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Role of Na^+/Ca^{2+} exchange in endothelin-1-induced increases in Ca^{2+} transient and contractility in rabbit ventricular myocytes: pharmacological analysis with KB-R7943

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1 The effects of endothelin-1 (ET-1) on intracellular Ca^{2+} ion level and cell contraction were simultaneously investigated in rabbit ventricular cardiac myocytes loaded with indo-1/AM. The role of Na^+/Ca^{2+} exchange in ET-1-induced positive inotropic effect (PIE) was examined by using KB-R7943 (2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl]isothiourea methanesulphonate), a selective inhibitor of reverse mode Na^+/Ca^{2+} exchange.

2 ET-1 at 0.3 pM – 1 nM increased cell contraction and Ca^{2+} transient (CaT) with EC_{50} values of 2.9 pM and 1.2 pM, respectively, and the increase in amplitude of CaT was much smaller relative to the PIE: ET-1 at 1 nM increased peak cell shortening by 237%, while it increased peak CaT by 167%. For a given level of PIE, ET-1-induced increase in CaT was much smaller than that induced by elevation of $[Ca^{2+}]_o$ and by isoprenaline. Therefore, ET-1 shifted the relationship between peak CaT and cell shortening to the left relative to the relationship for increase in $[Ca^{2+}]_o$, an indication that ET-1 increased myofibrillar Ca^{2+} sensitivity.

3 KB-R7943 at 0.1 μM and higher inhibited contraction and CaT induced by 0.1 nM ET-1 and at 0.3 μM it abolished the increase in CaT while inhibiting the PIE by 48.1%. Over concentration range of 0.1 – 0.3 μM, KB-R7943 neither inhibited baseline contraction and CaT nor the isoprenaline-induced response, although at 1 μM and higher it had a significant inhibitory action on these responses.

4 These results indicate that in rabbit ventricular myocytes both increases in CaT and myofibrillar Ca^{2+} sensitivity contribute to the ET-induced PIE, and the activation of reverse mode Na^+/Ca^{2+} exchange may play a crucial role in increase in CaT induced by ET-1 in rabbit ventricular cardiac myocytes.

Keywords: Endothelin-1; KB-R7943 (2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl]isothiourea methanesulphonate); cell contraction; Ca^{2+} transient; Na^+/Ca^{2+} exchanger; adult rabbit ventricular cardiac myocytes

Abbreviations: indo-1/AM, acetoxymethylester of indo-1; CaT, Ca^{2+} transient; DC, duration of Ca^{2+} transient (or cell shortening); EC_{50} value, half-maximal effective concentration; ET-1, endothelin-1; $[Ca^{2+}]_o$, extracellular calcium concentration, $[Ca^{2+}]_i$, intracellular free calcium concentration; PLC, phospholipase C; PIE, positive inotropic effect; PKC, protein kinase C; TD, time for Ca^{2+} transient (or cell shortening) to decline; TT, time to peak Ca^{2+} transient (or cell shortening)

Introduction

Endothelin-1 (ET-1) is a 21-amino acid vasoconstrictor/pressor peptide originally isolated from porcine aortic endothelial cells (Yanagisawa *et al*., 1988). This vasoactive polypeptide was soon proved to have a pronounced positive inotropic effect in cardiac muscle from several mammalian species, including rat (Hu *et al*., 1988; Moravec *et al*., 1989; Krämer *et al*., 1991), guinea-pig (Ishikawa *et al*., 1988), ferret (Shah *et al*., 1989), rabbit (Takanashi & Endoh, 1991, 1992) and human (Moravec *et al*., 1989) with a half-maximal effective concentration (EC_{50}) in the range of nM. The positive inotropic effect of ET-1 is shown to be associated with stimulation of phospholipase C (PLC) and the resultant acceleration of the hydrolysis of phosphoinositide (Vigne *et al*., 1989; Galron *et al*., 1990), leading to production of putative second messengers, 1,4,5-inositol trisphosphate and 1,2-diacylglycerol (Nishizuka, 1988; Berridge, 1993; Meyer *et al*., 1996). This signal transduction pathway is considered to be coupled to several subcellular mechanisms underlying the ET-induced positive inotropic effect (Rubanyi & Polokoff, 1994), such as an increase in the sensitivity of the myofilament to Ca^{2+} ions (Kelly *et al*., 1990; Wang *et al*., 1991; Qiu *et al*., 1992), an increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), by mobilization of intracellular Ca^{2+} ions and transmembrane Ca^{2+} currents (Kelly *et al*., 1990; Vigne *et al*., 1990), alkalinization of the intracellular milieu by stimulation of protein kinase C (PKC)-dependent Na^+/H^+ exchanger (Krämer *et al*., 1991). However, this transduction pathway varies widely among different mammalian species (Takanashi & Endoh, 1991; Del Monte *et al*., 1993; Yang & Endoh, 1997), which might be responsible for the species-dependent variations in the regulatory processes in ET-induced positive inotropic effect.

It has been established that Na^+/Ca^{2+} exchanger plays a major role in Ca^{2+}efflux and therefore it controls and regulates maintenance of Ca^{2+} homeostasis in the heart (Bridge *et al*., 1990; Schulze *et al*., 1993). It has recently been shown that ET-1 as well as phenylephrine and angiotensin II

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increase the Na^+/Ca^{2+} exchange activity in enriched sarcolemmal vesicles from rat heart *via* a PLC-PKC pathway, but isoprenaline does not regulate the Na^+/Ca^{2+} exchanger (Ballard & Schaffer, 1996). These results imply that the Na^+/Ca^{2+} exchanger might play an important role in the effects of agonists that facilitate the PLC-PKC pathway rather than those linked to cyclic AMP pathway. It is unclear, however, whether Na^+/Ca^{2+} exchanger contributes to the regulation by ET-1 of the myocardial contractility in intact myocardial cells, i.e., in acceleration of relaxation by Ca^{2+} extrusion *via* forward mode or in induction of positive inotropic effect by enhancing Ca^{2+} entry through reverse mode Na^+/Ca^{2+} exchanger. As Na^+/Ca^{2+} exchanger is coupled to a PLC-sensitive G protein-PKC-mediated pathway (Philipson *et al*., 1983; Pierce & Panagia, 1989; Ballard & Schaffer, 1996), it might play a role in the ET-induced inotropic regulation. Based on this hypothesis, we examined first the contractile regulation induced by ET-1 in relation to changes in Ca^{2+} transient. For this purpose the instantaneous relationship between the Ca^{2+} transient and cell shortening was investigated by means of phase-plane analysis to clarify whether ET-1 modulates the relation during the course of twitch contraction in addition to alteration of the peak relationship. Secondly the influence of a selective inhibitor of the reverse mode Na^+/Ca^{2+} exchange, KB-R7943 (2-[2-[4-(4-nitrobenzyloxy)phenyl] ethyl]isothiourea methanesulphonate; Iwamoto *et al*., 1996; Watano *et al*., 1996), on the ET-1-induced positive inotropic effect was studied in adult rabbit ventricular cardiac myocytes that had been loaded with a fluorescent dye acetoxymethylester of indo-1 (indo-1/AM).

Methods

The procedure for isolation of ventricular cardiac myocytes has been described previously (Fujita & Endoh, 1996). Briefly, adult male Japanese White rabbits (1.8–2.0 kg) were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.v.). The heart was excised and perfused by Langendorff method with Tyrode's solution for about 1 min at 37°C to washout the blood in the heart. Tyrode's solution contained (in mM): NaCl, 136.5; KCl, 5.4; $MgCl_2$, 0.53; $CaCl_2$, 1.8; NaH_2PO_4, 0.33; glucose, 5.0 and HEPES, 5.0 (pH 7.4) and was continuously bubbled with 100% O_2. Then the heart was perfused with nominally Ca^{2+} free Tyrode's solution for 5 min. Perfusion was switched to the solution containing 0.6 mg ml^{-1} collagenase (class II; Worthington Biochemical, Freehold, NJ, U.S.A.) and 0.12 mg ml^{-1} protease (type XIV; Sigma Chemical Co., St. Louis, MO, U.S.A.) for 18–24 min. Finally the heart was washed with Tyrode's solution that contained 0.2 mM $CaCl_2$ and then the ventricles were dispersed and filtered. The cell suspension was rinsed several times, with a gradual increase in the Ca^{2+} concentration up to 1.8 mM. The cells used in the study were rod shaped with well-defined striations and were without any spontaneous contractions.

Loading of myocytes with indo-1/AM

Myocytes were loaded with indo-1 by incubation with 5 μM indo-1/AM for 3 min at room temperature. After loading, the myocytes were plated on a superfusion chamber and superfused with bicarbonate buffer for at least 40 min to washout extracellular dye and allow intracellular indo-1 de-esterification. The bicarbonate buffer contained (in mM): NaCl, 116.4; KCl, 5.4; $MgSO_4$, 0.8; $CaCl_2$, 1.8; NaH_2PO_4, 1.0; $NaHCO_3$, 23.8 and glucose, 5.0 (pH 7.4) and had been equilibrated with 95% O_2 and 5% CO_2.

Cell shortening and fluorescence measurements

The chamber was placed on the stage of an inverted microscope (Diaphot TMD 300; Nikon, Tokyo, Japan) adapted for epifluorescence measurements. The cells were superfused with bicarbonate buffer in a rate of 1 ml min^{-1} at room temperature (25°C) and stimulated electrically by square-wave pulses with a voltage about 30–40% above the threshold at a frequency of 0.5 Hz.

