Endothelin-1 Regulates Cardiac L-Type Calcium Channels via NAD(P)H Oxidase-Derived Superoxide

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Received April 22, 2008; accepted June 4, 2008

ABSTRACT

It has been shown that reactive oxygen species (ROS) are involved in the intracellular signaling response to G-protein coupled receptor stimuli in vascular smooth muscle cells and in neurons. In the present study, we tested the hypothesis that NAD(P)H oxidase-derived ROS are involved endothelin-1 (ET-1)-induced L-type calcium channel activation in isolated cardiac myocytes. ET-1 (10 nM) induced a 2-fold increase in L-type calcium channel open-state probability (NPo). This effect of ET-1 was abolished by the ETA receptor antagonist cyclo(D-Trp-D-Asp-Pro-D-Val-Leu) [BQ-123 (1 μM)] but was not altered in the presence of an ETB receptor antagonist N-cis-2,6-dimethylpyridinocarbonyl-b-[Bu-Ala-D-Trp(1-methoxy carbonyl)-D-Nle-OH] [BQ-788 (1 μM)]. Pretreatment of cells with the ROS scavenger tempol (100 μM), polyethylene glycol-superoxide dismutase (SOD, 25 U/ml), or the NAD(P)H-oxidase inhibitor gp91ds-tat ([H]RKKRRQRRR-CSTRIRRQL[NH3]) (5 μM) significantly attenuated ET-1-induced increases in calcium channel NPo. Tempol, SOD, and gp91ds-tat alone had no effect on basal calcium channel activity. In addition, ET-1 significantly increased NAD(P)H oxidase activity and elevated intracellular superoxide levels in cultured cardiac myocytes. The superoxide generator, xanthine-xanthine oxidase (10 mM, 20 mU/ml), also increased calcium channel NPo in cardiac myocytes, mimicking the effect of ET-1. These observations provide the first evidence that ET-1 induces the activation of L-type Ca2+ channels via stimulation of NAD(P)H-derived superoxide production in cardiac myocytes.
characterization of $I_{Ca,L}$ without the alteration of intracellular components that occurs with whole-cell patch clamp technique. Therefore, it is essential to examine the effect of ET-1 on $I_{Ca,L}$ and the underlying mechanism in intact cardiac myocytes.

Reactive oxygen species (ROS) are well recognized as important mediators of cardiovascular pathology, including hypertrophy and heart failure (Dhalla et al., 2000; Byrne et al., 2003b). Both mitochondria and NAD(P)H oxidase are capable of participating directly in the reduction of oxygen and producing superoxide. In vascular tissue the NAD(P)H oxidase is recognized as the predominant source of superoxide (Griendling et al., 2000). Recent studies are providing evidence for a role for NAD(P)H oxidase-derived ROS in cardiac pathology. Mice lacking the pg91phox subunit do not develop angiotensin II-induced cardiac hypertrophy (Byrne et al., 2003a). With the exception of the importance of ROS in cardiac pathophysiology, it is still not clear whether NAD(P)H oxidase-derived ROS play a role in the rapid response to ET-1 in cardiac myocytes. Identifying the mechanisms responsible for the action of ET-1 could help identify a new therapeutic target for ET-1-associated cardiac disorders.

In this study, we examined whether ET-1 increases intracellular ROS production via increased NAD(P)H-oxidase activity and whether the elevated ROS subsequently modulates the function of L-type calcium channels in cardiac myocytes.

**Materials and Methods**

**Animals and Drugs.** Adult male Sprague-Dawley rats (200–250 g) were obtained from Charles River Farms (Wilmington, MA). Rats were housed individually and kept on a 12/12-h light/dark cycle in a climate-controlled room. Rat chow and water were provided ad libitum. All animal protocols were approved by the Institutional Animal Care and Use Committee of Northeast Normal University and by the North Dakota State University Institutional Animal Care and Use Committee.

