British Journal of Pharmacology (1997) 120, 1367–1375

Inhibitory effects of ω-3 polyunsaturated fatty acids on receptor-mediated non-selective cation currents in rat A7r5 vascular smooth muscle cells

*,†Michiko Asano, *,[1]Toshiaki Nakajima, *Kuniaki Iwasawa, *Hisanori Hazama, *Masao Omata, ‡Masaaki Soma, †Kamejiro Yamashita & †Yukichi Okuda

*Second Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113; †Department of Internal Medicine, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305 and ‡Mochida Pharmaceutical Co., Ltd, 7, Yotsuya 1-chome, Shinjuku-ku, Tokyo 160, Japan

1 The effects of ω-3 polyunsaturated fatty acids on receptor-mediated non-selective cation current (I_{cat}) and K^+ current were investigated in aortic smooth muscle cells from foetal rat aorta (A7r5 cells). The whole-cell voltage clamp technique was employed.

2 With a K^+-containing solution, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA, 30 μM) produced an outward current at a holding potential of −40 mV. This response was inhibited by tetraethylammonium (20 mM) or Cs^+ in the patch pipette solution, and the reversal potential of the EPA-induced current followed the K^+ equilibrium potential in a near Nernstian manner.

3 Under conditions with a Cs^+-containing pipette solution, both vasopressin and endothelin-1 (100 nM) induced a long-lasting inward current at a holding potential of −60 mV. The reversal potential of these agonist-induced currents was about +0 mV, and was not significantly altered by the replacement of the extracellular or intracellular Cl^- concentration, suggesting that the induced current was a cation-selective current (I_{cat}).

4 La^{3+} and Cd^{2+} (1 mM) completely abolished these agonist-induced I_{cat}, but nifedipine (10 μM) failed to inhibit it significantly.

5 ω-3 polyunsaturated fatty acids (3–100 μM), EPA, DHA and docosapentaenoic acids (DPA), inhibited the agonist-induced I_{cat} in a concentration-dependent manner. The potency of the inhibitory effect was EPA > DHA > DPA, and the half maximal inhibitory concentration (IC_{50}) of EPA was about 7 μM.

6 Arachidonic and linoleic acids (10, 30 μM) showed a smaller inhibitory effect compared to ω-3 fatty acids. Also, oleic and stearic acids (30 μM) did not show a significant inhibitory effect on I_{cat}.

7 A similar inhibitory action of EPA was observed when I_{cat} was activated by intracellularly applied GTPγS in the absence of agonists, suggesting that the site of action of ω-3 fatty acids is not located on the receptor.

8 These results demonstrate that ω-3 polyunsaturated fatty acids can activate a K^+ current and also effectively inhibit receptor-mediated non-selective cation currents in rat A7r5 vascular smooth muscle cells. Thus, the data suggest that ω-3 fatty acids may play an important role in the regulation of vascular tone.

Keywords: Vascular smooth muscle cells; A7r5 cells; ω-3 polyunsaturated fatty acids; eicosapentaenoic acid; docosahexaenoic acid; arachidonic acid; non-selective cation current; vasopressin; endothelin-1; K^+ currents

Introduction

ω-3 polyunsaturated fatty acids, such as eicosapentaenoic (EPA, C20:5 (ω-3) and docosahexaenoic (DHA, C22:6 (ω-3)) acid, which are the major components among the fatty acids contained in fish oil, have attracted much attention due to their biological activities. Dietary supplementation with fish oil reduces the incidence of coronary heart disease, and atherosclerosis (Bang & Dyerberg, 1972; Dyerberg *et al*., 1978; Kromann & Green, 1980; Kromhout *et al*., 1985) and also exerts antihypertensive effects (Puska *et al*., 1983; Hui *et al*., 1989; Knapp & FitzGerald, 199; Bonaa *et al*., 1990). Several mechanisms have been described to explain the antihypertensive effects of ω-3 fatty acids. Diets enriched in fish oils facilitate endothelium-dependent relaxations and reduce endothelium-dependent contractions in normal, atherosclerotic and hypercholesterolaemic arteries (Harris-Hooker *et al*., 1983; Shimokawa & Vanhoutte, 1988), and shift the balance of constrictor/dilatator prostaglandins to favour vasodilatation (Brox *et al*., 1981). An endothelium-independent vasorelaxant action of these fatty acids has also been shown (Juan *et al*., 1987; Engler *et al*., 1990; Engler, 1992a, b, c). ω-3 fatty acids, EPA or DHA, *per se* inhibited the contraction of rat aortic rings induced by agonists such as α-adrenoceptor agonists and angiotensin II (Juan *et al*., 1987; Engler *et al*., 1990; Engler, 1992a,b,c). Also, a diet rich in EPA can decrease systemic vascular reactivity to noradrenaline and lower systemic blood pressure in man (Lorenz *et al*., 1983). However, the precise mechanism of the hypotensive action of the ω-3 fatty acids remains to be clarified.

Polyunsaturated fatty acids are major components of phospholipids, the fundamental structure unit of biological membranes and play a key role in membrane function (Merrill & Schroeder, 1993). Dietary fats can modulate the composition of the fatty acid moiety of the membrane phospholipids in tissues and may influence the activity of membrane-bound proteins, such as enzymes and ion channels. Unsaturated free fatty acids such as arachidonic acid and polyunsaturated fatty acids, have been shown to activate K^+ channels in toad gastric smooth muscle cells (Ordway *et al*., 1989, 1991), rabbit pul-

[1] Author for correspondence.

monary artery (Kirber *et al*., 1992), aortic smooth muscle cells (Bregestovski *et al*., 1989) and canine coronary arterial cells (Xu & Lee, 1996). In vascular smooth muscle cells, hormones such as vasopressin, angiotensin II and endothelin induce Ca^{2+} release from internal stores and Ca^{2+} influx through receptor-operated Ca^{2+} channels and Ca^{2+} influx through the voltage-dependent Ca^{2+} channels. The receptor-activated Ca^{2+} entry is mediated partly by Ca^{2+}-permeable non-selective cation channels which are resistant to calcium antagonists, such as nifedipine (Benham & Tsien, 1987; Byrne & Large, 1988, Van Renterghem *et al*., 1988; Amedee *et al*., 1990; Wang & Large, 1991; Chen & Wagoner, 1991; Loirand *et al*., 1991; Inoue & Kuriyama, 1993; Van Renterghem & Lazdunski, 1994; Krautwurst *et al*., 1994; Nakajima *et al*., 1996). In addition, activation of these channels depolarizes the membrane, which may increase the probability of opening voltage-dependent L-type Ca^{2+} channels, and hence produce Ca^{2+} influx. Thus, Ca^{2+}-permeable non-selective cation channels are thought to play an important role in regulating vascular tone. Therefore, to clarify the mechanisms underlying the vasorelaxant effect of the ω-3 fatty acids, we examined their effects on receptor-mediated Ca^{2+}-permeable non-selective cation channels as well as K^+ currents. Here we provide novel evidence that ω-3 polyunsaturated fatty acids effectively inhibit the receptor-mediated Ca^{2+} permeable non-selective cation current (I_{cat}) in rat A7r5 aortic smooth muscle cells.

Cell preparation

A7r5 cells (ATCC-7), an established vascular smooth muscle cell line obtained from embryonic rat aorta (Kimes & Brandt, 1976; Ng *et al*., 1994), were purchased from the American Type Culture Collection through Dainippon Seiyaku (Kyoto, Japan). Cells were grown in Dulbecco's modified Eagle's Medium (DMEM, Nissui Seiyaku, Tokyo, Japan) supplemented with 10% foetal bovine serum (M.A. Bioproducts, Walkersville, MD), 50 units ml^{-1} penicillin and 50 μg ml^{-1} streptomycin at 35°C in a fully humidified atmosphere of 5% CO_2 in air. Cells subcultured to passage number 10–30 were grown as monolayers on glass slides, and confluent cell layers were serum-derived by culturing in DMEM containing 0.3% bovine serum albumin for 24 h. Cells were isolated by an enzymatic procedure with trypsin, and used for later experiments. All experiments were performed at 35–37°C.

