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Fatty Acid Block of the Transient Outward Current in Adult Human Atrium[1]

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ABSTRACT

Fatty acids represent an essential source of fuel for the heart and play an important role in the mechanical, electrical, and synthetic activities of cardiac cells. Under pathological conditions, such as ischemia followed by reperfusion, the myocardium is exposed to very high levels of fatty acids, in particular the monounsaturated fatty acid, oleic acid. Elevated plasma fatty acids have been linked to an increased risk for cardiac arrhythmias. In other species, fatty acids have been shown to modulate several cardiac ion channels, most notably potassium channels. Virtually nothing is known about the actions of oleic acid on potassium channels in human heart. We therefore characterized the effects of oleic acid on the transient outward current, sustained current, and inwardly rectifying current, some of the major potassium channels present in human atrium, using the whole-cell patch clamp method. Exposure of cells to oleic acid (5 μM) reduced the transient outward potassium current to 3.7 $\pm$ 0.8 pA/pF (n = 4) compared with 7.0 $\pm$ 0.7 pA/pF (n = 4) ($P < .05$) for cells not exposed. In contrast, oleic acid had little effect on either the sustained current (4.3 $\pm$ 0.3 pA/pF, n = 4 for oleic acid versus 4.8 $\pm$ 0.5, n = 5 for control) present after the decay of the transient outward current or on the amplitude of I_{K1} measured at -100 mV (1.4 $\pm$ 0.4 pA/pF, n = 4 for oleic acid versus 1.3 $\pm$ 0.4 pA/pF, n = 6 for control). In addition, oleic acid significantly slowed the rate of recovery of the transient outward current, which is predicted to result in a use-dependent reduction in current amplitude in the beating heart. These results suggest a possible contributing role for oleic acid block of the transient outward current in the pathological consequences of myocardial ischemia.

Voltage-gated potassium channels (K channels) play a crucial role in determining the resting potential, shape, and duration of the cardiac action potential. In human atrium, the transient outward K current (I_{to}), sustained current (I_{sus}), and inwardly rectifying K current (I_{K1}) represent the major inward and outward K currents and thus play an important role in determining the morphology of the action potential in human atrial myocytes (Escande et al., 1985; Shibata et al., 1989; Crumb et al., 1995).

Previous studies have shown that the physiological and pharmacological properties of K channels can be modulated by their lipid environment (Kim and Clapham, 1989; Rouzaire-Dubois et al., 1991; Kirber et al., 1992; Honore et al., 1994). A role for fatty acid regulation of K channels has been shown for K channels in neuroblastoma cells (Rouzaire-Dubois et al., 1991), the Ca-activated K channel in vascular smooth muscle (Kirber et al., 1992), the arachidonic acid-activated K channel in cardiac cells (Kim and Clapham, 1989), and the transfected Kv1.5 channels (Honore et al., 1994), a K channel cloned from human heart, which is believed to underlie a portion of I_{sus} recorded from human atrium.

Fatty acids represent an essential source of fuel for the heart and play an important role in the mechanical, electrical, and synthetic activities of cardiac cells. Under pathological conditions such as ischemia followed by reperfusion, the myocardium is exposed to very high levels of fatty acids, in particular the monounsaturated fatty acid, oleic acid (Svensson et al., 1990; Charnock, 1994; Finegan, 1994; Lopaschuk et al., 1994; al Makdessi et al., 1995). To date, nothing is known about the modulation of K channels in human heart by fatty acids. We therefore characterized the effects of oleic acid on some of the major K channels present in human atrium, I_{to}, I_{sus}, and I_{K1}.

Materials and Methods

Isolation of Cardiac Myocytes. Human myocytes were obtained from specimens of human right atrial appendage obtained during surgery from hearts of patients undergoing cardiopulmonary bypass for coronary artery disease (CAD). Tissue was obtained during the routine cannulation of the right atrial appendage for creation

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ABBREVIATIONS: I, current; K channels, potassium channels; I_{to}, transient outward K current; I_{K1}, inwardly rectifying K current; I_{sus}, sustained current; CAD, coronary artery disease.

