Potent block of inactivation-deficient Na\(^+\) channels by n-3 polyunsaturated fatty acids

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The activation (i.e., opening) of inwardly rectifying voltage-gated Na\(^+\) channels initiates the action potential in heart and other excitable tissues. The intracellular linker between domains 3 and 4 is essential for the fast inactivation (i.e., closing) of the Na\(^+\) channel, and deletion of this region causes persistence of a Na\(^+\) current (I\(_{Na}\)) during depolarization (2). The mutation IFM/QQQ in the linker between the third and fourth domains disables inactivation of Na\(^+\) channels (6, 16). IFM/QQQ expressed in human embryonic kidney (HEK)-293 cells has been used to test several clinically relevant Na\(^+\) channel blockers (6). However, we found that expression of this mutant (especially the persistent portion of I\(_{Na}\)) in HEK-293t cells was poor, which made it difficult to obtain meaningful results. We have been studying experimentally the antiarrhythmic action of the n-3 polyunsaturated fatty acids (PUFAs) in fish oils on cardiomyocytes and found that they were able to modulate the voltage-gated I\(_{Na}\). The PUFAs significantly enhance the transition of cardiac Na\(^+\) channels into the inactivation state and markedly shift the steady-state inactivation curve to the hyperpolarizing direction (19). These effects may eliminate the potential proarrhythmic effects of partially depolarized myocytes in ischemic cardiac tissues and prevent arrhythmias as we showed previously (19). With this strong effect on the inactivated state of heart cells in mind, we were interested in examining what effect, if any, the n-3 PUFAs would possess in inactivation-deficient cardiac Na\(^+\) channels. The usual preparation of the Na\(^+\) ion channel deficient in inactivation is the IFM3Q mutant; however, we found that the IFM3Q mutant expressed in HEK-293t cells exhibited I\(_{Na}\) that were too small to permit reliable measurement of I\(_{Na}\). We therefore investigated the effect of the PUFAs on persistent I\(_{Na}\) in HEK-293t cells transfected in the Na\(^+\) inactivation-deficient mutant L409C/A410W of the Na\(^+\) channel. The PUFAs significantly enhance the I\(_{Na}\) peak by 0.4 × 0.3 μM for I\(_{Na}\) peak (I\(_{Na}\) peak) and 0.9 ± 0.1 μM for I\(_{Na}\) late. EPA shifted the steady-state inactivation of I\(_{Na}\) peak by −19 mV in the hyperpolarizing direction. EPA accelerated the process of resting inactivation of the mutant channel and delayed the recovery of the mutated Na\(^+\) channel from resting inactivation.

Other polyunsaturated fatty acids, docosahexaenoic acid, linolenic acid, arachidonic acid, and linoleic acid, all at 5 μM concentration, also significantly inhibited I\(_{Na}\). In contrast, the monounsaturated fatty acid oleic acid or the saturated fatty acids stearic acid and palmitic acid at 5 μM concentration had no effect on I\(_{Na}\). Our data demonstrate that the double mutations at the 409 and 410 sites in the D1–S6 region of hH1,3 induce inactivation-deficient I\(_{Na}\) and that n-3 PUFAs inhibit mutant I\(_{Na}\).

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MATERIALS AND METHODS

Cell culture and transfection of cardiac Na\textsuperscript{+} channels. HEK-293t cells were cultured in DMEM containing 10% FBS, 1% penicillin and streptomycin solution, 3 mM taurine, and 25 mM HEPES as previously described (20). Cells were split twice per week. When HEK-293t cells were grown to ~50% confluence, transfection of the wild-type cardiac Na\textsuperscript{+} channel (hNav1.5; 4 \textmu}g) or a mutant (3 \textmu}g) of the \alpha-subunit of the human cardiac Na\textsuperscript{+} channel (hH1) plus the rat Na\textsuperscript{+} channel \beta1-subunit (20 \mu)g) and CD8 cDNA (1 \mu)g) was performed using a calcium phosphate precipitation method (20, 21). Expression of Na\textsuperscript{+} channels was adequate for current recording. The transfected cells were replated 15 h after transfection in 35-mm dishes (which also served as recording chambers) and were incubated at 37°C in a 5% CO\textsubscript{2} incubator. Transfection-positive cells were identified using immunobeads (CD8-Dynabeads M-450; Dynal, Oslo, Norway).

