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Electrophysiologic properties of lidocaine, cocaine, and n-3 fatty-acids block of cardiac Na^+ channels

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Abstract

Lidocaine and cocaine, two local anesthetics, and n-3 polyunsaturated fatty acids in fish oils, inhibit the voltage-gated Na^+ channels of cardiomyocytes. This inhibition by lidocaine and n-3 fish oil is associated with antiarrhythmic effects, whereas with cocaine lethal arrhythmias may occur. These electrophysiologic studies show that at the concentrations tested, the n-3 fish oil fatty acids and lidocaine share three actions on I_{Na}: a potent inhibition of I_{Na}; a strong voltage-dependence of this inhibition; and a large shift of the steady-state inactivation to hyperpolarized potentials. By contrast cocaine shares only the potent inhibition of I_{Na}. The voltage-dependence of the inhibition is much decreased with cocaine, which produces only a very small leftward shift of the voltage-dependence of inactivation. The large leftward shift of the steady-state inactivation seems very important in the prevention of fatal arrhythmias by the n-3 fatty acids. Thus, we suggest that it is lack of this effect by cocaine, which is one factor, that eliminates its ability to prevent fatal cardiac arrhythmias. Further we report that in cultured neonatal rat cardiomyocytes n-3 fish oil fatty acids terminate the tachycardia induced by the α_1 adrenergic agonist, phenylephrine, whereas cocaine accelerates the tachycardia and causes bouts of tachyarythmias.

Keywords: Fish oil; Polyunsaturated fatty acid; Arrhythmias; Adrenoceptor agonists; Eicosapentaenoic acid; Docosahexaenoic acid

1. Introduction

Local anesthetics, such as lidocaine and procaine, have long been used clinically as antiarrhythmic agents. Other local anesthetics, such as cocaine (Evans et al., 1996; Mouhaffel et al., 1995) and bupivacaine (Clarkson and Hondeghem, 1985), are cardiotoxic, associated with arrhythmias, which may be fatal. Recently, it has been shown that dietary long chain polyunsaturated fatty acids of the n-3 class in fish oils are potent antiarrhythmic agents in animals (Billman, 1994; Billman et al., 1994, 1997, 1999; McLennan et al., 1987, 1992; McLennan, 1993) and probably in humans. (GISSI-Prevenzione Investigators, 1999). The purpose of this study was to compare electrophysiologic effects of lidocaine, cocaine and the n-3 fatty acids, eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA), on human and rat cardiac Na^+ channels to learn how lidocaine and n-3 fatty acids are cardioprotective, whereas cocaine can be cardiotoxic.

In our studies on the mechanism of the antiarrhythmic actions of the n-3 fish oil fatty acids, we found that they modulate the conductance of several ion channels in the sarcolemma of cardiomyocytes. Of the ion channels so modulated, we think, it is their inhibitory actions on the voltage-gated fast Na^+ current (Xiao et al., 1995, 1998, 2000, 2001) and the L-type Ca^{2+} current (Xiao et al., 1997) that are the major actions of the PUFA, which prevent arrhythmias. In this study we will examine the electrophysiologic effects of lidocaine, cocaine and EPA on the fast voltage-gated Na^+ channel. Inhibition of the Na^+ current, I_{Na}, by EPA or DHA elicits a typical sigmoid curve with

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increasing concentrations of the fatty acid. In neonatal rat cardiomyocytes in culture the apparent IC_{50} for EPA was 4.8 μM (Xiao et al., 1995). In the human myocardial Na^+ channel α-subunit transiently expressed in human embryonic kidney (HEK293t) cells the IC_{50} for inhibition by EPA was 0.51 ± 0.06 μM (Xiao et al., 2000).

We have hypothesized (Leaf et al., 1998; Xiao et al., 2000) that in the presence of myocardial infarction or other ischemic stress, partially depolarized myocytes at the periphery of the ischemic zone create a risk for arrhythmias as it will take only a small further depolarizing stimulus, e.g., current of injury, to elicit an action potential. If this occurs during a vulnerable moment of the normal cardiac electrical cycle, it may induce a fatal arrhythmia. The antiarrhythmic action of the n-3 fatty acids, as relates to their effects on the Na^+ channel, results from their ability to remove from function such partially depolarized myocytes and this ability in turn results from the large shift to the left of the steady state inactivation to physiologically unattainably negative potentials.

In this study our data show three electrophysiologic effects for antiarrhythmic EPA or DHA. If a drug or agent causes: (a) inhibition of the voltage-gated Na^+ current of cardiomyocytes; (b) if such inhibition is voltage-dependent; (c) if a and b are associated with a large leftward shift of the inactivation potential, then the agent is likely to be antiarrhythmic. If, however, the agent inhibits the Na^+ current but the inhibition is only slightly voltage-dependent and causes only small leftward shift of the steady state inactivation, then the agent will not be antiarrhythmic. We will illustrate these criteria by a comparison of the actions of lidocaine and n-3 polyunsaturated fatty acids (two antiarrhythmic agents) as compared with cocaine (potentially cardiotoxic and arrhythmogenic) on the electrophysiology of the cardiac Na^+ channels.

2. Materials and methods

2.1. Materials

Lidocaine, cocaine, phenylephrine and methoxyamine (Sigma, St. Louis, MO) were dissolved biweekly in deionized water at 10 mM and stored at −20 °C before use. The experimental concentrations of lidocaine, cocaine, phenylephrinse and methoxyamine were obtained by dilution of the stocks. Fatty acids obtained from Sigma were dissolved weekly in ethanol at 10 mM and stored under nitrogen at −20 °C before use. The experimental concentrations of fatty acids were obtained by dilution of the stocks and contained negligible ethanol, which at the dilution applied had no effect on Na^+ currents. Our protocols used in this study were approved by our Institutional Animal Care Committee and the investigation conformed to the Guide for the *Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Cardiomyocyte isolation and culture

Primary cultures of ventricular myocytes were prepared from 1-day-old neonatal rats with a commercial isolation kit (Worthington Biochem, NJ). This kit utilizes purified enzyme preparations to produce healthy beating cells, which were seeded in 35-mm tissue culture dishes with an appropriate density. Isolated cells were incubated in F-10 Nutrient Mixture (Ham, 1X, GIBCO, Invitrogen, Gaitherburg, MD) with 10% donor horse serum and 5% fetal calf serum at 37 °C in air with 5% CO_2 added and 98% relative humidity (model 3123, Forma Scientific, Marietta, OH). The culture medium was changed every other day. Neonatal rat cardiomyocytes for patch-clamp experiments and for tachycardia studies were cultured for 3–7 days after isolation.

Single left ventricular myocytes of adult male rats (Wistar, 200–300 g body weight, Charles River, Wilmington, MA) were isolated with a modified method described previously (Xiao and Mcardle, 1994). Briefly, the heart was rapidly removed from an anesthetized (pentobarbital, 50 mg/kg, i.p.) rat. The aorta was cannulated and connected to a modified Langendorff system with a flow rate of 5–10 ml/min. The heart was initially perfused for 4 min with oxygenated 37 °C Tyrode's solution containing (in mM): NaCl 137, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, HEPES 10, glucose 10, pH 7.4. The heart was then perfused with a Ca^{2+}-free Tyrode's solution for 5 min, and recirculated with 50 ml of a Ca^{2+}-free Tyrode's solution containing 50 mg collagenase Type I and 2 mg protease Type XIV (Sigma) recirculated for 25 min. At the end of enzyme perfusion the heart was sequentially washed with 50 ml 0.2 mM $Ca^{2+}$and 50 ml 0.5 mM Ca^{2+} Tyrode's solution plus 1 mg/ml bovine serum albumin. After these treatments, several pieces were cut off from the left ventricle and minced to separate the cells. These ventricular myocytes were kept in 0.5 mM Ca^{2+} Tyrode's solution at room temperature (22–23 °C) for 1 h before use.

