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Role of nonselective cation channels as Ca^{2+} entry pathway in endothelin-1-induced contraction and their suppression by nitric oxide

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Abstract

The present study was carried out to clarify the role of nonselective cation channels as a Ca^{2+} entry pathway in the contraction and the increase in $[Ca^{2+}]_i$ induced by endothelin-1 in endothelium-denuded rat thoracic aorta rings, and their suppression by nitric oxide (NO). In Ca^{2+}-free medium, the endothelin-1-induced contraction was suppressed to about 20% of control values, although the increase in $[Ca^{2+}]_i$ became negligible. The contraction and the increase in $[Ca^{2+}]_i$ monitored by fura 2 fluorescence were unaffected by a blocker of L-type voltage-operated Ca^{2+} channels nifedipine. A blocker of nonselective cation channels 1-[β-[3-(4-methoxyphenyl)propoxyl]-4-methoxyphenethyl]-1*H*-imidazole · HCl(SK&F 96365) suppressed the endothelin-1-induced contraction and increase in $[Ca^{2+}]_i$ to the level similar to that after removal of extracellular Ca^{2+}. SK&F 96365 had no further effect on the endothelin-1-induced contraction in the absence of extracellular Ca^{2+}. The endothelin-1-induced contraction and increase in $[Ca^{2+}]_i$ were abolished by a donor of NO sodium nitroprusside. The effects of another NO donor 3-morpholinosydnonimine (SIN-1) were also tested and yielded essentially similar results to those for sodium nitroprusside on the endothelin-1-induced contraction. Furthermore, the inhibitory effects of sodium nitroprusside could be blocked with a guanylate cyclase inhibitor 1*H*-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ) at 30 μM. These findings suggest that Ca^{2+} entry through nonselective cation channels but not voltage-operated Ca^{2+} channels plays a critical role in the endothelin-1-induced increase in $[Ca^{2+}]_i$ and the resulting contraction and that inhibition by NO of the endothelin-1-induced contraction is mainly the result of blockade of Ca^{2+} entry through these channels.

Keywords: Endothelin-1; Cation channel, nonselective; Nitric oxide (NO); SK&F 96365; Nifedipine; Aorta, rat; Tension; Ca^{2+} level, cytosolic

1. Introduction

Endothelin-1 is a 21-amino acid peptide and is one of the most potent endogenous vasocontracting agent yet discovered (Yanagisawa et al., 1988). Endothelin-1 binds to receptors on vascular smooth muscle cells and subsequently raises the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), which is essential for cell contraction. It is generally accepted that the sustained contraction produced by endothelin-1 requires the persistent entry of extracellular Ca^{2+} (Masaki et al., 1991; Rubanyi and Polokoff, 1994). The voltage-operated Ca^{2+} channel is a well-known Ca^{2+} entry channel activated by endothelin-1 (Goto et al., 1989; Inoue et al., 1990), but the involvement of other channels permeable to Ca^{2+} has also been suggested (Huang et al., 1990; Simpson et al., 1990; Masaki et al., 1991). Several researchers have shown that the Ca^{2+}-permeable nonselective cation channel is activated by endothelin-1 in vascular smooth muscle cells (Van Renterghem et al., 1988; Chen and Wagoner, 1991). In this context, we have recently shown that both cloned endothelin ET_A receptor expressed in L*tk*$^-$cells and native endothelin ET_A receptor expressed in vascular smooth muscle cells are functionally coupled to a nonselective cation channel which is permeable to Ca^{2+} (Enoki et al., 1995; Minowa et al., 1997). In the same reports, we also indicated that the Ca^{2+}-permeable nonselective cation channel is functionally suppressed by 1-[β-[3-(4-methoxyphenyl)propoxyl]-4-methoxyphenethyl]-1*H*-imidazole · HCl (SK&F 96365) and mefenamic acid (Enoki et al., 1995; Minowa et al., 1997). Furthermore, we have

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recently shown that contractions of ring specimens of rabbit thoracic aorta induced by endothelin-1 are sensitive to mefenamic acid and SK&F 96365 (Komuro et al., 1997b). However, the causal relationship between the inhibition of the endothelin-1-induced contractions by these drugs and inhibition of Ca^{2+} entry is still uncertain.

Nitric oxide (NO) is an endothelium-derived vasorelaxing factor and is reported to suppress the vasocontraction induced by endothelin-1 (Yang et al., 1989; Jacoby et al., 1993; Pussard et al., 1995). Generally, NO induces vasorelaxation through guanosine-3′:5′-cyclic monophosphate (cyclic GMP)-dependent mechanisms, by (1) causing activation of K^+-channels and the resulting inhibition of voltage-operated Ca^{2+} channels (Archer et al., 1994), (2) directly inhibiting voltage-operated Ca^{2+} channels (Lorenz et al., 1994), (3) activating the Ca^{2+} pump on the plasma membrane (Rashatwar et al., 1987; Yoshida et al., 1991) and on the membrane of endoplasmic reticulum (Cornwell et al., 1991), and (4) decreasing the sensitivity of contractile elements to Ca^{2+} (Pfitzer et al., 1986; Karaki et al., 1988). Furthermore, we have recently shown that both an NO-generating agent, sodium nitroprusside, and 8-bromoguanosine-3′:5′-cyclic monophosphate (8-bromo-cyclic GMP) inhibit Ca^{2+} entry through endothelin-1-activated nonselective cation channels in rat aortic smooth muscle cells which are sensitive to SK&F 96365 (Minowa et al., 1997). Based on these findings, we now assume that NO induces vasorelaxation mainly by suppressing Ca^{2+} entry through nonselective cation channels.

