Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 12559–12563, October 1996
Neurobiology

Polyunsaturated fatty acids modulate sodium and calcium currents in CA1 neurons

(n-3, ω-3 polyunsaturated fatty acids/ion channels/anticonvulsant)

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Contributed by A. Leaf, August 15, 1996

ABSTRACT **Recent evidence indicates that long-chain polyunsaturated fatty acids (PUFAs) can prevent cardiac arrhythmias by a reduction of cardiomyocyte excitability. This was shown to be due to a modulation of the voltage-dependent inactivation of both sodium (I_{Na}) and calcium (I_{Ca}) currents. To establish whether PUFAs also regulate neuronal excitability, the effects of PUFAs on I_{Na} and I_{Ca} were assessed in CA1 neurons freshly isolated from the rat hippocampus. Extracellular application of PUFAs produced a concentration-dependent shift of the voltage dependence of inactivation of both I_{Na} and I_{Ca} to more hyperpolarized potentials. Consequently, they accelerated the inactivation and retarded the recovery from inactivation. The EC_{50} for the shift of the I_{Na} steady-state inactivation curve was 2.1 ± 0.4 μM for docosahexaenoic acid (DHA) and 4 ± 0.4 μM for eicosapentaenoic acid (EPA). The EC_{50} for the shift on the I_{Ca} inactivation curve was 2.1 ± 0.4 for DHA and >15 μM for EPA. Additionally, DHA and EPA suppressed both I_{Na} and I_{Ca} amplitude at concentrations >10 μM. PUFAs did not affect the voltage dependence of activation. The monounsaturated oleic acid and the saturated palmitic acid were virtually ineffective. The combined effects of the PUFAs on I_{Na} and I_{Ca} may reduce neuronal excitability and may exert anticonvulsive effects *in vivo*.**

Long chain polyunsaturated fatty acids (PUFAs) may affect excitable tissues, including neurons. They and their active metabolites modulate ion channels (for review see refs. 1 and 2). Attention has been given primarily to the effects of arachidonic acid (C20:4n-6) and its oxygenated metabolites, but recently it has been reported that the other class of essential PUFAs, n-3 PUFAs, also specifically modulate ion channels in cardiac myocytes and in cells of other tissues. In cardiac myocytes, voltage-dependent sodium currents are inhibited (3), as are L-type calcium channels (ref. 4; Y. F. Xiao, J. P. Morgan, and A.L., unpublished data). Outward potassium currents are also inhibited in cardiac cells (ref. 5; Y. F. Xiao, J. P. Morgan, and A.L., unpublished data) and in pinealocytes (6). The net physiologic effect of the PUFAs on the heart is to stabilize the myocytes electrically (7, 8), resulting in an antiarrhythmic action (9). Diets enriched with fish oils or n-3 fatty acids prevented fatal ventricular arrhythmias in rats (10, 11) and probably in humans (12–14). Furthermore, infusion of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intravenously just before an ischemic event prevented ventricular fibrillation in exercising dogs (15).

In cardiac myocytes the reduction in amplitude of the voltage-activated sodium current in heart cells occurs with a shift of its voltage dependence of inactivation in the hyperpolarizing direction (3). A comparable shift was shown for the calcium current inactivation (ref. 4; Y. F. Xiao, J. P. Morgan, and A.L., unpublished data). This modulating effect of the PUFAs shows remarkable similarities with the effect of local anesthetics like lidocaine (16) and anticonvulsant drugs active against partial seizures like phenytoin and carbamazepine (17, 18). Since PUFAs pass quickly through the blood–brain barrier, accumulating in neuronal membranes (19), and can be liberated by phospholipase A2 (20), we investigated the hypothesis that the PUFAs also play an important role in the regulation of neuronal excitability and tested their effects on sodium and calcium currents in freshly isolated hippocampal CA1 neurons of the rat.

MATERIALS AND METHODS

Materials. The polyunsaturated fatty acids, cis-4,7,10,13,16,19-DHA (C22:6n-3), cis-5,8,11,14,17-EPA (C20:5n-3), and cis-9,12-octadecadienoic acid (linoleic acid; C18:2n-6), the monounsaturated cis-9-octadecaenoic acid (oleic acid [OA]; C18:1n-9), and the saturated hexadecanoic acid (palmitic acid, C16:0) were obtained from Sigma. Fatty acids were dissolved in ethanol and stored at −20°C under nitrogen. The experimental concentration of fatty acids was obtained by dilution of the stocks. The final ethanol concentration was 0.2% in all experiments.