2.3. Testing the effect of DHA on the rhythms induced by the α_1-adrenoreceptor agonists, phenylephrine and methoxyamine

As previously reported (Kang and Leaf, 1994), a microscope coverslip with adherent cultured neonatal rat cardiac myocytes was placed in a perfusion chamber on an inverted microscope and the contraction of a single myocyte in a clump of spontaneously beating cells was monitored and its rate and amplitude of contraction recorded.

2.4. Cell culture and transfection of $hH1_\alpha NA^+$ channels

HEK293t cells were cultured with the method described previously (Xiao et al., 1998, 2000). Oligonucleotide-directed point mutations of $hH1_\alpha$ were performed by using the Transformer Site-Directed Mutagenesis Kit (Clontech Lab., Palo Alto, CA), with some modifications to increase the

yield of in vitro DNA synthesis. The method has been described in previous reports (Xiao et al., 1998; Wright et al., 1999). Cells transfected with the wildtype or its mutants of $hH1_{\alpha}Na^{+}$ channels were seeded with an appropriate density in 35-mm tissue culture dishes (which also served as recording chambers). Transfected cells were used for patch-clamp experiments within 6 days after transfection.

2.5. Electrophysiologic recordings

During an experiment cultured neonatal rat cardiomyocytes, isolated adult rat heart cells, or HEK293t cells transfected with $hH1_{\alpha}Na^{+}$ channels were continuously superfused (1–2 ml/min) with the Tyrode's solution. Recording glass electrodes (World Precision Instruments, Sarasota, FL) had a resistance of 0.5–1.2 MΩ when filled with one of the pipette solutions. The pipette solution used in cardiomyocytes contained (in mM): CsCl 100, CsOH 40, $MgCl_2$ 1, $CaCl_2$ 1, EGTA 11, MgATP 5, HEPES 10, and pH 7.3 with CsOH. The pipette solution used in HEK293t cells contained (in mM): NaF 100, NaCl 30, EGTA 10, and HEPES 10 (titrated with cesium hydroxide to pH 7.3). After forming a conventional "Gigaseal" the capacitance of an electrode was compensated. Additional suction was used to form the whole-cell configuration. After a measurement of whole-cell membrane capacitance compensation of cell capacitance and series resistance was then performed. The patched cells were dialyzed for 10 min and perfused with an extracellular solution before data collection. During an experiment extracellular solution was

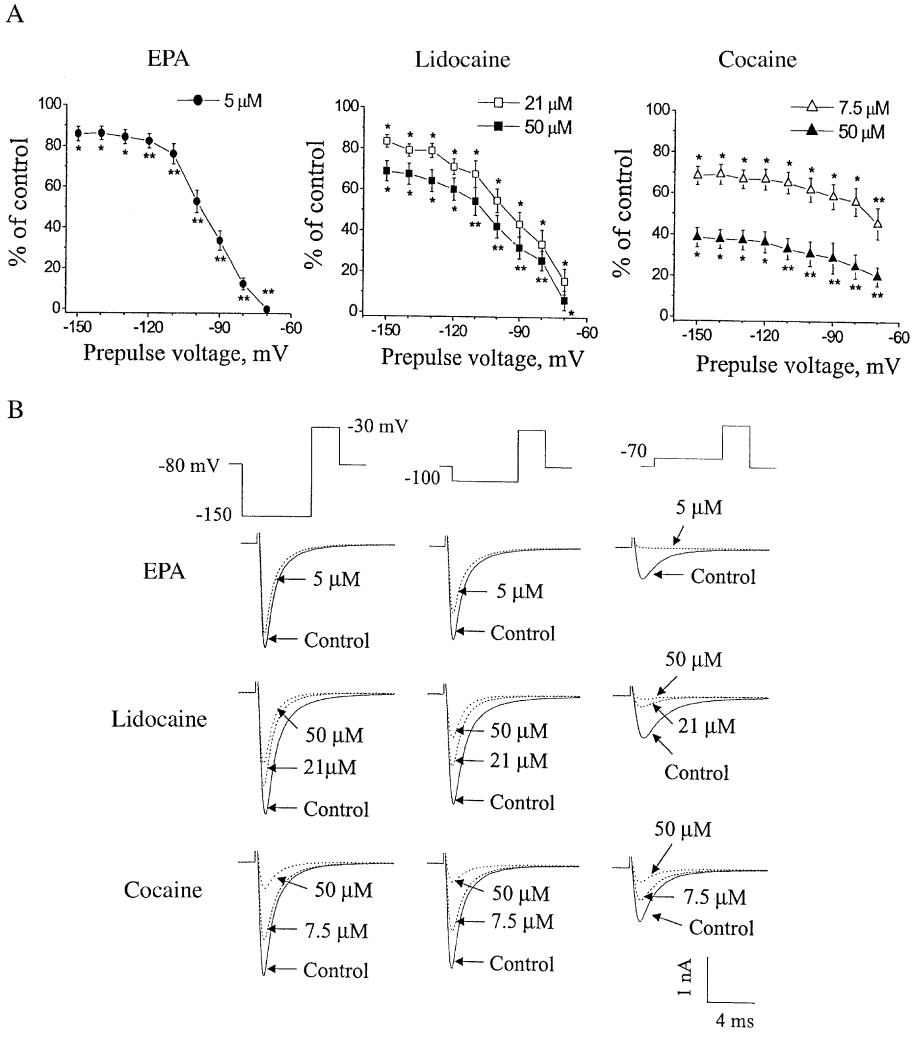

Fig. 1. Voltage-dependent inhibition of $I_{Na,nrat}$ in the presence of EPA, lidocaine, and cocaine. (A) The experimental protocol was composed of two pulses, a 500-ms prepulse and a 20-ms test pulse. Currents were elicited by test pulses to −30 mV following prepulses from −150 to −20 mV in 10-mV increments. The membrane holding potential was −80 mV and the pulse rate was 0.1 Hz. The inhibition was calculated as percentage ($I_{Na,nrat(Treatment)}/I_{Na,nrat(Control)} \times 100$) for their corresponding prepulse voltages for 5 μM EPA, 21 and 50 μM lidocaine, and 7.5 and 50 μM cocaine. $^{*}P<0.05$; $^{**}P<0.01$ versus their corresponding controls. (B) This also shows the voltage dependence of inhibition of the $I_{Na,nrat}$ by the three agents, but it uses single-step depolarizations from −150, −100, and −70 to −30 mV. Upper panels show the experimental protocols; lower panels show the original current traces in the absence (Control) and presence of EPA (5 μM), lidocaine (21 and 50 μM), and cocaine (7.5 and 50 μM).

