Differential Effects of Linoleic Acid Metabolites on Cardiac Sodium Current

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ABSTRACT

9,10-Epoxy-12-octadecenoic acid (EOA), a metabolite of linoleic acid, causes cardiac arrest in dogs. Other metabolites of linoleic acid also have toxic effects. This study investigates the mechanism of action of four of these compounds on cardiac Na⁺ current (I_{Na}). The whole-cell patch-clamp technique was used to investigate the effects of EOA, 9,10-dihydroxy-12-octadecenoic acid (DHOA), and their corresponding methyl esters (9,10-epoxy-12-octadecenoic methyl ester, EOM; and 9,10-dihydroxy-12-octadecenoic methyl ester, DHOM) on I_{Na} in isolated adult rat ventricular myocytes. Extracellular application of each compound elicited a concentration-dependent inhibition of I_{Na}. The dose-response curve yielded 50% inhibition concentrations of 301 ± 117 μM for DHOA, 41 ± 6 μM for DHOM, 34 ± 5 μM for EOA, and 160 ± 41 μM for EOM. Although there was no effect on activation, 50 μM DHOM, EOA, and EOM significantly hyperpolarized the steady-state inactivation curve by approximately −6 mV. Furthermore, EOM significantly increased the slope of the steady-state inactivation curve. These compounds also seemed to stabilize the inactivated state because the time for recovery from inactivation was significantly slowed from a control value of 12.9 ± 0.5 ms to 30.5 ± 3.3, 31.4 ± 1.4, and 20.5 ± 1.0 ms by 50 μM DHOM, EOA, and EOM, respectively. These compounds have multiple actions on Na⁺ channels and that despite their structural similarities their actions differ from each other. The steady-state block of I_{Na} suggests that either the pore is being blocked or the channels are prevented from gating to the open state. In addition, these compounds stabilize the inactivated state and promote increased population of a slower inactivated state.

The role of fatty acids in signaling processes is becoming increasingly important. Various fatty acids have been shown to alter several ion currents, including Na⁺ (Xiao et al., 1995; Kang and Leaf, 1996), Ca²⁺ (Huang et al., 1992; Hashimoto et al., 1999), K⁺ (Bogdanov et al., 1998; Crumb et al., 1999), and Cl⁻ (Ordway et al., 1991). Arachidonic acid (C20:4) has been widely studied for the actions of the parent compound as well as the extensive metabolic pathways, including the prostaglandins and leukotrienes. Although less widely studied, linoleic acid (C18:2) is a major component of cell membrane phospholipids and is subject to some of the same metabolic pathways. A linoleic acid metabolite (LAM) of interest is 9,10-epoxy-12-octadecenoic acid (EOA), also known as leukotxin. EOA is formed by lipid autoxidation in the lungs (Sevanian et al., 1979) and by spontaneous reaction of oxygen radicals with linoleic acid in neutrophil membranes (Hayakawa et al., 1996) (Fig. 1). Although undetectable in normal patients (Hayakawa et al., 1990), EOA has been associated with acute respiratory distress syndrome in burn patients where it can reach plasma concentrations up to 300 μM (mean peak plasma concentration of 99 ± 25 μM) (Kosaka et al., 1994). EOA has also been shown to cause cardiac arrest in dogs (Fukushima et al., 1988) and relax pulmonary artery smooth muscle (Takahashi et al., 1992). In pig experimentally induced cardiac ischemia caused EOA to increase from undetectable levels to on the order of 1 μg/g tissue (Dudda et al., 1996).

Recent studies have questioned whether EOA is the toxic agent involved, because it may be further metabolized by epoxide hydrolases to form 9,10-dihydroxy-12-octadecenoic acid (DHOA) (Moghaddam et al., 1997). Both EOA and DHOA have been reported to be toxic in humans and various animal preparations (Fukushima et al., 1988; Kosaka et al., 1994). During myocardial ischemia in pig hydroxy-metabolites of linoleic acid were found to increase from 1 to 3 μg/g tissue up to 4 to 22 μg/g tissue, depending on the metabolite formed (Dudda et al., 1996). Studies have suggested that EOA must be metabolized to DHOA before producing toxic effects.