In the present study, we attempted to clarify the role of Ca^{2+} entry through nonselective cation channels in the vasocontraction induced by endothelin-1 and in the vasorelaxation induced by NO. For this purpose, we used simultaneous measurement of tension and $[Ca^{2+}]_i$ in rat thoracic aorta rings and tested the effects of removal of extracellular Ca^{2+}, a blocker of L-type voltage-operated Ca^{2+} channels nifedipine, SK&F 96365 and sodium nitroprusside on these parameters. We also examined the effect of an inhibitor of phospholipase C 1-[6-[[(17β)-3-methoxyestra-1,3,5 (10)-trien-17-yl]-amino]hexyl]-1*H*-pyrrole-2,5 dione (U-73122) on the endothelin-1-induced contraction to assess the extent of involvement of phospholipase C in the contraction.

2. Methods

2.1. Tissue preparation and tension measurement

Preparation of rat thoracic aortic rings and measurement of isometric tension were performed as described previously (Komuro et al., 1997a). Briefly, male Wistar rats weighing 150–200 g were killed, and the thoracic aorta was removed quickly and placed into Krebs solution which contained (in mM): NaCl 120, KCl 5.4, $CaCl_2$ 2.2, $MgCl_2$ 1.0, $NaHCO_3$ 25, and glucose 5.6. Blood was rinsed from the lumen, adherent connective tissue was removed carefully and rings, approximately 1 to 2 mm in width, were cut from each aorta. Endothelial cells were removed from ring specimens by gently rubbing the intimal surface with a cotton bud moistened with Krebs solution. Successful removal of endothelial cells was confirmed by the inability of acetylcholine (1 μM) to induce relaxation. The aortic rings were mounted by use of a pair of stainless steel hooks under a resting tension of 1 g in organ baths containing 5 ml of Krebs solution which was maintained at 37°C and bubbled with a 95% O_2 and 5% CO_2 mixture: one of the hooks was connected to a force transducer (Orientec, Tokyo, Japan) and the developed tension was displayed on a Nihon Kohden (Tokyo, Japan) RJG4128 polygraph. During the whole experiment, the bath fluid was changed every 20 min.

Initially, resting force was readjusted after each wash-out until a stable baseline was attained (usually after about 60 min). After readjustment, the preparations were challenged at hourly intervals with 0.3 μM noradrenaline. When two noradrenaline contractions gave reproducible results, the actual experiment was started. At the beginning of each experiment, each preparation was challenged with 1 μM noradrenaline, which caused a maximal contraction, and the contractile responses to endothelin-1 are presented as percentages of the tension induced by 1 μM noradrenaline. It has been reported that because of tachyphylaxis, the same vascular ring cannot be repeatedly stimulated with endothelin-1 (Auguet et al., 1990). Therefore, the different concentrations of drugs (i.e., nifedipine, SK&F 96365 and sodium nitroprusside) were tested in different rings. For experiments in Ca^{2+}-free solution, Ca^{2+} was omitted from Krebs solution and 2 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) was added unless otherwise specified.

2.2. Measurement of $[Ca^{2+}]_i$ in fura 2-loaded preparation

The aortic rings were loaded with a Ca^{2+} indicator fura 2 as described previously (Ozaki et al., 1987). The rings were incubated with 5 μM fura 2/acetoxymethylester in modified physiological saline solution for 3 h at room temperature in the presence of 0.2% cremophor EL and rinsed with the same solution for 15 min. The modified physiological saline solution contained (in mM): NaCl 143, KCl 4.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, HEPES 20 (pH 7.4), and EDTA 0.01. After the incubation, the experiments were performed with a double wavelength excitation fluorimeter (CAF-110, Japan Spectroscopic, Tokyo, Japan) where the fura 2-loaded specimen was fixed horizontally in a bath that was bubbled with 95% O_2 and 5% CO_2 at 37°C. The mechanical activity was monitored isometrically as described in the tension study. Simultaneously, a part of the specimen was excited by light from a xenon high pressure lamp (75 W) equipped with a rotating filter wheel (48 Hz) which contained 340 nm and 380 nm interference

filters. The emitted light from the specimen was collected by a photomultiplier through a 500-nm filter. The ratio of the fluorescence due to excitation at 340 nm to that at 380 nm was calculated and regarded as an index of $[Ca^{2+}]_i$. At the beginning of each experiment, the specimens were challenged with 0.1 μM noradrenaline, and the contractions and increases in $[Ca^{2+}]_i$ are presented as percentages of the responses induced by 0.1 μM noradrenaline. A concentration of noradrenaline lower than that in the tension study was used to obtain a plateau within a short period, because it is known that fluorescence as an index of $[Ca^{2+}]_i$ decreases rapidly because of factors such as leakage of the dye from the cell and photobleaching.

