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Linoleic acid both enhances activation and blocks Kv1.5 and Kv2.1 channels by two separate mechanisms

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McKay, M. Craig, and Jennings F. Worley III. Linoleic acid both enhances activation and blocks Kv1.5 and Kv2.1 channels by two separate mechanisms. *Am J Physiol Cell Physiol* 281: C1277–C1284, 2001.—Linoleic acid (LA) had two effects on human Kv1.5 and Kv2.1 channels expressed in Chinese hamster ovary cells: an increase in the speed of current activation process (EC_{50} = 2.4 and 2.7 μM for Kv1.5 and Kv2.1, respectively) and current inhibition (IC_{50} = 6.6 and 7.4 for Kv1.5 and Kv2.1, respectively). LA affected the activation kinetics via two processes: a leftward shift in the instantaneous activation curves and an increase in the rate of current rise. Current inhibition by LA was time dependent but voltage independent. Hill slopes for plots of current inhibition (3.5 and 3.9 for Kv1.5 and Kv2.1, respectively) vs. dose of LA suggested that cooperativity was involved in the mechanism of current inhibition. A similar analysis of the effects of LA on current activation did not reveal cooperative interactions. The effects of LA were mediated from the external side of the channels, since addition of 10 μM LA to the patch pipette solution was without effect. Additionally, the methyl ester of LA was effective at enhancing peak current and promoting channel activation for Kv1.5 and Kv2.1 without inducing significant current inhibition.

potassium channel; activation kinetics; electrophysiology; unsaturated fatty acid

THE POLYUNSATURATED FREE FATTY acids (PUFFAs) have been intensively studied for their biophysical roles as triglyceride components in biomembranes and as biochemical elements of the metabolic pathways related to energy production and storage. Free fatty acids (FFAs), unsaturated or not, are constitutively synthesized de novo in the cytoplasm of the liver and other tissues, while the essential PUFFAs, linoleic acid (LA, C18n–2, Δ9) (12) and linolenic acid (C18n–3, Δ9) (12, 15), must be included in the diet and are the starting points for the synthesis of the n–3 and n–6 fatty acids.

A number of studies have shown that FFAs interact with a variety of cloned and native K^+ channels (1–5, 7–9, 11, 13, 15–17) and demonstrate a variety of effects. Many studies have reported that *cis*-polyunsaturated FFAs tended to act as blockers of voltage-gated delayed rectifier-type channels (1, 2, 5, 7–9, 16, 17), while saturated, monounsaturated, and *trans*-polyunsaturated FFAs have lesser or no effects (2, 5). On the other hand, FFAs activate the two-pore K^+ channels, TREK, TRAAK, and TWIK (4, 10, 11), as well as certain large-conductance Ca^{2+}-activated K^+ channels (2, 13). It has also been reported that activation or block of native mechanosensitive K^+ channels in smooth muscle by FFAs (and related derivatives) was determined primarily by head group charge and acyl chain length (15). With regard to voltage-gated K^+ channels, only the delayed rectifier channel, Kv1.1, has been explicitly reported to undergo an acceleration of activation time course by PUFFAs (7, 8), although a few studies have noted that some PUFFAs alter the activation kinetics of current development in addition to the phenomenon of K^+ channel current block (1, 9).

Given the range of effects reported for PUFFAs on various K^+ channels, it is not surprising that there is some uncertainty as to the site or sites of action by which PUFFAs exert their effects on ion channels. Consequently, the mechanism of action has remained obscure. On the basis of literature reports, it seems likely that the site and mechanism of action of PUFFAs on K^+ channels may vary by class.

Because of its dietary importance, we have characterized the effects of LA on the cloned human delayed rectifier channels Kv1.5 and Kv2.1. In particular, we have concentrated on the observed changes in the activation kinetics of Kv1.5 and Kv2.1 produced by LA. We found that LA accelerates the activation time course of Kv1.5 and Kv2.1 at all tested doses, even as it causes a time-dependent inhibition of current. In the case of Kv1.5, the effects of increased speed of activation and enhanced current can be seen independently of current inhibition at <5 μM LA. Our observations of the dual effects of LA on Kv1.5 and Kv2.1 (i.e., activation acceleration and current inhibition) as well as a putative extracellular site of action have led us to propose a model where LA has two binding sites on the Kv channels: a high-affinity site that alters the activation properties of the channels and a second, lower-affinity site at or near the pore that is responsible for the observed current inhibition. In addition to the interesting effects of LA on the biophysical properties

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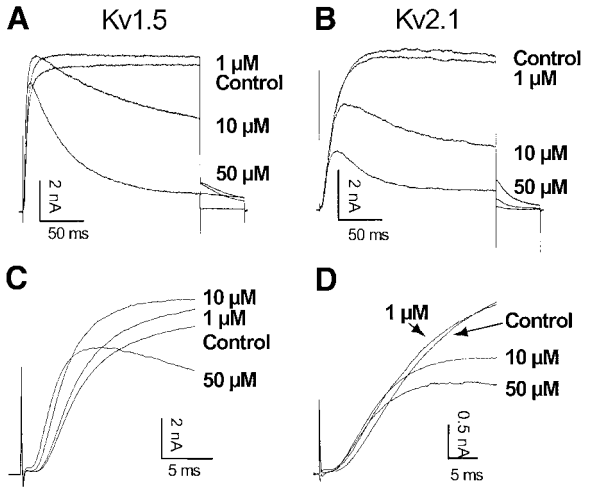