of the extracorporeal bypass circuit and was obtained in accordance with Tulane University School of Medicine Institutional Guidelines. All atrial specimens were described as grossly normal at the time of excision. The cell isolation procedure has been described previously (Crumb et al., 1995). Briefly, atrial samples were quickly immersed in a cardioplegia solution consisting of 50 mmol/l KH_2PO_4, 8 mmol/l $MgSO_4$, 10 mmol/l $NaHCO_3$, 5 mmol/l adenosine, 25 mmol/l taurine, 140 mmol/l glucose, and 100 mmol/l mannitol, titrated to a pH of 7.4 and bubbled with 100% O_2 at 0 to 4°C. Specimens were minced into 0.5-mm to 1.0-mm cubes and transferred to a 50-ml conical tube containing an ultralow calcium wash solution containing 137 mmol/l NaCl, 5 mmol/l KH_2PO_4, 1 mmol/l $MgSO_4$, 10 mmol/l taurine, 10 mmol/l glucose, 5 mmol/l HEPES, and 100 μM EGTA; pH = 7.4 (22–24°C). The tissue was gently agitated by continuous bubbling with 100% O_2 for 5 min. Next, the tissue was incubated in 5 ml of solution containing 137 mmol/l NaCl, 5 mmol/l KH_2PO_4, 1 mmol/l $MgSO_4$, 10 mmol/l taurine, 10 mmol/l glucose, and 5 mmol/l HEPES, supplemented with 0.1% bovine albumin, 2.2 mg/ml collagenase type V, and 1.0 mg/ml protease type XXIV (Sigma Chemical Co., St. Louis, MO), pH = 7.4 (37°C), and bubbled continuously with 100% O_2. The supernatant was removed after 40 min and discarded. The chunks were then incubated in a solution of the same ionic composition but supplemented with only collagenase and 100 μM $CaCl_2$. Microscopic examination of the medium was performed every 10 to 20 min to determine the number and quality of the isolated cells. When the yield appeared to be maximal, the cell suspension was centrifuged for 2 min, and the resulting pellet was resuspended in a modified Kraftbruhe solution (Anumonwo et al., 1990) containing 25 mmol/l KCl, 10 mmol/l KH_2PO_4, 25 mmol/l taurine, 0.5 mmol/l EGTA, 22 mmol/l glucose, 55 mmol/l glutamic acid, and 0.1% bovine albumin, pH = 7.3 (22–24°C). In general, the isolation procedure produced an initial yield of approximately 40% to 60% rod-shaped, calcium-tolerant cells. Cells were used within 8 h after isolation.

Solutions. All fatty acids (99% purity) were purchased from Sigma Chemical Co. The critical micelle concentration for oleic acid ranges from 0.7 to 3.5 mM (Murakami et al., 1986), indicating that the concentrations of oleic acid used in this study would not form micelles. When recording from human myocytes, cells were perfused with an "external" solution that consisted of 137 mmol/l NaCl, 4 mmol/l KCl, 1 mmol/l $MgCl_2$, 1.8 mmol/l $CaCl_2$, 11 mmol/l glucose, 10 mmol/l HEPES; adjusted to a pH of 7.4 with NaOH. Glass pipettes (electrodes) were filled with an "internal" solution that consisted of 120 mmol/l K-aspartate, 20 mmol/l KCl, 4 mmol/l Na-ATP, 5 mmol/l EGTA, 5 mmol/l HEPES; adjusted to a pH of 7.2 with KOH. Experiments were performed in the presence of 200 μM Cd^{2+} to block L-type calcium channels. All experiments were performed at room temperature (22–23°C).

Data Acquisition and Analysis. Acceptable atrial myocytes were rod-shaped and lacked any visible blebs on the surface. Currents were measured using the whole-cell variant of the patch clamp method (Hamill et al., 1981). Pipette tip resistance was approximately 1.0–2.0 MΩ when the pipettes were filled with the internal solution. Experiments were performed in cells in which the estimated voltage drop across the uncompensated series resistance was less than 3 mV.

For exponential fits of data (e.g., fits of time course of current decay and I_{to} recovery from inactivation), a single exponential fit was accepted as the fit of choice whenever the following criteria were met: 1) the amplitude parameters obtained from the least-squares fit were all of the same sign; and 2) a negative value for the asymptotic information criteria statistic was obtained when comparing a one- versus a two-exponential fit (Akaike, 1974; Horn, 1987). An unpaired Student's *t* test or ANOVA was used for statistical analysis. Data are presented as mean ± S.E.M.

In a preliminary series of experiments, it was noticed that there were time-dependent shifts in the voltage-dependence of I_{to} activation and inactivation parameters. For instance, the midpoints (V_{mid}) for steady-state activation and inactivation obtained from experiments performed approximately 5 min after entering the whole-cell mode were 21.8 ± 1.9 mV and −22.9 ± 1.3 mV (n = 8–10), respectively. However, after approximately 20 min there was a significant ($p < .05$) hyperpolarizing shift in the voltage dependence of activation (V_{mid} = 7.3 ± 1.5 mV) and inactivation (V_{mid} = −32.7 ± 1.7 mV) (n = 6). To avoid misinterpreting time-dependent shifts in I_{to} gating and changes in amplitude as effects of oleic acid exposure, group comparisons were made between cells exposed (pretreated with fatty acid for 20 min before starting experiment) and cells not exposed to fatty acids at an isochronal time point (approximately 5 min after entering whole-cell mode). The same protocol was used for the study of the congeners of oleic acid, oleic acid methyl ester, and stearic acid as well as the effects of these fatty acids on I_{sus} and I_{K1}.