2.3. Chemicals

Chemicals were obtained from the following sources: endothelin-1, from Peptide Institute (Osaka, Japan); fura 2/acetoxymethylester, HEPES, 3-morpholinosydnonimine (SIN-1) and EDTA, from Dojindo Laboratory (Kumamoto, Japan); EGTA and cremophor EL, from Nacalai Tesque (Kyoto, Japan); sodium nitroprusside, noradrenaline bitartrate and acetylcholine bromide, from Wako (Osaka, Japan); nifedipine, from Sigma (St. Louis, MO, USA); 1-[β-[3-(4-methoxyphenyl)propoxyl]-4-methoxyphenethyl]-1*H*-imidazole · HCl (SK&F 96365), 1-[6-[[(17β)-3-methoxyestra-1,3,5 (10)-trien-17-yl]-amino]hexyl]-1*H*-pyrrole-2,5 dione (U-73122) and 1*H*-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ), from Biomol Research (Plymouth Meeting, PA, USA). Nifedipine was dissolved in dimethyl sulphoxide (DMSO) and the final concentration of DMSO was 0.1%.

2.4. Statistical analysis

Data are presented as means ± S.E.M.. The EC_{50} (or IC_{50}) values and the maximal responses were estimated for individual concentration–response curves by use of nonlinear least-squares regression analysis. Pairs of means were compared using paired Student's *t*-test and groups of data were compared by using analysis of variance (ANOVA) followed by Newman–Keuls' multiple-range test. A probability level of $P < 0.05$ was considered statistically significant.

3. Results

Fig. 1 shows the effects of removal of extracellular Ca^{2+} and nifedipine on concentration–response relationships for the endothelin-1-induced contraction in endothelium-denuded rat thoracic aorta rings. In normal Krebs solution, endothelin-1 produced contractions of the rings in a concentration-dependent manner with an EC_{50} value of 1.00 ± 0.01 nM ($n = 8$) and a maximal response (E_{max}) amounting to 110 ± 4.2% ($n = 8$) of the 1 μM noradrenaline-induced contraction.

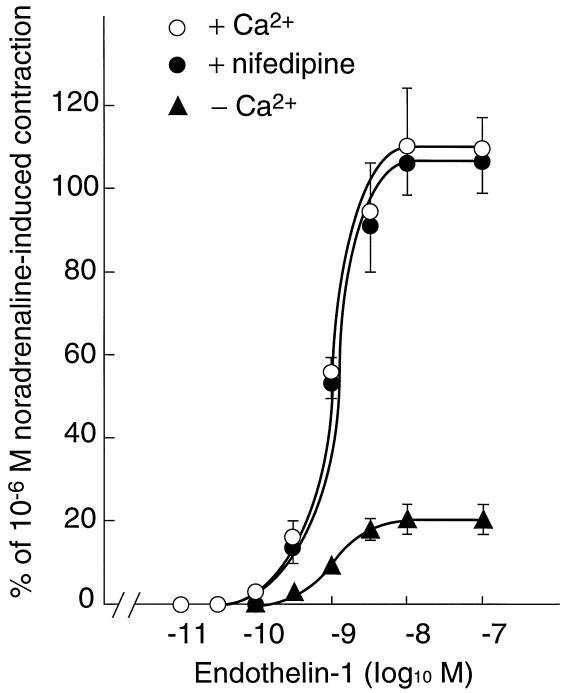

Fig. 1. Effects of removal of extracellular Ca^{2+} and nifedipine on concentration–response relationships for the endothelin-1-induced contraction in endothelium-denuded rat thoracic aorta rings. The rings were stimulated with various concentrations of endothelin-1 in normal Krebs solution (○, $n = 8$), Ca^{2+}-free Krebs solution (▲, $n = 8$) or normal Krebs solution containing 1 μM nifedipine (●, $n = 6$). The contractile responses to endothelin-1 are presented as percentages of the tension induced by 1 μM noradrenaline in each preparation. Each point represents the mean of *n* experiments with S.E.M. shown by vertical lines.

After removal of extracellular Ca^{2+} (2 mM EGTA added), the EC_{50} value was unchanged (1.01 ± 0.03 nM, $n = 8$), but the E_{max} was reduced to 20.7 ± 2.2% of the control value ($n = 8$, $P < 0.01$). Even when the concentration of EGTA was elevated to 5 mM, no further suppression of the contraction was observed (Table 1).