Fig. 1. Effects of bath-applied linoleic acid (LA) on Chinese hamster ovary cells expressing human Kv1.5 and Kv2.1. *A*: escalating doses of LA dose-dependently increased the speed of current activation and also induced a small dose-dependent increase in Kv1.5 current at low doses followed by a dose-dependent decrease in steady-state current at higher doses (see Fig. 4). LA also caused a dose-dependent slowing of the tail currents, irrespective of the degree of current activation or inhibition. *B*: LA induced a dose-dependent decrease in Kv2.1 current at all tested doses without the observed current enhancement seen with application of LA to Kv1.5. Expanded views of the time courses of Kv1.5 (*C*) and Kv2.1 (*D*) show increased speed of activation with application of LA.

of the ion channels we have studied, the effects of LA on these channels may be physiologically relevant to the dietary intake of PUFFAs or disease processes associated with fatty acid mobilization and transport. An enhanced understanding of the interactions of K^+ channels with the ubiquitous PUFFAs may form the basis of future ion channel-specific therapies.

MATERIALS AND METHODS

Cell maintenance. Stable cell lines expressing Kv2.1 or Kv1.5 were created by transfecting Chinese hamster ovary cells with the appropriate pcDNA3.1 vector containing a neoselectable marker with the CMV promoter using the Lipofectamine (GIBCO-BRL) method. Cells were maintained in 250-ml spinner culture bottles (Techne) containing Ex-Cell 301 (JRH Bioscience), 2% fetal bovine serum, 1% penicillin-streptomycin, and 1% G418 (GIBCO-BRL) at 37°C in 95% O_2-5% CO_2 and stirred at 60 rpm. One day before recording, aliquots of spinner culture were dropped onto ethanol-cleaned glass coverslips placed in 35-mm culture dishes containing DMEM-F-12 supplemented with 2% fetal bovine serum, 1% penicillin-streptomycin, and 1% G418 and incubated at 37°C in 95% O_2-5% CO_2.

Electrophysiology and solutions. Pieces of coverslip containing adherent cells were placed in a recording chamber (~200 μl volume) mounted on the stage of an inverted microscope. The chamber was continuously perfused at ~1 ml/min. All solutions and experiments were performed at room temperature (19–21°C). Patch pipettes were pulled using thin-wall glass capillaries (TWF-150, World Precision Instruments) and had resistances of <2 MΩ when filled with a recording solution consisting of (in mM) 145 KCl, 10 glucose, 10 K_2EGTA, 10 MOPS, 5 Mg-ATP, and 1 $CaCl_2$ (pH 7.2) and immersed in the bath solution consisting of (in mM) 145 NaCl, 10 HEPES, 5 KCl, 2.5 $CaCl_2$, and 1 $MgCl_2$ (pH 7.4).

LA (Sigma) was stored under N_2 at −20°C. A working stock solution of LA, prepared daily, was made by dissolving LA in absolute ethanol at a concentration of 10 mM. Test solutions were made by dilution of the working stock into the bath solution. Whole cell patch-clamp recordings were made using an Axopatch 200B amplifier and a Digidata 1200A analog-to-digital converter connected to a personal computer. Data acquisition was performed under the control of pCLAMP software (Axon Instruments). Data were analyzed and figures were constructed using a combination of pCLAMP (Axon Instruments), Excel (Microsoft), and Origin (Microcal).

RESULTS

The key findings of our study on the effects of LA on Kv1.5 and Kv2.1 are illustrated in Figs. 1 and 2. Application of increasing doses of LA (1–50 μM) to voltage-clamped Chinese hamster ovary cells expressing Kv1.5 or Kv2.1 had two effects: a dose-dependent increase in the speed of channel activation and a time-dependent inhibition of current. Figure 1, *A* and *B*, shows superimposed current traces of Kv1.5 and Kv2.1, respectively; control currents and the effects of increasing doses of LA are compared as the voltage was stepped from a holding potential of −70 to 50 mV for 200 ms at 10-s intervals. For Kv1.5, the effects of LA were complex and dependent on the dose of LA. At <5 μM, LA increased the speed of current activation and the magnitude of the initial peak current. At >5 μM, LA produced a time-dependent inhibition of current at all potentials examined, as well as acceleration in the time course of current activation. Regardless of the dose, LA always slowed the decay of Kv1.5 tail currents (repolarization to −40 mV), an effect that we did not examine further.

Application of LA to Kv2.1 under the conditions used for Kv1.5 caused a time-dependent inhibition of current at all doses of LA tested (Fig. 1*B*). Despite the current block of Kv2.1 induced by LA, we still observed a dose-dependent increase in the speed of the activation kinetics of this channel. In contrast to Kv1.5, LA had no obvious effect on the rate of decay of the tail currents of Kv2.1 (Fig. 2).

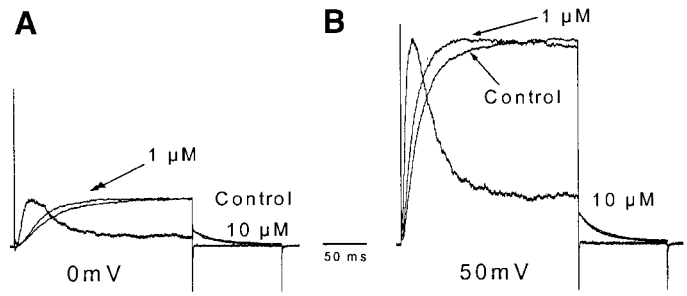

Fig. 2. Current block of Kv2.1 by LA obscures its effects on the speed of current activation. Effects of LA on the speed of Kv2.1 current activation in Fig. 1*B* are obscured by current block. When the currents were scaled by normalization to the peak current (at each voltage), acceleration in the activation of human Kv2.1 was obvious. *A* and *B*: effects of 1 and 10 μM LA on Kv2.1 when voltage was stepped from a holding potential of −70 mV to 0 and +50 mV, respectively.