Solutions and drugs

The composition of the control (Tyrode) extracellular solution was as follows (in mM): NaCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5 and HEPES-NaOH buffer 5.5. (pH 7.4). When the external concentration of K^+ ($[K^+]_o$) was changed, NaCl was replaced with equimolar KCl. Low external Cl^- $[Cl^-]_o$ solution was made by replacing NaCl (136.6 mM) into Na aspartate (136.5 mM). Low external $[Na^+]_o$ solution contained (in mM): TrisCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5 and HEPES-NaOH buffer 5.5 (pH 7.2). The Ca^{2+} bathing-solution contained (in mM): $CaCl_2$ 85, $MgCl_2$ 0.53, glucose 5.5 and HEPES 5, adjusted to pH 7.4 with $Ca(OH)_2$. The K^+-internal solution was as follows (in mM): KCl 130, EGTA 0.05, $MgCl_2$ 2, Na_2ATP 3, guanosine-5′-triphosphate (GTP, sodium salt) 1, and HEPES-KOH buffer 5 (pH 7.2). To block K^+ currents, the patch pipette contained the Cs^+-internal solution (in mM): CsCl 140, EGTA 0.15, $MgCl_2$ 2, Ma_2ATP 3, GTP 1, and HEPES-CsOH buffer 5 (pH 7.2). Low internal $[Cl^-]_i$ solution contained (in mM): Cs aspartate 120, CsCl 20, EGTA 0.15, $MgCl_2$ 2, Na_2ATP 3, GTP 1 and HEPES-CsOH buffer 5 (pH 7.2). In some experiments, guanosine-5′-o-(3-thiotriphosphate) (GTPγS, Boehringer and Mannhein, Germany) was added to the internal solution instead of GTP. [Arg^8]-vasopressin and endothelin-1 were obtained from Sigma Chemical Co. (St Louis, MO). *Cis*-5,8,11,14,17-eicosapentaenoic acid (EPA, Na salt and free acid), *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA, free acid), *cis*-7,10,13,16,19-docosapentaenoic acid (DPA, free acid), arachidonic acid (Na salt), oleic acid, stearic acid and linoleic acid (free acid) were purchased from Sigma Chemical Co. To prepare the salt of DHA or DPA (Na salt, 30 mM), 3 M NaOH was added to 3 M DHA or DPA (free acid). ω-3 fatty acids (EPA, DHA, DPA) (Na salt) and arachidonic acid (Na salt) were dissolved in the control bathing solution and were freshly made before use. Eicosapentaenoic acid (EPA, free acid) and the other fatty acids (free acid) were dissolved in ethanol at a concentration of 10 mM and stored under a nitrogen atmosphere at −20°C. The experimental concentration of fatty acids was obtained by diluting the stock solution. Indomethacin and nordihydroguaiaretic acid (NDGA) were also purchased from Sigma Chemical Co. and were dissolved in ethanol at a concentration of 10 mM.

Recording technique and data analysis

Membrane currents were recorded with glass pipettes by use of the whole-cell clamp configuration (Hamill *et al*., 1981; Nakajima *et al*., 1989), with a patch-clamp amplifier (EPC-7, List Electronics, Darmstadt, Germany). Heat-polished patch pipettes, filled with the artificial internal solution (for composition, see above), had a tip resistance of 2–4 MΩ. Membrane currents were monitored with a high-gain storage oscilloscope (COS 5020-ST, Kikusui Electronics, Tokyo). Capacitance currents were cancelled, by use of the automatic capacitance compensation. At the start of each experiment, the series resistance was compensated. The data were stored on video tapes by a PCM converter system (RP-880, NF electronic circuit design, Tokyo). Later, the data were reproduced, low-passed filtered at 2 kHz (−3 dB) with a Bessel filter (FV-665, NF, 48 dB/octave slope attenuation), sampled at 5 kHz and analysed off-line on a computer with p-Clamp software (Axon Instruments, CA). Voltage-ramp command pulses were used to generate current-voltage (I-V) relationships. In experiments of ramp pulses, nifedipine (10 μM) was added into the bathing solution. Data are expressed as the mean ± s.d. Student's *t* test was used for statistical analysis and $P<0.05$ was considered to be significant.

Results

Induction of K^+ current by ω-3 polyunsaturated fatty acids in rat A7r5 vascular smooth muscle cells

The effects of eicosapentaenoic acid (EPA, Na salt) on membrane currents were first investigated as shown in Figure 1a and Figure 2. Under conditions with a K^+-containing pipette solution, EPA (30 μM) induced an outward current at a holding potential of −40 mV (Figure 1a) and after washout, the current gradually returned to the control level. Tetraethylammonium (20 mM, not shown) or CsCl in the patch pipette (Figure 1c) abolished the activation of the outward current by EPA, suggesting that EPA activates a K^+ current. Similar results were obtained in eight out of ten cells tested. Docosahexaenoic acid (DHA, Na salt, 30 μM) (Figure 1b) or arachidonic acid (Na salt, 30 μM, not shown) also elicited an outward current. The current-voltage relationships of the EPA-induced current were examined with ramp voltage commands (Figure 2) and the EPA-induced current, defined as the difference between the control and the membrane current in the presence of EPA is presented in Figure 2b. The EPA-induced current displayed outward rectification (Figure 2b). With 5.4 mM K^+ in the extracellular solution, the current-voltage relationships of the EPA-induced current reversed at −81 ± 4 mV ($n=5$, Figure 2b and c). The reversal potential (E_{rev}) of the current was shifted by increasing $[K^+]_o$ from 5.4 mM to 20 mM (Figure 2b). The E_{rev} was −44 ± 4 mV ($n=5$) with 20 mM $[K^+]_o$ and −28 ± 3 mV ($n=5$) with 50 mM $[K^+]_o$ (Figure 2c). The E_{rev} changed about 55 mV per ten fold change of $[K^+]_o$, which is close to the theoretical shift of the

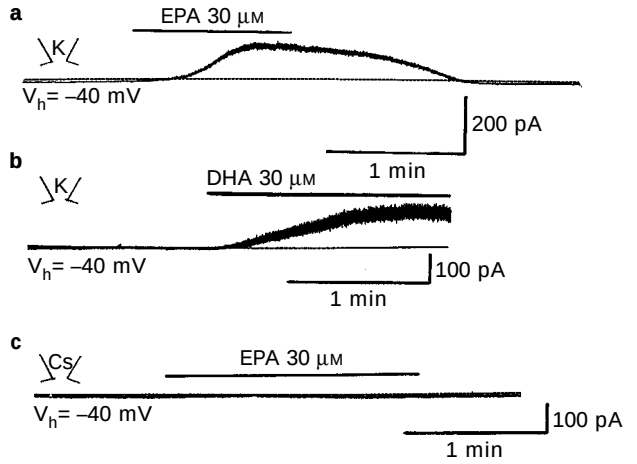

Figure 1 Effect of eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) on membrane currents in rat A7r5 aortic smooth muscle cells. The cells were held at −40 mV (V_h). The patch pipette contained K^+-(a, b) and Cs^+-internal solution (c). The zero current level is denoted by dotted lines. The drug sequences are illustrated with horizontal bars in this figure and the following figures above each trace.

equilibrium potential of K^+ (E_K). These results indicate that the EPA-induced currents are K^+ selective, which is compatible with data from earlier studies showing that polyunsaturated fatty acids, as well as arachidonic acid, activate K^+ channels in various kinds of smooth muscle cells including aorta (Bregestovski *et al*., 1989; Ordway *et al*., 1989; Kirber *et al*., 1992; Xu & Lee, 1996).