Recording of cardiac I\textsubscript{Na}. HEK-293t cells coated with CD8 beads were chosen for patch-clamp studies. The pipette solution contained (in mM) 30 NaCl, 100 CsOH, 1 MgCl\textsubscript{2}, 2 CaCl\textsubscript{2}, 11 EGTA, 5 MgATP, and 10 HEPES, pH 7.3 with CsOH. The bath solution contained (in mM) 30 NaCl, 100 N-methyl-D-glucamine, 1 MgCl\textsubscript{2}, 2 CaCl\textsubscript{2}, 10 HEPES, and 10 glucose (pH adjusted to 7.4 with HCl). Glass electrodes (World Precision Instruments, Sarasota, FL) had a resistance of ~1 M\textOmega when filled with the pipette solution. Whole cell current was recorded according to experimental protocols similar to those used in our previous study (20). Fatty acids (Sigma) were dissolved weekly in 100% ethanol at 10 mM concentration and stored in a nitrogen atmosphere at –20°C before use. The experimental concentration of fatty acids was obtained by diluting the stocks and contained a negligible amount of ethanol, which alone had no effect on the mutated I\textsubscript{Na}. Extracellular solution with various concentrations of fatty acids was exchanged with a rapid perfusion system (18). Experiments were conducted at 22–23°C.

Statistical analysis. I\textsubscript{Na} values were measured at the points of maximal activated current (I\textsubscript{Na peak}) and residual current near the end of each test pulse (I\textsubscript{Na late}). Activation and steady-state inactivation curves were fitted using a Boltzmann equation, {1/[1 + exp((V\textsubscript{m} – V\textsubscript{0})/k)]}, in which V\textsubscript{m} is the midpoint voltage of the function and k is the slope factor (in mV/\textdegree change in current). Concentration-dependent data were fitted using a logistical equation, {([A\textsubscript{1} – A\textsubscript{2}]/[1 + (x/x\textsubscript{0})\textsuperscript{p} + A\textsubscript{2}])}, in which x\textsubscript{0} is the center, p is power, A\textsubscript{1} is initial y-axis value, and A\textsubscript{2} is final y-axis value. The time constant (\tau) of inactivation was analyzed using least-squares fitting (y = A\textsubscript{0} + A\textsubscript{1}exp(-t/\tau)) (Origin version 6.0 software; Microcal Software, Northampton, MA) with a single exponential function. Data are presented as means ± SE. Results derived from two groups were analyzed using the unpaired Student’s t-test. Statistical differences among the results obtained from three or more experimental groups were determined using ANOVA. P < 0.05 was set as the level for statistical significance.

RESULTS

Voltage-gated, inactivation-deficient I\textsubscript{Na}. Voltage-activated, persistent I\textsubscript{Na} with fast activation and incomplete inactivation were evoked using depolarizing pulses from –90 mV to 50 mV in HEK-293t cells transiently transfected with the mutant L409C/A410W of hH1\textsubscript{α} plus \beta1-subunit (L409C/A410W + \beta1) (Fig. 1A). More than 65% of persistent I\textsubscript{Na} were observed at the end of 400-ms test pulses in HEK-293t cells transfected with inactivation-deficient mutants plus \beta1-subunits, whereas...
wild-type \( I_{Na} \) were almost completely inactivated at the end of 40-ms test pulses (wild-type \( \alpha + \beta_1 \)) (Fig. 1A). \( I_{Na} \) were activated at approximately \(-60 \) mV and reached maximal amplitude at \(-30 \) mV for both the mutant and wild-type hH1. To compare the current-voltage relationships between the mutant and the wild-type cardiac Na\(^+\) channels, the peak \( I_{Na} \) amplitudes were normalized to their corresponding maximal currents and plotted against different voltages. Figure 1B shows the similarities in the current-voltage relationship curves of the inactivation-deficient mutant (\( n = 15 \)) and the wild type (\( n = 7 \)) of hH1\(_{\alpha}\) plus \( \beta_1 \)-subunits. Normalized whole cell activation conductance curves calculated from peak \( \beta_{Na} \) remained comparable between the L409C/A410W mutant and wild-type hH1\(_{\alpha}\) (Fig. 1C). The average \( V_{1/2} \) and \( k \) (slope) values for the fitted functions were \(-42.2 \pm 0.17 \) mV and \( 8.6 \pm 0.30 \) mV, respectively, for the mutant (\( n = 15 \)) and \(-43.0 \pm 0.11 \) mV and \( 6.1 \pm 0.09 \) mV, respectively, for the wild type (\( n = 7 \)) \((P > 0.05)\). These results demonstrate that the double mutations at the 409 and 410 sites in the D1–S6 region of hH1\(_{\alpha}\) +\( \beta_1 \)-subunits. Extracellular application of \( 5 \) \( \mu \)M EPA significantly inhibited both \( I_{Na} \) peak and \( I_{Na} \) late within \( 10 \) s and reached the maximal effect within \( 7 \) min (Fig. 3). \( I_{Na} \) returned to the pretreatment level after washout of EPA with \( 0.2\% \) fatty acid-free BSA solution. Figure 3 shows the time course of inhibitory effects of \( 5 \) \( \mu \)M EPA on \( I_{Na} \) peak and \( I_{Na} \) late in a HEK-293t cell expressing L409C/A410W plus \( \beta_1 \)-subunits. \( I_{Na} \) late was more sensitive to the inhibitory effect of EPA and was almost completely inhibited, whereas \( I_{Na} \) peak was inhibited by \( 60\% \).