Figure 1, *C* and *D*, shows expanded views of the activation phase of current development for Kv1.5 and Kv2.1, respectively. Although the effects of LA on the speed of current activation and peak current of Kv1.5 are obvious, the large degree of current block of Kv2.1 caused by LA obscured the extent to which there was a change in the speed of activation of Kv2.1 with increasing doses of LA. To demonstrate the extent to which LA accelerated the activation time course of Kv2.1, we normalized the LA-affected currents to the peak control currents (no scaled data were used for quantitative analyses). Figure 2, *A* and *B*, shows these normalized current traces of Kv2.1 by comparing control with 1 and 10 μM LA at two different test potentials. Although LA induced a dose-dependent increase in the speed of current activation at the 0- and 50-mV test potentials, there was no sign that LA had an effect (in contrast to Kv1.5) on slowing the decay of the Kv2.1 tail currents.

To quantitate the changes in the speed of activation induced by LA, we measured the maximum slopes (nA/ms) of the activation phase of current development when the cells expressing Kv1.5 or Kv2.1 were voltage clamped at a holding potential of −70 mV and stepped to +20 mV at 10-s intervals. When expressed as the percent change vs. control, we found that 1 and 10 μM LA increased the maximum slope of the current activation of Kv1.5 by 16.3 ± 11.5% (SD) and 55.0 ± 18.7 (n = 5), respectively. The effects of 1 and 10 μM LA on the percent change in the maximum slopes of Kv2.1 current activation were smaller than those observed for Kv1.5 and produced a 10.4% ± 2.3 and 19.4 ± 5.9% (n = 5) change in slopes.

Changes in the 10–90% rise time (RT, nA/ms) have been used previously as a measure of current activation (12, 13). Because the values we measured for the slopes of the activation phase of current development were consistent with those obtained by measuring the 10–90% RT of current activation, we used the latter as a measure of the change in the current activation time course. RTs were measured at three voltages (−20, 0, and +50 mV), which were chosen to capture the range of activation of open channel probability from very low to maximal activation.

Figure 3, *A* and *B*, compares the fractional change in rise time of Kv1.5 and Kv2.1 currents in the absence and presence of 1, 5, and 10 μM LA. With the exception of 1 μM LA applied to Kv2.1 at −20 mV, all doses of LA decreased the RT of Kv1.5 and Kv2.1 compared with controls. The negative change observed for 1 μM LA on Kv2.1 at the −20-mV test potential may be attributed to the very small size of the elicited currents at this potential, which is close to the half-maximal activation potential ($V_{0.5}$) of this channel. Despite no clear pattern of voltage dependence, there was, at any given voltage, a dose-dependent decrease in RT with increasing doses of LA.

Values measured at 0 mV were used to plot $1 - (RT_{LA}/RT_{control})$ vs. log dose of LA (log[LA]) for Kv1.5 and Kv2.1 (Fig. 3, *C* and *D*). When fitted with a logistical equation of the form $y = ([LA]_{min} - [LA]_{max})/\{(1 + [LA]/[LA]_m)^p\} + \{([LA]_{min} + [LA]_{max})/2\}$, where $y = 1 - (RT_{LA}/RT_{control})$, $[LA]_m$ is the EC_{50} of the LA concentration, and p is the slope factor, we found that EC_{50} = 2.4 μM for LA and p = 1.1 for Kv1.5. A similar determination for Kv2.1 was made problematic, inasmuch as the impact of LA on reducing Kv2.1 currents was observed at the same doses that produced the alterations in channel activation kinetics. Despite this contaminating influence, from the data we estimate that EC_{50} = 2.7 μM and p = 1.9.

