Endothelin-1 increases intracellular Ca\(^{2+}\) in rabbit pulmonary artery smooth muscle cells through phospholipase C

Eun A. Ko,¹ Won Sun Park,¹ Jae-Hong Ko,¹ Jin Han,² Nari Kim,² and Yung E. Earm¹

¹Department of Physiology and National Research Laboratory for Cellular Signalling, Seoul National University College of Medicine, Seoul; and ²Mitochondrial Signaling Laboratory, Department of Physiology and Biophysics, College of Medicine, Biohealth Products Research Center, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

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ENDOTHELIN (ET)-1, the most potent vasoconstrictor described to date, is a 21-amino acid peptide secreted by the vascular endothelium (44). After release from the endothelium, ET-1 induces smooth muscle contraction by binding to ET receptor type A or B (ET\(_A\) or ET\(_B\)) receptors. ET\(_A\) receptors are present only on smooth muscle cells, whereas ET\(_B\) receptors are located on endothelial and smooth muscle cells (35). Studies of the roles of the two receptor subtypes in mediating the effects of ET-1 have produced highly variable results that range from exclusive mediation by one of the receptor subtypes to an equivalent contribution by both types (4, 5, 22, 29, 31, 33, 45). The ratio and coupling efficacy of ET\(_A\) and ET\(_B\) receptors to the contractile apparatus of muscle cells may vary across species, vascular regions, and vessel size, as well as with the concentration of ET-1 (1, 6, 13, 15). Fukuroda et al. (6) reported that ET\(_A\) receptors were dominant in the human pulmonary artery, whereas ET\(_B\) receptors were dominant in the rabbit pulmonary artery. Because both receptor types are coupled G proteins, they activate several signaling pathways, including phospholipase C (PLC), resulting in generation of inositol 1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG) (2, 23, 24). These molecules in turn cause an increase in the intracellular concentration of Ca\(^{2+}\) ([Ca\(^{2+}\)\(_i\)]) which activates protein kinase C (PKC) and leads to the contraction of smooth muscle (35, 42). However, the cellular mechanisms by which the ET-1 receptors induce an increase in [Ca\(^{2+}\)\(_i\)] in smooth muscle cells remain unclear. ET-1 could increase [Ca\(^{2+}\)\(_i\)] by stimulating Ca\(^{2+}\) influx through the plasma membrane (10, 45) and/or by causing Ca\(^{2+}\) release from internal Ca\(^{2+}\) stores (30, 41). The present study was designed to investigate 1) the source of the Ca\(^{2+}\) that is mobilized by ET-1, 2) the ET-1 receptor subtype(s) that mediates the effects of ET-1, and 3) the intracellular mechanisms that underlie the response of smooth muscle cells to ET-1.

MATERIALS AND METHODS

Cell isolation. The procedure was carried out in accordance with the guidelines of the European Community on the ethical use of animals, and all experimental procedures were reviewed and approved by the Committee for Animal Experiments of the Seoul National University College of Medicine. New Zealand White rabbits (2–2.5 kg) of either gender were anesthetized with pentobarbital sodium (50 mg/kg). The lungs were removed immediately and immersed in normal Tyrode solution containing 143 mM NaCl, 5.4 mM KCl, 0.33 mM NaHPO\(_4\), 1.8 mM CaCl\(_2\), 1.2 mM MgCl\(_2\), 5 mM HEPES, and 16.6 mM glucose (pH adjusted to 7.4 with NaOH). Small pulmonary arteries (\(<200 \mu M\)) were dissected out and placed in a 50-ml beaker where the tissue was kept in normal Tyrode solution to release the enzymes, and then incubated in Ca\(^{2+}\)-free normal Tyrode solution containing 1.0 mg/ml papain, 1.0 mg/ml collagenase, 1.0 mg/ml bovine serum albumin, and 1.0 mg/ml dithiothreitol) for 20 min and in the second digestion medium (Ca\(^{2+}\)-free Tyrode solution containing 1.5 mg/ml collagenase, 1.0 mg/ml bovine serum albumin, and 1.0 mg/ml dithiothreitol) for 25 min. The samples were rinsed with Ca\(^{2+}\)-free normal Tyrode solution to release the enzymes, and the released cells were detached by gentle agitation with a fire-polished glass pipette. The isolated cells were suspended in the intracellular concentration of Ca\(^{2+}\) in rabbit pulmonary artery smooth muscle cells through phospholipase C

Address for reprint requests and other correspondence: Y. E. Earm, Dept. of Physiology, College of Medicine, Seoul National Univ., Seoul 110-799, Korea (E-mail: earmye@sun.ac.kr).