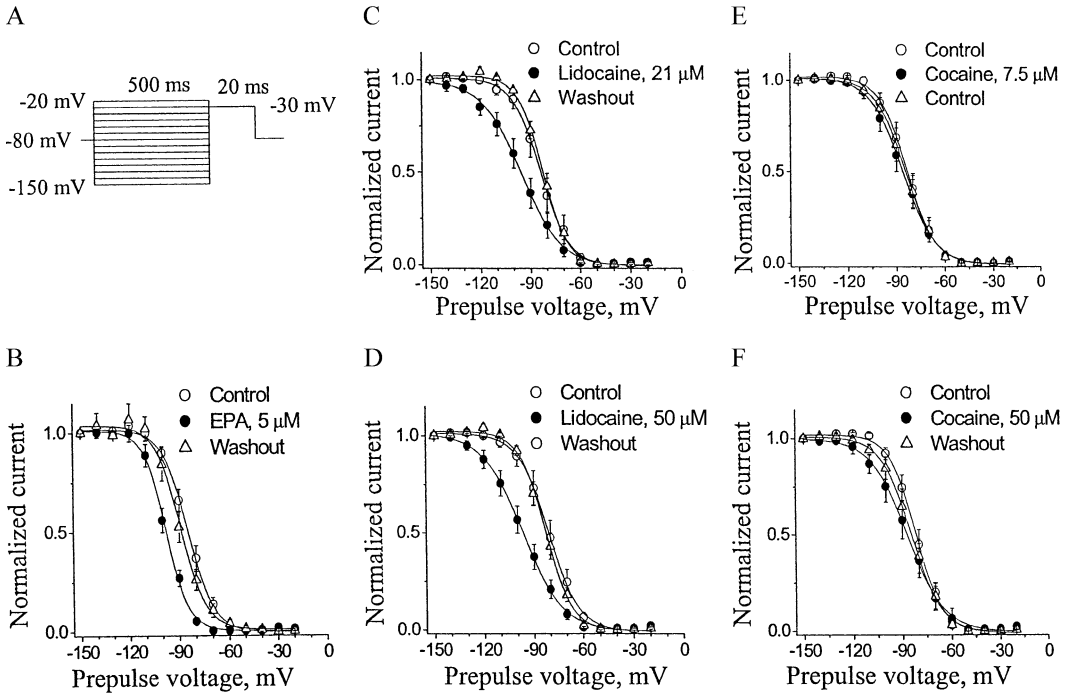

Fig. 2. Effects of EPA, lidocaine, and cocaine on the steady-state inactivation of $I_{Na,nrat}$ in cultured neonatal rat cardiomyocytes. The experimental protocol is shown (A). Currents were elicited by 20-ms test pulses to −30 mV following 500-ms prepulses varying from −150 to −20 mV with 10-mV increments. The membrane potential was held at −80 mV and the pulse rate was 0.1 Hz. Currents were normalized by their maximal currents elicited at the prepulse voltage of −150 mV. Normalized inactivation data points were fitted by the Boltzmann equation in the absence, presence and washout of 5 μM EPA (B, $n=10$), 21 (C, $n=8$) and 50 (D, $n=9$) μM lidocaine, and 7.5 (E, $n=11$) and 50 (F, $n=10$) μM cocaine. See Table 1 for the values of the $V_{1/2}$ shifts.

rapidly exchanged by use of a puffer-pipette system (Konnerth et al., 1987). In cardiomyocytes the external solution contained (in mM): NaCl 20, *N*-methyl-*D*-glucamine 120, $MgCl_2$ 1, $CaCl_2$ 2, and HEPES 10, and glucose 10 (pH adjusted to 7.4 with HCl). With the internal and external recording solutions in cardiomyocytes, maintaining a tight seal for a relative long period became difficult at holding potentials more negative than −80 mV. Therefore, the membrane potential was held at −80 mV in most experiments to ensure that myocytes remained stable long enough for us to examine $I_{Na,rat}$ from the same cell before, during, and after exposure to a compound. In addition, the amplitude and the current–voltage relationship curve were not altered when $I_{Na,rat}$ was elicited by pulses from a holding potential of −150 mV or from −80 mV with a 500-ms hyperpolarizing prepulse to −150 mV. Therefore, a 500-ms hyperpolarizing prepulse to −150 mV was likely sufficient to remove fast and slow inactivation of cardiac Na^+ channels (Xiao et al., 2000). However, in HEK293t cells the external perfusion solution contained (in mM): NaCl 65, choline chloride 85, $CaCl_2$ 2, and HEPES 10 (titrated with tetramethyl ammonium hydroxide to pH 7.4), so that the patch was more stable and Na^+ currents could be measured in the outward direction from the cell in reverse of their normal inward movement (Wright et al., 1999). Various concentrations of cocaine, lidocaine or fatty acids were applied by the puffer-pipette system. Experiments were conducted at 22–23 °C.

2.6. Data analysis

Currents were acquired and analyzed as before (Xiao et al., 1995, 1998, 2000, 2001). Data are presented as mean ± S.E.M. Activation, inactivation, and voltage-dependent block of $hH1_\alpha Na^+$ channels were fit by the Boltzmann equation, $\{1/[1+\exp(V_{1/2}-V)/k]\}$, where $V_{1/2}$ is the midpoint voltage of the function and k is the slope factor (in mV/*e*-fold change in current). The paired or unpaired Student's *t*-test was used to determine statistical differences between two experimental groups. Data derived from three or more experimental groups were examined by one-way analysis of variance (ANOVA). Statistical significance was set at $P<0.05$.

3. Results

3.1. Effects of eicosapentaenoic acid (EPA), lidocaine, and cocaine on Na^+ currents in cultured neonatal rat cardiomyocytes[1] $I_{Na\ nrat}$

Our previous study showed that the n-3 fatty acids significantly inhibited Na^+ currents in cultured neonatal rat cardiomyocytes (Xiao et al., 1995). To compare the effects

[1] Note when referring to the Na^+ currents of neonatal or adult rat heart cells, $I_{Na\ nrat}$ or $I_{Na\ arat}$, respectively, will appear in the subscript.

of EPA, lidocaine, and cocaine on Na^+ currents, cultured neonatal rat cardiomyocytes were used. In the following experiments, 5 μM EPA, 21 and 50 μM of lidocaine, and 7.5 and 50 μM of cocaine were tested. The concentration of EPA was selected, because the IC_{50} of the inhibition of $I_{Na,nrat}$ is 4.8 μM in neonatal rat cardiomyocytes (Xiao et al., 1995). The concentrations of lidocaine (21 μM) and cocaine (7.5 μM) were selected as they represent concentrations in the upper range to which heart cells might be exposed clinically (Evans et al., 1996; Katzung, 1987). Fig. 1A shows the voltage-dependent inhibition of the rat cardiac Na^+ current ($I_{Na,nrat}$) in the presence of EPA, lidocaine, and cocaine. In the presence of 5 μM EPA, $I_{Na,nrat}$ was inhibited by $15 \pm 3\%$ ($n=10$, $P<0.05$) and by 100% ($n=10$, $P<0.01$) with the prepulses of -150 and -70 mV, respectively. The inhibitions of $I_{Na,nrat}$ caused by 21 and 50 μM lidocaine were $17 \pm 3\%$ ($n=8$, $P<0.05$) and $32 \pm 4\%$ ($n=9$, $P<0.05$) with the prepulse of -150 mV, and $86 \pm 6\%$ ($n=8$, $P<0.05$) and $94 \pm 4\%$ ($n=9$, $P<0.05$) with the prepulse of -70 mV, respectively. However, the inhibitions of $I_{Na,nrat}$ caused by 7.5 and 50 μM cocaine were $32 \pm 4\%$ ($n=11$, $P<0.05$) and $62 \pm 4\%$ ($n=10$, $P<0.05$) with the prepulse of -150 mV, and $54 \pm 7\%$ ($n=11$, $P<0.01$) and $80 \pm 4\%$ ($n=10$, $P<0.01$) with the prepulse of -70 mV, respectively. After washout of EPA, lidocaine, or cocaine, $I_{Na,nrat}$ recovered more than 90% of control (data not shown). These results suggest that compared to EPA and lidocaine, cocaine produced a much smaller voltage-dependent inhibition of $I_{Na,nrat}$.