The fluorescence of indo-1 was excited with light from a xenon lamp (150 W) at a wavelength of 355 nm, reflected by a 380 nm long-pass dichroic mirror, and detected by a fluorescence spectrophotometer (CAM-230; Japan Spectroscopic Co., Tokyo, Japan). Excitation light was applied to myocytes through a neutral-density filter to minimize the photobleaching of indo-1. The emitted fluorescence was collected by an objective lens (CF Fluor DL40, Nikon, Japan) and then separated by a 580 nm long-pass dichroic mirror (Omega Optical, Brattleboro, VT, U.S.A.) after passing through a 380 nm long-pass dichroic mirror. The fluorescence light was subsequently split with a 425 nm dichroic mirror to permit simultaneous measurements of light at both 405 and 500 nm wavelengths through band-pass filters. The emission field was restricted to a single cell with the aid of an adjustable window. The fluorescence ratio (405/500 nm) was used as an indicator of $[Ca^{2+}]_i$ (Grynkiewicz *et al*., 1985).

Cells were simultaneously illuminated with red light (wavelength above 620 nm) through the normal bright-field illumination of the microscope and the bright-field images of one myocyte were collected by an objective lens and separated first by a 580 nm long-pass dichroic mirror (Omega Optical). The bright-field image of a cell was projected onto a photodiode array of an edge detector (C6294-01, Hamamatsu Photonics, Hamamatsu, Japan) with 5 ms temporal resolution and the cell length was monitored by images simultaneously with fluorescent dye indo-1.

Experimental protocol

To determine a concentration-response curve for ET-1, extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) or KB-R7943, the perfusate was switched to a solution that contained higher concentration of an agent, when the effects of the agent reached a steady state. To observe the influence of KB-R7943 on the cardiotonic effects of the agonists, KB-R7943 was added cumulatively in the presence of the agonists, and at each concentration it was allowed to act for 10 min to reach a steady level. The cell length was monitored continuously throughout the experiment, while the fluorescence of indo-1 was monitored only intermittently to reduce the possibility of photobleaching. Simultaneous recordings were made at the baseline state and in the presence of the drugs when the response had reached a steady level.

Data recording and analysis

Cell length and fluorescence signals were acquired by computer (Power Macintosh 8100/100AV, Apple Computer Inc., Cuptertino, CA, U.S.A.) with an A/D converter (MP-100A, BIOPAC Systems, Santa Barbara, CA, U.S.A.) at 200 Hz and analysed after low-pass filtering (cutoff frequency, 20 Hz) and averaging of five successive signals.

The baseline diastolic cell length and the indo-1 fluorescence ratio were assigned to a value of zero, and the baseline peak systolic cell length and indo-1 fluorescence ratio were assigned to a value of 100, respectively. The changes in cell shortening and in indo-1 fluorescence ratio were estimated from the changes in each peak systolic value. Parameters representing the time course of Ca^{2+} transient or cell shortening, i.e., the duration (DC), time to decline (TD) and time to peak (TT) were measured from actual tracings. To reduce noise, Ca^{2+} transients were measured after smoothing by curve fitting with an equation of higher degree (polynomial 9th order) by means of CA-Cricket Graph III (Computer Associates International Inc., Islandia, NY, U.S.A.). Corresponding parameters of cell shortening were measured at the level of 10% of the maximum shortening without smoothing. KB-R7943 had no effect on the emission of fluorescence from indo-1.

Drugs

The drugs and reagents used were ET-1 (Peptide Institute, Osaka, Japan), (−)-isoprenaline hydrochloride and protease type XIV (Sigma, St. Louis, MO, U.S.A.); indo-1 AM (Dojin Chemical, Kumamoto, Japan); collagenase (Worthington Biochemical, Freehold, NJ, U.S.A.); pentobarbitone sodium (Tokyo Kasei Kogyo, Tokyo, Japan); KB-R7943, 2-[2-[4-(nitrobenzyloxy)phenyl]ethyl]isothiourea methanesulphonate (Kanebo, Osaka, Japan). Other reagents used were of the highest grade commercially available.

Statistics

Data are presented as means ± s.e.mean. Significance of differences was estimated by a repeated measures analysis or one-way analysis of variance followed by application of the Bonferroni/Dunn method. Significant differences between the two mean values were estimated by use of Student's *t*-test. A P value less than 0.05 was considered as an indication of significance.

Results

Effects of elevation of $[Ca^{2+}]_o$ and ET-1 in ventricular cardiac myocytes loaded with indo-1

This series of experiments was performed to elucidate the characteristics of ET-1 on the Ca^{2+} transient and contraction. Comparisons were made between the effects of ET-1 and those from the elevation of $[Ca^{2+}]_o$. In single rabbit ventricular myocytes, the increase in peak cell shortening was consistently accompanied by an increase in the amplitude of Ca^{2+} transient as $[Ca^{2+}]_o$ was elevated from 1.8 mM – 14.4 mM (Figure 1a). Compared with the basal values at 1.8 mM $[Ca^{2+}]_o$, the duration of Ca^{2+} transient was shortened at high concentrations of $[Ca^{2+}]_o$ (e.g., 14.4 mM), while the duration of the cell shortening was unaffected (Figure 1b). The duration of Ca^{2+} transient (DC) and the time for Ca^{2+} transient to decline (TD) were significantly ($P<0.001$) shortened to 78.3 ± 4.6% and 75.2 ± 5.2% compared with the respective control values ($n=7$ each), whereas the time to peak Ca^{2+} transient (TT) was not altered significantly (93.1 ± 4.1%; $n=7$). Changes in the corresponding parameters of cell shortening were not significant (DC: 108.2 ± 3.7%; TD: 121.1 ± 9.3%; TT: 102.7 ± 4.6% of the corresponding control values at 1.8 mM $[Ca^{2+}]_o$; $n=7$, each). After washout for 20 min, the peak cell shortening and peak Ca^{2+} transient returned to basal levels (Figure 1a).

Actual tracings and summarized data of concentration-dependent effects of ET-1 on indo-loaded rabbit ventricular cardiac myocytes are shown in Figures 2a and 3, respectively.

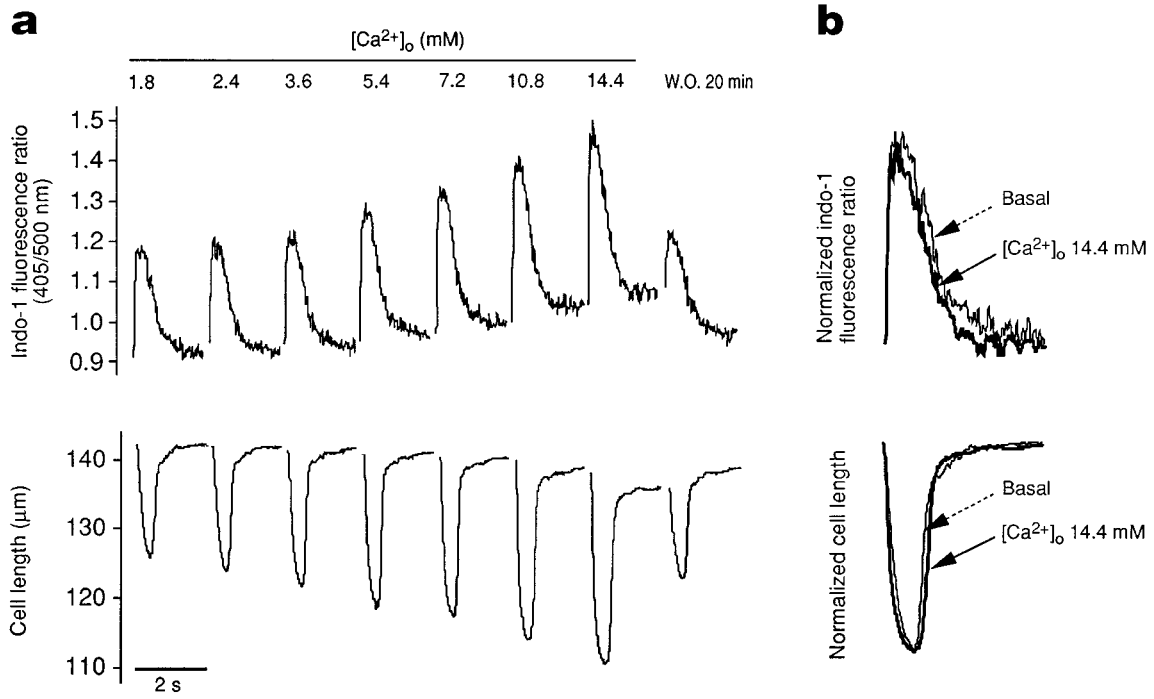


Figure 1 (a) Effects of elevation of $[Ca^{2+}]_o$ on the simultaneous recordings of indo-1 fluorescence ratio (upper traces) and cell shortening (lower traces) in a single adult rabbit ventricular cardiac myocyte. $[Ca^{2+}]_o$ was increased stepwise from 1.8 mM to 14.4 mM. (b) To facilitate the comparison of changes in time-course induced by elevation of $[Ca^{2+}]_o$ tracings at 1.8 mM and 14.4 mM $[Ca^{2+}]_o$ were normalized and superimposed. W.O.: washout. Individual tracings were obtained by means of signal averaging of five successive signals.

