) (*3*), ATP ($I_{K\cdot ATP}$) (*4*), or guanine nucleotide binding protein (G) α and βγ subunits ($I_{K\cdot ACh}$) (*5*). The outwardly rectifying transient outward current (I_{TO}) (*6*) and the delayed rectifier current (I_K) are regulated by phosphorylation via protein kinase A or protein kinase C (*7*). We now report two outwardly rectifying K^+ channels in heart that open in response to certain fatty acid derivatives. We propose that lipid-soluble, intramembranous second messenger pathways may play an important role in channel gating.

Kim *et al.* showed that the βγ subunits of G proteins activate the inwardly rectifying, muscarinic-gated K^+ channel ($I_{K\cdot ACh}$) via stimulation of phospholipase A_2 (*2*). During our experiments with arachidonic acid, we noted the appearance of previously undescribed K^+ currents. These channels were distinct from atrial $I_{K\cdot ACh}$. Figure 1A shows whole-cell records of a rat neonatal atrial cell before and after intracellular perfusion with arachidonic acid. The current-voltage (*I-V*) relation immediately after breaking into the cell was similar to that measured in control cells (Fig. 1B) ($n = 8$). As the arachidonic acid diffused into the cells, both inward and outward currents developed within 2 to 4 min, increasing the slope conductance from 0.8 to 2.8 nS at 40 mV and from 0.9 to 1.7 nS at −120 mV. The increase in slope conductance was not due to nonspecific leakage. The inwardly rectifying current was completely blocked, and the outwardly rectifying current was partially blocked by 1 m*M* extracellular $BaCl_2$ (Fig. 1C). The outwardly rectifying current was not observed when intracellular CsCl replaced KCl, suggesting that arachidonic acid activated K^+-selective ionic currents. To determine the relative contributions from the many K^+ currents present, we studied currents at the single-channel level.

When an inside-out patch was formed in the presence of 10 μ*M* arachidonic acid in the bath, a high conductance channel ($I_{K\cdot AA}$) often appeared within 3 min. We rarely (<2%) observed this channel in control patches ($n > 100$) not exposed to arachidonic acid. Figure 2A shows the gradual increase in the opening frequency of the