Fig. 1B shows the inhibition of Na^+ currents with the single step protocol from -150, -100, -70 each to -30 mV for EPA (5 μM), lidocaine (21 and 50 μM), and cocaine

Table 1
Effects of EPA, lidocaine, and cocaine on the steady-state inactivation of cardiac Na^+ currents

Current	Compound	$V_{1/2}$ (mV) Control	$V_{1/2}$ (mV) Treated	$V_{1/2}$ (mV) Delta	n	P
$I_{Na,nrat}$	EPA: 5 μM	−84	−98	−13.7	10	<0.001
	Lidocaine: 21 μM	−83	−94	−11.5	8	<0.05
	50 μM	−82	−96	−13.2	9	<0.01
	Cocaine: 7.5 μM	−83	−86	−2.7	11	>0.05
	50 μM	−82	−87	−5.5	10	<0.05
$I_{Na,arat}$	EPA: 5 μM	−84	−104	−20.2	9	<0.001
	Lidocaine: 21 μM	−83	−92	−8.4	8	<0.01
	50 μM	−83	−101	−17.9	8	<0.01
	Cocaine: 7.5 μM	−82	−82	−0.1	7	>0.05
	50 μM	−82	−86	−3.9	9	>0.05
$I_{Na,hH1\alpha}$	EPA: 5 μM	−95	−122	−27.1	13	<0.001
	Lidocaine: 50 μM	−92	−105	−13.6	11	<0.01
	Cocaine: 50 μM	−91	−98	−6.8	9	<0.05

Values are means. $V_{1/2}$, voltage at the midpoint of the steady-state inactivation curve fitted by a Boltzmann equation; $V_{1/2}$ Delta, difference of the $V_{1/2}$ in the absence and presence of one of the compounds; n, number of individual cells patched for each group; $I_{Na,nrat}$, Na^+ current recorded from cultured neonatal rat cardiomyocytes; $I_{Na,arat}$, Na^+ current recorded from isolated adult rat cardiomyocytes; $I_{Na,hH1\alpha}$, Na^+ current recorded from HEK293t cells transfected with $hH1_\alpha$ Na^+ channels.

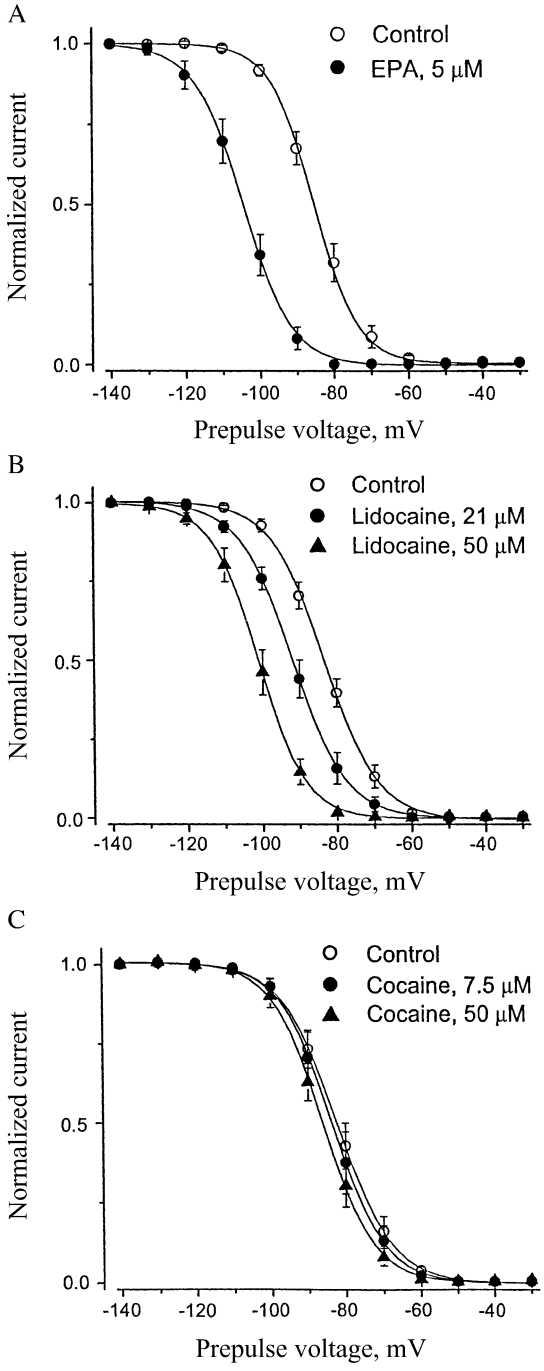

Fig. 3. Effects of EPA, lidocaine, and cocaine on the steady-state inactivation of $I_{Na,arat}$ in isolated adult rat cardiomyocytes. The experimental protocol is similar to the one in Fig. 2, except prepulse voltages varying from -140 to -30 mV. Currents were normalized by their corresponding controls at the prepulse voltage of -140 mV. A, B, and C show the steady-state inactivation of $I_{Na,arat}$ in the absence (open circles) and presence of 5 μM EPA (solid circles, $n=9$, A), 21 (solid circles, $n=8$) and 50 (solid triangles, $n=8$) μM lidocaine (B), and 7.5 (solid circles, $n=7$) and (solid triangles, $n=9$) μM cocaine (C). Normalized inactivation of $I_{Na,arat}$ was fitted by the Boltzmann equation. See Table 1 for the values of the $V_{1/2}$ shifts.

(7.5 and 50 μM). Both in the absence and presence of the agents the amplitude of the elicited $I_{Na,nrat}$ decreased as the holding potential was more depolarized. However, this effect was the most profound for EPA, so that at the holding potential of −70 mV, 5 μM EPA essentially eliminated detectable Na^+ current. In contrast, even in the presence of 50 μM cocaine, more than 20% of $I_{Na,nrat}$ was elicited at the holding potential of −70 mV. The averaged percent of $I_{Na,nrat}$ remaining at the holding potential of −70 mV for 5 μM EPA and with 21 and 50 μM lidocaine was 2%, 16%, and 6%, respectively. Whereas the remaining $I_{Na,nrat}$ was 46% for 7.5 μM and 21% for 50 μM cocaine, respectively. The important consequence is that at the low holding potential of −70 mV, the remaining $I_{Na\ nrat}$ is larger in the presence of both 7.5 and 50 μM cocaine than for either EPA or lidocaine.