ET-1 at concentrations of 0.3 pM and higher increased likewise systolic and diastolic levels of indo-1 ratio and cell shortening in a concentration-dependent manner. The maximal response to ET-1 of indo-1 ratio was achieved at 10 pM, while that of cell shortening was obtained at 0.1 nM in a representative myocyte (Figure 2a), indicating that the response of indo-1 ratio reached the maximum with ET-1 at a lower concentration. This tendency was found also in summarized data, in which the percentage changes in indo-1 ratio and cell shortening induced by ET-1 at increasing concentrations were compared (Figure 3). Up to 3 pM ET-1 the percentage increases in indo-1 ratio and shortening were equivalent, but at 10 pM and higher the increase in cell shortening was significantly higher than that in indo-1 ratio (Figure 3). Therefore the EC_{50} value for ET-1, that was calculated from cumulative concentration-response curves in individual myocytes, to increase indo-1 ratio (1.2 ± 0.2 pM) was significantly lower than that for cell shortening (2.9 ± 0.9 pM; $n = 7$, each). It is noteworthy that the extent of increase in cell shortening induced by ET-1 was associated with less elevation of systolic and diastolic levels of indo-1 fluorescence ratio (Figure 2a) compared with the increase in cell shortening induced by elevation of $[Ca^{2+}]_o$ (Figure 1a), which implies that ET-1 may increase the myofibrillar sensitivity to Ca^{2+} ions at concentrations of 10 pM and higher (Figure 3).

This possibility was examined by comparing the effects of ET-1 at 0.1 nM on systolic and diastolic levels of cell shortening and indo-1 ratio with those of elevation of $[Ca^{2+}]_o$ from 1.8–14.4 mM. While elevation of $[Ca^{2+}]_o$ (14.4 mM) and ET-1 (0.1 nM) increased systolic levels of cell shortening to an identical extent, ET-1 increased systolic levels of indo-1 ratio significantly less than elevation of $[Ca^{2+}]_o$, an indication that ET-1 may increase the myofibrillar Ca^{2+} sensitivity. While ET-1 tended to increase diastolic levels of cell shortening more and those of indo-1 ratio less than elevation of $[Ca^{2+}]_o$, the differences were statistically not significant. ET-1 at 0.1 nM prolonged the duration of cell shortening without affecting the duration of Ca^{2+} transient, being consistent with the effect of ET-1 to prolong the duration of isometric contractions in a concentration-dependent manner in rabbit papillary muscle (Takanashi & Endoh, 1991), which support also the Ca^{2+} sensitizing effect of ET-1. The duration of cell shortening (DC) and the time for cell shortening to decline (TD) were

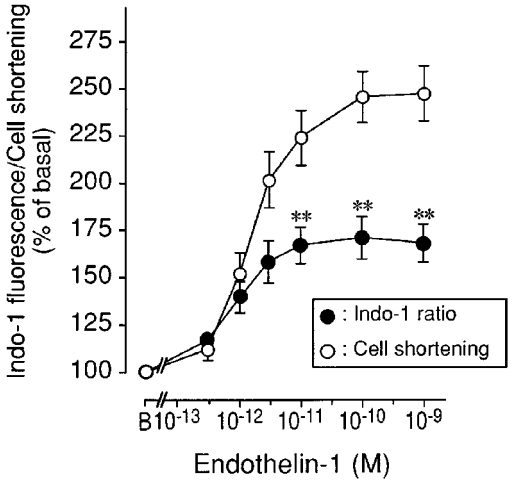


Figure 3 Concentration-response curves for the effects of ET-1 administered in a cumulative manner on the cell shortening and indo-1 fluorescence ratio in isolated rabbit ventricular myocytes. B: basal level before the addition of ET-1 was assigned to 100%. Numbers of myocytes are nine ($n = 9$) except for the highest concentration of ET-1 at which two myocytes had deteriorated and excluded from the calculation; therefore numbers of myocytes at 1.0 nM ET-1 are seven ($n = 7$). $^{**}P < 0.01$ vs the percentage increase in cell shortening. Statistical analysis was carried out by means of a repeated measures analysis of variance followed by Student's t-test.

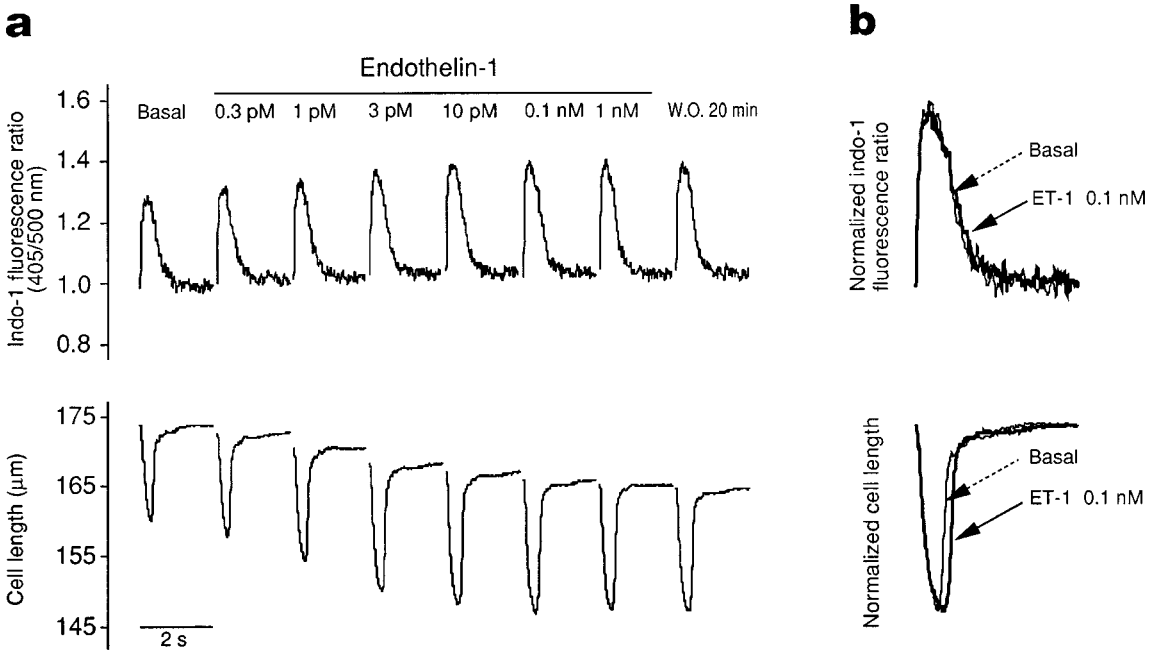


Figure 2 (a) Effects of ET-1 on the simultaneous recordings of indo-1 fluorescence ratio (upper traces) and cell shortening (lower traces) in a single adult rabbit ventricular myocyte. ET-1 was increased from 0.3 pM to 1 nM. (b) To facilitate the comparison of changes in time-course induced by ET-1, tracings at 0.1 nM ET-1 were compared to those at baseline level. W.O.: washout. Individual tracings were obtained by means of signal averaging of five successive signals.

significantly ($P<0.001$) prolonged to 112.3 ± 6.1 and $122.3\pm10.3\%$ compared with the respective control values ($n=7$, each), whereas the time to peak cell shortening (TT) was not significantly altered ($106.5\pm4.6\%$; $n=7$). Changes in the corresponding parameters of Ca^{2+} transient were not significant (DC: $96.0\pm3.2\%$; TD: $96.4\pm3.2\%$; TT: $94.9\pm4.8\%$ of the corresponding control values in the absence of ET-1; $n=7$, each). Another difference in the response to ET-1 and elevation of $[Ca^{2+}]_o$ is that the effect of ET-1 lasted long even during washout period: after 20 min washout, the effects of ET-1 remained unchanged (Figure 2a), while the increase induced by elevation of $[Ca^{2+}]_o$ was abolished by washout (Figure 1a).

The phase-plane diagrams of the time-dependent changes in cell shortening and indo-1 fluorescence ratio during the course of twitch contractions are shown in Figure 4. The indo-1 ratio-cell shortening trajectory was shifted to the right and upwards keeping substantially a similar figure when $[Ca^{2+}]_o$ was elevated from 1.8–14.4 mM (Figure 4a). While ET-1 also shifted the indo-1 ratio-cell shortening trajectory to the right and upwards, the shift was more upwards: ET-1 at 1 pM and higher cell shortening was increased in association with a relatively small increase in indo-1 ratio (Figure 4b). It is also evident that diastolic cell length for a given diastolic level of indo-1 ratio is shortened by ET-1 in a concentration-dependent manner (Figure 4b). Although there was a quite wide range of variations in the shape of the trajectory among individual cardiac myocytes, the shift of the trajectory caused by elevation of $[Ca^{2+}]_o$ or ET-1 in other myocytes was essentially consistent with those shown in Figure 4.

Figure 5 shows the relationship between the peak Ca^{2+} transient and the peak shortening induced by addition of ET-1 or elevation of $[Ca^{2+}]_o$. There was no significant difference in baseline cell shortening and Ca^{2+} transient between these two groups (data not shown). The relationship for the positive inotropic effect induced by elevation of $[Ca^{2+}]_o$ was linear with a slope of unity, while ET-1 shifted the relationship to the left, an indication that in addition to an increase in $[Ca^{2+}]_i$, ET-1 might increase sensitivity of myofilament to Ca^{2+} ions.