Table 1
Effects of removal of extracellular Ca^{2+} and U-73122 on the 10 nM endothelin-1-induced contraction of rat thoracic aorta

	Tension (%)	*n*
Endothelin-1 (10 nM, control)	110.8 ± 2.7	8
− Ca^{2+} (+2 mM EGTA)	20.2 ± 0.4 *	8
− Ca^{2+} (+5 mM EGTA)	22.2 ± 0.7 *	8
+ U-73122 (10 μM)	109.2 ± 0.5	6
+ U-73122 (30 μM)	112.7 ± 2.2	6
+ U-73122 (100 μM)	109.9 ± 1.7	6
+ U-73122 (10 μM)		
− Ca^{2+} (+2 mM EGTA)	21.2 ± 0.7 *	6

Developed tension is expressed as a percentage of the tension induced by 1 μM noradrenaline.
Values are expressed as the means ± S.E.M. of *n* experiments.
* $P < 0.01$, significantly different from control values.

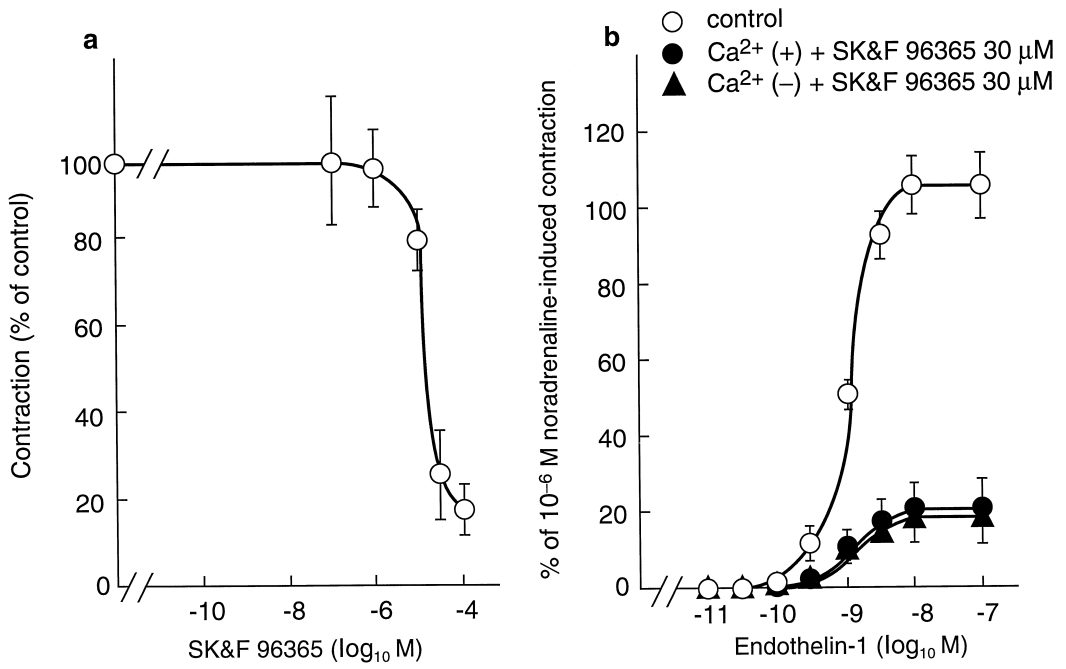

Fig. 2. Effects of SK&F 96365 on contractions of endothelium-denuded rat thoracic aorta rings induced by endothelin-1. (a) A concentration–response relationship for inhibition by SK&F 96365 of contractions of the rings induced by 10 nM endothelin-1. (b) Concentration–response relationships for contractions of the rings induced by endothelin-1 in either normal Krebs solution without (○) or with 30 μM SK&F 96365 (●) or Ca^{2+}-free Krebs solution with 30 μM SK&F 96365 (▲). Nifedipine at 1 μM was added to the media except for the Ca^{2+}-free Krebs solution. Contractions are presented as percentages of the tension induced by either 10 nM endothelin-1 in the absence of SK&F 96365 (a) or 1 μM noradrenaline (b). Each point represents the mean of six experiments with S.E.M. shown by vertical lines.