Dihydroethidium (DHE) was purchased from Molecular Probes (Carlsbad, CA). Dulbecco’s modification of Eagle’s medium was obtained from GibCo (Carlsbad, CA). Bay K 8644 was purchased from Calbiochem (San Diego, CA). The selective NADPH oxidase inhibitor gp91ds-tat ([H]RKKRRQRRR-CSTRIRRQL[NH$_3$]) and its control, scrambled gp91ds-tat ([H]RKKRRQRRR-CLRTRQR[S][NH$_3$]), were synthesized by Tufts University Core Facility (Medford, MA). BQ-123, BQ-788, ET-1, ATP, GTP, HEPES, and others were purchased from Sigma-Aldrich (St. Louis, MO).

**Ventricular Myocytes Isolation.** Cardiac myocytes were enzymatically isolated from hearts obtained from rats anesthetized with sodium pentobarbitone (30 mg/kg i.p. injection). Each heart was rapidly excised and cooled in ice-cold Tyrode’s solution containing Na$_2$CO$_3$. The potential of the electrode was adjusted to zero before using for electrophysiological recording.

**Measurement of Intracellular ROS Level.** ROS generation was determined using the oxidant-sensitive fluorogenic probe DHE (excitation wavelength, 488 nm; emission wavelength, 610 nm) essentially as described elsewhere (Hoel et al., 2005). In brief, primary cultured cardiomyocytes were loaded with 100 nM DHE for 30 min at 37°C. The cells were then incubated with PBS for 5 min, followed by 10 nM ET-1 for 5 min. In another set of cardiomyocytes, cells were pretreated with BQ-123 (1 μM) or vehicle control for 10 min before ET-1 treatment. Ethidium fluorescence within cardiomyocytes was detected by a fluorescent microscope (Nikon, Melville, NY), and its intensity in individual cells was analyzed using Quantity One Software (Bio-Rad, Hercules, CA). Each treatment condition was run in triplicate within experiments, and each set of experiments was performed using three separate culture dishes.

**Measurement of NAD(P)H Oxidase Activity.** The lucigenin-derived chemiluminescence method was used to measure ET-1-induced NAD(P)H oxidase activity in primary cultured cardiomyocytes. Cardiomyocytes were treated with ET-1 (10 nM) or PBS for 5 min and washed in ice-cold PBS, and cells were scraped and then sonicated for 1 s. Ten minutes before recording luminescence, NAD(P)H (100 μM) and lucigenin (5 μM) were added and light emission was recorded during the next 10 s by a Wallace 1450 MicroBeta JET Lumimeter (PerkinElmer Life and Analytical Sciences, Waltham, MA). Protein concentrations were determined using a Bio-Rad protein assay kit with bovine serum albumin standards. Data were presented as counts per minute per milligram of protein.

**Data Analysis.** All data are expressed as mean ± S.E. Comparisons between experimental groups were performed using analysis of variance followed by a Newman-Keuls test. Differences were considered significant at $P < 0.05$.

**Results**

ET-1 Stimulates L-type Calcium Channel. ET-1 has positive inotropic and chronotropic actions in a variety of cardiac preparations (Kramer et al., 1991). To investigate the role of $I_{Ca,L}$ in these responses to ET-1, we used cell-attached patch-clamp single channel recording to assess the effect of ET-1 on $I_{Ca,L}$ in isolated rat cardiac myocytes without disrup-
tion of intracellular environment. Application of ET-1 (10 nM) evoked a significant increase in the calcium channel NPo (Fig. 1A and B). The channel open time was increased from $2.95 \pm 0.33$ to $4.74 \pm 0.42$ ms ($n = 11$, $P < 0.05$), and the channel close time was decreased from $88.40 \pm 14.73$ to $35.31 \pm 12.41$ ms ($n = 11$, $P < 0.05$) by application of ET-1 (10 nM). However, the perfusion of cardiomyocytes with ET-1 (10 nM) did not significantly alter the unitary current amplitude, as shown in Fig. 1A ($1.42 \pm 0.11$ and $1.40 \pm 0.18$ pA in cells before and after treatment with ET-1, respectively, $n = 11$, $P > 0.05$). The increases in NPo of IC$_{CaL}$ evoked by ET-1 were partially reversed after 10-min washout with fresh bath solution (from $0.021 \pm 0.002$ to $0.017 \pm 0.002$, $n = 4$, $P < 0.05$; the baseline NPo was $0.014 \pm 0.03$). In another series of experiments, the addition of a specific IC$_{CaL}$ activator Bay K 8644 (100 nM) significantly increased NPo of IC$_{CaL}$, without changing the unitary current amplitude, mimicking the effect of ET-1 on this channel in cardiac myocytes (Fig. 1, C and D). The Bay K 8644-induced increases in NPo of IC$_{CaL}$ were completely reversed after 10-min washout with fresh bath solution (from $0.026 \pm 0.03$ to $0.017 \pm 0.01$, $n = 4$, $P < 0.05$; the baseline NPo was $0.015 \pm 0.02$).