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ability to respond to depolarization with 100 mM KCl by contracting and increasing [Ca\(^{2+}\)]. Cells that did not exhibit a Ca\(^{2+}\) response to K\(^{+}\) depolarization were not used.

**Measurement of intracellular Ca\(^{2+}\).** Intracellular Ca\(^{2+}\) was measured using the membrane-permeant (acetoxymethyl ester) form of the Ca\(^{2+}\)-sensitive fluorescent dye indo 1 (indo 1-AM). The cells were loaded by incubation in 3 μM indo 1-AM for 30 min at 37°C, and the extracellular indo 1-AM was then rinsed off with normal Tyrode solution. Monochromatic excitation light (355 nm) was delivered to the cell using a filter wheel (Life Science Resources, Cambridge, UK) via a liquid light guide and an oil-immersion objective lens (×4/0, NA 1.3; Nikon). The light emitted through an aperture slightly larger than the cell was measured simultaneously at 405 and 490 nm, and Ca\(^{2+}\) concentration was estimated from the ratio of the fluorescence signals (405/490) obtained from the two photomultipliers (Life Science Resources). [Ca\(^{2+}\)], was calculated using the equation described by Grynkiewicz et al. (12)

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[\text{Ca}^{2+}] = K_B(R - R_{\text{min}})/(R_{\text{max}} - R)
\]

where R is the ratio of the fluorescence signals (405/490), R_{\text{max}} is 405/490 in Ca\(^{2+}\)-free medium containing 10 mM EGTA (ratio = 0.8), R_{\text{min}} is 405/490 in the presence of saturating Ca\(^{2+}\) (ratio = 7.5), and B is the ratio of the 490-nm fluorescence signal measured in a Ca\(^{2+}\)-free solution to that measured in a Ca\(^{2+}\)-replete solution (ratio = 8.2). The Ca\(^{2+}\) dissociation constant (K\(_D\)) was determined to be 250 nM at 37°C for this dye and optical system. All experiments were carried out at room temperature (22–25°C).

**Perforated-patch recordings.** Membrane currents were recorded using a patch-clamp amplifier (Axopatch 1C, Axon Instrument, Union, CA). Pulse protocols and data acquisition were performed by a digital interface (Digidata 1200, Axon Instrument) coupled to an IBM-compatible microcomputer. The current signals were filtered at 0.5–1 kHz, and the sampling rate was 1–3 kHz. For the perforated-patch recordings of the voltage-dependent Ca\(^{2+}\) currents, the cells were bathed in a solution containing 120 mM NaCl, 2 mM CaCl\(_2\), 5 mM CsCl, 20 mM TEA-Cl, 0.5 mM MgCl\(_2\), 10 mM HEPES, and 10 mM glucose (pH adjusted to 7.4 with NaOH). The patch pipettes were filled with a solution containing 130 mM CsCl, 10 mM HEPES, 10 mM EGTA, and 5 mM Mg-ATP (pH adjusted to 7.2 with CsOH). Nystatin was added to a fresh aliquot of the pipette solution every 2 h to give a final concentration of 200 μg/ml. Inward currents through voltage-gated Ca\(^{2+}\) channels were recorded by application of a depolarizing pulse to 0 mV for 300 ms from the holding potential of −80 mV every 10 s. Membrane capacitance was determined using 10-mV voltage-clamp steps from a holding potential of −60 mV, and the current amplitudes were normalized using the membrane capacitance. The average cell capacitance was 14.9 ± 1.59 (SE) pF (n = 24).

**Western blot analysis.** Sections of the rabbit pulmonary arteries were homogenized in a hand-held microtissue grinder (Pyrex, Corning Life Sciences, Acton, MA) in two volumes of ice-cold storage buffer (100 mM KPO\(_4\), 1 mM EDTA, 1 mM diethiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, and 30% glycerol, pH 7.25). The homogenates were centrifuged at 3,500 g for 15 min at 4°C. The supernatants were colleted and stored at −80°C until use in Western blot analysis. Aliquots of pulmonary artery homogenates containing 20 μg of protein were separated by electrophoresis on 10% SDS-polyacrylamide gels. The gels were transferred to Immobilon-P membranes (Millipore), which were blocked overnight in Tris-buffered saline (20 mM Tris and 150 mM NaCl, pH 8.0) containing 5% nonfat dry milk and then probed with antiserum to β-tubulin, ET\(_A\) receptor, or ET\(_B\) receptor at 1:200 dilution for 1 h at room temperature. The membranes were then incubated with horseradish peroxidase-conjugated goat anti-mouse (for β-tubulin) or mouse anti-goat (for ET\(_A\) and ET\(_B\) receptors) antibody at 1:1,000 dilution for 1 h at room temperature. The bands were visualized using an enhanced chemiluminescence Western blotting detection kit (Amersham Biosciences, Piscataway, NJ).