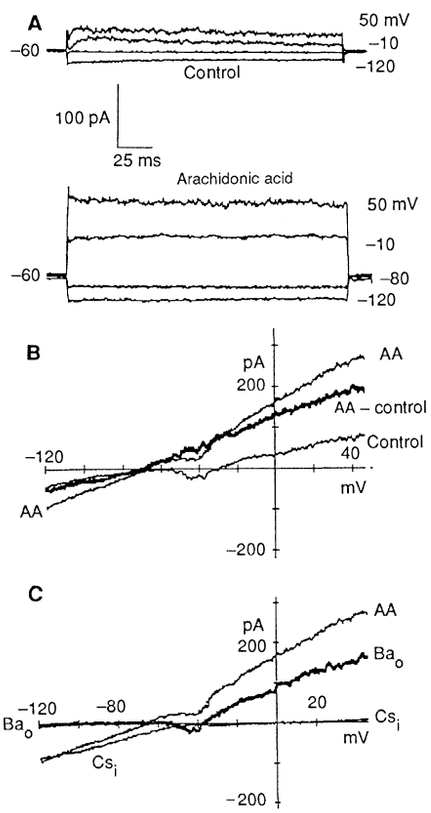

Fig. 1. Whole-cell currents produced by arachidonic acid. The pipette contained 140 m*M* K^+, 140 m*M* Cl^-, 5 m*M* EGTA, 2 m*M* Mg^{2+}, 10 m*M* Hepes (*p*H 7.2), and 10 μ*M* arachidonic acid. Extracellular solution contained 5 m*M* K^+, 140 m*M* Na^+, 1.0 m*M* Co^{2+}, 10 m*M* Hepes, 2 m*M* Mg^{2+}, and 3μ*M* tetrodotoxin (*p*H 7.2). (**A**) Control and arachidonic acid–induced currents in response to steps from a holding potential of −60 mV. (**B**) *I-V* relations. Immediately after the initiation of whole-cell recording, voltage ramps from −120 to 50 mV were made every 5 s from a holding potential of −60 mV. The *I-V* relations were taken 10 s (control) and 3 min (AA) after the initiation of whole-cell recording. The subtracted *I-V* relation (AA − control) is shown as the thick solid line. (**C**) $BaCl_2$ (1 m*M*) applied extracellularly, blocked the inwardly rectifying component of $I_{K\cdot AA}$. Intracellular recording with CsCl replacing KCl blocked the outward component of $I_{K\cdot AA}$ (separate recording). Rat heart cells were prepared by collagenase digestion of atria from 1- to 2-day-old newborn rat (*12*). Isolated single atrial cells were plated on glass cover slips and incubated in an atmosphere of 5% CO_2 and 95% air at 37°C until use 12 to 26 hours later. All experiments were performed at 22° ± 2°C. Currents were recorded with a LIST model EPC7 patch-clamp amplifier and recorded on a digital tape recorder. Records were filtered with an eight-pole Bessel filter at 2 kHz and analyzed with an INDEC 11/73 computer (*5*).

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large conductance channel at a holding potential of 40 mV. These channels appeared in 70% of inside-out patches exposed to 10 μM arachidonic acid (n = 30). Arachidonic acid did not activate the channels when added extracellularly or to the pipette in cell-attached patches. Once activated, $I_{K\cdot AA}$ remained active for the duration of the patch recording (up to 30 min). The limiting single-channel conductance was 160 pS in the outward-current passing direction and 70 pS in the inward-current passing direction (Fig. 2, B, C, and D). By comparison, $I_{K\cdot ACh}$ and I_{K1}, two common cardiac K^+ channels, had conductances of 35 ± 5 pS in symmetrical 145 mM K^+ at −80 mV. The mean open time was ~1 ms at −60 mV (Fig. 2C, lower) and did not vary significantly with voltage. $I_{K\cdot AA}$ was K^+-selective; varying the intracellular K^+ concentration from 140 to 35 mM by substituting choline for K^+ shifted the single-channel reversal potential according to the expected Nernst relation (Fig. 2D). A plot of the reversal potential versus the intracellular K^+ concentration had a slope of −56 mV per decade, close to the expected slope of −58 mV per decade at 22°C (Fig. 2E). Replacement of K^+ by Cs^+ (intracellular surface) blocked the outward current.

In contrast to $I_{K\cdot ACh}$, $I_{K\cdot AA}$ was not activated [no increase in mean channel activity, Np_o, the product of the number of channels (N) and the probability of channel opening (p_o)] by products of arachidonic acid metabolism. 5-Hydroxyeicosatetraenoic acid (5-HETE), 5-hydroperoxyeicosatetraenoic acid (5-HPETE), 15-HETE, 12-HPETE, 12-HETE, and leukotrienes B_4, C_4, D_4, and E_4 (10 μM) did not activate $I_{K\cdot AA}$ in inside-out patches ($n \geq 5$). Indomethacin (10 μM) and nordihydroguaiaretic acid (10 μM), blockers of the cyclooxygenase and lipoxygenase pathways, respectively, did not prevent activation. Channel activity was not affected by the addition of the detergent 3-[3-cholamidopropyldimethylammonio]-1-propanesulfonate (CHAPS) up to 200 μM, ATP (4 mM), Mg^{2+} (0 to 10 mM), guanosine thiotriphosphate (GTPγS, 1 to 10 μM), guanosine triphosphate (1 to 100 μM), or by increasing the free Ca^{2+} concentrations of the bath to 100 μM. Many other lipid-soluble membrane components have structures related to arachidonic acid, which is normally generated by the action of phospholipase A_2 (PLA_2) on membrane phospholipids. We examined whether other fatty acids also activated the channel. Linoleic acid (10 μM) (n = 4) but not oleic, palmitic, stearic, or myristic acid (10 to 50 μM) (n = 4) activated the channel in inside-out patches of membrane. These results suggest that the opening of these latent K^+ channels is not a nonspecific effect of hydrophobic compounds on the channel.