It has been shown that both ET$_A$ and ET$_B$ receptors are expressed in cardiomyocytes (Wainwright et al., 2005). Thus, we examined the endothelin-receptor subtype(s) involved in the L-type calcium current response to ET-1 by pretreatment of the cells with specific receptor antagonists. Data are shown in Fig. 2A, indicating that the ET$_A$ receptor antagonist BQ-123 (1 $\mu$M) completely blocked the ET-1-induced increase in calcium channel NPo. By contrast, the ET$_B$ receptor antagonist BQ-788 (1 $\mu$M) did not alter the calcium channel responsiveness to ET-1 under the same treatment conditions. In these experiments, neither BQ-123 (1 $\mu$M) nor BQ-788 (1 $\mu$M) alone significantly changed the basal calcium channel activity.

**NAD(P)H Oxidase-Derived ROS Are Involved in IC$_{CaL}$ Activity to ET-1**. Initially, we used tempol, a superoxide scavenger, to determine the role of ROS in ET-1-induced increases in L-type calcium channel NPo in freshly isolated cardiac myocytes. Figure 2B shows that, under control conditions, ET-1 (10 nM) caused a 2-fold increase in NPo of IC$_{CaL}$ and that treatment with tempol (100 $\mu$M) resulted in an 80% attenuation of the ET-1-induced increase in NPo of IC$_{CaL}$ ($P < 0.05$ compared with ET-1 alone). However, in the presence of tempol (100 $\mu$M), Bay K 8644 (100 nM) increased the NPo of IC$_{CaL}$ from $0.014 \pm 0.002$ to $0.026 \pm 0.004$ ($n = 6$, $P < 0.01$), indicating that tempol has no effect on Bay K 8644-induced IC$_{CaL}$. Tempol (100 $\mu$M) alone did not significantly change the basal calcium channel activity in isolated cardiac myocytes (Fig. 2B). Next, we used gp91ds-tat to confirm the involvement of ROS and further to identify the source of ROS in the ET-1 action. Pretreatment of cardiomyocytes with gp91ds-tat (5 $\mu$M) significantly attenuated ET-1-induced increases in NPo of IC$_{CaL}$ ($P < 0.05$, compared to ET-1 alone; Fig. 2B). In the presence of gp91ds-tat (5 $\mu$M), Bay K 8644 (100 nM) increased the NPo of IC$_{CaL}$ from $0.015 \pm 0.01$ to $0.025 \pm 0.03$ ($n = 6$, $P < 0.01$). In contrast, scrambled gp91ds-tat (5 $\mu$M) did not alter the ET-1-induced activation of IC$_{CaL}$ under the same treatment conditions (data not shown). Neither gp91ds-tat nor scrambled gp91ds-tat alone had any significant effect on the basal IC$_{CaL}$ activity in cardiac myocytes.
ET-1 Increases ROS Production in Cardiomyocytes.

