Effects of fatty acids on BK channels in GH₃ cells

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Denson, Donald D., Xiaoping Wang, Roger T. Worrell, and Douglas C. Eaton. Effects of fatty acids on BK channels in GH₃ cells. Am J Physiol Cell Physiol 279: C1211–C1219, 2000.—Ca²⁺-activated K⁺ (BK) channels in GH₃ cells are activated by arachidonic acid (AA). Because cytosolic phospholipase A₂ can produce other unsaturated free fatty acids (FFA), we examined the effects of FFA on BK channels in excised patches. Control recordings were made at several holding potentials. The desired FFA was added to the bath solution, and the voltage paradigm was repeated. AA increased the activity of BK channels by 3.6 ± 1.6-fold. The cis FFA, palmitoleic, oleic, linoleic, linolenic, eicosapentaenoic, and the triple bond analog of AA, eicosatetraynoic acid, all increased BK channel activity, whereas stearic (saturated) or the trans isomers elaidic, linolealaidic, and linolene-laidic had no effect. The cis unsaturated FFA shifted the open probability vs. voltage relationships to the left without a change in slope, suggesting no change in the sensitivity of the voltage sensor. Measurements of membrane fluidity showed no correlation between the change of membrane fluidity and the change in BK channel activation. In addition, AA effects on BK channels were unaffected in the presence of the voltage sensor. Our data imply a direct interaction between cis FFA and the BK channel protein.

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of BK channels have been described within the CNS (28, 33). BK channels in the neurosecretory GH3 cell line have been well characterized by ourselves and others (8–11, 15, 18, 23) and are sensitive to arachidonic acid produced by PLA2 (10, 19, 32, 40, 42). In the case of GH3 cells, BK channels appear to respond to arachidonic acid itself rather than one of its metabolites (9). On the other hand, a clonal cell line derived from GH3 cells, GH3.C1, does not respond to arachidonic acid itself, presumably because it contains an alternative BK splice variant (although it does seem to respond to lipoygenase metabolites of arachidonic acid; see Ref. 12). Thus a careful examination of the effects of fatty acids on BK channels in GH3 cells could provide information about the mechanism of fatty acid activation of BK channels. Therefore, as a first step, we evaluated the effects of fatty acids on BK channels in GH3 cells. We sought to confirm that the effects of fatty acids were not due to secondary metabolic products and to understand whether there were structural characteristics that were required if a fatty acid was to have an effect on BK channel activity in GH3 cells. We investigated the effects of a number of fatty acids on the membrane fluidity in GH3 cells in an effort to understand whether fatty acid effects arise via a direct interaction with the channel protein or associated structure within the plasma membrane or whether the changes in BK channel activity could be explained simply by alterations in the membrane phospholipid order. We also examined whether the effect of arachidonic acid on BK channels was due to the formation of reactive oxygen species by using a free radical scavenger, N-acetylcysteine, to reduce or eliminate any such products. Finally, in an effort to gain some insight into the sidedness of the FFA effect, we used arachidonyl-CoA as a membrane-impermeable source of arachidonate.

MATERIALS AND METHODS

Cell culture. The GH3 cell line was obtained from American Type Culture Collection (ATCC, Rockville, MD). GH3 cells were grown at 37°C in a 5% CO2 atmosphere in DMEM supplemented with 15% heat-activated horse serum, 2.5% FBS, and 2 mM glutamine. Cells for electrophysiological experiments were plated on polylysine-coated petri dishes to which a polycarbonate recording chamber with a volume of 0.2 ml had been previously affixed with Sylgard. Cells were used 1–3 days after plating, and cells from passages 22 to 40 were used in the experiments described in this study.

Recording solutions. The solutions used in all experiments were (in mM) 150 KCl, 2 MgCl2, and 10 HEPES (pH 7.30) or 140 KCl; 5 HEPES, 5 K2EGTA, and 4.55 CaCl2 (100 μM free ionized Ca2+; pH 7.30) for the pipette and 140 KCl, 15 HEPES, 5 K2EGTA, and 0.1, or 1 μM intracellular Ca2+ (Ca2+; pH 7.4) for the bath.

Fatty acid solutions. Stock solutions for all fatty acids (10–100 mM) were prepared in the following manner. A 15-ml screw cap tube was flame-dried under N2. The appropriate volume of absolute ethanol that had been degassed with N2 was introduced. The solution was thoroughly mixed and then stored −20°C under an N2 blanket between experiments. Fresh solutions were made daily. For anisotropy measurements, fatty acid solutions were prepared daily in anhydrous DMSO.