**Chemicals and drugs.** Indo 1-AM was purchased from Molecular Probes (Eugene, OR), collagenase from Wako Pure Chemical Industries (Osaka, Japan), and antisera to ET\(_A\) and ET\(_B\) and mouse anti-goat antibody from Santa Cruz Biotechnology (Santa Cruz, CA). All other chemicals were obtained from Sigma Chemical (St. Louis, MO).

**Statistics.** Values are means ± SE. Differences were examined for significance using Student’s t-test. *P* < 0.05 was considered statistically significant.

**RESULTS**

**Transient Ca\(^{2+}\) response to ET-1 does not involve extracellular Ca\(^{2+}\).** Exposure of rabbit pulmonary artery smooth muscle cells to ET-1 caused a rapid rise in [Ca\(^{2+}\)], followed by a return to baseline Ca\(^{2+}\) levels. The magnitude of this transient peak was concentration dependent, with maximum [Ca\(^{2+}\)] of 28.6 ± 2.8 mM (n = 8), 46.9 ± 2.5 mM (n = 11), and 82.4 ± 3.7 mM (n = 15) at 0.25, 0.5, and 5 nM ET-1, respectively (Fig. 1A and B). [Ca\(^{2+}\)] increased to 176.1 ± 5.6 mM (n = 18) in cells that were depolarized with 100 mM KCl (Fig. 1D), indicating that the cells were responsive and that the sarcoplasmic reticulum was loaded with Ca\(^{2+}\) (35, 38). After they were washed for 15 min, the cells were exposed to ET-1. To determine whether the ET-1-induced increase in [Ca\(^{2+}\)], required Ca\(^{2+}\) influx through voltage-dependent channels, 5 nM ET-1 was applied 10 min after the start of exposure to 5 μM nicardipine, an L-type Ca\(^{2+}\) channel antagonist. Nicardipine had no effect on the ET-1-induced increase in [Ca\(^{2+}\)], (n = 6; Fig. 1C). To further evaluate the role of extracellular Ca\(^{2+}\) influx, 5 mM ET-1 was applied to cells that were superfused with Ca\(^{2+}\)-free buffer containing 2 mM EGTA for 7 min before stimulation with ET-1. Removal of extracellular Ca\(^{2+}\) with EGTA did not affect the transient [Ca\(^{2+}\)], increase induced by ET-1 (n = 7; Fig. 1D). Therefore, we concluded that, in pulmonary artery smooth muscle cells, ET-1-induced [Ca\(^{2+}\)], increases are not mediated by influx of extracellular Ca\(^{2+}\).

**ET-1 induces Ca\(^{2+}\) release from intracellular stores.** Given that the removal of extracellular Ca\(^{2+}\) did not abolish ET-1-induced increases in [Ca\(^{2+}\)], the contribution of the release of Ca\(^{2+}\) from intracellular stores was examined. To determine which intracellular Ca\(^{2+}\) stores were involved in the ET-1-induced increase in [Ca\(^{2+}\)], we used ryanodine to block the ryanodine-sensitive intracellular Ca\(^{2+}\) stores (20) or thapsigargin (38) to block the endoplasmic reticulum Ca\(^{2+}\)-ATPase pump. The ET-1-induced [Ca\(^{2+}\)], increase was attenuated in the presence of 10 μM ryanodine (Fig. 2A). In addition, pretreatment of the cells with 10 μM thapsigargin completely abolished the ET-1-induced [Ca\(^{2+}\)], increase (Fig. 2B). These results (summarized in Fig. 2C) suggested that intracellular ryanodine- and thapsigargin-sensitive stores of Ca\(^{2+}\) were mobilized by ET-1 stimulation.