The fluorogenic probe, DHE, was used to assess the effect of ET-1 on ROS production in primary cultured cardiomyocytes. Under control conditions, ethidium fluorescence was low in PBS-treated cardiomyocytes (Fig. 3B); however, treatment of these same cells with 10 nM ET-1 resulted in a significant increase in the density of ethidium fluorescence within cardiomyocytes (Fig. 3, C and G). The ET-1-induced increase in fluorescence was completely inhibited by the ETA endothelin receptor antagonist BQ-123 (1 μM; Fig. 3, E and G). BQ-123 (1 μM) alone did not alter the basal ethidium fluorescence (Fig. 3, E and G). In contrast, the ETB endothelin receptor antagonist BQ-788 (1 μM) had no significant effect on this action of ET-1 (Fig. 3G). In addition, the ET-1-induced increase in ethidium fluorescence was attenuated by gp91ds-tat (5 μM) (845 ± 61 and 926 ± 77 ethidium fluorescence intensity of cells treated with gp91ds-tat and gp91ds-tat ET-1, respectively, n = 13, P < 0.05). However, the scrambled control gp91ds-tat did not alter the stimulatory action of ET-1 on ROS production (751 ± 79 and 1522 ± 98 ethidium fluorescence intensity of cells treated with scrambled control gp91ds-tat and ET-1, respectively, n = 13, P < 0.01).

**Fig. 2.** Role of ETA receptor, ETB receptor, and superoxide in ET-1-induced increases in ICaL in isolated cardiomyocytes. A, bar graphs showing the effect of an ETA receptor antagonist, BQ-123 (BQ123, 1 μM), and an ETB receptor antagonist, BQ-788 (BQ788, 1 μM), on ET-1-induced increases in ICaL in cell-attached patches. Data are mean ± S.E. channel open probability in cardiomyocytes (n = 7 and 8) treated under the following conditions: Control, ET-1 (10 nM), BQ-123 or BQ-788, and BQ-123 + ET-1 or BQ-788 + ET-1. *, P < 0.05 compared with the respective control. B, bar graphs showing the effect of a superoxide scavenger, tempol (TP, 100 μM), and a NAD(P)H oxidase inhibitor, gp91ds-tat (GP, 5 μM), on ET-1-induced increases in ICaL in cell-attached patches. Data are mean ± S.E. of channel open probability in cardiomyocytes (n = 9 and 7) treated under the following conditions: control, ET-1 (10 nM), TP or GP, and TP + ET-1 or GP + ET-1. *, P < 0.05 compared with control.

**Fig. 3.** Effects of ET-1 on superoxide production within cardiomyocytes. Superoxide levels were detected using the fluorogenic probe DHE in primary cultured cardiomyocytes as detailed under Materials and Methods. A through C, cardiomyocytes that were treated under the following conditions: cardiomyocytes in normal optical phase (A); fluorescence micrograph of cardiomyocytes loaded with DHE and treated with PBS (B); and fluorescence micrograph of the same cardiomyocytes as shown in B (C), following treatment with ET-1 (10 nM). D through F, cardiomyocytes that were treated under the following conditions: cardiomyocytes in normal optical phase (D); fluorescence micrograph of cardiomyocytes loaded with DHE and treated with PBS (B); and fluorescence micrograph of the same cardiomyocytes as shown in B (C), following treatment with ET-1 (10 nM). Bar = 20 μm. G, bar graphs summarizing ethidium fluorescence intensity before and after treatment with ET-1 in the presence of vehicle control (PBS), BQ-123 (BQ123, 1 μM), or BQ-788 (BQ788, 1 μM). In each treatment condition, 20 to 23 cardiomyocytes were used for quantification of fluorescence. They were derived from three experiments and at least three dishes in each experiment. Data are mean ± S.E. *, significant difference from the respective control (P < 0.01).
fluorescence intensity of cells treated with scrambled gp91ds-tat and scrambled gp91ds-tat + ET-1, respectively, \( n = 11, \ P < 0.01 \). These results indicate that, via activation of \( \text{ET}_A \) receptors, ET-1 increases superoxide production, which is blocked by inhibition of NAD(P)H oxidase.

