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Inhibitory effect of n-3 fish oil fatty acids on cardiac Na^+/Ca^{2+} exchange currents in HEK293t cells

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Abstract

Abnormal activity of the cardiac Na^+/Ca^{2+} exchanger (NCX1) can affect intracellular Ca^{2+} homeostasis and cause arrhythmias. The n-3 polyunsaturated fatty acids (PUFAs), however, may prevent arrhythmias. To test the effect of PUFAs on the cardiac NCX1 current (I_{NCX1}), the canine NCX1 cDNA was expressed in human embryonic kidney (HEK293t) cells. The average density of I_{NCX1} was 10.9 ± 2.6 pA/pF ($n = 44$) in NCX1-transfected cells and eicosapentaenoic acid (EPA, C20:5n-3) significantly inhibited I_{NCX1}. The suppression of I_{NCX1} by EPA was concentration-dependent with an IC_{50} of $0.82 \pm 0.27\,\mu M$. EPA had a similar effect on outward or inward I_{NCX1}. Docosahexaenoic acid (DHA, C22:6n-3) and arachidonic acid (AA, C20:4n-6) also significantly inhibited I_{NCX1}, whereas the saturated fatty acid, stearic acid (SA, C18:0), did not. Our data demonstrate that the n-3 PUFAs significantly suppress cardiac I_{NCX1}, which is probably one of their protective effects against lethal arrhythmias.

Keywords: Na^+/Ca^{2+} exchanger; Eicosapentaenoic acid; Docosahexaenoic acid; Arrhythmia; Human embryonic kidney cells

A major cause of death in patients with chronic heart failure is sudden cardiac death that is actually more common among patients with a less severe degree of the disease and accounts for more than half of total mortality from chronic heart failure [1,2]. Several studies have shown that NCX1 is elevated at the transcript, protein, and activity levels in several models of cardiac hypertrophy and/or heart failure [3–5]. Increased activity of NCX1 may contribute to cardiac arrhythmogenesis, particularly in its reverse mode. Delayed after-depolarizations (DADs) and early after-depolarizations (EADs) have been observed in failing hearts. In the rabbit and dog heart failure models, enhanced NCX1 activity may lead to the increased occurrence of DADs and triggered arrhythmias [6,7]. EADs, however, mainly result from the loss of repolarizing K^+ currents, whereas enhanced NCX currents are beneficial [8]. Intracellular Ca^{2+} overload is one of the major causes of ischemia-reperfusion injury and arrhythmias. In Langendorff-perfused whole rabbit hearts, the NCX1 inhibitor KB-R7943 almost completely blocked ventricular arrhythmias (tachycardia and fibrillation) associated with both ischemia and reperfusion, indicating a critical role for NCX1 in the events [9]. Therefore, it will be a great benefit to heart failure patients if the natural n-3 fish oil fatty acids inhibit the NCX1 currents.

We have found that the n-3 long-chain polyunsaturated fatty acids in fish oil, namely eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA), are anti-arrhythmic in animals [10–14] and probably in humans [15–19]. Because of the importance of NCX in the heart, it behooves us to learn if these fish oil fatty acids will modulate the actions of NCX, as this

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may be an important role of these fatty acids in their beneficial actions on the heart. Therefore, we have utilized the availability of the dog NCX1 cDNA [20] expressed in HEK293t cells to learn if polyunsaturated fatty acids specifically modulate the activity of NCX.

Materials and methods

Cell culture and transient transfection of NCX1 cDNA. HEK293t cells were cultured in Dulbecco's modified Eagle's medium (DMEM) according to the method described previously [21,22]. Cloned canine cardiac CNX1 cDNA of the Na^+/Ca^{2+} exchanger (generously provided by Dr. Philipson's laboratory) was transfected into HEK293t cells with a calcium phosphate precipitation method [21,22]. A reporter plasmid CD8-pih3m (1 μg, cell surface antigen) and NCX1 cDNA clone (3 μg) in the pcDNA1/amp vector were prepared in 250 mM $CaCl_2$, added to a test tube containing 0.36 ml HBS (2×) solution (in mM): 274 NaCl, 40 Hepes, 12 dextrose, and 10 KCl, 1.4 Na_2HPO_4, pH 7.05, and incubated at 22 °C for 20 min. The DNA solution was then dripped over a cell culture of a TI-25 flask containing 7 ml of the culture solution. The transfection was satisfactory under these conditions [22]. The transfected cells were trypsinized and replated 15 h later to an appropriate density in 35-mm tissue culture dishes (which also served as recording chambers) containing 2 ml of fresh DMEM. Transfected cells were incubated at 37 °C in air with 5% CO_2 added and 98% relative humidity and used within 4 days. Transfection-positive cells which were identified by binding immunobeads (CD8-Dynabeads M-450, Dynal A.S., Oslo, Norway) coated with a monoclonal antibody (ITI-5C2) specific for CD8 antigen were selected for patch-clamp experiments.