For statistical analysis, the number (n) of atrial preparations used for a particular experiment is given. Results obtained from multiple cells from the same atrial preparation were averaged and counted as n = 1.

Human Atrial Specimens. Myocytes were obtained from the right atrial appendages of 21 adult patients between 45 and 69 years of age (see Table 1). Tissue was considered free from significant pathology if the following criteria were met: 1) the tissue appeared grossly normal upon removal; 2) there was no evidence of right atrial enlargement (e.g., by P-wave amplitudes greater than 2.5 mm or by examination on echocardiogram).

Results

Figure 1A illustrates the effects of oleic acid on the transient outward potassium current (I_{to}), sustained current (I_{sus}) remaining after the decay of I_{to}, and the inwardly rectifying potassium current (I_{K1}) recorded from a human atrial myocyte. As illustrated, in the presence of 5 μM oleic acid (see *Materials and Methods*), I_{to} amplitude was dramatically reduced, while the amplitudes of I_{sus} and I_{K1} were not changed. Exposure of cells to oleic acid (5 μM) produced no change in the amplitude of I_{sus} measured at the end of an 800 ms voltage pulse to +60 mV (I_{sus}) (4.3 ± 0.3 pA/pF, n = 4 for oleic acid versus 4.8 ± 0.5, n = 5 for control), or in the amplitude of I_{K1} measured at −100 mV (1.4 ± 0.4 pA/pF, n = 4 for oleic acid versus 1.3 ± 0.4 pA/pF, n = 6 for control) (see *Materials and Methods* regarding sample number). To more clearly see the effects of drug on I_{to}, steady-state current was

TABLE 1
Patient population characteristics

Diagnosis	Age	Sex	Medications
	yr		
CAD	51	Male	Digoxin, nifedipine
CAD	56	Male	None
CAD	58	Male	Digoxin, amiodarone
CAD	61	Male	Diltiazem
CAD	62	Male	None
CAD	64	Male	Digoxin
CAD	68	Female	Metoprolol
CAD	45	Male	Digoxin, nifedipine
CAD	57	Female	None
CAD	51	Male	Digoxin, amiodarone
CAD	69	Male	Metoprolol, nifedipine
CAD	62	Female	None
CAD	64	Male	Digoxin
CAD	66	Male	Digoxin
CAD	50	Male	Digoxin, nifedipine
CAD	49	Male	None
CAD	48	Male	Digoxin, nicardipine
CAD	64	Male	Digoxin
CAD	61	Female	None
CAD	63	Male	Digoxin
CAD	68	Female	Digoxin