Drugs and chemicals. With the exception of eicosatetraenoic acid (ETYA), obtained from Biomol (Plymouth Meeting, PA), all fatty acids and other chemicals were obtained from Sigma Chemical (St. Louis, MO).

Drug exposure paradigm. For all electrophysiological experiments, drug exposure was effected using a gravity perfusion/suction removal technique with a perfusion rate of 2.0 ml/min and a dead volume of 1.0 ml. Previous experiments showed that exchange was 90 ± 7% complete after 0.5 min (8). After obtaining a high-resistance (>25 GΩ) seal, patches were excised in a 1 μM Ca2+ solution and depolarized to +20 mV. Control recordings were obtained in K+EGTA-buffered solutions containing the desired [Ca2+]. After control recordings (typically 2–5 min), the patch was perfused with a second solution containing 2.5 μM of the fatty acid being studied, and recordings continued for an additional 10 min. For experiments involving voltage dependence measurements, 2- to 3-min control recordings were made at +10, +20, +30, +40, and +50 mV. The patch was then depolarized to +20 mV, and the fatty acid solution was introduced. After allowing any effect of the fatty acid to stabilize (5–10 min), the voltage paradigm described above was repeated.

Electrophysiological recordings. All experiments in this study used the excised patch configuration of the patch-clamp technique. Electrodes were fabricated from Corning 7052 glass (Garner Glass, Fullerton, CA) in two steps on a Narishige PP-83 electrode puller (Narishige, Tokyo, Japan). Electrodes were fire polished to a final tip resistance between 3 and 5 MΩ. Recordings were performed at room temperature with a Dagan model 3900 patch-clamp amplifier (Dagan, Minneapolis, MN). All experiments were conducted with the patch depolarized initially to +20 mV. Single channel data were stored on digital audio tape using a Sony model DAS-75 digital audio tape recorder (Dagan).

Data analysis. Single channel data were digitized using Axoscope-7 software (Axon Instruments, Foster City, CA) at a sampling rate of 5 kHz and were filtered at 2 kHz using a four-pole low-pass Bessel filter. The digitized single channel data were analyzed in 1-min segments to generate Np (the open probability (Po) times the number of channels (N)) vs. time plots using Fetchan and P-Stat software programs (Axon Instruments). Po was determined from the amplitude histograms by fitting each amplitude histogram to the appropriate sum of Gaussian distribution functions using iterative nonlinear regression software (PeakFit, SPSS, Chicago, IL) after correction of the baseline to make zero current coincident with the state in which all channels were closed. Np values were first calculated from the amplitude histogram. The Po was then calculated as Np/N.

Fluorescence anisotropy measurements. Relative plasma membrane fluidity was determined by changes in fluorescence anisotropy using the dye trimethylammonium-diphenylhexatriene (TMA-DPH, 1.0 μM). The anisotropy value, r, was measured with an excitation wavelength of 340 nm and emission wavelength of 425 nm on a SLM8000 fluorescence spectrophotometer (SLM Instruments, Urbana, IL; see Refs. 5 and 41). For each experiment 4 × 106 GH3 cells mixed with TMA-DPH in a final volume of 2 ml PBS was used. Initial values for the instrument correction factor (G) and anisotropy obtained once steady state had been reached (typically <8 min) were 0.665 ± 0.014 and 0.265 ± 0.012, respectively. Step concentrations of 25, 50, 75, and 100 μM FFA were then added, and r was calculated after allowing for equilibration between each step (3–5 min for each dose). Data were then
plotted as the percentage change in anisotropy corrected for the effect of DMSO vehicle for each fatty acid.

Statistical analysis. A t-test for repeated measures was used for within-group comparisons for two treatments. In all cases, a P value < 0.05 was required to reject the null hypothesis. Intergroup comparisons for fatty acid effects were made using an ANOVA followed by a post hoc Scheffé’s test for multiple comparisons. Multiple linear regression was used to determine whether a significant relationship existed between chain length, number of double bonds, and increase in P_o.

Linear regression was used to analyze the relationship between the number of double bonds and the increase in P_o. All data are presented as means ± SD unless specified otherwise.

