ELSEVIER

European Journal of Pharmacology 379 (1999) 199–209

ejp

www.elsevier.nl/locate/ejphar

Eicosapentaenoic acid inhibits vasopressin-activated Ca^{2+} influx and cell proliferation in rat aortic smooth muscle cell lines

Michiko Asano [a,c], Toshiaki Nakajima [a,*], Kuniaki Iwasawa [a], Yukari Asakura [c], Toshihiro Morita [a], Fumitaka Nakamura [a], Takanobu Tomaru [a], Yuepeng Wang [a], Atsuro Goto [a], Teruhiko Toyo-oka [a], Masaaki Soma [b], Seiji Suzuki [c], Yukichi Okuda [c]

[a] *Second Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*
[b] *Mochida Pharmaceutical, 7, Yotsuya 1-chome, Shinjuku-ku, Tokyo 160-8515, Japan*
[c] *Department of Internal Medicine, Institute of Clinical Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-0005, Japan*

Received 29 October 1998; received in revised form 26 May 1999; accepted 29 June 1999

Abstract

The purpose of this study was to clarify how eicosapentaenoic acid (EPA), an ϖ-3 polyunsaturated fatty acid, modulates the vascular action of vasopressin in rat aortic smooth muscle cell lines. The effects of EPA on Ca^{2+} mobilization and DNA synthesis elicited by vasopressin were investigated and compared to those of Ca^{2+} channel blocking agents, by means of Ca^{2+} measurements and the incorporation of [^{3}H]thymidine. Patch-clamp techniques were also employed. Vasopressin (100 nM) elicited an initial peak of intracellular Ca^{2+} ($[Ca^{2+}]_i$), followed by a sustained phase due to Ca^{2+} entry. Nifedipine or nicardipine (1 μM), a potent L-type Ca^{2+} channel blocker, partly inhibited the sustained phase, but La^{3+} completely abolished it. EPA (10 μM) also inhibited it even in the presence of nicardipine. Under voltage-clamp conditions with CsCl-internal solution, depolarizing pulses positive to −30 mV from a holding potential of −40 mV elicited a slow inward current. The inward current was blocked by La^{3+}, nicardipine, and nifedipine (1 μM), suggesting that the inward current mainly consisted of the voltage-dependent L-type Ca^{2+} channel ($I_{Ca.L}$). EPA (1–30 μM) also inhibited $I_{Ca.L}$ in a concentration-dependent manner. The inhibitory effect of EPA was observed at concentrations higher than 1 μM, and its half-maximal inhibitory concentration (IC_{50}) was 7.6 μM. Vasopressin induced a long-lasting inward current at a holding potential of −40 mV. The vasopressin-induced current was considered as a non-selective cation current (I_{cat}) with a reversal potential of approximately +0 mV. Both nifedipine and nicardipine (10 μM) failed to inhibit it significantly, but La^{3+} completely abolished I_{cat}. EPA also inhibited vasopressin-induced I_{cat} in a concentration-dependent manner; its IC_{50} value was 5.9 μM. Vasopressin (100 nM) stimulated [^{3}H]thymidine incorporation. Exclusion of extracellular Ca^{2+} with EGTA or La^{3+} markedly inhibited it. EPA (3–30 μM) also inhibited the incorporation induced by vasopressin, while nifedipine and nicardipine (1 μM) only partly inhibited it. These results suggested that EPA, unlike nifedipine and nicardipine, inhibited vasopressin-induced Ca^{2+}-entry and proliferation in rat vascular smooth muscle cells, where the inhibitory effects of EPA on I_{cat} as well as $I_{Ca.L}$ might be involved. Thus, EPA would exert hypotensive and antiatherosclerotic effects.

Keywords: Eicosapentaenoic acid; Ca^{2+} current, voltage-dependent L-type; Vasopressin; Ca^{2+}-permeable non-selective cation channel; Ca^{2+} entry, receptor-mediated; Smooth muscle cell, vascular; Cell proliferation; La^{3+}

1. Introduction

Eicosapentaenoic acid (EPA), an ϖ-3 polyunsaturated fatty acid extracted from fish oil, has been shown to exert antihypertensive and antiatherosclerotic effects (Dyerberg et al., 1978; Puska et al., 1983; Hui et al., 1989; Knapp and Fitzgerald, 1989; Bonna et al., 1990). These basic mechanisms include inhibition of platelet aggregation (Siess et al., 1980; Brox et al., 1981), and decrease in serum lipids and enhancement of the production of vasodilator substrates including prostaglandin I_2 (Abeywardena et al., 1989). EPA has also been shown to reduce vascular smooth muscle contractility (Juan et al., 1987; Engler, 1992a,b,c), which may partially account for the reduction in cardiovascular risk. In addition, EPA suppresses the proliferation and migration of vascular smooth

* Corresponding author. Tel.: +81-3-3815-5411; fax: +81-3-3814-0021

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00476-8

muscle cells (Shiina et al., 1993; Terano et al., 1996; Mizutani et al., 1997; Asano et al., 1998), which may contribute to its antiatherosclerotic effects. However, the mechanisms by which EPA inhibits the proliferation and reduces vascular smooth muscle contractility are not well understood. Since both growth (Pardee, 1989; Sperti and Colucci, 1991; Mogami and Kojima, 1993; Short et al., 1993; Berridge, 1995) and contractility depend in part on entry of extracellular Ca^{2+}, reduction of Ca^{2+} entry may be involved in these vascular action of EPA. Actually, DNA synthesis and cell proliferation have been reported to be inhibited by Ca^{2+} channel antagonists such as verapamil and nifedipine (Block et al., 1989; Sperti and Colucci, 1991; Asakura et al., 1997).

Vasopressin induces the breakdown of phosphatidylinositol that results in the formation of inositol 1,4,5-triphosphate and diacylglycerol in vascular smooth muscle cells, subsequently increasing intracellular Ca^{2+} ($[Ca^{2+}]_i$) and stimulating protein kinase C which are closely related to cell contraction and proliferation (Capponi et al., 1985; Doyle and Ruegg, 1985; Caramelo et al., 1989; Thibbonnier et al., 1991). Furthermore, vasopressin induces c-fos protein (Nambi et al., 1989) and activates mitogen-activated protein (MAP) kinase (Granot et al., 1993; Kribben et al., 1993), which plays an essential role in signal transduction, and acts as a growth factor in normal and abnormal cell development. In vascular smooth muscle cells, vasoactive agents such as vasopressin increase $[Ca^{2+}]_i$ in a biphasic manner (Capponi et al., 1985; Doyle and Ruegg, 1985; Wallnofer et al., 1987; Thibbonnier et al., 1991; Nakajima et al., 1996; Minowa et al., 1997). The initial transient increase in $[Ca^{2+}]_i$ is mainly elicited by intracellular release from Ca^{2+} storage sites, while the second phase is considered to be due to Ca^{2+} entry across the plasma membrane via receptor-operated Ca^{2+} entry pathways such as capacitative Ca^{2+} entry (CRAC) (Putney, 1990; Byron and Taylor, 1995; Iwasawa et al., 1997) and second messenger-operated channels (SMOCs) (Meldolesi and Poz-