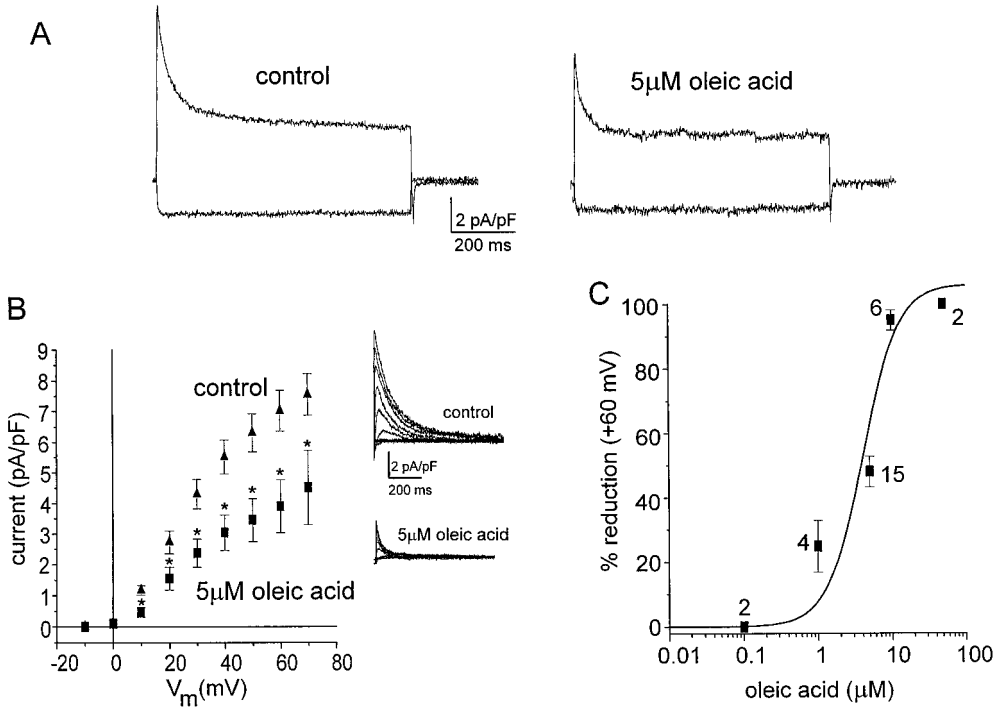


Fig. 1. Effects of oleic acid (5 μM) on an isolated adult human atrial myocyte. A, currents were elicited in response to an 800 ms voltage pulse to +60 mV (upper current trace) and −100 mV (lower current trace) from a holding potential of −40 mV. Currents are shown from cells exposed and not exposed to oleic acid. B, family of I_{to} currents elicited by a series of 800 ms voltage pulses from −10 to +70 mV from a holding potential of −40 mV (0.2Hz). Steady-state current has been subtracted from peak current. Current/voltage relationship for cells in the presence and absence of oleic acid is also shown. Values have been normalized to cell capacitance (pA/pF) (n = 4 atrial preparations). Asterisks indicate value was significantly different from cells not exposed to oleic acid. C, dose-response curve for I_{to} block by oleic acid. I_{to} was elicited by a voltage pulse to +60 mV. Numbers indicate number of cells tested at each concentration. Dose-response relationship was fit with a Hill equation. Symbols represent mean ± S.E.

subtracted from peak current amplitude (Fig. 1B). In the absence of drug, I_{to} amplitude measured at +60 mV was 7.0 ± 0.7 pA/pF (n = 4). In cells exposed to 5 μM oleic acid, the amplitude of I_{to} measured at +60 mV was reduced approximately 2-fold to 3.7 ± 0.8 pA/pF (n = 4). The current-voltage relationship for I_{to} is shown in Fig. 1B and indicates a significant reduction in I_{to} amplitude at potentials positive to threshold (0 mV) in cells exposed to 5 μM oleic acid ($P <$ .01). A fit of the mean dose-response relationship for oleic acid indicates an inhibition of I_{to} with an IC_{50} of 4.1 μM (Fig. 1C).

To determine whether the oleic acid-induced decrease in I_{to} density was the result of a shift in the voltage dependence of inactivation and/or activation, the steady-state activation and inactivation parameters of I_{to} were characterized in control cells and in cells exposed to oleic acid (Fig. 2). The relationship between prepulse potential and tail current amplitude (Fig. 2A), which defines the voltage dependence of I_{to} activation, could be well described by a Boltzmann distribution of the form: $I = I_{max}/\{1 + \exp[(V_{0.5} - V_m)/k]\}$, where I is tail current amplitude at a given prepulse potential, I_{max} is the maximum current amplitude at positive potentials, $V_{0.5}$ is the voltage at half-maximal activation, V_m is the membrane potential, and k is the slope factor. When comparing mean values for $V_{0.5}$ and k between cells exposed ($V_{0.5}$ = 22.9 ± 1.8 mV, k = 8.8 ± 1.3 mV, n = 4) and not exposed ($V_{0.5}$ = 22.3 ± 5.3 mV, k = 7.9 ± 1.5 mV, n = 4) to oleic acid, no significant differences were found. A two-pulse protocol was used to define the voltage dependence of steady-state inactivation (Fig. 2B). The $V_{0.5}$ for steady-state inactivation obtained from Boltzmann fits was −22.8 ± 2.4 mV (n = 4) for cells exposed to oleic acid and −19.8 ± 2.1 mV (n = 4) for control cells (P not significant). However, k was significantly different with values of 8.7 ± 0.9 mV and 5.6 ± 0.6 mV for cells exposed to oleic acid and control cells, respectively ($P <$ .05).

As illustrated in Fig. 1B, in cells exposed to oleic acid, I_{to} decayed more rapidly compared with control cells. To define the effects of oleic acid on current decay, the voltage-dependence of decay was examined. Figure 3A illustrates examples of the decaying portion of I_{to} elicited by voltage pulses from a holding potential of −40 mV to +20 mV, +40 mV, and +60 mV in a cell exposed to 5 μM oleic acid. The smooth lines through the current traces are fits to a single exponential function. At voltages between +20 mV and +60 mV, current decay in cells exposed to oleic acid was significantly faster than in control cells (Fig. 3B). There was no obvious voltage dependence for the time constant of current decay over this voltage range for either control or oleic acid exposed cells (Fig. 3B).