The inhibitory effect of EPA on the mutant channel was concentration dependent. The IC\(_{50}\) of EPA for \( I_{Na} \) peak of the inactivation-deficient mutant and wild-type hH1\(_{\alpha}\) +\( \beta_1 \)-subunits in HEK-293t cells was similar: \( 4.0 \pm 0.4 \) \( \mu \)M for L409C/A410W and \( 3.9 \pm 0.3 \) \( \mu \)M for the wild type, respectively. However, \( I_{Na} \) late of the mutant was more sensitive to EPA, with IC\(_{50}\) of \( 0.9 \pm 0.1 \) \( \mu \)M (Fig. 4).

Effects of EPA on activation and inactivation of \( I_{Na} \). To evaluate the effects of EPA on the activation of the mutant channel, \( I_{Na} \) were activated in the absence or presence of \( 5 \) \( \mu \)M EPA (Fig. 5). \( I_{Na} \) peak was profoundly inhibited, and \( I_{Na} \) late was almost completely suppressed (Fig. 5B). The inhibition was reversible after washout of EPA with bath solution containing \( 0.2\% \) BSA (Fig. 5C). The current-voltage relationship of \( I_{Na} \) peak (Fig. 5D) or \( I_{Na} \) late (Fig. 5F) was not altered in the presence of \( 5 \) \( \mu \)M EPA. The current traces of L409C/A410W showed phenotypic restoration of the inactivation property in the presence of EPA (Fig. 5B). The activation curves of \( I_{Na} \) peak were calculated from normalized conductance and were superimposed in the absence or presence of \( 5 \) \( \mu \)M EPA \((n = 8); P > 0.05)\).

**Fig. 2.** Steady-state inactivation of \( I_{Na} \) in HEK-293t cells transfected with either the inactivation-deficient mutant or wild-type hH1\(_{\alpha}\) plus \( \beta_1 \)-subunits. Superimposed original \( I_{Na} \) traces for mutant (A, L409C/A410W + \( \beta_1 \)) and wild-type Na\(^+\) channels (B; wild type + \( \beta_1 \)) elicited by 200-ms (mutant) or 10-ms test pulse (wild type) to \(-30 \) mV after 500-ms conditional prepulses that were varied from \(-160 \) to \(+80 \) mV (mutant) in 10-mV increments or to \(-40 \) mV (wild type) in 5-mV increments. The membrane potential of the cells was held at \(-90 \) mV, and the pulse rate was 0.1 Hz. A, inset, voltage-pulse protocol. Dotted lines in A and B represent zero current. C: normalized peak currents of fast steady-state inactivation averaged for mutant (\( n = 17 \)) and wild-type Na\(^+\) channels (\( n = 9 \)). Data were fitted using a Boltzmann equation.
The 50% channel availability of activation data were 43.3 ± 0.28 mV with a $k$ value of 7.1 ± 0.31 mV for the control and 42.6 ± 0.15 mV with a $k$ value of 6.8 ± 0.12 mV for 5 M EPA.