To determine whether the different inhibition of $I_{Na,nrat}$ caused by EPA, lidocaine, and cocaine resulted from their effects on the fast steady-state inactivation, a two-pulse protocol was used in cultured neonatal rat cardiomyocytes (Fig. 2A). Currents were elicited by 20-ms test pulses to −30 mV following 500-ms prepulses ranging from −150 to −20 mV (in 10 mV increments). Cells were held at a membrane potential of −80 mV. The delta shift of the $V_{1/2}$ of $I_{Na,nrat}$ caused by 5 μM EPA (Fig. 2B) was -13.7 ± 1.3 mV ($n = 10$), see Table 1 for details. After 5 min washout of EPA with 0.2 % fatty acid-free bovine serum albumin, the steady-state inactivation curve was shifted back to −89 at the $V_{1/2}$ point. The values of the $V_{1/2}$ of the steady-state inactivation curves between control and washout were not significantly different ($P>0.05$), but the difference between EPA and washout was statistically significant ($P<0.05$). Fig. 2C and D shows that lidocaine at 21 or 50 μM also caused a significant shift of the steady-state inactivation, see Table 1. The delta shifts of the $V_{1/2}$ of $I_{Na,nrat}$ were -11.5 ± 1.7 and -13.2 ± 1.3 mV for 21 and 50 μM lidocaine, respectively. However, the shifts of the $V_{1/2}$ of $I_{Na,nrat}$ were significantly less for cocaine at 7.5 and 50 μM. Fig. 2E and F shows the curves of the voltage-dependence of inactivation in the absence and presence of cocaine, see Table 1. The delta shifts of the $V_{1/2}$ of $I_{Na,nrat}$ were -2.7 ± 0.8 and -5.5 ± 2.3 mV for 7.5 and 50 μM cocaine, respectively. The delta shift for 7.5 μM cocaine was not significant. After 3 min washout of lidocaine or cocaine, the inactivation curves of $I_{Na,nrat}$ returned toward the control levels (open triangles in Fig. 2B, C, D, E, and F). The differences of the delta shifts of the $V_{1/2}$ of $I_{Na,nrat}$ were not significant between 5 μM EPA (−13.7 mV) and 21 μM

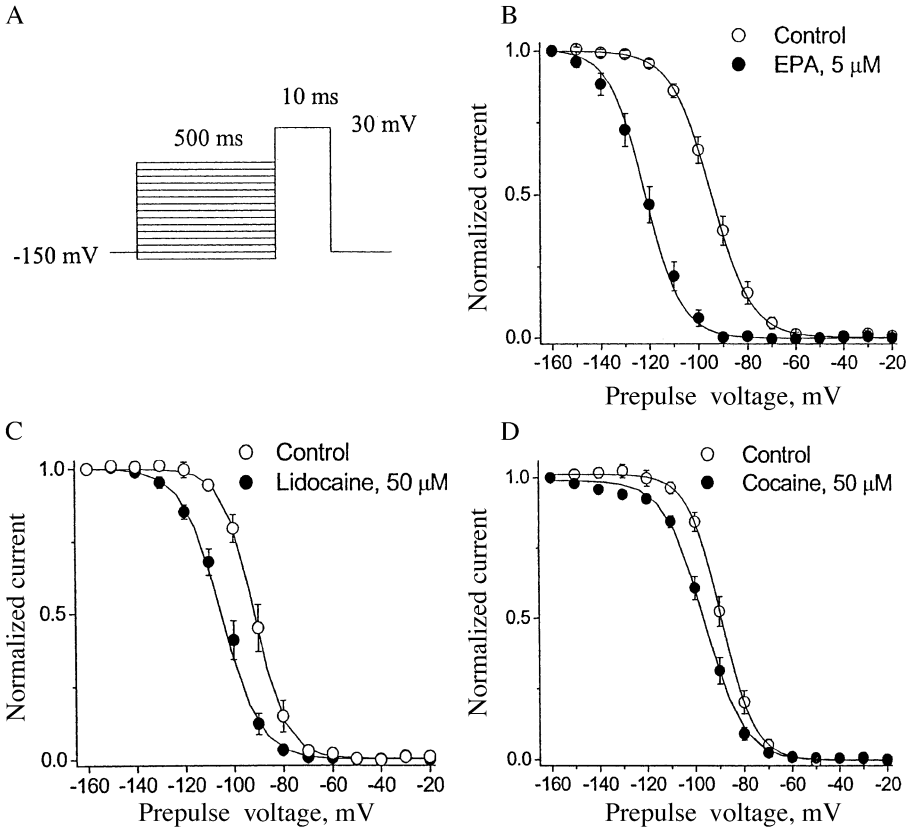

Fig. 4. Effects of EPA, lidocaine, and cocaine on the steady-state inactivation of $I_{Na,hH1\alpha}$ in HEK293t cells transfected with the α-subunit of the human cardiac Na^+channel ($hH1_\alpha$). The experimental protocol is shown (A). Currents ($I_{Na,hH1\alpha}$) were elicited by 10-ms test pulses to 30 mV following 500-ms conditional prepulses varying from −160 to −20 mV with 10-mV increments. The membrane potential was held at −150 mV and the pulse rate was 0.1 Hz. Normalized steady-state inactivation was fitted by the Boltzmann equation in the absence and presence of 5 μM EPA (B), 50 μM lidocaine (C), and 50 μM cocaine (D). See Table 1 for the values of the $V_{1/2}$ shifts.

(−11.5 mV) or 50 μM (−13.2 mV) lidocaine. However, the delta shifts of the $V_{1/2}$ of $I_{Na,nrat}$ in the presence of 5 μM EPA and 21 or 50 μM lidocaine were significantly greater ($P<0.05$) than those in the presence of 7.5 (−2.7 mV) and 50 μM (−5.5 mV) cocaine. The shifts in $V_{1/2}$ of the steady state inactivation for $I_{Na,nrat}$ are summarized in Table 1. Clearly the delta shifts were nil at 7.5 μM and minimal at 50 μM cocaine.

3.2. Effects of EPA, lidocaine, and cocaine on Na^+ currents in adult rat cardiomyocytes, $I_{Na,arat}$

The expression of β_1 subunits of Na^+ channels in the brain is developmentally regulated after birth. The mature Na^+channel, including β_1, contributes to the development of circuitry that supports complex patterns of electrogenesis (Sashihara et al., 1996, 1995). Since there is no report to show neonatal rat cardiomyocytes express mature Na^+ channels containing the β1-subunit, we used isolated adult rat cardiomyocytes to examine the effects of EPA, lidocaine, and cocaine on mature cardiac Na^+channels. The same experimental protocol as in Fig. 2A was applied. The average $V_{1/2}$ of the fast steady-state inactivation curve of $I_{Na,arat}$ was -84 ± 1.7 mV. Extracellular application of 5 μM EPA shifted the $V_{1/2}$ to -104 ± 2.1 mV ($n=9$, $P<0.001$, Fig. 3A). The delta shift of the $V_{1/2}$ of $I_{Na,arat}$ caused by 5 μM EPA was -20.2 ± 2.0 mV ($n=9$). Thus, in the adult rat cardiomyocytes the shift of the steady state inactivation at $V_{1/2}$ with 5 μM EPA was even larger than in the neonatal rat cardiac myocytes—see Table 1.

In another series of experiments, lidocaine at 21 or 50 μM also caused significant shifts of the steady-state inactivation (Fig. 3B). The delta shifts of the $V_{1/2}$ of $I_{Na,arat}$ were -8.4 ± 1.7 and -17.9 ± 1.6 mV for 21 and 50 μM lidocaine, respectively, see Table 1.