Receptor-mediated Ca^{2+}-permeable non-selective cation currents

Figure 3 shows the effects of vasopressin on membrane currents in rat aortic smooth muscle cells (A7r5 cells) with Cs^+-containing pipette solution. Vasopressin (100 nM) induced a long-lasting inward current which was associated with an increase in membrane 'noise' at a holding potential of −60 mV (Figure 3a). The response to vasopressin was observed in 86% of cells (12 of the 14 cells). The current-voltage relationship of the inward current were examined with the voltage ramps. In the control bathing solutions, the current-voltage relationship of the vasopressin-induced current reversed at -2 ± 3 mV ($n=12$, Figure 3a). The reversal potential (E_{rev}) of the current was unaffected by decreasing $[Cl^-]_o$ or $[Cl^-]_i$ (Figure 3b (ii), (iii), Table 1). Alternatively, when Na^+ was replaced by $Tris^+$, the E_{rev} shifted to negative potentials (Figure 3b (i), Table 1). These results suggest that vasopressin activates a non-selective cation current (I_{cat}) with no contribution of a chloride current (Hazama *et al*., 1996). Similarly, vasopressin induced an inward current with 10 mM EGTA or BAPTA in the patch pipette to chelate intracellular Ca^{2+}, which agrees with previous results (Van Renterghem *et al*., 1988; Inoue & Kuriyama 1993; Van Renterghem & Lazdunski, 1994; Krautwurst *et al*., 1994; Nakajima *et al*., 1996). In addition, even when extracellular Na^+ was totally replaced by Ca^{2+} (Figure 3c), vasopressin (100 nM) still activated I_{cat} with a negative shift of E_{rev} (Table 1), suggesting that Ca^{2+} is also a charge carrier of the vasopressin-induced I_{cat} as shown previously (Van Renterghem *et al*., 1988; Nakajima *et al*., 1996).

Figure 4 shows the effects of nifedipine, Cd^{2+} and La^{3+} on vasopressin-induced I_{cat}. Nifedipine (10 μM) did not significantly affect the holding current or inhibit the vasopressin-induced I_{cat} (Figure 4a). However, Cd^{2+} (1 mM) (Figure 4b) and La^{3+} (1 mM) (Figure 4c) completely inhibited the vasopressin-induced I_{cat}. The reduction of I_{cat} by these agents was associated with a decrease in the noise level and the inhibitory effect of these agents was reversible. Endothelin-1 (100 nM) also activated the non-selective cation current and the effects of endothelin-1 were also abolished by La^{3+} (1 mM) (Figure 4d).

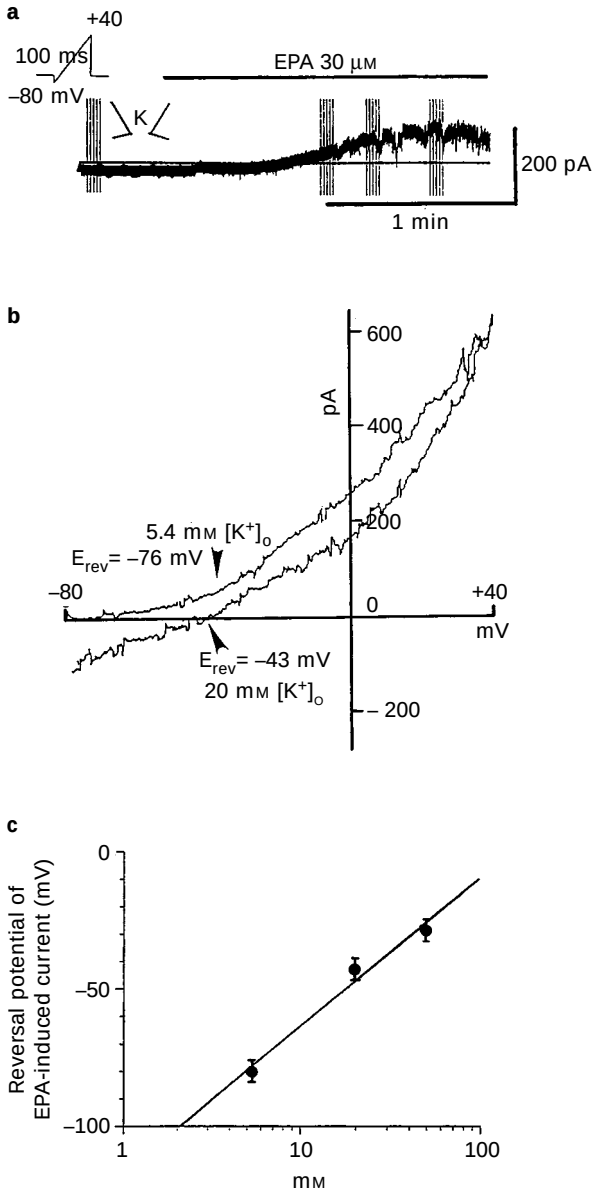

Figure 2 Activation of K^+ current by eicosapentaenoic acid (EPA). (a) The patch pipette contained a K^+-containing solution. The cell was held at −40 mV, and ramp commands from −80 mV to +40 mV (100 ms in duration) were applied before and during the application of EPA (30 μM). The zero current level is denoted by dotted lines. The current-voltage (*I*-V) relationships of the EPA-induced current, defined as the difference between the control and the membrane current in the presence of EPA, were obtained by subtracting the control current from that in the presence of EPA during the ramp pulses. In (b) a typical *I*-V relationship of the EPA-induced current with 5.4 mM K^+ bath solution and 20 mM K^+ bath solution are shown. The reversal potential (E_{rev}) of the EPA-induced current was −76 mV with 5.4 mM K^+, and −43 mV with 20 mM K^+ in the bathing solution. (c) Dependence of the reversal potential of EPA-induced currents on $[K^+]_o$. The reversal potential is plotted as a function of $[K^+]_o$. The mean values are shown from five different cells in each $[K^+]_o$; vertical lines show s.d.

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Figure 5 illustrates the effects of EPA (30 μM) on the vasopressin-induced I_{cat}. With a Cs^+-containing pipette solution,

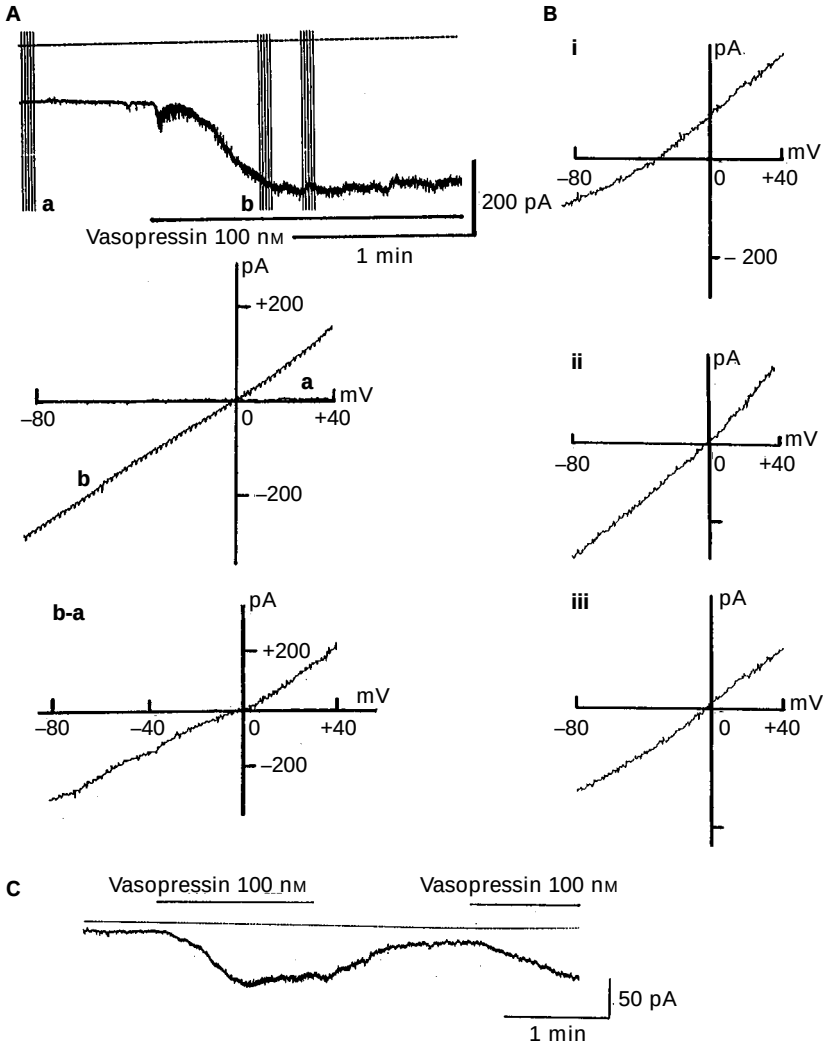

Figure 3 Activation of Ca^{2+}-permeable non-selective cation channels by vasopressin in rat A7r5 aortic smooth muscle cells. (A) Activation of the inward current by vasopressin. The cells were held at −60 mV and the patch pipette was filled with the Cs^+-containing pipette solution. Ramp voltage commands from −80 mV to +40 mV (100 ms in duration) were applied before and during the application of vasopressin (100 nM). The current traces of ramp pulses (a, b) recorded at the times indicated in the upper part of the trace are shown in the graph. The current-voltage relationships of (a) and (b) are shown, after the leakage current determined by linear approximation of current amplitudes measured during 5 mV hyperpolarizing steps from −80 mV had been subtracted. The current-voltage relationships of the subtraction current from (b−a) are shown in the lower part of (A). (B) Effect of altering the Na^+ or Cl^- concentration on the reversal potential (E_{rev}) of the vasopressin-induced current. (i) Extracellular Na^+ was replaced by Tris. (ii, iii) Effect of changing extracellular ($[Cl^-]_o$) or intracellular ($[Cl^-]_i$) Cl^- on E_{rev} where extracellular (ii) or intracellular Cl^- (iii) was replaced by aspartate. The typical *I*-V relationships of the vasopressin-induced current are indicated in (i–iii). (C) Activation of the inward current by vasopressin in a bathing solution containing 85 mM $CaCl_2$; the cell was held at −60 mV.