Ischemia increases arachidonic acid and other free fatty acids, depresses pH_i, and increases the K+ conductance in cardiac cells (*8*). $I_{K\cdot AA}$ activity increased with a decrease in intracellular pH (Fig. 3). After induction of $I_{K\cdot AA}$ with 10 mM arachidonic acid, the pH was changed sequentially from 7.2 to 6.0 and back to 7.2 in 0.2 pH unit steps. Mean channel activity increased tenfold when the pH was lowered to 6.4. Activity was only slightly increased when the pH was lowered further to 6.0. The acidification-induced rise in channel activity reversed when normal pH was restored. Channel activity is plotted against pH in Fig. 3B with half-maximal activation occurring at pH 6.7. The pH dependence of channel activity was not affected by addition of ATP, addition of Ca^{2+} (0.1 mM), or removal of Mg^{2+}. All solutions were pH-adjusted in Hepes buffer after addition of fatty acid. Thus, $I_{K\cdot AA}$ was not simply H^+-gated.

Free fatty acids are generated from phos-

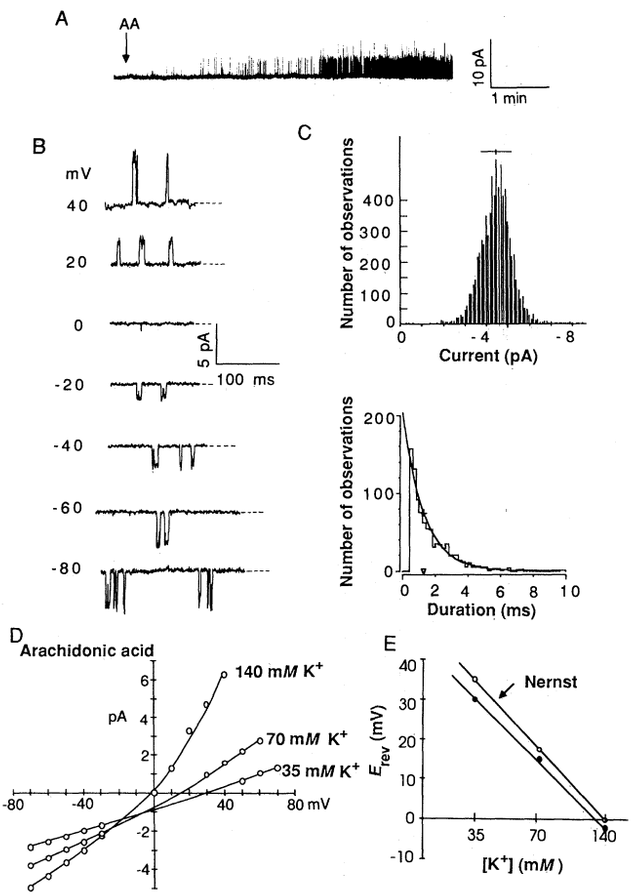

Fig. 2. Single-channel currents activated by arachidonic acid in inside-out patches of neonatal rat atrial cells ($I_{K\cdot AA}$). The pipette and bath solution contained 140 mM K^+, 140 mM Cl^-, 5 mM EGTA, 2mM Mg^{2+}, and 10 mM Hepes (pH 7.2). (**A**) Single-channel currents induced by 10 μM arachidonic acid applied to the inside surface of the patch. Holding potential, +40 mV; cutoff frequency, 2.5 kHz. (**B**) Single-channel records at patch potentials ranging from −80 to +40 mV. Dotted lines indicate zero-current level. Reversal potential is ~0 mV. (**C**) Amplitude (4.5 pA) and open time histograms [time constant (τ) = 1.13 ms; binned maximum likelihood fit] of channel openings observed at −60 mV. (**D**) Single-channel I-V relations at three different intracellular K^+ concentrations as indicated (fitted by eye). (**E**) Semilogarithmic plot of the single-channel reversal potential versus intracellular K^+ concentration. The fitted line (solid circles) has a slope of −56 mV per decade and is compared to the theoretical (Nernst) relation (open circles). A small offset was present in this set of experiments.

