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# Endothelin-1 Stimulates the Expression of L-Type $Ca^{2+}$ Channels in Neonatal Rat Cardiomyocytes via the Extracellular Signal–Regulated Kinase 1/2 Pathway

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**Abstract** The cardiac L-type $Ca^{2+}$ channel current ($I_{Ca,L}$) plays an important role in controlling both cardiac excitability and excitation–contraction coupling and is involved in the electrical remodeling during postnatal heart development and cardiac hypertrophy. However, the possible role of endothelin-1 (ET-1) in the electrical remodeling of postnatal and diseased hearts remains unclear. Therefore, the present study was designed to investigate the transcriptional regulation of $I_{Ca,L}$ mediated by ET-1 in neonatal rat ventricular myocytes using the whole-cell patch-clamp technique, quantitative RT-PCR and Western blotting. Furthermore, we determined whether the extracellular signal–regulated kinase 1/2 (ERK1/2) pathway is involved. ET-1 increased $I_{Ca,L}$ density without altering its voltage dependence of activation and inactivation. In line with the absence of functional changes, ET-1 increased L-type $Ca^{2+}$ channel pore-forming $\alpha_{1C}$-subunit mRNA and protein levels without affecting the mRNA expression of auxiliary β- and $\alpha_2/\delta$-subunits. Furthermore, an actinomycin D chase experiment revealed that ET-1 did not alter $\alpha_{1C}$-subunit mRNA stability. These effects of ET-1 were inhibited by the $ET_A$ receptor antagonist BQ-123 but not the $ET_B$ receptor antagonist BQ-788. Moreover, the effects of ET-1 on $I_{Ca,L}$ and $\alpha_{1C}$-subunit expression were abolished by the ERK1/2 inhibitor (PD98059) but not by the p38 MAPK inhibitor (SB203580) or the c-Jun N-terminal kinase inhibitor (SP600125). These findings indicate that ET-1 increased the transcription of L-type $Ca^{2+}$ channel in cardiomyocytes via activation of ERK1/2 through the $ET_A$ receptor, which may contribute to the electrical remodeling of heart during postnatal development and cardiac hypertrophy.

**Keywords** Endothelin-1 · L-type $Ca^{2+}$ channel · Cardiomyocyte · Mitogen-activated protein kinase

## Introduction

Although endothelin-1 (ET-1) plays important physiological roles in vasoconstriction, cell proliferation and blood pressure, it can also exert pathophysiological effects: previous studies have suggested that ET-1 is involved in the development of various cardiovascular diseases such as hypertension, ischemic heart disease, congestive heart failure (Kirkby et al. 2008), cardiac hypertrophy (Schneider et al. 2007) and arrhythmias. The arrhythmogenic effects of ET-1 have been suggested by the following studies. First, it has been shown that exogenous ET-1 may exert intrinsic arrhythmogenic effects that are not solely attributable to myocardial ischemia (Yorikane and Koike 1990). Second, ET-1 administration decreases the conduction velocity in cultured neonatal rat ventricular myocytes (Reisner et al.

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2009). Third, ET-1 receptor antagonists have exhibited antiarrhythmic properties (Garjani et al. 1995; Geshi et al. 1999). Furthermore, it has been reported that ET-1 antagonists prevent electrical remodeling in an animal model of congestive heart failure, resulting in a reduction of ventricular arrhythmias (Matsumoto et al. 2002). However, the ionic and subcellular mechanisms of the arrhythmogenic actions of ET-1 are unclear.

A possible ionic mechanism underlying cardiac arrhythmias is alterations in the function and expression of various ion channels (including L-type $Ca^{2+}$ channels) and transporters in the heart, which is known as electrical remodeling. Upregulation of $I_{Ca,L}$ has been reported in some cardiovascular diseases such as hypertension (Xiao and McArdle 1994), cardiac hypertrophy (Wang et al. 2001) and heart failure (Wang et al. 2008). Increased activity of L-type $Ca^{2+}$ channels may lead to $Ca^{2+}$ overload in cardiomyocytes, which results in delayed afterdepolarizations (DADs) (Hiraoka and Kawano 1984) and, thereby, contributes to arrhythmias and to sudden death. Moreover, an increase in calcium influx through $I_{Ca,L}$ prolongs action potential durations and triggers early afterdepolarizations (EADs) (Gaur et al. 2009; Ming et al. 1994). EADs and triggered activity can induce reentrant arrhythmias. In addition, postnatal development is associated with alterations in cardiac channels (Cerbai et al. 1999; Grandy et al. 2007; Stocker and Bennett 2006), including L-type $Ca^{2+}$ channels (Huang et al. 2006). However, the possible role of ET-1 in electrical remodeling in postnatal and diseased hearts remains unknown. Therefore, the present study was designed to investigate ET-1 regulation of L-type $Ca^{2+}$ channel gene expression in neonatal rat ventricular myocytes using the whole-cell patch-clamp technique, quantitative RT-PCR and Western blotting.

Once ET-1 is generated, it exerts its physiological and pathophysiological effects primarily by binding to the ET types A ($ET_A$) and B ($ET_B$) receptors (Schneider et al. 2007). Activation of these receptors can lead to activation of various tyrosine and serine/threonine protein kinases. ET-1 regulates the function of ion channels through direct phosphorylation by protein kinases such as PKA and PKC (Kiesecker et al. 2006; Zeng et al. 2009). In addition, ET-1 stimulates gene expression through a variety of signal-transduction pathways that include the extracellular signal–regulated kinase 1/2 (ERK1/2) pathway (Cullingford et al. 2008). The ERK1/2 pathway reportedly regulates the expression of different ion channels in cardiomyocytes (Jia and Takimoto 2006; Marni et al. 2009) and other cell types (Dey et al. 2011; Mustafa et al. 2008). Therefore, we also determined whether ET ($ET_A$ and $ET_B$) receptors and ERK1/2 are involved in the modulation of $I_{Ca,L}$ by ET-1.

## Materials and Methods

### Cell Isolation and Culture

Primary cultures of cardiomyocytes were obtained from neonatal ventricles of 1- to 2-day-old neonatal Sprague-Dawley rats; all animal procedures were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH publication 85-23, revised 1996) and approved by the institutional animal care and use committee at the Hubei University of Science and Technology, China. Subsequently, the hearts were dissected and ventricles separated from atria; single ventricular myocytes were isolated using enzymatic dissociation procedures adapted from previous reports (Fink et al. 2000; Webster et al. 1993). Briefly, ventricular tissues were minced into $\sim$1 $mm^3$ pieces, collected in $Ca^{2+}$- and magnesium-free Hanks' balanced salt solution (HBSS) containing 0.1 % trypsin (Sigma, St. Louis, MO) and 0.1 % collagenase type II (Sigma) and agitated for 7 min at 37 °C to dissociate cardiomyocytes. Remaining tissues were incubated in a fresh enzyme solution and allowed to dissociate for 7 min. This procedure was repeated five times. Cell suspensions were collected, centrifuged and resuspended in Dulbecco's modified Eagle medium (DMEM containing 1.05 mmol/l $Ca^{2+}$; GIBCO BRL, Gaithersburg, MD) supplemented with 5 % fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin and 4 mM L-glutamine. Dissociated cells were preplated for 1 h at 37 °C to selectively remove nonmuscle cells. Nonadherent cells (mostly cardiomyocytes) were then plated into 35-mm dishes at a density of $1 \times 10^5$ cells/ml for patch-clamp experiments and into 100-mm dishes at a density of $5 \times 10^5$ cells/ml for molecular experiments. Dishes were maintained in an incubator at 37 °C with 5 % $CO_2$. Medium was refreshed daily. After 48 h cultivation, the medium was replaced by FBS-free medium and incubated for 10 h before drug application. Subsequently, cardiomyocytes were incubated with or without ET-1 (10 nM) in culture medium containing 5 % FBS for 24 h before measurements. In some experiments, ET-1 antagonists (BQ-123, BQ-788) or MAPK inhibitors (PD98059, SB203580 and SP600125) were included in the culture medium. ET-1, BQ-123, BQ-788, PD98059, SB203580 and SP600125 were purchased from Sigma-Aldrich (St. Louis, MO).

### Voltage-Clamp Experiments

Control and ET-1-treated cardiomyocytes were cultured on glass coverslips and transferred to a temperature-controlled recording chamber, which was mounted on the stage of an inverted microscope (Zeiss, Oberkochen, Germany).