Table 1 Reversal potential of the vasopressin-induced currents in rat A7r5 vascular smooth muscle cells

Ionic conditions		
Bath solution	*Pipette solution*	*Reversal potential*
Control Tyrode solution (136 mM Na^+, 145 mM Cl^-)	CsCl – internal solution (140 mM Cs^+, 145 mM Cl^-)	−2±3 mV (n=12)
Control Tyrode solution (136 mM Na^+, 145 mM Cl^-)	Cs aspartate – internal solution (140 mM Cs^+, 24 mM Cl^-)	−3±4 mV (n=5)
Low Cl^- solution (136 mM Na^+, 10 mM Cl^-)	CsCl – internal solution (140 mM Cs^+, 145 mM Cl^-)	−4±3 mV (n=5)
Low Na^+ solution (136 mM $Tris^+$, 145 mM Cl^-)	CsCl – internal solution (140 mM Cs^+, 145 mM Cl^-)	−37±6 mV (n=4)*
Ca^{2+} bathing solution (85 mM Ca^{2+})	CsCl – internal solution (140 mM Cs^+, 145 mM Cl^-)	−9±4 mV (n=4)*

Extracellular Na^+ was replaced by $Tris^+$, and extracellular or intracellular Cl^- was replaced by aspartate. The detailed compositions are shown in the Methods section. The data represent the mean±s.d. and the number of cells tested (n) is shown in parentheses. *$P<0.05$ when compared with the control (145 mM $[Cl^-]_o$/145 mM $[Cl^-]_i$).

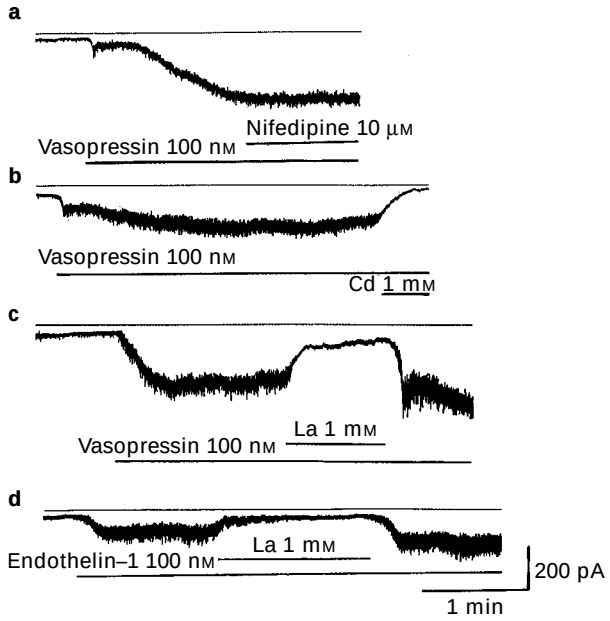

Figure 4 Effect of (a) nifedipine (10 μM), (b) Cd^{2+} (1 mM) and (c) La^{3+} (1 mM) on the vasopressin-activated non-selective cation current. The cells were held at −40 mV. The zero current level is illustrated by the dotted lines. (d) Activation of the inward current by endothelin-1. Endothelin-1 (100 nM) activated the inward current.

EPA (Na salt, 10–100 μM) did not affect the holding current significantly as shown in Figure 1c. However, EPA (Na salt, 30 μM) did inhibit the vasopressin-activated I_{cat} and this was partially reversible (Figure 5Aa). On the other hand, after EPA was washed out with a bath solution containing 1 mg of bovine serum albumin ml^{-1}, the amplitude of I_{cat} returned rapidly to the control level (Figure 5Ab). The EPA-induced suppression of the currents was observed at all voltages studied (Figure 5b). The reversal potential of the current was not altered by EPA (Na salt, 30 μM) (Figure 5B), which was approximately +0 mV. The inhibitory effect of EPA was also observed in the free acid form of EPA (Figure 5Ac).

Figure 6 shows the effects of various concentrations of ω-3 fatty acids on the vasopressin-activated I_{cat}. EPA (3–100 μM) inhibited the vasopressin-induced current in a concentration-dependent manner (Figure 6Aa). DHA (100 μM, Figure 6Ab) and DPA (100 μM, Figure 6Ac) also suppressed the current and the potency of the inhibitory effect of the ω-3 fatty acids was EPA > DHA > DPA. Figure 6B summarizes the concentration-dependence of the ω-3 fatty acid-induced suppression of the vasopressin-activated I_{cat}. The half-maximal inhibitory concentration (IC_{50}) of EPA was 7.0 μM, and 100 μM EPA almost completely inhibited the currents. EPA (30 μM) inhibited the endothelin-induced I_{cat} in a similar manner (Figure 7A). Taken together, these results indicate that the ω-3 fatty acids inhibit the receptor (vasopressin and endothelin-1)-activated I_{cat} in rat A7r5 vascular smooth muscle cells.

Inhibitory effect of ω-3 fatty acids on GTPγS-induced I_{cat}

To investigate the site of action of EPA on I_{cat}, the effect of EPA on GTPγS-induced non-selective cation currents was examined. For this purpose, the patch pipette was filled with the non-hydrolysable GTP analogue (GTPγS, 1 mM). Immediately after the rupture of the membrane (Figure 7B), the holding current gradually increased in the inward direction at a holding potential of −40 mV even in the absence of agonist (vasopressin or endothelin-1). The reversal potential of the GTPγS-induced current was -1 ± 3 mV ($n = 4$), and was unaffected by

