Two Novel Cardiac Atrial $K^+$ Channels, $I_{k,aa}$ and $I_{k,pc}$

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ABSTRACT Two $K^+$-selective channels in neonatal rat atrial cells activated by lipophilic compounds have been characterized in detail. The arachidonic acid-stimulated channel ($I_{k,aa}$) had a slope conductance of $124 \pm 17 \mu S$ at $+30 \text{ mV}$ in symmetrical $140 \text{ mM}$ potassium and a mean open time of $\sim 1 \text{ ms}$, and was relatively voltage independent. $I_{k,aa}$ activity was reversibly increased by lowering pH to 6.0. Arachidonic acid was most effective in activating this channel, although a number of lipophilic compounds resulted in activation. Surprisingly, choline, a polar molecule, also activated the channel. A second $K^+$ channel was activated by $10 \mu M$ phosphatidylcholine applied to the intracellular surface of inside-out atrial patches. This channel ($I_{k,pc}$) had a slope conductance of $60 \pm 6 \mu S$ at $+40 \text{ mV}$ and a mean open time of $\sim 0.6 \text{ ms}$, and was also relatively voltage independent. Fatty acids are probably monomeric in the membrane under the conditions of our recording; thus detergent effects are unlikely. Since a number of compounds including fatty acids and prostaglandins activated these two channels, an indirect, channel-specific mechanism may account for activation of these two cardiac $K^+$ channels.

INTRODUCTION

The modulation of ion channels by lipophilic second messengers now appears to be common among several cell types (Ordway, Singer, and Walsh, 1991). Lipophilic second messengers for ion channels, such as fatty acids, potentially act in four ways: (a) by direct channel protein–messenger interaction; (b) indirectly, via initiation of metabolic pathways to produce other active messengers that then interact with the channel (e.g. lipoxygenase, cyclooxygenase pathways); (c) indirectly, by altering the lipid environment surrounding a channel, a potentially physiologic mechanism; and (d) artifactually, at micellar concentrations, by disrupting the membrane as a detergent. Direct fatty acid activation of purified enzymes has been demonstrated for several important cellular processes. Purified protein kinase C (Seifert, Schachtele, Rosenthal, and Schultz, 1988) and guanylate cyclase (Braughler, Mittal, and Murad, 1984) have been activated by micellar fatty acids.

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1979; Ignarro and Wood, 1987) are both activated by a number of free fatty acids. Isoprenylation is common for G protein α and γ subunits and may play a role in associating these subunits with either the membrane or membrane protein targets (Maltese, 1990). Fatty acids such as arachidonic acid (AA) have also been shown to modulate channel activity. Two types of K⁺ channels in cardiac atrial cells (Kim and Clapham, 1989), gastric smooth muscle cells (Ordway, Clapp, Singer, and Walsh, 1989a), as well as Ca²⁺-activated K⁺ channels in pulmonary artery smooth muscle cells (Ordway, Walsh, and Singer, 1989b) were activated by fatty acids. Conversely, decreases in Ca²⁺, Na⁺, and Cl⁻ currents are induced by fatty acids in guinea pig atrial, GH₄, and epithelial cells (Anderson and Welsh, 1990; Cohen, Bale, and Leibowitz, 1990; Katz, Roy-Contancin, Bale, and Reuben, 1990; Leibowitz, Bale, and Cohen, 1990).

The second type of activation (indirect, metabolic) has been most convincingly demonstrated in cases that involve the lipoxygenase metabolites of AA. A number of different lipoxygenase metabolites have been shown to be involved in the presynaptic inhibition of Aplysia sensory neurons (Piomelli, Volterra, Dale, Siegelbaum, Kandel, Schwartz, and Belardetti, 1987). Specifically, in inside-out patches, 12-hydroperoxy-eicosatetraenoic acid (12-HPETE) increased the activity of S-type K⁺ channels in Aplysia neurons (Belardetti, Campbell, Falck, Demontis, and Rosolowsky, 1989; Buttner, Siegelbaum, and Volterra, 1989). Lipoxygenase products have also been shown to activate K⁺ channels in neonatal rat atria (Kim and Clapham, 1989) and guinea pig atria (Kurachi, Ito, Sugimoto, Shimizu, Miki, and Ui, 1989).

Indirect, but potentially physiologically relevant, modulation of ion channels through an alteration of membrane composition has been proposed. This membrane destabilization is a possible mechanism of fatty acids in modifying the activity of squid giant axon Na⁺ (Takenaka, Horie, Hori, and Kawakami, 1988) and aortic smooth muscle K⁺ channels (Bregestovski, Bolotina, and Serebryakov, 1989). An important caveat is the possibility of a detergent effect of lipophilic compounds at concentrations close to the critical micellar concentration, where aggregates of monomers disrupt membranes and can apparently create lipidic pores (Sawyer and Anderson, 1989).

In this paper we present more detailed information on two neonatal rat atrial, outwardly rectifying K⁺ channels activated by lipophilic compounds as originally described by Kim and Clapham (1989). Originally, one channel was activated by AA (Iₖ,AA) and the second channel by phosphatidylcholine (PC) (Iₖ,PC). Here we characterize the kinetics of these channels, the sensitivity of the channels to intracellular pH, and the ability of a number of compounds, including fatty acids and prostaglandins, to activate these two channels.