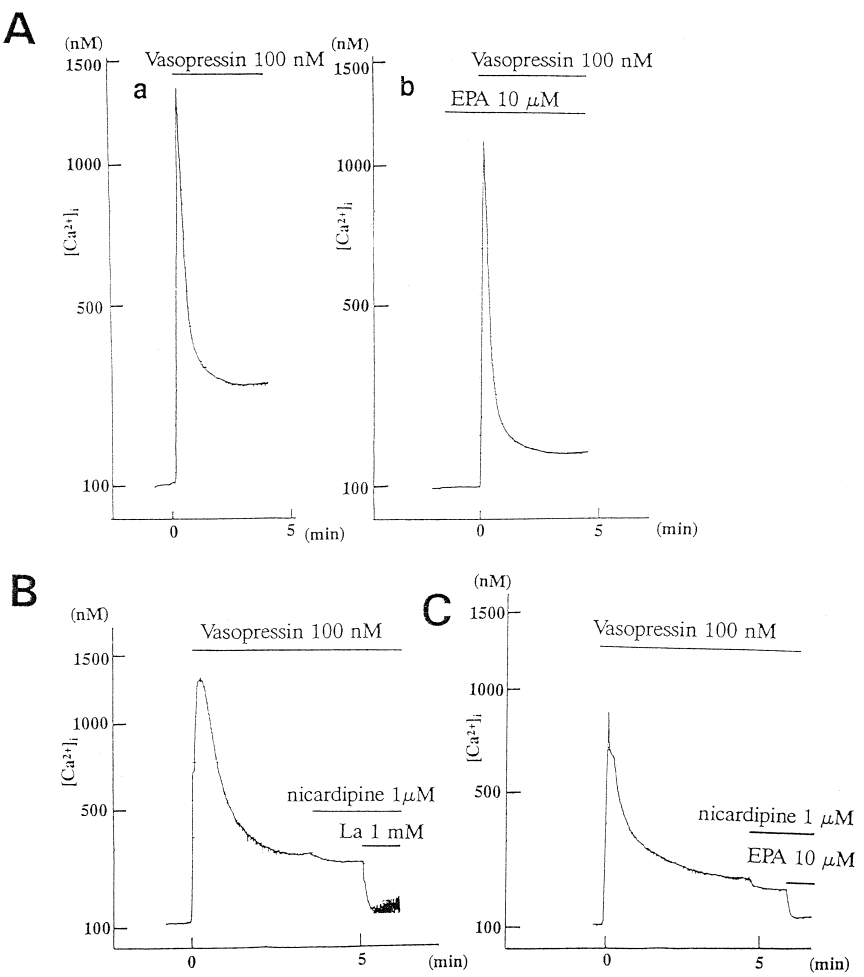

Fig. 1. Effects of EPA on $[Ca^{2+}]_i$ concentration in aortic smooth muscle cells (A7r5 cells). (A) Effects of vasopressin (a) and EPA (10 μM) (b) on $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+}. Results are representative of five similar experiments. (B) Effects of nicardipine (1 μM) and La^{3+} (1 mM) on vasopressin-induced $[Ca^{2+}]_i$ rise. Results are representative of 15 similar experiments. (C) Effects of EPA (10 μM) on the sustained rise in $[Ca^{2+}]_i$ elicited by vasopressin (100 nM) in the presence of nicardipine (1 μM). Results are representative of seven similar experiments. $[Ca^{2+}]_i$ with calibration was obtained from the ratio signal of the emission light at 500 nm by excitation at 340 and 380 nm.

zan, 1987; Van Renterghem and Lazdunski, 1994), or voltage-dependent Ca^{2+} channels. Also, non-selective cation channels that allow the influx of several cations such as Na^{+} and Ca^{2+} (Byrne and Large, 1988; Van Renterghem et al., 1988; Amedee et al., 1990; Wang and Large, 1991; Inoue and Kuriyama, 1993; Krautwurst et al., 1994; Nakajima et al., 1996; Minowa et al., 1997) play an important role in the receptor-mediated Ca^{2+} entry and opening the voltage-dependent Ca^{2+} channels. Thus, Ca^{2+}-permeable non-selective cation channels (I_{cat}) as well as the voltage-dependent L-type Ca^{2+} channels ($I_{Ca.L}$) play an essential role in regulating vascular functions including cell proliferation as shown in other types of cells (Jung et al., 1992). Recently, we have demonstrated that ϖ-3 polyunsaturated fatty acids inhibit receptor-activated non-selective cation currents (I_{cat}) in vascular smooth muscle cells (Asano et al., 1997), but the detailed effects of EPA on vascular actions of vasopressin including Ca^{2+} mobilization and cell proliferation and their underlying mechanisms have not been investigated in detail.

Therefore, the present study was undertaken to clarify how EPA modulates the cellular action of vasopressin in vascular smooth muscle cells. The effects of EPA on Ca^{2+} mobilization and DNA synthesis induced by vasopressin were investigated and compared to those of Ca^{2+} channel blocking agents. The effects of EPA on the $I_{Ca.L}$ and I_{cat} were also compared to Ca^{2+} blocking agents, by means of the whole-cell voltage clamp technique.

2. Materials and methods

2.1. Cell preparation

A7r5 cells (ATCC-7), a well-established vascular smooth muscle cell line obtained from embryonic rat aorta (Kimes and Brandt, 1976; Standley et al., 1991), were purchased from the American Type Culture Collection through Dainippon Seiyaku (Kyoto, Japan). Cultured cells were fed every second day with Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 50 μg/ml streptomycin at 35°C in a fully humidified atmosphere of 5% CO_2. Cells subcultured to passage number 12–18 were grown as monolayers on glass slides, and confluent cells were detached from the culture flasks with 0.25% trypsin in 0.02% EDTA, and used for later experiments. Cell viability, as determined by trypan blue exclusion, was

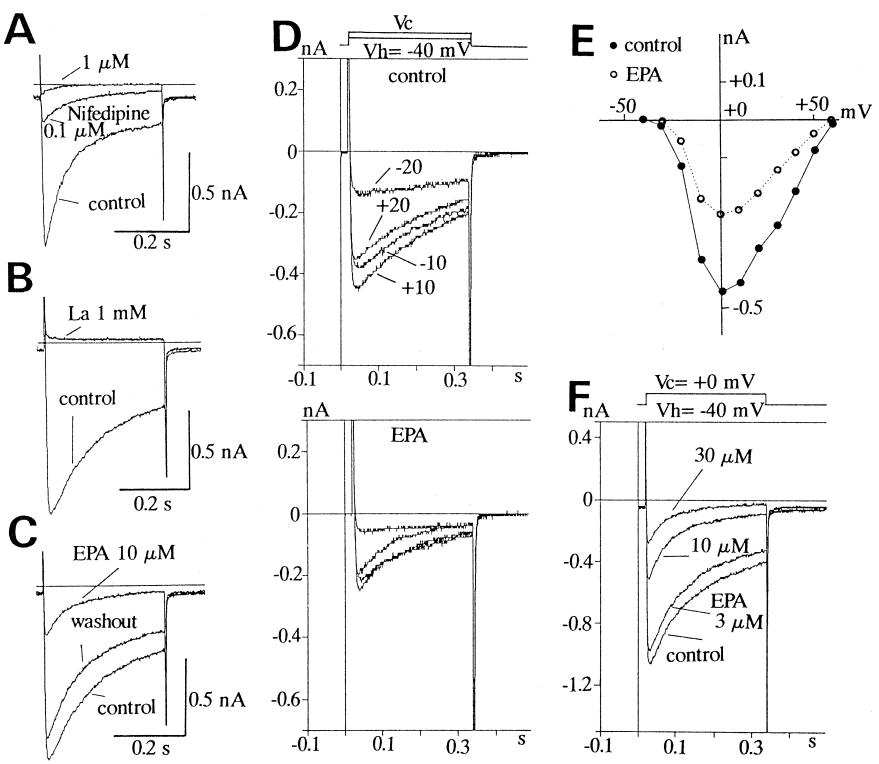

Fig. 2. Effects of EPA on $I_{Ca.L}$. In A–C, the cells were held at −40 mV, and command voltage steps to +0 mV (320 ms in duration) were applied at 0.2 Hz. The zero current level is indicated by the line. (A and B) Effects of nifedipine (0.1–1 μM) (A) and La^{3+} (1 mM) (B) on $I_{Ca.L}$. (C) Effects of EPA on $I_{Ca.L}$. The current traces in the control, in the presence of EPA (10 μM), and after the washout with bathing solution containing albumin (0.1%) are shown. In (D) the cells were held at −40 mV, and command voltage pulses to various membrane potentials were applied at 0.2 Hz. The original current traces in the control (upper trace) and in the presence of EPA (10 μM, lower trace) are shown. The current–voltage relationships at the peak I_{Ca} are plotted in (E). (F) Concentration-dependent inhibition of $I_{Ca.L}$ by EPA. Various concentrations of EPA were tested, and results are representative of six similar experiments.

approximately 91%. All experiments were performed at 35–37°C.