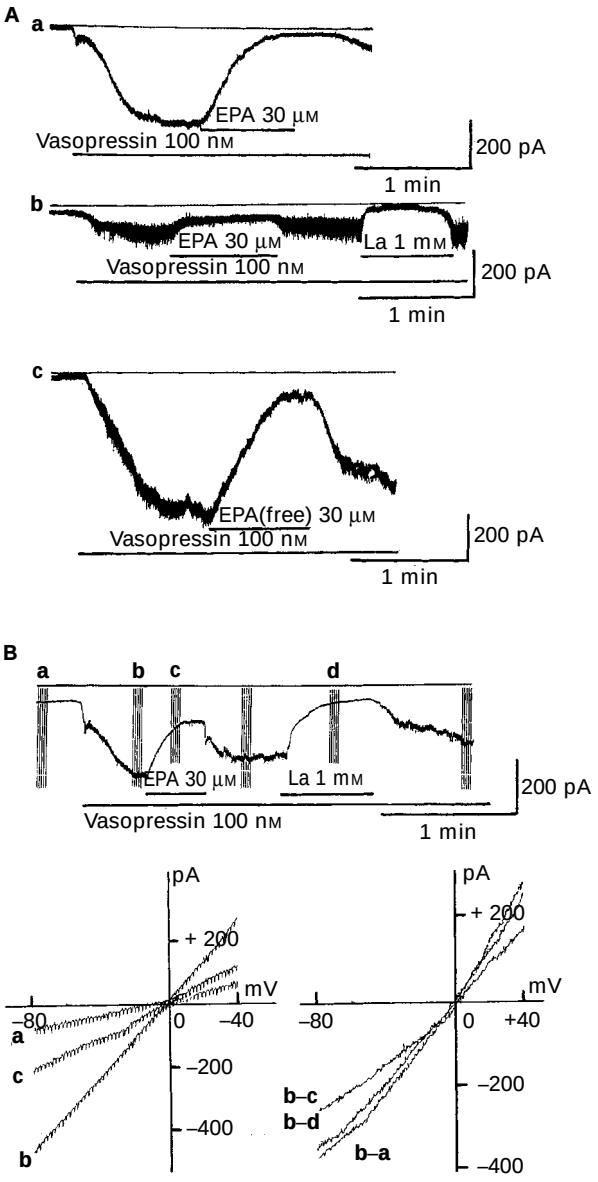

Figure 5 Effect of EPA (eicosapentaenoic acid) on vasopressin-activated non-selective cation current in rat A7r5 aortic smooth muscle cells. The cells were held at −40 mV. In (Aa), EPA was washed out with control bath solution. In (Ab) and (Ac), EPA was washed out with bath solution containing 1 mg bovine serum albumin (BSA) ml^{-1}. The dotted lines represent the zero current level. EPA (Na salt) was used in (Aa) and (Ab), and EPA (free acid) was used in (Ac). In the subsequent figures, the fatty acids were washed out with bath solution containing 1 mg ml^{-1} (BSA). (B) Effect of EPA on the current-voltage (*I*-V) relationship of the vasopressin-induced current. The cell was held at −60 mV, and ramp voltage commands (−80 mV to +40 mV in 100 ms duration) were applied under control conditions (a), during the application of vasopressin (100 nM) (b), vasopressin plus EPA (Na salt, 30 μM) (c), and vasopressin plus La (1 mM) (d). The original current traces of (a)–(c) are shown on the left side of the lower part. The current-voltage relationships of the subtraction currents of (b) − (a), (b) − (c) and (b) − (d) are shown on the right side of the lower part.

changes in $[Cl^-]_o$ or $[Cl^-]_i$. These results suggest that GTPγS activates I_{cat} even in the absence of agonists, possibly by direct activation of the GTP-binding proteins coupled to the receptors (vasopressin and endothelin receptors). EPA (3–100 μM) inhibited the GTPγS-activated I_{cat} in a concentration-dependent manner (Figure 7B). The concentration-dependence of the relationships for the inhibitory effect of EPA on the vasopressin-induced I_{cat} and GTPγS-induced currents (Table 2) were similar, suggesting that the site of action of EPA is not located on the pharmacological receptor.

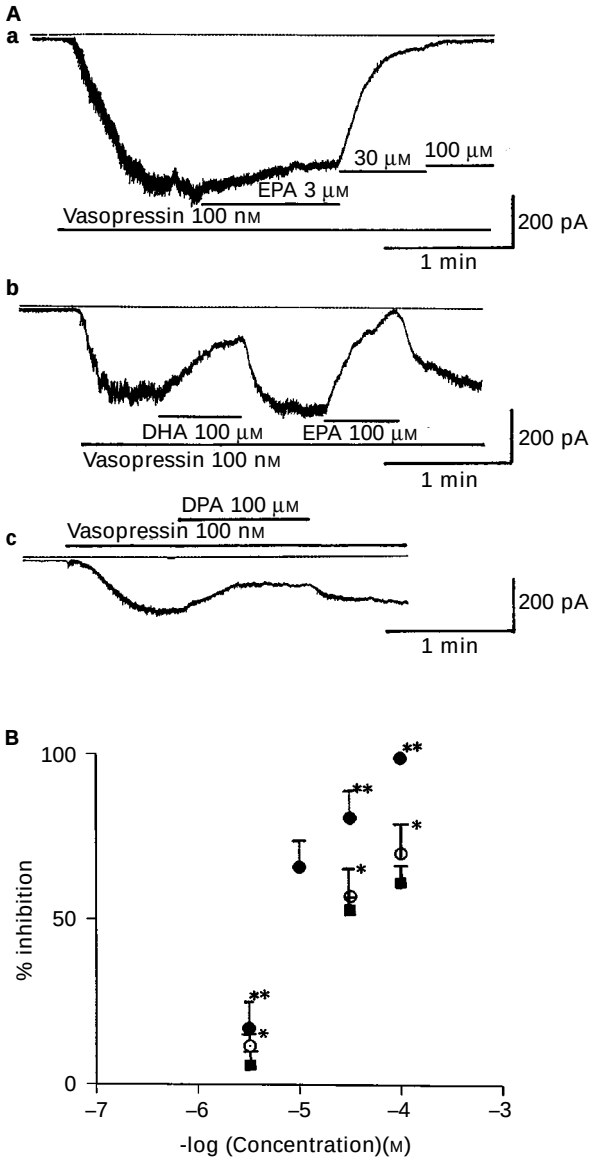

Figure 6 Effects of the ω-3 fatty acids on the vasopressin-activated non-selective cation current I_{cat}. The cells were held at −40 mV. (A) Effects of EPA (a), DHA (b) and DPA (c) on I_{cat}. The zero current level is indicated by dotted lines. (B) Dose-response curve for ω-3 fatty acid-induced suppression of the vasopressin-induced non-selective cation current; EPA (●), DHA (○) and DPA (■) were examined at various concentrations. The vasopressin-induced current was measured in the presence of vasopressin (100 nM) alone and plus an ω-3 fatty acid. The % inhibition by the ω-3 fatty acid of I_{cat} is illustrated. Each point represents the mean of data obtained from five different cells; vertical lines show s.d. EPA (eicosapentaenoic acid, Na salt), DHA (docosahexanoic acid, Na salt) and DPA (docosapentaenoic acid, Na salt). $^{**}P<0.05$ vs DHA at various concentrations. $^{*}P<0.05$ vs DPA at various concentrations.

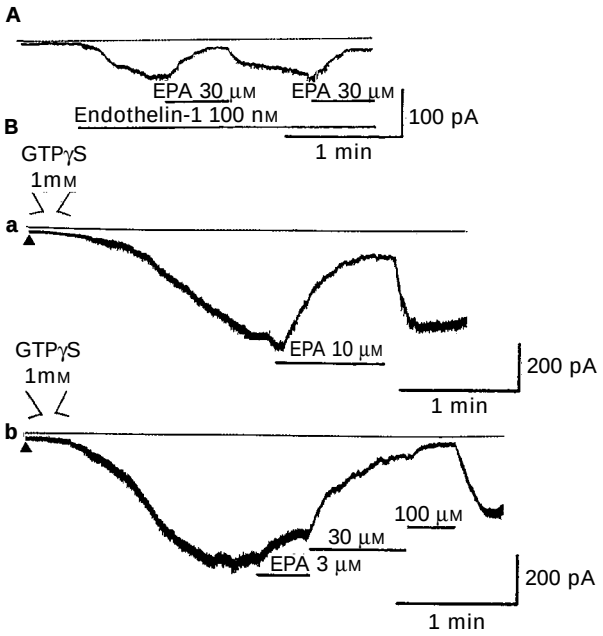

Figure 7 Effect of eicosopentaenoic acid (EPA) on endothelin-1- and GTPγS-induced non-selective cation currents. The cells were held at −40 mV. (A) Effect of EPA (Na salt, 30 μM) on endothelin-1 -induced non-selective cation current. Endothelin-1 (100 nM) was added to the bath solution and EPA (Na salt) was washed out with bath solution containing 1 mg ml^{-1} BSA. (B) Effect of EPA (Na salt) on GTPγS-induced non-selective cation current. The patch pipette was filled with Cs^{+}-internal solution containing GTPγS (1 mM). Rupture of the membrane occurred at the arrowhead (▲).

Table 2 Inhibition of EPA of vasopressin or GTPγS-induced currents in aortic smooth muscle cells

	% inhibition	
EPA concentration	*Vasopressin*	*GTPγS*
3 μM	16±10 (n=6)	18±8 (n=6)
10 μM	65±9 (n=6)	70±17 (n=6)
30 μM	82±8 (n=6)	85±9 (n=6)
100 μM	97±3 (n=6)	98±2 (n=6)

The % inhibition by eicosapentaenoic acid (EPA, 3–100 μM) of vasopressin- or GTPγS-induced non-selective cation currents is indicated. The mean±s.d. value is shown and the number of cells tested (n) is shown in parentheses. No statistically significant difference between the % inhibition by EPA on vasopressin-induced non-selective cation currents and that by EPA on GTPγS-induced non-selective cation currents at any concentration was observed.