**METHODS**

**Cell Preparation**

Atrial heart cells were isolated from 2-3-d-old neonatal rats by collagenase digestion. Briefly, hearts of 2-3-d-old rats were removed from the animals via thoracotomy. The atria were removed and minced with a scalpel blade. The minced tissue was incubated in Hank's balanced salts media containing collagenase (type I; Worthington Biochemical Corp., Freehold, NJ) and trypsin (Gibco Laboratories, Grand Island, NY) on a 37°C shaking water bath. Cells were
harvested every 10 min until the tissue was fully digested. Cells were then placed in trypsin inhibitor and filtered through a 200-μm nylon mesh. The filtered cells were pelleted, suspended in DMEM (Sigma Chemical Co., St. Louis, MO) containing 25 mM HCO₃⁻, plated onto glass coverslips, and maintained in a 95% O₂/5% CO₂ incubator at 37°C for use 12–24 h later.

**Solutions**

The majority of the experiments using inside-out patches were performed in buffer containing (in mM): 140 K⁺, 144 Cl⁻, 10 HEPES, 5 EGTA, and 2 Mg²⁺, pH 7.2 (K-140). The standard bath solution for whole-cell experiments contained (in mM): 140 Na⁺, 5 K⁺, 10 HEPES, 2 Mg²⁺, 1 Co²⁺, and 151 Cl⁻, pH 7.2.

Fatty acids (Nu Chek Prep, Elysian, MN) and prostaglandins (Cayman Chemical Co. Inc., Ann Arbor, MI) were prepared using the guidelines specified by the supplier. Generally, the fatty acids or prostaglandins were dissolved in chloroform or absolute ethanol and separated into aliquots. The solvent was then evaporated under argon gas and the container sealed. Finally, the fatty acid or prostaglandin was dissolved in the appropriate experimental buffer, gassed with argon, and sonicated for 30 s to 2 min before use.

**Electrophysiology**

Standard whole-cell and inside-out patch clamp techniques were applied to measure channel activity (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981). Currents were measured using a LIST (Darmstadt, Germany) model EPC-7 patch clamp amplifier and recorded using a PCM/VCR (Neurocorder DR 2-84) digital tape recorder. Records were filtered with an eight-pole Bessel filter at 3 kHz. The data were analyzed on an INDEC 11/73 computer (Sunnyvale, CA).

Whole-cell currents were measured using electrodes whose tips alone were filled with K-140. The remainder of the pipette was filled with K-140 containing the appropriate concentration of a lipophilic compound; this allowed time for seal formation before the test compound reached the pipette tip. The current–voltage (I–V) relation immediately after breaking into the cell was comparable to control determinations in the absence of activator. Each cell served as its own control. The I–V relation was determined again 2–5 min after breaking into the cell, giving time for the agonists to diffuse into the cell. Current amplitudes were normalized by measured cell capacitance.

Various compounds were added to the bath containing inside-out patches by addition from a concentrated stock solution. The bath concentration was changed by flowing several volumes of buffer of the desired composition through the bath. To determine the effects of pH on the channel, activity was stimulated by addition of the desired compound to the bath. Once channel activity had reached a steady state, the bathing solution was changed to the desired experimental conditions and the new channel activity measured. Channel activity data for the pH experiments were presented as the product \( Np_o \), where \( N \) is the number of channels in the patch and \( p_o \) is the open probability of the channel. Channel current was integrated over time (on-line) and divided by single channel current amplitude \( i \) and time to obtain \( Np_o (i = Np_o j; \text{see Nanavati, Clapham, Ito, and Kurachi, 1990}).\)

**RESULTS**

**AA-stimulated Current**

Intracellular perfusion of neonatal rat atrial cells with AA led to an increase in whole-cell current. Atrial cells were voltage-clamped in the whole-cell configuration. The patch pipette contained K-140 with 10 μM AA. The bathing solution was the
standard bath solution with 1 mM Co²⁺ replacing Ca²⁺ (to eliminate inward Ca²⁺ current). The I-V relation was determined 10–20 s after breaking into the cell (Fig. 1 A, I). The initial I-V relation was the same as that determined for control cells in the absence of AA. After 3 min there was an increase in both inward and outward

![Image of Figure 1](https://example.com/image1.png)