2.2. Solutions and drugs

The composition of the standard Tyrode's solution was as follows (in mM): NaCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5, HEPES–NaOH buffer 5 (pH 7.4). The patch pipette contained (in mM): CsCl 130, EGTA 0.15, $MgCl_2$ 2, Na_2ATP 3, guanosine-5′-triphosphate (sodium salt, Sigma, St. Louis, MO) 0.1 and HEPES–CsOH buffer 5 (pH 7.2). The Ba^{2+}-containing Tyrode's solution was the same as the control bathing solution with the exception that $CaCl_2$ was replaced by $BaCl_2$ (5 mM). When extracellular Cl^- ($[Cl^-]_o$) or intracellular Cl^- ($[Cl^-]_i$) concentration was changed, Cl^- was replaced by aspartic acid. [Arg^8]vasopressin and *cis*-5,8,11,14,17-EPA (EPA, Na salt) were purchased from Sigma.

2.3. Recording technique and data analysis

Membrane currents were recorded with glass pipettes under whole-cell clamp conditions (Hamill et al., 1981; Nakajima et al., 1989), using a patch-clamp amplifier (EPC-7, List Electronics, Darmstadt, Germany). The heat-polished patch electrode, filled with the artificial internal solution (for composition, see above), had a tip resistance of 3–5 MΩ. The series resistance was compensated. Membrane currents were continuously monitored with a high-gain storage oscilloscope (COS 5020-ST, Kikusui Electronic, Tokyo, Japan). The data were stored on a videotape using the PCM converter system (RP-890, NF, Electronic Circuit Design, Tokyo). The data were reproduced, low-passed, filtered at 1 kHz (−3 dB) with a Bessel filter (FV-625, NF, 48 dB/octave slope attenuation), sampled at 5 kHz and analyzed off-line on a computer using pClamp software (Axon Instruments, CA).

2.4. Measurement of DNA synthesis

Cells were grown to subconfluence in 24-well tissue culture dishes and growth was arrested for 48 h in serum-free DMEM. The DMEM medium was employed to maintain the vascular smooth muscle cells in a quiescent, but not catabolic state, a condition resembling that of healthy cells in the normal arterial wall in vivo. The medium was then removed, and fresh DMEM containing vasopressin (100 nM) was added to the quiescent cells. The cells were subsequently incubated for 18 h in the absence or presence of EPA with α-tocopherol acetate (1 μM), nifedipine, nicardipine or La^{3+}. In several experiments, 0.5 mM EGTA was added to the control solution to reduce extracellular Ca^{2+} concentration. The cells were then incubated with methyl-[^{3}H]thymidine (0.5 μCi/ml, Amersham Pharmacia Biotech UK, Bucks, UK) for 4 h in the absence or presence of these agents. The medium was then removed, the cells were washed twice with ice cold 5% trichloroacetic acid and then incubated in 5% trichloroacetic acid on ice for 15 min. The cells were solubilized by adding 0.5 ml of 0.5 N NaOH. The 0.2-ml aliquots were then neutralized and counted in scintillation fluid. Cellular proteins were measured using Bradford's method.

2.5. Determination of cytosolic free Ca^{2+} concentration

Cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) was determined using the fura-2 fluorescence method as described previously (Grynkiewicz et al., 1985; Asano et al., 1998). The Ca^{2+}-free bathing solution was the same as the normal

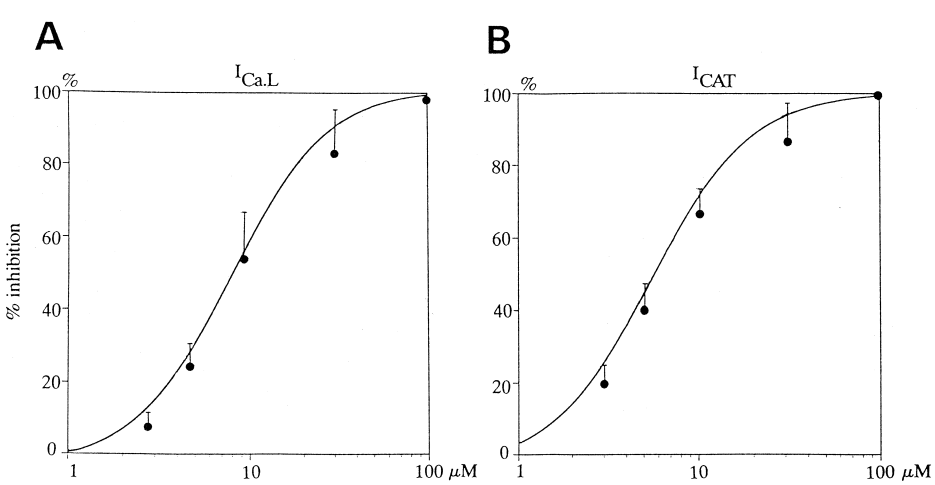

Fig. 3. Effects of EPA on $I_{Ca.L}$ and vasopressin-activated non-selective cation currents (I_{cat}). (A) Concentration-dependent inhibitory effects of EPA on $I_{Ca.L}$. The amplitude of the peak $I_{Ca.L}$ during application of EPA was compared with the control value. The percent inhibition induced by EPA on $I_{Ca.L}$ (mean ± S.D. value) is shown. The data were obtained from six different cells including Fig. 2F. (B) Concentration-dependent inhibitory effects of EPA on vasopressin-activated I_{cat}. The data were obtained from 10 different cells including Fig. 4F.

Tyrode's solution except that $CaCl_2$ was omitted and 0.5 mM EGTA was added to the solution (pH 7.4). Fura-2 acetoxymethylester (fura-2 AM) was obtained from Dojin Chemicals (Japan). Cells were trypsinized, washed twice with the standard solution, adjusted to a cell density of 10^6 cell/ml and loaded with 1 μM fura-2 AM for 60 min in a 20°C-shaking water bath. After incubation, the medium containing fura-2 was removed, and fluorescent cells in suspension were measured at 37°C while being continuously stirred in a cuvette placed by a spectrofluorometer (CAF-100, JASCO, Tokyo). The excitation wavelengths were 340 and 380 nm, and emission was 500 nm. Fluorescence signals were calibrated using 0.5% Triton W-100 for maximum fluorescence, 300 mM EGTA pH < 8.0 for minimum fluorescence. $[Ca^{2+}]_i$ was determined using the method of Grynkiewicz et al. (1985).

2.6. Statistical analysis

Data were expressed as the mean ± S.D., and analysis of variance (ANOVA) and Fisher's protected least significance difference (PLSD) for multiple comparisons and Student's *t*-test were performed. Differences with a *P* value of < 0.05 were considered significant.