**ET-1 Stimulates NAD(P)H Oxidase in Cardiomyocytes.** Treatment of primary cultured cardiomyocytes with ET-1 (10 nM) resulted in a 2-fold increase in NAD(P)H oxidase activity (Fig. 4A). This effect of ET-1 was completely blocked by preincubation of cardiomyocytes with the \( \text{ET}_A \) endothelin receptor antagonist BQ-123 (1 \( \mu \)M). However, the \( \text{ET}_B \) endothelin receptor antagonist, BQ-788 (1 \( \mu \)M), has no effect on ET-1-induced increases in NAD(P)H oxidase activity (Fig. 4A). In addition, preincubation of cardiomyocytes with gp91ds-tat (5 \( \mu \)M), but not with scrambled gp91ds-tat (5 \( \mu \)M), significantly attenuated the ET-1-induced activation of NAD(P)H oxidase (Fig. 4B).

**Superoxide Contributes to ET-1-Induced ICaL Activation.** To identify whether superoxide contributes to ET-1-induced ICaL activation, we recorded ICaL activity in the presence or absence of a cell-permeable superoxide scavenger, PEG-SOD (25 U/ml), in isolated cardiac myocytes. The results are shown in Fig. 5A, indicating that pretreatment of cardiomyocytes with PEG-SOD (25 U/ml) significantly attenuated ET-1-induced ICaL activation and that PEG-SOD alone had no effect on the basal ICaL activity. In addition, in the presence of PEG alone, ET-1 (10 nM) still increased ICaL NPo from 0.012 ± 0.002 to 0.019 ± 0.003 (\( n = 5, \ P < 0.01 \)), indicating that PEG itself has no effect on ET-1-induced ICaL activation. Moreover, superfusion of cardiomyocytes with a superoxide generator, xanthine-xanthine oxidase, also increased ICaL activity, mimicking the effects of ET-1 (Fig. 5A). These results suggest that superoxide is involved in the ET-1-induced ICaL activation in cardiomyocytes. Next, we examined the role of \( \text{H}_2\text{O}_2 \) in the action of ET-1 on ICaL in cardiac myocytes. Pretreatment of cells with PEG-catalase (250 U/ml) did not significantly alter ET-1-induced ICaL activation. Superfusion of cardiomyocytes with \( \text{H}_2\text{O}_2 \) (1 \( \mu \)M) had no effect on ICaL. Taken together, these data suggest that the stimulatory effect of ET-1 on ICaL is mediated by intracellular superoxide production in cardiac myocytes.

**Discussion**

The present studies provides the first evidence that ET-1-induced activation of L-type calcium channels (ICaL) is mediated by NAD(P)H oxidase-derived calcium production in cardiac myocytes. This conclusion is supported by the following observations that 1) ET-1 increased the open-state probability of ICaL recorded in cell-attached patches of cardiac myocytes and that this effect of ET-1 was diminished by a specific \( \text{ET}_A \) receptor antagonist; 2) the ET-1-induced increase in ICaL was attenuated by the superoxide scavengers, tempol and PEG-SOD; 3) superfusion of cardiac myocytes with a superoxide generator, xanthine-xanthine oxidase, also increased ICaL activity, mimicking the action of ET-1; 4) incubation of cultured cardiomyocytes with ET-1 increased intracellular superoxide levels and NAD(P)H oxidase activity; and 5) pretreatment of cardiac myocytes with the NAD(P)H oxidase inhibitor, gp91ds-tat, attenuated ET-1-induced superoxide production and increases in ICaL open-state probability.