The shifts, however, of the $V_{1/2}$ of $I_{Na,arat}$ were not significant and much less for cocaine at 7.5 and 50 μM. Fig. 3C shows the inactivation curves in the absence and presence of 7.5 μM or 50 μM cocaine. The delta shifts of the $V_{1/2}$ of $I_{Na,arat}$ were -1.7 ± 1.1 and -3.8 ± 1.7 mV for 7.5 and 50 μM cocaine, respectively, neither significant, see Table 1. After 3 min washout of lidocaine or cocaine, the inactivation curves of $I_{Na,arat}$ returned toward the control levels (data not shown). The differences of the delta shifts of the $V_{1/2}$ of $I_{Na,arat}$ were not significant between 5 μM EPA (−20.2 mV) and 50 μM lidocaine (−17.9 mV). However, the delta shifts of the $V_{1/2}$ of $I_{Na,arat}$ in the presence of 5 μM EPA and 50 μM lidocaine were significantly greater ($P<0.05$) than that in the presence of 50 μM cocaine (−3.8 mV). The shift of 3.8 mV with 50 μM cocaine was not itself significant ($P>0.05$). The effects of EPA, lidocaine and cocaine on the steady state inactivation of $I_{Na,arat}$ are summarized in Table 1. Clearly the delta shifts with 5 μM EPA and with 21 and 50 μM lidocaine are much larger than the nonsignificant delta shifts with 7.5 or 50 μM cocaine in the adult rat myocyte with a mature Na^+ channel.

3.3. Inhibition of human cardiac NA^+ currents by EPA, lidocaine, and cocaine

Our previous studies showed that EPA strongly inhibited the human cardiac Na^+ current ($I_{Na,hH1\alpha}$) expressed in HEK293t cells (Xiao et al., 1998, 2000, 2001). Therefore, we compared the effects of EPA, lidocaine, and cocaine on $I_{Na,hH1\alpha}$ in HEK293t cells transfected with the α-subunit of the human cardiac Na^+ channel ($hH1_\alpha$). Fig. 4 compares the effects of EPA (5 μM), lidocaine (50 μM), and cocaine (50 μM) on the inactivation relationship of $I_{Na,hH1\alpha}$ in HEK293t cells transfected with the wildtype of $hH1_\alpha Na^+$ channels. The experimental protocol is illustrated in Fig. 4A and described in the Fig. 4 legend. The delta shift of the $V_{1/2}$ decreases in the order of 27.1>13.6>6.8 mV for EPA>lido-

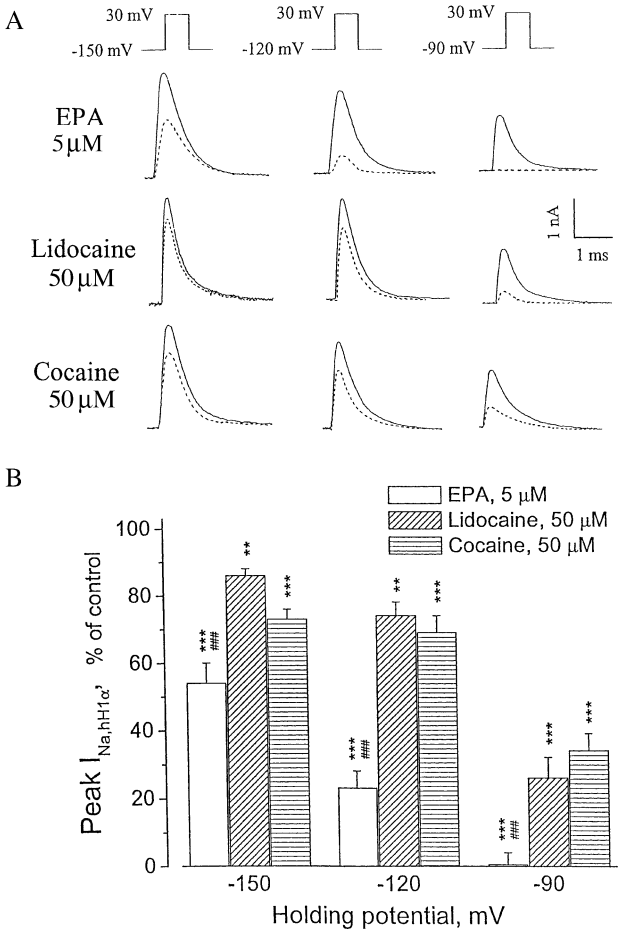

Fig. 5. Effects of EPA, lidocaine, and cocaine on $I_{Na,hH1\alpha}$ recorded in HEK293t cells expressing $hH1_\alpha$ Na^+channels. (A) Single step depolarizations from −150, −100, and −90 to +30 mV each in the absence (solid line) and presence (dotted line) of EPA (5 μM), lidocaine (50 μM), and cocaine (50 μM). (B) A bar graph of averaged data shows the percent remaining $I_{Na,hH1\alpha}$ by each agent: EPA (5 μM, open bar, $n=8$); lidocaine (50 μM, slanting striped bar, $n=13$); cocaine (50 μM, horizontal striped bar, $n=9$) for the holding potentials of −150, −100, and −90 mV. $^{**}P<0.01$; $^{***}P<0.001$ versus their corresponding controls. $^{\#\#\#}P<0.001$ versus their corresponding values of $I_{Na,hH1\alpha}$ in the presence of lidocaine and cocaine.

caine>cocaine, respectively, $P<0.001$ for each decrement, see Table 1.

To determine what these differences in shifts of channel inactivation for each of the agents tested might mean for their respective, potential, antiarrhythmic potency, the voltage dependence of the amplitudes of I_{Na} in the absence (solid line) or presence of EPA (5 μM), lidocaine (50 μM), or cocaine (50 μM) (dotted line) were measured in HEK293 cells transiently expressing the α-subunit of the human Na^+ channel, as shown in Fig. 5A. Top panels of Fig. 5A show the experimental protocols in which a single step depolarization was made from the holding potential of −150, −120, or −90 to 30 mV. Both in the absence and presence of the agents the peak amplitude of the elicited $I_{Na,hH1\alpha}$ decreased as the holding potential was reduced. In the presence of EPA this effect was greatest, so that at −90 mV holding potential 5 μM EPA essentially eliminated detectable sodium current. This effect is further examined in Fig. 5B, which shows the percent of $I_{Na,hH1\alpha}$ remaining at each holding potential for each of the three agents tested. At −150 and −120 mV holding potentials the order of % of $I_{Na,hH1\alpha}$ remaining was lidocaine>cocaine>EPA. At −90 mV holding potential it is clear that in the presence of EPA (5 μM) there is insufficient current elicited to produce an action potential, which would propagate through out the myocardium to cause a contraction of the heart. At −90 mV lidocaine (50 μM) with some 26% of the $I_{Na,hH1\alpha}$ remaining, lidocaine would often be unable to elicit a self propagating action potential through the myocardium with a resultant cardiac contraction. In the presence of cocaine (50 μM) causing 35% of $I_{Na,hH1\alpha}$ still remaining, this remaining 35% of the current will be sufficient to elicit an action potential to propagate and cause a cardiac contraction, which is likely to cause a fatal arrhythmia in the presence of ischemic myocardium. Thus it should be expected that cocaine may have effects on cardiac function which differ from those we expect from lidocaine or EPA and other n-3 fatty acids. Table 1 summarizes the effects of EPA, lidocaine and cocaine on the steady state inactivation of $I_{Na,hH1\alpha}$.