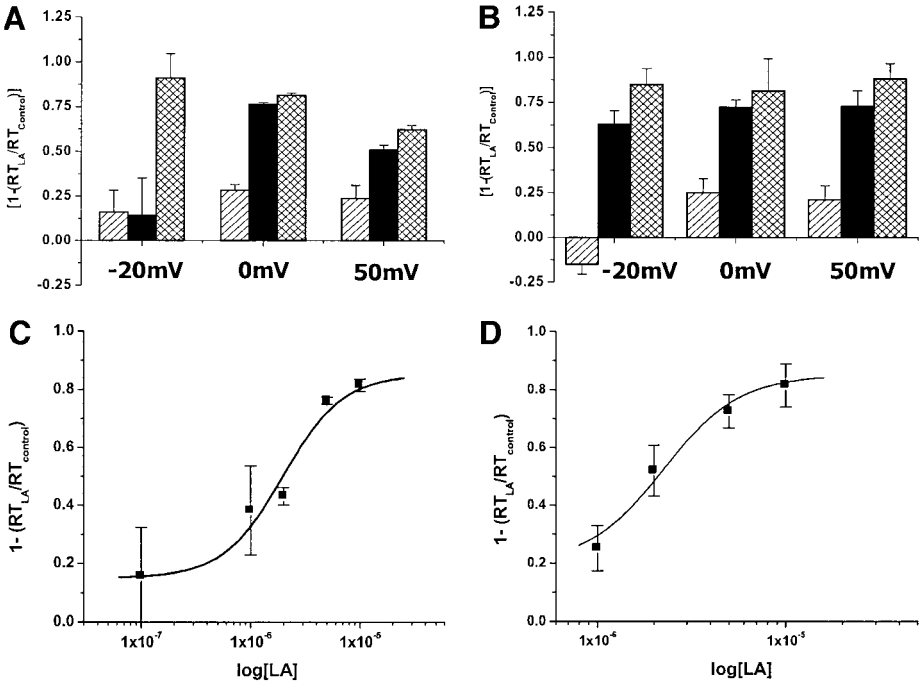

Fig. 3. Effect of LA on 10–90% rise times (RTs) of peak current of Kv1.5 and Kv2.1. RTs of current activation of Kv1.5 (*A*) and Kv2.1 (*B*) were measured at −20, 0, and 50 mV and 1 μM (hatched bars), 5 μM (solid bars), and 10 μM (cross-hatched bars) LA. Comparison of the fractional RTs for Kv1.5 and Kv2.1 show that all test doses of LA at all potentials caused an increase in current RT that, with the exception of 1 μM LA at −20 mV, was without voltage dependence. Error bars, SE; each bar represents 5 measurements. *C* and *D*: fractional RT vs. log dose of LA (log [LA]) for Kv1.5 and Kv2.1. In *C*, LA affected Kv1.5 with EC_{50} = 2.4 μM and slope factor (p) = 1.1 (n = 5). In *D*, a similar determination for Kv2.1 gave EC_{50} = 2.7 μM and p = 1.9 (n = 4).

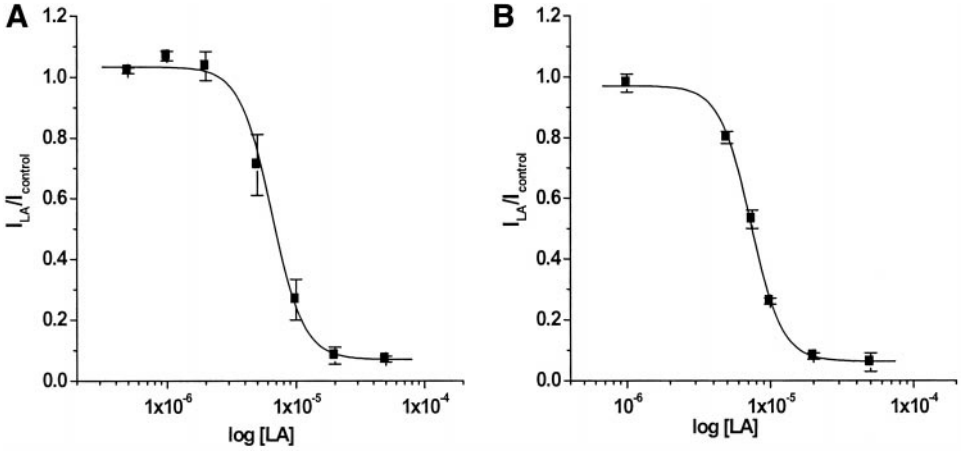

Fig. 4. Effects of LA on the steady-state block of Kv1.5 and Kv2.1 currents. Currents were measured by averaging the last 10 ms of the test pulse. Results were expressed as fractional current ($I_{LA}/I_{control}$) plotted against log [LA] and then fitted using a Hill equation. Error bars, SE; $n = 5$. *A*: LA induced a small dose-dependent increase in steady-state Kv1.5 current at <5 μM followed by a dose-dependent decrease (IC_{50} = 6.6 μM, $p = 3.4$) in current at higher doses. *B*: LA induced only a dose-dependent decrease in steady-state current (IC_{50} = 7.4 μM, $p = 3.9$).

In addition to the increase in the speed of current activation, LA also caused an obvious time-dependent inhibition of Kv1.5 and Kv2.1 currents. The effects of LA on current block of Kv1.5 and Kv2.1 appeared to saturate at the highest tested doses (50 μM). Figure 4 shows the results of these dose-response experiments on the changes in current amplitude of Kv1.5 and Kv2.1 measured at the end of the current pulse. Currents were expressed as the fractional current, f = $I_{LA}/I_{control}$, and plotted against the dose of LA. When fitted with a Hill equation of the form $f/(1 - f) = ([LA]/IC_{50})^{n_H}$, LA blocked Kv1.5 with an IC_{50} of 6.6 μM and a Hill slope (n_H) of 3.5 (5 determinations). Similarly, increasing doses of LA blocked Kv2.1 currents with an IC_{50} of 7.4 μM and a Hill slope of 3.9 (5 determinations), values very close to those derived for Kv1.5. Comparison of the EC_{50} values and slope factors of the effects of LA on the RTs of Kv1.5 and Kv2.1 (EC_{50} = 2.4 μM and $p = 1.1$ for Kv1.5; EC_{50} = 2.7 μM and $p = 1.9$ for Kv2.1) with the corresponding values obtained from Hill plots of current block (IC_{50} = 6.6 μM and $n_H = 3.5$ for Kv1.5; IC_{50} = 7.4 μM and $n_H = 3.9$ for Kv2.1) suggest that LA is affecting these channels by different processes.