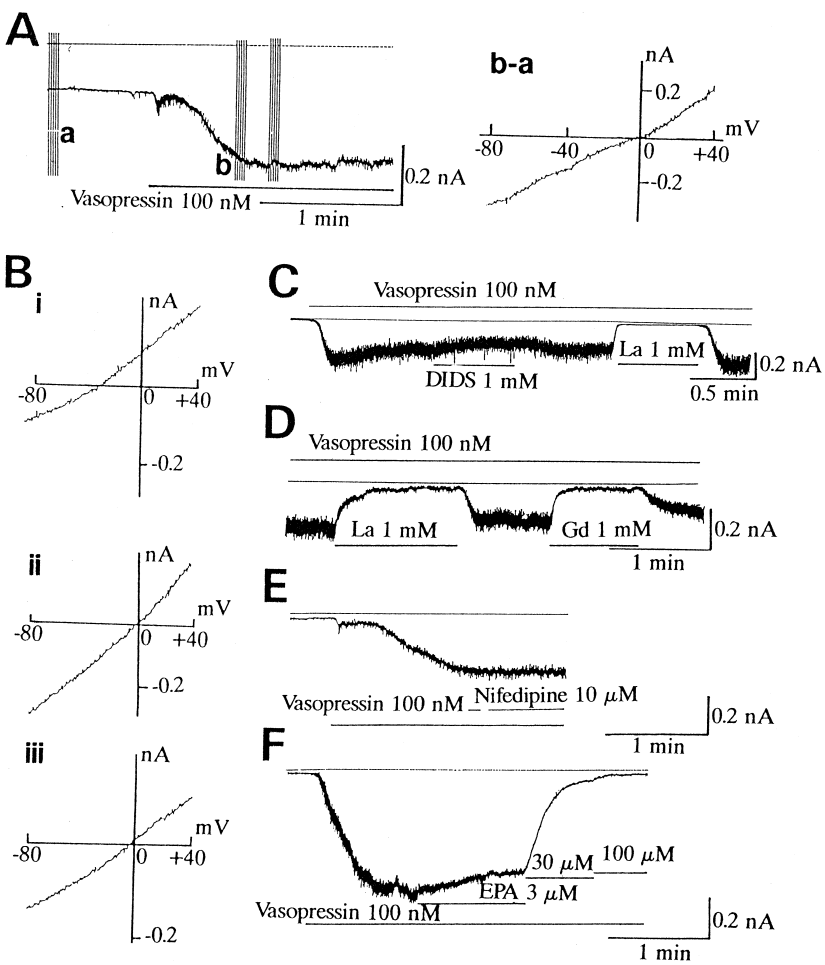

Fig. 4. Effects of La^{3+}, nifedipine and EPA on vasopressin-activated non-selective cation currents (I_{cat}). (A) Activation of I_{cat} by vasopressin. The cells were held at −60 mV. Ramp voltage pulses from −80 mV to +40 mV (100 ms in duration) were applied before and during application of vasopressin (100 nM). The zero current level is denoted by dotted lines. The current–voltage relationships of the subtraction current from the current trace in the presence of vasopressin to control trace are shown in the right part of (A). Results are representative of 12 similar experiments. (B) Effect of altering the Na^+ or Cl^- concentration on the reversal potential (E_{rev}) of the vasopressin-induced current, (i) extracellular Na^+ (136 mM) was totally replaced by $Tris^+$ (136 mM). (ii, iii) Effect of changing $[Cl^-]_o$ (10 mM Cl^- in bath solution) or $[Cl^-]_i$ (24 mM Cl^- in the pipette solution) on E_{rev}, where equimolar concentration of $[Cl^-]_o$ (ii) or $[Cl^-]_i$ (iii) was replaced by aspartate. The typical current–voltage relationships of the vasopressin-induced current are indicated in (i–iii). (C) Effects of 4,4′-diisothiocyanotostilbene-2,2′-disulphonic acid (DIDS, 1 mM) and La^{3+} (1 mM) on the vasopressin-activated currents. (D and E) Effects of nifedipine, La^{3+} and Gd^{2+} on vasopressin-activated I_{cat}. The cells were held at −40 mV. Note that La^{3+} and Gd^{2+} (1 mM) completely abolished the current. The zero current level is shown by dotted lines. (F) Effects of EPA on vasopressin-activated I_{cat}. In C–F, the cells were held at −40 mV, and results are representative of four to five similar experiments.

3. Results

3.1. Effects of eicosapentaenoic acid on Ca^{2+} mobilization elicited by vasopressin

Fig. 1 shows the effects of vasopressin and EPA on $[Ca^{2+}]_i$ concentration in rat aortic smooth muscle cell lines (A7r5 cells). In the presence of extracellular Ca^{2+}, vasopressin (100 nM) induced a biphasic increase of $[Ca^{2+}]_i$ (Fig. 1Aa). The first transient increase of $[Ca^{2+}]_i$ elicited by vasopressin resulted mainly from Ca^{2+} release from intracellular store sites, and the persistent elevation of $[Ca^{2+}]_i$ resulted from the entry of extracellular Ca^{2+}. Fig. 1B and C show the effects of nicardipine and La^{3+} on vasopressin-induced Ca^{2+} mobilization. After the $[Ca^{2+}]_i$ rise elicited by vasopressin (100 nM, Fig. 1B and C) reached the steady state, nicardipine (1 μM, Fig. 1B and C) and nifedipine (1 μM, data not shown) partly decreased the final sustained phase of $[Ca^{2+}]_i$ by about 12% of the control level (12 ± 10% of the control, $n = 15$, $P < 0.05$). Alternatively, La^{3+} (1 mM) completely eliminated the sustained phase of $[Ca^{2+}]_i$, suggesting that vasopressin-induced Ca^{2+} entry occurred via a dihydropyridine-insensitive Ca^{2+} channel as well as the $I_{Ca.L}$. Fig. 1Ab shows the effects of EPA on resting $[Ca^{2+}]_i$ and Ca^{2+} mobilization induced by vasopressin. EPA (10 μM) did not affect the resting $[Ca^{2+}]_i$. However, in the presence of EPA (10 μM, Fig. 1Ab), the sustained rise in $[Ca^{2+}]_i$ elicited by vasopressin significantly decreased, in comparison with the control cell (Fig. 1Aa). Fig. 1C shows the effects of EPA on the sustained rise in $[Ca^{2+}]_i$ elicited by vasopressin. After the $[Ca^{2+}]_i$ rise elicited by vasopressin reached the steady state, nicardipine (1 μM) slightly decreased the sustained phase of $[Ca^{2+}]_i$ by 18% of the control level, whereas EPA (10 μM) markedly inhibited it. EPA suppressed the sustained rise in $[Ca^{2+}]_i$ induced by vasopressin by about 80% of the control level (76 ± 14%, $n = 7$) in the presence of nicardipine or nifedipine. Similarly, EPA (10 μM) inhibited it by about 80% of the control level (80 ± 13%, $n = 6$, data not shown) in the absence of nicardipine. These results suggest that EPA inhibited the dihydropyridine-insensitive Ca^{2+} entry induced by vasopressin in aortic smooth muscle cells.

3.2. Effects of eicosapentaenoic acid on the voltage-dependent L-type Ca^{2+} current

In vascular smooth muscle cells, vasoactive agents such as vasopressin induce Ca^{2+} influx through voltage-dependent Ca^{2+} channels and/or receptor-operated Ca^{2+} chan-

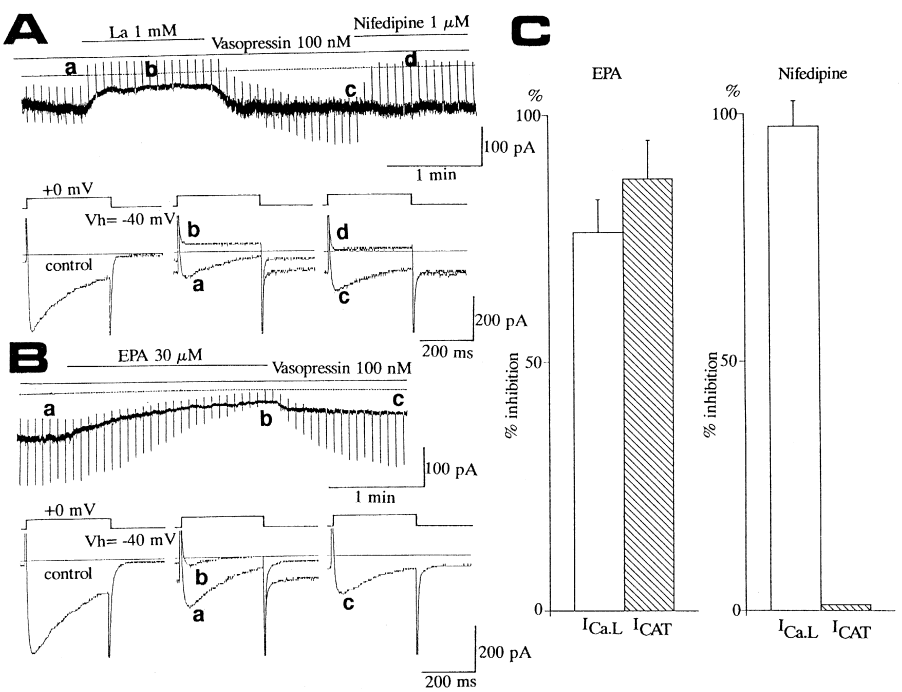

Fig. 5. Effects of La^{3+}, nifedipine and EPA on $I_{Ca.L}$ and vasopressin-activated non-selective cation currents (I_{cat}). The cells were held at −40 mV, and command voltage pulses to +0 mV (320 ms in duration) were applied at 0.2 Hz. Effects of nifedipine (1 μM) and EPA (30 μM) on $I_{Ca.L}$ and vasopressin-activated I_{cat} are shown in (A) and (B). The current traces (a–d) shown in the lower part of (A) and (B) were obtained at the times indicated in the upper part (a–d). In (A) and (B), the original current traces in the control are shown in the left part. Note that vasopressin increased the holding current in the inward direction, which reflected the activation of I_{cat} and concomitantly inhibited $I_{Ca.L}$ (Aa and Ba), in comparison with the control trace. (C) Comparative effects of EPA and nifedipine on the $I_{Ca.L}$ and vasopressin-activated non-selective cation currents (I_{cat}). The cells were held at −40 mV, and command voltage steps to +0 mV were applied to evoke $I_{Ca.L}$. The percent inhibition of nifedipine and EPA on $I_{Ca.L}$ and I_{cat} (mean ± S.D.) is shown, in comparison to the inhibition by La^{3+} (1 mM), since La^{3+} (1 mM) completely inhibited $I_{Ca.L}$ and vasopressin-activated I_{cat}. The data were obtained from six different cells including (A) and (B).