ABBREVIATIONS: LAM, linoleic acid metabolite; EOA, 9,10-epoxy-12-octadecenoic acid; DHOA, 9,10-dihydroxy-12-octadecenoic acid; EOM, 9,10-epoxy-12-octadecenoic methyl ester; DHOM, 9,10-dihydroxy-12-octadecenoic methyl ester.
effects (Moghaddam et al., 1997; Greene et al., 2000). In accordance with this evidence, in isolated rat cardiac myocytes 9,10-dihydroxy-12-octadecenoic methyl ester (DHOM) (a methyl ester of DHOA) has been shown to block both Na⁺ current (I_{Na}) and transient outward K⁺ current (I_{to}), whereas 9,10-epoxy-12-octadecenoic methyl ester (EOM) (a methyl ester of EOA) had no significant effect (Stimers et al., 1999).

This study further investigated the effects of EOA, DHOA, and their corresponding methyl esters (EOM and DHOM) on sodium current in adult rat ventricular myocytes. This study was designed to investigate the role of each of these four LAM in blocking Na⁺ channels and identify the possible mechanism of action. A kinetic analysis of the actions of each compound on I_{Na} in adult rat cardiac myocytes using whole-cell patch-clamp techniques was performed. Although it has been suggested that these compounds, being lipid soluble, may have actions on cell membranes that indirectly alter I_{Na}, we show herein for the first time that each compound has direct actions on Na⁺ channels that suggest selective interaction with the channels. A preliminary report of this work was published previously (Harrell and Stimers, 2002).

Materials and Methods

Chemicals. EOA, EOM, DHOA, and DHOM were all synthesized and purified from linoleic acid as described previously (Moran et al., 2000). Ethanol stock solutions were prepared before use and stored at −40°C. Unless otherwise noted, all other chemicals were purchased from either Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Fair Lawn, NJ).

Solutions. The techniques used to isolate and measure I_{Na} were similar to those described previously (Stimers et al., 1999). All intracellular (pipette) solutions contained 120 mM N-methyl-D-glucamine, 1 mM MgCl₂, 0.2 mM CdCl₂, 2 mM BaCl₂, 10 mM HEPES, 5.55 mM dextrose, and 11.69 mM sucrose, pH 7.4. These solutions block all major ion currents other than I_{Na} and reduce the magnitude of I_{Na} so that good voltage control could be maintained in each cell. Linoleic acid metabolites tested were dissolved in ethanol and added to the 30 Na⁺ extracellular solutions. Ethanol concentration did not exceed 0.5%, which had no effect on I_{Na}.

Cell Isolation and Culture. Cardiac myocytes from adult male Sprague-Dawley rats were isolated by Langendorff perfusion of 0.05% collagenase (type II; Worthington Biochemicals, Freehold, NJ), as described previously (Dobretsov et al., 1998). Myocytes were then plated in a KB buffer that contained the following: 85 mM KCl, 5 mM HEPES, 5 mM Tris, 25 mM KH₂PO₄, 0.5 mM EGTA, 5 mM MgSO₄, 5 mM K-pyruvate, 5 mM Na₂-creatine phosphate, 20 mM taurine, 0.1 mM CaCl₂, and 20 mM dextrose, pH 7.4. After allowing cells to attach to the culture dishes, KB buffer was replaced with culture media of M199 (Invitrogen, Carlsbad, CA) with 4% fetal bovine serum and 1% penicillin-streptomycin. Cells were then incubated at 37°C under 95% air, 5% CO₂ for 1 to 4 days until use. As shown in Fig. 2 there was no significant change in the magnitude or voltage dependence of peak I_{Na} over this time in culture.

Patch Clamp. Individual rat myocytes were patch clamped in the whole-cell configuration with an integrating patch clamp (3900A; Dagan, Minneapolis, MN) using standard techniques (Dobretsov and Stimers, 1996; Stimers and Dobretsov, 1998; Dobretsov et al., 1998). Culture dishes were placed on the stage of an inverted microscope (Diaphot; Nikon, Melville, NY) and perfused with solutions at a rate of 0.5 ml/min. Patch pipettes were produced from borosilicate glass using a Flaming-Brown P77 puller (Sutter Instrument Co., Novato, CA). The pipettes were then fire polished to produce pipettes with a final resistance of 1 to 3 MΩ. After forming a seal, the holding potential was set at −100 mV. Upon formation of a giga-ohm seal, the electrode capacitance was compensated. A brief electrical pulse was used to achieve the whole-cell configuration. Cell capacitance, input resistance, and series resistance were measured in every cell by applying a 10-mV pulse. Capacitance and series resistance compensation were used before a voltage-clamp protocol to improve the quality of the voltage clamp. Pulses were generated and collected using pClamp software and a Digidata 1200 computer acquisition system (Axon Instruments, Union City, CA). In all pulse protocols used in this study, a P4/4 protocol was used to subtract capacity.
Cardiac Na\(^+\) Current and Linoleic Acid Metabolites