Solutions. The neurons were dissociated in a solution containing the following: 120 mM NaCl, 10 mM piperazine-*N,N'*-bis[2-ethanesulfonic acid], 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM D-glucose, and 1 mg of bovine trypsin (type XI) per ml, pH 7.0. The patch pipet solution used for sodium and calcium current measurements contained the following: 110 mM CsF, 25 mM tetraethylammonium chloride, 20 mM phosphocreatine, 50 units of phosphocreatine kinase per ml, 10 mM EGTA, 10 mM Hepes, 5 mM NaCl, 2 mM $MgCl_2$, 0.5 mM $CaCl_2$, 0.5 mM $BaCl_2$, 2 mM MgATP, 0.1 mM NaGTP, and 0.1 mM leupeptin; pH set at 7.3. The bath solution for recording sodium currents contained the following: 90 mM choline chloride, 25 mM tetraethylammonium chloride, 20 mM NaCl, 10 mM Hepes, 5.4 mM KCl, 5 mM 4-aminopyridine, 1mM $CaCl_2$, 1 mM $MgCl_2$, 0.1 mM $CdCl_2$, and 25 mM D-glucose; pH was set at 7.4. For recording calcium currents, the bath solution contained the following: 110 mM NaCl, 10 mM Hepes, 25 mM tetraethylammonium chloride, 5.4 mM KCl, 5 mM $CaCl_2$, 5 mM 4-aminopyridine, 1 mM $MgCl_2$, 25 mM D-glucose, and 1 μM tetrodotoxin; pH was set at 7.4. All chemicals were obtained from Sigma.

Cell Preparation. CA1 pyramidal neurons were isolated enzymatically from male Wistar rats (150–200 g) as described in detail previously (21). From both hippocampi, 500-μm thick

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Abbreviations: PUFA, polyunsaturated fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; OA, oleic acid.

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slices were made from which the CA1 area was dissected. These tissue pieces were incubated for 85 min at 32°C in oxygenated dissociation solution containing 0.1% trypsin. Finally they were washed twice and kept in the dissociation solution without trypsin (20°C). CA1 subslices were dispersed in bath solution by trituration through Pasteur pipets of decreasing diameter and brought into a perfusion chamber. Fusiform neurons with a bright and smooth appearance and no visible organelles were selected for recording.

Current Recording. Currents were measured under whole-cell voltage-clamp conditions at 23°C, using patch pipets of 1.5- to 2.5-MΩ resistance. After seal formation (>0.5 GΩ) and cell membrane rupture, series resistance was compensated (>75%) and the capacitance transients were minimized. Selected cells were compact and had no signs of space-clamp errors. Sodium current was recorded with a List EPC-7 amplifier (List Electronics, Darmstadt, Germany) in fatty acid-free condition, with 1 mg of delipidated bovine serum albumin (BSA) per ml. Next the cells were exposed to fatty acids in the absence of BSA. Recordings started at least 5 min after introduction of the fatty acids and after wash with BSA. Holding potential was −70 mV. Calcium current was recorded with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA), before, during, and, if possible, after washout of the fatty acids. Holding potential for calcium currents was −80 mV.

Current Analysis. Currents were corrected for linear, non-specific leak and capacitive transients. Current decay was fit with an exponential equation: $I(t) = A_0 + A_1 \cdot \exp[(t_0 - t)/\tau_i)]$, where τ_i is the time constant of current inactivation, t is the time after the start of the pulse at t_0, and $A_0 + A_1$ is the amplitude at the time of the step. Sodium conductances were calculated from the equation: $g_{ion} = I/(V_m - E_{ion})$, where V_m is the membrane potential and E_{ion} is the reversal potential for Na^+ or Ca^{2+}. Voltage dependence of activation and inactivation was fit with a Boltzmann equation: $A(V) = A_{max}/\{1 + e[(V - V_h)/V_c]\}$, where V_h is the potential of half-maximal amplitude and V_c is proportional to the slope at V_h. Dose–response relationships were fit with the Hill equation: $\Delta(c) = \Delta_{max}/[1 + (c/EC_{50})^h]$, where Δ_{max} is the maximal effect, c is the concentration of the drug, EC_{50} is the concentration of half-maximal effect, and h is the Hill coefficient.

Statistics. Data are given as the mean ± standard error of the mean (SEM). Statistical comparisons were made with Student's t test.

RESULTS

Sodium Currents in CA1 Neurons. Voltage-dependent sodium currents (I_{Na}) were activated by stepwise varied depolarizations. Fig. 1*A* shows a representative experiment. It can be seen that I_{Na} has an activation threshold of −55 mV and reached its maximal amplitude around −20 mV. The inactivation of I_{Na} as a function of time could be described by a single exponential decay. The time constant of inactivation (τ_i) was voltage dependent and equal to 1.7 ± 0.1 ms (n = 123) at −25 mV. To quantify I_{Na} activation, the sodium conductance (g_{Na}) was calculated from I_{Na} amplitude and the driving force for Na^+. g_{Na} as a function of membrane potential (V_m) was fit by the Boltzmann equation (see Fig. 1*E*, open circles). In 86 neurons, g_{Na} was 120 ± 5 nS, the potential of half-maximal activation ($V_{h,a}$) was −31.1 ± 0.4 mV, and the slope factor ($V_{c,a}$) was −5.1 ± 0.1 mV. Voltage dependence of steady-state inactivation was determined using a double-pulse protocol: I_{Na} was activated by a test pulse to −25 mV, preceded by a conditioning prepulse of stepwise varied potential. Fig. 1*B* shows that less I_{Na} can be activated at increasing prepulses, indicating the increasing level of steady-state inactivation at the prepulse voltage. The amplitude as a function of V_m (Fig. 1*E*, open squares) was fit with the Boltzmann equation (mean