To further characterize the voltage dependence of oleic acid block, the relative current $I_{oleic}/I_{control}$ was plotted as a function of voltage (Fig. 4). Block increased steeply between 0 mV and +20 mV, coinciding with the voltage dependence of channel opening. At potentials positive to +40 mV, where channel opening has reached an apparent steady state, block exhibited no clear voltage dependence.

The effects of oleic acid on the time course of I_{to} recovery were also defined. As illustrated in Fig. 5, the time course of recovery of I_{to} was significantly slower in cells exposed to oleic acid (237.5 ± 42.5 ms, n = 4) compared with cells not exposed to oleic acid (136.9 ± 13.2 ms, n = 4) ($P <$ .05). This

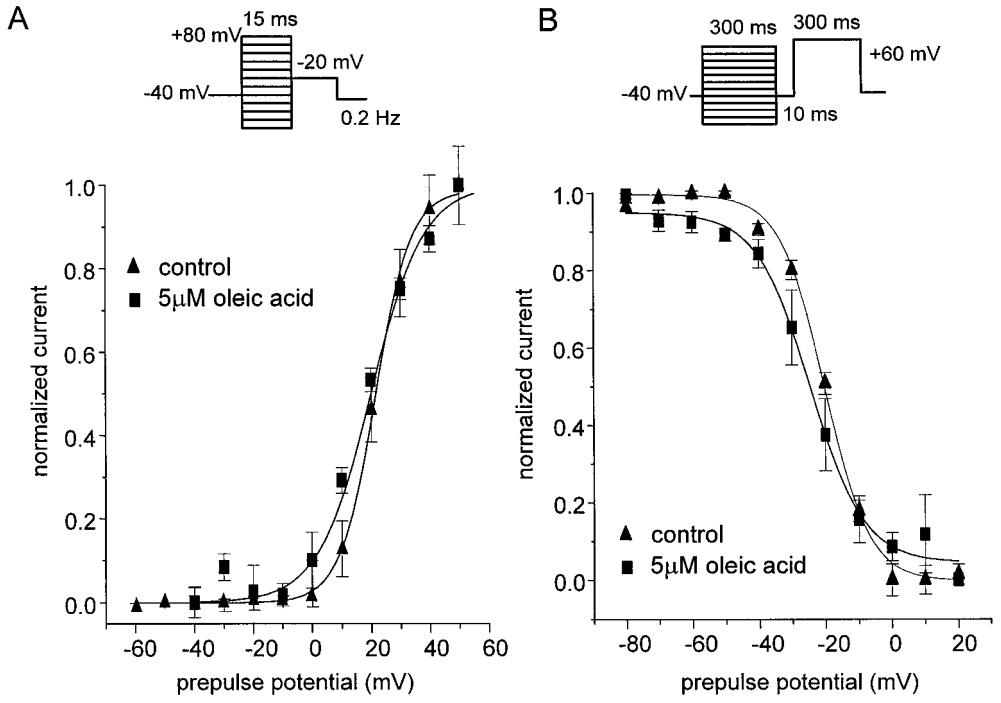


Fig. 2. Voltage dependence of steady-state activation and inactivation for control and oleic acid-exposed cells. A, steady-state activation elicited by illustrated voltage protocol. Upon repolarization to −20 mV, tail currents were observed that were taken as a measure of activation of the transient outward current. These values were normalized to the largest tail current and plotted as a function of prepulse potential. Values indicate mean ± S.E.M. Curves shown are fits of mean data to a Boltzmann distribution. B, steady-state inactivation relationship. Voltage protocol is shown above. Values measured at +60 mV were plotted as the ratio of current at the respective potential to the largest test current. Values indicate mean ± S.E.M.

oleic acid-induced slowing of I_{to} recovery kinetics would be expected to result in a greater use-dependent reduction in I_{to} amplitude at faster heart rates.