Cardiomyocytes were first superfused with normal Tyrode solution containing (mmol/L) NaCl 135, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, $NaH_2PO_4$ 0.33, glucose 10 and HEPES 10 (pH 7.4) with NaOH. Thereafter, the extracellular bath solution was replaced by test solution for recording $I_{Ca,L}$ currents. Test solution consisted of (mmol/L) choline-Cl 140, TEA-Cl 20, $CaCl_2$ 2, glucose 10, HEPES 10 and tetrodotoxin 0.03 (pH 7.4 with TEA-OH). The pipette solution was composed of the following (mmol/L) (Avila et al. 2007): Cs-Asp 135, $MgCl_2$ 10, EGTA 10, $CaCl_2$ 1, HEPES 10, glucose 5, ATP-Mg 5 and GTP-$Na_2$ 0.05 (pH 7.4 with CsOH).

$I_{Ca,L}$ currents were recorded from single ventricular myocytes using the tight-seal whole-cell voltage-clamp technique (Hamill et al. 1981) with an Axopatch 700B amplifier (Molecular Devices, Foster City, CA). Command generation and data acquisition were performed with pClamp 8.0 software and a Digidata 1320 (Molecular Devices). Current signals were filtered at 2 kHz and digitized at 10 kHz. The recording pipettes were pulled from borosilicate glass capillaries and had tip resistances of 2–5 MΩ when filled with the pipette solution. The liquid junction potential between the pipette and bath solutions was always corrected before the formation of a giga-ohm seal. The series resistance was electrically compensated by 40–80 %. Whole-cell recordings were started 5 min after seal disruption, to allow for cell dialysis. Experiments were conducted at 36.5 ± 0.5 °C.

To measure $I_{Ca,L}$, the holding potential ($V_h$) was kept at −80 mV. A 50-ms prepulse to −40 mV was applied before the voltage protocol to inactivate all sodium channels, and total calcium currents were then elicited by a series of 200-ms depolarizing pulses in 10-mV increments from −40 to +60 mV. The bath solution was then changed to nifedipine (L-type $Ca^{2+}$ channel antagonist, 3 μmol/L)–containing solution, and nifedipine-insensitive calcium currents were measured 5–10 min later. $I_{Ca,L}$ was obtained by subtracting nifedipine-insensitive calcium currents from total calcium currents recorded from the same cells with the same protocol before nifedipine infusion. The $I_{Ca,L}$ current size was defined as the difference between the peak inward current and the current remaining at the end of the 200-ms depolarizing pulse.

To evaluate the steady-state activation of $Ca^{2+}$ currents, cell membrane potential was held at −80 mV. $I_{Ca,L}$ was elicited by 200-ms test pulses from −40 to +60 mV in 10-mV increments after a 50-ms prepulse to −40 mV. To study the steady-state inactivation of $Ca^{2+}$ currents, cell membrane potential was held at −40 mV, stepped to levels of −50 to 20 mV for 2,000 ms in 10-mV increments and then stepped to the test potential of 0 mV for 200 ms. Stimulation frequency was 0.2 Hz. These measurements were made about 20 min after establishment of the whole-cell mode to minimize the contribution of time-dependent shifts of steady-state gating parameter measurements. Current density was calculated by dividing the current amplitude by the cell capacitance ($C_m$). $C_m$ was measured at the beginning of each experiment as described previously (Yuan et al. 1996). For each cell, a capacitance transient due to a 5-mV step was measured and analyzed as follows: $C_m = \tau_c I_0/[\Delta V_m(1 - I_\infty/I_0)]$, where $\tau_c$ is the time constant of the capacitative current relaxation, $I_0$ is the peak capacitative current determined by single exponential fit and extrapolation to the first sample point after the voltage step $\Delta V_m$ and $I_\infty$ is the amplitude of the steady-state current following the voltage step.

To evaluate the steady-state activation, we calculated the peak conductance ($g$) for each cell according to the equation $g = I/(V_m - V_{rev})$, where $g$ is the conductance calculated at the membrane potential $V_m$, $I$ is the current amplitude and $V_{rev}$ is the reversal potential derived from $I$–$V$ curve extrapolation for each cell. Activation curves were fitted with the Boltzmann equation, $g = g_{max}/\{1 + \exp[(V_m - V_a)/k_a]\}$, where $g$ is the peak conductance at test voltage $V_m$, $g_{max}$ is the maximum conductance, $V_a$ is the voltage of half-maximal activation and $k_a$ is the slope factor. Steady-state inactivation curves were fitted with a Boltzmann function, $I = I_{max}/\{1 + \exp[(V_m - V_i)/k_i]\}$, where $I$ is the peak current at test voltage $V_m$, $V_i$ is the voltage of half-maximal inactivation and $k_i$ is a slope factor.

Voltage-clamp data analysis was performed with Origin 7.5 software (Microcal Software, Northampton, ME) and Clampfit 10.0 software (Axon Instruments, Weatherford, TX).

## Quantitative RT-PCR

Primary cultures of cardiomyocytes were treated with ET-1 or vehicle (control) in the presence or absence of specific inhibitors for 24 h. Total RNA was extracted from cardiomyocytes using TRIzol Reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Single-stranded cDNA was synthesized from 2 μg total RNA using oligo d(T) primer and ReverTra Ace reverse transcriptase (Toyobo, Osaka, Japan). SYBR Green PCR amplifications were performed using an iCycler iQ Real-Time Detection System (Bio-Rad, Hercules, CA) associated with the iCycler Optical System Interface software (version 2.3, Bio-Rad). All PCR experiments were carried out in triplicate with a reaction volume of 25 μl using SYBR Green (Roche, Indianapolis, IN) as a detection reagent. Oligonucleotide primer sequences and optimal reaction conditions are summarized in Table 1. Amplification involved one cycle at 95 °C for 5 min for initial denaturation, followed by 35 cycles of denaturation at

95 °C for 30 s, primer annealing at the optimal temperatures (see Table 1) for 30 s and extension at 72 °C for 45 s. Amplification data acquisition and analysis were carried out using the iCycler Optical System Interface software. Data were calculated by the $2^{-\Delta\Delta CT}$ method and normalized to GAPDH, compared with those of control. The size of the PCR products was confirmed using a 2 % agarose ethidium bromide–stained gel under UV irradiation.

To investigate the possible effects of ET-1 on L-type $Ca^{2+}$ channel $\alpha_{1C}$-subunit mRNA stability, the transcriptional inhibitor actinomycin D (5 μg/ml) was added alone or simultaneously with ET-1 into the medium and then the cells were harvested after 0, 1, 2, 12 and 24 h.

## Western Blotting

Western blotting was used to measure the protein expression of the L-type $Ca^{2+}$ channel $\alpha_{1C}$-subunit (also named $Ca_v1.2$). Cultured cardiomyocytes were washed with ice-cold PBS twice. Subsequently, cells were collected by scraping and lysed at 4 °C in RIPA buffer consisting of 150 mM NaCl, 20 mM Tris (pH 7.5), 1 mM EDTA, 1 mM EGTA, 1 % Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM $Na_3VO_4$, 1 μg/ml leupeptin and 1 mM phenylmethanesulfonyl fluoride. After centrifugation at 12,000×*g* for 30 min at 4 °C, the supernatants were collected and served as total cell extracts. The protein concentration of the supernatant was determined using the BCA assay (Pierce, Rockford, IL). After normalization, equal amounts of protein (30 μg) were subjected to 7.5 % (for $\alpha_{1C}$-subunit) or 12 % (for ERK1/2, p38 or JNK) SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore, Billerica, MA). Nonspecific binding was blocked with 5 % nonfat milk prepared in Tris-buffered saline with Tween-20 (TBST) containing 150 mM NaCl, 100 mM Tris and 0.1 % Tween-20 (pH 7.4 adjusted with HCl). Subsequently, the membranes were incubated with the primary antibodies (anti-$Ca_v1.2$ antibody from Alomone Labs, Jerusalem, Israel; anti-phospho ERK1/2, anti-phospho p38 or anti-phospho JNK from Cell Signaling, Boston, MA; anti-ERK1/2, anti-p38 or anti-JNK, from Santa Cruz Biotechnology, Santa Cruz, CA) at 4 °C overnight. After being washed three times with TBST, membranes were incubated with horseradish peroxidase (HRP)–conjugated anti-rabbit IgG antibody (Beyotime Institute of Biotechnology, Haimen, Jiangsu, China) diluted 1:1,000 in TBST for 1 h at 37 °C. Specific protein bands were visualized using an enhanced chemiluminescence (ECL) Western blotting kit (Beyotime Institute of Biotechnology) according to the manufacturer's instructions and quantified with the gel analysis program of Image J software (NIH, Bethesda, MD). β-Actin was used to normalize the protein sample loading.