nels. So, we first examined the effects of EPA on $I_{Ca.L}$ in A7r5 cells as shown in Fig. 2. The bath was superfused into Ba^{2+}-containing solution instead of Ca^{2+}, and the patch pipette contained with EGTA and ATP. The command voltage pulses to +0 mV elicited the inward current. Nifedipine (0.1 and 1 μM, Fig. 2A), nicardipine (1 μM, data not shown) and La^{3+} (1 mM, Fig. 2B) inhibited $I_{Ca.L}$, proposing that it mainly consisted of $I_{Ca.L}$. EPA (10 μM) also reduced the amplitude of $I_{Ca.L}$ by 55 ± 13% ($n = 6$) (Fig. 2C). After washing out the cells with bathing solution containing albumin (0.1%), $I_{Ca.L}$ returned to a near control level. Fig. 2D shows the effects of EPA (10 μM) on current–voltage relationships of $I_{Ca.L}$. $I_{Ca.L}$ elicited by depolarizing command steps from −40 mV was reduced by EPA (10 μM, Fig. 2D, lower part). The amplitude of the peak inward current was plotted at each command potential (Fig. 2E). EPA consistently reduced the amplitude of $I_{Ca.L}$ at each command potential without affecting the shape of the current–voltage relationships of $I_{Ca.L}$. Fig. 2FFig. 3A indicate the relationships between concentrations of EPA and percent inhibition of $I_{Ca.L}$ at +0 mV from a holding potential of −40 mV. EPA suppressed $I_{Ca.L}$ in a concentration-dependent manner. The half-maximal inhibitory concentration of EPA was approximately 7.6 μM (Fig. 3A). These results indicated that EPA inhibited $I_{Ca.L}$ in vascular smooth muscle cells, which may partly contribute to the inhibitory effects of EPA on the vasopressin-induced sustained rise of $[Ca^{2+}]_i$.

3.3. Effects of eicosapentaenoic acid on vasopressin-activated non-selective cation currents

In vascular smooth muscle cells, the contractile agonists such as endothelin-1 and vasopressin induce Ca^{2+} influx through receptor-operated Ca^{2+} channels such as CRAC and SMOCs, in which I_{cat} is also involved (Byrne and Large, 1988; Van Renterghem et al., 1988; Amedee et al., 1990; Wang and Large, 1991; Krautwurst et al., 1994; Nakajima et al., 1996; Iwasawa et al., 1997; Minowa et al., 1997). Therefore, we next examined the effects of EPA on vasopressin-activated I_{cat}, and compared them with those of Ca^{2+}-blocking agents. Fig. 4 shows the effects of vasopressin on membrane currents in rat aortic smooth muscle cells (A7r5 cell). Under the conditions with Cs^+-internal solution, vasopressin (100 nM) induced a long-lasting inward current with a high noise level at a holding potential of −60 mV. The vasopressin-activated current reversed at −2 ± 3 mV ($n = 12$, Fig. 4A). The reversal potential (V_{rev}) of the current was not affected when $[Cl^-]_o$ or $[Cl^-]_i$ was decreased (Fig. 4B), suggesting that vasopressin activated I_{cat}, but not a Cl^- current under these conditions, as previously described (Van Renterghem et al., 1988; Krautwurst et al., 1994; Nakajima et al., 1996; Iwasawa et al., 1997). 4,4′-Diisothiocyanotostilbene-2,2′-disulphonic acid (DIDS, 1 mM, Fig. 4C), a Ca^{2+}-activated Cl^- channel blocker, party inhibited the vasopressin-activated currents only by 25 ± 10% ($n = 4$), which also

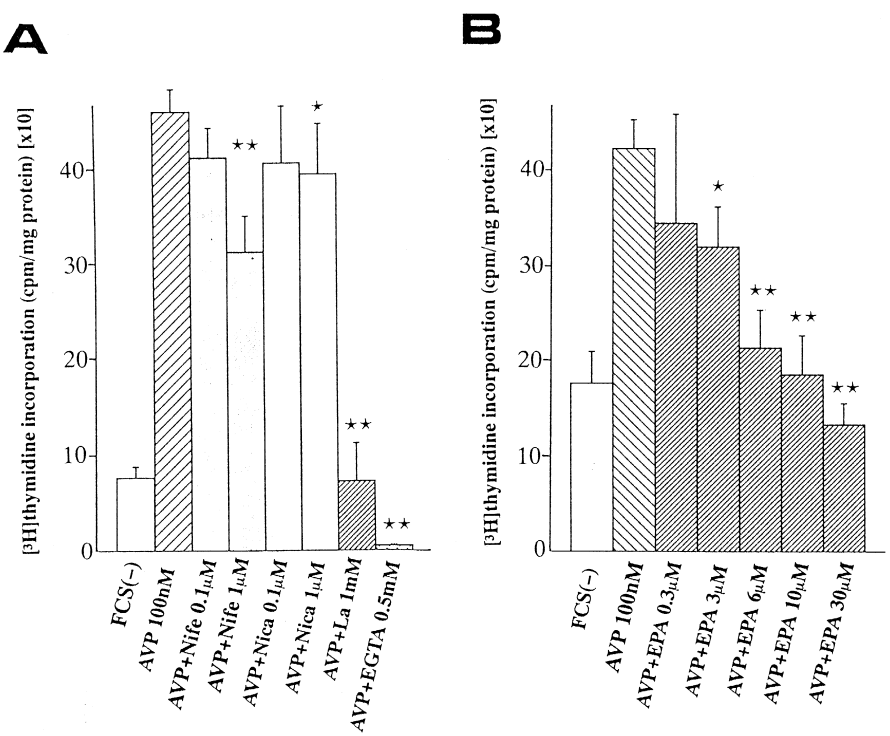

Fig. 6. Effects of removal of extracellular Ca^{2+}, Ca^{2+} channel antagonists and EPA on vasopressin (AVP)-induced proliferation of A7r5 cells. Cell proliferation was measured by the incorporation of [^{3}H]thymidine. Each mean ± S.D. value was obtained from five different experiments. $^*P < 0.05$ vs. controls $^{**}P < 0.01$ vs. controls. (A) Effects of nifedipine (0.1–1 μM), nicardipine (0.1–1 μM), La^{3+} (1 mM) and removal of extracellular Ca^{2+} on vasopressin (100 nM)-induced [^{3}H]thymidine incorporation. (B) Effects of EPA (0.3–30 μM) on vasopressin-induced [^{3}H]thymidine incorporation.

suggests that the vasopressin-induced current mainly consisted of I_{cat}. Fig. 4C–F show the effects of nifedipine, La^{3+}, Gd^{2+} and EPA on the vasopressin-activated I_{cat}. The current was not significantly affected by nifedipine (10 μM, Fig. 4E), nor by nicardipine (10 μM, data not shown), but it was completely abolished by La^{3+} (1 mM, Fig. 4C and D) and Gd^{2+} (1 mM, Fig. 4D). EPA (3–30 μM, Fig. 4F) also inhibited the vasopressin-activated I_{cat} in a concentration-dependent manner. The half-maximal inhibitory concentration of EPA on I_{cat} was 5.9 μM as indicated in Fig. 3B.