RESULTS

Electrophysiological characteristics of BK channels. BK channels in these experiments had a unit conductance of 222 ± 35 pS. The channels had a normal response to [Ca^{2+}], with a P_o of 0.24 ± 0.05 in 0.1 μM [Ca^{2+}]. The channels had a normal voltage dependence over the range from 0 to +50 mV, with a 35 ± 8 mV change producing a 10-fold change in P_o. All kinetic and other biophysical characteristics of the BK channels were similar to those previously reported by many other investigators in a variety of tissues (1, 20, 21, 24).

The cis unsaturated fatty acids activate BK channels. The cis unsaturated fatty acids palmitoleic (C16:1, cis-9), oleic (C18:1, cis-9), linoleic (C18:2, cis-9,12), linolenic (C18:3, cis-9,12,15), arachidonic (C20:4, cis-5,8,11,14), and eicosapentaenoic (C20:5, cis-5,8,11,14,17) resulted in highly significant increases in BK channel activity (P < 0.001 in all cases), increasing P_o by 1.36 ± 0.19-, 1.91 ± 0.56-, 2.08 ± 0.50-, 2.23 ± 0.54-, 3.60 ± 1.10-, and 4.01 ± 0.14-fold, respectively. Interestingly ETYA [C20:4, yne-5,8,11,14 (triple bonds)] resulted in an increase in BK channel activity of 3.27 ± 0.16-fold (P < 0.001). Conversely, neither the saturated fatty acid, stearic (C18:0), nor the trans unsaturated acids, elaidic (C18:1, trans-9), linolealaidic (C18:2, trans-9,12), or linolenalaidic (C18:3, trans-9,12, 15), resulted in any significant change from control activity. Figure 1 shows typical BK channel activity in response to the cis unsaturated acid, oleic (C18:1, cis-9), and the corresponding trans isomer, elaidic (C18:1, trans-9) acid. Although there was a significant increase in P_o after treatment with oleic acid (C18:1, cis-9), there was no significant change in the unit conductance of the channel.

Fatty acid activation of BK channels does not alter the slope of the voltage dependence. BK channels are sensitive to both Ca^{2+} and voltage, which often makes...
it difficult to separate changes in Ca\(^{2+}\) sensitivity from changes in voltage sensitivity. The increases in \(P_o\) described above could be due to a change in the sensitivity of the channel to Ca\(^{2+}\) or to a shift in the \(P_o\) vs. [Ca\(^{2+}\)] curve to lower voltages. One measure of the voltage sensitivity of the channel is the slope of the \(P_o\) vs. voltage curve at constant Ca\(^{2+}\) concentration. Even though changes in Ca\(^{2+}\) may shift the curves along the voltage axis, if the slopes of \(P_o\) vs. voltage curves are the same under two treatment conditions, then the voltage-gating components of the channel are no more or no less sensitive to changes in the membrane potential field. We evaluated the effect of increasing voltage on BK channels from cells treated with fatty acids at 0.1 \(\mu\)M [Ca\(^{2+}\)]. These data show that 2.5 \(\mu\)M fatty acids containing at least one double bond in the cis configuration result in a highly significant increase in \(P_o\) (\(P < 0.001\); as expected from our results above), but the slope of the \(P_o\) vs. voltage relationship is unchanged from control. Figure 2A is a representative example using linolenic acid (C18:3, cis-9,12,15) as the model compound. For comparison, Fig. 2B shows BK channel activity from control cells and cells treated with 2.5 \(\mu\)M linolenelaidic acid (C18:3, trans-9,12,15) in 0.1 \(\mu\)M Ca\(^{2+}\). In these cells, the trans unsaturated fatty acid produces no change in either \(P_o\) or in the slope of the \(P_o\) vs. voltage relationship. Because of variability in the control values for \(P_{o,0}\), responses were normalized to the \(P_{o,0}\) obtained at +30 mV under control conditions for each of the 12 cells. \(P_o\) vs. voltage experiments with arachidonic, oleic, linoleic, elaidic, and linolelaidic acids produced similar results. Because the slopes of the \(P_o\) vs. voltage relationship for control cells and cells treated with any fatty acid were not significantly different, we conclude that fatty acid treatment is not altering the voltage sensitivity of the channel but possibly is altering the intrinsic Ca\(^{2+}\) sensitivity.