## Statistical Analysis

All data are expressed as means ± SEM. Statistical analysis was performed using unpaired Student's *t*-tests and one-way ANOVA where appropriate. Differences were considered significant when $P < 0.05$.

**Table 1** Oligonucleotide primer sequences and optimal reaction conditions for real-time quantitative RT-PCR

| Gene | Accession number | Primer sequence (5′–3′) | Annealing temperature (°C) | Product size (bp) |
|---|---|---|---|---|
| *α1C* | NM012517 | F: 5′-CCAGCCCAGAAAAGAAACAG-3′ | 60 | 271 |
| | | R: 5′-ACTGCCTTTTCCTTAAGGTGCA-3′ | | |
| *β1* | NM017346 | F: 5′-AGGACTTCCTGCACATCAAGG-3′ | 60 | 175 |
| | | R: 5′-TTGTCACCTGACTTGCTGGAG-3′ | | |
| *β2* | NM053851 | F: 5′-ATCCATCACAAGAGTCACTGC-3′ | 55 | 526 |
| | | R: 5′-GGTGGGGCTCAGAGGTAAAG-3′ | | |
| *β3* | NM012828 | F: 5′-TCAGCCGACTCCTACACCAG-3′ | 55 | 602 |
| | | R: 5′-GACGCGGGTGATGGAGATC-3′ | | |
| *α2/δ1* | NM001110847 | F: 5′-GAAAGGCTTTAGCTTCGCGTTT-3′ | 60 | 103 |
| | | R: 5′-TCTCTCTTCTCCTCCATCCGTG-3′ | | |
| *α2/δ2* | NM175592 | F: 5′-CAGTGGTGGGTGTCAAAC-3′ | 55 | 134 |
| | | R: 5′-TACCTCGCAGTCCATCTC-3′ | | |
| *α2/δ3* | NM175595 | F: 5′-TCCGAACGCACCATCAAG-3′ | 55 | 114 |
| | | R: 5′-ACTGTCCACCACCACCAT-3′ | | |
| *GAPDH* | NM017008 | F: 5′-AGTTCAACGGCACAGTCAAG-3′ | 60 | 148 |
| | | R: 5′-GTGGTGAAGACGCCAGTAGA-3′ | | |

*F* forward, *R* reverse

## Results

### ET-1 Upregulated the L-type $Ca^{2+}$ Channel $\alpha_{1C}$-Subunit of Neonatal Ventricular Myocytes

Cardiac L-type $Ca^{2+}$ channels are heterotetramers constituted of at least four distinct subunits ($\alpha_{1C}$, β, $\alpha_2$, δ) (Colecraft et al. 2002). The $\alpha_{1C}$-, β-, and $\alpha_2$-subunits are products of distinct genes, while $\alpha_2$ and δ are encoded by the same gene (De Jongh et al. 1990). In the present study, we examined the modulation of mRNA expression of these subunits by ET-1. Quantitative PCR revealed the expression of these subunit mRNAs in control and in ET-1-treated cardiomyocytes. Application of ET-1 for 24 h significantly increased L-type $Ca^{2+}$ channel $\alpha_{1C}$-subunit mRNA in a concentration- and time-dependent manner (Fig. 1a, b). Furthermore, treatment of cells with the transcriptional inhibitor actinomycin D completely blocked ET-1 stimulation of $\alpha_{1C}$-subunit mRNA (Fig. 1d). These results suggested that the increase in $\alpha_{1C}$-subunit mRNA might be due to a transcriptional mechanism.

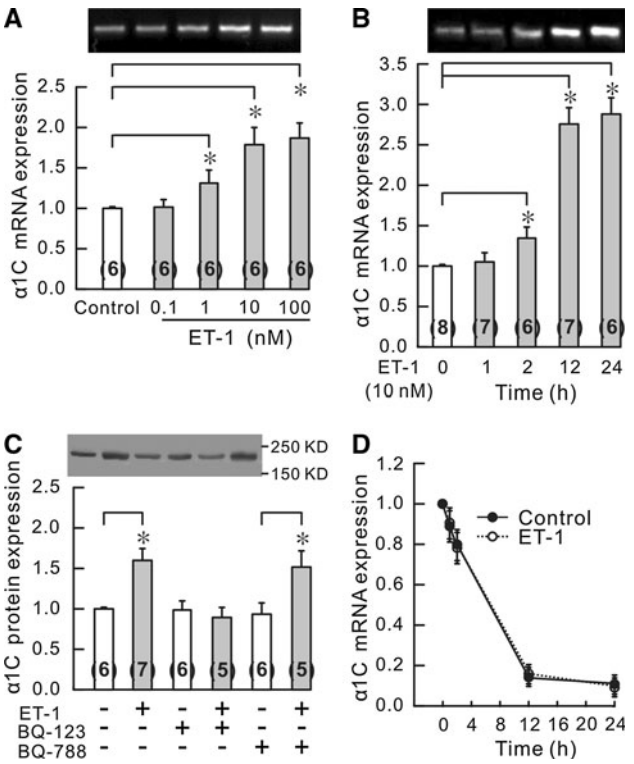


**Fig. 1** Effects of ET-1 on L-type $Ca^{2+}$ channel $\alpha_{1C}$-subunit mRNA and protein. **a** ET-1 (0.1–100 nM) exposure for 24 h resulted in a dose-dependent increase in the level of L-type $Ca^{2+}$ channel $\alpha_{1C}$-subunit mRNA in neonatal cardiomyocytes. **b** Treatment with ET-1 (10 nM) stimulated the expression of L-type $Ca^{2+}$ channel $\alpha_{1C}$-subunit mRNA in a time-dependent manner. **c** The increase in L-type $Ca^{2+}$ channel $\alpha_{1C}$-subunit protein by ET-1 (10 nM) was prevented by BQ-123 (1 μM) but not BQ-788 (1 μM). Data are presented as the mean value ± SEM. The mean value of each control is defined as 1. **d** The actinomycin D (5 μg/ml) chase experiment revealed that ET-1 did not alter $\alpha_{1C}$-subunit mRNA stability. $*P < 0.05$ versus each control. The number of experiments is indicated in *parentheses*

Additionally, we found that the $ET_A$ receptor antagonist BQ-123 (1 μM) prevented the increase of $\alpha_{1C}$-subunit protein induced by ET-1, whereas the $ET_B$ receptor antagonist BQ-788 (1 μM) did not (Fig. 1c). ET-1 did not affect the mRNA expression of L-type $Ca^{2+}$ channel β- and $\alpha_2$/δ-subunits (Fig. 2).

### ET-1 Increased $I_{Ca,L}$ Density in Ventricular Myocytes via $ET_A$ Receptor without Altering the Kinetic Properties of $I_{Ca,L}$

Neonatal rat ventricular myocytes exhibited a typical nifedipine-sensitive $I_{Ca,L}$ (Fig. 3a), the density of which was significantly increased at test potentials between −30 and +30 mV under ET-1 stimulation (10 nM, 24 h) ($P < 0.01$) (Fig. 3b). The effects of ET-1 on $I_{CaL}$ density were markedly attenuated by a specific $ET_A$ receptor antagonist, BQ-123 (1 μM), but not by a specific $ET_B$ receptor antagonist, BQ-788 (1 μM). Thus, the effects of ET-1 were mediated through the $ET_A$ receptor.

Next, the voltage dependence of nifedipine-sensitive $I_{Ca,L}$ activation (Fig. 3c) and inactivation (Fig. 3d) was also measured in control and in ET-1-treated cardiomyocytes. Similar values for half-maximal activation ($V_a$) and inactivation ($V_i$) voltages and slope factors ($k_a$ and $k_i$) were obtained in both groups (Table 2).