ET-1 has a positive inotropic effect in many isolated cardiac preparations (Kramer et al., 1991). It is well known that an increase in the intracellular calcium transient contributes to this positive inotropic effect and that the basis for the increased \( \text{Ca}^{2+} \) transient is stimulation of ICaL. The results of the present study are consistent with this hypothesis, inasmuch as ET-1 caused an increase in the open-state probability of ICaL in rat cardiac myocytes. However, previous investigations examining the effect of ET-1 on ICaL have produced conflicting results, with decreases (Lauer et al., 1992; Boixel et al., 2001), increases (Cheng et al., 1995; Ono et al., 1995),

![Fig. 4. Effects of ET-1 and gp91ds-tat on NAD(P)H oxidase activity in cardiomyocytes. A, effect of ET-1 and receptor involvement. Primary cultured cardiomyocytes were pretreated under the following conditions: vehicle control, BQ-123 (BQ123 1 \( \mu \)M), or BQ-788 (BQ788 1 \( \mu \)M) for 10 min. This was followed by incubation with 10 nM ET-1 for an additional 5 min. Cells were collected, and NAD(P)H activity was measured and expressed as mean light emission (counts per milligram of protein per minute). Data are mean ± S.E. (\( n = 7 \), *), significantly different from respective control treatment \( (P < 0.05) \). B, effect of gp91ds-tat. Primary cultured cardiomyocytes were pretreated under the following conditions: vehicle control, gp91ds-tat (GP, 5 \( \mu \)M), or scrambled gp91ds-tat control (ScrGP, 5 \( \mu \)M) for 10 min before treatment with 10 nM ET-1 for 5 min as described in A. Data are mean ± S.E. (\( n = 9 \), *), significantly different from respective control \( (P < 0.05) \).](https://jpet.aspetjournals.org)
and no effect (Tohse et al., 1990) on basal $I_{Ca,L}$ reported. These discrepancies may be due to differences in experimental conditions, particularly differences in the electrophysiological recording methods used (e.g., rupture of the cell membrane). One important reason for the discrepant findings regarding the effects of ET-1 on $I_{Ca,L}$ in cardiac myocytes may be related to the expression of ET receptors. Cardiac myocytes express both ET$_A$ and ET$_B$ endothelin receptors (Moe et al., 2003; Wainwright et al., 2005). In general, the ET$_A$ receptor subtype is more abundant (90%) and is considered more important for the cardiac stimulatory effects of ET-1 on $I_{Ca,L}$ as demonstrated in the present study. Although ET$_B$ receptors may not contribute to the action of ET-1 on $I_{Ca,L}$, as shown in the present study in rat cardiac myocytes, they may contribute to inhibitory effects of ET-1 (Kedzierski and Yanagisawa, 2001). Thus, the ratio between ET$_A$ and ET$_B$ receptors in cardiac myocytes from different animal species could play a role in the observed action of ET-1 on $I_{Ca,L}$. Another important reason for these discrepant findings may be related to the interaction with other regulatory factors and intracellular second messengers, such as cyclic AMP, β-adrenergic receptor agonists, protein kinase A, and protein kinase C (PKC). It has been shown that the effect of ET-1 on cardiac contractility and L-type calcium current varies depending on the presence of norepinephrine, β-adrenergic receptor agonists, or cAMP analogs (Watanabe and Endoh, 1999; Chu et al., 2003). For example, in canine ventricular myocardium, ET-1 induces either a positive inotropic effect or a negative inotropic effect, which is determined by the presence of a low concentration or high concentration of norepinephrine, respectively. Moreover, in rabbit cardiomyocytes, ET-1 has a biphasic effect on calcium current, causing a transient decrease followed by a long-lasting increase in current (Watanabe and Endoh, 1999). The ET-1-induced inhibition of L-type calcium current was enhanced in the presence of an activated cAMP-dependent pathway. This interaction between ET-1 and norepinephrine in the regulation of cardiomyocyte contractility and calcium channel current could play an important role in the pathophysiology of heart failure, which is associated with increased sympathetic nerve activity and elevated plasma norepinephrine levels. Nevertheless, studying the effect of ET-1 on calcium channel current under different neurotransmotional conditions will help improve our understanding of the cellular mechanisms underlying the pathogenesis of cardiac diseases, such as heart failure and cardiac remodeling.