Effects of KB-R7943 on the increase in peak cell shortening and peak Ca^{2+} transient induced by ET-1 or isoprenaline

In this series of experiments, the influence of KB-R7943, a novel inhibitor of reverse-mode Na^+/Ca^{2+} exchange on the ET-1- and isoprenaline-induced response of indo-1 ratio and cell shortening was examined. It is important to show that the response to these agonists is not altered without desensitization during the course of experiments, because KB-R7943 was administered in a cumulative manner during induction of the PIE by these agonists. Figure 6A shows the effects of ET-1 at 0.1 nM that was allowed to act for 60 min continuously. The ET-1-induced increases reached a stable level at 10–15 min and were maintained for at least 60 min: the increase in Ca^{2+} transient did not decrease during the course of investigation. The systolic level of cell shortening also remained unaltered, while the diastolic cell length decreased gradually during the

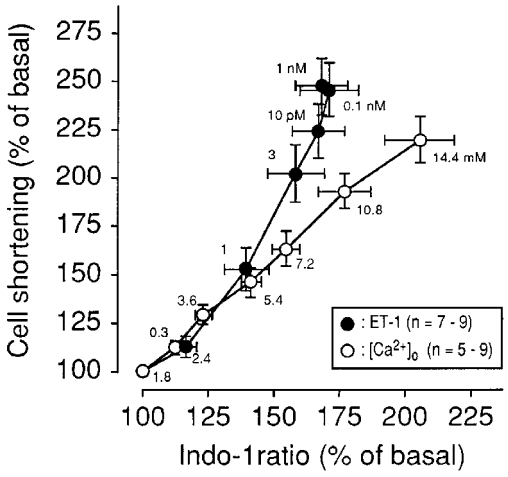


Figure 5 The relationship between the peak Ca^{2+} transient and peak shortening induced by elevation of $[Ca^{2+}]_o$ and ET-1 at various concentrations in rabbit ventricular cardiac myocytes.

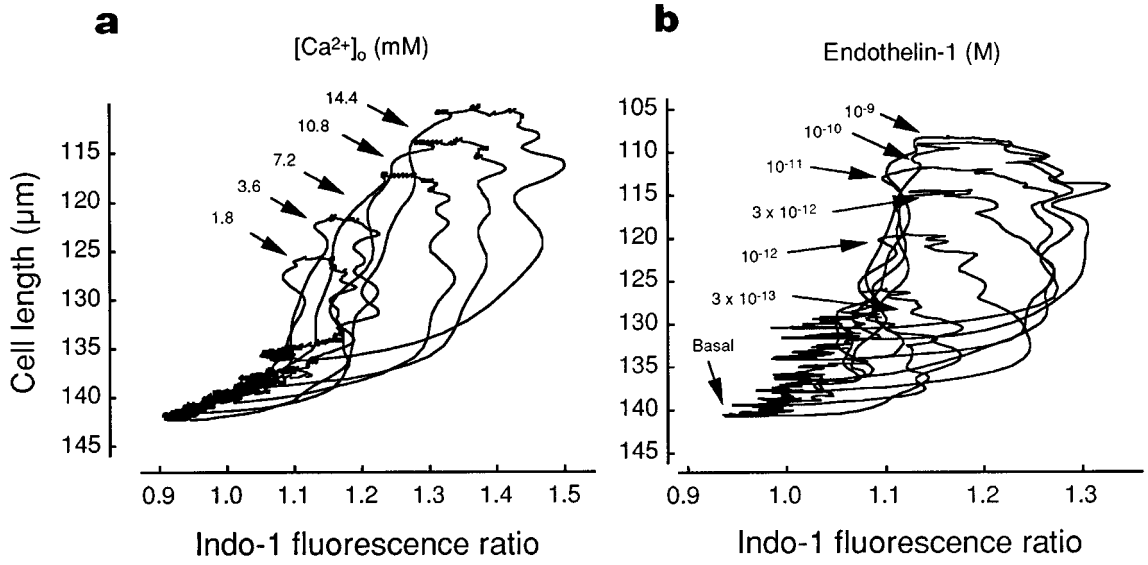
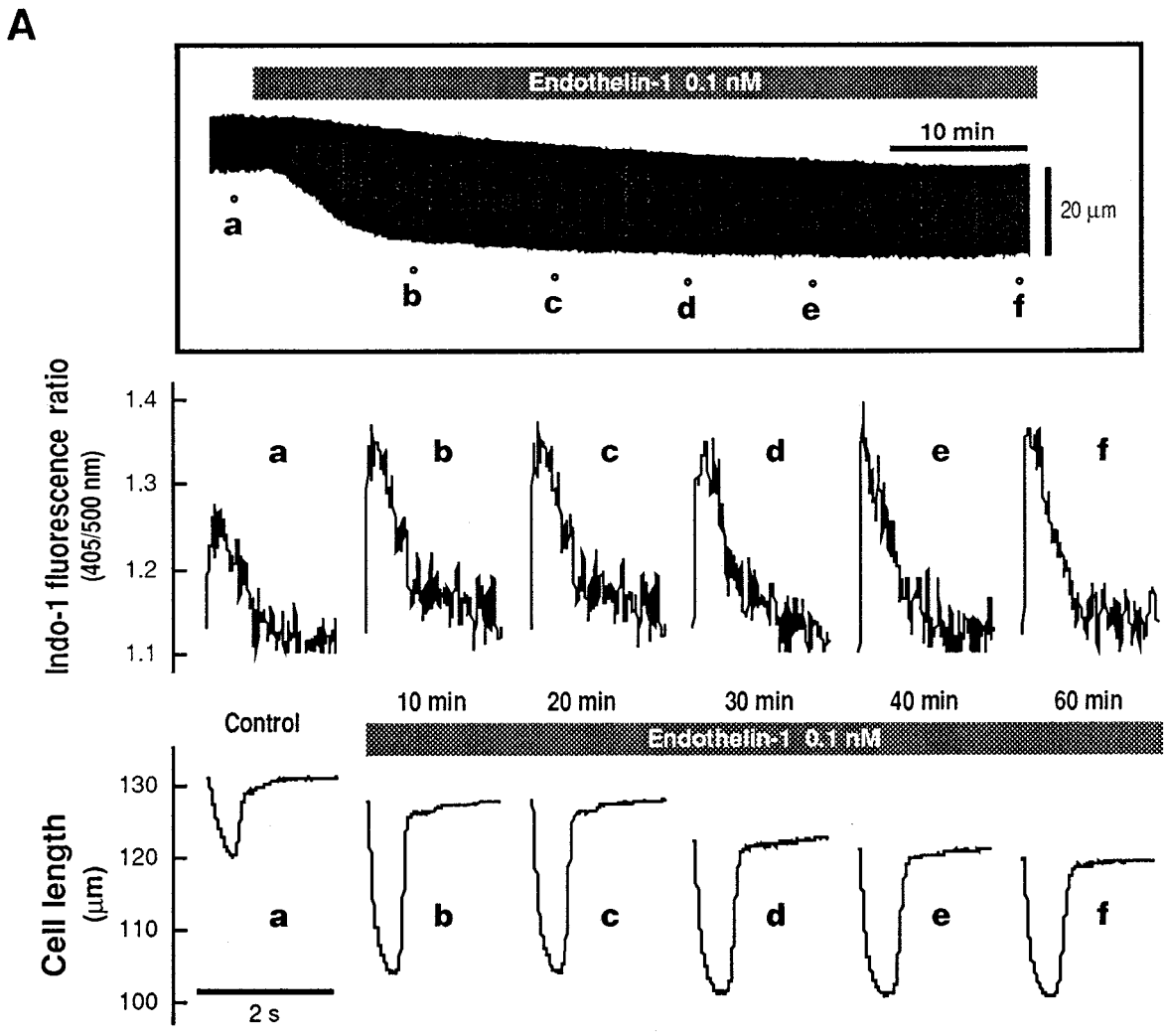


Figure 4 The indo-1 ratio-cell shortening trajectory obtained at different concentrations of $[Ca^{2+}]_o$ (a) and ET-1 (b) in adult rabbit ventricular cardiac myocytes. Each trajectory was obtained by means of signal averaging of five successive signals.

course, probably partly because of continuous increase in cell shortening and partly due to the mechanism of action of ET-1, i.e., by an increase in myofibrillar Ca^{2+} sensitivity (Table 1). Figure 6B shows the effects of isoprenaline at 10 nM that was allowed to act for 35 min on Ca^{2+} transient and cell shortening. Both parameters that were increased by isoprenaline did not decline during the course of investigation. These observations indicate that neither the response to ET-1 (0.1 nM) nor that to isoprenaline (10 nM) show desensitization during the course of administration of KB-R7943.

KB-R7943, a novel inhibitor of reverse-mode Na^+/Ca^{2+} exchange, did not affect the baseline cell shortening and Ca^{2+} transient (1.8 mM $[Ca^{2+}]_o$) at 0.1 and 0.3 μM, but at 1–3 μM it inhibited them significantly in a concentration-dependent manner (Figure 7). After washout for 15 min, the inhibitory effects of KB-R7943 on these parameters were almost reversed to the baseline level (Figure 7). ET-1 at 0.1 nM increased the cell shortening and Ca^{2+} transient to 208.4±13.5% and 145.7±5.0% of baseline levels ($n=7$), respectively (Figure 8). KB-R7943 at concentrations of 0.1 and 0.3 μM, which did not affect the baseline levels of parameters (Figure 7), inhibited the increases in the peak cell shortening and peak Ca^{2+} transient induced by 0.1 nM ET-1 in a concentration-dependent manner. The increase in peak Ca^{2+} transient induced by ET-1 was more susceptible than the increase in cell shortening to the inhibitory action of KB-R7943 (Figure 9a). KB-R7943 at 0.1 μM inhibited the ET-1-induced peak cell shortening and peak Ca^{2+} transient by 16.0±4.3% and 43.6±4.4%, respectively, and at 0.3 μM by 48.1±5.6% and 91.2±12.0%, respectively (Figure 9a). KB-R7943 at 1 μM abolished the increase in cell shortening induced by ET-1 (0.1 nM) and decreased indo-1 ratio to a level even lower than the baseline value prior to administration of ET-1 (Figure 9a), being consistent with the finding that KB-R7943 at this concentration decreases the baseline level of indo-1 ratio and cell shortening (Figure 7). However, when the percentage inhibition of indo-1 ratio and cell shortening induced by KB-R7943 in the absence and presence of ET-1 at 0.1 nM was compared (Table 2), in the presence of ET-1 the indo-1 ratio was significantly attenuated by KB-R7943 at 0.1 μM and higher and cell shortening at 0.3 μM and higher, while in the absence of ET-1 these parameters were decreased significantly by KB-R7943 first at 1 μM (Table 2). These findings indicate that the response to ET-1 is more susceptible to the inhibitory action of KB-R7943 than the baseline levels of indo-1 ratio and cell shortening.