Recording of NCX1 currents. During an experiment HEK293t cells plated in a culture dish were continuously superfused (1–2 ml/min) with Tyrode's solution (in mM): NaCl 137, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1.5, Hepes 10, and glucose 10, pH 7.4. Recording glass electrodes had a resistance of 0.7–1.2 MΩ when filled with the pipette solution and were connected via Ag–AgCl wire to an Axopatch 200B amplifier (Axon Instruments, CA). Cells coated with CD-8 beads were chosen for patch clamps. After forming a conventional "Gigaseal" the capacitance of an electrode was compensated. Additional suction was used to form the whole-cell configuration. The capacitance (26.3 ± 1.2 pF, $n = 96$) of cells was measured with the pCLAMP software (Version 8.02, Axon Instruments, CA). Correction of cell capacitance and series resistance was performed before data collection. Experimental voltage-clamp protocols were similar to those used in other studies [23,24]. With a membrane potential at 0 mV, outward or inward I_{NCX1} was triggered by changing extracellular concentration of Ca^{2+} from 0 to 1 mM or Na^+ from 0 to 145 mM, respectively. The ramp NCX1 current was recorded by pulses from a holding potential of −40 mV to a 100-ms step depolarization to +60 mV, then to a descending voltage ramp to −100 mV (from +60 to −100 mV at 100 mV/s) and a step back to −40 mV. The descending portion of the ramp (from +60 to −100 mV) was used to plot the current–voltage (I–V) relation curve. The magnitudes of I_{NCX1} were measured at the voltages near +60 mV and −100 mV and compared in the absence or presence of fatty acids. External solutions with or without fatty acids were exchanged rapidly with a perfusion system described previously [21,22]. Experiments were conducted at 22–23 °C.

Materials and solutions. The experimental concentrations of fatty acids (Sigma, St. Louis, MO) were obtained by dilution of the stocks that were prepared weekly in ethanol at 10 mM and stored under a nitrogen atmosphere at −20 °C. The dilution contained negligible ethanol which had no effect on NCX1 currents. The pipette solution for recording the reverse (outward) exchange currents contained (in mM): NaCl 120, KCl 5, $MgCl_2$ 2, TEA-Cl 20, Hepes 10, glucose 8, Na_2ATP 1, pH 7.3, and EGTA 5 plus $CaCl_2$ 4.28 (1 μM free Ca^{2+}) and the bath solution contained (in mM): LiCl 145, $MgCl_2$ 2, TEA-OH 10, Hepes 10, and glucose 10, pH 7.4, with either 0.5 mM EGTA (<1 nM free Ca^{2+}) or 1 mM $CaCl_2$ [16]. The pipette solution for recording forward (inward) exchange currents contained (in mM): NaCl 20, CsOH 100, KCl 5, $MgCl_2$ 2, TEA-Cl 20, Hepes 10, glucose 8, Na_2ATP 1, pH 7.3, and EGTA 5 plus $CaCl_2$ 4.94 (5 μM free Ca^{2+}) and the bath solution contained (in mM): NaCl 145, $MgCl_2$ 1, $CaCl_2$ 1.0, EGTA 0.5, Hepes 10, and glucose 10, pH 7.4 (0.5 mM free Ca^{2+}). The pipette solution for recording ramp I_{NCX1} was the same as that for recording forward (inward) exchange currents and the bath solution contained (in mM): NaCl 137, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1.5, Hepes 10, and glucose 10, pH 7.4.

Data analysis and statistics. The amplitude of peak I_{NCX1} in forward or reverse mode was measured and analyzed. Current density, pA/pF, of I_{NCX1} of each cell was calculated by dividing the amplitude of peak current by the cell membrane capacitance. The normalized I_{NCX1} after the treatment with PUFAs was calculated as $I_{(PUFAs)}/I_{(Control)}$ from the same cell. The reversal potential of I_{NCX1} was calculated by the equation, $E_{NaCa} = 3E_{Na} - 2E_{Ca}$. Data are presented as means ± SEM and were analyzed by one-way analysis of variance (ANOVA) or by un-paired Student's t test. Statistical significance was set at the level of $P < 0.05$.