Furthermore, time-dependent effects of ET-1 on L-type $Ca^{2+}$ current density were observed. As shown in Fig. 3e, f, ET-1 (10 nM) increased the nifedipine-sensitive $I_{Ca,L}$ peak densities in cardiomyocytes in a time-dependent manner. The $I_{Ca,L}$ peak density at 1 h of ET-1 treatment was similar to that at 0 h. Thus, though ET-1 reportedly increases $I_{Ca,L}$ through direct phosphorylation of L-type $Ca^{2+}$ channel by PKC (He et al. 2000), the effects of direct phosphorylation on $I_{Ca,L}$ peak density under our experimental conditions seemed to be eliminated by the long-lasting washout of the extracellular perfusate before whole-cell recording. In our voltage-clamp experiments, cardiomyocytes grown on coverslips were mounted on the stage of an inverted microscope (Zeiss) and continuously perfused with normal Tyrode solution for 20 min to remove the culture medium and nonbound cells. Subsequently, the extracellular bath solution was replaced by test solution for recording $I_{Ca,L}$ currents. Thus, an increase in $I_{Ca,L}$ peak density at 2, 12 or 24 h of ET-1 treatment may not be due to direct phosphorylation of L-type $Ca^{2+}$ channels. In addition, we cannot role out the possibility that the lack of short-term effect of ET-1 might be due to the configuration of the patch-clamp technique (conventional whole-cell vs. perforated patch). A previous study has demonstrated that the $I_{Ca,L}$ response to angiotensin II is

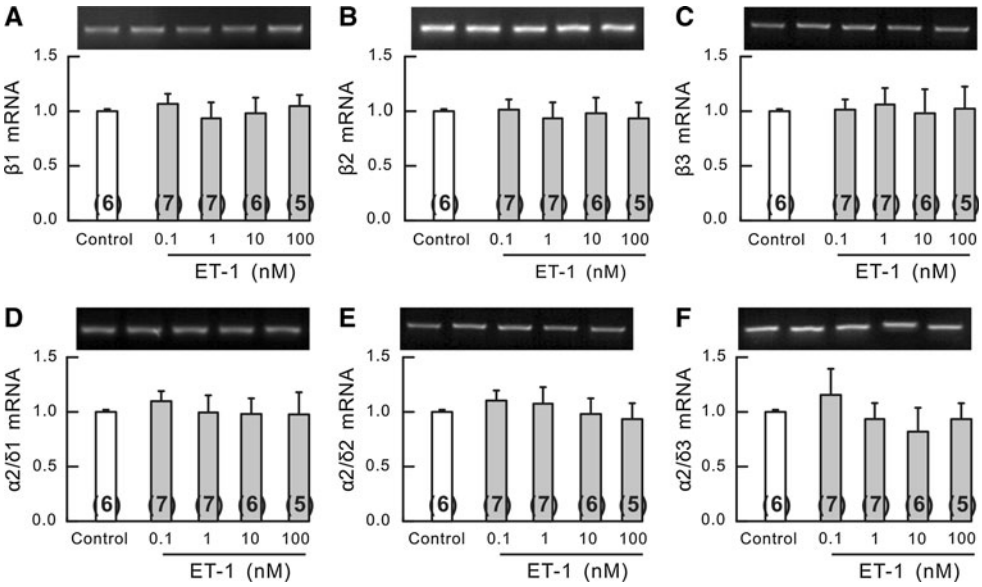


**Fig. 2** Effects of ET-1 on L-type $Ca^{2+}$ channel β- and $\alpha_2$/δ-subunit mRNA expression. ET-1 (0.1–100 nM) exposure for 24 h did not alter L-type $Ca^{2+}$ channel β1–3 (**a–c**) and $\alpha_2$/δ1–3 (**d–f**) subunit mRNA in neonatal cardiomyocytes. Data are presented as the mean value ± SEM. The mean value of each control is defined as 1. The number of experiments is indicated in *parentheses*

significantly modified by rupture of the membrane patch by means of the conventional patch-clamp technique (Ichiyanagi et al. 2002). The perforated patch-clamp technique, which maintains the integrity of the physiological intracellular milieu, has been suggested as a more appropriate method for evaluating the drug-induced effects on $I_{Ca,L}$.

### ERK1/2 Signaling Pathway Was Involved in ET-1-Induced Upregulation of $I_{Ca,L}$

It is well known that ET-1 activates the mitogen-activated protein kinase (MAPK) subfamilies such as ERK1/2, c-Jun N-terminal kinase (JNK) and p38 (Sugden and Clerk 2005). In the present study, the ability of ET-1 to induce the phosphorylation of ERK1/2, p38 and JNK in neonatal cardiomyocytes was evaluated by Western blotting. As shown in Fig. 4, ET-1 triggered ERK1/2, p38 and JNK phosphorylation. Subsequently, PD98059, SB203580 and SP600125, inhibitors of ERK1/2, p38 and JNK, respectively, were used to evaluate the precise role of each MAPK in the regulation of $I_{Ca,L}$. To check the specificity of each inhibitor, cardiomyocytes were preincubated for 30 min with inhibitors (10 μM) and then stimulated for 2 h with ET-1 (10 nM). Levels of phosphorylated ERK1/2, p38 and JNK were measured by Western blotting analysis. Pretreatment of cardiomyocytes with PD98059 (10 μM) significantly inhibited ET-1-induced ERK1/2 activation and was without significant effect on p38 and JNK phosphorylation (Fig. 4). SB203580 (10 μM) inhibited ET-1-induced p38 phosphorylation without affecting ERK1/2 and JNK activation, while SP600125 (10 μM) prevented the phosphorylation of JNK after ET-1 stimulation without affecting ERK1/2 and p38 activation.

After having shown that these inhibitors were specific for each MAPK, we used them to evaluate the role of these MAPKs in the modulation of L-type $Ca^{2+}$ channel expression. As shown in Fig. 5, the relative expression of $\alpha_{1C}$-subunit mRNA was upregulated by ET-1 (10 nM) in the presence or absence of SP600125 (10 μM) and SB203580 (10 μM). Importantly, expression of $\alpha_{1C}$-subunit mRNA was not altered by SB203580 or SP600125 alone. However, cotreatment with PD98059 (10 μM) markedly blocked ET-1-induced upregulation of $\alpha_{1C}$-subunit mRNA (Fig. 5). These results suggested that ET-1 enhanced functional expression of $I_{Ca,L}$ channels by means of ERK1/2 pathway activation.

To further confirm the interaction of the ERK1/2 signaling pathway on ET-1 modulation of nifedipine-sensitive $I_{Ca,L}$, some electrophysiological experiments were carried out. Figure 6a–c exhibits representative nifedipine-sensitive $I_{Ca,L}$ traces and their modulation by ET-1 in the presence or absence of three different MAPK inhibitors. The p38 MAPK inhibitor (SB203580) and the JNK inhibitor (SP600125) did not affect ET-1 stimulation of $I_{Ca,L}$ (Fig. 6d, e). However, as shown in Fig. 6f, ET-1 failed to increase nifedipine-sensitive $I_{Ca,L}$ in the presence of PD98059, suggesting that ET-1-induced $I_{Ca,L}$ upregulation was mediated by ERK1/2 activation. In addition, we showed that $I_{Ca,L}$ density remained unchanged with each MAPK inhibitor alone. These electrophysiological findings were highly consistent with our RT-PCR data in Fig. 5. Therefore, we concluded that the ERK1/2-dependent signaling pathway was involved in ET-1-induced $I_{Ca,L}$ upregulation in neonatal cardiomyocytes.

## Discussion

In neonatal ventricular myocytes, ET-1 increased the transcriptional expression of L-type $Ca^{2+}$ channel $\alpha_{1C}$-subunit and $I_{Ca,L}$ density, which were both mediated by the $ET_A$ receptor.