To further exclude the possible nonselective inhibitory effect of KB-R7943 over this concentration range that might contribute to the inhibitory action of the compound on the ET-1-induced responses, the influence of KB-R7943 on the isoprenaline-induced increases in peak cell shortening and peak Ca^{2+} transient was investigated. It has been shown that isoprenaline has no effect on Na^+/Ca^{2+} exchange activity (Ballard & Schaffer, 1996). Isoprenaline at 10 nM increased the peak cell shortening and peak Ca^{2+} transient to 202.2±18.5% and 194.0±12.6% of baseline levels ($n=5$), respectively (Figure 9b). The extent of increase in cell shortening induced by



isoprenaline at 10 nM was equivalent to that induced by 0.1 nM ET-1, whereas isoprenaline-induced increase in Ca^{2+} transient was much higher than that induced by 0.1 nM ET-1 (Figure 9). The isoprenaline-induced increases in both parameters reached the steady level at 4–10 min after the administration and were maintained stable for 40 min (Figure 6B). The increases in cell shortening and Ca^{2+} transient induced by isoprenaline were not attenuated by KB-R7943 at 0.1–0.3 μM (Figure 9b), while KB-R7943 inhibited markedly the responses of ET-1 over this concentration range as shown in Figures 8 and 9a. KB-R7943 at 1 μM inhibited also the isoprenaline-induced cell shortening and Ca^{2+} transient (Figure 9b).

Discussion

In single rabbit adult ventricular cardiac myocytes loaded with indo-1/AM, ET-1 (0.3 pM–1 nM) elicited a concentration-dependent positive inotropic effect in association with an increase in the amplitude of Ca^{2+} transient. Although ET-1 increased the indo-1 ratio and cell shortening over the same concentration range and the percentage increase induced by ET-1 in both parameters were equivalent at concentrations of 3 pM and lower, the percentage increase in indo-1 ratio was significantly lower than that of cell shortening at 10 pM and higher (Figure 3). The lower percentage increase in indo-1 ratio

Table 1 Peak systolic and end-diastolic cell shortening and indo-1 fluorescence ratio changes (%) in response to an increase in $[Ca^{2+}]_o$ and ET-1

			Cell shortening		*Indo-1 ratio*	
		n	*Diastolic*	*Systolic*	*Diastolic*	*Systolic*
$[Ca^{2+}]_o$	14.4 mM	5	33.8±2.5	219.2±12.0	40.6±12.9	205.6±13.1
ET-1	0.1 nM	10	44.1±5.4	235.7±15.3	27.0±7.1	169.0±10.2*

Values presented are means±s.e.mean of percentage changes of basal levels of the respective parameters. *$P<0.05$ vs the corresponding value at $[Ca^{2+}]_o$ 14.4 mM. Statistical analysis was carried out by means of one-way analysis of variance.

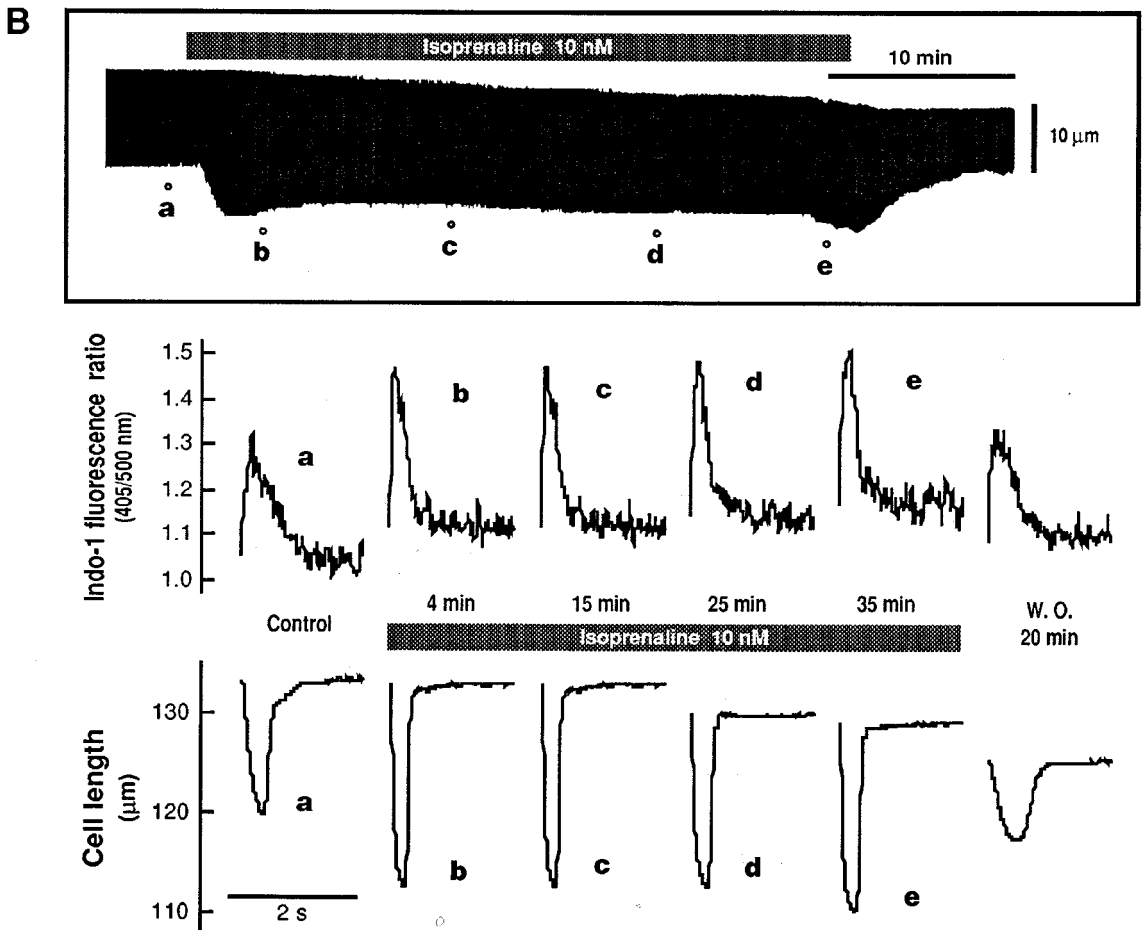


Figure 6 (A) Effects of ET-1 at 0.1 nM on the simultaneous recordings of indo-1 fluorescence ratio (middle traces) and cell shortening (upper and lower traces) in a single adult rabbit ventricular myocyte. Upper tracings represent the continuous recordings of cell length. Middle and lower tracings show individual signals recorded at a–f in upper tracings. (B) Effects of isoprenaline at 10 nM on the simultaneous recordings of indo-1 fluorescence ratio (middle traces) and cell shortening (upper and lower traces) in a single adult rabbit ventricular myocyte. Upper tracings represent the continuous recordings of cell length. Middle and lower tracings show individual signals recorded at a–e in upper tracings. W.O.: washout. Individual tracings were obtained by means of signal averaging of five successive signals.

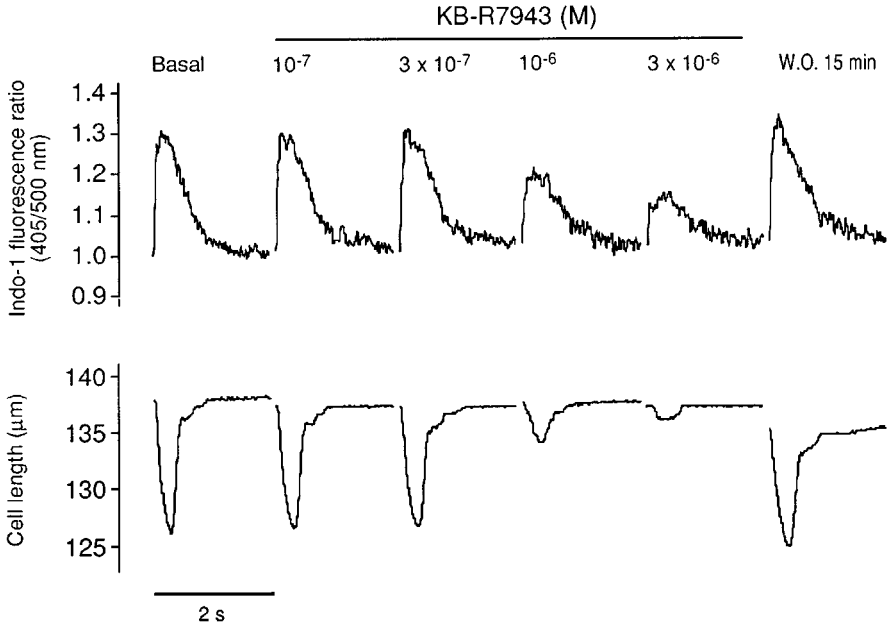


Figure 7 Influence of KB-R7943 on the simultaneously recorded indo-1 fluorescence ratio and cell shortening in the presence of 1.8 mM $[Ca^{2+}]_o$ in an isolated rabbit ventricular myocyte. Individual tracings were obtained by means of signal averaging of five successive signals.