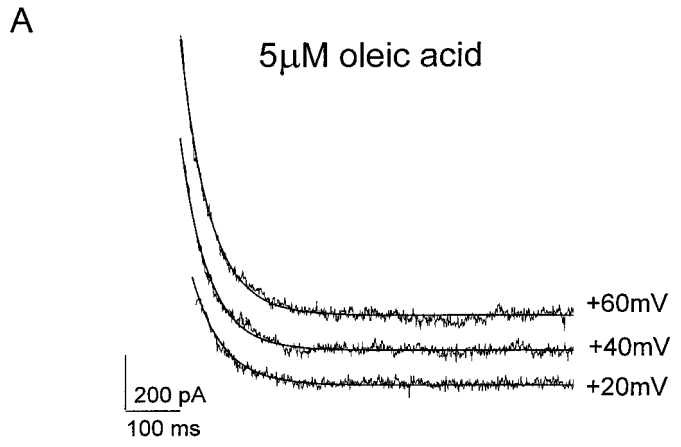
Because oleic acid has been shown to be an activator of protein kinase C (Murakami et al., 1986; Khan et al., 1993), a series of experiments were performed to determine whether the blockade of I_{to} was mediated by protein kinase C. For these experiments, cells were bathed in the standard external solution (see *Materials and Methods*) to which 100 nM staurosporine was added. After exposure to 10 μM oleic acid, the reduction in the amplitude of I_{to} (92.5 ± 6.7%, n = 3) was similar to that observed in cells not exposed to staurosporine (95.5 ± 3.8%, n = 4), suggesting that oleic acid's ion channel blocking properties are not mediated by protein kinase C activation.

The structural features required to block I_{to} were examined by comparing the effects of oleic acid with two of its congeners, oleic acid methyl ester and stearic acid. Whereas 10 μM oleic acid produced near maximal inhibition of I_{to}, at similar concentrations, the esterified (oleic acid methyl ester) and the saturated (stearic acid) forms were without effect (Fig. 6). As illustrated, oleic acid (10 μM) virtually abolished I_{to}, while having no effect on I_{sus}. Oleic acid methyl ester and stearic acid had no effect on either current. Similar results were obtained in four additional experiments. These findings suggest that the double bond and the hydroxyl group present in oleic acid are important features in the blocking properties of oleic acid.

Discussion

Elevated plasma fatty acids have been linked to an increased risk for cardiac arrhythmias (Oliver et al., 1968; McLennan, 1993; Charnock, 1994). Previous studies have shown activation and/or inhibition of ion channels in a variety of cell types by several fatty acids including oleic acid (Hwang et al., 1990; Kim and Duff, 1990; Rouzaire-Dubois et al., 1991; Honore et al., 1994). The present study is the first to demonstrate the fatty-acid block of an ion channel in human cardiac myocytes. Oleic acid selectively blocked I_{to} and dramatically slowed I_{to} recovery kinetics in human atrial myocytes, while having no effect on either I_{sus} or I_{K1}.

Because oleic acid is lipophylic, it is possible that its ion channel blocking properties are mediated through an indirect, membrane effect. A similar mechanism of action has been proposed for the hydrocarbon block of the sodium current in squid axon (Haydon and Urban, 1983). Haydon and Urban (1983) hypothesized that some of the changes observed in sodium channel parameters in response to hydrocarbon exposure could be explained by a thickening of the nonpolar region of the membrane. Evidence for a thickening of the membrane was given by a decrease in membrane capacitance. In contrast, fatty acids decrease the thickness of cell membranes (Ashcroft et al., 1980), a finding that is supported in the present study by an observed increase in the membrane capacitance of cells exposed to oleic acid (92.5 ± 7.9 pF, n = 7) versus control cells (64.9 ± 4.7 pF, n = 12). Although oleic acid appears to alter the lipid bilayer, the oleic acid block of I_{to} is most consistent with a direct interaction with the I_{to} channels, as both oleic acid methyl ester and stearic acid increased membrane capacitance (77.5 ± 6.9 pF, n = 3 and 92.6 ± 8.9 pF, n = 3, respectively), but neither blocked I_{to} (Fig. 6). Furthermore, oleic acid block was specific for I_{to}; neither I_{K1} nor I_{sus} were blocked. The block produced by oleic acid was consistent with an interaction with the open state of the channel because the block occurred mainly after channel opening (Fig. 1B), and block increased sharply in the voltage range of channel activation (Fig. 4). Interestingly, this block was not voltage dependent (see Fig. 4). Oleic acid is a weak base with a pK_a = 7.8. At a pH of 7.3–7.4, oleic acid exists predominantly in the protonated, uncharged form. The lack of voltage dependence could, therefore, reflect the interaction of the uncharged form at a site within the transmem-