Figure 6 shows the effects of EPA on $I_{Na}$ inactivation for HEK-293t cells transfected with the wild-type or inactivation-deficient mutant of hH1 Na$^+$ channels (Fig. 6) ($n$ = 19). The inactivation of the mutated currents elicited by pulses in a range from 25 mV to 40 mV were significantly reduced in the presence of 5 M EPA ($n$ = 12). The decreased inactivation $\tau$ of $I_{Na}$ of the mutant in the presence of EPA, however, were still much greater than those of wild-type $I_{Na}$. The effects of 5 M EPA on the inactivation were not obvious for wild-type $I_{Na}$ ($n$ = 6). These results suggest that EPA enhances phenotypic inactivation of inactivation-deficient Na$^+$ channels but not that of the wild type.

To evaluate the effects of EPA on fast steady-state inactivation of mutant, persistent $I_{Na}$ were elicited using a double-pulse protocol (Fig. 7, A and B). The steady-state inactivation curve in the absence of EPA showed the nonactivated persistent portion (~25%) of $I_{Na}$ peak with prepulses depolarized...
significantly shifted the steady-state inactivation of BSA solution, the steady-state inactivation curve was shifted to a mV level, whereas the activation conductance of activated persistent portion (Fig. 7, B). Extracellular perfusion of 5 μM EPA on I_Na late measured near end of each pulse in control (C) and EPA conditions (A). I_Na late was inhibited almost completely by 5 μM EPA (B and F), whereas I_Na peak was inhibited ~50% (B and D). Data in E were fitted using a Boltzmann equation.

above ~50 mV, which inactivated all wild-type Na^+ channels (Fig. 2C). Extracellular perfusion of 5 μM EPA significantly reduced I_Na peak, including complete inhibition of the noninactivated persistent portion (Fig. 7, B and C). The normalized steady-state inactivation curve of I_Na peak was significantly shifted to the negative direction in the presence of 5 μM EPA. The V_0.5 of the steady-state inactivation curve was shifted from −90.3 ± 1.7 mV for the control (k = 9.6 ± 1.1 mV, n = 16) to −109.3 ± 0.5 mV for EPA (k = 10.1 ± 0.4 mV, n = 9) (P < 0.001). After washout of EPA with 0.2% fatty acid-free BSA solution, the steady-state inactivation curve was shifted back toward the control. These results demonstrate that EPA significantly shifted the steady-state inactivation of I_Na peak by ~19 mV, which is similar to our previous finding of a ~22-mV shift for the wild-type hH1α Na^+ channel (20). In addition, EPA eliminates the noninactivated persistent portion of the steady-state inactivation curve of the mutant channel.

Development of resting inactivation of I_Na. Resting inactivation of voltage-gated cardiac Na^+ channels is referred to as direct transition of the resting state to the inactivated state without opening of the channel (3, 7, 9). To assess the effects of L409C/A410W double mutations on the development of resting inactivation of the mutant (Fig. 8A), we selected ~65 mV as the conditioning voltage because this depolarization level was enough to inactivate the channels with minimal channel activation. Figure 8B shows that the amplitudes of I_Na dramatically decreased as the duration (Δt) of conditioning pulses was prolonged, indicating that an increasing proportion of channels was entering the inactivated state. However, an ~50% portion of I_Na was not inactivated even with the longest conditioning pulse tested, 120 ms (Fig. 8C) (n = 5), at which the I_Na of wild-type hH1α Na^+ channels were completely inactivated (Fig. 8C). Our results indicate that the L409C/A410W mutant of the hH1α Na^+ channel significantly alters the development of resting inactivation and induces a significant portion of noninactivated currents.