Effects of some other polyunsaturated and saturated fatty acids on the vasopressin-induced non-selective cation current

Figure 8 shows the effects of EPA (10, 30 μM), arachidonic acid (C20:4 (ω-6)) (10, 30 μM), linoleic acid (C18:2 (ω-6)) (10, 30 μM), oleic acid (C18:1 (ω-9)) (30 μM) and stearic acid (C18:0) (30 μM) on the vasopressin-induced I_{cat}. Arachidonic acid (Figure 8a) and linoleic acid (10, 30 μM) inhibited the current, but their inhibitory effects were smaller than that of EPA and the other ω-3 fatty acids (Figure 8a and c). By contrast, oleic acid (Figure 8b), a monounsaturated fatty acid, and stearic acid, a saturated fatty acid, had no significant inhibitory effects on the vasopressin-induced non-selective cation current (Figure 8c). To exclude the possible involvement of the cyclo-oxygenase and lipoxygenase pathways, the effects of EPA on the vasopressin-induced I_{cat} were investigated in the presence of both indomethacin (10 μM), a cyclo-oxygenase inhibitor, and NDGA (10 μM), a lipoxygenase inhibitor. Indomethacin (10 μM, Figure 9a) and NDGA (10 μM, Figure 9b) did not inhibit the vasopressin-induced I_{cat} and the inhibitory effects of EPA were still observed in all cells tested (n=3), as shown in Figure 9c.

Discussion

The major findings of the present study are: (1) ω-3 polyunsaturated fatty acids (EPA or DHA) induced a K^{+} current in rat A7r5 aortic smooth muscle cells. (2) ω-3 polyunsaturated fatty acids, EPA, DHA and DPA, at concentrations of 3–100 μM inhibited I_{cat} activated by vasopressin and endothelin.

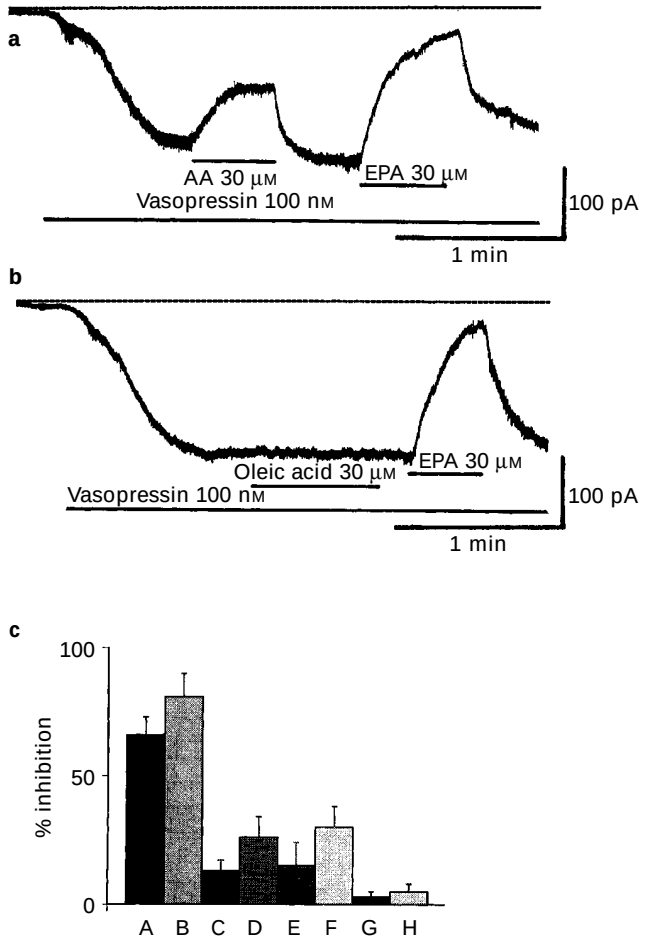

Figure 8 Comparison of the blocking effects of fatty acids on the vasopressin-induced non-selective cation currents (I_{cat}). (a and b) Effects of various fatty acids on the I_{cat}. The cells were held at −40 mV. (c) Comparison of the blocking effects of fatty acids on the I_{cat}: column (A) EPA 10 μM, (B) EPA 30 μM, (C) AA 10 μM, (D) AA 30 μM, (E) linoleic acid 10 μM, (F) linoleic acid 30 μM, (G) oleic acid 30 μM and (H) stearic acid 30 μM. Each column represents the mean % inhibition ± s.d. The data were obtained from five different cells in each case. EPA (eicosapentaenoic acid, Na salt), AA (arachidonic acid, Na salt), linoleic acid (free acid), oleic acid (free acid) and stearic acid (free acid).

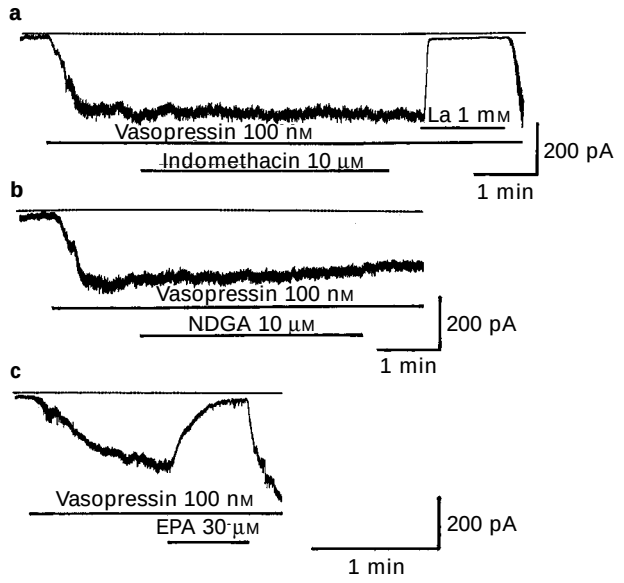

Figure 9 Effects of eicosapentaenoic acid (EPA) on the vasopressin-induced non-selective cation currents in the presence of (a) indomethacin (10 μM) and (b) nordihydroguaiaretic acid (NDGA, 10 μM). (c) Effects of EPA (30 μM) on the vasopressin-induced non-selective cation currents in a cell treated with indomethacin (10 μM) and NDGA (10 μM) for approximately 30 min.

(3) The site of action of ω-3 fatty acids is not located on the receptors.

It has been shown that fish oil and ω-3 fatty acids, eicosapentaenoic and docosahexaenoic acids (EPA and DHA), induce relaxation of α-adrenoceptor agonist- and angiotensin II-induced contraction in aortic smooth muscle (Juan *et al*., 1987; Engler *et al*., 1990; Engler, 1992a, b, c). The relaxant effects of the ω-3 fatty acids were not modified by the use of indomethacin or NDGA (Engler *et al*., 1990), suggesting that cyclo-oxygenase/lipoxygenase pathway products, e.g. vasoactive eicosanoids and docosanoids, are not involved in the relaxant action. Furthermore, the vasoactive effects of EPA are not attributed to elevated levels of cyclic nucleotides (cyclic AMP, cyclic GMP) (Juan *et al*., 1987). In addition, EPA or DHA produced a concentration-dependent relaxant effect in vessels contracted with noradrenaline, an α-adrenoceptor agonist, without substantially affecting KCl-induced contraction (Engler, 1992a, b, c), suggesting that the voltage-dependent L-type Ca^{2+} channel is not a primary site of the inhibitory action of the ω-3 fatty acids. Moreover, DHA and EPA did not affect the initial phase of the noradrenaline-induced contraction in Ca^{2+}-free solution (Engler 1992b), which is mediated by inositol 1,4,5-triphosphate (IP_3) formation and subsequent release of intracellular calcium. Taking these previous findings into account, it is suggested that the relaxant effects of the ω-3 fatty acids may not be attributed to an inhibition of IP_3 formation, but to a preferential inhibition of calcium influx associated with receptor-operated channels. In vascular smooth muscle cells, contractile agonists such as noradrenaline, vasopressin and endothelin induce Ca^{2+} release from internal stores and Ca^{2+} influx through receptor-operated Ca^{2+} channels or from Ca^{2+} influx through voltage-dependent Ca^{2+} channels. The receptor-operated Ca^{2+} entry is partly mediated by Ca^{2+}-permeable non-selective cation channels, which are resistant to calcium antagonists, such as nifedipine (Benham & Tsien, 1987; Byrne & Large, 1988; Van Renterghem *et al*., 1988; Amedee *et al*., 1990; Wang & Large, 1991; Chen & Wagoner 1991; Loirand *et al*., 1991; Inoue & Kuriyama, 1991; Van Renterghem & Lazdunski, 1994; Krautwurst *et al*., 1994; Nakajima *et al*., 1996). The present results demonstrate that the ω-3 unsaturated fatty acids inhibit the receptor (vasopressin and endothelin-1)-mediated Ca^{2+}-permeable non-selective cation current. The inhibitory effect of the ω-3 fatty acids was observed at concentrations of 3–10 μM, indicating that the inhibitory action of the ω-3 fatty acids on I_{cat} may contribute to the vasorelaxant actions. Actually, the ω-3 fatty acids at concentrations more than 1 μM have been shown to produce a concentration-dependent relaxation in vessels contracted with noradrenaline (Engler, 1992a, b).