Results

Na^+/Ca^{2+} exchange currents in HEK293t cells transfected with NCX1 cDNA

To determine whether there was an endogenous I_{NCX1} in HEK293t cells, we recorded the outward whole-cell I_{NCX1} at a holding potential of 0 mV. Non-transfected HEK293t cells had no obvious outward currents when the external solution was switched from 0 mM $[Ca^{2+}]_o$ to 1 mM $[Ca^{2+}]_o$ (Fig. 1A, control cell). The average density of I_{NCX1} in the control HEK293t cells was 0.06 ± 0.02 pA/pF ($n = 16$, Fig. 1B, control). However, in the HEK293t cells with transfection of the canine NCX1 cDNA, significant outward currents were elicited when the extracellular solution was switched from 0 mM Ca^{2+} to 1 mM Ca^{2+} (Fig. 1A, NCX1-transfected cell). The average density of I_{NCX1} in the transfected HEK293t cells was 10.9 ± 2.6 pA/pF ($n = 44$, $P < 0.001$, versus the control; Fig. 1B). These results demonstrate that after NCX1-cDNA transfection, the HEK293t cells expressed significant amount of Na^+/Ca^{2+} exchange currents.

Inhibition of I_{NCX1} by EPA

Our previous data show that the n-3 polyunsaturated fatty acids (PUFAs) prevent ventricular arrhythmias in dogs [25] and inhibit Na^+, Ca^{2+}, and K^+ currents in isolated mammalian cardiomyocytes [26–28]. Cardiac Na^+/Ca^{2+} exchanger is the primary Ca^{2+} extrusion mechanism in the heart [29] and dramatically affects membrane action potentials [30]. To assess the effects of PUFAs on I_{NCX1}, we transfected the canine NCX1 cDNA into HEK293t cells. Fig. 2 shows that outward I_{NCX1} at a

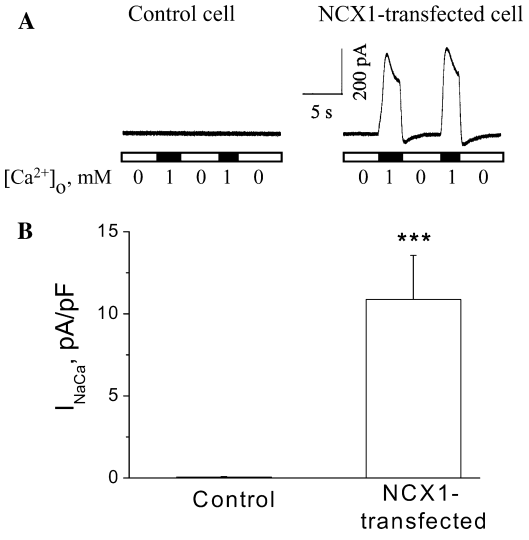

Fig. 1. Whole-cell Na^+/Ca^{2+} exchange current (I_{NCX1}) in HEK293t cells. (A) The original current traces recorded from a non-transfected cell (control cell) and a cell transfected with canine cardiac NCX1 cDNA (NCX1-transfected cell). The components of the pipette and bath solutions for recording outward I_{NCX1} were described in Materials and methods. To elicit outward I_{NCX1}, the 0 mM Ca^{2+}external solution was switched to one with 1 mM $[Ca^{2+}]_o$. The membrane potential was held at 0 mV. (B) The averaged amplitudes of peak outward I_{NCX1} in the control ($n=16$) and NCX1-transfected ($n=44$) HEK293t cells. Current was normalized to the capacitance of each individual cell. ***$P<0.001$; versus control.

holding potential of 0 mV was elicited by switching the external solution from 0 mM $[Ca^{2+}]_o$ to 1 mM $[Ca^{2+}]_o$ in a control (panel A) and an EPA-treated (panel B) HEK293t cells. In the absence of EPA, outward I_{NCX1} did not show a significant rundown during the 5-min observation. In the presence of 30 μM EPA, however, outward I_{NCX1} was significantly inhibited (Fig. 2B). The inhibition of outward I_{NCX1} initiated within 30 s and reached the maximal effect in 4 min after external application of 30 μM EPA. Outward I_{NCX1} recovered toward the control level after washout of EPA with the bath solution containing 0.2% bovine serum albumin (Figs. 2B and C).