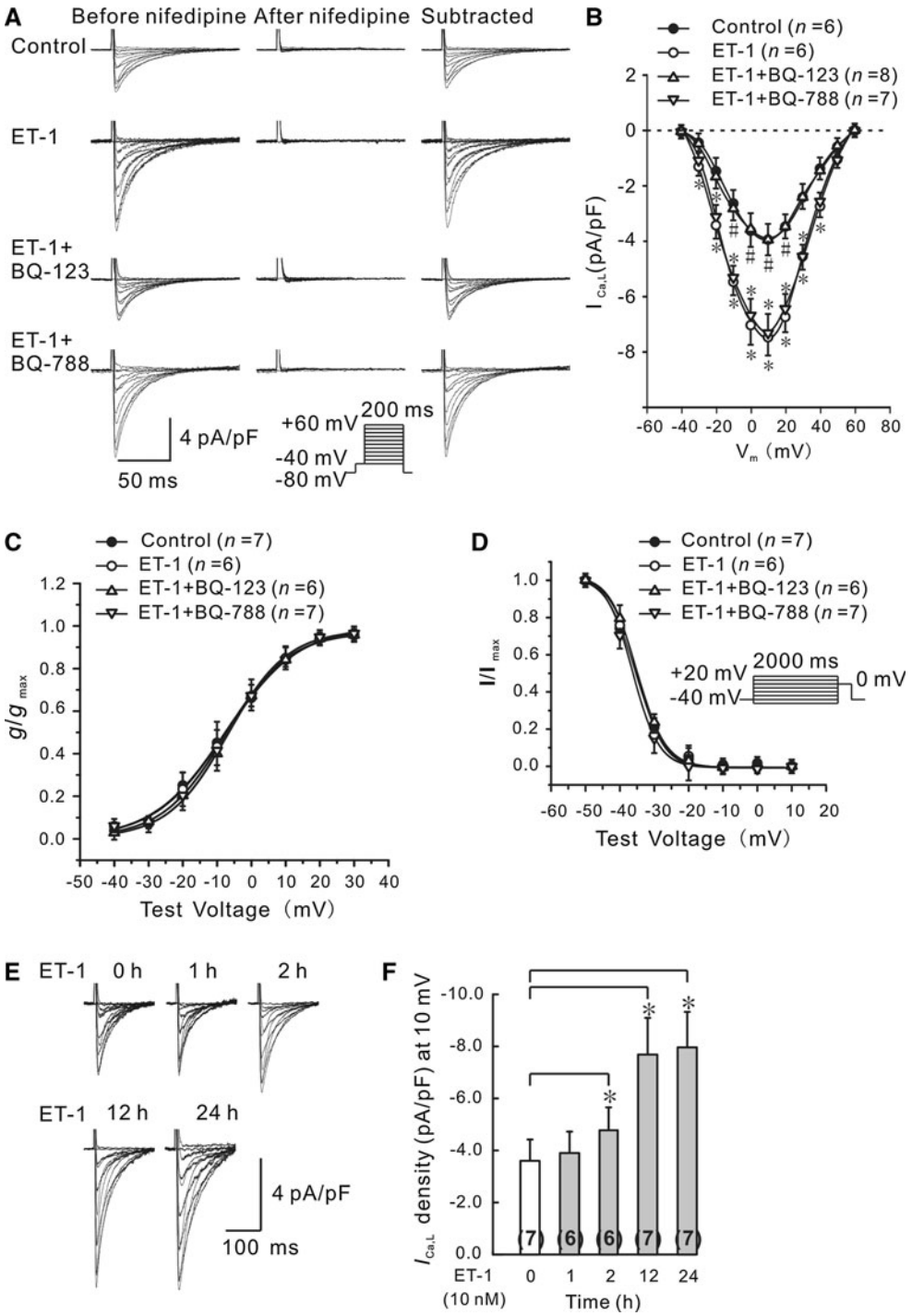


**Fig. 3** Effects of ET-1 on $I_{Ca,L}$ density, activation and inactivation parameters. **a** Typical $I_{Ca,L}$ traces in control (*top*), 10 nM ET-1 (*middle-up*), 10 nM ET-1 plus 1 μM BQ-123 (*middle-down*) and 10 nM ET-1 plus 1 μM BQ-788 (*bottom*) treatment for 24 h. $I_{Ca,L}$ was measured as the nifedipine-sensitive current. The voltage protocol is shown in the *inset*. **b** Averaged current density–voltage relationships of peak nifedipine-sensitive $I_{Ca,L}$ in response to various depolarizing pulses in control and 10 nM ET-1, 10 nM ET-1 plus 1 μM BQ-123 and 10 nM ET-1 plus 1 μM BQ-788 treatments for 24 h. **c** Normalized activation curves obtained from the current–voltage (*I–V*) curves shown in **b**. **d** Normalized inactivation curves. The voltage protocol is shown in the *right inset*. The corresponding kinetic parameters describing the voltage dependence of activation and inactivation are shown in Table 2. **e** Representive nifedipine-sensitive $I_{Ca,L}$ traces for time-dependent effects of ET-1 (10 nM). **f** A time-course analysis of nifedipine-sensitive $I_{Ca,L}$ amplitudes at 10 mV in cardiomyocytes after ET-1 (10 nM) treatment for the indicated times. Data are presented as the mean value ± SEM. $^{*}P < 0.05$ versus control, $^{\#}P < 0.05$ versus ET-1. *n* represents the number of experiments

**Table 2** Kinetic parameters of $I_{Ca,L}$ activation and inactivation

| | Activation | | | Inactivation | | |
|---|---|---|---|---|---|---|
| | $V_a$ | $k_a$ | *n* | $V_i$ | $k_i$ | *n* |
| Control | –6.7 ± 0.7 | 8.3 ± 0.6 | 7 | –34.6 ± 1.4 | 4.2 ± 0.7 | 7 |
| ET-1 | –7.6 ± 1.0 | 9.2 ± 1.3 | 6 | –36.2 ± 2.2 | 5.0 ± 0.8 | 6 |
| BQ-123 | –7.0 ± 0.9 | 7.9 ± 0.7 | 6 | –33.8 ± 1.9 | 5.2 ± 0.8 | 6 |
| BQ-788 | –6.0 ± 0.9 | 9.6 ± 0.8 | 7 | –37.5 ± 1.8 | 4.0 ± 0.6 | 7 |

In addition, ERK1/2 activation was critical for the ET-1-induced increase in transcription of the $\alpha_{1C}$-subunit gene.

## ET-1 Might Increase Transmembranous $I_{Ca,L}$ Current through Stimulating the Expression of the L-Type $Ca^{2+}$ Channel $\alpha_{1C}$-Subunit

Although several studies have demonstrated that ET-1 increases $I_{Ca,L}$ through direct phosphorylation of the

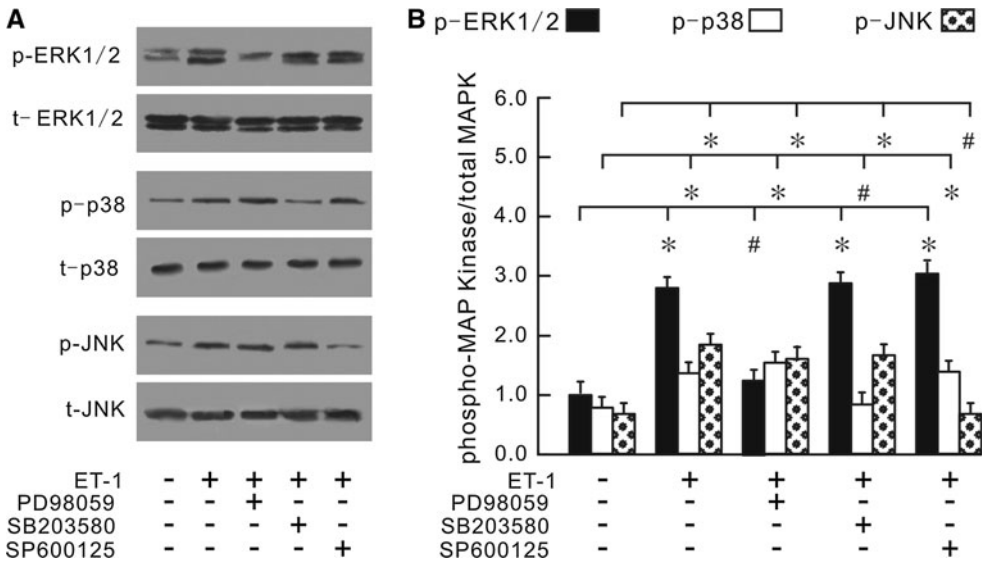


**Fig. 4** Specific inhibition of MAPK activation by PD98059, SB203580 and SP600125. **a** Western blotting bands showing total and phosphorylated ERK1/2, p38 and JNK. **b** Densitometric analysis of effects of blockers on phosphorylation of ERK1/2, p38 and JNK. Each band was normalized to β-actin. Data are presented as the mean value ± SEM of six to eight independent trials. $^{*}P < 0.05$ versus control, $^{\#}P < 0.05$ versus ET-1