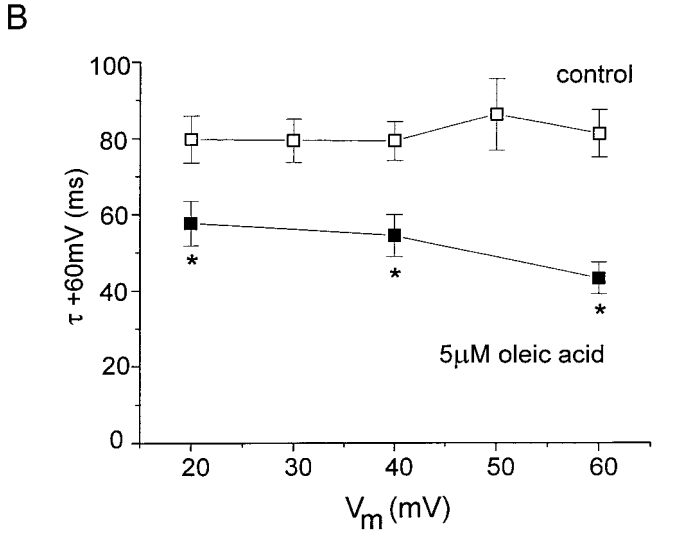


Fig. 3. Voltage dependence of I_{to} decay in response to oleic acid. A, examples of single exponential fits to data obtained at test potentials of +20, +40, and +60 mV from a cell exposed to 5 μM oleic acid. Currents were elicited by 800-ms voltage pulses from a holding potential of −40 mV. Calculated time constants were 61.4 ms (+20 mV), 51.9 ms (+40 mV), and 54.5 ms (+60 mV) for data in A. B, effect of voltage on current decay in control and in oleic acid-exposed cells. Values represent mean ± S.E.M. (n = 4–7). *Value was significantly different from cells not exposed to oleic acid.

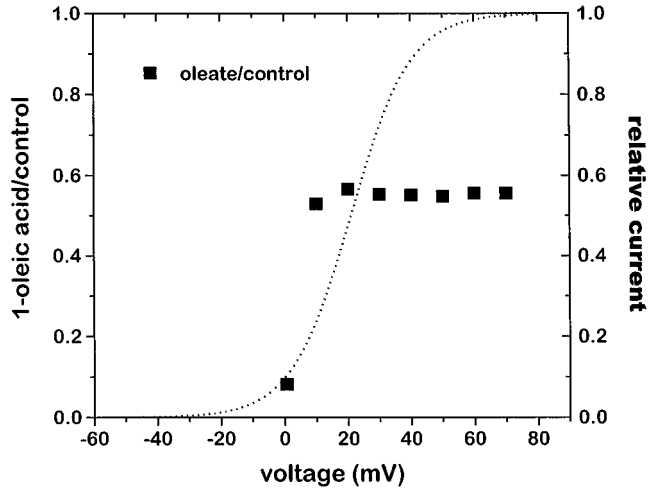


Fig. 4. Voltage dependence of I_{to} block by oleic acid (5 μM). Relative current (oleic acid/control) was obtained from mean data shown in Fig. 1. Mean steady-state activation curve is shown for comparison (fit line). Block increased sharply between 0 mV and +20 mV. Over a voltage range where activation was maximal (positive to +40 mV), there was no voltage dependence to oleic acid block.

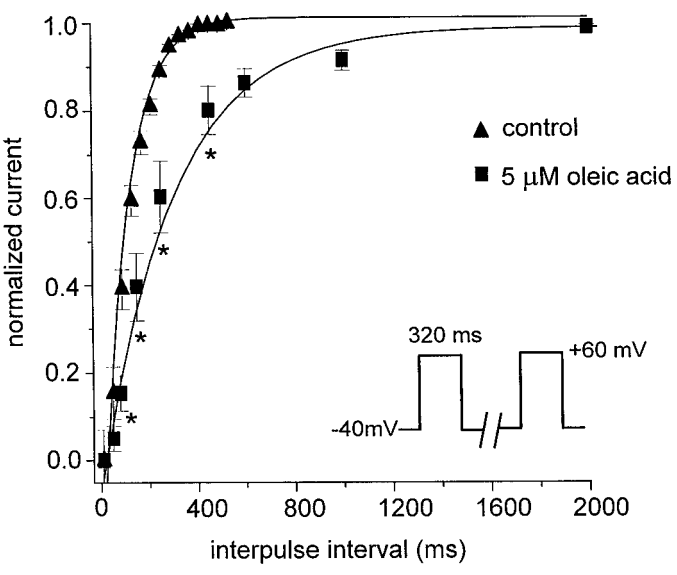


Fig. 5. Slowing of I_{to} recovery kinetics in response to oleic acid. Recovery kinetics for I_{to} are shown in the absence and presence of 5 μM oleic acid. Current was elicited by the protocol shown in inset. Currents recorded during the second voltage pulse to +60 mV were normalized to the maximum current elicited by that voltage pulse. Symbols are mean ± S.E. Values were fit with a single exponential equation. *Value was significantly different from cells not exposed to oleic acid.

brane electric field or an interaction of oleic acid at a site outside the transmembrane electric field. Taken together these data suggest that oleic acid blocks I_{to} by interacting directly with the open state of the channel, most likely through a membrane delimited pathway.