Fig. 2. Effect of time in culture on voltage dependence of peak \(I_{\text{Na}}\). The data were collected as described in Fig. 3. Peak \(I_{\text{Na}}\) measured for voltage pulses from a holding potential of \(-100\) mV to potentials between \(-80\) and \(+60\) mV in 10-mV increments. Data were collected from two cultures that were examined on all 4 days. There are no differences between peak currents on any day (\(n = 7, 7, 10,\) and 11 on days 1–4, respectively).

Results

Activation. To examine the effect of LAMs on \(I_{\text{Na}}\) activation, \(I_{\text{Na}}\) was measured using whole-cell patch-clamp techniques. Membrane potential was held at \(-100\) mV and pulsed to potentials between \(-80\) and \(+60\) mV in 10-mV increments for 25 ms (Fig. 3). Typical records of Na\(^+\) currents from single cells are shown after exposure to control solution and 50 \(\mu\)M DHOA or DHOM (Fig. 3). In this cell, DHOA had no effect on \(I_{\text{Na}}\), whereas DHOM blocked about 50% of the inward current. Other experiments applied 50 \(\mu\)M EOA or EOM (data not shown). Chemicals were applied for 3 to 5 min before data were collected. Control currents displayed typical activation and inactivation kinetics and voltage dependence for \(I_{\text{Na}}\). Peak current measurements were made in each cell, normalized for cell capacitance, averaged between cells with like treatments, and plotted in Fig. 4. Figure 4A shows that on average in these experiments DHOA had no significant effect on \(I_{\text{Na}}\), at any of the tested potentials; however, 50 \(\mu\)M DHOM significantly inhibited \(I_{\text{Na}}\) at potentials between \(-40\) and \(+30\) mV (\(P < 0.05\)). In contrast, both 50 \(\mu\)M EOA and EOM significantly inhibited \(I_{\text{Na}}\) between \(-30\) and \(+30\) mV (\(P < 0.05\); data not shown for clarity). Figure 4B shows the percentage of inhibition caused by each compound with respect to control measured at \(-30\) mV, the peak of the current-voltage relation. DHOM showed the greatest inhibition of all tested compounds. When DHOM was included in the patch pipette solution, there was no inhibition of \(I_{\text{Na}}\) (data not shown). There was no apparent shift in the voltage dependence for \(I_{\text{Na}}\) activation. Such a shift would indicate that LAMs affected the potentials necessary to activate the sodium channels in rat ventricular myocytes. However, no such shift occurred; therefore, LAMs have no effect on steady-state activation.

Steady-State Inactivation. Steady-state inactivation was measured to examine the effect of EOA, EOM, DHOA, and DHOM. Myocytes were voltage clamped at \(-100\) mV and a two-pulse protocol was applied in control and in the presence of 50 \(\mu\)M of each compound (Fig. 5). Similar to the activation protocol used above, a prepulse was applied from the holding potential to potentials between \(-80\) and \(-10\) mV in 5-mV increments for 20 ms to induce Na\(^+\) channel inacti-
vation. After a 2.5-ms repolarization to −100 mV, a second depolarizing pulse to −10 mV was applied for 10 ms to measure the amount of \( I_{\text{Na}} \) that was available for activation. This protocol is designed to measure the number of \( \text{Na}^+ \) channels not inactivated by the prepulse. Results from a typical experiment are shown in Fig. 5 where the protocol was applied in control conditions and after exposure to 50 μM DHOA and 50 μM DHOM. For clarity pulses are shown in steps of 20 mV; however, data were collected every 10 mV. Cell capacitance was 94 pF. Time scale indicates from the start of data acquisition.