To assess the effects of EPA on the development of resting inactivation of the L409C/A410W mutant, the same conditioning pulses as those described above were applied. In the presence of 5 μM EPA, increases in the duration of conditioning pulses enhanced the portion of mutant channels into a resting inactivated state (Fig. 8B), whereas the fitting slope (Fig. 8C) was similar to that found in the absence of EPA (Fig. 8C). Compared with control, only ~15% of mutant channels in the presence of EPA were not inactivated when the duration of the conditioning pulse was set at 120 ms (Fig. 8C). The slope of the resting inactivation of the mutant was superimposed with that of the wild-type Na^+ channel in the presence of 5 μM EPA (Fig. 8C), except for the noninactivated portion of the mutant. The data suggest that the double mutations of hH1α result in incomplete resting inactivation of the channel and that EPA decreases the noninactivated portion of the current.
Delayed recovery from inactivation of \( I_Na \) by EPA. To determine whether the mutations at the 409 and 410 sites of hH1\( _{\alpha} \) affect recovery from resting inactivation, the available currents elicited by 50-ms test pulses to \(-30\) mV were measured (Fig. 9, A and B). The \( \Delta \) recovery from inactivation of \( I_Na \) was fitted using a single exponential function (Fig. 9C). The \( \tau \) for recovery from inactivation of the mutant current was 600.7 \pm 48.0 ms for control (\( A_1 = -1.04 \)) (Fig. 9C) and 927.1 \pm 54 ms for 5 \( \mu \)M EPA (\( A_1 = -1.05 \)) (Fig. 9C) \( n = 5; P < 0.01 \). Compared with the mutant, the \( \tau \) values for wild-type hH1\( _{\alpha} \) \( Na^+ \) channels were significantly \( (P < 0.01) \) smaller: 10.8 \pm 1.8 ms for control (\( A_1 = -0.96 \)) (Fig. 9C) and 120.0 \pm 13.6 ms for 5 \( \mu \)M EPA (\( A_1 = -0.81 \)) (Fig. 9C) \( n = 8 \). These results indicate that the mutant of L409C/A410W delays recovery from resting inactivation and that EPA further slows recovery.

Effects of other fatty acids on \( I_Na \). To evaluate the effects of other saturated or unsaturated fatty acids on mutant channels, docosahexaenoic acid (DHA; C22:6n-3, \( n = 10 \)), linolenic acid (LNA; C18:3n-3, \( n = 6 \)), arachidonic acid (AA; 20:4n-6, \( n = 12 \)), and linoleic acid (LA; C18:2n-6, \( n = 5 \)) were evaluated in HEK-293t cells transfected with the inactivation-deficient mutant of hH1\( _{\alpha} \) plus \( \beta_1 \)-subunits. Figure 10 shows that extracellular application of one of the PUFAs at 5 \( \mu \)M concentration significantly blocked the mutant channel. In contrast, the monounsaturated fatty acid oleic acid (OA; C18:1n-9, \( n = 6 \)) or either of the saturated fatty acids stearic acid (SA; C18:0, \( n = 6 \)) or palmitic acid (PA; C16:0, \( n = 9 \)) at 5 \( \mu \)M concentration had no significant inhibitory effect on the mutant channel in HEK-293t cells. These results are consistent with our previous findings that only PUFAs, not monounsaturated or saturated fatty acids, have inhibitory effects on cardiac \( I_Na \) (19, 20).

**DISCUSSION**

The main findings of this study are that the mutant L409C/A410W of the \( \alpha \)-subunit of human cardiac \( Na^+ \) channels causes a long-lasting, persistent \( I_Na \) and that n-3 PUFAs significantly inhibited \( I_Na \) in HEK-293t cells transfected with the inactivation-deficient mutant. The effect of PUFAs on \( I_Na \) late was even greater than that on \( I_Na \) peak (Figs. 3, 5, and 7). The persistent \( I_Na \) current has been observed in adult mammalian ventricular cardiomyocytes (14), and hypoxia has been shown to enhance its amplitude (5). Increased \( Na^+ \) influx during hypoxia increases intracellular \( Na^+ \) concentration ([\( Na^+ \)])i), which in turn activates the reversal mode of the \( Na^+ \)/\( Ca^{2+} \) exchanger so that intracellular \( Ca^{2+} \) concentration ([\( Ca^{2+} \])i) level increases as well. An increase in the persistent \( I_Na \) and [\( Ca^{2+} \])i] level can cause arrhythmias and irreversible cell damage (5). Blockade of voltage-gated \( Na^+ \) channels has long been accepted an effective therapy for patients with many types of cardiac arrhythmia. A recent study showed that blocking persistent \( I_Na \) late in ventricular cardiomyocytes of patients with heart failure ceased soon after depolarization (10). The inhibition of \( I_Na \) late by n-3 PUFAs thus might have potential therapeutic value in certain patients with ischemia-induced arrhythmia.