The present observations demonstrate that ET-1 increases intracellular superoxide levels by stimulation of NAD(P)H oxidase and that increased superoxide production is involved in the stimulatory effect of ET-1 on $I_{Ca,L}$ in cardiac myocytes. Although superoxide may be rapidly converted to hydrogen peroxide, it is unlikely that the stimulatory effect of ET-1 on $I_{Ca,L}$ is mediated by hydrogen peroxide because the hydrogen peroxide scavenger, catalase, had no effect on the response to ET-1 and authentic hydrogen peroxide itself had no effect on $I_{Ca,L}$. These results are supported by data from others in vascular smooth muscle cells showing that ET-1 increases superoxide production via ET$_A$-receptor/NAD(P)H oxidase pathways (Callera et al., 2003; Li et al., 2003). Given that extracellular calcium influx plays essential roles in ET-1-induced vascular contraction and cell proliferation, it is not surprising that the ET-1-ROS signaling pathway is involved in the action of other cardiovascular regulatory factors, such as angiotensin II-induced positive inotropic effects, leptin-induced cardiac hypertrophy, and atrial natriuretic peptide-mediated antihypertrophic effects (Xu et al., 2004; Cingolani et al., 2006; Laskowski et al., 2006). However, the nature of the intracellular signaling mechanisms by which ROS increases $I_{Ca,L}$ in cardiac myocytes remains to be clarified. One possibility may be a direct redox-dependent mechanism, because the pore-forming α$_{1C}$ subunit of the cardiac L-type Ca$^{2+}$ channel contains more than 10 cysteine residues (Mikami et al., 1989) that can potentially undergo redox modification. Indeed, oxidation of Src homology groups causes stimulation of $I_{Ca,L}$, whereas GSH and dithiothreitol, which reduce disulfide bonds, inhibit this current in ferret ventricular myocytes (Campbell et al., 1996).

Another major question that arises from our studies con-
cerns the mechanisms that are involved in the ET-1-stimu-
lated NAD(P)H oxidase activation in cardiac myocytes. At
present, little is known with regard to such mechanisms in
cardiac myocytes. However, several clues are emerging from
other research areas that may provide future direction for
addressing this question. Activation of PKC is involved in
ET-1-induced increases in cardiac ICaL via an unknown
mechanism (He et al., 2000), and PKC-dependent NAD(P)H
oxidase activation is the main source of intracellular ROS
production in response to angiotensin II in vascular smooth
muscle cells (Touyz and Schiffrin, 2001; Yasunari et al., 2002;
Ungvari et al., 2003) and in neurons (Wang et al., 2006).
Therefore, future studies examining whether PKC-mediated
phosphorylation of NAD(P)H oxidase contributes to the ET-
1-induced ROS elevation in cardiac myocytes should provide
important information regarding intracellular signaling.

In summary, ET-1 increases ICaL activation via stimula-
tion of ETA receptors in cardiac myocytes. The ET-1-induced
elevation in cardiac ICaL is mediated by NAD(P)H oxidase-derived
superoxide production. Given that activation of L-type cal-
cium channels is involved in ET-1-related cardiac pathophys-
iological conditions (Chu et al., 2003; Hirotani et al., 2004;
Angerio, 2005; Sugden and Clerk, 2006), ROS-dependent ac-
tivation of ICaL may contribute to ET-1-induced intracellular
calcium mobilization, cardiac excitation-contraction, myocyte
proliferation, and consequently disorders, such as heart fail-
ure and cardiac hypertrophy.