The effects of La^{3+}, nifedipine and EPA on $I_{Ca.L}$ as well as on vasopressin-activated I_{cat} were compared as shown in Fig. 5. The cells were held at −40 mV, and command voltage pulses to +0 mV were applied at 0.2 Hz. Vasopressin (100 nM) increased the holding current in the inward direction, which reflected the activation of I_{cat}, and decreased the amplitude of $I_{Ca.L}$ in comparison with the control (Fig. 5Aa and Ba) as previously described (Van Renterghem et al., 1988; Nakajima et al., 1995). La^{3+} (1 mM) completely abolished vasopressin-activated I_{cat} as well as $I_{Ca.L}$ (Fig. 5Ab). On the other hand, nifedipine (1 μM) almost completely abolished $I_{Ca.L}$, but it failed to significantly inhibit vasopressin-activated I_{cat} (Fig. 5Ad). EPA also inhibited $I_{Ca.L}$, and antagonized the vasopressin-evoked I_{cat} (Fig. 5Bb). Fig. 5C summarizes the effects of nifedipine and EPA on $I_{Ca.L}$ and vasopressin-activated I_{cat}. EPA (30 μM) inhibited $I_{Ca.L}$ and I_{cat}, while nifedipine did not inhibit I_{cat} at all.

3.4. Effects of eicosapentaenoic acid on vasopressin-induced mitogenesis

Fig. 6 illustrates the effects of vasopressin on the proliferation of rat aortic smooth muscle cells. Vasopressin (100 nM) elicited an increase in the incorporation of [^{3}H]thymidine into cells. Fig. 6A shows the effects of extracellular Ca^{2+} and Ca^{2+} channel antagonists on vasopressin-induced mitogenesis. Removal of extracellular Ca^{2+} with EGTA dramatically suppressed the vasopressin-induced incorporation of [^{3}H]thymidine. Nifedipine (1 μM) or nicardipine (1 μM) partly inhibited the vasopressin-induced thymidine incorporation, while La^{3+} (1 mM) suppressed it remarkably. These observations suggest that extracellular Ca^{2+} may play a role in the mitogenic effects of vasopressin. The effects of EPA on vasopressin-induced mitosis are shown in Fig. 6B. EPA (0.3–30 μM) decreased the incorporation of [^{3}H]thymidine elicited by vasopressin in a concentration-dependent manner.

4. Discussion

The major findings of the present study are the following. (1) EPA inhibited the sustained rise of $[Ca^{2+}]_i$ elicited by vasopressin in aortic smooth muscle cells, while nifedipine and nicardipine partly inhibited it. (2) EPA inhibited vasopressin-elicited I_{cat} as well as the $I_{Ca.L}$, while nifedipine and nicardipine failed to inhibit I_{cat}. (3) Vasopressin stimulated mitosis as indicated by the incorporation of [^{3}H]thymidine. Removal of extracellular Ca^{2+} and La^{3+} reduced it more effectively than nifedipine and nicardipine. (4) EPA also inhibited proliferation induced by vasopressin in a concentration-dependent manner. These results suggest that EPA inhibits vasopressin-induced Ca^{2+} entry, and thereby the proliferation of vascular smooth muscle cells, where the inhibitory effects of EPA on I_{cat} as well as $I_{Ca.L}$ might be involved.

4.1. Eicosapentaenoic acid inhibits vasopressin-induced $[Ca^{2+}]_i$ mobilization

Vasocontractile agonists such as vasopressin induce the sustained rise in $[Ca^{2+}]_i$ due to Ca^{2+} entry across the plasma membrane. Since vasopressin increases $I_{Ca.L}$ in certain types of smooth muscle cells (Bonev and Isenberg, 1992) or depolarizes the membrane potential (Van Renterghem et al., 1988), it is speculated that Ca^{2+} entry through $I_{Ca.L}$ may be involved in the sustained rise in $[Ca^{2+}]_i$ elicited by vasopressin. Actually, as illustrated in Figs. 1 and 2, nifedipine and nicardipine (1 μM), a potent $I_{Ca.L}$ channel antagonist, which almost completely blocked $I_{Ca.L}$, inhibited the sustained rise in $[Ca^{2+}]_i$ induced by vasopressin by approximately 12% of the control. However, removal of extracellular Ca^{2+} and La^{3+} (1 mM) suppressed it more effectively than the Ca^{2+} channel-blocking agents. Thus, it is likely that Ca^{2+} entry through $I_{Ca.L}$ contributes in part to the sustained rise in $[Ca^{2+}]_i$ elicited by vasopressin, but vasopressin mainly evokes Ca^{2+} entry via a nifedipine-insensitive and La^{3+}-sensitive mechanism as previously reported (Wallnofer et al., 1987; Thibbonnier et al., 1991; Nakajima et al., 1996; Iwasawa et al., 1997). EPA inhibited the sustained rise in $[Ca^{2+}]_i$ elicited by vasopressin even in the presence of nicardipine, suggesting that EPA inhibited the nifedipine-insensitive Ca^{2+} entry pathways. It has been reported that fish oil and EPA induce relaxation of α-adrenoceptor agonist- and angiotensin II-induced contraction in aortic smooth muscle cells (Juan et al., 1987; Engler, 1992a,b,c). In the present study, 10 μM EPA markedly suppressed the sustained rise in $[Ca^{2+}]_i$ elicited by vasopressin. On the other hand, EPA (10 μM) did not inhibit the transient rise in $[Ca^{2+}]_i$ induced by vasopressin in the absence of extracellular Ca^{2+} (data not shown, $n = 4$), indicating that the inhibitory effects of EPA are not located proximal to inositol 1,4,5-triphosphate production. These findings are compatible with those of the previous studies in that EPA did not affect the initial phase of the noradrenaline-induced contraction in Ca^{2+}-free solution (Engler, 1992a), which is mediated by inositol 1,4,5-triphosphate formation and subsequent release of $[Ca^{2+}]_i$. Taking these findings into

accounts, we may assume that the inhibitory effects of EPA on vasopressin-induced Ca^{2+} mobilization may not be attributed to an inhibition of inositol 1,4,5-triphosphate formation, but to a preferential inhibition of Ca^{2+} entry associated with receptor-operated channels. Recently, we have shown that chronic treatment of vascular smooth muscle cells with EPA for 7 days modulated Ca^{2+} mobilization induced by vasoactive agents (Asano et al., 1998). Under long-term treatment with EPA (30 μM), both the peak and the sustained rise in $[Ca^{2+}]_i$ induced by vasopressin, endothelin-1 and platelet derived-growth factor (PDGF) decreased, in contrast with the findings of the present study. Chronic treatment with EPA for several days incorporated the EPA into the plasma membrane, possibly inhibiting receptor-inositol 1,4,5-triphosphate signalling pathways, and subsequently suppressing the formation of inositol 1,4,5-triphosphate as previously described (Locher et al., 1988; Locher et al., 1989). Thus, it is interesting to note that EPA can modulate $[Ca^{2+}]_i$ mobilization induced by vasoactive agents in several ways.

4.2. Eicosapentaenoic acid inhibited vasopressin-induced non-selective cation currents and voltage-dependent L-type Ca^{2+} currents

ϖ-3 Polyunsaturated fatty acids such as EPA have been shown to have a variety of effects on ionic channels in excitable cells including smooth muscle cells. They activate K^+ channels in toad gastric smooth muscle cells (Ordway et al., 1989, 1991), pulmonary arterial cells (Kirber et al., 1992) and canine coronary arterial cells (Xu and Lee, 1996). Recently, we have shown that ϖ-3 polyunsaturated fatty acids such as EPA inhibit receptor (vasopressin and endothelin-1)-activated I_{cat} (Asano et al., 1997). EPA has also been reported to inhibit $I_{Ca.L}$ in rat ventricular myocytes and guinea-pig tracheal myocytes (Xiao et al., 1997; Hazama et al., 1998). In the present study we also showed that EPA modulated $I_{Ca.L}$ as well as I_{cat} in vascular smooth muscle cells. The half-maximal inhibitory concentration of EPA was approximately 7.6 μM for $I_{Ca.L}$ and 5.9 μM for I_{cat}. Thus, EPA inhibited both types of Ca^{2+}-permeable channels in vascular smooth muscle cells.