**Figure 1.** Intracellular AA increases whole-cell currents in rat atrial cells. Tight seal whole-cell recordings were made as indicated below in standard bath solution. (A) Whole-cell current traces. Whole-cell recordings were made with the pipette containing K-140 and 10 μM AA. Holding potential, −80 mV. The inward current traces were obtained by stepping the voltage to −120 mV for 200 ms. Outward traces were obtained by stepping the voltage to +20 mV. The control curves (1) were determined 15 s after breaking into the cell. (Note that the Na⁺ current is present.) Currents were measured again 3 min after break-in (2). During this time the AA had diffused into the cells and both the inward and outward currents had increased. Currents were then obtained after adding 1 mM Ba²⁺ to the bath solution (3). The Ba²⁺ completely blocked the increase in inward current and partially blocked the increase in outward current. (B) I-V relation for AA-activated whole-cell currents (C = 13 pF). The control curve (circles) was taken 15 s after breaking into the cell. AA diffusing into the cell leads to an increase in current in both the inward and outward direction (squares). The increase in inward current was completely blocked and outward current was partially blocked by adding 1 mM Ba²⁺ to the bath solution (triangles). (C) Whole-cell I-V relation for AA-activated currents with CsCl in the pipette (C = 15 pF). Whole-cell recordings were made with CsCl replacing KCl in the pipette (10 μM AA present). The control curve (circles) was taken 20 s after breaking into the cell. A small increase in both the inward and outward current direction was seen 4 min after break-in (squares). Addition of 1 mM Ba²⁺ to the bathing solution completely blocked the inward current and partially blocked the outward current.
currents as AA diffused into the cell (Fig. 1 A, 2). The AA-induced increase in inward and outward currents was blocked significantly by the addition of 1 mM Ba$^{2+}$ to the bathing solution (Fig. 1 A, 3 and 1 B). The block by Ba$^{2+}$ suggested that the current was due to an increase in K$^+$ conductance (see Kim and Clapham, 1989). In experiments in which CsCl was used in place of KCl in the pipette, the amplitude of the AA-induced outward current was smaller, while the inward current increased over control levels similar to when KCl was present. The addition of 1 mM Ba$^{2+}$ to the bathing solution once again completely blocked the inward current and most of the small outward current (Fig. 1 C).

The AA-induced current was characterized at the single channel level using inside-out patches. Addition of 10 μM AA (CMC > 70 μM) to the intracellular surface of the membrane induced an increase in single channel current in 43 of 59 attempts, with a delay of 1–5 min (Fig. 2 A). AA did not increase single channel currents when present in the pipette solution of inside-out patches or when added to
the bath solution under cell-attached patch conditions (n > 80). Choline also consistently activated this channel (see below, Table I). In symmetrical K-140 solution with the membrane voltage at +30 mV the channel amplitude was 3.7 ± 0.5 pA (±SD, n = 16, Fig. 2 B). The I-V relation reversed at ~ 0 mV under these conditions (Fig. 2, C and D). The slope conductance of the channel was 124 ± 17 pS (n = 16) at +30 mV and 70 ± 5 pS (n = 10) at −50 mV.

The kinetics for the opening and closing of IKAA were studied using inside-out patches in symmetrical K-140. An open time histogram was obtained from the distribution of channel openings in an inside-out patch at +30 mV that had been exposed to 10 μM AA. The histogram was best fit by a single exponential with a mean open time of 1.1 ± 0.4 ms (n = 16, Fig. 3 A). The mean open time and channel activity (Npo) were independent of membrane voltage over the range from −70 to +50 mV (Fig. 3 B). Mean open time was 1.05 ± 0.37 ms. A maximum likelihood method (Sigworth and Sine, 1987) was used to fit the closed time distribution. Three exponential time constants, ð1 = 0.7 ms, ð2 = 9 ms, and ð3 = 53 ms, were derived from the best fits (Fig. 3 C). Analysis of burst durations gave mean burst durations of 9 and 26 ms (Fig. 3 D).

A basic kinetic model for IKAA was devised with the assumptions that the three closed time durations reflect at least three closed states. For simplicity, the only transition to the open state is through the first closed state represented by the shortest dwell time (τ = 0.7 ms).

\[
\begin{align*}
&\begin{array}{c}
\text{C}\_1 \\
\text{C}\_2 \\
\text{C}\_3 \\
\text{C}\_4 \\
\text{C}\_5 \\
\text{C}\_6 \\
\text{C}\_7 \\
\text{C}\_8 \\
\text{C}\_9 \\
\text{C}\_10 \\
\text{C}\_11 \\
\text{C}\_12 \\
\text{C}\_13 \\
\text{C}\_14 \\
\text{C}\_15 \\
\text{C}\_16
\end{array} \\
&\begin{array}{c}
\text{B}\_1 \\
\text{B}\_2 \\
\text{B}\_3 \\
\text{B}\_4 \\
\text{B}\_5 \\
\text{B}\_6 \\
\text{B}\_7 \\
\text{B}\_8 \\
\text{B}\_9 \\
\text{B}\_10 \\
\text{B}\_11 \\
\text{B}\_12 \\
\text{B}\_13 \\
\text{B}\_14 \\
\text{B}\_15 \\
\text{B}\_16
\end{array}
\end{align*}
\]

An initial approximation to the rate constants was determined by solving algebraically for six rate constants based on the open and closed times, and burst durations assuming that the exponential time constants reflect the dwell times (see, for example, Clapham and Neher, 1984). To more explicitly determine the rate constants between states, a transition rate matrix (Colquhoun and Hawkes, 1981) of the form below was derived, where the submatrix Q_{AA} represents the closed state distribution of eigenvalues.

\[
\text{matrix } Q = \begin{pmatrix}
-k_{01} & k_{01} & 0 & 0 \\
-\left(k_{10} + k_{12}\right) & k_{12} & 0 & 0 \\
0 & k_{21} & -\left(k_{21} + k_{25}\right) & k_{25} \\
0 & 0 & -k_{32} & 0
\end{pmatrix}
\]