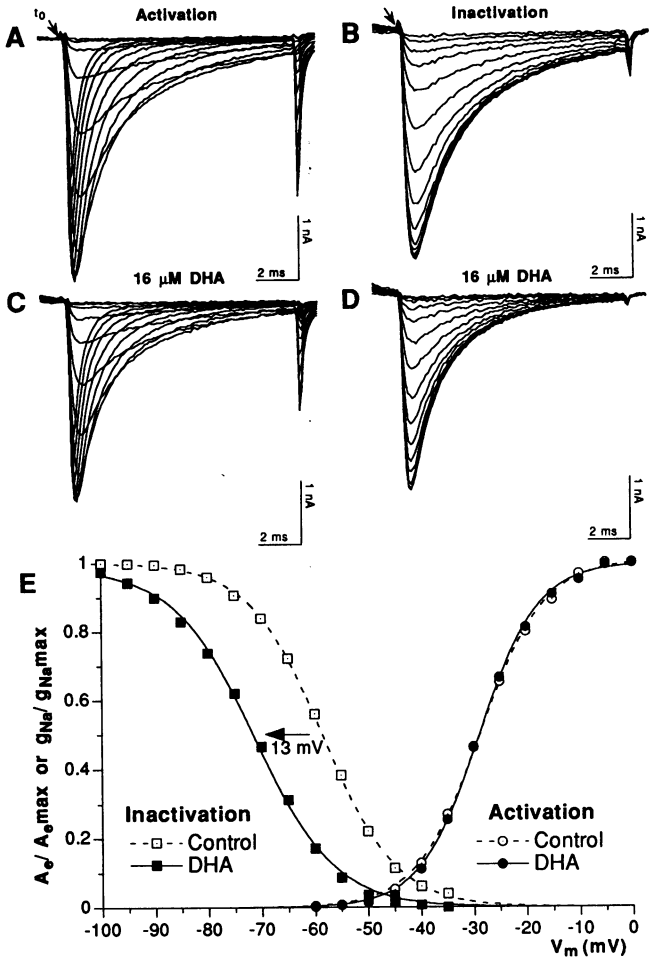

FIG. 1. Sodium currents (I_{Na}) in a single isolated CA1 neuron. (*A*) I_{Na} was activated by 10-ms depolarizing potential steps (ranging from −60 mV to 5 mV, with 5-mV increments), preceded by a 0.5-s prepulse at −140 mV. (*B*) Steady-state inactivation of the I_{Na} was measured by a test pulse to −25 mV, preceded by 0.5-s conditioning prepulse (ranging from −140 mV to −35 mV, with 10-mV increments). (*C*) I_{Na} activation in 16 μM DHA. (*D*) Steady-state inactivation of I_{Na} in 16 μM DHA. DHA suppressed I_{Na} and increased inactivation. (*E*) Current decay was fit with an exponential equation and sodium conductance (g_{Na}) was calculated by dividing the amplitude at t_0 (A_e) by the driving force for Na^+. Normalized g_{Na} and A_e given against V_m before DHA was added (○ and □) and in DHA (● and ■). Lines represent Boltzmann fits. DHA (16 μM) shifted the voltage dependence of steady state to more hyperpolarized potentials (in this neuron, by −13 mV).

control values: $V_{h,i}$, −60.4 ± 0.4 mV; $V_{c,i}$, 5.7 ± 0.1 mV; n = 123). Inactivation was measured twice in control solutions for each cell before the determination of $V_{h,i}$ for that cell to quantify shift in $V_{h,i}$ with time. Subsequent measurements of $V_{h,i}$ for each cell were corrected for this nonspecific time-dependent shift in the baseline. The overall mean value of the time-dependent shift was −2.6 ± 0.3 mV per 10 min (n = 123). Recovery from inactivation was determined using a double-pulse protocol; I_{Na} was inactivated during a first 20-ms step to −25 mV. After a recovery period of increasing length (at −70 or −80 mV), the degree of recovery was measured by a second pulse of 10 ms to −25 mV. Recovery as a function of interval duration was fit with an exponential function that provided the time constant of recovery (mean control values: τ_r = 11.5 ± 0.4 ms at −70 mV and τ_r = 6.1 ± 0.3 ms at −80 mV; n = 112).