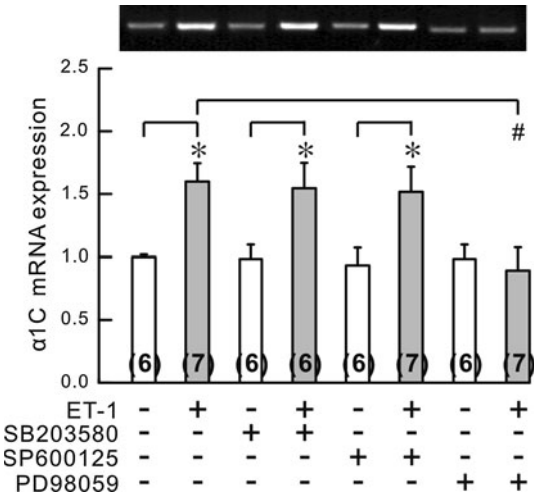


**Fig. 5** Upregulation of L-type $Ca^{2+}$ channel $\alpha_{1C}$-subunit mRNA by 10 nM ET-1 was prevented by 10 μM PD98059 but not by 10 μM SB203580 or 10 μM SP600125. *Inset* Agarose gel electrophoresis showing amplified fragments. Data are presented as the mean value ± SEM. The mean value of control group is defined as 1. $^{*}P < 0.05$ versus each control, $^{\#}P < 0.05$ versus ET-1. *Numbers* in *parentheses* indicate the number of experiments

channel in cardiomyocytes (He et al. 2000), transcriptional regulation of the $I_{Ca,L}$ channel by ET-1 has never been reported.

In the present study, we demonstrated that ET-1 increased expression of the L-type $Ca^{2+}$ channel $\alpha_{1C}$-subunit in neonatal cardiomyocytes but did not affect expression of the L-type $Ca^{2+}$ channel β- and $\alpha_2/\delta$-subunits. The $\alpha_{1C}$-subunit forms the membrane pore and voltage sensor and is the major determinant of channel identity and pharmacology, whereas the other subunits modulate the voltage dependence, kinetics of activation and inactivation and current amplitude (Benitah et al. 2010; Letts et al. 1998). Meanwhile, we showed that $I_{Ca,L}$ density in ET-1-treated cardiomyocytes was increased but that the voltage dependence of activation and inactivation and the kinetics of $I_{Ca,L}$ inactivation did not change. The transcriptional inhibitor actinomycin D completely blocked ET-1 stimulation of $\alpha_{1C}$-subunit mRNA. Therefore, the increase in $I_{Ca,L}$ density may be due to ET-1-induced transcriptional activation of the pore-forming $\alpha_{1C}$-subunit gene rather than an increase of mRNA stability.

## ERK1/2 Was Involved in the ET-1-Induced Upregulation of $I_{Ca,L}$ in Neonatal Cardiomyocytes

ERK1/2 is known to modulate the expression of genes involved in cardiac cell growth, proliferation (Li et al. 2011), differentiation (Zhai et al. 2007) and apoptosis (Yeh et al. 2010); and ERK1/2 activation has been reported in several cardiac diseases such as cardiac hypertrophy (Lorenz et al. 2009), heart failure (Takeishi et al. 2001) and myocardial infarction (Yeh et al. 2010). This study is the first to implicate ERK1/2 signaling in modulation of the L-type $Ca^{2+}$ channel. We demonstrated in this study that ET-1 treatment of cardiomyocytes stimulated ERK1/2 activity, resulting in upregulation of $I_{Ca,L}$ channel expression. Furthermore, the ERK1/2 pathway has been reported to regulate the expression of other ion channels in cardiomyocytes (Jia and Takimoto 2006; Marni et al. 2009) and other cell types (Dey et al. 2011; Mustafa et al. 2008). In addition, other hypertrophic factors, such as angiotensin II, isoprenaline and aldosterone, reportedly activate the ERK1/2 pathway (Lu et al. 2012; Okoshi et al. 2004; Tang et al. 2011) and regulate ion channels (Alvin et al. 2011; Carrillo et al. 2011; Martin-Fernandez et al. 2009) in cardiomyocytes. Thus, the results of our study and others suggest that ERK1/2 may occupy a central regulatory

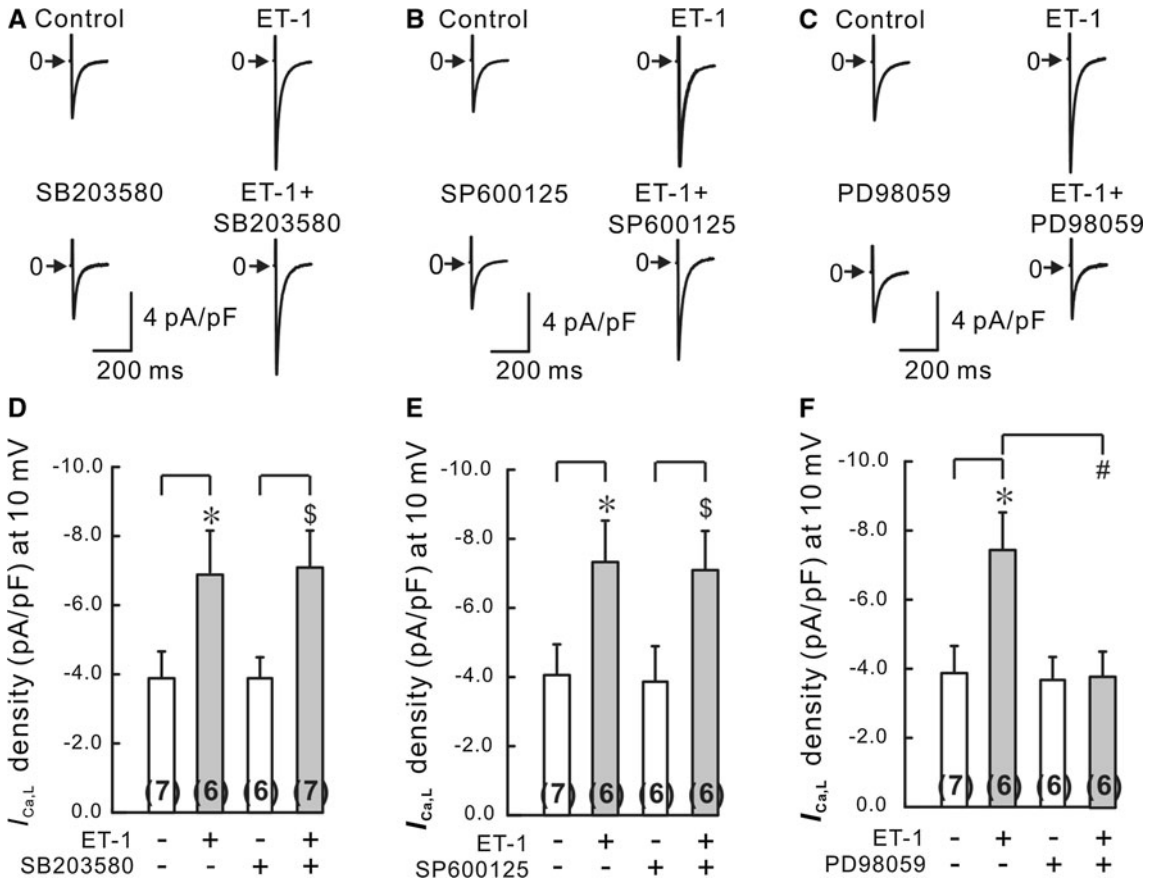


**Fig. 6** Effects of ET-1 and three different MAPK inhibitors on $I_{Ca,L}$ density. **a–c** Representative nifedipine-sensitive $I_{Ca,L}$ traces at 10 mV from neonatal cardiomyocytes treated with vehicle and ET-1 (10 nM) with or without the following MAPK inhibitors for 24 h: **a** SB203580 (10 μM), **b** SP600125 (10 μM), **c** PD98059 (10 μM). **d–f** Summarized data for nifedipine-sensitive $I_{Ca,L}$ amplitudes at 10 mV in vehicle and ET-1 (10 nM) treatment for 24 h with or without SB203580 (**d**), SP600125 (**e**) and PD98059 (**f**). Data are presented as the mean value ± SEM. $^{*}P < 0.05$ versus control, $^{\$}P < 0.05$ versus SB203580 or SP600125, $^{\#}P < 0.05$ versus ET-1. *Numbers* in *parentheses* indicate the number of experiments

position in the signaling mechanism of ion channel remodeling of the diseased heart.