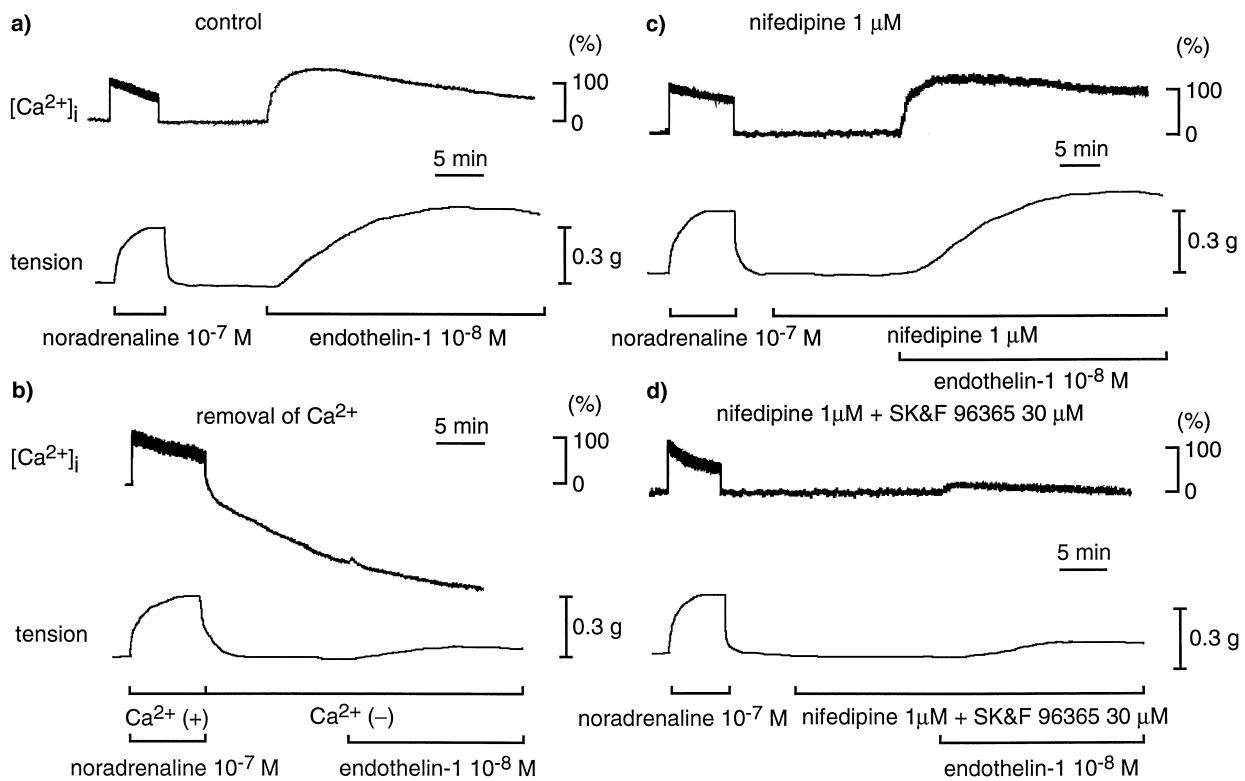

Fig. 3. Typical tracings showing effects of removal of extracellular Ca^{2+} (b), 1 μM nifedipine (c) and 1 μM nifedipine plus 30 μM SK&F 96365 (d) on the endothelin-1-induced contractions (lower trace) and increases in $[Ca^{2+}]_i$ (upper trace) measured simultaneously in endothelium-denuded rat thoracic aorta rings loaded with fura 2. (a) Control represents responses of $[Ca^{2+}]_i$ and tension to 10 nM endothelin-1 in the absence of nifedipine and SK&F 96365.

In contrast, a specific blocker of L-type voltage-operated Ca^{2+} channels nifedipine at 1 μM had no effect on the endothelin-1-induced contractions of the rings (EC_{50}, 0.99 ± 0.02 nM; E_{max}, $109.3 \pm 6.3\%$, $n = 6$).

We also examined the effect of an inhibitor of phospholipase C U-73122 on the endothelin-1-induced contraction to clarify the extent of involvement of increased formation of inositol-1,4,5-trisphosphate (IP_3) and subsequent mobilization of Ca^{2+} from intracellular stores. As shown in Table 1, the contraction was totally unaffected by U-73122 up to the concentration of 100 μM. Furthermore, the contraction remaining after removal of extracellular Ca^{2+} was also resistant to treatment with U-73122.

To test whether the endothelin-1-induced contraction depends on Ca^{2+} entry through nonselective cation channels, we examined the effects of various concentrations of a blocker of nonselective cation channels SK&F 96365 on the contraction induced by 10 nM endothelin-1 (Fig. 2a). Since SK&F 96365 is known to block L-type voltage-operated Ca^{2+} channels in addition to nonselective cation channels (Merritt et al., 1990; Blayney et al., 1992), experiments were performed in the presence of 1 μM nifedipine to completely block L-type voltage-operated Ca^{2+} channels. SK&F 96365 inhibited the endothelin-1-induced contraction in a concentration-dependent manner with an IC_{50} value of 13.3 ± 1.20 μM ($n = 6$), and nearly complete inhibition was obtained at 30 μM: the extent of inhibition was similar to that seen after removal of extracellular Ca^{2+}. Conversely, 30 μM SK&F 96365 suppressed the E_{max} value of the endothelin-1-induced contraction to $19.3 \pm 2.9\%$ ($n = 6$, $P < 0.01$) with the EC_{50} value being unchanged (0.96 ± 0.03 nM, $n = 6$) (Fig. 2b). Notably, 30 μM SK&F 96365 had no effect on the contractions induced in the absence of extracellular Ca^{2+} (Fig. 2b).

To confirm whether SK&F 96365 actually suppresses Ca^{2+} entry into vascular smooth muscle cells, we examined the effects of SK&F 96365 on the endothelin-1-induced increase in $[Ca^{2+}]_i$ by simultaneously measuring $[Ca^{2+}]_i$ and tension in the aortic rings loaded with fura 2.