References

559.
Boixel C, Dinanian S, Lang-Lazdunski L, Mercier JD, and Hatem SN (2001)
Characterization of effects of endothelin-1 on the L-type Ca2+
Byrne JA, Grieve DJ, Bendall JK, Li JM, Gove C, Lambeth JD, Cave AC, and Shah
AM (2003a) Contrasting roles of NAD(P)H oxidase isoforms in pressure-overload
Byrne JA, Grieve DJ, Cave AC, and Shah AM (2003b) Oxidative stress and heart
Callera GE, Touyz RM, Texeira SA, Moseuna NR, Carvalho MH, Fortes ZB, Nigro
D, Schiffrin EL, and Tostes RC (2003) ET1 receptor blockade decreases vascular
channels in ferret ventricular myocytes. Dual mechanism regulation by
Cheng TH, Chang CY, Wei J, and Lin CI (1995) Effects of endothelin 1 on calcium and
sodium currents in isolated human cardiac myocytes. Can J Physiol Pharma-
coL 73:1774–1783.
Chu L, Takahashi R, Norota I, Miyamoto T, Takeishi Y, Ishii K, Kubota I, and Endoh
95:829–834.
Cingolani HE, Villa-Abrille MC, Cornelli M, Nolly A, Ennism IL, Garciarena C,
Morana MT, and Martin U (2006) No evidence of transdifferentiation of human endothelial
progenitor cells into cardiomyocytes after coculture with neonatal rat cardiomyo-
dicylglycerol increase L-type Ca2+
current by activation of protein kinase C
Hirotani S, Hayuchi Y, Nishida K, Nakayama H, Yamaguchi O, Hikosso S, Takeda K,
Pyk2/CARK beta-dependent signaling is essential for G-protein-coupled receptor
Hool LC, Di Maria CA, Visla HM, and Arthur PG (2005) Role of NAD(P)H oxidase in the
regulation of cardiac L-type Ca2+
Ito H, Hirota Y, Adachi S, Tanaka M, Tsujino M, Koike A, Nogami A, Murumo F, and
Hirse M (1995) Endothelin-1 is an autocrine/paracrine factor in the mechanism of
angiogenesis II-induced hypertrophy in cultured rat cardiomyocytes. J Clin Invest
95:396–403.
Ito H, Hirota Y, Hiroe M, Tsujino M, Adachi S, Takamoto T, Nitta M, Taniguchi K,
and Marumo F (1991) Endothelin-1 induces hypertrophy with enhanced expres-
sion of muscle-specific genes in cultured neonatal rat cardiomyocytes. Circ Res
Laskowski A, Woodman OL, Cao AH, Drummond GR, Marshall T, Kaye DM, and
Ritchie RH (2006) Antioxidant actions contribute to the antihypertrophic effects of
Laurer MR, Gunn MD, and Clusin WT (1992) Endothelin activates voltage-dependent
Ca2+
channel by a G protein-dependent mechanism in rabbit cardiac myocytes.
Li L, Fink GD, Watts SW, Northcott CA, Galignij JJ, Pagano PJ, and Chen AF
(2003) Endothelin-1 increases vascular superoxide via endothelin A/NADPH oxidase
Primary structure and functional expression of the cardiac dihydroxypridine-
Moe GW, Rouleau JL, Nguyen QT, Cancee P, and Stewart DJ (2003) Role of
Ono K, Eto K, Sakamoto A, Masaki T, Shibata K, Sada T, Hashimoto K, and
Tsujimoto G (1995) Negative chronotrophic effect of endothelin 1 mediated through
Cell Cardiol 38:877–886.
signaling pathways in cardiac myocytes. Antioxid Redox Signal 8:1111–2124.
signaling: recent advances in cardiovascular medicine. Antioxid Redox Signal
7:929–934.
increase Ca2+
Touyz RM and Schiffrin EL (2001) Increased generation of superoxide by angiotensin
II in smooth muscle cells from resistance arteries of hypertensive patients: role of
phospholipase D-dependent NAD(P)H oxidase-sensitive pathways. J Hypertens
19:1245–1254.
Increased levels of angiotensin II promote oxidant stress in the arterial wall.
Wainwright CL, McCabe C, and Kane KA (2005) Endothelin and the ischaemic
Iadecola C (2006) Nrxz2, Ca2+
and protein kinase C play a role in angiotensin
II-induced free radical production in nucleus tractus solitarius. Hypertension 48:
452–458.
Watanabe T and Endoh M (1999) Characterization of the endothelin-1-induced
regulation of L-type Ca2+
current in rabbit ventricular myocytes. Naunyn
induces hypertrophy via endothelin-1-reactive oxygen species pathway in cultured
Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Koyama M, Mitsu Y, Yazaki Y,
angiogenesis II-mediated migration of human coronary smooth muscle cells

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