At least one cis-oriented double bond is required for fatty acid activation of BK channels in GH3 cells. Table 1 provides a structural summary of the fatty acids studied along with the corresponding effect on the \(P_o\) of BK channels in excised patches. Figure 3 summarizes the effects of each fatty acid studied on BK channel activity presented as a fraction of the effect elicited by arachidonic acid. All fatty acids containing cis double bonds resulted in significant activation of BK channels compared with control values, and longer-chain fatty acids appeared to produce a slightly larger response than shorter chain acids. However, multiple linear regression fitting the effect on \(P_o\) to chain length and the number of double bonds suggests that, although the regression coefficient is 0.983, most of the variation in the \(P_o\) values can be attributed to the effect of changes in the number of double bonds (\(P = 0.003\)) while the correlation with chain length is marginal (\(P = 0.09\)). If the best-fit linear regression relationship is calculated for relative \(P_o\) vs. number of double bonds by combining data from different chain lengths (Fig. 4), the regression coefficient is 0.792, and the linear regression predicts that the addition of each double bond increases \(P_o\) by 60 \(\pm\) 7.7% relative to control levels (slope is significantly different from 0, \(P < 0.001\)). ETPYA (C:20-triple bonds 5,8,11,14), the triple-bond analog of arachidonic acid, has a similar molecular structure to arachidonic acid and was capable of activating BK channels in GH3 cells to nearly the same extent as arachidonic acid.

**The magnitude of activation of BK channels is not related to the changes in membrane fluidity.** In an attempt to determine whether BK channel activation by cis unsaturated fatty acids was a direct interaction with the channel protein or some closely associated regulatory protein or whether the changes in BK channel activity could be explained simply by alterations in

![Fig. 2. Representative effects of a polyunsaturated cis (linolenic C18:3, cis-9,12,15) and trans (linolenelaidic C18:3, trans-9,12,15) fatty acid on the \(P_o\) vs. mV relationship for BK channels in GH3 cells. These experiments were conducted using the excised (inside-out) patch configuration of the voltage-clamp technique with the patch initially depolarized to +20 mV. Both the bath and pipette contained 150 mM K\(^+\), and the bath contained 0.1 \(\mu\)M [Ca\(^{2+}\)]. After control recordings were made at +20, +30, +40, and +50 mV, a bath solution containing 2.5 \(\mu\)M of the desired fatty acid was introduced by continuous perfusion. After any effect was allowed to stabilize, recordings were obtained again at each of the voltages. Control; \(\bullet\), fatty acid treatment. Because of variability in the control values for \(P_{o,0}\), responses were normalized to the \(P_{o,0}\) obtained at +30 mV under control conditions for each of the 12 cells. A: significant increase in \(P_o\) in response to linolenic acid (C18:3, cis-9,12,15) but without a change in the slope of the \(P_o\) vs. mV relationship. B: linolenelaidic acid (C18:3, cis-9,12,15) did not result in a change in either the \(P_o\) or the slope of the \(P_o\) vs. mV relationship.**
FFAs do not play a major role in the activation of BK or saturated fatty acids. FFA effect on BK channel order does not correlate with the order observed for the response than shorter-chain acids. ETYA, eicosatetraynoic acid.

Longer-chain fatty acids appeared to produce a slightly larger re- cative activation of BK channels compared with control values, and acid. All fatty acids containing activity presented as a fraction of the effect elicited by arachidonic acid. The effects of each fatty acid studied on BK channel Fig. 3. Effect of structure on the degree of BK channel activation by fatty acids. The effects of each fatty acid studied on BK channel activity presented as a fraction of the effect elicited by arachidonic acid. All fatty acids containing cis double bonds resulted in significant activation of BK channels compared with control values or unsaturated fatty acids. cis-double bonds produced a significant activation of ETYA. This series order does not correlate with the order observed for the FFA effect on BK channel $P_o$ (Figs. 3 and 4), which is 20:4 > ETYA > 18:1,2 or 3 cis and no effect of the trans or saturated fatty acids.