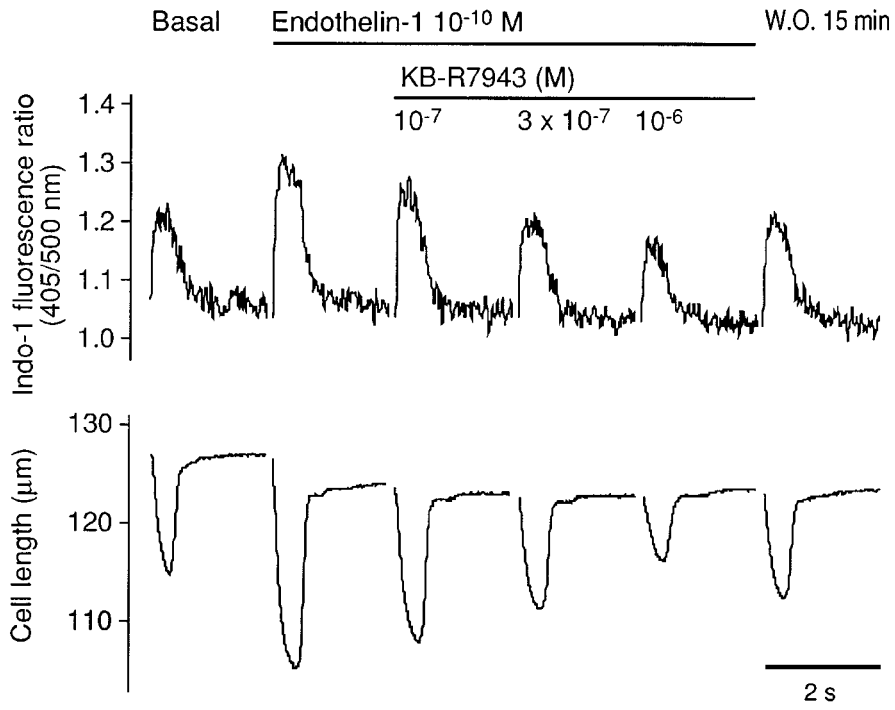


Figure 8 Influence of KB-R7943 on 0.1 nM ET-1-induced increase in indo-1 fluorescence ratio (upper tracings) and cell shortening (lower tracings) in an isolated rabbit ventricular myocyte. Individual tracings were obtained by means of signal averaging of five successive signals.

induced by ET-1 is not considered to be due to saturation of detection system because elevation of $[Ca^{2+}]_o$ and isoprenaline are able to increase the indo-1 ratio to a level much higher than that achieved by ET-1 (Fujita & Endoh, 1996). The EC_{50} value for ET-1 to increase indo-1 ratio was lower than that to increase cell shortening (Figure 3). These findings indicate that ET-1 may induce a positive inotropic effect mainly by a facilitation of Ca^{2+} transient at lower concentrations (0.3–3 pM) but it acts by an increase in myofibrillar Ca^{2+} sensitivity over higher concentration range (10 pM and higher). The shift of the relationship between the extent of cell shortening and the amplitude of Ca^{2+} transient by ET-1 to the left compared with that of elevation of $[Ca^{2+}]_o$ (Figure 5) and the alteration of the shape of phase-plane diagrams of indo-1 ratio and cell shortening relationship (Figure 4) support an increase in myofibrillar Ca^{2+} sensitivity by ET-1 at higher concentrations. These results are essentially consistent with the previous findings in the ferret (Wang *et al.*, 1991; Qiu *et al.*, 1992) and

rat (Kelly *et al*., 1990; Krämer *et al*., 1991) that ET-1 produced an increase in myofibrillar sensitivity to Ca^{2+} ions in the ventricular myocardium of these species. The changes in phase-plane diagrams induced by elevation of $[Ca^{2+}]_o$ indicate that indo-1 ratio and cell shortening are increased in parallel during graded elevation of $[Ca^{2+}]_o$, which is consistent with the findings in aequorin-loaded rabbit papillary muscle (Endoh & Blinks, 1988). By contrast, it could be seen that the shape of phase-plane diagrams is altered in a manner that peak levels of cell shortening were maintained over a wider range of increased levels of indo-1 ratio during administration of ET-1, which are the changes that imply the increase in myofibrillar sensitivity to Ca^{2+} ions.

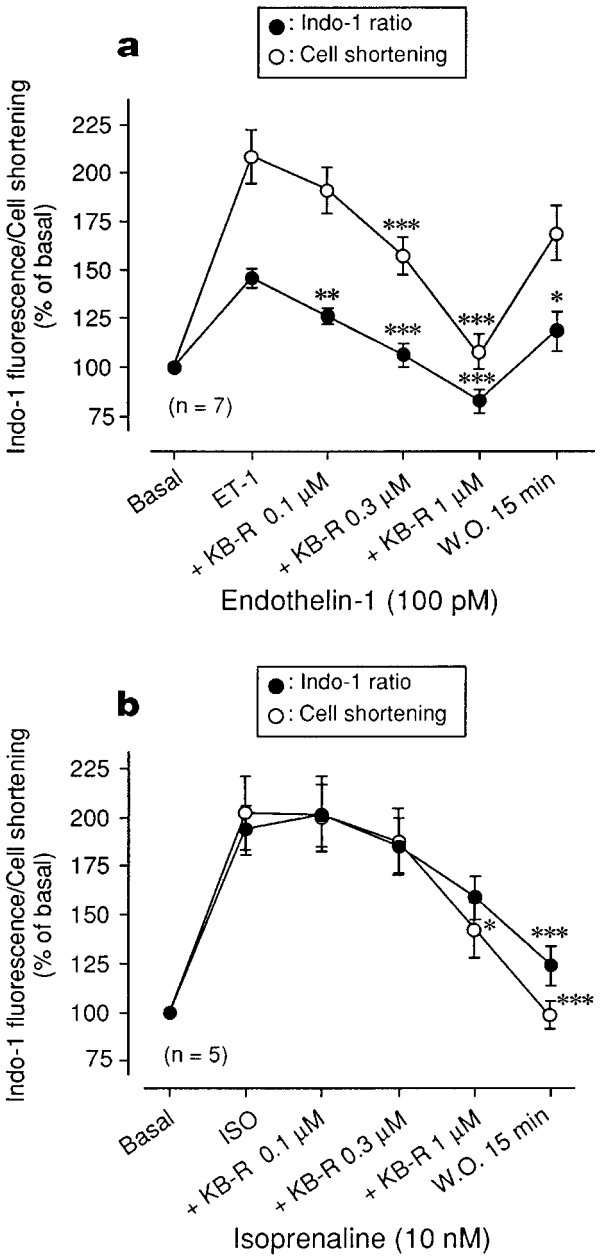


Figure 9 Influence of KB-R7943 on the increases in indo-1 fluorescence ratio and cell shortening induced by ET-1 at 0.1 nM (a) and by isoprenaline at 10 nM (b). Numbers in parentheses represent numbers of myocytes. KB: KB-R7943. $*P<0.05$; $**P<0.01$ and $***P<0.001$ vs the values with ET-1 or isoprenaline alone, respectively. Statistical analysis was carried out by means of one-way analysis of variance.

It is noteworthy that in rabbit ventricular cardiac myocytes angiotensin II and phenylephrine (α_1-adrenoceptor agonist) modulate the relationship between the peak Ca^{2+} transient and inotropism in a similar manner as ET-1 does, i.e., they consistently shifted the relationship to the left over the higher concentration range (Fujita & Endoh, 1996). Because activation of receptors by these agonists leads to stimulation of the hydrolysis of phosphoinositide in cardiac muscle (Fedida *et al*., 1993; Endoh, 1994), these agonists might share a common signal transduction pathway that involves diacylglycerol that activates PKC (Nishizuka, 1988) and inositol 1,4,5-trisphosphate that releases Ca^{2+} ions from intracellular stores (Berridge, 1993). In most mammalian cardiac myocytes including the rat, guinea-pig, rabbit and human (Vigne *et al*., 1990; Tohse *et al*., 1990; Furukawa *et al*., 1992; Lauer *et al*., 1992; Cheng *et al*., 1995; Delpech *et al*., 1997), the effects of ET-1 and α-adrenoceptor agonists to activate L-type Ca^{2+} channels are relatively small (or even absent) compared to the effect of activation of β-adrenoceptors. Furthermore, the increase in the amplitude of Ca^{2+} transients induced by these receptor agonists that stimulate the hydrolysis of phosphoinositide is much less than that produced by β-adrenoceptor agonists (Endoh & Blinks, 1988; Fujita & Endoh, 1996). These observations indicate that the Ca^{2+} release induced by inositol 1,4,5-trisphosphate if any in the regulation of myocardial Ca^{2+} mobilization to lead to an increase in contractility in intact ventricular myocardium may be much less compared with that produced by β-adrenoceptor activation.