Traditional local anesthetics act on common structural determinants at the D4–S6 segment of the \( Na^+ \) channel \( \alpha \)-subunit (13). Certain mutations (F1760K and Y1767K) in this region of hH1\( _{\alpha} \) \( Na^+ \) channels were found eliminate the inhibitory effects of lidocaine and cocaine on cardiac \( I_Na \) in HEK-293t cells transfected with these mutants, but they did not alter the inhibition of \( I_Na \) by n-3 PUFAs. In contrast, the mutant N406K in the D1–S6 region greatly attenuated the effects of the n-3 PUFAs on cardiac \( I_Na \) (21). These results indicate that EPA may bind to a region (D1–S6) different from the one to which local anesthetics bind (D4–S6).

Because the sites of the mutant L409C/A410W are close to N406, EPA might possibly bind to a region near the mutation determinants at the D4–S6 segment of the \( Na^+ \) channel. The main effects of this study are that the mutant L409C/A410W of the \( \alpha \)-subunit of human cardiac \( Na^+ \) channels causes a long-lasting, persistent \( I_Na \) and that n-3 PUFAs significantly inhibited \( I_Na \) in HEK-293t cells transfected with the inactivation-deficient mutant. The effect of PUFAs on \( I_Na \) late was even greater than that on \( I_Na \) peak (Figs. 3, 5, and 7). The persistent \( I_Na \) current has been observed in adult mammalian ventricular cardiomyocytes (14), and hypoxia has been shown to enhance its amplitude (5). Increased \( Na^+ \) influx during hypoxia increases intracellular \( Na^+ \) concentration ([\( Na^+ \)])i), which in turn activates the reversal mode of the \( Na^+ \)/\( Ca^{2+} \) exchanger so that intracellular \( Ca^{2+} \) concentration ([\( Ca^{2+} \])i) level increases as well. An increase in the persistent \( I_Na \) and [\( Ca^{2+} \])i] level can cause arrhythmias and irreversible cell damage (5). Blockade of voltage-gated \( Na^+ \) channels has long been accepted an effective therapy for patients with many types of cardiac arrhythmia. A recent study showed that blocking persistent \( I_Na \) late in ventricular cardiomyocytes of patients with heart failure ceased soon after depolarization (10). The inhibition of \( I_Na \) late by n-3 PUFAs thus might have potential therapeutic value in certain patients with ischemia-induced arrhythmia.

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Because the sites of the mutant L409C/A410W are close to N406, EPA might possibly bind to a region near the mutation sites of the inactivation-deficient \( Na^+ \) channels and thus modulate the behavior of the inactivation gate so that it more closely resembles a normal inactivation process.

Our present results show that the fish oil n-3 PUFAs significantly shifted the curve of the steady-state inactivation in a hyperpolarizing direction and eliminated the portion of noninactivated currents (Fig. 7). EPA at 5 \( \mu \)M concentration inhibited \( I_Na \) peak values by 50%, but \( I_Na \) late was essentially abolished. This finding shows that EPA essentially abolishes the...
Fig. 7. Effects of EPA on the fast steady-state inactivation of persistent $I_{\text{Na}}$ in HEK-293t cells. Superimposed original current traces in absence (A, Control) or presence of 5 μM EPA (B) were elicited using 200-ms test pulses to −30 mV after 500-ms conditional prepulses that we varied from −160 mV to +30 mV in 10-mV increments. Membrane holding potential of cells was −90 mV, and pulse rate was 0.1 Hz. Dotted lines in A and B represent zero current. C: normalized $I_{\text{Na peak}}$ of fast steady-state inactivation averaged in absence (■) or presence of 5 μM EPA (○, n = 16). Steady-state inactivation curve was significantly shifted in the hyperpolarizing direction. In the presence of 5 μM EPA, $I_{\text{Na peak}}$ was inhibited by 50% with prepulses from −160 mV to −130 mV and was suppressed almost completely with prepulses more positive than −70 mV (●, relative inhibition). Data in C were derived using a Boltzmann equation. A portion (~25%) of $I_{\text{Na peak}}$ in absence of EPA was not inactivated even with highly depolarized prepulses, but EPA at 5 μM concentration abolished the noninactivated portion almost completely.