The vasopressin-activated Ca^{2+}-entry is mediated through the $I_{Ca.L}$ and/or receptor-mediated Ca^{2+} channels such as CRAC and SMOCs. The receptor-activated Ca^{2+} channel is also mediated partly by I_{cat} (Byrne and Large, 1988; Van Renterghem et al., 1988; Amedee et al., 1990; Wang and Large, 1991; Inoue and Kuriyama, 1993; Krautwurst et al., 1994; Nakajima et al., 1996; Minowa et al., 1997). Nifedipine and nicardipine (1 μM) partly inhibited the sustained rise in $[Ca^{2+}]_i$ elicited by vasopressin, suggesting that EPA may inhibit the vasopressin-induced sustained rise in $[Ca^{2+}]_i$ by inhibiting $I_{Ca.L}$. However, EPA further inhibited the sustained rise in $[Ca^{2+}]_i$ elicited by vasopressin even in the presence of nicardipine or nifedipine as in case of La^{3+}. These effects of EPA on $[Ca^{2+}]_i$ unrelated to $I_{Ca.L}$ may be partly due to the blockade of I_{cat} elicited by vasopressin. However, the contribution of the effects of EPAs on the other receptor-mediated Ca^{2+} entry pathway such as CRAC and SMOCs cannot be ruled out in the present study. In rat aortic smooth muscle cells, it has been reported that nitric oxide donors and cyclic GMP inhibited endothelin-1-activated non-selective cation currents, inhibiting the sustained rise in $[Ca^{2+}]_i$ induced by endothelin-1 (Minowa et al., 1997). Therefore, it has to be clarified whether the inhibitory effects of EPA on I_{cat} are related to the production of nitric oxide or cyclic GMP. However, the inhibitory effects of EPA on I_{cat} were not inhibited by N^G-monomethyl-L-arginine (L-NAME, 1 mM), a nitric oxide synthetase inhibitor (data not shown), indicating that the involvement of nitric oxide might be ruled out.

4.3. Eicosapentaenoic acid inhibited vasopressin-induced cell proliferation

In vascular smooth muscle cells, it has been shown that eicosapentaenoic acid inhibits [^{3}H]thymidine incorporation induced by PDGF (Shiina et al., 1993; Terano et al., 1996). The present findings also showed that eicosapentaenoic acid inhibited vasopressin-stimulated [^{3}H]thymidine incorporation into rat aortic smooth muscle cells as previously reported (Jones et al., 1994). $[Ca^{2+}]_i$ is considered to play an essential role in cell proliferation as well as contractility (Kleine et al., 1986; Pardee, 1989; Sperti and Colucci, 1991; Mogami and Kojima, 1993; Short et al., 1993; Berridge, 1995). The removal of extracellular Ca^{2+} markedly reduced the incorporation of [^{3}H]thymidine induced by vasopressin as shown in Fig. 6, suggesting that extracellular Ca^{2+} mediates the mitogenic effects of vasopressin. Nifedipine and nicardipine partly decreased the incorporation of [^{3}H]thymidine as previously reported (Block et al., 1989; Sperti and Colucci, 1991; Asakura et al., 1997), suggesting that the influx of Ca^{2+} through the $I_{Ca.L}$ might contribute to the mitogenic effects of vasopressin in vascular smooth muscle cells. However, the inhibitory effects of nifedipine or nicardipine were much less that those of La^{3+} or the removal of extracellular Ca^{2+}. These findings indicate that Ca^{2+} entry pathways other than $I_{Ca.L}$ activated by vasopressin may play essential roles in the mitogenic actions of vasopressin. These results are somewhat compatible with those of the present study showing that nifedipine and nicardipine (1 μM) almost completely inhibited $I_{Ca.L}$, but only partly decreased the sustained rise in $[Ca^{2+}]_i$ elicited by vasopressin. On the other hand, La^{3+} and eicosapentaenoic acid markedly reduced the sustained rise in $[Ca^{2+}]_i$ induced by vasopressin as well as $I_{Ca.L}$,and blocked the mitogenic effects of vasopressin. Thus, it is likely that eicosapentaenoic acid inhibits vasopressin-induced cell proliferation, in which the inhibitory effects of eicosa-

pentaenoic acid on I_{cat} as well as $I_{Ca.L}$ might be involved. In fact, it has been reported that blockers of PDGF-activated non-selective cation channel inhibit cell proliferation in mouse fibroblasts (Jung et al., 1992). However, the effects of eicosapentaenoic acid on mitogenesis might be partly be due to the chronic effects of eicosapentaenoic acid after incorporating cell membrane, since eicosapentaenoic acid rapidly incorporates into cell membrane as previously described (Asano et al., 1998).

In summary, eicosapentaenoic acid inhibited the vascular action of vasopressin including Ca^{2+} mobilization and cell proliferation in rat aortic smooth muscle cells, in which the inhibitory effects of eicosapentaenoic acid on I_{cat} as well as $I_{Ca.L}$ might be involved. The inhibitory effects of eicosapentaenoic acid on cell proliferation may contribute to the suppressive effects of eicosapentaenoic acid on smooth muscle cell growth observed in patients with hypertension or atherosclerosis. Thus, eicosapentaenoic acid appears to exert antihypertensive and antiatherosclerotic effects.

References

Abeywardena, M.Y., Fischer, S., Schweer, H., Charnock, J.S., 1989. In vivo formation of metabolites of prostaglandins I_2 and I_3 in the marmoset monkey (*Callithrix jacchus*) following dietary supplementation with tuna fish oil. Biochem. Biophys. Acta 1003, 161–166.

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.

Asakura, Y., Okuda, Y., Nakajima, T., Asano, M., Suzuki, S., Yamashita, K., 1997. Effects of benedipine hydrochloride on DNA synthesis and cytosolic free Ca^{2+} concentrations of rat aortic smooth muscle cells induced by insulin. Diabetic Res. 32, 237–244.

Asano, M., Nakajima, T., Iwasawa, K., Hazama, H., Omata, M., Soma, M., Yamashita, K., Okuda, Y., 1997. Inhibitory effects of omega-3 polyunsaturated fatty acids on receptor-mediated non-selective cation currents in rat A7r5 vascular smooth muscle cells. Br. J. Pharmacol. 120, 1367–1375.

Asano, M., Nakajima, T., Hazama, H., Iwasawa, K., Tomaru, T., Omata, M., Soma, M., Asakura, Y., Mizutani, M., Suzuki, S., Yamashita, K., Okuda, Y., 1998. Influence of cellular incorporation of $n-3$ eicosapentaenoic acid on intracellular Ca^{2+} concentration and membrane potential in vascular smooth muscle cells. Atherosclerosis 138, 117–127.

Berridge, M.J., 1995. Calcium signalling and cell proliferation. BioEssays 17, 491–500.

Block, L.H., Emmons, L.R., Vogt, E., Sachinidis, A., Vetter, W., Hoppe, J., 1989. Ca^{2+}-channel blockers inhibit the action of recombinant platelet-derived growth factor in vascular smooth muscle cells. Proc. Natl. Acad. Sci. U.S.A. 86, 2388–2392.

Bonev, A., Isenberg, G., 1992. Arginine–vasopressin induces mode-2 gating in L-type Ca^{2+} channels (smooth muscle cells of the urinary bladder of the guinea-pig). Pfluegers Arch. 420, 219–222.

Bonna, 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.

Brox, J.H., Killie, J.E., Gunnes, S., Nordoy, A., 1981. The effect of cod liver oil and corn oil on platelets 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.

Byron, N.G., Taylor, C.W., 1995. Vasopressin stimulation of Ca^{2+} mobilization, two bivalent cation entry pathways and Ca^{2+} efflux in A7r5 rat smooth muscle cells. J. Physiol. 485, 455–468.

Capponi, A.M., Lew, P.D., Vallotton, M.B., 1985. Cytosolic free calcium levels in monolayers of cultured rat aortic smooth muscle cells: effects of angiotensin II and vasopressin. J. Biol. Chem. 260, 7836–7842.

Caramelo, C., Okada, K., Tsai, P., Schrier, R.W., 1989. Phorbol esters and arginine vasopressin in vascular smooth muscle cell activation. Am. J. Physiol. 256, F875–F881.

Doyle, V.M., Ruegg, U.T., 1985. Vasopressin induced production of inositol triphosphate and Ca^{2+} efflux in a smooth muscle cell line. Biochem. Biophys. Res. Commun. 131, 469–476.

Dyerberg, J., Bang, H.O., Stoffersen, E., Moncada, S., Vane, J.R., 1978. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis?. Lancet II, 117–119.

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.