**ET\(_A\) and ET\(_B\) receptors mediate the Ca\(^{2+}\) response to ET-1.** We investigated the involvement of the two ET-1 receptor subtypes in the ET-1-induced increase in [Ca\(^{2+}\)], by using BQ-123 and BQ-788 to antagonize ET\(_A\) and ET\(_B\) receptors, respectively (17, 19), as well as IRL-1620, a specific agonist of ET\(_B\) receptors (37). The ET-1-induced [Ca\(^{2+}\)], increase was partially blocked in the presence of 1 μM BQ-123 (n = 5; Fig. 3A), which suggested that the ET\(_A\) receptor partially mediated the Ca\(^{2+}\) response to ET-1. The ET-1-induced [Ca\(^{2+}\)], increase was completely inhibited by 1 μM BQ-788, although 100 mM KC1 could still evoke an increase in [Ca\(^{2+}\)], (n = 8; Fig. 3B),
sustained elevation of [Ca\(^{2+}\)] is mediated mainly via ETB receptors. To exclude the possibility, we pretreated the cells with 1 \(\mu\)M nicardipine and removed extracellular Ca\(^{2+}\) with EGTA. ET-1 failed to induce an increase in [Ca\(^{2+}\)] in response to ET-1. IRL-1620 failed to induce an increase in [Ca\(^{2+}\)] in cells pretreated with 1 \(\mu\)M nicardipine and removal of extracellular Ca\(^{2+}\) with EGTA. These observations indicated that the absence of a sustained increase in [Ca\(^{2+}\)] in response to ET-1 might be caused by ET\(_A\) receptor activation, which causes inhibition of the influx of extracellular Ca\(^{2+}\) through Ca\(^{2+}\) channels activated by the ET\(_B\) receptor. Using a perforated patch-clamp technique, we examined the effect of ET-1 on changes in [Ca\(^{2+}\)] current (\(I_{\text{Ca}}\)) evoked by IRL-1620. IRL-1620 (100 nM) increased \(I_{\text{Ca}}\) by 65.4 ± 2.8% (n = 18; Fig. 4A), but the increase in \(I_{\text{Ca}}\) caused by 100 nM IRL-1620 was markedly attenuated by 30 nM ET-1 alone had no significant effect on \(I_{\text{Ca}}\) (n = 12; Fig. 4B), but the increase in \(I_{\text{Ca}}\) induced by 100 nM IRL-1620 was markedly attenuated by 30 nM ET-1 alone had no significant effect on \(I_{\text{Ca}}\) (n = 12; Fig. 4B). Addition of IRL-1620 increased \(I_{\text{Ca}}\) mainly via activation of ETB receptors. In contrast, the effect of 10 nM IRL-1620 on \(I_{\text{Ca}}\) was completely inhibited by 1 \(\mu\)M BQ-123 (data not shown). These observations indicated that the absence of a sustained increase in [Ca\(^{2+}\)] in response to ET-1 may be the result of activation of ET\(_A\) receptors, which masks the Ca\(^{2+}\) influx through Ca\(^{2+}\) channels activated by ETB receptors. To exclude the possibility that IRL-1620 activates \(I_{\text{Ca}}\) directly, rather than through the ETB receptor, we examined the effect of ET-1 on \(I_{\text{Ca}}\) in the presence of the ET\(_A\) antagonist BQ-123. ET-1 increased \(I_{\text{Ca}}\) by 45.3 ± 4.3% (n = 5; Fig. 4, H and I) in the absence of ET\(_A\) activity.
with ETA and ETB receptors to increase [Ca$^{2+}$], in rabbit pulmonary artery smooth muscle cells. Incubation of cells with 3 μM U-73122 caused a complete inhibition of the ET-1-induced [Ca$^{2+}$] increase (n = 4; Fig. 6A), which suggested that the ET-1-induced increase in [Ca$^{2+}$] involved activation of PLC. In addition, incubation of cells with U-73122 caused a complete inhibition of the IRL-1620-induced [Ca$^{2+}$] increase (n = 4; Fig. 6C). Also, treatment with U-73122 completely inhibited the I$_{Ca}$ activated by IRL-1620 (n = 5; Fig. 6E). Basal I$_{Ca}$ was not affected by treatment with U-73122 (data not shown), which suggested that effects on [Ca$^{2+}$], and I$_{Ca}$ induced by ET$_B$ receptor activation involved PLC. Stimulation of PLC in response to the occupation of ET-1 receptors is associated with generation of IP$_3$ and DAG; the latter activates multiple effectors via PKC phosphorylation, including ryanodine receptors and L-type Ca$^{2+}$ channels (11, 43).