Effects of DHA on Sodium Currents. Fig. 1 *C* and *D* show that addition of 16 μM DHA to the bath reduced I_{Na} (in this neuron by ≈18%). In 12 neurons, 16 μM DHA reduced g_{Na} by

Table 1. Effects of fatty acids (16 mM) on I_{Na} activation and inactivation in CA1 neurons

		Activation		Inactivation		
Fatty acid	n	Δg_{Na}, %	$\Delta V_{h,a}$, mV	$\Delta V_{h,i}$, mV	$\Delta V_{c,j}$, mV	$\Delta\tau_r$ (−80 mV), ms
DHA	14	−35 ± 7*	1.0 ± 1.6	−11.1 ± 1.0†	0.8 ± 0.2*	6.2 ± 0.9†
EPA	7	−10 ± 6*	2.5 ± 1.2‡	−11.2 ± 1.1†	1.3 ± 0.2†	4.0 ± 0.8*
Linoleic acid	7	3 ± 5	0.2 ± 0.8	−4.0 ± 1.0†	0.6 ± 0.2*	3.4 ± 0.9‡
OA	7	−9 ± 2‡	−0.9 ± 0.6	−0.7 ± 0.5	−0.1 ± 0.2	1.1 ± 0.8
Palmitic acid	4	2 ± 3	−0.1 ± 0.6	−0.4 ± 1.0	0.1 ± 0.1	1.4 ± 0.8

Values (mean ± SEM) give the change of I_{Na} properties induced by the exposure to fatty acids at 16 μM. Statistical significance of differences from values obtained by perfusion with BSA-free solution was tested with the unpaired t test.
*$P < 0.001$; †$P < 0.01$; and ‡$P < 0.05$.

−33 ± 7% ($P < 0.01$) without affecting the voltage dependence of activation (Fig. 1*E*, solid circles). In contrast to activation, DHA clearly modifies I_{Na} inactivation properties. DHA accelerated I_{Na} inactivation (Fig. 1*D*) and shifted the voltage dependence of inactivation to more negative potentials (Fig. 1*E*; filled squares). Furthermore, DHA slowed down the recovery from inactivation (Table 1). In 14 neurons, 16 μM DHA induced a shift of the voltage dependence of inactivation ($\Delta V_{h,i}$) of −13.4 ± 0.9 mV ($P < 0.001$), increased the slope at V_h by 1.1 ± 0.2 mV ($P < 0.001$) and increased τ_r at −80 mV by 7.5 ± 0.6 ms ($P < 0.001$).

Effects of Other Fatty Acids. Apart from DHA, the polyunsaturated fatty acids, EPA (C20:5n-3) and linoleic acid (C18:2n-6, LA), the monounsaturated OA (C18:1n-9), and the saturated palmitic acid (16:0) were tested. Table 1 summarizes the effects of fatty acids at 16 μM on I_{Na} properties. Since PUFAs were tested in BSA-free medium, the effects of BSA-free medium on the determinations of ΔV_h and $\Delta\tau_r$ of the cells were evaluated in the absence of the PUFAs. Washout of BSA in eight cells induced a $\Delta V_{h,i}$ of −2.3 ± 0.4 mV, a $\Delta V_{h,a}$ of 0.5 ± 0.4, and a $\Delta\tau_r$ of −1.3 ± 0.7 ms. The values in Table 1 are differences from perfusions with control solutions without BSA. Only the polyunsaturated fatty acids shift $V_{h,i}$ to more negative values. Furthermore, DHA and EPA suppress g_{Na} at this concentration, but linoleic acid did not. In all measurements, the negative shift in $V_{h,i}$ was correlated with an increase in $V_{c,i}$. In contrast to the polyunsaturated fatty acids, the monounsaturated OA and the saturated palmitic acid had no noticeable effects.

Dose–Response Relationship of DHA and EPA. We determined the dose–response relationship (with respect to control solutions) between $\Delta V_{h,i}$ and DHA or EPA (0, 1, 1.3, 2, 4, 8, 16, or 32 μM). The PUFA-induced $\Delta V_{h,i}$ as a function of fatty acid concentration was fit with the Hill equation (Fig. 2). Maximal shift was −11.2 ± 0.9 mV for DHA and −11.8 ± 0.7 for EPA; EC_{50} was 2.1 ± 0.4 μM for DHA and 4.0 ± 0.4 for EPA; and the Hill coefficient was −2.0 ± 0.5 for DHA and −1.9 ± 0.3 for EPA. Only the EC_{50} showed a significant difference between DHA and EPA. g_{Na} suppression was only observed at doses of DHA and EPA >8 μM. Upon washout with BSA-containing solution, g_{Na} recovered fast and completely. The recovery of the $V_{h,i}$ shift was complete at doses <8 μM but less complete with higher doses of fatty acids.