In summary, oleic acid blocks I_{to} and slows the recovery kinetics of I_{to} in human atrial cells. Blockade of I_{to} by oleic acid may be arrhythmogenic, as a reduction in the amplitude of I_{to} in human atrium has been shown to shorten atrial action potential duration (Escande et al., 1985; Shibata et al., 1989). Indeed, a shortening of the action potential duration in atrial myocytes is believed to be a precipitating factor in the genesis of atrial fibrillation (Janse, 1997). These effects of oleic acid may contribute to the pathology observed after ischemia/reperfusion, where the myocardium can be exposed to abnormally high levels of fatty acids. Such an instance occurs during coronary artery bypass surgery, where oleic acid accounts for approximately 40% of the 2- to 3-fold increase in total plasma fatty acid levels (Svensson et al., 1990). Interestingly, block of I_{to} by oleic acid occurs over a concentration range that is predicted to be achieved during and after bypass surgery (1 μM) (Sorrentino et al., 1989). It is intriguing to speculate that blockade of I_{to} in human atrial myocytes may play a role in the incidence of atrial arrhythmias that have been observed in as many as 30% of patients undergoing coronary artery bypass surgery (Fuller et al., 1989; Crosby et al., 1990; Frost et al., 1992, 1995; Chew and Ong, 1993).

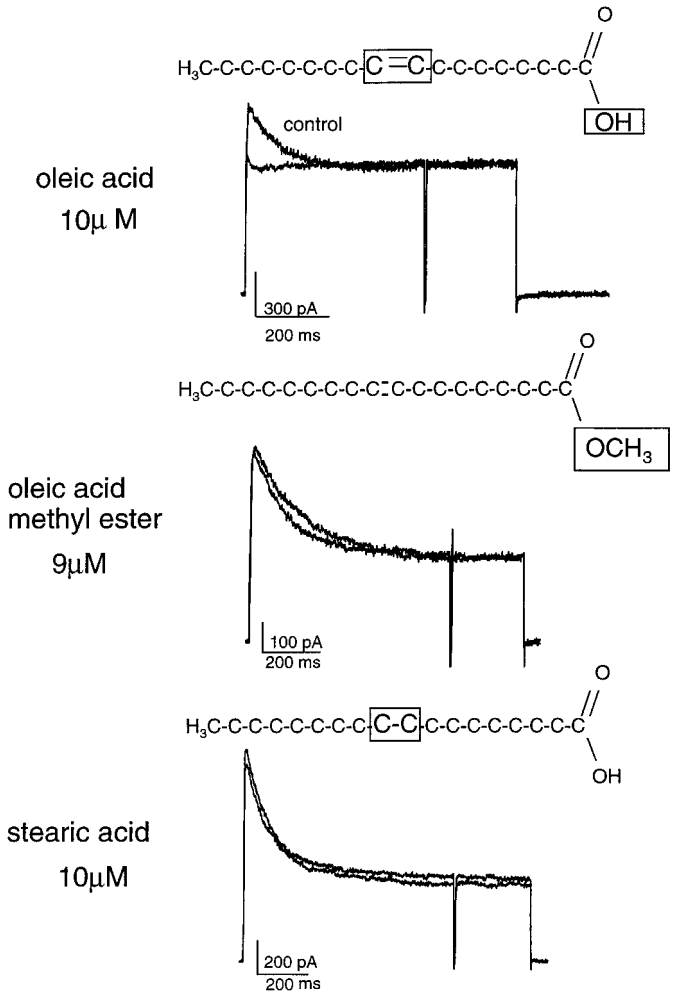


Fig. 6. Structural requirements for oleic acid block of human atrial I_{to}. Currents recorded in the absence and presence of oleic acid (10 μM), oleic acid methyl ester (9 μM), or stearic acid (10 μM). Currents were elicited by a voltage pulse to +60 mV (800 ms), followed by a second voltage pulse to +60 mV after a brief return (4 ms) to the holding potential (−40 mV). Structures of oleic acid and its congeners are shown. Portions of the molecule that are different from oleic acid are enclosed in a box. Note the lack of effect of both oleic acid methyl ester and stearic acid on either I_{to} or I_{sus} current amplitude.

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