**DISCUSSION**

We characterized the cellular mechanisms that underlie ET-1-induced increases in [Ca$^{2+}$], in rabbit pulmonary artery smooth muscle cells. ET-1 induced a transient increase in [Ca$^{2+}$], that was followed by a return to baseline levels. Our results revealed patterns of changes in [Ca$^{2+}$], that are distinct from those reported elsewhere. For example, ET-1 was reported to induce a large transient [Ca$^{2+}$] increase followed by a sustained plateau that remained elevated above baseline (8, 34), and other studies reported oscillations in [Ca$^{2+}$], in smooth muscle cells in response to ET-1 (3, 16). This discrepancy is most likely attributable to differences in the wide variety of models used to study Ca$^{2+}$ regulation, which include freshly isolated cells, cultured cells, and different cell types. Godfraind (9) suggested that ET$_A$ receptors mediate constriction in smaller, preresistance arteries, whereas more than one ET receptor is involved in the larger segments. In contrast, Adner and colleagues (1) indicated a dominance of ET$_B$ receptors in the human peripheral lung. Therefore, participation of ET-1 receptor subtypes might differ according to artery diameter, which is attributed to the different pattern of [Ca$^{2+}$], response to ET-1. In our study, we clarified the effect of ET-1-induced [Ca$^{2+}$], increases on <200-μm third- to fourth-order pulmonary resistance arteries. We found that ET$_A$ and ET$_B$ receptors coexist in this preparation, as shown in Western blot analysis, and the ET-1-induced [Ca$^{2+}$], increases were mediated mainly through ET$_B$ receptors.

Our results clearly showed that the ET-1-induced [Ca$^{2+}$], response in rabbit pulmonary artery smooth muscle cells was the result of the release of [Ca$^{2+}$], from ryanodine- and IP$_3$-sensitive intracellular stores, inasmuch as the response was not altered by nicardipine or removal of Ca$^{2+}$ from the bath solution but was inhibited by pretreatment of the cells with ryanodine or thapsigargin. The mechanisms that mediate the effects of ET-1 on smooth muscle cells are unclear. An earlier study using patch-clamp techniques revealed that voltage-gated Ca$^{2+}$ channels were activated by ET-1; removal of extracellular Ca$^{2+}$ and pretreatment of cells with verapamil reduced the ET-1-induced transient increase in [Ca$^{2+}$], in vascular smooth
However, other evidence suggested that inhibitors of L-type voltage-gated Ca\(^{2+}\) channels did not inhibit the ET-1-induced [Ca\(^{2+}\)]\(_i\) increases or the contraction of vascular smooth muscle (25, 32).

In the present study, BQ-788 completely inhibited the ET-1-induced [Ca\(^{2+}\)]\(_i\) increase (Fig. 3B), which suggested that the activation of ET\(_B\) receptors is necessary for the ET-1-induced increase in [Ca\(^{2+}\)]\(_i\). However, the patterns of changes in [Ca\(^{2+}\)]\(_i\) induced by IRL-1620 from those induced by ET-1. IRL-1620 induced a sustained, rather than a transient, [Ca\(^{2+}\)]\(_i\) increase. The sustained component resulted from Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels, because it was abolished when cells were treated with nicardipine (Fig. 3E).

Using the nystatin-perforated patch-clamp technique, we found that the activation of ET\(_B\) receptors increased I\(_{Ca}\) and that I\(_{Ca}\) was inhibited by the activation of ET\(_A\) receptors. Our data suggested that the two subtypes of ET-1 receptors produce increases in [Ca\(^{2+}\)]\(_i\), that are the net result of a complex interaction between the regulation of Ca\(^{2+}\) channels by each receptor subtype. The opposing effects of ET\(_A\) and ET\(_B\) receptors on [Ca\(^{2+}\)]\(_i\) lead to the elimination of a sustained [Ca\(^{2+}\)]\(_i\) increase when both receptors are activated.

In this study, we found that IRL-1620 had no effect on I\(_{Ca}\) measured using the whole cell patch-clamp technique (data not shown). Such differences in experimental techniques have contributed to the apparent discrepancies in the reported results. The perforated patch-clamp recording allows I\(_{Ca}\) to be studied without the movement of large intracellular molecules. 