Figure 5 illustrates the lack of effect on the activation kinetics (measured as RT) or current inhibition (measured at the end of the voltage step) of Kv1.5 or Kv2.1 when 10 μM LA was included in the patch pipette filling solution. Current records were obtained by holding cells at −70 mV and stepping to +20 mV for 200 ms every 10 s. Currents recorded with LA in the pipette solution were indistinguishable from those recorded without LA in the pipette solution. Measurements of RT and current were normalized to values obtained before addition of LA to the bath solution. We could, however, affect the alterations in kinetics and current block (seen without LA in the patch pipettes) by adding LA to the bath solution and reverse the effects by washing with 0.1% BSA-containing solution ($n = 3$ each), implying that the effects of LA were mediated at the extracellular surface of the channel. Also illustrated in Fig. 5 are two further observations: *1*) the onset of changes in the current activation always preceded changes in the magnitude of the currents

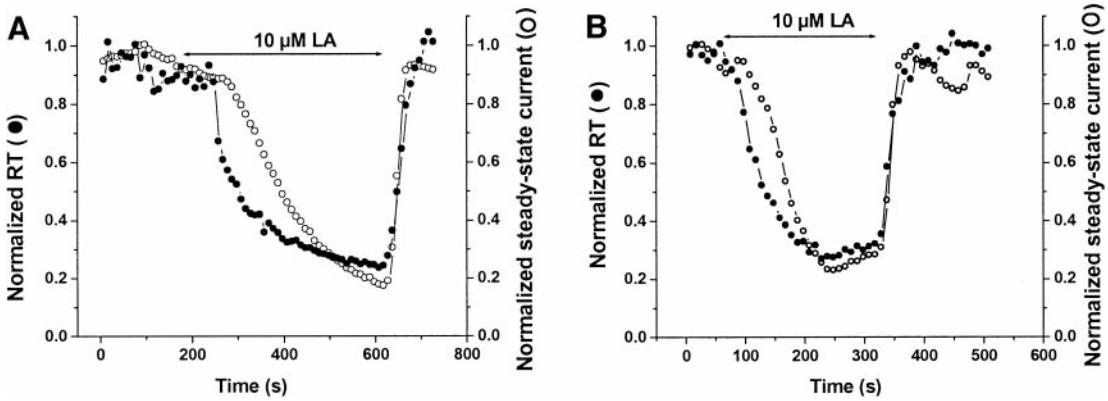

Fig. 5. Time course for effects of LA on changes in RTs and steady-state current block with LA in the patch pipette in Kv1.5 (*A*) and Kv2.1 (*B*). Inclusion of 10 μM LA in the patch pipette solution does not change activation kinetics or induce current block of Kv1.5 or Kv2.1. At indicated times, 10 μM LA was infused into the bathing solution. The post-LA bath contained 0.1% BSA. Raw currents were normalized to values obtained before addition of LA to the bath. Similarly, RTs were normalized to values measured before addition of LA to the bath. ●, RT current; ○, current measured at the end of the voltage step.

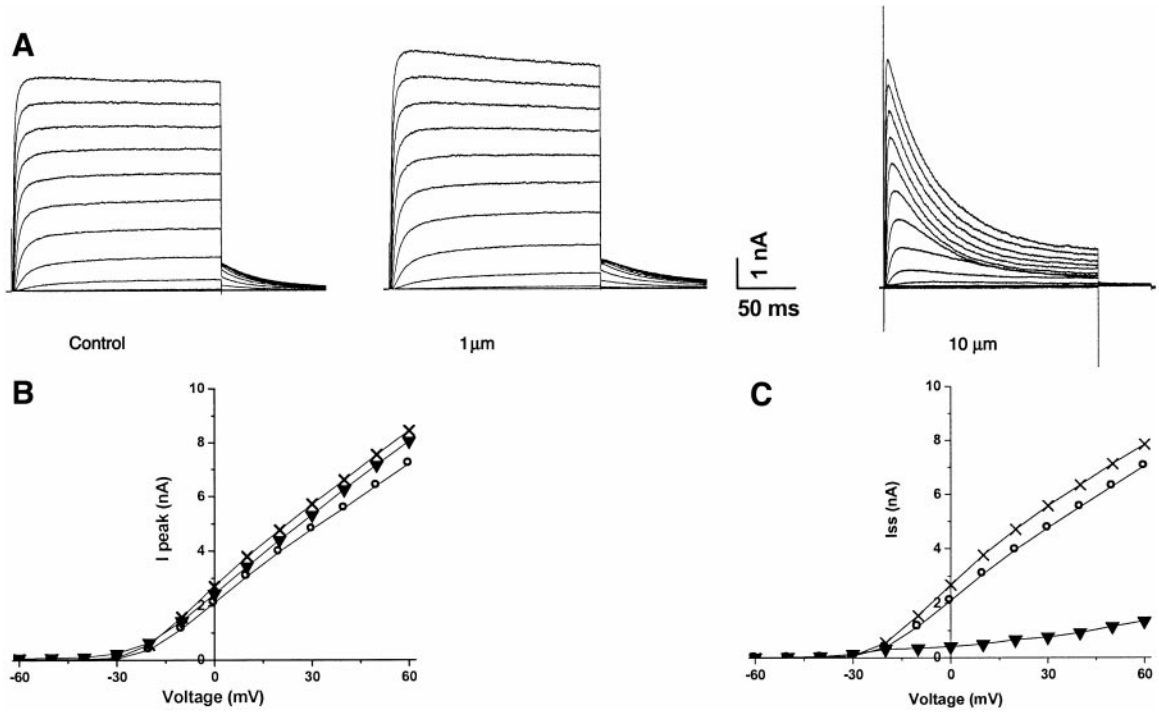

Fig. 6. Current-voltage (*I-V*) relationships for effects of LA on Kv1.5. *I-V* plots were constructed by measuring peak (measured during the first 50 ms after the voltage step) or late current (average of the last 10 ms of current flowing during the voltage step) and plotting those values against membrane potential. *A*: control vs. 1 and 10 μM LA in family of Kv1.5 currents. Note increase in current at 1 μM and time-dependent nature of the block at 10 μM. *B* and *C*: *I-V* plots of peak (I_{peak}) and late (steady-state) currents (I_{ss}), respectively. ○, Control; ×, 1 μM; ▼, 10 μM.

after addition of LA to the bath solution; and *2*) both processes returned to control values with the same time course during the washout phase. These two observations were also the case for experiments when there was no LA in the patch pipette solution.