It has been shown that activation of PKC stimulates Na^+/Ca^{2+} exchanger in the *in vitro* experiments by use of cardiac sarcolemma vesicles (Ballard & Schaffer, 1996). Since activation of receptors that are linked to stimulation of the hydrolysis of phosphoinositide generates diacylglycerol that activates PKC (Nishizuka, 1988; Berridge, 1993; Meyer *et al*., 1996), it is likely that activation of Na^+/Ca^{2+} exchanger contributes to the positive inotropic effect of ET-1 that stimulates the hydrolysis of phosphoinositide in rabbit ventricular myocardium (Takanashi & Endoh, 1992). In addition, it has been shown that the stimulation of Na^+/H^+ exchanger is likewise induced by activation of PKC in myocardial cells (Grinstein & Rothstein, 1986; Iwakura *et*

Table 2 Effects of KB-R7943 on indo-1 ratio and cell shortening (% inhibition by KB-R7943) in the absence and presence of ET-1 at 0.1 nM

			% inhibition by KB-R7943					
KB-R7943			*0.1*		*0.3*		*1*	
(μM)		n	*Indo-1 ratio*	*Shortening*	*Indo-1 ratio*	*Shortening*	*Indo-1 ratio*	*Shortening*
ET-1	–	7	−2.2±3.52	3.1±3.36	5.1±4.29	14.7±3.53	24.9±5.23*	60.9±3.31**
ET-1	0.1 nM	7	46.7±4.36***	16.0±4.29	91.2±11.97***	48.1±5.60**	145.4±15.56***	94.1±8.35***

Values presented are means±s.e.mean of percentage inhibition of indo-1 ratio and cell shortening induced by KB-R7943. $*P<0.05$; $**P<0.01$; $***P<0.001$ vs the corresponding values in the absence of KB-R7943. Statistical analysis was performed by means of one-way analysis of variance followed by Bonferroni/Dunn method.

al., 1990), which may lead to intracellular alkalinization and accumulation of intracellular Na^+ ions. It has been postulated that the intracellular alkalinization may be partially responsible for the increase in myofibrillar Ca^{2+} sensitivity resulting from activation of receptors that belong to this class, while accumulation of intracellular Na^+ ions may indirectly lead to an increase in $[Ca^{2+}]_i$ *via* Na^+/Ca^{2+} exchanger (Krämer *et al*., 1991; Gambassi *et al*., 1992; Matsui *et al*., 1995). Thus, activation of Na^+/Ca^{2+} exchanger through diverse pathways might be involved in cardiac excitation-contraction coupling by an increase in $[Ca^{2+}]_i$ and/or facilitation of Ca^{2+}-induced Ca^{2+} release mechanism (LeBlanc & Hume, 1990; Bouchard *et al*., 1993; Levesque *et al*., 1994) through reverse mode operation of Na^+/Ca^{2+} exchanger in intact myocardium, namely under activation of receptors that stimulates the hydrolysis of phosphoinositide.

Therefore, the possible contribution of activation of Na^+/Ca^{2+} exchanger in the positive inotropic effect of ET-1 was examined by use of a pharmacological tool, KB-R7943, which has been reported to inhibit selectively the reverse mode of Na^+/Ca^{2+} exchanger (Iwamoto *et al*., 1996; Watano *et al*., 1996). It was shown in the present study that KB-R7943 at 0.1 and 0.3 μM inhibited the ET-1-induced positive inotropic effect and the increase in Ca^{2+} transient selectively without affecting the baseline levels of Ca^{2+} transient and cell shortening or the response to isoprenaline (Figures 7–9). These observations indicate that KB-R7943 over an appropriate concentration range exerts a selective inhibitory action on the ET-1-induced response, which may be ascribed to an inhibition of the ET-1-stimulated reverse-mode Na^+/Ca^{2+} exchange. It is noteworthy that the ET-1-induced increase in cell shortening remains less inhibited even when the ET-1-induced increase in Ca^{2+} transient has been abolished by 0.3 μM KB-R7943 (Figure 8). This implies that an increase in myofibrillar Ca^{2+} sensitivity induced by ET-1 is less susceptible to the inhibitory action of KB-R7943 over the concentration range of 0.1–0.3 μM where that compound elicits a selective action of Na^+/Ca^{2+} exchanger.

Since it has been shown that ET-1 is involved in induction of myocardial hypertrophy and heart failure (Sakai *et al*., 1996; Colucci, 1997), and that the expression of cardiac Na^+/Ca^{2+} exchanger is increased during cardiac hypertrophy (Kent *et al*., 1993; Menick *et al*., 1996) and failing human heart (Studer *et al*., 1994; Flesch *et al*., 1996), KB-R7942 may have potential to be a useful pharmacological tool to analyse the pathophysiological relevance of ET-1 in relation to activation of Na^+/Ca^{2+} exchanger under these cardiac disorders.

KB-R7943 at 1 μM inhibited the baseline levels of cell shortening and Ca^{2+} transient, and the responses to isoprenaline (Figures 7 and 9b). These findings indicate that KB-R7943 elicits nonspecific inhibitory action at higher concentrations, which is in accordance with the previous report that KB-R7943 suppresses the sarcolemmal ion channel activity, including Na^+, Ca^{2+} and K^+ channels at higher concentrations: it has been reported that KB-R7943 inhibits the reverse-mode and forward-mode Na^+/Ca^{2+} exchange with IC_{50} values of 0.32 and 17 μM, respectively, while it inhibited also Na^+, Ca^{2+} and K^+ channel activities with IC_{50} values of 14, 8 and 7 μM, respectively (Watano *et al*., 1996).

In conclusion, the present findings indicate that in adult rabbit ventricular cardiac myocytes, ET-1 elicits a positive inotropic effect *via* an increase in Ca^{2+} transient and an increase in myofibrillar Ca^{2+} sensitivity. Activation of reverse-mode Na^+/Ca^{2+} exchange may play an important role in mediating the ET-1-induced increase in Ca^{2+} transient, which is inhibited selectively by a novel Na^+/Ca^{2+} exchange inhibitor, KB-R7943 at concentrations of 0.1–0.3 μM.

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References

BALLARD, C. & SCHAFFER, S. (1996). Stimulation of the Na^+/Ca^{2+} exchanger by phenylephrine, angiotensin II and endothelin-1. *J. Mol. Cell. Cardiol.,* **28,** 11–17.

BERRIDGE, M.J. (1993). Inositol trisphosphate and calcium signaling. *Nature,* **361,** 315–325.

BOUCHARD, R.A., CLARK, R.B. & GILES, W.R. (1993). Regulation of unloaded cell shortening by sarcolemmal sodium-calcium exchange in isolated rat ventricular myocytes. *J. Physiol.,* **469,** 583–599.

BRIDGE, J.H.B., SMOLLEY, J.R., SPITZER, K.W. (1990). The relationship between charge movements associated with I_{Ca} and $I_{Na\text{-}Ca}$ in cardiac myocytes. *Science,* **248,** 376–378.

CHENG, T.H., CHANG, C.Y., WEI, J. & LIN, C.I. (1995). Effects of endothelin 1 on calcium and sodium currents in isolated human cardiac myocytes. *Can. J. Pharmacol.,* **73,** 1774–1783.

COLUCCI, W.S. (1997). Molecular and cellular mechanisms of myocardial failure. *Am. J. Cardiol.,* **80,** 15L–25L.

DEL MONTE, F., MYNETT, J.R., SUGDEN, P.H., POOLE-WILSON, P.A. & HARDING, S.E. (1993). Subcellular mechanism of the species difference in the contractile response of ventricular myocytes to endothelin-1. *Cardioscience,* **4,** 185–191.

DELPECH, N., SOUSTRE, H. & POTREAU, D. (1997). Endothelin-1 inhibits L-type Ca^{2+} current enhanced by isoprenaline in rat atrial myocytes. *J. Cardiovasc. Pharmacol.,* **29,** 136–143.

ENDOH, M. (1994). The effects of various drugs on the myocardial inotropic response. *Gen. Pharmacol.,* **26,** 1–31.

ENDOH, M. & BLINKS, J.R. (1988). Actions of sympathomimetic amines on the Ca^{2+} transients and contractions of rabbit myocardium: reciprocal changes in myofibrillar responsiveness to Ca^{2+} mediated through α- and β-adrenoceptors. *Circ. Res.,* **62,** 247–265.

FEDIDA, D., BRAUN, A.P. & GILES, W.R. (1993). Alpha 1-adrenoceptors in myocardium: functional aspects and transmembrane signaling mechanisms. *Physiol. Rev.,* **73,** 469–487.

FLESCH, M., PUTZ, F., SCHWINGER, R.H. & BÖHM, M. (1996). Functional relevance of an enhanced expression of the Na^+-Ca^{2+} exchanger in the failing human heart. *Ann. N.Y. Acad. Sci.,* **779,** 539–542.

FUJITA, S. & ENDOH, M. (1996). Effects of endothelin-1 on $[Ca^{2+}]_i$-shortening trajectory and Ca^{2+} sensitivity in rabbit single ventricular cardiomyocytes loaded with indo-1/AM: comparison with the effects of phenylephrine and angiotensin II. *J. Cardiac. Fail.,* **2,** (suppl 4): S45–S57.

FURUKAWA, T., ITO, H., NITTA, J., TSUJINO, M., ADACHI, S., HIROE, M., MARUMO, F., SAWANOBORI, T. & HIRAOKA, M. (1992). Endothelin-1 enhances calcium entry through T-type calcium channels in cultured neonatal rat ventricular myocytes. *Circ. Res.,* **71,** 1242–1253.