## Direct Phosphorylation and Enhanced Expression of the L-Type $Ca^{2+}$ Channel: An Apparent Paradox of ET-1 Actions on Cardiomyocytes

The results presented here demonstrate that ET-1 stimulates the expression of cardiomyocyte L-type $Ca^{2+}$ channels, which seems to contradict previous findings that ET-1 increases $I_{Ca,L}$ through direct phosphorylation of L-type $Ca^{2+}$ channels (He et al. 2000). A possible explanation for this apparent paradox is that different PKC isoforms might mediate these actions of ET-1. These include conventional (cPKC-α, cPKC-β), novel (nPKC-δ, nPKC-ε) and atypical (aPKC-λ, aPKC-ζ) isoforms. These different PKC isoforms can independently phosphorylate $\alpha_{1C}$-subunit $Ser^{1928}$, a common phosphorylation site for PKA and PKC (Yang et al. 2005). Phosphorylation of $\alpha_{1C}$ is believed to lead to increased channel activity. Among these PKC isoforms, novel PKC isoforms (nPKC-δ, nPKC-ε) have been demonstrated to be activated by ET-1 through the $ET_A$ receptor in neonatal cardiomyocytes (Clerk et al. 1994; Heidkamp et al. 2001). ET-1 did not induce activation of cPKC-α and aPKC-ζ (Clerk et al. 1994). Activation of nPKC-δ and nPKC-ε reportedly led to ET-1-induced activation of the downstream ERK1/2 pathway (Clerk et al. 1994; Heidkamp et al. 2001). Thus, our results and the above findings prompted us to speculate that novel PKC isoforms (nPKC-δ, nPKC-ε) might mediate these different actions of ET-1. On the one hand, ET-1 might increase the activity of the L-type $Ca^{2+}$ channel through direct phosphorylation by different PKC isoforms. On the other hand, chronic ET-1 treatment might also stimulate gene expression of the L-type $Ca^{2+}$ channel through novel PKC isoform–mediated activation of the ERK1/2 pathway. Because novel PKC isoforms are activated through $Ca^{2+}$-independent pathways (Mackay and Mochly-Rosen 2001), direct phosphorylation of L-type $Ca^{2+}$ channels might not be responsible for the subsequent increase in expression of L-type $Ca^{2+}$ channels. Future studies

examining whether PKC-dependent mechanisms contribute to ET-1-stimulated expression of the L-type $Ca^{2+}$ channel in cardiomyocytes should provide important information regarding intracellular signaling.

In conclusion, the results of this study have elucidated that functional activity and expression of the L-type $Ca^{2+}$ channel is upregulated by ET-1 treatment in neonatal cardiomyocytes, which is $ET_A$ receptor–dependent, and that this regulation is mediated by the ERK1/2 pathway. ET-1 acts to upregulate the L-type $Ca^{2+}$ channel in cardiomyocytes to potentially promote L-type $Ca^{2+}$ channel–associated electrical remodeling and arrhythmias in diseased hearts. Furthermore, previous work of Solti et al. (1998) showed that preconditioning with the $Ca^{2+}$ channel blocker verapamil prevented ET-1-induced characteristic ventricular tachycardias and ventricular fibrillation in anesthetized dogs, which supports a key role of the L-type $Ca^{2+}$ channel in ET-1-associated arrhythmias. In addition to disease, our findings are of physiological relevance. The ET-1 signaling system is important for heart development (Asai et al. 2010; Brand et al. 2002). In fact, several components of the ET-1 signaling system are involved in cardiac development (Brand et al. 2002; Kurihara et al. 1995) and differentiation (Chen et al. 2010; Gassanov et al. 2004). The corresponding effects might involve induction of cardiac-specific genes including the L-type $Ca^{2+}$ channel. Developmental changes in the function and expression of the L-type $Ca^{2+}$ channel have been demonstrated in rabbit cardiomyocytes during postnatal development (Huang et al. 2006). Increased gene expression of endothelin receptors has been reported in the murine heart after birth (Adur et al. 2003). Within this context, our present study can be interpreted to suggest that ET-1 may contribute to the electrophysiological maturation of cardiomyocytes during postnatal development. It will be interesting to investigate the potential effects of ET-1 on the functional expression of ion channels in neonatal cardiomyocytes in the near future. Our findings also highlight an additional action of ET-1 that is directly linked to transcriptional regulation of the cardiovascular system.

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**Conflict of Interest** No conflicts of interest are declared by the authors.

## References

Adur J, Takizawa S, Quan J et al (2003) Increased gene expression and production of murine endothelin receptors after birth. Biochem Biophys Res Commun 305:700–706

Alvin Z, Laurence GG, Coleman BR et al (2011) Regulation of L-type inward calcium channel activity by captopril and angiotensin II via the phosphatidyl inositol 3-kinase pathway in cardiomyocytes from volume-overload hypertrophied rat hearts. Can J Physiol Pharmacol 89:206–215

Asai R, Kurihara Y, Fujisawa K et al (2010) Endothelin receptor type A expression defines a distinct cardiac subdomain within the heart field and is later implicated in chamber myocardium formation. Development 137:3823–3833

Avila G, Medina IM, Jimenez E et al (2007) Transforming growth factor-beta1 decreases cardiac muscle L-type $Ca^{2+}$ current and charge movement by acting on the Cav1.2 mRNA. Am J Physiol Heart Circ Physiol 292:H622–H631

Benitah JP, Alvarez JL, Gomez AM (2010) L-type $Ca^{2+}$ current in ventricular cardiomyocytes. J Mol Cell Cardiol 48:26–36

Brand M, Kempf H, Paul M et al (2002) Expression of endothelins in human cardiogenesis. J Mol Med (Berl) 80:715–723

Carrillo ED, Escobar Y, Gonzalez G et al (2011) Posttranscriptional regulation of the beta2-subunit of cardiac L-type $Ca^{2+}$ channels by microRNAs during long-term exposure to isoproterenol in rats. J Cardiovasc Pharmacol 58:470–478

Cerbai E, Pino R, Sartiani L et al (1999) Influence of postnatal-development on *I*(f) occurrence and properties in neonatal rat ventricular myocytes. Cardiovasc Res 42:416–423

Chen M, Lin YQ, Xie SL et al (2010) Mitogen-activated protein kinase in endothelin-1-induced cardiac differentiation of mouse embryonic stem cells. J Cell Biochem 111:1619–1628

Clerk A, Bogoyevitch MA, Anderson MB et al (1994) Differential activation of protein kinase C isoforms by endothelin-1 and phenylephrine and subsequent stimulation of p42 and p44 mitogen-activated protein kinases in ventricular myocytes cultured from neonatal rat hearts. J Biol Chem 269:32848–32857

Colecraft HM, Alseikhan B, Takahashi SX et al (2002) Novel functional properties of $Ca^{2+}$ channel beta subunits revealed by their expression in adult rat heart cells. J Physiol 541:435–452

Cullingford TE, Markou T, Fuller SJ et al (2008) Temporal regulation of expression of immediate early and second phase transcripts by endothelin-1 in cardiomyocytes. Genome Biol 9:R32

De Jongh KS, Warner C, Catterall WA (1990) Subunits of purified calcium channels. Alpha 2 and delta are encoded by the same gene. J Biol Chem 265:14738–14741

Dey D, Shepherd A, Pachuau J et al (2011) Leukemia inhibitory factor regulates trafficking of T-type $Ca^{2+}$ channels. Am J Physiol Cell Physiol 300:C576–C587

Fink C, Ergun S, Kralisch D et al (2000) Chronic stretch of engineered heart tissue induces hypertrophy and functional improvement. FASEB J 14:669–679

Garjani A, Wainwright CL, Zeitlin IJ et al (1995) Effects of endothelin-1 and the $ET^A$-receptor antagonist, BQ123, on ischemic arrhythmias in anesthetized rats. J Cardiovasc Pharmacol 25:634–642

Gassanov N, Er F, Zagidullin N et al (2004) Endothelin induces differentiation of ANP-EGFP expressing embryonic stem cells towards a pacemaker phenotype. FASEB J 18:1710–1712