To address the potential mechanism of action and to explain these apparently different effects of LA, we examined several properties of LA action. We first investigated the voltage dependence of LA block by generating current-voltage (*I-V*) curves (Figs. 6 and 7). Figure 6*A* shows a representative example of the effects of 1 and 10 μM LA on a family of Kv1.5 currents compared with control. The *I-V* relationships (Fig. 6, *B* and *C*) are for the peak currents (measured during the first 50 ms) after the voltage step (Fig. 6*B*) and for the late currents, measured as the average of the last 10 ms of the voltage step (Fig. 6*C*). Even at 10 μM LA, where there was considerable block of the steady-state currents, there was no indication that the effects of LA were voltage dependent. Figure 7 illustrates a representative example of the effects of 1 and 5 μM LA on a family of Kv2.1 currents compared with control. As observed for Kv1.5, changes in the peak and late currents (Fig. 7, *C* and *D*, respectively) between control and LA treatments do not show evidence of voltage-dependent block, although block is evident at both doses. A plot of the fractional currents, f ($I_{LA}/I_{control}$), against voltage (not shown) confirmed the absence of voltage dependence to the current block induced by LA on Kv1.5 or Kv2.1 currents, implying that the site of inhibition is located outside the membrane electric field.

Next, we investigated the effects of LA on the characteristics of current activation by measuring the instantaneous activation curves derived from an analysis of tail currents in the presence and absence of LA (Fig. 8). Tail currents were measured at −40 mV after first being stepped from −60 to 60 mV in 10-mV increments from a constant holding potential of −80 mV. In control and LA-treated cells, a single-exponential function was adequate to describe the current deactivation, suggesting that no additional open or blocked state was associated with the effects of LA on these channels. This function was extrapolated to *time 0*, where the current amplitude was measured. After normalization, the *time 0* current amplitudes were plotted against the eliciting voltage step, and the result was fit with a Boltzmann equation of the form $I_{norm} = 1/\{1 + \exp[(V_{0.5} - V_h)/V_s]\}$, where $V_{0.5}$ is the voltage at which I_{norm} (normalized current) = 0.5, V_h is the holding potential, and V_s is the slope factor. LA at 1 and 5 μM shifted the $V_{0.5}$ of Kv1.5 to the left by −5.9 and −17 mV, respectively, compared with control. In a similar fashion, the $V_{0.5}$ of Kv2.1 was leftward shifted, compared with control, by −4.7 and −10.8 mV by 2 and 10 μM LA, respectively. Thus, with different potencies, LA causes a dose-dependent leftward shift in the instantaneous activation curves for Kv1.5 and Kv2.1. Although low doses of LA increased the activation of Kv1.5 without causing current inhibition, the same

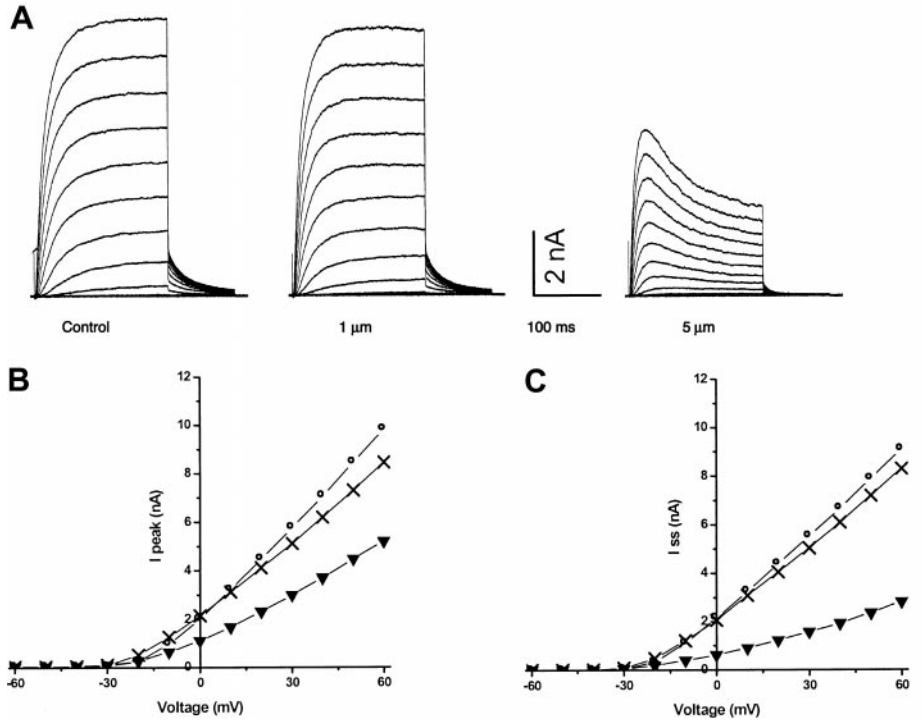

Fig. 7. *I-V* relationships for effects of LA on Kv2.1. *I-V* plots were constructed by measuring peak or late currents (average of the last 10 ms of current flowing during the voltage step) and plotting those values against membrane potential. *A*: control vs. 1 and 5 μM LA in a family of Kv1.5 currents. *B* and *C*: *I-V* plots of peak and late currents, respectively. ○, Control; ×, 1 μM; ▼, 5 μM.