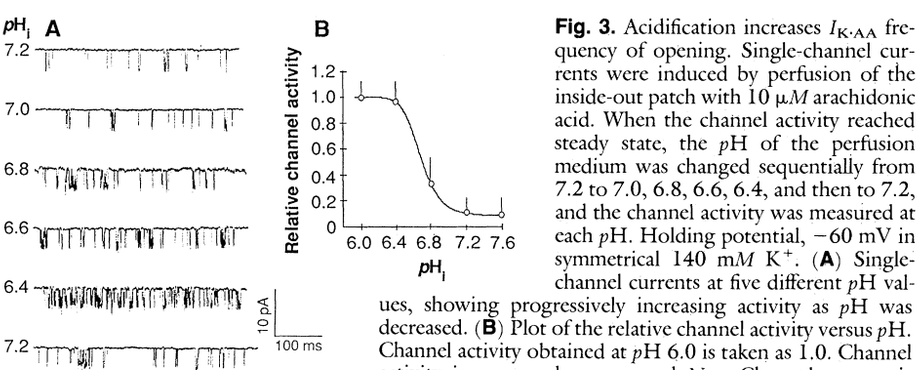

Fig. 3. Acidification increases $I_{K\cdot AA}$ frequency of opening. Single-channel currents were induced by perfusion of the inside-out patch with 10 μM arachidonic acid. When the channel activity reached steady state, the pH of the perfusion medium was changed sequentially from 7.2 to 7.0, 6.8, 6.6, 6.4, and then to 7.2, and the channel activity was measured at each pH. Holding potential, −60 mV in symmetrical 140 mM K^+. (**A**) Single-channel currents at five different pH values, showing progressively increasing activity as pH was decreased. (**B**) Plot of the relative channel activity versus pH. Channel activity obtained at pH 6.0 is taken as 1.0. Channel activity is expressed as averaged Np_o. Channel current is integrated over time and divided by current amplitude (i) to obtain Np_o. Each point is the mean of four experiments (+ SD), and points were fitted with a sigmoid curve.

pholipids by the action of PLA_2. During our investigation of the role of phospholipids in activating $I_{K\cdot AA}$, we found that 10 μM phosphatidylserine (PS) or phosphatidylcholine (PC) activated a second K^+ channel when applied to the intracellular surface of the patch. This channel was K^+-selective, as judged by a −60 mV per decade change in slope in the plot of intracellular K^+ concentration versus reversal potential. The limiting single-channel conductance was 68 pS in the outward direction and 44 pS in the inward direction with a mean open time of ~1 ms. In cell-attached patches, 10 to 50 μM PC or PS did not cause channel activation when added to the bath or to the pipette. Lysophosphatidylcholine, a product of the action of PLA_2 on PC, activated this channel in one patch out of six. Arachidonic acid and its metabolites mentioned above (HPETEs, HETEs, and leukotrienes) had no effect on channel activation. It is unlikely that protein kinase C was involved in channel activation because phorbol esters at 10 μM [phorbol myristate acetate (PMA) and 1-oleoyl-2-acetylglycerol (OAG)] ($n \geq 183$) did not open either of these K+ channels.