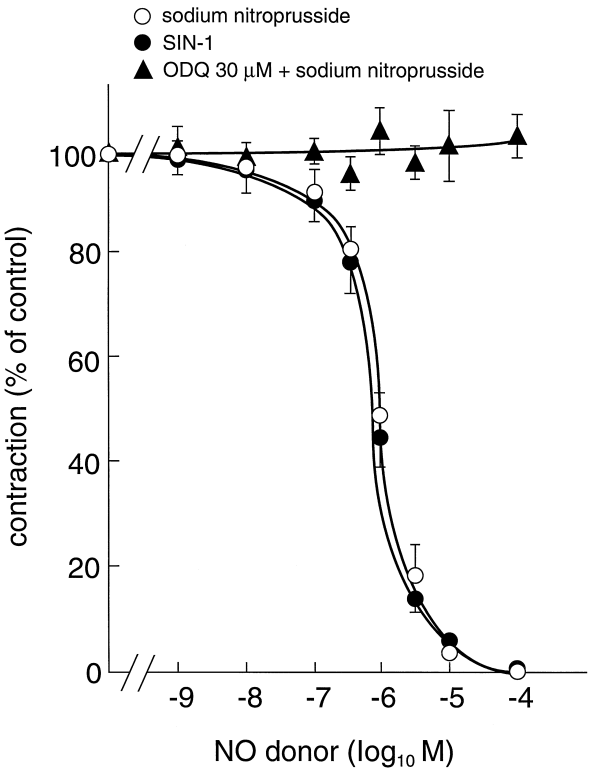

Fig. 4. Effects of various concentrations of NO donors such as sodium nitroprusside and SIN-1 on 10 nM endothelin-1-induced contractions in endothelium-denuded rat thoracic aorta rings in the presence or absence of 30 μM ODQ. Sodium nitroprusside (○) or SIN-1 (●) was added to the bath solution 15 min before stimulation with 10 nM endothelin-1. ODQ at 30 μM was added to the bath solution 15 min before application of sodium nitroprusside (▲). Contractions are presented as percentages of the tension induced by 10 nM endothelin-1 in normal Krebs solution containing 1 μM nifedipine. Each point represents the mean of 6 experiments with S.E.M. shown by vertical lines.

As reported previously (Kai et al., 1989; Takuwa et al., 1990), endothelin-1 at 10 nM induced an increase in $[Ca^{2+}]_i$ in vascular smooth muscle cells, amounting to 110% of that induced by 0.1 μM noradrenaline (Fig. 3a

Table 2
Effects of removal of extracellular Ca^{2+}, nifedipine, SK&F 96365 and sodium nitroprusside on the endothelin-1-induced tension and increases in $[Ca^{2+}]_i$ measured simultaneously in rat thoracic aorta loaded with fura 2

	$[Ca^{2+}]_i$ (%)	Tension (%)
Endothelin-1 (10 nM, control)	120.4 ± 1.5	112.3 ± 1.6
$-Ca^{2+}$ (+2 mM EGTA)	ND	21.2 ± 0.8*
+ Nifedipine (1 μM)	120.8 ± 1.2	108.5 ± 1.2
+ Nifedipine (1 μM) + SK&F 96365 (30 μM)	11.3 ± 0.4*	24.2 ± 0.3*
+ Nifedipine (1 μM) + sodium nitroprusside (1 μM)	54.1 ± 0.4*	45.1 ± 0.9*
+ Nifedipine (1 μM) + sodium nitroprusside (100 μM)	ND	ND

Developed tension and increases in $[Ca^{2+}]_i$ are presented as percentages of responses induced by 0.1 μM noradrenaline.
Values are expressed as the means ± S.E.M. of 6 experiments.
* $P < 0.01$, significantly different from control values.
ND, not detectable.