3.4. Inhibition in neonatal rat heart cells by n-3 fatty acids of the tachycardia induced by the α_1-adrenoreceptor agonist, phenylephrine, and induction of tachyarrythmias by cocaine

It has been reported (Billman, 1995) that cocaine enhances catecholamine effects on the heart, that can produce tachycardias, which may be important factors causing cocaine to initiate fatal arrhythmias. Therefore, we have tested the effect of phenylephrine and methoxyamine, two α_1-adrenergic agonists, to induce tachycardia in cultured neonatal rat cardiomyocytes and then determined the effect of DHA and cocaine on the tachycardia. This is shown in Fig. 6 with phenylephrine (20 μM). Following the regular control beating rate, the addition of the phenylephrine to the superfusate in a short time accelerated the beating rate of the myocyte ($n=11$ at 20 μM; $n=2$ at 10 μM, $n=1$ at 30 μM and $n=2$ at 40 μM phenylephrine.). In the presence of

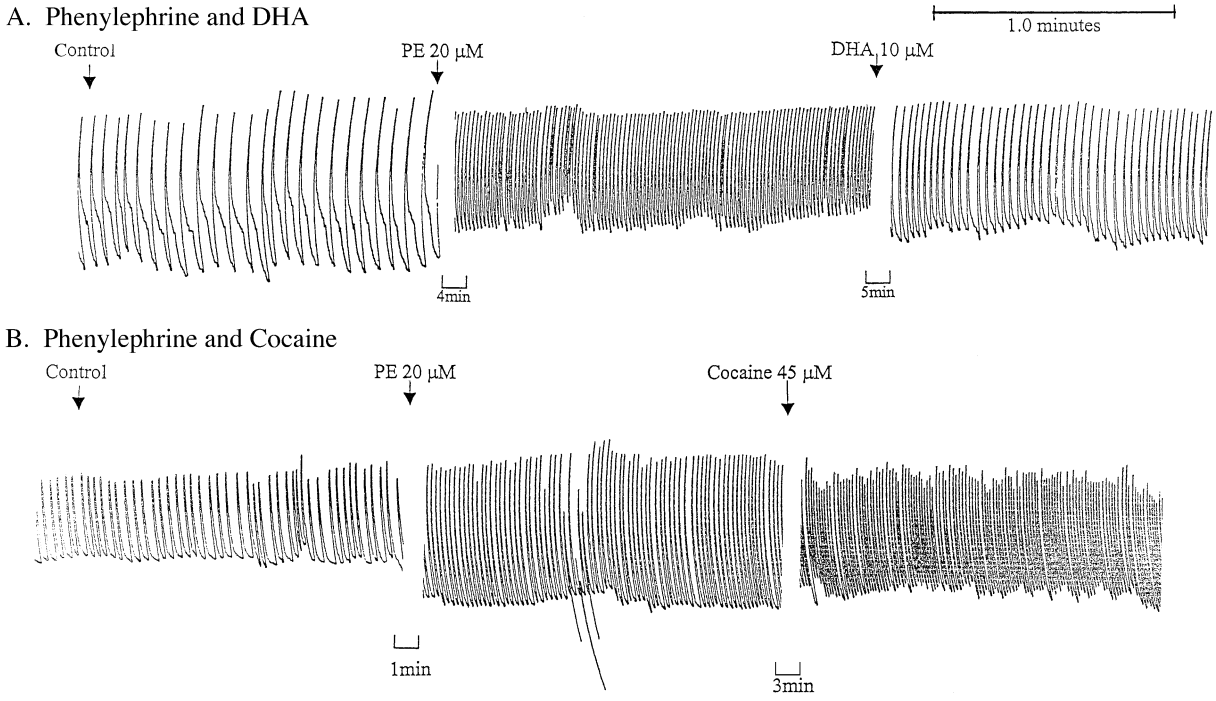

Fig. 6. Effect of DHA and cocaine on the tachycardia induced by the α_1-adrenergic agonist, phenylephrine, on the beating rate of cultured neonatal rat cardiomyocytes. (A) shows the tachycardia induced by phenylephrine (20 μM) and the action of DHA still in the presence of the phenylephrine to terminate the tachyarrhythmia. (B) shows in other cells the tachycardia induced by the same concentration of phenylephrine and the action of cocaine (45 μM) to further accelerate the beating rate and to induce bouts of tachyarrhythmias when added still in the presence of the phenylephrine.

this rapid beating rate and the continued presence of phenylephrine the addition of DHA (10 μM) to the superfusate slowed the beating rate back toward its initial control rate. At all concentrations of phenylephine tachycardia was similarly elicited and the major effect of the higher concentrations was to prolong the time for the 10 μM DHA to slow the tachycardia back to the control beating rate. As controls to determine if the effect of DHA were simply a nonspecifc effect of fatty acids, stearic acid (18:0), a saturated fatty acid, and oleic acid (18:1n-9), a monounsaturated fatty acid, were tested and neither had a slowing effect on the beating rate following phenylephrine. The other α_1-adreneroreceptor agonist, methoxyamine had no effect on the beating rate of the neonatal cardiomyocytes over a concentration range of methoxyamine from 30 to 200 μM ($n=11$). Thus, the n-3 DHA prevents the tachycardia caused by the α_1-adreneroreceptor agonist, phenylephrine. By contrast cocaine (45 μM), when added in the presence of the phenylephrine (20 μM), accelerated the tachycardia and induced bouts of tachyarrhythmias ($n=5$). This is shown in Fig. 6B. Cocaine at (45 μM) would be a lethal concentration in humans, so we tested a lower concentration of 7.5 μM ($n=4$), which is thought to be at the upper limit tolerated in humans (Mouhaffel et al., 1995; Evans et al., 1996). In one out of four experiments at the lower concentration bouts of tachyarrhythmias were induced, suggesting that even at this concentration a lethal arrhythmia might occur.

4. Discussion

The present study was undertaken to examine and compare the electrophysiologic properties of lidocaine, cocaine and n-3 fish oil fatty acids to determine a possible basis for the fact that lidocaine and n-3 fatty acids can prevent fatal ventricular arrhythmias, whereas cocaine may be cardiotoxic and associated with arrhythmias. Lidocaine and procaine are local anesthetics with a long clinical use as antiarrhythmic agents. Fish oil fatty acids are unrelated structurally to local anesthetics, but have been shown to be potent antiarrhythmic agents in animals (McLennan et al., 1987, 1992; McLennan, 1993; Billman et al., 1994, 1997, 1999) and probably in humans (Burr et al., 1989; GISSI-Prevenzione Investigators, 1999). But some local anesthetics, such as cocaine or bupivacaine, may be proarrhythmic and cause malignant ventricular arrhythmias. We report whole cell patch clamp studies of I_{Na} of adult and neonatal rat cardiomyocytes and of the human myocardial hH1 α-subunit transiently expressed in HEK293t cells comparing the effects of lidocaine, cocaine and n-3 fatty acids on the I_{Na} to find if electrophysiologic differences might contribute an explanation of why lidocaine and fish oil fatty acids are antiarrhythmic, whereas cocaine may be associated with fatal arrhythmias.