Calcium Currents in CA1 Neurons. High voltage-activated calcium currents (I_{Ca}) were activated with depolarizing voltage steps. Fig. 3*A* shows I_{Ca} of a representative neuron with an activation threshold of −35 mV and a peak value at 0 mV. Calcium conductance (g_{Ca}) was calculated from I_{Ca} amplitude and the driving force for Ca^{2+}. g_{Ca} was plotted as a function of V_m (Fig. 3*E*, open circles) and fit by Boltzmann equation (mean control values: g_{Ca}, 32.2 ± 1.8 nS; $V_{h,a}$, −5.2 ± 0.7 mV; $V_{c,a}$, −6.1 ± 0.1 mV; $n = 49$). Voltage dependence of steady-state inactivation was determined by a double-pulse protocol with a test pulse to 10 mV, preceded by a conditioning pulse from levels between −90 to 15 mV. Fig. 3*B* shows that, in the same neuron, I_{Ca} becomes inactivated by depolarizing prepulses. Peak amplitude (A_p) was plotted as a function of V_m (Fig. 3*E*, open squares) and fit with the Boltzmann equation (mean control values: $V_{h,i}$, −30 ± 1.0 mV; $V_{c,i}$, 9.0 ± 0.2 mV; $n = 50$).

Effects of DHA on Calcium Currents. Fig. 3 *C* and *D* show that perfusion with 10 μM DHA reduced g_{Ca} by −27% in this cell. The mean reduction in five cells was −26 ± 11%, which was twice as large as the normally observed rundown observed with perfusion of control solutions (−14 ± 9%; $n = 6$). The voltage dependence of activation was unaffected ($\Delta V_{h,a}$ was 0.7 ± 1.0 mV; not significant). In contrast, the inactivation of the calcium current was accelerated (Fig. 3*D*), and a prominent shift of $V_{h,i}$ to more hyperpolarized potentials occurred (Fig. 3*E*, solid squares). In the five neurons tested, 10 μM DHA shifted $V_{h,i}$ by −8.2 ± 1.9 mV ($n = 5$), which was significantly different ($P < 0.01$) from the $\Delta V_{h,i}$ with time in control solution (−1.0 ± 0.9 mV; $n = 6$). Furthermore, the DHA-induced $\Delta V_{c,i}$ was different from that in control solutions (2.0 ± 0.9 mV and 0.1 ± 0.2, respectively; $P < 0.05$).

Dose–Response Relationship of DHA and EPA. We determined the dose–response relationship between $\Delta V_{h,i}$ and different concentrations of DHA or EPA (0, 2, 4, 10, 20, or 40 μM; Fig. 4). The small values obtained with perfusions without PUFAs were subtracted from those with PUFAs. The PUFA-induced $\Delta V_{h,i}$ as a function of fatty acid concentration was fit with the Hill equation. For DHA, the maximal effect was −6.6 ± 0.6 mV, the EC_{50} was 2.1 ± 0.5 μM, and the Hill coefficient was 2.2 ± 1.1. Solubility problems with high concentrations of EPA (>40 μM) prevented the acquisition of the full dose–response curve. The data, therefore, were fit up to 40 μM with the Hill coefficient set at 2 and a maximal effect

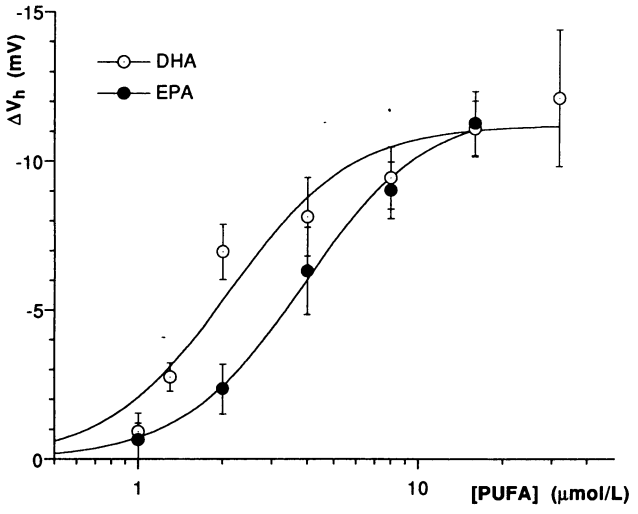

FIG. 2. The dose–response relationship between shift in inactivation ($\Delta V_{h,i}$) and [DHA] (5–12 cells, ○) or [EPA] (4–8 cells, ●). Lines represent the fits with the Hill equation. The EC_{50} was 2.1 μM for DHA and 4.0 μM for EPA.