This matrix Q was solved for the eigenvalues using a computer algorithm to determine the roots of the cubic equations (Crouzy and Sigworth, 1990). The predicted eigenvalues were iteratively compared with the eigenvalues derived from the exponential fits of the open and closed time distributions until they converged, yielding the rate constants between states. The dwell times are then simply the reciprocal of the rate constants leaving the state (Colquhoun and Hawkes, 1983).
FIGURE 3. Kinetic characterization of $I_{KAA}$. The kinetics of channel opening and closing were determined for $I_{KAA}$ in symmetrical K-140. (A) Open time histogram. The channel open time was plotted and fit to a single exponential. The figure shows the data from one experiment in which the mean open time of the channel was determined to be 0.98 ms at +30 mV. The mean open time of the channel at +30 mV for the 16 experiments used in Fig. 2 was $1.1 \pm 0.4$ ms. (B) Voltage dependence of open time and channel activity ($N_{po}$). The mean open time and channel activity did not vary significantly with voltage from −70 to +50 mV ($n \geq 4$, ±SEM). (C) Closed time histogram. The closed times for the channel were binned and fit using a maximum likelihood method (Sigworth and Sine, 1986). The closed times were best fit by three exponentials with values of 0.7, 9, and 53 ms. The main figure shows the fit for the first two exponentials and the inset shows the fit to the longer closed times. (D) Burst duration histogram. The time between consecutive openings was determined and fit using the maximum likelihood method to determine burst duration. The best fit yielded two time constants with values of 9 and 25 ms.

$$\tau_0 = (k_{o1})^{-1} = (909 \text{ s}^{-1})^{-1} = 1.1 \text{ ms}$$

$$\tau_{c1} = (k_{1.0} + k_{1.3})^{-1} = 0.7 \text{ ms}$$

$$\tau_{c2} = (k_{2.1} + k_{2.3})^{-1} = 10.8 \text{ ms}$$

$$\tau_{c3} = (k_{3.0})^{-1} = 20.8 \text{ ms}$$

From the analysis of burst durations and the above derived kinetic data, we can make an approximation of the mean number of openings/burst ($M_o$), as well as the
mean number of bursts/cluster ($M_b$). The first burst duration ($r_{Bl}$) is the time the channel spends shuttling between the open state (O) and first closed state ($C_1$) before the transition to the second closed state ($C_2$):

$$r_{Bl} = M_o r_o + (M_o - 1) r_{C1}$$

The mean number of openings/burst was approximately five. The second burst duration ($r_{B2}$) reflects the time the channel spends either bursting ($r_{B1}$) or in the second closed state ($C_2$) before the transition to the third closed state ($C_3$):

$$r_{B2} = M_b r_{B1} + (M_b - 1) r_{C2}$$

The mean number of bursts/cluster was approximately two.

In previously reported experiments (Kim and Clapham, 1989), decreasing pH in the presence of AA led to an increase in the activity of $I_{K,AA}$. It was not clear if the increase in activity was due to a change in the amount of the protonated form of AA. Here the effect of changes in intracellular pH on the activity of $I_{K,AA}$ was tested with choline used to activate the channel. Inside-out patches were formed in symmetrical K-140 solution at pH 7.2. $I_{K,AA}$ was activated by adding 10 μM choline to the intracellular surface of the membrane and the steady-state channel activity ($Npo$) was determined. The bath solution was then changed to K-140 with 10 μM choline at the desired pH and the new steady-state activity of the channel was determined. Changing pH from 7.2 to 6.8 led to a marked increase in channel activity. Decreasing the pH to 6.0 caused a further increase in channel activity, yielding a net threefold increase in $Npo$ from that at 7.2 (Fig. 4A). In five experiments decreasing pH to 6.0 led to a three- to sevenfold increase in channel activity (Fig. 4B). Increasing the pH to 7.6 reversibly decreased channel activity below control at 7.2. This increase in activity was not due to activation of the channel by increasing H$^+$ ion concentration alone, as decreasing the pH in the absence of AA or choline did not activate the channel. It is also unlikely that the increase in activity was due to an increase in access of the charged or uncharged form of the activator to a regulatory site. Decreasing intracellular pH could differentially affect choline versus AA, as the pK$_a$ of AA is acidic (pK$_a$ ~ 4.7) while the pK$_a$ of choline is nearly neutral (pK$_a$ ~ 7.1). However, this does not rule out the possibility that these two agents may act via separate and independent mechanisms susceptible to pH changes.

**PC-stimulated Currents**

Intracellular perfusion of neonatal rat atrial cells with PC caused an increase in whole-cell current similar to that caused by AA. In whole-cell patch clamp experiments with 10 μM PC in the pipette, current was increased in both the inward and outward directions after a delay of 2–5 min. The increase in inward current could be completely blocked by the addition of 1 mM Ba$^{2+}$ to the bathing solution, while the outward current was partially blocked (Fig. 5, A and B). When KCl was replaced by CsCl in the pipette, the amplitude of the PC-induced outward current was markedly decreased, while the increase in inward current was affected only slightly (Fig. 5C). These data suggest that PC gates a K$^+$ conductance in atrial cells.
Atrial Cardiac \( K^+ \) Channels