was not true for Kv2.1, where the onset of current inhibition was concomitant with the increase in the speed of activation. To further examine the hypothesis that LA may have two separate mechanisms by which it exerts its effects on Kv1.5 and Kv2.1, we tested a derivative of LA, where the carboxylic acid of LA was replaced by a methyl ester group [LA methyl ester (LAME)]. Traditionally, separate sites of action of a modulator may be revealed by small alterations in the molecule. To this end, the effects of LAME on current levels and the activation properties of Kv1.5 and Kv2.1 were examined. Figure 9 shows representative current recordings of the effects of LAME on Kv1.5 and Kv2.1. We found that 1 μM LAME increased the speed of current activation and the current magnitude of Kv1.5 (Fig. 9*A*) and Kv2.1 (Fig. 9*B*), while the 20 μM dose of LAME increased the speed of activation of Kv1.5 and Kv2.1 with only a small of amount of current inhibition. Apparently, the carboxyl group of LA is critical to the development of current inhibition in Kv1.5 and Kv2.1 but does not participate in alterations of channel activation. These results further support the idea that LA alters the activation properties and induces current inhibition by two separate mechanisms.

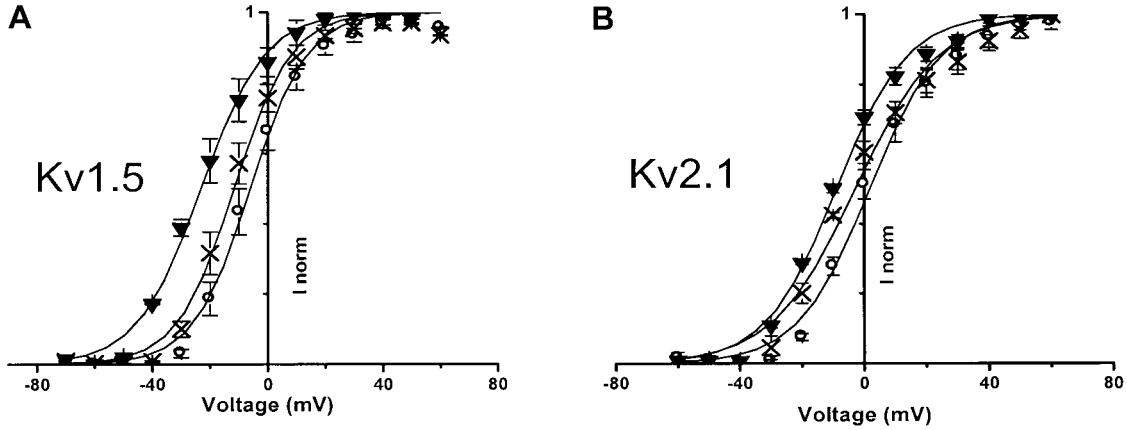

Fig. 8. Instantaneous activation curves derived from tail current analyses of Kv1.5 and Kv2.1 before and after treatment with LA. LA caused a dose-dependent leftward shift in activation curves. Tail currents were fitted with single-exponential equations extrapolated to *time 0,* where amplitudes of the tail currents were measured. A Boltzmann equation (see RESULTS) was fitted to data and used to determine half-maximal activation voltage ($V_{0.5}$). *A*: 1 and 5 μM LA caused a leftward shift in $V_{0.5}$ of the instantaneous activation curve of human Kv1.5 (5.9 and 17 mV, respectively) compared with control ($V_{0.5} = -6$ mV). ○, Control; ×, 1 μM; ▼, 5 μM. *B*: 2 and 10 μM LA also caused a leftward shift in $V_{0.5}$ of the instantaneous activation curves of human Kv2.1 (4.7 and 10.8 mV, respectively) compared with control ($V_{0.5} = 1.8$ mV). ○, Control; ×, 2 μM; ▼, 10 μM.

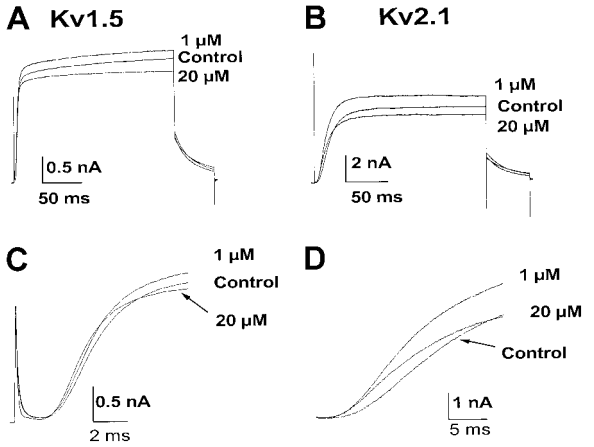

Fig. 9. LA methyl ester (LAME) alters activation kinetics of Kv1.5 and Kv2.1 without substantial current block. Cells were voltage clamped as described in Fig. 1 legend. *A*: 1 and 20 μM LAME accelerated activation kinetics of Kv1.5; 1 μM LAME increased peak and steady-state current, while 20 μM LAME caused a small reduction in current (see Fig. 1*A*) compared with the same dose of LA. *B*: 5 μM LAME increased the peak current of Kv2.1 and accelerated activation kinetics of Kv2.1, while 20 μM LAME produced a small reduction in current compared with control. *C*: expanded time course of the activation phase of Kv1.5 in *A*. *D*: expanded time course of the activation phase of Kv2.1 in *B*.