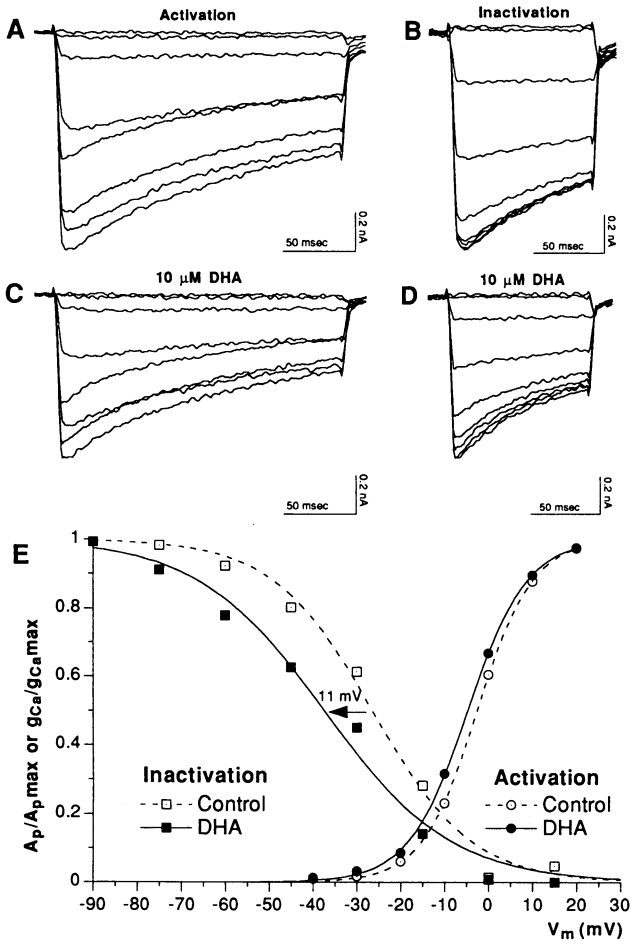

FIG. 3. Calcium currents (I_{Ca}) in a single isolated neuron. (*A*) I_{Ca} was activated by 200-ms depolarizing potential steps (ranging from −50 mV to 50 mV, with 10-mV increments), preceded by a 3-s prepulse to −80 mV. (*B*) Steady-state inactivation of I_{Ca} was measured by a 100-ms test pulse to 10 mV, preceded by 3-s conditioning prepulse (ranging from −90 mV to 15 mV, with 15-mV increments). (*C*) Activation in 10 μM DHA. (*D*) Steady-state inactivation in 10 μM DHA. DHA suppresed I_{Ca} and increased inactivation. (*E*) Normalized g_{Ca} and A_p as a function of V_m before DHA was added (○ and □) and in DHA (● and ■). Data were fit with a Boltzmann equation. DHA (10 μM) shifted the steady-state inactivation functions to more hyperpolarized potentials (in this neuron, by −11 mV).

of −8.3 ± 0.1 mV and an EC_{50} of 17.8 ± 0.2 μM were found, the latter of which is probably better stated as simply >15 μM. The differences between the EC_{50} of DHA and EPA for I_{Ca} were qualitatively the same but considerably larger in I_{Ca} compared with I_{Na}. The effect of PUFA on I_{Ca} was slow and, especially at high doses (≥20 μM), often not complete within 10 min. Recovery of I_{Ca} upon washout with BSA-containing solution was poor and variable. As with the sodium currents, the monounsaturated fatty acid OA (even at 20 μM) had no effect on I_{Ca} ($\Delta V_{h,i}$ was −2.3 ± 1.0 mV, $n = 5$; not significant).

DISCUSSION

The polyunsaturated fatty acids tested shift the voltage dependence of inactivation of the sodium and calcium currents. At higher doses, they also suppress the conductance of these two currents in CA1 neurons. Monounsaturated and saturated fatty acids at comparable concentrations are virtually ineffective.

The PUFA-induced shift of I_{Na} inactivation to more hyperpolarized potentials is qualitatively similar, although slightly

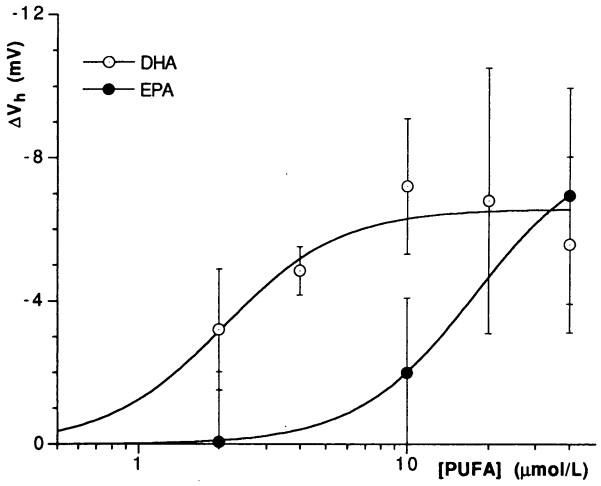

FIG. 4. The dose–response relationship between the shift in ΔV_{hi} and [DHA] (4–6 cells, ○) or [EPA] (4–6 cells, ●). Lines represent the fit with the Hill equation (for EPA, the Hill coefficient was fixed at −2).

smaller, to that seen in cardiomyocytes (3). The increase in time constants of recovery from inactivation could be due to a comparable shift in the voltage dependence of recovery from inactivation. In contrast to cardiomyocytes (3), low concentrations of PUFAs hardly affected g_{Na}. This difference with the effects on I_{Na} in cardiomyocytes is either due to tissue-specific differences or also could be related to methodological differences.