Inhibition of I_{NCX1} by EPA was concentration-dependent. Fig. 3 shows the inhibition of outward I_{NCX1} in NCX1-transfected HEK293t cells treated with different concentrations of EPA. The IC_{50} of EPA for $I_{NCX1}$was 0.82 ± 0.27 μM. These results indicate that EPA is a significant and highly potent inhibitor of the outward current of cardiac Na^+/Ca^{2+} exchangers.

EPA-induced inhibition of I_{NCX1} in both forward and reverse modes

As our above results show that EPA significantly inhibited the outward current, a reverse mode of I_{NCX1} (Figs. 1–3), we applied a ramp protocol (upper panels in Figs. 4A and B) to elicit voltage-dependent I_{NCX1} and to assess whether EPA inhibited both reverse (outward current) and forward (inward current) modes of the current. Fig. 4A shows that the amplitude of the control ramp-activated I_{NCX1} was significantly reduced in the presence of 5 μM EPA (Fig. 4A, EPA). The inhibition initiated within 20 s and reached the maximal effect around 4 min after external application of 5 μM EPA. EPA at 5 μM reduced I_{NCX1} by 82.5 ± 2.3% ($n=13$, $P<0.001$) for the reverse mode and 80.0 ± 3.4% ($n=13$, $P<0.01$) for the forward mode in NCX1-transfected HEK293t cells (Fig. 4B). Recovery of I_{NCX1} toward the control level was observed after washout of EPA for 3–5 min with the external solution containing 0.2% bovine fetal albumin (Figs. 4A and B, washout).

To evaluate the effect of the NCX blocker nickel on I_{NCX1}, we added 5 mM $NiCl_2$ into the extracellular solution. I_{NCX1} was significantly inhibited after the perfusion with Ni^{2+} in NCX1-transfected HEK293t cells (Fig. 4C). $NiCl_2$ at 5 mM inhibited I_{NCX1} by 90.0 ± 2.6% ($n=9$, $P<0.001$) for the reverse mode and 87.8 ± 2.9% ($n=9$, $P<0.01$) for the forward mode of NCX1 (Fig. 4D). The inhibited current was returned to the control level after washout of Ni^{2+}. Compared with the effect of EPA on I_{NCX1}, Ni^{2+} produced a similar level of inhibition, but more recovery of the inhibited I_{NCX1} was observed in HEK293t cells treated with 5 mM Ni^{2+} than with 5 μM EPA (Figs. 4B and D).

To further test the effects of EPA on the forward mode of I_{NCX1} (inward), we held the membrane potential at 0 mV and switched the external solution from 0 mM $[Na^+]_o$ to 145 mM $[Na^+]_o$ in NCX1-transfected HEK293t cells. Fig. 5 shows that a significant inward current was elicited when the extracellular solution was switched from 0 to 145 mM Na^+ (Fig. 5A). The amplitude of inward I_{NCX1} was significantly inhibited after extracellular application of 10 μM EPA (Fig. 5B). The inhibition of inward I_{NCX1} initiated within 30 s and reached the maximal effect in 4 min after extracellular perfusion of 10 μM EPA. The average inhibition of inward I_{NCX1} caused by 10 μM EPA was 77.8 ± 5.0% (Fig. 5C, $n=13$, $P<0.001$), which was similar to that (88.9 ± 4.9%, Fig. 3) of outward I_{NCX1} in the presence of 10 μM EPA. Significant recovery of inhibited inward I_{NCX1} by EPA was observed after washout of EPA with the external solution containing 0.2% bovine fetal albumin (Fig. 5C, 71.8 ± 24.5%).