GALRON, R., BDOLAH, A., KLOOG, Y. & SOKOLOVSKY, M. (1990). Endothelin/sarafotoxin receptor induced phosphoinositide turnover: effects of pertussis and cholera toxins and of phorbol ester. *Biochem. Biophys. Res. Commun.,* **71,** 949–954.

GAMBASSI, G., SPURGEON, H.A., LAKATTA, E.G., BLANK, P.S. & CAPOGROSSI, M.C. (1992). Different effects of alpha- and beta-adrenergic stimulation on cytosolic pH and myofilament responsiveness to Ca^{2+} in cardiac myocytes. *Circ. Res.,* **71,** 870 – 882.

GRINSTEIN, S. & ROTHSTEIN, A. (1986). Mechanism of regulation of the Na^+/H^+ exchanger. *J. Memb. Biol.,* **90,** 1 – 12.

GRYNKIEWICZ, G., POENIE, M. & TSIEN, R.Y. (1985). A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.,* **260,** 3440 – 3450.

HU, J.R., VON, H.R. & LANG, R.E. (1988). Endothelin has potent inotropic effects in rat atria. *Eur. J. Pharmacol.,* **158,** 275 – 278.

ISHIKAWA, T., YANAGISAWA, M., KIMURA, S., GOTO, K. & MASAKI, T. (1988). Positive inotropic action of novel vasoconstrictor peptide endothelin on guinea pig atria. *Am. J. Physiol.,* **255,** H970 – H973.

IWAKURA, K., HORI, M., WATANABE, Y., KITABATAKE, A., CRAGOE, JR, E.J., YOSHIDA, H. & KAMADA, Y. (1990). α_1-Adrenoceptor stimulation increases intracellular pH and Ca^{2+} in cardiomyocytes through Na^+/H^+ and Na^+/Ca^{2+} exchange. *Eur. J. Pharmacol.,* **186,** 29 – 40.

IWAMOTO, T., WATANO, T. & SHIGEKAWA, M. (1996). A novel isothiourea derivative selectively inhibits the reverse model of Na^+-Ca^{2+} exchange in cell expressing NCX1. *J. Biol. Chem.,* **271,** 22391 – 22397.

KELLY, R.A., EID, H., KRÄMER, B.K., O'NEILL, M., LIANG, B.T., REERS, M. & SMITH, T.W. (1990). Endothelin enhances the contractile responsiveness of adult rat ventricular myocytes to calcium by a pertussis sensitive pathway. *J. Clin. Invest.,* **86,** 1164 – 1171.

KENT, R.L., ROZICH, J.D., MCCOLLAM, P.L., THACKER, U.F., MENICK, D.R., MCDERMOTT, P.J. & COOPER, G. (1993). Rapid expression of the Na^+-Ca^{2+} exchanger in response to cardiac pressure overload. *Am. J. Physiol.,* **265,** H1024 – H1029.

KRÄMER, B.K., SMITH, T.W. & KELLY, R.A. (1991). Endothelin and increased contractility in adult rat ventricular myocytes: role of intracellular alkalosis induced by activation of the protein kinase C-dependent Na^+-H^+ exchanger. *Circ. Res.,* **68,** 269 – 279.

LAUER, M.R., GUNN, M.D. & CLUSIN, W.T. (1992). Endothelin activates voltage-dependent Ca^{2+} current by a G protein-dependent mechanism in rabbit cardiac myocytes. *J. Physiol.,* **448,** 729 – 747.

LEBLANC, N. & HUME, J.R. (1990). Sodium current-induced release of calcium from cardiac sarcoplasmic reticulum. *Science,* **248,** 372 – 376.

LEVESQUE, P.C., LEBLANC, N. & HUME, J.R (1994). Release of calcium from guinea pig cardiac sarcoplasmic reticulum induced by sodium-calcium exchange. *Cardiovasc. Res.,* **28,** 370 – 378.

MATSUI, H., BARRY, W.H., LIVSEY, C. & SPITZER, K.W. (1995). Angiotensin II stimulates sodium-hydrogen exchange in adult rabbit ventricular myocytes. *Cardiovasc. Res.,* **29,** 215 – 221.

MENICK, D.R., BARNES, K.V., DAWSON, M.M., KENT, R.L. & COOPER, G. (1996). Gene expression of the Na-Ca exchanger in cardiac hypertrophy. *J. Cardiac. Fail.,* **2** (Suppl 4): S69 – S76.

MEYER, M., LEHNART, S., PIESKE, B., SCHLOTTAUER, K., MUNK, S., HOLUBARSCH, C., JUST, H. & HASENFUSS, G. (1996). Influence of endothelin-1 on human atrial myocardium – myocardial function and subcellular pathways. *Basic Res. Cardiol.,* **91,** 86 – 93.

MORAVEC, C.S., REYNOLDS, E.E., STEWART, R.W. & BOND, M. (1989). Endothelin is a positive inotropic agent in human and rat heart in vitro. *Biochem. Biophys. Res. Commun.,* **159,** 14 – 18.

NISHIZUKA, Y. (1988). The molecular heterogeneity of protein kinase C and its implications for cellular regulation. *Nature,* **334,** 661 – 665.

PHILIPSON, K.D., FRANK, J.S. & NISHIMOTO, A.Y. (1983). Effects of phospholipase C on the Na^+-Ca^{2+} exchange and Ca^{2+} permeability of cardiac sarcolemmal vesicles. *J. Biol. Chem.,* **258,** 5905 – 5910.

PIERCE, G.N. & PANAGIA, V. (1989). Role of phosphatidylinositol in cardiac sarcolemmal membrane sodium-calcium exchange. *J. Biol. Chem.,* **264,** 15344 – 15350.

QIU, Z., WANG, J., PERREAULT, C.L., MEUSE, A.J., GROSSMAN, W. & MORGAN, J.P. (1992). Effects of endothelin on intracellular Ca^{2+} and contractility in single ventricular myocytes from the ferret and human. *Eur. J. Pharmacol.,* **214,** 293 – 296.

RUBANYI, G.M. & POLOKOFF, M.A. (1994). Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. *Pharmacol. Rev.,* **46,** 325 – 415.

SAKAI, S., MIYAUCHI, T., KOBAYASHI, M., YAMAGUCHI, I., GOTO, K. & SUGISHITA, Y. (1996). Inhibition of myocardial endothelin pathway improves long-term survival in heart failure. *Nature,* **384,** 353 – 355.

SCHULZE, D., KOFUJI, P., HADLEY, R., KIRBY, M.S., KIEVAL, R.S., DOERING, A., NIGGLI, E. & LEDERER, W.J. (1993). Sodium/calcium exchanger in heart muscle: molecular biology, cellular function, and its special role in excitation-contraction coupling. *Cardiovasc. Res.,* **27,** 1726 – 1734.

SHAH, A.M., LEWIS, M.J. & HENDERSON, A.H. (1989). Inotropic effects of endothelin in ferret ventricular myocardium. *Eur. J. Pharmacol.,* **163,** 365 – 367.

STUDER, R., REINECKE, H., BILGER, J., ESCHENHAGEN, T., BÖHM, M., HASENFUSS, G., JUST, H., HOLTZ, J. & DREXLER, H. (1994). Gene expression of the cardiac Na^+-Ca^{2+} exchanger in end-stage human heart failure. *Circ. Res.,* **75,** 443 – 453.

TAKANASHI, M. & ENDOH, M. (1991). Characterization of positive inotropic effect of endothelin on mammalian ventricular myocardium. *Am. J. Physiol.,* **261,** H611 – H619.

TAKANASHI, M. & ENDOH, M. (1992). Concentration- and time-dependence of phosphoinositide hydrolysis induced by endothelin in relation to the positive inotropic effect in the rabbit ventricular myocardium. *J. Pharmacol. Exp. Ther.,* **262,** 1189 – 1194.

TOHSE, N., HATTORI, Y., NAKAYA, H., ENDOU, M. & KANNO, M. (1990). Inability of endothelin to increase Ca^{2+} current in guinea-pig heart cells. *Br. J. Pharmacol.,* **99,** 437 – 438.

VIGNE, P., BREITTMAYER, J.P., MARSAULT, R. & FRELIN, C. (1990). Endothelin mobilizes Ca^{2+} from a caffeine- and ryanodine-insensitive intracellular pool in rat atrial cells. *J. Biol. Chem.,* **265,** 6782 – 6787.

VIGNE, P., LAZDUNSKI, M. & FRELIN, C. (1989). The inotropic effect of endothelin-1 on rat atria involves hydrolysis of phosphatidylinositol. *FEBS Lett.,* **249,** 143 – 146.

WANG, J.X., PAIK, G. & MORGAN, J.P. (1991). Endothelin-1 enhances myofilament Ca^{2+} responsiveness in aequorin-loaded ferret myocardium. *Circ. Res.,* **69,** 582 – 589.

WATANO, T., KIMURA, J., MORITA, T. & NAKANISHI, H. (1996). A novel antagonist, No. 7943, of the Na^+-Ca^{2+} exchange current in guinea-pig cardiac ventricular cells. *Br. J. Pharmacol.,* **119,** 555 – 563.

YANAGISAWA, M., KUSUMOTO, H., KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature,* **332,** 411 – 415.

YANG, H.-T. & ENDOH, M. (1997). Species-dependent differences in inotropic effects and phosphoinositide hydrolysis induced by endothelin-3 in mammalian ventricular myocardium. *Br. J. Pharmacol.,* **120,** 1497 – 1504.

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