Free radical production or oxygen metabolites of FFAs do not play a major role in the activation of BK channels. Some ion channels are activated by oxidation products of fatty acids rather than by the parent fatty acid. These products can arise through the action of the enzymes cyclooxygenase or lipoxygenase on fatty acids or by the reaction of unsaturated fatty acids and superoxide. Cyclooxygenase or lipoxygenase products are unlikely to be involved, since ETYA produced an effect on channel $P_o$ similar to that of arachidonic acid even though ETYA cannot be metabolized by either cyclooxygenase or lipoxygenase. However, superoxide can react with both ETYA or arachidonic acid as well as other

![Graph showing the effect of number of double bonds on $P_o$](https://example.com/fig4)

Fig. 4. Relationship between the effect on BK channel $P_o$ and the number of double bonds in the fatty acids applied to a patch. All fatty acids containing cis double bonds produced a significant activation of BK channels compared with control values or unsaturated fatty acids with more double bonds producing a larger response. In addition, longer-chain fatty acids produced to appear a slightly larger response than shorter-chain acids with the same number of double bonds. However, multiple linear regression fitting the effect on $P_o$ to chain length and the number of double bonds suggests that, although the regression coefficient is 0.983, most of the variation in the $P_o$ values can be attributed to the effect of changes in the number of double bonds ($P = 0.003$), whereas the correlation with chain length is marginal ($P = 0.09$). When the contribution of chain length is ignored, the best-fit linear regression relationship for relative $P_o$ vs. number of double bonds is shown on top. The regression coefficient is 0.792 with an intercept of 1.0 ± 0.23 and a slope of 0.60 ± 0.079, implying that the addition of each double bond increases the $P_o$ by 60 ± 7.9% relative to control levels (slope is significantly different from 0, $P < 0.001$). FFA, free fatty acid.

### Table 1. Effect of fatty acid structure on increases in BK channel activity

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>Designation</th>
<th>Change in Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂H</td>
<td>Stearic</td>
<td>C18:0</td>
<td>1.07 ± 0.13*</td>
</tr>
<tr>
<td>CO₂H</td>
<td>Elaidic</td>
<td>C18:1, trans-9</td>
<td>1.05 ± 0.16*</td>
</tr>
<tr>
<td>CO₂H</td>
<td>Linoleic</td>
<td>C18:2, trans-9,12</td>
<td>1.08 ± 0.26*</td>
</tr>
<tr>
<td>CO₂H</td>
<td>Linolenelaidic</td>
<td>C18:3, trans-9,12,15</td>
<td>0.96 ± 0.13*</td>
</tr>
<tr>
<td>CO₂H</td>
<td>Palmitoleic</td>
<td>C16:1, cis-9</td>
<td>1.36 ± 0.19†</td>
</tr>
<tr>
<td>CO₂H</td>
<td>Oleic</td>
<td>C18:1, cis-9</td>
<td>1.91 ± 0.86†</td>
</tr>
<tr>
<td>CO₂H</td>
<td>Linoleic</td>
<td>C18:2, cis-9,12</td>
<td>2.05 ± 0.44†</td>
</tr>
<tr>
<td>CO₂H</td>
<td>Linolenic</td>
<td>C18:3, cis-9,12,15</td>
<td>2.23 ± 0.94</td>
</tr>
<tr>
<td>CO₂H</td>
<td>Arachidonic</td>
<td>C20:4, cis-5,8,11,14</td>
<td>3.6 ± 0.86</td>
</tr>
<tr>
<td>CO₂H</td>
<td>Eicosapentaenoic</td>
<td>C20:5, cis-5,8,11,14,17</td>
<td>4.01 ± 0.14</td>
</tr>
<tr>
<td>CO₂H</td>
<td>ETYA</td>
<td>C20:4, yne-5,8,11,14</td>
<td>3.27 ± 0.16</td>
</tr>
</tbody>
</table>

Data are means ± SD. ETYA, eicosatetraynoic acid. *Not significantly different from control; †significantly different from arachidonic acid.
unsaturated fatty acids. To determine whether superoxide was playing a role in the activation of BK channels by fatty acids by spontaneous reaction with the double bonds, we examined the effect of arachidonic acid on BK channels in excised patches that had been exposed to N-acetylcysteine, a known scavenger of free radicals, including superoxide (6). Patches were excised in 1 mM [Ca\textsuperscript{2+}]\textsubscript{i} and depolarized to +20 mV. After control recordings had been made, 2 mM N-acetylcysteine was added. After allowing the patch to stabilize and after recording activity in the presence of N-acetylcysteine, 2.5 μM arachidonic acid was added in the presence of N-acetylcysteine, and the maximum activation was recorded. N-acetylcysteine applied by itself does not result in a significant change in \( P_o \) (0.18 ± 0.04 under control conditions to 0.16 ± 0.04 in the presence of N-acetylcysteine). On the other hand, 2.5 μM arachidonic acid applied in the presence of N-acetylcysteine resulted in a significant increase in \( P_o \) to 0.53 ± 0.15 in all three patches examined (\( P < 0.05 \)); however, the extent of arachidonic acid activation in the presence of N-acetylcysteine (\( P_o = 0.58 ± 0.18 \)) was not significantly different from that noted in the absence of N-acetylcysteine, implying that reactive oxidation products are not significant contributors to the fatty acid activation of BK channels.