To make such comparisons it seems there could be at least two ways to proceed. A concentration of EPA, lidocaine and cocaine might be found at which all three inhibited I_{Na} to the same extent. At these concentrations each agent could be tested for voltage-dependence of the inhibition and the magnitude of shift of the inactivation to more hyperpolarized potentials–shift to the left. A difficulty, however, arises in proceeding in this manner. Because the magnitude of the inhibition of I_{Na} by each agent is voltage-dependent, there may be a match at one holding potential, but when that changes so does the amount of inhibition of I_{Na} by each agents—see Figs. 1 and 5. Therefore, we chose concentrations for lidocaine and cocaine, which clinically were shown to be at the upper limit at which each is used or tolerated (Katzung, 1987; Mouhaffel et al., 1995; Evans et al., 1996). This is where the concentration of lidocaine (21 μM) and cocaine (7.5 μM) came from. Even the suitability of this approach, we realize, may be questioned since the concentrations were derived by titrating two different effects having different clinical endpoints.

We have hypothesized (Leaf et al., 1998; Xiao et al., 2000) that the antiarrhythmic effect of EPA and DHA results from their inhibition of the I_{Na} with an associated large shift of the steady state inactivation to very hyperpolarized potentials. Here we report that lidocaine causes a slightly lesser shift and cocaine an even smaller shift in our in vitro conditions of study. These effects on the steady state inactivation are summarized in Table 1.

These results with cocaine in the adult rat cardiomyocytes are particularly important in this study for two reasons: (a) Despite the lack of significant shifts with 7.5 and 50 μM cocaine, the shifts with EPA μM (-20.2 ± 2.0 mV, $n=9$) and 21 μM lidocaine (-8.4 ± 1.7 mV, $n=8$) and with 50 μM lidocaine (-17.9 ± 1.6 mV, $n=8$) in the adult rat cardiomyocytes were large and significant. (b) The adult rat has a complete and fully developed Na^+ channel with both α and β1 subunits. The latter is, of course, not present with the human $hH1_\alpha$ expressed in the HEK293t cells and not known to be present in the neonatal rat cardiomyocytes.

In the presence of cardiac ischemia we have proposed, that partially depolarized cardiomyocytes initiate arrhythmias. The resting membrane potential in such partially depolarized myocytes is closer to the threshold for the gating of the inwardly directed fast Na^+ current so that any further small depolarizing stimulus, e.g., currents of injury, might initiate an action potential, which, if it falls during a vulnerable moment in the electrical cycle of the cardiomyocyte, may induce an action potential and set off a fatal arrhythmia. But in the presence of EPA or DHA the resultant shift in the steady state inactivation to physiologically unobtainable hyperpolarized potentials can eliminate the partially depolarized cells from function and prevent arrhythmias (Xiao et al., 2000).

The potential antiarrhythmic effectiveness of an agent in preventing fatal arrhythmias in this manner would vary directly with the magnitude of shift to the left in the steady state inactivation that it produced. EPA or DHA, which inhibit at -90 mV virtually 100% of the peak I_{Na}, as shown

in Fig. 5, will prevent any residual I_{Na} from eliciting a self propagating action potential with a resultant cardiac contraction. Lidocaine (50 μM—a very high concentration clinically) with still some 25–27% remaining I_{Na}, will cause sufficient shift to often prevent arrhythmias, assuming that some 20% or less remaining I_{Na} is insufficient to elicit an action potential. By contrast, cocaine produced such a small shift that it left some 35% of the peak I_{Na} uninhibited. Unfortunately, this would be sufficient I_{Na} to elicit an action potential, which could propagate throughout the myocardium and in the presence of the nonhomogeneous conduction rates of action potentials through ischemic tissue, induce fatal ventricular arrhythmias. Thus though PUFA, lidocaine and cocaine all inhibit I_{Na}, cocaine, in contrast to EPA and lidocaine, causes insufficient shift of the steady state inactivation to eliminate production of propagating action potentials and fatal cardiac arrhythmias.

We realize that, if we had prolonged the prepulse for 10 or 20 s, rather than the 500 ms at normal or hyperpolarized membrane potentials used in our in vitro experiments, cocaine would also have shifted the steady state inactivation of the Na^+ channel to the left like lidocaine (Wright et al., 1999). Such long time intervals in the resting state for cocaine to be freed from the inactivated state of the Na^+ channel are not applicable to the in vivo situation, where pulse rates are much above 3 or 6 beats per minute. Therefore, our reported findings are more physiologic and representative of the in vivo situation than using conditioning pulse duration of 10 or 20 s for the Na^+ channel inactivation to study the blocking effects of cocaine (Wright et al., 1999) or bupivacaine (Clarkson and Hondeghem, 1985). It is this minimal leftward shift in the inactivation potential, not a lesser inhibition of I_{Na} by cocaine than by lidocaine or EPA that is important to the inability of cocaine to prevent arrhythmias.

There have been many studies examining the cause of the serious arrhythmias associated with cocaine usage. Several investigators have concluded that there are two basic mechanisms that would account for cardiac toxicity from cocaine: one is the local anesthetic effects, which account for the Na^+ channel effects of cocaine and the other is its sympathomimetic actions, which inhibit neuronal uptake of norepinephrine producing an excess of neurotransmitters (Billman, 1990, 1995; Kloner et al., 1992; Chakko, 2002). In this study we have shown that the local anesthetic effect of cocaine may not just be its inhibition of I_{Na}, but importantly the small voltage dependence of this inhibition and the failure to shift the steady state inactivation to the left as do n-3 fatty acids and lidocaine. We also show that the sympathomimetic effect of cocaine, which has been attributed largely to the α-adreneroreceptor agonists are countered by DHA but enhanced to a proarrhythmic action by cocaine as shown in Fig. 6. It has been shown (Kang and Leaf, 1995) that the n-3 fatty acids will prevent the arrhythmias induced by β-adrenergoreceptor agonist isoproterenol or by cyclic-AMP in neonatal rat cardiomyocytes. They also reduce conductivity of the L-type Ca^{2+} channel ($I_{Ca,L}$) preventing cytosolic Ca^{2+} overload with delayed after potentials and arrhythmias (Xiao et al., 1997). Cocaine activates and augments adrenergic over-activity (Mouhaffel et al., 1995; Evans et al., 1996), whereas n-3 fatty acids, we have now shown, prevent both α_1 and β-adrenergic overactivity.

As indicated in the Introduction, our data show three electrophysiologic effects for antiarrhythmic n-3 fish oil fatty acids: (1) They are potent inhibitors of I_{Na}. (2) This inhibition is strongly voltage-dependent. (3) This inhibition is associated with large leftward shifts of the steady-state inactivation. The n-3 fish oil fatty acids are known cardiac antiarrhythmic agents and they exhibit these three electrophysiologic actions on the Na^+ channel conductance. By contrast cocaine inhibits I_{Na}, but the voltage dependence of this inhibition is much less than with EPA or lidocaine (Fig. 1A), and the shift it produces in the inactivation at physiologic pulse rates is very much less than the shifts produced by EPA and lidocaine (Figs. 2, 3 and 4). Thus, cocaine shares only its ability to inhibit I_{Na} with n-3 fatty acids or lidocaine. As a consequence, cocaine will not prevent arrhythmias, but will be likely to induce arrhythmias in ischemic myocardium in which the resting membrane potential is partially depolarized and close to the gating potential for I_{Na} , and the conduction rate of action potentials is nonhomogeneous in the ischemic myocardium. In addition we report that in cultured neonatal rat cardiomyocytes n-3 DHA terminates the tachycardia induced by the α_1 adreneroreceptor agonist phenylephrine, whereas cocaine accelerates the tachycardia and causes bouts of tachyarythmias. Thus, as Billman stated in 1995, the adrenergic and local anesthetic properties of cocaine could act synergistically to elicit toxic actions on the heart.

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