**Figure 4.** \( I_{K_{AA}} \) activity was sensitive to intracellular pH. Inside-out patches were formed in symmetrical K-140 at pH 7.2. \( I_{K_{AA}} \) activity was induced by addition of 10 \( \mu \)M choline to the bath. After the channel activity had reached steady state, \( N_p \) was determined and the bathing solution was changed to K-140 at the desired pH. (A) \( I_{K_{AA}} \) was sensitive to pH in a reversible fashion. An inside-out patch was formed and steady-state activity of the channel determined. The pH was then decreased from 7.2 to 6.0 sequentially. The increasing in activity with decreasing pH was reversible. Upon returning the pH to 7.2 the channel activity returned to control levels. \( N_p \) was set to zero during solution changes. Values > 100% are due to the presence of more than one channel in the patch. (B) Relative channel activity of \( I_{K_{AA}} \). The activity (\( N_p \)) of \( I_{K_{AA}} \) is shown at various internal pH levels. The data are presented as the mean and SD of five experiments. The values were normalized to activity at pH 7.2 (=1) for each experiment.
The characteristics of the channel responsible for the PC-induced increase in whole-cell currents were studied using inside-out patches. Exposure of inside-out patches to 10 μM PC led to the activation of a channel after 1–4 min (Fig. 6A). The mean current amplitude for this channel was 2.40 ± 0.28 pA (±SD, n = 5) at +40 mV (Fig. 6B). Single channel current measurements used to determine the $I-V$ relation showed that the current reversed near 0 mV in symmetrical K-140 (Fig. 6, C and D). The single channel conductance for the PC-induced channel ($I_{K_{PC}}$) was 66 ± 6 pS (n = 5) at +30 mV and 55 ± 4 pS (n = 4) at −50 mV. Further evidence for the

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Intracellular PC-induced whole-cell currents in atrial cells. Whole-cell recordings were made in standard bath solution. Holding potential, −80 mV. (A) A whole-cell recording was made using an intracellular solution containing K-140 with 10 μM PC. Current traces for −120 and +20 mV are shown (1). A second $I-V$ relation was determined 3.5 min after breaking into the cell. The membrane currents increased as PC diffused into the cell (2). Addition of 1 mM Ba$^{2+}$ to the bath solution completely blocked the increase in inward current and partially blocked the increase in outward current (3). The fast transient is the Na$^+$ current. (B) $I-V$ relation for PC-stimulated whole-cell currents (C = 10 pF). The control curve (circles), PC-induced currents (squares), and Ba$^{2+}$ blocked currents (triangles) are shown. Note that extracellular Ba$^{2+}$ completely blocked the inward currents and partially blocked the outward currents. (C) $I-V$ relation for PC-activated currents (10 μM PC) with CsCl substituted for KCl in the pipette (C = 14 pF). The control curve (circles) was determined 15 s after breaking into the cell. An increase in inward and outward currents was seen at 4 min after breaking into the cell (squares). The increase in outward currents was greatly reduced compared to the increase when K-140 was used as the pipette solution. Addition of 1 mM Ba$^{2+}$ to the bath completely blocked both the inward and outward currents under these conditions (triangles).
Figure 6. PC activation of single channels in inside-out patches. (A) PC increases single channel activity. Addition of 10 μM PC to the inside surface of rat atrial membranes leads to an increase in channel activity after a 2–4-min delay. Holding potential, -60 mV. (B) Sample amplitude histogram for $I_{K,PC}$ at +40 mV. The mean current amplitude for the channel at +40 mV was 2.40 ± 0.28 pA ($n = 5$). (C) Single channel current traces at membrane voltages ranging from -60 to +40 mV. (D) $I-V$ relation for $I_{K,PC}$. The values are the means ± SEM ($n$ is in parenthesis).

Figure 7. $I_{K,PC}$-activated channel is K$^+$ selective. (A) Dependence of the $I-V$ relation on intracellular K$^+$ concentration. The current voltage relationship was determined for $I_{K,PC}$ at 140, 70, and 35 mM [K$^+$]. (B) Semilog plot of reversal potential versus intracellular K$^+$ concentration. The log of the reversal potentials from the experiment in A were plotted versus intracellular [K$^+$]. The slope of the fitted line, -60 mV/decade, is in good agreement with the predicted -58 mV/decade slope calculated from the Nernst potential.
K⁺ selectivity of this channel was demonstrated by conducting experiments at various intracellular K⁺ concentrations. I₁₅₆⁺ was activated by adding 10 μM PC to the bathing solution. The bath solution was changed to varying K⁺ concentrations and an I-V relation was determined (Fig. 7A). These data showed that the reversal potential varied with changes in K⁺ concentration in a manner predicted by the Nernst potential (Fig. 7B). In addition, I₁₅₆⁺ activity was independent of pH between 6.0 and 7.6.

The kinetics for the opening of I₁₅₆⁺ were studied using inside-out patches in symmetrical K-140. The mean open time of the channel was 0.6 ± 0.16 ms (n = 5) at +40 mV (Fig. 8A) and was relatively constant over the voltage range from −60 to +60 mV. The mean open time for all events was 0.62 ± 0.11 (n ≥ 5 ± SEM) and was relatively voltage independent. The net channel activity (Nₚₒ) was not significantly voltage-dependent (n ≥ 4 ± SEM). (C) Closed time histogram. The distribution of closed times for the channel was best fit by two exponentials having time constants of 0.8 and 46 ms. The main figure shows the fit to the shorter closed time and the inset shows the fit to the longer closed time. (D) Burst duration histogram. The distribution of bursts for the channel was fit using the maximum likelihood method giving a time constant of 45 ms.
+50 mV (Fig. 8B). The closed time histogram was best fit by a double exponential with time constants of 0.8 and 46 ms (Fig. 8C). Analysis of burst durations yielded a single exponential process with a time constant of 45 ms (Fig. 8D). The kinetic model for $I_{K,PC}$ was similar to that of $I_{K,AA}$ with the exception that $I_{K,PC}$ has only three states. As described above, the rate constants were calculated by solving the transition rate matrix and were:

$$
\begin{align*}
C_s & \frac{k_1 = 1.212}{C_t} \\
C_t & \frac{k_1 = 1.695}{C_s}
\end{align*}
$$