Effects of other fatty acids on I_{NCX1}

To evaluate the effects of other polyunsaturated or saturated fatty acids on I_{NCX1}, docosahexaenoic acid (DHA, C22:6n-3), arachidonic acid (AA, C20:4n-6), and stearic acid (SA, C18:0) were applied to NCX1-transfected HEK293t cells. Fig. 6 shows that other polyunsaturated fatty acids also significantly reduced outward I_{NCX1} in HEK293t cells transfected with

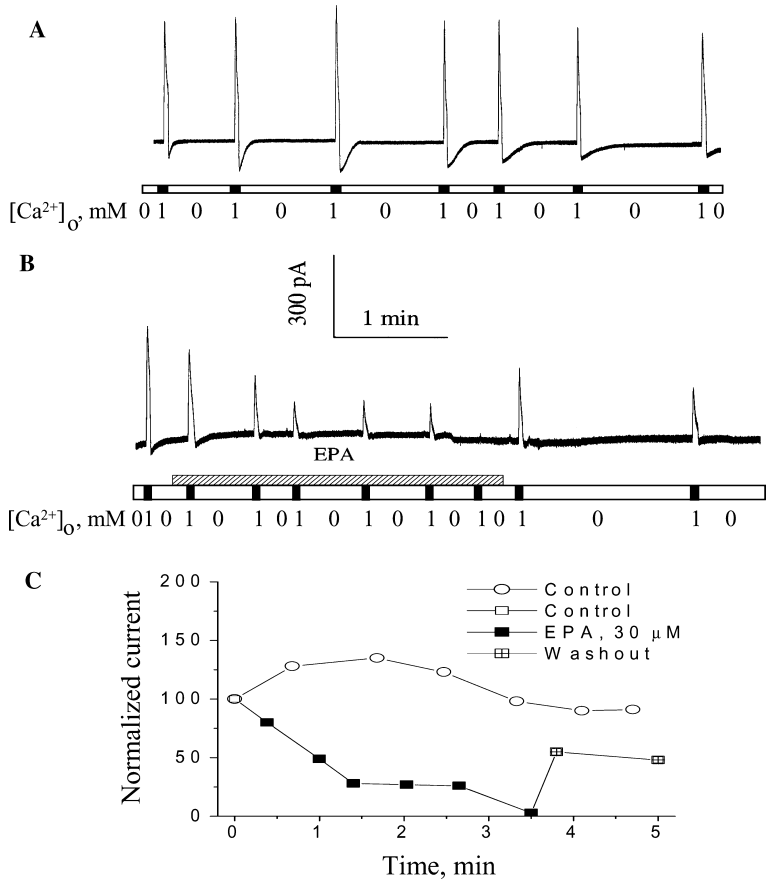

Fig. 2. Inhibition of outward I_{NCX1} by EPA in HEK293t cells transfected with cardiac NCX1 cDNA. The original current traces were recorded from the NCX1-transfected cells in the absence (A) and presence (B) of 30 μM EPA. Outward I_{NCX1} was elicited at a holding potential of 0 mV by switching the external solution from 0 mM $[Ca^{2+}]_o$ to 1 mM $[Ca^{2+}]_o$ as described in Fig. 1. Outward I_{NCX1} did not significantly run down during 5-min observation (A). However, I_{NCX1} was significantly inhibited in the presence of 30 μM EPA (B). (C) The time course of peak outward I_{NCX1} recorded from the NCX1-transfected HEK293t cells shown in (A,B). The values of outward I_{NCX1} in (C) were normalized to their corresponding control amplitudes. Recovery toward the control level was observed after washout of EPA with the bath solution containing 0.2% bovine serum albumin.

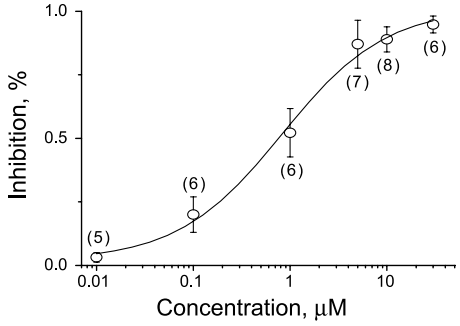

Fig. 3. Concentration-dependent inhibition of outward I_{NCX1} by EPA in NCX1-transfected HEK293t cells. In parentheses are the numbers of individual cells treated with different concentrations of EPA. Outward I_{NCX1} was elicited with the method described in the legend of Fig. 1 and peak I_{NCX1} was measured and analyzed.

NCX1 cDNA. DHA and AA at 10 μM inhibited I_{NCX1} by 96.3 ± 1.5% ($n = 6$, $P < 0.001$) and 74.1 ± 11.1% ($n = 9$, $P < 0.05$), respectively. In contrast, SA at 10 μM did not significantly alter outward I_{NCX1} (6.9 ± 17.5% inhibition, $n = 5$, $P > 0.05$). These results indicate that PUFAs could significantly and specifically inhibit I_{NCX1} in NCX1-transfected HEK293t cells.