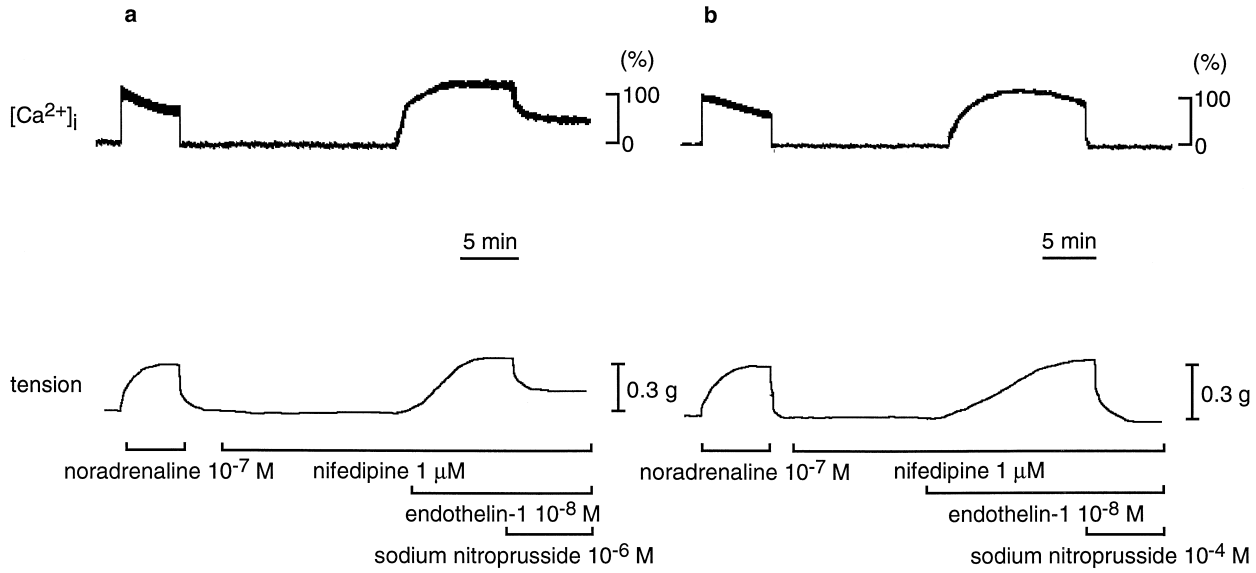

Fig. 5. Typical tracings showing effects of 1 μM (a) or 100 μM (b) sodium nitroprusside on the endothelin-1-induced contractions (lower trace) and increases in $[Ca^{2+}]_i$ (upper trace) measured simultaneously in endothelium-denuded rat thoracic aorta rings loaded with fura 2 in the presence of 1 μM nifedipine. Nifedipine was added to the bath solution at 1 μM 15 min before stimulation with 10 nM endothelin-1. After the contractions reached a plateau, sodium nitroprusside was added at a final concentration of 1 μM or 100 μM, respectively.

and Table 2). In this system, endothelin-1-induced contractions of aortic rings which were smaller than the contractions of the rings not loaded with fura 2 as reported (Sakata et al., 1989; Kodama et al., 1994). After removal of extracellular Ca^{2+}, the increase in $[Ca^{2+}]_i$ seemed to be negligible, although it is difficult to measure the extent of suppression accurately because of continuous drifting of the baseline of $[Ca^{2+}]_i$: the contraction was reduced to about 20% of the corresponding control values (Fig. 3b and Table 2). Nifedipine at 1 μM produced no significant effect on the increase in $[Ca^{2+}]_i$ and the contractions induced by 10 nM endothelin-1 (Fig. 3c and Table 2).

As shown in Fig. 3d, SK&F 96365 at 30 μM in combination with 1 μM nifedipine was without effect on the resting $[Ca^{2+}]_i$ and the resting tension, but this treatment suppressed a major part of the endothelin-1-induced increase in $[Ca^{2+}]_i$ as well as the endothelin-1-induced contraction (Fig. 3d and Table 2).

We tested the effects of NO donors such as sodium nitroprusside and 3-morpholinosydnonimine (SIN-1) on the endothelin-1-induced contraction and the endothelin-1-induced increase in $[Ca^{2+}]_i$. Both sodium nitroprusside and 3-morpholinosydnonimine (SIN-1) inhibited the endothelin-1-induced contraction in a concentration-dependent manner with IC_{50} values of 1.19 ± 0.22 μM ($n = 6$) and 1.12 ± 0.14 μM ($n = 6$), respectively, and complete inhibition was obtained at concentrations higher than 10 μM. Furthermore, the inhibition of the endothelin-1-induced contraction by sodium nitroprusside was completely reversed by a guanylate cyclase inhibitor 1*H*-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ) at 30 μM (Fig. 4).

In accordance with the tension study, the endothelin-1-induced increases in $[Ca^{2+}]_i$ were suppressed to about 50% and 0% by 1 μM and 100 μM sodium nitroprusside, respectively (Fig. 5 and Table 2).

4. Discussion

4.1. Dependence on extracellular and intracellular Ca^{2+} sources

A major part of the endothelin-1-induced contraction and of the endothelin-1-induced increase in $[Ca^{2+}]_i$ was suppressed by removal of extracellular Ca^{2+}. These results are essentially similar to those of previous reports for endothelin-1-induced contractions (Kasuya et al., 1989; Sakata et al., 1989) and increases in $[Ca^{2+}]_i$ (Goto et al., 1989; Takuwa et al., 1990; Simonson and Dunn, 1990; Huang et al., 1990), and indicate that the endothelin-1-induced sustained contraction depends mainly on the entry of extracellular Ca^{2+}, although the remaining small part of the contraction (resistant to removal of extracellular Ca^{2+}) is the result of a mechanism other than Ca^{2+} influx. Judging from the lack of a significant effect of an inhibitor of phospholipase C 1-[6[[(17β)-3-methoxyestra-1,3,5(10)-trien-17yl]-amino]hexyl]-1*H*-pyrrole-2,5 dione (U-73122) on the endothelin-1-induced contractions in the absence of extracellular Ca^{2+}, mobilization of Ca^{2+} from intracellular stores via increased formation of IP_3 does not play a significant role in the contraction. Therefore, the contraction in the absence of Ca^{2+} might be due to an

increase in the Ca^{2+}-sensitivity of contractile elements (Sakata et al., 1989).