**Recovery from Steady-State Inactivation.** Fig. 7 shows the protocol used and typical results obtained from a single myocyte to measure the rate of recovery from inactivation. A two-pulse protocol was used with both pulses being identical steps from the holding potential of −100 to 0 mV for 10 ms. The interpulse interval was varied from 2 to 142 ms in 10-ms increments to determine the rate at which the \( \text{Na}^+ \) channels recover from inactivation induced by the first pulse. In the figure, the first trial of the protocol is bolded. In subsequent trials, the first pulse is repeated followed by the second pulse with a greater delay. Peak currents measured during the test (second) pulse were normalized by dividing by the peak current during the prepulse. The figure shows that under control conditions the test pulse currents returned to control levels rapidly. DHOM reduced the size of the currents but recovery from inactivation was clearly seen in this cell. Results for all cells were averaged for each treatment and plotted versus interpulse interval in Fig. 8. Although control records (half-filled squares) showed a rapid recovery of current, recovery was only 91% complete in these experiments, suggesting that some slower inactivation process is not fully measured in this time scale. The time between trials in these
experiments was set to 10 s to allow full recovery between each trial. Data were plotted versus interpulse interval and fit to a single exponential function. Parameters are given in Table 2. Although results similar to control were obtained in cells treated with 50 μM DHOA, those cells treated with DHOM, EOA, and EOM all showed a significant slowing in the rate of recovery. EOM, although significantly different from control, was also significantly different from both DHOM and EOA. In addition, significant differences in the extent of recovery were found between control and DHOM-, EOA-, and EOM-treated cells. This suggests that these compounds are also affecting this slower inactivation process.

Dose-Response Relationship. The dose dependence of the $I_{Na}$ inhibition displayed by LAMs was determined by applying a single repeating pulse from the holding potential of −100 to −10 mV for 10 ms to a whole-cell patch-clamped myocyte. After the $I_{Na}$ reached equilibrium, the solution was changed to a higher concentration of drug and allowed to reach a new equilibrium. This process was completed for concentrations of 0.1, 1, 3, 10, 20, and 50 μM LAMs. Higher concentrations were not used because the myocytes either became leaky or contracted into balls in such conditions. This may be reflective of our previous observation that these compounds have multiple effects (Stimers et al., 1999; Moran et al., 2001; Ha et al., 2002). The peak $I_{Na}$ from each pulse was plotted against time (data not shown). This plot was used to identify the average peak $I_{Na}$ of the various concentrations. These peak $I_{Na}$ values were divided by the control peak $I_{Na}$ of each cell and expressed as percentage of control in Fig. 9. The curves represent the best fit of the Hill equation to each data set assuming that at high enough concentrations all of $I_{Na}$ would be blocked and a Hill slope of 1. The half-inhibitory concentration (IC$_{50}$) values obtained from these fits are given in Fig. 9. As can be seen, DHOM and EOA showed lower IC$_{50}$ values than did DHOA or EOM. It should be noted that if different assumptions are made to generate the fit curve...
Discussion

Linoleic acid metabolites have been shown to be very toxic in a variety of cell types, in several species of animals, and most importantly in humans. Effects include cell death, cardiac arrhythmias, multiple organ failure, and cardiac arrest. In a clinical study, it was found that severely burned patients’ mean peak plasma concentrations of EOA were 99 ± 25 μM and that the level was correlated with mortality (Kosaka et al., 1994). This study was undertaken to identify the mechanism by which these compounds affect electrical activity in the heart. Previously, we have shown that DHOM prolongs the cardiac action potential and slows its rate of rise (Stimers et al., 1999). This suggested that Na⁺ channels were involved. In this study, we have investigated in detail the effects of four structurally similar linoleic acid metabolites on I_{Na}. Significant new findings in this study have shown in adult rat ventricular myocytes a pronounced dose-dependent reduction in Na⁺ current density caused by DHOM and EOA especially. Current inhibition was not due to shifts in activation kinetics; however, a significant shift in steady-state inactivation was measured. Interestingly, of the four tested compounds only EOM caused a significant change in the slope of the steady-state inactivation curve. Recovery from fast inactivation was slowed by EOA, EOM, and DHOM, suggesting an interaction with the inactivation gates or a stabilization of the inactivated state.

**Structure-Related Effects.** Our previous study used only the methyl esters of these compounds and showed that Na⁺ current was sensitive to DHOM but not to EOM (Stim-

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**Table 1**

Boltzmann fit to steady-state inactivation curves

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Midpoint (mV)</th>
<th>Slope (mV/e-fold change)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-51.4 ± 0.9</td>
<td>5.9 ± 0.7</td>
<td>12</td>
</tr>
<tr>
<td>DHOA</td>
<td>-50.1 ± 0.4</td>
<td>4.8 ± 0.2</td>
<td>7</td>
</tr>
<tr>
<td>DHOM</td>
<td>-57.5 ± 0.6*</td>
<td>5.1 ± 0.3</td>
<td>6</td>
</tr>
<tr>
<td>EOA</td>
<td>-56.8 ± 0.8*</td>
<td>4.9 ± 0.7</td>
<td>7</td>
</tr>
<tr>
<td>EOM</td>
<td>-57.0 ± 0.6*</td>
<td>3.6 ± 0.7*</td>
<td>6</td>
</tr>
</tbody>
</table>

* Significantly different from control.