Discussion

The results clearly demonstrate that the n-3 polyunsaturated fatty acids in fish oils modulate the function of NCX1 and that this is a very potent action of these fatty acids, $IC_{50} = 0.82 \pm 0.27$ μM for I_{NCX1} in HEK293t cells with transfection of canine NCX1 cDNA, $P < 0.001$. With increasing concentrations of EPA a typical sigmoid inhibition curve was created (see Fig. 3). But we found that EPA inhibited I_{NCX1} not only when it was operating in the forward mode. The inhibition was equally great when I_{NCX1} was operating in the reverse mode. The NCX currents were inhibited by the polyunsaturated fatty acids EPA, DHA, also by

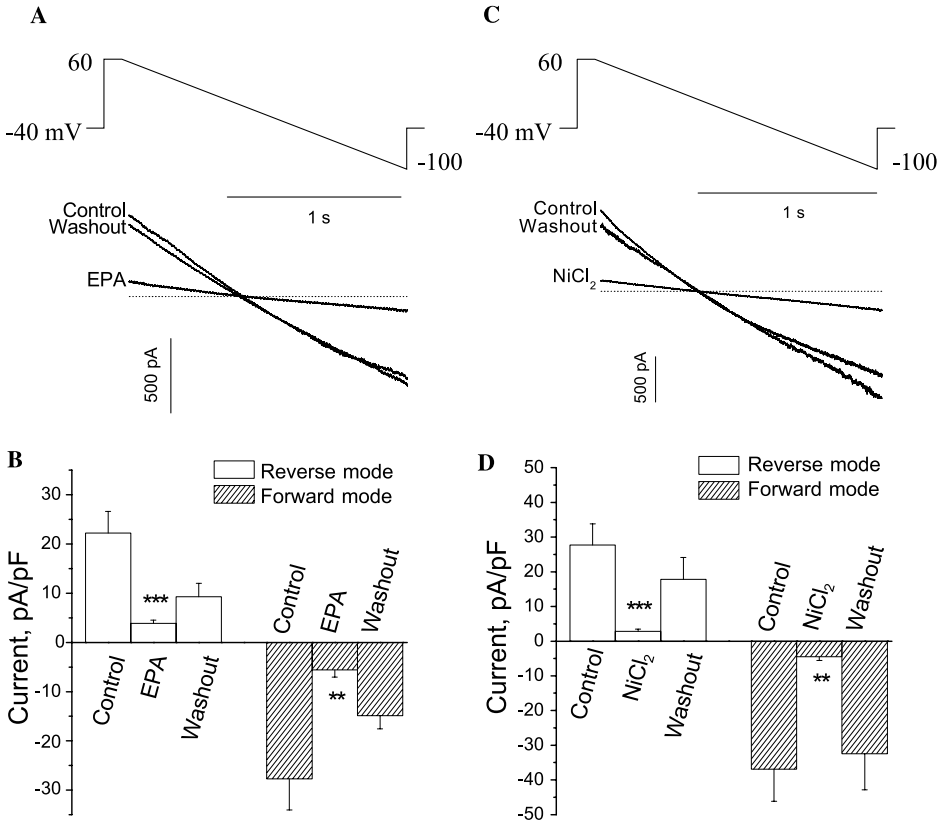

Fig. 4. Inhibition of voltage ramp-activated I_{NCX1} by EPA in HEK293t cells. (A) The ramp protocol (upper panel) and original current traces in the absence (control and washout) or presence (EPA) of 5 μM EPA in a NCX1-transfected HEK293t cell. The pipette and bath solutions for recording ramp I_{NCX1} (see the Materials and methods) were used. The ramp protocol was composed of a 100-ms step depolarization from a holding potential of −40 to +60 mV and a descending voltage ramp to −100 mV (at 100 mV/s). The dotted lines in (A,B) are the level of 0 pA. (B) The averaged amplitudes of I_{NCX1} in the absence or presence of 5 μM EPA in the NCX1-transfected HEK293t cells ($n=13$). The magnitudes of I_{NCX1} were measured and analyzed at the voltages near +60 and −100 mV. Current was normalized to the capacitance of each individual cell. (C) The original current traces in the absence (control and washout) or presence ($NiCl_2$) of 5 mM Ni^{2+} in a NCX1-transfected HEK293t cell. The pipette solution, bath solution, and ramp protocol were the same as those used for the recordings of I_{NCX1} in (A). (D) The averaged amplitudes of I_{NCX1} in the absence or presence of 5 mM Ni^{2+} in the NCX1-transfected HEK293t cells ($n=9$). $^{**}P<0.01$; $^{***}P<0.001$; versus control.