Gaur N, Rudy Y, Hool L (2009) Contributions of ion channel currents to ventricular action potential changes and induction of early afterdepolarizations during acute hypoxia. Circ Res 105:1196–1203

Geshi E, Nomizo A, Arata Y et al (1999) Effect of non-selective endothelin blockade, TAK-044, on the ischemic cellular injury of rat heart. Basic Res Cardiol 94:94–101

Grandy SA, Trepanier-Boulay V, Fiset C (2007) Postnatal development has a marked effect on ventricular repolarization in mice. Am J Physiol Heart Circ Physiol 293:H2168–H2177

Hamill OP, Marty A, Neher E et al (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 391:85–100

He JQ, Pi Y, Walker JW et al (2000) Endothelin-1 and photoreleased diacylglycerol increase L-type $Ca^{2+}$ current by activation of protein kinase C in rat ventricular myocytes. J Physiol 524(Pt 3): 807–820

Heidkamp MC, Bayer AL, Martin JL et al (2001) Differential activation of mitogen-activated protein kinase cascades and apoptosis by protein kinase C epsilon and delta in neonatal rat ventricular myocytes. Circ Res 89:882–890

Hiraoka M, Kawano S (1984) Regulation of delayed afterdepolarizations and aftercontractions in dog ventricular muscle fibres. J Mol Cell Cardiol 16:285–289

Huang J, Xu L, Thomas M et al (2006) L-type $Ca^{2+}$ channel function and expression in neonatal rabbit ventricular myocytes. Am J Physiol Heart Circ Physiol 290:H2267–H2276

Ichiyanagi O, Ishii K, Endoh M (2002) Angiotensin II increases L-type $Ca^{2+}$ current in gramicidin D–perforated adult rabbit ventricular myocytes: comparison with conventional patch-clamp method. Pflugers Arch 444:107–116

Jia Y, Takimoto K (2006) Mitogen-activated protein kinases control cardiac KChIP2 gene expression. Circ Res 98:386–393

Kiesecker C, Zitron E, Scherer D et al (2006) Regulation of cardiac inwardly rectifying potassium current $I_{K1}$ and Kir2.x channels by endothelin-1. J Mol Med (Berl) 84:46–56

Kirkby NS, Hadoke PW, Bagnall AJ et al (2008) The endothelin system as a therapeutic target in cardiovascular disease: great expectations or bleak house? Br J Pharmacol 153:1105–1119

Kurihara Y, Kurihara H, Oda H et al (1995) Aortic arch malformations and ventricular septal defect in mice deficient in endothelin-1. J Clin Invest 96:293–300

Letts VA, Felix R, Biddlecome GH et al (1998) The mouse stargazer gene encodes a neuronal $Ca^{2+}$-channel gamma subunit. Nat Genet 19:340–347

Li P, Cavallero S, Gu Y et al (2011) IGF signaling directs ventricular cardiomyocyte proliferation during embryonic heart development. Development 138:1795–1805

Lorenz K, Schmitt JP, Vidal M et al (2009) Cardiac hypertrophy: targeting Raf/MEK/ERK1/2-signaling. Int J Biochem Cell Biol 41:2351–2355

Lu M, Wang H, Yang Y et al (2012) The function of calcineurin and ERK1/2 signal in the antihypertrophic effects of kappa-opioid receptor stimulation on myocardial hypertrophy induced by isoprenaline. Pharmazie 67:182–186

Mackay K, Mochly-Rosen D (2001) Localization, anchoring, and functions of protein kinase C isozymes in the heart. J Mol Cell Cardiol 33:1301–1307

Marni F, Wang Y, Morishima M et al (2009) 17 Beta-estradiol modulates expression of low-voltage-activated $Ca_V3.2$ T-type calcium channel via extracellularly regulated kinase pathway in cardiomyocytes. Endocrinology 150:879–888

Martin-Fernandez B, Miana M, De Las Heras N et al (2009) Cardiac L-type calcium current is increased in a model of hyperaldosteronism in the rat. Exp Physiol 94:675–683

Matsumoto Y, Aihara H, Yamauchi-Kohno R et al (2002) Long-term endothelin a receptor blockade inhibits electrical remodeling in cardiomyopathic hamsters. Circulation 106:613–619

Ming Z, Nordin C, Aronson RS (1994) Role of L-type calcium channel window current in generating current-induced early afterdepolarizations. J Cardiovasc Electrophysiol 5:323–334

Mustafa SB, Castro R, Falck AJ et al (2008) Protein kinase A and mitogen-activated protein kinase pathways mediate cAMP induction of alpha-epithelial $Na^+$ channels (alpha-ENaC). J Cell Physiol 215:101–110

Okoshi MP, Yan X, Okoshi K et al (2004) Aldosterone directly stimulates cardiac myocyte hypertrophy. J Card Fail 10:511–518

Reisner Y, Meiry G, Zeevi-Levin N et al (2009) Impulse conduction and gap junctional remodelling by endothelin-1 in cultured neonatal rat ventricular myocytes. J Cell Mol Med 13:562–573

Schneider MP, Boesen EI, Pollock DM (2007) Contrasting actions of endothelin ET(A) and ET(B) receptors in cardiovascular disease. Annu Rev Pharmacol Toxicol 47:731–759

Solti F, Toth M, Merkely B et al (1998) Verapamil reduces the arrhythmogenic effect of endothelin. J Cardiovasc Pharmacol 31(Suppl 1):S386–S387

Stocker PJ, Bennett ES (2006) Differential sialylation modulates voltage-gated $Na^+$ channel gating throughout the developing myocardium. J Gen Physiol 127:253–265

Sugden PH, Clerk A (2005) Endothelin signalling in the cardiac myocyte and its pathophysiological relevance. Curr Vasc Pharmacol 3:343–351

Takeishi Y, Huang Q, Abe J et al (2001) Src and multiple MAP kinase activation in cardiac hypertrophy and congestive heart failure under chronic pressure-overload: comparison with acute mechanical stretch. J Mol Cell Cardiol 33:1637–1648

Tang W, Wei Y, Le K et al (2011) Mitogen-activated protein kinases ERK 1/2- and p38-GATA4 pathways mediate the Ang II–induced activation of FGF2 gene in neonatal rat cardiomyocytes. Biochem Pharmacol 81:518–525

Wang Z, Kutschke W, Richardson KE et al (2001) Electrical remodeling in pressure-overload cardiac hypertrophy: role of calcineurin. Circulation 104:1657–1663

Wang Y, Tandan S, Cheng J et al (2008) $Ca^{2+}$/calmodulin-dependent protein kinase II–dependent remodeling of $Ca^{2+}$ current in pressure overload heart failure. J Biol Chem 283:25524–25532

Webster KA, Discher DJ, Bishopric NH (1993) Induction and nuclear accumulation of fos and jun proto-oncogenes in hypoxic cardiac myocytes. J Biol Chem 268:16852–16858

Xiao YF, McArdle JJ (1994) Elevated density and altered pharmacologic properties of myocardial calcium current of the spontaneously hypertensive rat. J Hypertens 12:783–790

Yang L, Liu G, Zakharov SI et al (2005) Ser 1928 is a common site for Cav1.2 phosphorylation by protein kinase C isoforms. J Biol Chem 280:207–214

Yeh CC, Li H, Malhotra D et al (2010) Distinctive ERK and p38 signaling in remote and infarcted myocardium during post-MI remodeling in the mouse. J Cell Biochem 109:1185–1191

Yorikane R, Koike H (1990) The arrhythmogenic action of endothelin in rats. Jpn J Pharmacol 53:259–263

Yuan W, Ginsburg KS, Bers DM (1996) Comparison of sarcolemmal calcium channel current in rabbit and rat ventricular myocytes. J Physiol 493(Pt 3):733–746

Zeng Q, Li X, Zhong G et al (2009) Endothelin-1 induces intracellular [$Ca^{2+}$] increase via $Ca^{2+}$ influx through the L-type $Ca^{2+}$ channel, $Ca^{2+}$-induced $Ca^{2+}$ release and a pathway involving $ET_A$ receptors, PKC, PKA and $AT_1$ receptors in cardiomyocytes. Sci China C Life Sci 52:360–370

Zhai P, Gao S, Holle E et al (2007) Glycogen synthase kinase-3 alpha reduces cardiac growth and pressure overload–induced cardiac hypertrophy by inhibition of extracellular signal–regulated kinases. J Biol Chem 282:33181–33191