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**Fig. 6.** Voltage dependence of inactivation. A, mean ± S.E. values obtained from experiments like that shown in Fig. 5. Data are normalized to the maximum current obtained in each condition. This shows that DHOM, EOA, and EOM all cause a significant shift of the steady-state inactivation curve to hyperpolarized potentials by about -6 mV. The data points for DHOM, EOA, and EOM significantly differ from control values between -60 and -40 mV (P < 0.05). B, isolates the same data for control and EOM to more clearly show the significant increase in slope caused by EOM. Lines are best-fit Boltzmann relations to each data set. Fit parameters and number of cells are given in Table 1.

**Fig. 7.** Recovery from inactivation. Top, protocol used to measure the rate of recovery of I_{Na} from fast inactivation. From a holding potential of -100 mV a 10-ms prepulse to 0 mV was followed 2 to 140 ms later by an identical test pulse. Bottom, current records from a single cell under control conditions and after exposure to 50 μM DHOM. Peak currents were measured during each test pulse and normalized to the current magnitude during the prepulse. Cell capacitance was 136 pF.
ers et al., 1999). It was assumed that the cell’s intrinsic esterase activity would convert these compounds to their free acid counterparts (DHOA and EOA). Results in this study suggest that this is not the case. Because DHOA was found to be almost completely without activity against \( I_{\text{Na}} \), it is very unlikely that DHOM was converted to DHOA in either the previous study or the present one. In addition, in this study EOA was found to be more effective than EOM in its effects on most of the parameters measured. This further supports the idea that these compounds are not being de-esterified during these experiments.

Because all of these compounds are highly lipid soluble, it may be possible that these compounds exert their effects via interactions in the membrane that change membrane fluidity and indirectly alter \( I_{\text{Na}} \). In another study, both EOA and DHOA have also been shown to inhibit Na\(^+\)/H\(^+\) pump current in oligodendrocytes (Ha et al., 2002). This inhibition was also shown to have no correlation to membrane fluidity (Ha et al., 2002).

Results presented herein also suggest a direct interaction between the channel protein and LAM. As noted above, these compounds seem to not be significantly metabolized in these experiments and their effects occur rapidly, within 2 min after bath exchange, and wash out rapidly (3–5 min) (Stimers et al., 1999). Taken together, these results suggest their effects are exerted extracellularly. In addition, because of the significant differences between the four compounds in their effects on \( I_{\text{Na}} \) channels, we speculate that they are having a direct interaction with the Na\(^+\)/H\(^+\) channel proteins. Previous experiments have shown that polyunsaturated fatty acids can bind to sodium channel proteins (Kang and Leaf, 1996), and it would seem that the four tested compounds act similarly.

**Voltage Dependence of Inactivation.** Inhibition of \( I_{\text{Na}} \) by any compound can occur by a single mechanism or by a combination of effects. Peak \( I_{\text{Na}} \) can be inhibited by blocking the conductance pathway, by shifting the voltage dependence of activation to more depolarized potentials, or by shifting the voltage dependence of inactivation to more hyperpolarized potentials. In the data shown in Figs. 3 and 4 there is no evidence of a shift in the voltage dependence of activation as the current first activates between \(-60\) and \(-50\) mV and it reaches its maximum at \(-30\) mV under all tested conditions. However, the same is not true for inactivation. As shown in Figs. 5 and 6, steady-state inactivation is shifted about 6 mV in the hyperpolarizing direction by 50 \( \mu \)M DHOM, EOA, and EOM. Furthermore, EOM caused a significant increase in the slope of the steady-state inactivation curve. This suggests that the charge on this compound may be interacting with the voltage sensor or the membrane field in these experiments. Despite the shift in inactivation to more hyperpolarized potentials, this is not sufficient to explain the decreased magnitude, because \( I_{\text{Na}} \) was inhibited even with a steady-

**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time Constant (ms)</th>
<th>Fractional Recovery</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.9 ± 0.5</td>
<td>0.91 ± 0.01</td>
<td>23</td>
</tr>
<tr>
<td>DHOA</td>
<td>14.6 ± 0.4</td>
<td>0.94 ± 0.01</td>
<td>7</td>
</tr>
<tr>
<td>DHOM</td>
<td>30.5 ± 3.3*</td>
<td>0.73 ± 0.02*</td>
<td>4</td>
</tr>
<tr>
<td>EOA</td>
<td>31.4 ± 1.4*</td>
<td>0.74 ± 0.01*</td>
<td>7</td>
</tr>
<tr>
<td>EOM</td>
<td>20.5 ± 1.0**</td>
<td>0.78 ± 0.01**</td>
<td>4</td>
</tr>
</tbody>
</table>

* Significantly different from control.