4.2. Involvement of voltage-operated Ca^{2+} channel

The endothelin-1-induced contraction was totally resistant to specific blockers of L-type voltage-operated Ca^{2+} channels such as nifedipine. The increase in $[Ca^{2+}]_i$ was also totally resistant to nifedipine. Our data on nifedipine are similar to those of previous reports on the endothelin-1-induced contraction of rat aorta (Chabrier et al., 1989; D'Orleans-Juste et al., 1989; Turner et al., 1989; Nakajima et al., 1996) and the endothelin-1-induced increase in $[Ca^{2+}]_i$ in isolated rat aorta (Huang et al., 1990) and cultured vascular smooth muscle cells (Takuwa et al., 1990).

Thus, the present study indicates that L-type voltage-operated Ca^{2+} channels play a minor role in the contraction of rat aorta induced by endothelin-1, although endothelin ET_A receptor is reported to be coupled to voltage-operated Ca^{2+} channel (Goto et al., 1989; Inoue et al., 1990). Taken together with the dependence of the contraction on extracellular Ca^{2+}, these results strongly indicate that the endothelin-1-induced contraction of rat aorta depends exclusively on Ca^{2+} entry through channels other than L-type voltage-operated Ca^{2+} channels.

4.3. Sensitivity of endothelin-1-induced contraction and increase in $[Ca^{2+}]_i$ to a blocker of nonselective cation channels SK&F 96365

In the present study, the major part of the endothelin-1-induced contraction and the endothelin-1-induced increase in $[Ca^{2+}]_i$ was eliminated by SK&F 96365. The inhibitory effect of SK&F 96365 seems to be mainly the result of inhibition of entry of extracellular Ca^{2+} based on the following observations: (1) The magnitude of the suppression of the contraction produced by SK&F 96365 was similar to that after removal of extracellular Ca^{2+}; (2) SK&F 96365 had little effect on the endothelin-1-induced contraction in the absence of extracellular Ca^{2+}; and (3) Removal of extracellular Ca^{2+} (by addition of 2 mM EGTA) was without effect on the endothelin-1-induced contraction in the presence of SK&F 96365.

In our recent work using whole-cell recordings and the patch clamp technique, we have shown that both cloned endothelin ET_A receptors expressed in L tk^- cells and native endothelin ET_A receptors expressed in vascular smooth muscle cells are functionally coupled to Ca^{2+}-permeable nonselective cation channels, which are sensitive to SK&F 96365 (Enoki et al., 1995; Minowa et al., 1997). It is reported that SK&F 96365 has inhibitory effects on L-type voltage-operated Ca^{2+} channels in addition to nonselective cation channels (Merritt et al., 1990). However, because L-type voltage-operated Ca^{2+} channels do not play a significant role in the endothelin-1-induced contraction and the increase in $[Ca^{2+}]_i$, and because a maximally effective concentration of a blocker of L-type voltage-operated Ca^{2+} channels nifedipine, was present in medium to completely block voltage-operated Ca^{2+} channels, it follows that the inhibitory effect of SK&F 96365 on the endothelin-1-induced contraction and the increase in $[Ca^{2+}]_i$ is mainly derived from blockade of Ca^{2+} entry through not voltage-operated Ca^{2+} channels but nonselective cation channels. These results taken together demonstrate that endothelin-1 causes an increase in $[Ca^{2+}]_i$ and the resulting contraction of rat aorta mainly by activating nonselective cation channels as a dominant Ca^{2+} entry pathway.

4.4. Effects of nitric oxide on the endothelin-1-induced contraction and the endothelin-1-induced increase in $[Ca^{2+}]_i$

As previously reported, both the endothelin-1-induced contraction and increase in $[Ca^{2+}]_i$ were completely inhibited by an NO generator sodium nitroprusside (Sakata et al., 1989; Jacoby et al., 1993; Pussard et al., 1995; Yang et al., 1989). Essentially similar results were obtained for SIN-1 on the endothelin-1-induced contraction. Furthermore, the inhibitory effects of sodium nitroprusside could be blocked by a guanylate cyclase inhibitor ODQ. These data indicate that NO itself suppresses the endothelin-1-induced contraction. Several mechanisms have so far been proposed for inhibition of an agonist-induced vasocontraction by NO (see Section 1). The present finding that nonselective cation channels but not voltage-operated Ca^{2+} channels play a critical role in the endothelin-1-induced vasocontraction and our recent data that NO functionally suppresses Ca^{2+} entry through nonselective cation channels, as monitored by whole-cell recordings (Minowa et al., 1997), strongly indicate that NO inhibits the endothelin-1-induced increase in $[Ca^{2+}]_i$ and vasocontraction mainly by suppressing Ca^{2+} entry through nonselective cation channels.

In addition, sodium nitroprusside suppressed the part of the endothelin-1-induced contraction which is independent of Ca^{2+} entry. It is likely that the effect of sodium nitroprusside results from suppression of the increased Ca^{2+}-sensitivity of the contractile elements (Karaki et al., 1988).

5. Conclusion

In summary, the present study showed that Ca^{2+} entry through nonselective cation channels plays a major role in the endothelin-1-induced vasocontraction and also that NO suppresses the endothelin-1-induced vasocontraction mainly by inhibiting Ca^{2+} entry through these nonselective cation channels.

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