**Arachidonyl-CoA activates BK channels in excised patches.** In an effort to determine whether FFAs have to cross the cell membrane to produce their effects, we employed arachidonyl-CoA as others have done (29) to deliver a membrane-impermeable form of arachidonic acid to the cytosolic surface of excised patches. Arachidonyl-CoA (2.5 μM) resulted in a 1.92 ± 0.45-fold increase (\( n = 5 \)) in BK channel \( P_o \). Because arachidonyl-CoA does not cross the cell membrane, we conclude that the observed increases in BK channel activity resulting from treatment with FFAs involve an action of the fatty acid in the inner leaflet of the membrane or in the aqueous phase close to the cytosolic surface of the membrane (29).

**DISCUSSION**

The major findings from this investigation were 1) BK channel activation in GH\textsubscript{3} cells by fatty acids requires a fatty acid with at least one double bond in the cis configuration; 2) there is a significant correlation between the degree of cis unsaturation and the extent of BK channel activation; 3) there is not a significant correlation between changes in membrane fluidity and extent of BK channel activation in GH\textsubscript{3} cells; 4) neither superoxide nor fatty acid oxidation products play a major role in the activation of BK channels by fatty acids; and 5) experiments with arachidonyl-CoA suggest that the activation of BK channels by fatty acids results from an action in or near the cytosolic membrane. These results imply that cis unsaturated fatty acids are interacting directly with the channel protein itself.

**Fatty acid activation does not change the voltage or Ca\textsuperscript{2+} sensitivity of BK channels.** The cis unsaturated fatty acids all increase the \( P_o \) of BK channels. They could increase \( P_o \) in a variety of ways, including...
changes in the voltage or Ca²⁺ sensitivity of the channels. We have previously shown that application of arachidonic acid or ETYA does not alter the slope of the Ca²⁺ vs. P_o relationship (9, 10). In this paper, we show that, in all cases where fatty acid treatment resulted in the activation of BK channels, the resultant increase in P_o was not accompanied by changes in the slope of the voltage dependence or changes in the unit conductance of the channel. Similarly, for the saturated or trans fatty acids that did not effect P_o, no changes in the slope of the voltage dependence or unit conductance were noted. These data suggest that the voltage-sensing residues of BK channels are unaffected by fatty acid treatment and that the effects of fatty acids on BK channels are through a direct and specific interaction of the fatty acid with the channel protein rather than a nonspecific lipid interaction as has been noted for a number of ion channels (26, 30).

At least one cis double bond is required for fatty acid activation of BK channels in GH3 cells. The effects of fatty acids on ion channels appear to be ion channel specific. For example, a number of ion channels, including GABA_A channels, only respond (activation or inhibition) to fatty acids that have cis double bonds, whereas others are equally effected by cis, trans, or saturated fatty acids (14, 17, 35). Some ion channels that are affected by arachidonic acid do not respond to ETYA, whereas others do (14, 16, 26). These data suggest that, for some ion channels, there is a nonspecific effect of fatty acids, whether it is by alterations in membrane fluidity or via other mechanisms (26, 34). Other ion channels, including BK channels from other neuronal cell lines, are affected by oxidation products of arachidonic and/or other fatty acids susceptible to metabolism by cyclooxygenase and/or lipoxygenase (12). In the present study, saturated fatty acids or those containing one or more double bonds in the trans configuration had no effect on BK channels. On the other hand, those fatty acids containing one or more double bonds in the cis configuration consistently activated BK channels, with more double bonds leading to more potent activation. These data show that there are structural features that must be satisfied for a given fatty acid to increase the activity of BK channels. Such requirements suggest the possibility of direct binding or interaction with the channel protein. The cis FFA form a “hairpin” like structure that results in two long hydrocarbon tails, one having a hydrophilic and one having a hydrophobic terminus. Such configurations may optimize the possibility of II-orbital bonding between the FFA and elements on the channel protein. Such an interaction could produce changes in the channel protein conformation, resulting in a conformation with a lower energy state. The hydrophilic and hydrophobic tails may serve to stabilize such an interaction. This explanation would also be consistent with the activation of BK channels by ETYA, since the electronic structures (particularly the capacity for strong Π bonding) of ETYA and arachidonic acid are extremely similar (5). Although a putative fatty acid binding site has been described on the N-methyl-d-aspartate receptor (31), we were unable to identify the consensus sequence for that binding site in either the α- or β-subunit sequence of the rat BK channels. This, of course, does not rule out the possibility that such a site does exist but rather that such a site cannot be identified by simple homology.