The dwell times were $\tau_0 = 0.6$, $\tau_{C1} = 0.8$, and $\tau_{C2} = 45$ ms. The mean number of openings/burst was $\sim 33$.

| TABLE 1 |
| --- | --- | --- |
| **Ability of Various Compounds to Activate $I_{K,AA}$ and $I_{K,PC}$** | **Activated/attempts (%)** |  |
| **Fatty acids (10 $\mu$M)** |  |  |
| AA (20:4 cis-5,8,11,14) | 43/59 (73) | 3/59 (5) |
| Linoleic acid (18:3 cis-9,12,15) | 1/4 (25) | 0/4 (0) |
| Linoleic acid (18:2 cis-9,12) | 4/8 (50) | 0/8 (0) |
| Oleic acid (18:1 cis-9) | 0/8 (0) | 0/8 (0) |
| Palmitoleic acid (16:1 cis-9) | 3/9 (33) | 2/9 (22) |
| Palmitelaidic acid (16:1 trans-9) | 0/4 (0) | 0/4 (0) |
| Palmitic acid (16:0) | 2/9 (22) | 1/9 (11) |
| Pentadecanoic acid (15:0) | 0/4 (0) | 0/4 (0) |
| Myristic acid (14:0) | 3/7 (43) | 2/7 (29) |
| Lauric acid (12:0) | 0/4 (0) | 0/4 (0) |
| Decanoic acid (10:0) | 1/4 (25) | 0/4 (0) |
| **Prostaglandins (10 $\mu$M)** |  |  |
| PGA$_2$ | 0/4 (0) | 0/4 (0) |
| PGB$_2$ | 0/4 (0) | 0/4 (0) |
| PGD$_2$ | 0/4 (0) | 0/4 (0) |
| PGE$_2$ | 0/4 (0) | 0/4 (0) |
| PGF$_{2\alpha}$ | 0/4 (0) | 0/4 (0) |
| 6-keto PGF$_{1\alpha}$ | 3/7 (43) | 1/7 (14) |
| PGF$_{3\alpha}$ | 1/11 (9) | 6/11 (54) |
| PGH$_2$ | 2/8 (25) | 2/8 (25) |
| PGI$_2$ | 0/4 (0) | 0/4 (0) |
| 12-HHT | 4/8 (50) | 3/8 (37) |
| TxB$_2$ | 0/4 (0) | 0/4 (0) |
| **Others (10 $\mu$M)** |  |  |
| Choline | 84/142 (59) | 45/142 (32) |
| PC | 2/24 (8) | 16/24 (67) |
| Phosphocholine | 8/16 (50) | 5/16 (31) |
| Phospholipase D | 2/21 (10) | 2/21 (10) |
| Palmitoylcarnitine | 0/4 (0) | 0/4 (0) |
| Myristoylcarnitine | 0/4 (0) | 0/4 (0) |
| Phosphatidic acid | 0/4 (0) | 0/4 (0) |
**Activation of I_{KAA} and I_{KPC}**

To determine whether AA and PC were directly responsible for channel activation, or if activation was due to metabolites of these compounds, a number of agents were tested for their ability to activate the channel (Table I). As described previously, lipoxygenase derivatives did not activate either of these channels (Kim and Clapham, 1989). The best activator of I_{KAA} was AA (73% of patches at 10 μM). A number of unsaturated, monounsaturated, and polyunsaturated fatty acids were tested. Other fatty acids that activated I_{KAA} were: linoleic acid (18:2, four of eight attempts), palmitoleic acid (16:1, three of nine attempts), and myristic acid (14:0, three of seven attempts). Other longer chain unsaturated fatty acids (>C18:0) were not easily solubilized by our methods and were not tested. Organic solvents were not used to enhance solubilization since they would easily enter the membrane. Carriers such as albumin were also not used due to the potential introduction of contaminants. To avoid oxidation, fatty acids were not heated above 25°C. Prostaglandins (cyclooxygenase metabolites of AA) were also tested for their ability to activate the channels. 12-hydroxyheptadecatrienoic acid (12-HHT) activated each of the channels in two of four patches. Choline caused activation of I_{KAA} in 59% of attempts at 10 μM and stimulated activity at concentrations as low as 100 nM. Remarkably, choline also activated I_{KPC} in 45 of 142 patches (39%) but was not as effective as PC, which activated I_{KPC} in 16 of 24 patches (67%). However, it is important to note that it is unlikely that significant amounts of PC are incorporated into the membrane since the solubility of PC is extraordinarily small although finite (Small, 1986). Thus the effect of PC is due to the small amount of PC incorporated in the bilayer, to contaminating choline, or to contamination by unknown fatty acids. It does not appear that choline was liberated from PC by the action of phospholipase D, since inside-out patches exposed to phospholipase D rarely resulted in channel activity. PGF$_2$ was the only prostaglandin to consistently activate I_{KPC} (6 of 11 attempts at 10 μM) with activation occurring at concentrations as low as 500 nM.