**Fig. 8.** Effect of linoleic acid metabolites on recovery from inactivation. Data from experiments like that shown in Fig. 7 were collected from multiple cells (n given in Table 2). Symbols represent mean ± S.E. for each treatment. Cells exposed to 50 \( \mu \)M DHOM and EOA show a recovery time that was significantly slower than control, and 50 \( \mu \)M EOM was significantly slower than control but significantly faster than DHOM and EOA. Data points for DHOM, EOA, and EOM are significantly different from control values for recovery times greater than 10 ms (\( p < 0.05 \)). Lines represent best fit of a single exponential function to each data set. Fit parameters are given in Table 2.
state holding potential of $-100$ mV, at which there was no
detectable steady-state inactivation. Thus, although the shift
in inactivation could contribute to the block of $I_{Na}$, it is likely
that there is also a significant block of the channel pore by
these compounds. Of course, this could be due to a block of
channel gating rather than a physical block of the pore.

**Kinetics of Recovery from Inactivation.** One mecha-
nism for inhibiting Na$^+$ current that is exhibited by local
anesthetics is a stabilization of the inactivated state (Jia et
al., 1993). This is characterized by a slowing of the rate of
recovery of $I_{Na}$ from inactivation. The data shown in Figs. 7
and 8 confirm that three of the compounds do significantly
slow the rate of recovery from inactivation. In addition, there
was a slower component (not slow inactivation) not measured
by these experiments that was also slowed, or at least its
extent was enhanced, because the fractional recovery over
this time period (140 ms) was significantly reduced. Note
that this slowing of recovery was in addition to the steady-
state block produced by these agents, because these data
were normalized to the prepulse current amplitude, not con-
trol amplitude. Together, the shift of the steady-state inactiva-
tion curve and the increased recovery time suggest that
these compounds inhibit $I_{Na}$ by stabilizing the inactivated
state of the cardiac sodium channel.

**Physiological Significance.** These data are of physiolog-
ical significance because slight changes in cardiac sodium
current can, over time, develop into arrhythmias and possibly
lead to cardiac arrest. Furthermore, the characteristics of
these LAMs on $I_{Na}$ are similar to the effects of class I an-
arrhythmic agents such as lidocaine and others. This sug-
gests caution should be used in patients with elevated circu-
lating fatty acids (e.g., severely burned or ischemic patients)
needing treatment for arrhythmias. Further studies in this
area could test the interaction of these compounds with lido-
caine effects on $I_{Na}$.

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**References**

Bogdanov KY, Spurgeon GA, Vinogradova TM, and Lakatta EG (1998) Modula-
tion of the transient outward current in adult rat ventricular myocytes by polyunsatu-

Crumb WJ Jr, Munfakh N, Heck HA, and Harrison LH (1999) Fatty acid block of the
transient outward current in adult rat cardiac myocytes. *J Pharmacol Exp Ther* 298:
386–391.

$\beta$-adrenergic stimulation of adult rat cardiac myocytes. *J Physiol (Lond)* 507(3):
527–539.

Dobretsov M and Stimers JR (1996) Characterization of the Na/K pump current in


Fukushima A, Hayakawa M, Sugiyama S, Masayoshi A, Ito T, Satake T, and Ozawa
T (1988) Cardiovascular effects of leukotoxin (9,10-epoxy-12-octadecenoate) and

fatty acids and related compounds to cells expressing human soluble epoxide

acid metabolites on Na$^+$–K$^+$ pump current in N20.1 oligodendrocytes: role of

Harrell MD and Stimers JR (2002) Effects of linoleic acid metabolites on cardiac Na$^+$

Hashimoto M, Shinozuka K, Gamoh S, Tanabe Y, Hossain MS, Kwon YM, Hata N,
sahexaenoic acid is associated with the enhanced release of ATP from the caudal artery of aged rats. *J Nutr* **129**:70–76.


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