The inhibitory effect of the ω-3 fatty acids on the channels could occur at any of the steps that exist between receptor binding and channel opening. High concentrations of EGTA or BAPTA (10 mM) in the patch pipette did not block the activation of the current by vasopressin or endothelin-1, indicating that I_{cat} is not directly mediated by a rise in the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$). These results are consistent with those of earlier reports (Byrne & Large, 1988; Van Renterghem *et al*., 1988; Wang & Large, 1991; Inoue & Kuriyama, 1993; Van Renterghem & Lazdunski, 1994; Krautwurst *et al*., 1994; Nakjima *et al*., 1996). The treatment of smooth muscle by free fatty acids such as EPA and DHA has been shown to inhibit binding or pressor hormones (including angiotensin II) to vascular smooth muscle with consequent reduction in IP_3 formation and in calcium mobilization (Ullian, 1993). However, it is also unlikely that a direct inhibitory effect on receptors (vasopressin and en-

dothelin-1) is primarily involved in ω-3-induced inhibition of I_{cat}. As illustrated in Figure 7, and shown in previous studies (Krautwurst *et al*., 1994; Nakjima *et al*., 1996), activation of I_{cat} by vasopressin and endothelin is mediated by GTP-binding proteins. Inclusion of GDPβS in the patch pipette solution abolished activation of the current, and the addition of GTPγS, a non-hydrolysable GTP analogue, activated I_{cat} in the absence of agonists, probably due to the direct activation of GTP-binding proteins coupled to receptors (Krautwurst *et al*., 1994; Nakajima *et al*., 1996). ω-3 fatty acids inhibited both the agonist-dependent activation of I_{cat} and the activation of I_{cat} by GTPγS in a similar manner (Figure 7B, Table 2), suggesting that the receptor is not the primary site of the inhibitory action of ω-3 fatty acids. As illustrated in Figure 3, immediately after wash-out with albumin, the suppressed current rapidly increased within several seconds. The reason for this early rapid wash-out effect is not clear. However, the inhibitory effects of ω-3 fatty acids on kinase activity, i.e. protein kinase C as previously shown (Holian & Nelsen, 1992), may not be involved in the actions of ω-3 which appear to modulate directly the channel protein itself or act at some site near the channels.

Lipoxygenase-dependent metabolites of arachidonic acid regulate Na^+ channels in A6 cells (Cantiello *et al*., 1990) and play an important role in the modulation of activity of the 5-HT-gated channels in *Aplysia* sensory neurone (Piomelli *et al*., 1987), and the GTP-binding protein-coupled muscarinic receptor gated K^+ channels in cardiac myocytes (Kim *et al*., 1989; Kurachi *et al*., 1989). In the present study, neither indomethacin, a cyclo-oxygenase inhibitor, nor NDGA, a lipoxygenase inhibitor, prevented the inhibitory effects of ω-3 fatty acids on I_{cat}, suggesting that the metabolites of cyclo-oxygenase or lipoxygenase pathways are not involved in the action of ω-3 fatty acids.

Fatty acids are known to have a wide variety of effects on ionic channels in excitable cells including smooth muscle. In toad stomach smooth muscle cells, fatty acids such as arachidonic acid activate a K^+ channel with a conductance of approximately 40 pS (Ordway *et al*., 1989). In vascular smooth muscle cells of mammalian origin (Bregestovski *et al*., 1989; Kirber *et al*., 1992; Xu & Lee, 1996), a Ca^{2+}-activated or ATP-sensitive K^+ channel is activated by certain fatty acids. The present study confirmed that ω-3 polyunsaturated fatty acids induce K^+ currents in aortic smooth muscle cell lines, but it remains to be clarified which type of K^+ channel is activated by ω-3 fatty acids. Therefore, the hyperpolarizing action of the fatty acids by activating the K^+ channel may also contribute to the relaxant action of the ω-3 fatty acids.

Recently, Xiao *et al*. (1995) showed that ω-3 fatty acids block Na^+ channels of neonatal rat ventricular myocytes with an IC_{50} value of 4.8 μM, which is very similar to that of EPA (7 μM) on I_{cat} in the present study. Thus, ω-3 polyunsaturated fatty acids may play an important role in regulating vascular tone as well as cardiac excitability.

This work was supported in part by grants from Ministry of Education, Science and Culture of Japan and a Grant-in-Aid for Scientific Research on Priority Areas of 'Channel-Transporter Correlation' to T.N.

References

AMEDEE, T., BENHAM, C.D., BOLTON, T.B., BYRNE, N.G. & LARGE, W.A. (1990). Potassium, chloride and non-selective cation conductances opened by noradrenaline in rabbit ear artery cells. *J. Physiol*., **423,** 551–568.

BANG, H.O. & DYERBERG, J. (1972). Plasma lipids and lipoproteins in Green-landic west coast Eskimos. *Acta Med. Scand.*, **192,** 85–94.

BENHAM, C.D. & TSIEN, R.W. (1987). A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle. *Nature*, **328,** 275–278.

BONAA, K.H., BJERVE, K.S., STRAUME, B., GRAM, I.T. & THELLE, D. (1990). Effect of eicosapentaenoic and docosahexaenoic acids on blood pressure in hypertension. *N. Engl. J. Med.*, **322,** 795–801.

BREGESTOVSKI, P.D., BOLOTINA, V.M. & SEREBRYAKOV, V.N. (1989). Fatty acid modifies Ca^{2+}-dependent potassium channel activity in smooth muscle cells from the human aorta. *Proc. R. Soc. Lond. B.*, **237,** 259–266.

BROX, J.M., KILLIE, J.E., GRUNNES, S. & NORDOY, A. (1981). The effect of cod liver oil and corn oil on platelet and vessel wall in man. *Thromb. Haemostasis*, **46,** 604–611.

BYRNE, N.G. & LARGE, W.A. (1988). Membrane ionic mechanisms activated by noradrenaline in cells isolated from the rabbit portal vein. *J. Physiol*., **404,** 557–573.

CANTIELLO, H.F., PATENAUDE, C.R., CODINA, J., BIRNBAUMER, L. & AUSIELLO, D.A. (1990). $G\alpha_i$-3 regulates epithelial Na channels by activation of phospholipase A_2 and lipoxygenase pathways. *J. Biol. Chem*., **265,** 21624–21628.

CHEN, C. & WAGONER, K. (1991). Endothelin induces a non-selective cation current in vascular smooth muscle cells. *Circ. Res*., **69,** 447–454.

DYERBERG, J., BANG, H.O., STOFFERSON, E., MONCADA, S. & VANE, J.R. (1978). Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet*, **II,** 117–119.

ENGLER, M.B., KARANIAN, J.W. & SALEM, N. (1990). Docosahexaenoic acid (22: 6n3)-induced relaxation of the rat aorta. *Eur. J. Pharmacol*., **185,** 223–226.

ENGLER, M.B. (1992a). Vascular relaxation to omega-3 fatty acids: comparison to sodium nitroprusside, nitroglycerin, papaverine, and D600. *Cardiovasc. Drugs Ther*., **6,** 605–610.

ENGLER, M.B. (1992b). Effect of omega-3 fatty acids, docosahexaenoic and eicosapentaenoic, on norepinephrine-induced contractions. *Can. J. Physiol. Pharmacol*., **70,** 675–679.