the n-6 arachidonic acid (AA), but not by the saturated fatty acid, stearic acid. It had been shown [31] that polyunsaturated fatty acids of both the n-3 and n-6 classes are antiarrhythmic, but AA proved to be anomalous. In about a third of the experiments it proved to be proarrhythmic. However, the arrhythmias were not the direct result of the AA, but rather were caused by cyclooxygenase products of AA. When cycloxygenase was inhibited by indomethacin, the AA was only antiarrhythmic. Later it was shown [32] that the prostaglandins formed from AA were all proarrhythmic, whereas the prostaglandins formed from EPA were not. This is the reason we have stressed the importance of the n-3 fish oil fatty acids for clinical use and not the n-6 fatty acids.

We had previously shown that the n-3 fish oil fatty acids were potent inhibitors of the L-type calcium current ($I_{Ca,L}$) [27]. We had been assuming that the action of these fatty acids in preventing triggered non-reentrant ventricular arrhythmias resulting from delayed after potentials was based on the action of the fatty acids on $I_{Ca,L}$. But the effect of the n-3 fish oil fatty acids on inhibiting I_{NCX} is much too potent to ignore. The effect of these fatty acids to prevent fatal arrhythmias was clearly masking their effect on I_{NCX1}, as well. Perhaps the effects of these fatty acids combine their actions on both $I_{Ca,L}$ and on I_{NCX1} to produce the beneficial action of these fatty acids to prevent fatal ventricular arrhythmias. How much either effect might contribute to the beneficial action, we do not now know.

There is a similar issue now with the actions of the fatty acids on the cardiac toxicity from digitalis-like glycosides. One of our earliest experiments with the n-3 fish oil fatty acids showed they prevented ouabain-induced arrhythmias in neonatal rat cardiomyocytes [33]. After we found that the fish oil fatty acids potently inhibited the L-type Ca^{2+} channel currents, the prevention of cardioglycoside toxicity was attributed to inhibition of $I_{Ca,L}$. But it is equally logical to attribute this to inhibi-

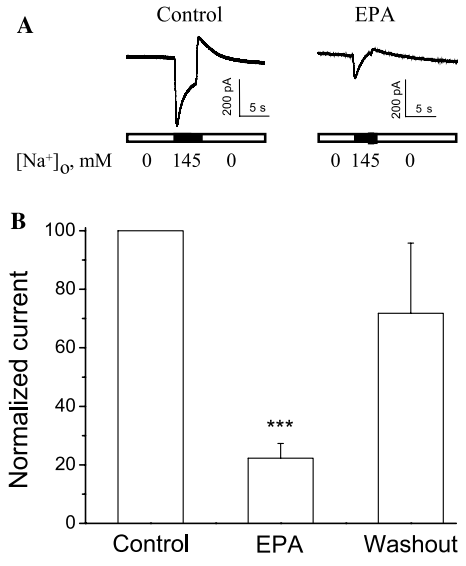

Fig. 5. Inhibition of inward (forward mode) I_{NCX1} by EPA in HEK293t cells. (A) The original current traces in the absence (control) and presence (EPA) of 10 μM EPA in a NCX1-transfected HEK293t cell. The membrane potential was held at 0 mV. The pipette and bath solutions for recording inward I_{NCX1} (see Materials and methods) were used. To elicit inward I_{NCX1}, the external solution was switched from 0 mM $[Na^+]_o$ to 145 mM $[Na^+]_o$. (B) The inhibition of peak inward I_{NCX1} in the absence or presence of 10 μM EPA in the NCX1-transfected HEK293t cells ($n = 13$). Currents were normalized to their control values of individual cells. ***$P < 0.001$; versus control.