Granot, Y., Erikson, E., Fridman, H., Van Putten, V., Williams, B., Schrier, R.W., Maller, J.L., 1993. Direct evidence for tyrosine and threonine phosphorylation and activation of mitogen-activated protein kinase by vasopressin in cultured rat vascular smooth muscle cells. J. Biol. Chem. 268, 9564–9569.

Grynkiewicz, G., Poenie, M., Tsien, R.Y., 1985. A new generation of calcium indicators with greatly improved fluorescence properties. J. Biol. Chem. 260, 3440–3450.

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. Pfluegers Arch. 391, 85–100.

Hazama, H., Nakajima, T., Asano, M., Iwasawa, K., Morita, T., Igarashi, K., Nagata, T., Horiuchi, T., Suzuki, J., Soma, M., Okuda, Y., 1998. Omega-3 polyunsaturated fatty acids-modulation of voltage-dependent L-type Ca^{2+} current in guinea-pig tracheal smooth muscle cells. Eur. J. Pharmacol. 355, 257–266.

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.

Iwasawa, K., Nakajima, T., Hazama, H., Goto, A., Shin, W.S., Toyo-oka, T., Omata, M., 1997. Effects of extracellular pH on receptor-mediated Ca^{2+} influx in A7r5 rat smooth muscle cells: involvement of two different types of channel. J. Physiol. 503, 237–251.

Jones, L.G., Ella, K.M., Bradshaw, C.D., Gause, K.C., Dey, M., Wisehart-Johnson, A.E., Spivey, E.C., Meier, K.E., 1994. Activation of mitogen-activated protein kinases and phospholipase D in A7r5 vascular smooth muscle cells. J. Biol. Chem. 269, 23790–23799.

Juan, H., Sutter, D., Sametz, W., 1987. Influence of eicosapentaenoic acid on noradrenaline and angiotensin-induced contractions of the rabbit aorta: mode of action. Prog. Clin. Biol. Res. 242, 57–62.

Jung, F., Selvaraj, S., Gargus, J.J., 1992. Blockers of platelet-derived growth factor-activated nonselective cation channel inhibit cell proliferation. Am. J. Physiol. 262, C1464–C1470.

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.

Kleine, L.P., Whitfield, J.F., Boynton, A.L., 1986. Ca^{2+}-dependent cell surface protein phosphorylation may be involved in the initiation of DNA synthesis. J. Cell. Physiol. 129, 303–309.

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 LOE 908 selectively blocks vasopressin-activated nonselective cation currents in A7r5 aortic smooth muscle cells. Naunyn-Schmiedeberg's Arch. Pharmacol. 349, 301–307.

Kribben, A., Wieder, E.D., Li, X., Putten, V., Granot, Y., Schrier, R.W., Nemenoff, R.A., 1993. AVP-induced activation of MAP kinase in vascular smooth muscle cells is mediated through protein kinase C. Am. J. Physiol. 265, C939–C945.

Locher, R., Vogt, E., Steiner, A., Vetter, W., 1988. The phosphoinositide turnover of vascular smooth muscle cells is influenced by fish oil. J. Hypertens. 6, S222–S224, Suppl. 4.

Locher, R., Sachinidis, A., Steiner, A., Vogt, E., Vetter, W., 1989. Fish oil affects phosphoinositide turnover and thromboxane A_2 metabolism in cultured vascular smooth muscle cells. Biochim. Biophys. Acta 1012, 279–283.

Meldolesi, J., Pozzan, T., 1987. Pathways of Ca^{2+} influx at the plasma membrane: voltage-, receptor-, and second messenger-operated channels. Exp. Cell. Res. 171, 271–283.

Minowa, T., Miwa, S., Kobayashi, S., Enoki, T., Zhang, X.F., Komuro, T., Iwamuro, Y., Masaki, T., 1997. Inhibitory effect of nitrovasodilators and cyclic GMP on ET-1-activated Ca^{2+}-permeable nonselective cation channel in rat aortic smooth muscle cells. Br. J. Pharmacol. 120, 1536–1544.

Mizutani, M., Asano, M., Roy, S., Nakajima, T., Soma, M., Yamashita, K., Okuda, Y., 1997. Omega-3 polyunsaturated fatty acids inhibit migration of human vascular smooth muscle cells in vitro. Life Sci. 61, 269–274.

Mogami, H., Kojima, I., 1993. Stimulation of calcium entry is prerequisite for DNA synthesis induced by platelet-derived growth factor in vascular smooth muscle cells. Biochim. Biophys. Res. Commun. 29, 650–658.

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.

Nakajima, T., Kitazawa, T., Hamada, E., Hazama, H., Omata, M., Kurachi, Y., 1995. 17β-estradiol inhibits the voltage-dependent L-type Ca^{2+} currents in aortic smooth muscle cells. Eur. J. Pharmacol. 294, 625–635.

Nakajima, 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.

Nambi, P., Watt, R., Whitman, M., Aiyar, N., Moore, J.P., Evan, G.I., Crooke, S., 1989. Induction of c-fos protein by activation of vasopressin receptors in smooth muscle cells. FEBS Lett. 245, 61–64.

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.

Ordway, R.W., Singer, J.J., Walsh, J.V. Jr., 1991. Direct regulation of ion channels by fatty acids. Trends Neurosci. 14, 96–100.

Pardee, A.B., 1989. G_1 events and regulation of cell proliferation. Science 246, 603–608.

Puska, P., Iacono, J.M., Nissinen, A., Korhonen, H.J., Vartianinen, E., Pietinen, P., Dougherty, R., Leino, U., Mutanen, M., Moisio, S., Huttunen, J., 1983. Controlled, randomized trial of the effect of dietary fat on blood pressure. Lancet I, 1–5.

Putney, J.W. Jr., 1990. Capacitative calcium entry revisted. Cell Calcium 11, 611–624.

Shiina, T., Terano, T., Saito, J., Tamura, Y., Yoshida, S., 1993. Eicosapentaenoic and docosahexaenoic acid suppress the proliferation of vascular smooth muscle cells. Atherosclerosis 104, 95–103.

Short, A.D., Bian, J., Ghosh, T.K., Waldron, R.T., Rybak, S.L., Gill, D.L., 1993. Intracellular Ca^{2+} pool content is linked to control of cell growth. Proc. Natl. Acad. Sci. U.S.A. 90, 4986–4990.

Siess, W., Roth, P., Scherer, B., Kurzman, I., Bohlig, B., Weber, P.C., 1980. Platelet-membrane fatty acids, platelet aggregation, and thromboxane formation during a mackerel diet. Lancet 1, 441–444.

Sperti, G., Colucci, W.S., 1991. Calcium influx modulates DNA synthesis and proliferation in A7r5 vascular smooth muscle cells. Eur. J. Pharmacol. 206, 279–284.

Standley, P.R., Zhang, F., Ram, J.L., Zemel, M.B., Sowers, J.R., 1991. Insulin attenuates vasopressin-induced calcium transients and a voltage-dependent calcium response in rat vascular smooth muscle cells. J. Clin. Invest. 88, 1230–1236.

Terano, T., Shiina, T., Tamura, Y., 1996. Eicosapentaenoic acid suppresses the proliferation of vascular smooth muscle cells through modulation of various steps of growth signals. Lipids 31, S301–S304.

Thibbonnier, M., Bayer, A.L., Simonson, M.S., Kester, M., 1991. Multiple signaling pathways of V-1 vascular vasopressin receptors of A7r5 cells. Endocrinology 129, 2845–2856.

Van Renterghem, C., Lazdunski, K., 1994. Identification of the Ca^{2+} current activated by vasoconstrictors in vascular smooth muscle cells. Pfluegers 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.

Wallnofer, A., Cauvin, C., Ruegg, U.T., 1987. Vasopressin increases $^{45}Ca^{2+}$ influx in rat aortic smooth muscle cells. Biochem. Biophys. Res. Commun. 148, 273–278.

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., Gomez, A.M., Morgan, J.P., Lederer, W.J., Leaf, A., 1997. Suppression of voltage-gated L-type Ca^{2+} currents by polyunsaturated fatty acids in adult and neonatal rat ventricular myocytes. Proc. Natl. Acad. Sci. U.S.A. 94, 4182–4187.

Xu, X., Lee, K.S., 1996. Dual effects of arachidonic acid on ATP-sensitive K^+ current of coronary smooth muscle cells. Am. J. Physiol. 270, H1957–H1962.