**DISCUSSION**

This paper describes two distinct K' -selective channels in neonatal rat atrial cells. Both lipophile-activated channels (I_{KAA} and I_{KPC}) are slightly outwardly rectifying channels with I_{KAA} having nearly twice the conductance as I_{KPC}. Both channels display complex kinetics. The AA-sensitive channel (I_{KAA}) shows a pattern of clusters of bursts and a closed time distribution suggesting at least three closed states as opposed to only two closed states for I_{KPC}. Interestingly, both channels appear to be relatively voltage independent. Finally, the channels differed in their sensitivities to intracellular pH. I_{KPC} activity was insensitive to pH, while I_{KAA} activity was reversibly activated by lowering intracellular pH.

The marked modulation of channel activity by AA at low intracellular pH arouses suspicion that I_{KAA} may play a role in ischemia. During ischemia atrial intracellular pH has been shown to decrease from pH 7.2 to ~6.4, while levels of nonesterified fatty acids (particularly AA) were increased (Prinzen, Van Der Vusse, Arts, Roemen, Coumans, and Teneman, 1984). Under these conditions I_{KAA} should be maximally activated (see Fig. 5 B), reducing the action potential duration and heart rate. I_{KAA}
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has also been found in rat ventricular cells, where a role in ischemia would be more relevant (Kim and Duff, 1990). Estimates of current density in atrial cells (Kim and Clapham, 1989) suggest that \(I_{KAA}\) might contribute as much current for repolarization as \(I_{KATP}\). Experiments are currently underway to test this hypothesis.

A number of compounds have been tested for their ability to activate \(I_{KAA}\) and \(I_{KPC}\) in an effort to determine the nature of channel activation. Previous experiments from this laboratory have demonstrated that the lipoxygenase metabolites of AA did not activate either of these channels (Kim and Clapham, 1989). This was in sharp contrast to the acetylcholine-activated \(K^+\) channel (\(I_{KACH}\)) in these same cells where a series of lipoxygenase pathway products including leukotrienes \(B_4\) and \(C_4\), 5-hydroperoxyeicosatetraenoic acid (5-HPETE), and 12-hydroxyeicosatetraenoic acid (12-HETE) activated \(I_{KACH}\) (Kim, Lewis, Graziadei, Neer, Bar-Sagi, and Clapham, 1989). This demonstrated the existence of an indirect (metabolic) activation pathway for \(I_{KACH}\) via the oxygenated metabolites of arachidonic acid. \(I_{KAA}\) and \(I_{KPC}\) were found only in inside-out patches or in whole-cell recordings of perfused atrial cells and after many seconds of exposure to either AA or PC. Application of these agents to the extracellular surface of membranes did not activate these channels, a puzzling finding if AA is freely diffusible. However, it may be that enzymes (cyclooxygenase, lipoxygenase) in intact cells metabolize AA, leaving too little AA in the membrane for activation of this channel. Our conclusion is that this channel is not easily activated by simple exposure to AA and that cell disruption is required, perhaps allowing diffusible inhibitors of channel activation or metabolic enzymes to escape. Another question is why \(G\) protein \(\beta\gamma\) subunits (\(G_\beta\)) applied in previous studies to atrial patches did not activate these channels in inside-out patches if \(G_\beta\)-activated phospholipase \(A_2\): \(G_\beta\), caused full activation of often three to five \(I_{KACH}\) channels/patch after only a few seconds of exposure. \(I_{KAA}\) activity averages less than one channel per patch and is activated late in the time course after AA exposure, obscuring these channels in records where \(I_{KACH}\) is fully activated. Second, given the long time required for activation by AA, it is unlikely that enough \(G_\beta\)-stimulated AA is produced from the small membrane patch to activate \(I_{KAA}\).

At an early stage in this work we suspected that \(I_{KAA}\) would be activated by a series of cyclooxygenase metabolites in much the same way as \(I_{KACH}\) was activated by lipoxygenase metabolites. Our data did not support this hypothesis, however, as only two cyclooxygenase metabolites consistently activated the channel, 6-keto prostaglandin \(F_1\) (6-keto PGF\(_1\)) and 12-HHT. 12-HHT was almost equally effective at activating \(I_{KPC}\) as \(I_{KAA}\). Prostaglandin \(F_2\) (PGF\(_2\)) activated \(I_{KPC}\) as well, and was the only other cyclooxygenase metabolite to affect either channel. These three active compounds are not members of a specific metabolic reaction pathway and are not directly interconvertible. This suggests that these compounds are acting simply as 20 carbon fatty acids, similar to AA, and are not part of a metabolic activation pathway.