It was reported that, in neonatal rat cardiomyocytes, DHA had no direct effect on the L-type calcium channels (22). Recently, however, the PUFA arachidonic acid (C20:4n-6) was shown in frog cardiomyocytes to suppress the isoproterenol-stimulated I_{Ca} amplitude with an EC_{50} of 2 μM and shift its voltage dependence of inactivation to more hyperpolarized potentials, whereas the saturated myristic acid had no effect (4). Even without isoproterenol stimulation, the n-3 PUFAs also are potent inhibitors of the L-type Ca^{2+} channels and shift the voltage dependence of inactivation to more hyperpolarized potentials in neonatal and adult rat cardiomyocytes (Y. F. Xiao, J. P. Morgan, and A.L., unpublished data). The modulating effect of PUFAs on whole-cell calcium currents may be complex, since, in the neuronal preparation, the current consists of several components with different voltage-dependent properties.

The fast onset and recovery (following perfusion of cells with delipidated BSA) of the effects of the PUFAs on I_{Na} suggests a direct action of the PUFAs without metabolic alteration. The strength of the effect of the fatty acids on voltage dependence increases with the number of C=C unsaturated bonds. Since esterified PUFAs were without effect *in vitro* (9) and *in vivo* (15), the apparent structural requirements for affecting I_{Na} have been proposed to be a long hydrocarbon chain with two or more unsaturated C=C bonds and a free carboxylic acid group at one end (23).

Further, it was speculated that the lipophilic chain is essential to partition the PUFAs among the acyl chains of the membrane phospholipids, the unsaturated C=C bonds to bind the PUFAs to the protein of the ion channel so that the negatively charged carboxyl group, anchored at the lipid–water interface, comes close to the voltage sensor of the sodium channel and changes the local electric field. In the CA1 neurons, the action of the PUFA seems manifest primarily on its inactivation properties. The binding site for the PUFAs to the α subunit of the sodium channel remains to be determined.

The effect of PUFAs, especially DHA on I_{Na} inactivation properties, will reduce the ability of neurons to fire from

relatively depolarized levels, increase their refractory period, and reduce the maximal firing rate. The effect of the PUFAs on I_{Ca} inactivation tends to reduce calcium-dependent neurotransmission and the postsynaptic calcium influx in neurons. This combined action on sodium and calcium currents is characteristic of drugs effective against partial seizures, like carbamazepine and phenytoin (18, 24). A concentration in the therapeutic range of 8 μM carbamazepine will achieve a specific shift of $V_{h,i}$ by -3 mV of I_{Na} inactivation without affecting the conductance (M.V., unpublished observation), while this effect can be achieved by a 6-fold lower concentration of DHA. This concentration has a qualitatively similar effect on the calcium current. The PUFA EPA is slightly less potent than DHA but much more specific in affecting the sodium over the calcium conductance.

PUFAs, in the same concentration range as tested in the present study, are reported to enhance potassium currents (25). In the neonatal rat, adult rat, and ferret cardiomyocytes, however, these PUFAs primarily inhibit the main repolarizing potassium channels, I_{to}, the transient outward rectifier current, and I_k, the delayed outward rectifier current, but have no effect on I_{k1}, the inward rectifier current (ref. 4; Y. F. Xiao, J. P. Morgan, and A.L., unpublished data). Suppression of γ-aminobutyric acid (GABA) responses by low micromolar concentrations of DHA has been reported recently (26), and enhancement of *N*-methyl-D-aspartate (NMDA) responses—but with much higher concentrations of DHA (27)—could increase the excitability of a neuronal network. However, the changes in I_{Na} and I_{Ca} may reduce the firing rate and thus counteract the changes in those transmitter responses. Furthermore, liberation of incorporated PUFAs from the membrane by activity-dependent lipase (20) may act as a negative feedback mechanism in synaptic transmission.

These effects on intrinsic membrane properties give PUFAs, especially DHA, a potential anticonvulsive profile (18, 24). *In vivo* experiments indeed demonstrated anticonvulsive properties of PUFAs, since a preparation of essential fatty acids raised the seizure threshold in rats (28), and, when administered intravenously in rats, DHA and EPA increased the seizure threshold by 25% in a cortical stimulation model (ref. 29; R.A.V., A. Cleton, I. Postel, and A.L., unpublished data). It is well established that DHA, which is present in mothers' milk, is essential for the early, normal development of the brain and retina (30–32). Since PUFAs are essential components of the human diet and can pass the blood–brain barrier promptly (19), they may be of therapeutic relevance in preventing epileptic discharges. One wonders if the efficacy of the classic, high-fat, "ketogenic" diet in some epilepsy patients may not be due to its content of these PUFAs.