Fig. 8. Development of resting inactivation of mutated $I_{\text{Na}}$ by EPA. A: voltage-pulse protocol was composed of a prepulse from holding potentials of −150 mV to −65 mV with increasing durations, followed by 50-ms test pulse to −30 mV. B: original current traces of $I_{\text{Na}}$ elicited by prepulses at time 0 and at 10, 20, and 120 ms in absence (Control) or presence of 5 μM EPA. C: development of resting inactivation of mutated Na+ channel in absence (●, Control) and presence of 5 μM EPA (○, n = 5). Time courses of resting inactivation in the mutant were fitted using a single-exponential decay function. Resting inactivation τ of mutated $I_{\text{Na}}$ were 6.5 ± 0.02 ms for control (●, $A_1 = 0.47$) and 6.9 ± 0.03 ms for 5 μM EPA (○, $A_1 = 0.84$) (n = 5). Dotted and dashed lines represent resting inactivation of wild-type $I_{\text{Na}}$ in absence or presence of 5 μM EPA with τ (fitted using a single exponential function) of 32.8 ± 0.14 ms ($A_1 = 1.05$) for control (dotted line) and 8.5 ± 0.06 ms ($A_1 = 1.06$) for 5 μM EPA (dashed line). n = 7; P < 0.05 vs. control.
I of the mutant and results in phenotypic restoration of the inactivation in the inactivation-deficient mutant. Therefore, in the presence of EPA, the inactivation-deficient Na\(^+/H\) channel behaves similarly to inactivation in wild-type Na\(^+/H\) channels. The EPA-induced inhibition of the mutant I\(_{\text{Na}}\) had no effect on the activation of mutant Na\(^+/H\) channels (Fig. 5).

The antiarrhythmic drug flecainide also inhibited the mutant current without altering the activation of inactivation-deficient mutants of skeletal muscle Na\(^+/H\) channels (15). EPA, however, significantly shifted the steady-state inactivation of the mutant I\(_{\text{Na}}\) by 19 mV, which is similar to our previous finding of a 22-mV shift for wild-type hH1\(^+/H\) plus β1-subunits of Na\(^+\) channels (20). Typically, this action is limited to PUFAs and is not produced by monounsaturated or saturated fatty acids as shown in Fig. 10 (19, 20).

It seems that any cardiac dysfunction that results in prolonged I\(_{\text{Na}}\) enhances the opportunity for cardiac arrhythmias to occur. Long QT-3 syndromes, e.g., LQT-3/ΔKPQ, have persistent I\(_{\text{Na}}\) late (12). Patients with these presentations, too, might potentially benefit from treatment with n-3 fatty acids, which block persistent I\(_{\text{Na}}\) late. After binding, the fatty acids may block Na\(^+\) channels or induce the channels to enter an inactive state and stabilize. The ability to inhibit persistent I\(_{\text{Na}}\) and stabilize Na\(^+\) channels in their inactivated state has clinical implications for potential therapeutic use of fish oil n-3 PUFAs.
Arrhythmias that arise from enhanced persistent $I_{\text{Na}}$ in patients with ischemia can cause sudden cardiac death (5). We have shown that the n-3 PUFAs, by blocking persistent $I_{\text{Na}}$, may be able to prevent these fatal arrhythmias as has been shown in clinical trials (1, 11). The beneficial effects of n-3 PUFAs on certain cardiac arrhythmias may result from the inhibition of persistent $I_{\text{Na}}$ by enhancement of channel inactivation and stabilization of the inactivation gate.

The results of the present study indicate that the blocking action of fish oil n-3 PUFAs on Na$^+$ channels in a mutant produced an inactivation-deficient channel, presumably by disabling the receptor of the inactivation particle, so that the inactivation particle was unable to close the Na$^+$ channel. The intracellular linker between domains 3 and 4 is known to be essential for the fast inactivation of the Na$^+$ channel, and deletion of this region also causes persistence of $I_{\text{Na}}$ during depolarization (2). We do not know whether the n-3 PUFAs have any or no blocking effect on a disabled, inactivated Na$^+$ channel such as that produced in the mutant IFM/3Q and did not address that issue in this study.

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