ENGLER, M.B. (1992c). Effects of omega-3, omega-6 and omega-9 fatty acids on vascular smooth muscle tone. *Eur. J. Pharmacol*., **215,** 325–328.

HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch*., **391,** 85–100.

HARRIS-HOOKER, S.A., GAJDUSEK, S.M., WIGHT, T.N. & SWARTZ, S.M. (1983). Neovascularization responses induced by cultured aortic endothelial cells. *J. Cell. Physiol*., **114,** 302–310.

HAZAMA, H., NAKAJIMA, T., HAMADA, E., OMATA, M. & KURACHI, Y. (1996). Neurokinin A and Ca^{2+} current induce Ca^{2+}-activated Cl^- currents in guinea-pig tracheal myocytes. *J. Physiol*., **492,** 377–393.

HOLIAN, O. & NELSON, R. (1992). Action of long-chain fatty acids on protein kinase C activity: comparison of omega-6 and omega-3 fatty acids. *Anticancer Res*., **12,** 975–980.

HUI, R., ST LOUIS, J. & FALARDEAU, P. (1989). Antihypertensive properties of linoleic acid and fish oil omega-3 fatty acids independent of the prostaglandin system. *Am. J. Hypertens*., **2,** 610–617.

INOUE, R. & KURIYAMA, H. (1993). Dual regulation of cation-selective channels by muscarinic and α_1-adrenergic receptors in the rabbit portal vein. *J. Physiol*., **465,** 427–448.

JUAN, H., SUTTER, D. & SAMETZ, W. (1987). Influence of eicosapentaenoic acid on noradrenaline and angiotensin-induced contractions of the rabbit aorta: mode of action. *Prostaglandins Clin. Res*., 52–62.

KIM, D., LEWIS, D.L., CRAZIADEI, L., NEER, E.J., BAR-SAGI, D. & CLAPHAM, D.E. (1989). G-protein βγ-subunits activate the cardiac muscarinic K channel via phospholipase A_2. *Nature*, **337,** 557–560.

KIMES, B.W. & BRANDT, B.L. (1976). Characterization of two putative smooth muscle cell lines from rat thoracic aorta. *Exp. Cell. Res*., **98,** 349–366.

KIRBER, M.T., ORDWAY, R.W., CLAPP, L.H., WALSH, J.V. & SINGER, J.J. (1992). Both membrane stretch and fatty acids directly activate large conductance Ca^{2+}-activated K^+ channels in vascular smooth muscle cells. *FEBS Lett*., **297,** 24–28.

KNAPP, H.R. & FITZGERALD, G.A. (1989). The antihypertensive effects of fish oil. *N. Engl. J. Med*., **320,** 1037–1043.

KRAUTWURST, D., DEGTIAR, V.E., SCHULTZ, G. & HESCHELER, J. (1994). The isoquinoline derivative LEO 908 selectively blocks vasopressin-activated nonselective cation currents in A7r5 aortic smooth muscle cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **349,** 301 – 307.

KROMANN, N. & GREEN, A. (1980). Epidemiological studies in the Upernavik district, Greenland: incidence of some chronic disease 1950-74. *Acta Med. Scand.*, **208,** 401 – 406.

KROMHOUT, D., BOSSCHIETER, E.B. & DE LEZENENE, C.C. (1985). The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N. Engl. J. Med.*, **312,** 1205 – 1209.

KURACHI, Y., ITO, H., SUGIMOTO, T., SHIMIZU, T., MIKI, I. & UI, M. (1989). Arachidonic acid metabolites as intracellular modulation of the G protein-gated cardiac K^+ channel. *Nature*, **337,** 555 – 557.

LOIRAND, J., PACAUD, P., BARON, A., MIRONNEAU, C. & MIRONNEAU, J. (1991). Large conductance calcium-activated non-selective cation channel in smooth muscle cells isolated from rat portal vein. *J. Physiol.*, **437,** 461 – 475.

LORENZ, R., SPENGLER, U., FISHER, S., DUHM, J. & WEBER, P.C. (1983). Platelet function, thromboxane formation and blood pressure control during supplementation of the Western diet with cod liver oil. *Circulation*, **67,** 504 – 511.

MERRILL, A.H. & SCHROEDER, J.J. (1993). Lipid modulation of cell function. *Ann. Rev. Nutr.*, **13,** 539 – 559.

MAKAJIMA, T., HAZAMA, H., HAMADA, E., WU, S.N., IGARASHI, K., YAMASHITA, T., SEYAMA, Y., OMATA, M. & KURACHI, Y. (1996). Endothelin-1 and vasopressin activate Ca^{2+}-permeable non-selective cation channels in aortic smooth muscle cells: mechanism of receptor-mediated Ca^{2+} influx. *J. Mol. Cell. Cardiol.*, **28,** 707 – 722.

NAKAJIMA, T., KURACHI, Y., ITO, H., TAKIKAWA, R. & SUGIMOTO, T. (1989). Anti-cholinergic effects of quinidine, disopyramide, and procainamide in isolated atrial myocytes: mediation by different molecular mechanisms. *Circ. Res.*, **64,** 297 – 303.

NG, L.L., DAVIES, J.E. & WOJCIKIEWICZ, R.J.H. (1994). 3-Hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibition modulates vasopressin-stimulated Ca^{2+} responses in rat A_{10} vascular smooth muscle cells. *Circ. Res.*, **74,** 173 – 181.

ORDWAY, R.W., SINGER, J.J. & WALSH, J.V. (1991). Direct regulation of ion channels by fatty acids. *Trends Neurosci.*, **14,** 96 – 100.

ORDWAY, R.W., WALSH, J.V. & SINGER, J.J. (1989). Arachidonic acid and other fatty acids directly activate potassium channels in smooth muscle cells. *Science*, **244,** 1176 – 1179.

PIOMELLI, D., VOLTERRA, A., DALE, N., SIEGELBAUM, S.A., KANDEL, E.R., SCHWARTZ, J.H. & BELARDETTI, F. (1987). Lipoxygenase metabolites of arachidonic acid as second messengers for presynaptic inhibition of Aplysia sensory cells. *Nature*, **328,** 38 – 43.

PUSKA, P., NISSINEN, A., VARTIAINEN, E., DOUGHERTY, R., MUTANEN, M., IACONA, J.M., KARHONEN, H.J., PIETINEN, P., LEINO, U. & MOISIO, S. (1983). Controlled, randomized trail of the effect of dietary fat on blood pressure. *Lancet*, **I,** I – 5.

SHIMOKAWA, H. & VANHOUTTE, P.M. (1988). Dietary cod-liver oil improves endothelial-dependent response in hypercholesterolemic and atherosclerotic porcine coronary arteries. *Circulation*, **78,** 1421 – 1430.

ULLIAN, M.E. (1993). Fatty acid inhibition of angiotensin II-stimulated inositol phosphates in smooth muscle cells. *Am. J. Physiol.*, **264,** H595 – H603.

VAN RENTERGHEM, C. & LAZDUNSKI, M. (1994). Identification of the Ca^{2+} current activated by vasoconstrictors in vascular smooth muscle cells. *Pflugers Arch.*, **429,** 1 – 6.

VAN RENTERGHEM, C., ROMEY, G. & LAZDUNSKI, M. (1988). Vasopressin modulates the spontaneous electrical activity in aortic cells (line A7r5) by acting on three different types of ionic channels. *Proc. Natl. Acad. Sci. U.S.A.*, **85,** 9365 – 9369.

WANG, Q. & LARGE. W.A. (1991). Noradrenaline-evoked cation conductance recorded with the nystatin whole-cell method in rabbit portal vein cells. *J. Physiol.*, **435,** 21 – 39.

XIAO, Y.F., KANG, J.X., MORGAN, J.P. & LEAF, A. (1995). Blocking effects of polyunsaturated fatty acids on Na^+ channels of neonatal rat ventricular myocytes. *Proc. Natl. Acad. Sci. U.S.A.*, **92,** 11000 – 11004.

XU, X. & LEE, K.A. (1996). Dual effects of arachidonic acid on ATP-sensitive K^+ current of coronary smooth muscle cells. *Am. J. Physiol.*, **270,** H1957 – H1962.

(Received December 10, 1996
Revised January 2, 1997
Accepted January 3, 1997)