We thank F. H. Lopes da Silva and O. W. Witte for helpful discussions. This study was supported by Netherlands Organization for Scientific Research Grant 900–53-091 and Deutsche Forschungsgemeinschaft Grant Br-1617/1-1, and it was supported in part by National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases Grant DK38165.

1. Ordway, R. W., Singer, J. J. & Walsh, J. V. (1991) *Trends Neurosci.* **14,** 96–100.
2. Meves, H. (1994) *Prog. Neurobiol.* **43,** 175–186.
3. Xiao, Y. F., Kang, J. X., Morgan, P. J. & Leaf, A. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 11000–11004.
4. Petit-Jacques, J. & Hartzell, H. C. (1996) *J. Physiol. (London)* **493,** 67–81.
5. Honore, E., Barhanin, J., Attali B., Lesage, F. & Lazdunski, M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1937–1941.
6. Poling, J. S., Karanian, J. W., Salem N. Jr., & Stefano, V. (1995) *Mol. Pharmacol.* **47,** 381–390.
7. Kang, J. X., Xiao, Y. F. & Leaf, A. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3997–4001.
8. Kang, J. X. & Leaf, A. (1996) *Eur. J. Pharmacol.* **297,** 97–106.
9. Kang, J. X. & Leaf, A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 9886–9890.
10. McLennan, P. L., Abeywardena, M. Y. & Charnock, J. S. (1985) *Can. J. Physiol. Pharmacol.* **63,** 1411–1417.
11. Hock, C. E., Beck, L. D., Bodine, L. C. & Reibel, D. K. (1990) *Am. J. Physiol.* **259,** H1518–H1526.
12. Burr, M., Gilbert, J. F., Holliday, R. M., Elwood, P. C., Fehily, A. M., Rogers, S., Sweetnam R. S. & Deadman, N. M. (1989) *Lancet* **334,** 757–761.
13. de Logeril, M., Renaud, S., Mamelle, N., Salen, P., Martin, J.-L., Monjaud, I., Guidollet, J., Touboul, P. & Delaye, J. (1994) *Lancet* **343,** 1454–1459.
14. Siscovick, D. S., Raghunathan, T. E., King, I., Weinmann, S., Wicklund, K. G., Albright, J., Bovbjerg, V., Arbogast, P., Smith, H. & Kushi, L. H. (1995) *J. Am. Med. Assoc.* **274,** 1363–1367.
15. Billman, G. E., Hallaq, H. & Leaf, A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4427–4430.
16. Bean, B. P., Cohen, C. J. & Tsien R. W. (1983) *J. Gen. Physiol.* **81,** 613–642.
17. Schwartz, J. R. & Grigat, G. (1989) *Epilepsia* **30,** 286–294.
18. Rogawski, M. A. & Porter, R. J. (1990) *Pharmacol. Rev.* **42,** 223–286.
19. Robinson, P. J. & Rapoport, S. I. (1986) *Am. J. Physiol.* **251,** R1212–R1220.
20. Dumuis, A., Sebben, M., Haynes, L., Pin, J. P. & Brockaert, J. (1988) *Nature (London)* **336,** 68–70.
21. Vreugdenhil, M. & Wadman, W. J. (1992) *Neuroscience* **49,** 373–381.
22. Pepe, S., Bogdanov, K., Hallaq, H., Spurgeon, H., Leaf, A. & Lakatta, E. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 8832–8836.
23. Kang, J. X. & Leaf, A. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 3542–3546.
24. Elliott, P. (1990) *Eur. J. Pharmacol.* **175,** 155–163.
25. Kim, D., Sladek, C. D., Aguado-Velasco, C. & Mathiasen, J. R. (1995) *J. Physiol. (London)* **484,** 661–660.
26. Hamano, H., Nabekura, J., Nishikawa, M. & Ogawa, T. (1996) *J. Neurophysiol.* **75** 1264- 1270.
27. Nishikawa, M., Kimura, S. & Akaike N. (1994) *J. Physiol. (London)* **475,** 83–93.
28. Yehuda, S., Carasso R. & Mostofsky, D. I. (1994) *Eur. J. Pharmacol.* **254,** 193–198.
29. Voskuyl, R. A., Dingemanse, J. & Danhof, M. (1989) *Epilepsy Res.* **3,** 120–129.
30. Connor, W. E., Neuringer, M. & Reisbeck, S. (1992) *Nutr. Rev.* **50,** 21–29.
31. Uauy, R., Pierano, P., Hoffman, D., Mena, P., Birch, D. & Birch, E. (1996) *Lipids* **31,** S167–S176.
32. Makrides, M., Neumann, M., Simmer, K. & Gibson, R. A. (1995) *Lancet* **345,** 91–97.