The magnitude of activation of BK channels is not related to the changes in membrane fluidity. One hypothesis that has been suggested for the mechanism by which fatty acids alter ion channel activity is that the fatty acids alter ion channel activity in proportion to their alteration in membrane fluidity. Such changes in membrane fluidity are thought to alter the conformation of the channel protein, making transitions from the closed to the open state more energetically favorable. This hypothesis has resulted in a dichotomy in the literature (26, 34). In an effort to determine whether alterations in membrane fluidity could explain the results in the present investigation, we measured changes in anisotropy for all of the acids under investigation except ETYA (C20:4, yne-5,8,11,14). Our results show that membrane fluidity is affected by all fatty acids studied. The cis isomers resulted in a slightly greater (although not significant) alteration in membrane fluidity compared with the corresponding trans isomers. Interestingly, ETYA produced a significantly smaller change in membrane fluidity than arachidonic and a number of the other fatty acids while producing an increase in P_o that was not different from that seen with arachidonic acid. Overall, there was no correlation between the extent of fatty acid alterations in membrane fluidity and the corresponding effects on P_o. These data strongly support our hypothesis that fatty acid changes in P_o are not related to corresponding changes in membrane fluidity.

Products arising from the action of lipoxygenase, cyclooxygenase, or superoxide are not responsible for the fatty acid activation of BK channels. Cyclooxygenase and lipoxygenase products have been shown to activate and inactivate a variety of ion channels while having no effects on others (12, 26). Cyclooxygenase and lipoxygenase metabolism of fatty acids requires that a 1,4-pentadiene structure be present (30). Although a number of the fatty acids tested did not have such a structure, we were unable to identify the consensus sequence for that binding site in either the α- or β-subunit sequence of the rat BK channels. This, of course, does not rule out the possibility that such a site does exist but rather that such a site cannot be identified by simple homology.
significantly different from arachidonic acid. ETYA is susceptible to oxidation by superoxide. In an effort to rule out oxidation products produced by superoxide, we conducted a series of experiments in which excised patches were pretreated with 2 mM N-acetylcysteine, an efficient scavenger of superoxide (6). Treatment of patches with N-acetylcysteine had no effect on $P_g$, although subsequent treatment with 2.5 μM arachidonic resulted in a increase in $P_g$ that was indistinguishable from cells not pretreated with N-acetylcysteine. Taken together, these results support the hypothesis that products of fatty acid oxidation by superoxide are not major contributors to the activation of BK channels.

Arachidonyl-CoA activates BK channels in excised patches. The treatment of excised patches with arachidonyl-CoA, which has a large head group and does not cross the cell membrane, resulted in a strong activation of BK channels, as others have reported (29). The magnitude of this increase was ~50% of that noted for arachidonic acid itself. This is not unexpected, since the binding efficiency of arachidonate delivered as arachidonyl-CoA would not be expected to be nearly as great as with arachidonic acid itself. This observation suggests that the effects of FFAs on BK channels are via an action on the cytosolic side of the membrane and is consistent with published reports regarding the actions of FFAs on other types of ion channels (29, 38). This result also suggests a possible site for interaction of FFAs. We have previously shown that the primary effect of arachidonic acid is to increase the Ca$^{2+}$ sensitivity of BK channels (10). The Ca$^{2+}$ binding site of BK channels is purportedly near the inner surface of the membrane in the cytosolic tail of BK channels (36). Binding of arachidonic acid or arachidonoyl-CoA to this domain could be responsible for the change in Ca$^{2+}$ affinity.

The data presented here strongly suggest that the activation of BK channels in GH$_3$ cells by fatty acids is specific rather than nonspecific and that the activation arises via a direct interaction between the fatty acid and the channel protein on the cytosolic surface of the membrane, which alters the Ca$^{2+}$ sensitivity of the channel.

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