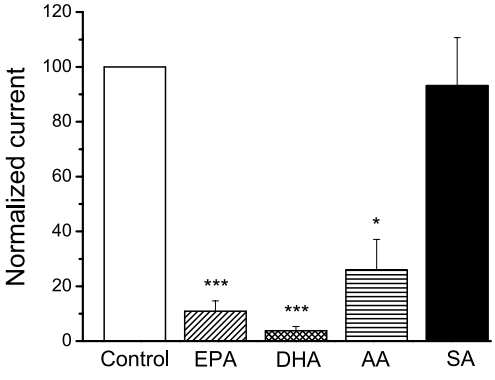

Fig. 6. Effects of other fatty acids on outward I_{NCX1} in NCX1-transfected HEK293t cells. Outward I_{NCX1} was elicited by the method described as in the legend of Fig. 1. The amplitude of peak outward I_{NCX1} was measured and compared. Normalized current was calculated by $I_{EPA}/I_{control}$. Each fatty acid at 10 μM was applied to the external solution. EPA, eicosapentaenoic acid ($n = 8$); DHA, docosahexaenoic acid ($n = 6$); AA, arachidonic acid ($n = 9$); and SA, stearic acid ($n = 5$). *$P < 0.05$; ***$P < 0.001$; versus the control.

tion of I_{NCX}. Indeed, it is generally accepted that cardioglycoside toxicity is caused by the action of Na^+/Ca^{2+} exchangers.

With the occurrence of a myocardial infarction there occurs a zone of ischemia. Cells in the periphery of the ischemic zone will be partially depolarized, but viable. Such partially depolarized cells are potentially proarrhythmic, as any further small depolarization can initiate an action potential, which may induce an arrhythmia. Ischemia has been shown to be associated with an increased concentration of Na^+ in the myocytes. This has been attributed to two factors: (1) Inhibition of the Na–K-ATPase sodium pump because of lack of oxygen and substrates will directly lead to increased $[Na^+]_i$. (2) Ischemia is associated with intracellular acidosis due to lactic acid accumulation. The increased intracellular H^+ has been shown to exchange for extracellular Na^+[34], which would further increase intracellular Na^+. This elevated intracellular Na^+ and the decreased membrane potential in the partially depolarized ischemic myocyte should favor removal of the high Na^+ in exchange for Ca^{2+} entering the myocyte via the NCX operating in the reverse mode. It seems that n-3 fish oil would act to reduce intracellular calcium, and thereby help to prevent overload of cytosolic Ca^{2+} and triggered arrhythmias. If so, this might act to complement the action of the PUFAs to inhibit the L-type calcium current, which accomplishes the same beneficial antiarrhythmic action [27].

It seems that any cardiac dysfunction which resulted in prolonged action potentials would enhance the opportunity for NCX operating in reverse mode to increase $[Ca^{2+}]_i$ and promote triggered cardiac arrhythmias. Just such conditions may occur in certain long QT syndrome-associated mutations of SCN5A and lead to maintained depolarizing currents and action potential prolongation, setting up a substrate for arrhythmias [35]. These mutant Na^+ channels can reopen during the plateau of the action potential. The effect is to maintain a small depolarizing I_{Na} to persist during the plateau phase and thus prolong the action potential duration [36]. Again the prolonged action potential with the depolarized state of the membrane potential would favor action of NCX in its reverse mode causing $[Ca^{2+}]_i$ overload and possible triggered arrhythmias, which can be prevented by the n-3 fatty acids.

A recent study [37] reported the effects of the open versus closed states of the gap junctures in the myocardium during reperfusion injury. Blockade of L-type Ca^{2+} channels did not modify propagation of high Ca^{2+} between adjacent cells with contracture and death of myocytes, but inhibition of the Na^+/Ca^{2+} exchanger in its reverse mode did. It seems that the n-3 PUFAs might have therapeutic benefit by suppressing myocardial damage from reperfusion injury.

It has been pointed out [38] that the lipid environment surrounding the Na^+/Ca^{2+} exchanger may also be modified during ischemia and affect the transporter. The accumulation of lysophosphatidyl-choline early following myocardial infarction increases sarcolemmal permeability to Ca^{2+}has been shown to cause arrhythmias [39] and was shown to influence Na^+/Ca^{2+} exchange

activity [40]. Other changes in the sarcolemmal lipids and fatty acid composition may have secondary effects on the Na^+/Ca^{2+} exchanger [40,41].

There is increasing interest for a possible role of Na^+/Ca^{2+} exchange in the pathophysiology of the failing heart and numerous studies have been recently reported. Whether in this failing state of the heart or in other cardiac conditions the finding that the Na^+/Ca^{2+} exchanger is strongly inhibited in both its forward and reverse modes of operation by n-3 polyunsaturated fatty acids will help in the understanding or treatment of these maladies remains to be seen.

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