DISCUSSION

We have shown that LA induces dose-dependent changes in the peak and steady-state currents, time to peak current, and steady-state activation curves of two voltage-dependent K^+ channels, Kv1.5 and Kv2.1. In agreement with other reports (5, 9, 16, 17) on the effects of PUFFAs on voltage-dependent K^+ channels, the site of action was on the extracellular side of the channel, since the addition of LA to the contents of the patch pipette was uniformly without effect on Kv1.5 or Kv2.1.

Our data suggest that the increase in the speed of activation was due to a reduction in the energy necessary for the channels to open (i.e., the $V_{0.5}$ for channel opening was leftward shifted) and that, once opened, the Kv1.5 channel, but not the Kv2.1 channel, deactivated more slowly. A similar shift in the activation voltage was noted for the effects of arachidonic acid (AA) on Kv1.5 (9) and Kv.1.1 (7, 8), and a leftward shift in the activation voltage was also reported when docosahexaenoic acid was applied to Kv1.2 (17).

With respect to the mechanism of action of LA on the K^+ channels that we have studied, our results suggest two distinct mechanisms of action. Like others who have reported on the effects of various PUFFAs on delayed rectifier channels (5, 8, 9, 16, 17), we found that LA caused a dose-dependent inhibition of Kv1.5 and Kv2.1 currents. The inhibition of Kv1.5 and Kv2.1 current by LA was time dependent (i.e., block accumulated during the voltage step) but without obvious voltage dependence. The time course of the inhibition of Kv1.5 and Kv2.1 was well fit by a single-exponential function (data not shown). Taken together, our findings lead us to conclude that LA causes open channel block of Kv1.5 and Kv2.1. A similar conclusion was reached for the effects of AA on Kv1.5 (9) and docosahexaenoic acid on Kv1.2 and Kv3.2 (17).

Our comparative dose-response data for current activation and current block clearly show that the effects of LA on Kv1.5 and Kv2.1 could be separated into two effects: current activation and time-dependent current block (Figs. 5 and 6). The values for EC_{50} and slope (2.4 μM and 1.1, respectively, for Kv1.5; 2.7 μM and 1.9, respectively, for Kv2.1) for the acceleration of activation and the values for IC_{50} and slope (6.6 μM and 3.5, respectively, for Kv1.5; 7.4 μM and 3.9, respectively, for Kv2.1) for current inhibition were different. The fact that we were able to isolate the changes in the activation time course from current block using LAME adds substantially to our hypothesis that the current activation and current inhibition induced by LA are separate (and separable) effects. Others have reported that although AA induced current block, the methyl ester of AA was without effect (7, 8, 17), suggesting that the carboxylic acid moiety is responsible for current block. Interestingly, Gubitosi-Klug and Gross (7) also reported that whereas AA methyl ester was without effect on Kv1.1, the ethyl, propyl, and butyl esters of AA enhanced the speed of current activation without the time-dependent block seen with AA alone. They concluded that AA and fatty acid ethyl esters interacted with Kv1.1 at independent sites.

Although much effort in understanding the voltage dependence of activation in K^+ channels has focused on the transmembrane domains, especially S4 (see Ref. 12 for review), Swartz and MacKinnon (18) demonstrated that the effects of hanatoxin on the activation kinetics of drk1 (Kv2.1) could be reduced by mutations in the S3-S4 linker region. With the use of *Shaker*, it was reported (6) that the activation time course of this channel was slowed as increasing numbers of residues were deleted from the S3-S4 region of this channel. Thus we do not consider it unlikely that LA can interact with a putative activation domain located on an extracellular loop in such a way as to increase the speed of activation.

The steepness of the Hill plots for Kv1.5 and Kv2.1 (n = 3.4 and 3.9, respectively) suggests a large amount of cooperativity in the current blocking effects of LA on these channels and supports the ideas that multiple molecules of LA are needed to cause channel block. The corresponding slopes of the activation curves of Kv1.5 and Kv2.1 (1.1 and 1.9, respectively) indicate that little cooperatively is required to alter current activation. There are several possible interpretations for the effects we have observed. One explanation is that there may be a single binding site for LA on each of the four subunits, and it may take only a single molecule of LA binding to a single subunit to begin to alter the activation properties of Kv1.5 and Kv2.1. As successive molecules of LA bind to a channel, a blocking particle is created via the cooperative interaction of multiple carboxylic acid groups. In this interpretation, the carboxylate ends of LA become oriented toward the channel pore and coordinate or trap K^+ ions exiting the pore.

A second explanation for our observations would be the existence of two separate binding sites for LA: one site for activation and the other for channel block. In this view, binding of LA to the higher-affinity site would affect the activation properties, and the carboxylic acid groups would act as tethered blocking particles near or in the mouth on the external face of the channels. Our data on the different affinities for the two processes support both hypotheses, as does our observation that LAME is able to remove or diminish current block, with little or no effect on the acceleration of activation. Further experiments involving the creation of mutant channels may be necessary to resolve these questions.

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