**Fig. 3.** ET-1-induced [Ca\(^{2+}\)]\(_i\) increases are mediated by ET type A and B (ET\(_A\) and ET\(_B\)) receptors. A and B: changes in [Ca\(^{2+}\)]\(_i\) induced by ET-1 after pretreatment with the ET\(_A\) receptor blocker BQ-123 (1 μM; A) or the ET\(_B\) receptor blocker BQ-788 (1 μM; B). C and D: changes in [Ca\(^{2+}\)]\(_i\) induced by repeated addition of 5 nM ET-1 (C) or the ET\(_B\) receptor agonist IRL-1620 (10 nM; D). E: sustained component of the [Ca\(^{2+}\)]\(_i\) increase induced by 5 nM ET-1 was inhibited by 1 μM nicardipine in cells pretreated with 1 μM BQ-123. F: sustained component of the [Ca\(^{2+}\)]\(_i\) increase induced by 10 nM IRL-1620 was inhibited by 1 μM nicardipine. Vertical bars indicate 20-min gap in recording. Traces are representative of 5–12 experiments. G: summarized data normalized to initial peak [Ca\(^{2+}\)]\(_i\) on the first stimulation by ET-1 and IRL-1620. *P < 0.01; #P < 0.001 vs. ET-1.
and structures that occurs with the rupture of the cell membranes required in the whole cell patch-clamp technique (14, 21). Our results indicated that ETA and ETB receptors cooperate positively to mediate the transient component of the ET-1-induced \( \text{Ca}^{2+} \) increase and cooperate negatively to lead to the elimination of the sustained \( \text{Ca}^{2+} \) increases induced by ETB receptor activation in rabbit pulmonary artery smooth muscle cells. In agreement with our results, it has been reported that both ET receptors can contribute to ET-1-induced contraction; for example, in pulmonary arteries, both receptors coexist and, thus, could cooperate to mediate contraction (6, 7, 22).

Fig. 4. ET-1 inhibits changes in \( \text{Ca}^{2+} \) current (\( I_{\text{Ca}} \)) evoked by IRL-1620. Original current traces recorded from a holding potential of −80 to 0 mV show changes in \( I_{\text{Ca}} \) evoked by application of 100 nM IRL-1620 (A) and 30 nM ET-1 (B). C: current density of \( I_{\text{Ca}} \) evoked by IRL-1620. *\( P < 0.01 \) vs. baseline. D: changes in \( I_{\text{Ca}} \) induced by 30 nM ET-1 in the presence of 100 nM IRL-1620. E: current density-time relations of \( I_{\text{Ca}} \). F: changes in \( I_{\text{Ca}} \) evoked by application of 30 nM ET-1 in the presence of 100 nM IRL-1620 after pretreatment with 1 \( \mu \)M BQ-123. G: current density-time relations of \( I_{\text{Ca}} \). H: increases in \( I_{\text{Ca}} \) induced by 30 nM ET-1 in the presence of BQ-123. I: current density-time relations of \( I_{\text{Ca}} \). Traces are representative of 5–14 independent experiments.
In general, the ET-1-induced increase in \([\text{Ca}^{2+}]_i\) involves different \([\text{Ca}^{2+}]_i\)-mobilizing mechanisms, including release of \([\text{Ca}^{2+}]_i\) from intracellular stores via a PLC-mediated activation of IP3 receptors, activation of L-type \([\text{Ca}^{2+}]_i\) channels, and \([\text{Ca}^{2+}]_i\)-activated Cl\(^{-}\) currents (27, 28, 34, 40, 43). In our study, the ET-1-induced increases in \([\text{Ca}^{2+}]_i\) were not inhibited by the \([\text{Ca}^{2+}]_i\) channel blocker nicardipine, and ET-1 did not affect I\(_{\text{Ca}}\). Therefore, we believe that \([\text{Ca}^{2+}]_i\) release from intracellular stores is the main mechanism by which ET-1 elicits an increase in \([\text{Ca}^{2+}]_i\). Furthermore, ET-1-induced \([\text{Ca}^{2+}]_i\) increases were completely abolished by the PLC blocker U-73122, which suggested that the ET-1-induced \([\text{Ca}^{2+}]_i\) responses involve activation of PLC. Previous reports have shown that PLC plays a crucial role in ET-1-induced \([\text{Ca}^{2+}]_i\) increases in cultured ciliary muscle and bronchial smooth muscle cells (26, 27). However, further investigations should explore whether activation of the PLC pathway results in the generation of two second messengers, IP3 and DAG, which are involved in intracellular \([\text{Ca}^{2+}]_i\) release and PKC activation, respectively.

In conclusion, we have demonstrated that ET-1 induces the release of \([\text{Ca}^{2+}]_i\) from intracellular stores in rabbit pulmonary...
artery smooth muscle cells via activation of ET\textsubscript{A} and ET\textsubscript{B} receptors. In addition, ET-1 was functionally coupled to a rise in [Ca\textsuperscript{2+}i] through activation of PLC.

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