The K^+ channels found in this study may play an important role in protecting against cell damage caused by ischemia, which is known to elevate intracellular levels of certain fatty acids, including arachidonic acid (*9*). Ischemia or hypoxia can reduce the duration of the action potential and thereby cause early repolarization of cardiac cells (*10*). Opening of the K^+ channels reported here would cause rapid hyperpolarization of the cell and limit additional entry of Ca^{2+} via voltage-sensitive Ca^{2+} channels as well as minimize energy consumption by conserving ATP. A decrease in intracellular *p*H together with the opening of the K^+ channels by arachidonic acid would contribute to more rapid repolarization of the cells by increasing the K^+ channel activity, further protecting the cells from ischemic damage. We cannot yet generalize our results to adult human cardiac cells, so the importance of these channels in ischemia remains speculative. Alternatively, these K^+ channels may be part of newly discovered second messenger pathways involving lipophilic compounds, as suggested by other reports of arachidonic acid metabolites in gating channels (*1, 2*). The role of these compounds as part of a physiological second messenger system is supported by the fact that the existence of these K^+ channels may not be limited to heart cells. Arachidonic acid (5 to 40 μM) and certain fatty acids activate two types of K^+ channels in gastric smooth muscle cells (*11*) when added extracellularly. We do not know whether our channels are activated directly by fatty acids such as arachidonic acid. The pathway is apparently independent of $G_{\beta\gamma}$-dependent $I_{K\cdot ACh}$ activation (*2*).

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Arachidonic Acid and Other Fatty Acids Directly Activate Potassium Channels in Smooth Muscle Cells

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Arachidonic acid, as well as fatty acids that are not substrates for cyclooxygenase and lipoxygenase enzymes, activated a specific type of potassium channel in freshly dissociated smooth muscle cells. Activation occurred in excised membrane patches in the absence of calcium and all nucleotides. Therefore signal transduction pathways that require such soluble factors, including the NADPH-dependent cytochrome P450 pathway, do not mediate the response. Thus, fatty acids directly activate potassium channels and so may constitute a class of signal molecules that regulate ion channels.

ARACHIDONIC ACID (AA) AFFECTS the behavior of biological systems in two ways. First, the liberation of this fatty acid (FA) from cell membrane phospholipid, via receptor-mediated activation of phospholipases, leads to the generation of biologically active AA metabolites (that is, products of cyclooxygenase, lipoxygenase, and cytochrome P450 metabolic pathways) (*1*). Such metabolic conversion of AA can result in the activation of K^+ channels (*2*). A second class of responses is elicited by AA itself and by other FAs. These responses do not proceed through the metabolic pathways for AA and are referred to as "direct" FA effects. The most convincing demonstration of this second class of responses is the direct activation of purified enzymes by FAs (*3–6*). FA modulation of cellular processes (*7–11*) may reflect similar direct interactions of FAs with proteins. Here we report that both AA and certain other FAs, at concentrations similar to those required for both metabolically mediated (*1, 2, 12*) and direct (*4–6, 7–10*) effects of FAs, directly activate specific K^+ channels in smooth muscle cells. Thus certain FAs, liberated by receptor-regulated lipases or by other processes, may be second messenger molecules for the regulation of ion channels. Some of these results have appeared in preliminary form (*13*).

The effects of FAs were examined in smooth muscle cells, isolated from the stomach of the toad *Bufo marinus*, by using tight-seal, whole-cell and patch recording techniques (*14*). In the whole-cell configuration, AA (10 to 40 μM) rapidly and reversibly activated an outwardly rectifying K^+ current (Fig. 1). In a typical experiment, the membrane potential was intermittently stepped to values negative and then positive to the calculated equilibrium potential for K^+ (E_K). The activated K^+ current was seen as a divergence in the current trace with inward current generated at −110 mV (negative to E_K) and outward current at −60 mV (positive to E_K) (Fig. 1A). At a constant internal (patch pipette) K^+ concentration, current-voltage (*I-V*) relations for AA-activated current were obtained at different external K^+ concentrations (Na^+ exchanged for K^+) (Fig. 1B). A plot of the reversal potentials for AA-activated current as a function of the extracellular K^+ concentration (Fig. 1C) approximated that predicted for a K^+ current.

To determine whether activation of K^+ current required metabolism of AA by cyclooxygenase or lipoxygenase, we took ad-

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