\(I_{KAA}\) and \(I_{KPC}\) also varied in their responsiveness to fatty acids. While several different fatty acids could induce \(I_{KAA}\) activity, none of the fatty acids used consistently activated \(I_{KPC}\). In testing fatty acids for their ability to activate \(I_{KAA}\), fatty acids of various chain length, degree of saturation, and types of double bonds were used in an effort to identify certain characteristics that were required for activation. AA, the fatty acid that most consistently activated \(I_{KAA}\), contains four \textit{cis}-1,4-pentadiene groups.
Four other fatty acids containing cis double bonds were tested. Two of these fatty acids effectively activated the channel (linoleic and palmitoleic), while two did not (linolenic and oleic). All were soluble under the conditions of our experiment. Although not as effective as AA, the effectiveness of these compounds in activating channels was comparable; no pattern was found from variations in cis double bonds and chain length of unsaturated fatty acids to predict their effectiveness. Of the saturated fatty acids, only myristic and palmitic acid, in the series of 10-16 carbon long fatty acids tried, activated $I_{K,AA}$. Insoluble fatty acids such as arachidic, stearic, and nondecanoic acid were not included in our data. Thus, no common structural pattern for the fatty acids that activate $I_{K,AA}$ has emerged; fatty acids from 14 to 20 carbons long, saturated, monounsaturated, and polyunsaturated, were effective in channel activation. Similarly, some fatty acids from each of these groups were ineffective in channel activation.

This nonspecificity of channel activation is also found in work with similar moieties in another cell system. Ordway et al. (1989b) identified six different fatty acids that activated K⁺ channels in gastric smooth muscle cells. In their studies, effective fatty acids included two cis-polysaturated fatty acids (AA and linolenic acid), a trans-polyunsaturated fatty acid (linoelaidic acid 18:2 trans-9,12), two cis-monounsaturated fatty acids (palmitoleic and oleic acids), and a saturated fatty acid (myristic acid). Additionally, they identified fatty acids that did not activate K⁺ channels, two of which are of particular interest. Palmitic acid, with two more carbons than myristic acid and lacking the cis double bond found in palmitoleic acid, was ineffective in channel activation. Myristoleic acid (14:1 cis-9) with the same chain length as myristic acid and with a cis double bond in the same position as palmitoleic and oleic acids, was also inactive. The authors concluded that the effective fatty acids activated the channels either through interaction with the channel protein or alteration of the lipid environment of the channel. A structural requirement for the effective fatty acids was not identified. Interactions of other types of membrane proteins with fatty acids have yielded similar nonspecific results to those of K⁺ channel activation. Fatty acids were found to induce closure of gap junction channels in rat lacrimal glands (Giaume, Randriamampita, and Trautmann, 1989). Takenaka et al. (1988) showed that Na⁺ currents in squid giant axon were inhibited by both medium and long chain fatty acids. Saturated, monounsaturated, and polyunsaturated fatty acids were all effective over a broad concentration range. Fatty acids with less than eight carbons and those containing double bonds in certain positions were found to be ineffective. The ED₅₀ for medium chain length fatty acids decreased with increased chain length from 8 to 13 carbons, but the concentrations of fatty acids used were quite high (in the millimolar range). Inhibition increased with chain length up to 22 carbons, with docosatetraenoic acid (22:4) being the most potent. As discussed previously, no clear structural requirement was found for Na⁺ current inhibition, but many fatty acids were effective at various concentrations.

Four mechanisms for lipophile activation of these two cardiac channels were proposed in the Introduction and should be discussed in light of our results. Since concentrations of all compounds used were at least 10-fold below their critical micelle concentration at room temperature in low calcium and at pH 7.2, it is unlikely that nonspecific detergent effects accounted for channel activation. The fatty acids used
would certainly be in equilibrium with the membrane as monomers (see Jain, 1972; Small, 1986), not as micelles. Another more serious concern is that compounds, although suspended at 10 μM concentrations, are certainly not equivalent in the equilibrium they reach with the membrane and that negative results may merely represent lower solubility in the bath solution or in the membrane. This problem is not easily solved without methods for assaying the amount and state of aggregation of monomers in the patch membrane, a problem beyond current techniques. A dose–response curve, with dose representing monomer concentration in the patch, is needed to compare membrane lipid–protein interactions. This potential mechanism, the direct, specific interaction of lipophile with channel, seems unlikely given the wide range of compounds activating this channel. Another potential mechanism, the interaction of metabolic products of AA with the channel, also appears unlikely given the range and characteristics of activating compounds. Thus, the third mechanism, the interaction of lipophile with the membrane surrounding the channel seems the most logical explanation of the results.

Modulation of channel activity by fatty acids does not fall into categories similar to those of traditional second messengers or of direct protein binding to receptors. Of course the interactions between fatty acids and channels in a lipid environment are fundamentally different, although not necessarily less significant. As second messengers, lipophilic compounds have the advantage of remaining in the plane of the membrane, where the channels operate, rather than diffusing into the relatively large cytoplasmic space. No pattern has been identified for fatty acid binding to a specific binding site or sites on a channel, or for that matter, on many other proteins where physiologic actions are suspected. This lack of specificity at the structural level does not mean that specificity cannot be achieved by other means, such as restriction of these compounds in domains in the membranes. On the other hand, we have shown that in cardiac atrial patches where a wealth of K channels are present and activated by a variety of mechanisms, only these two channels respond to the agents tested. Other abundant channel types such as \( I_{K_{ATP}} \), \( I_{K_{ACh}} \), and \( I_{K} \) were present but not activated. This relative specificity, or sensitivity to activation by lipophilic compounds and choline, distinguishes \( I_{K_{AA}} \) and \( I_{K_{PC}} \).

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