Characterization of effects of endothelin-1 on the L-type Ca\(^{2+}\) current in human atrial myocytes

CHRISTOPHE BOIXEL, SYLVIE DINANIAN, LOÎC LANG-LAZDUNSKI, JEAN-JACQUES MERCADIER, AND STEPHANE N. HATEM. Characterization of effects of endothelin-1 on the L-type Ca\(^{2+}\) current in human atrial myocytes. Am J Physiol Heart Circ Physiol 281: H764–H773, 2001.—The effects of endothelin-1 (ET-1) on the L-type Ca\(^{2+}\) current (\(I_{\text{Ca}}\)) were examined in whole cell patch-clamped human atrial myocytes. Depending on the initial current density, ET-1 (10 nM) increased the amplitude of \(I_{\text{Ca}}\) by 99 ± 7% or decreased it by 33 ± 2%. The stimulatory effect predominated on current of low density (2.3 ± 0.2 pA/pF), whereas \(I_{\text{Ca}}\) of higher density (5.8 ± 0.3 pA/pF) was inhibited by ET-1. After \(I_{\text{Ca}}\) stimulation by 1 \(\mu\)M isoproterenol, ET-1 always inhibited the current by 32 ± 7% (\(P < 0.05\)), an effect that was suppressed by pretreating myocytes with pertussis toxin. Atrial natriuretic peptide (ANP) inhibited \(I_{\text{Ca}}\) (41 ± 3%) by reducing intracellular cAMP concentration. In ANP-treated myocytes, the stimulatory effect of ET-1 on \(I_{\text{Ca}}\) predominated (52 ± 7%). The inhibitory effect of ET-1 on \(I_{\text{Ca}}\) was blocked by the ET\(\alpha\) antagonist BQ-123, whereas the stimulatory effect was suppressed by the ET\(\beta\) agonist BQ-788. We conclude that ET-1 has opposite effects on \(I_{\text{Ca}}\), depending on the baseline amplitude of current, and both subtype ET receptors are implicated in the signal transduction pathways.

human cardiac cells; whole cell patch clamp

ENDOTHELIN-1 (ET-1) is a 21-amino acid peptide originally isolated from porcine aortic endothelial cells (42) as a vasoconstricting agent. It has since emerged as one of the main regulators of cardiac function both in physiological conditions and during chronic heart failure, both in humans (40) and in animal models (29, 19). However, its effects on the heart are complex, multiple, and poorly defined. Depending on the species, the stage of ontogenic development and the type of myocyte studied, ET-1 acts as a positive (32, 10) or negative inotropic agent (27). It is reported to accelerate or to fail to accelerate the heart (9, 21) and usually stimulates atrial natriuretic peptide (ANP) secretion by cardiac myocytes (33). ET-1 is also one of the growth factors participating in the hypertrophic response of the heart to changes in its working conditions (31). This diversity of ET-1 actions on the myocardium is largely due to the fact that receptor subtypes are coupled to distinct G protein-linked signal transduction pathways, such as adenyl cyclase and phospholipase-dependent pathways (30), which regulate cardiac function and phenotype by acting on a number of intracellular targets, including ion channels.

In cardiac myocytes, the L-type Ca\(^{2+}\) current (\(I_{\text{Ca}}\)) is the main depolarizing current contributing to the plateau-shape of the action potential and governing Ca\(^{2+}\) release from the sarcoplasmic reticulum (4). Channels carrying \(I_{\text{Ca}}\) are regulated by a number of second messengers, which explains why they mediate the action of a number of hormones and neuromediators on cardiac electrical and contractile properties. This is the case of ET-1, which has been found to modulate \(I_{\text{Ca}}\) in cardiac myocytes. Because inhibition and stimulation of the current have both been described (1, 14, 22, 34, 38, 39), it is possible that the effects of ET-1 on \(I_{\text{Ca}}\) depend on the species, the stage of ontogenic development, the type of myocyte studied, and the experimental conditions (11).

In human atrial myocytes also, \(I_{\text{Ca}}\) is the main current that activates during the plateau phase and that regulates the excitation-contraction process (7). Interestingly, \(I_{\text{Ca}}\) regulation by second messengers differs in several respects between human atrial myocytes and myocytes from other tissues or species, as illustrated by the regulation of \(I_{\text{Ca}}\) by serotonin (23) and, more recently, by its tonic inhibition by tyrosine kinase (2). In human atrial myocytes, phosphodiesterases (PDE) inhibit \(I_{\text{Ca}}\) in the absence of \(\beta\)-adrenergic stimulation, indicating that in this tissue there is a basal production and degradation of cAMP (13, 26). All of these aspects of Ca\(^{2+}\) channel regulation by second messengers in human atrial myocytes may reflect the functional specialization of these cells, for instance, the constitutive capacity of these cells to secrete ANP.
These phenomena may also be due to pathophysiolog-
al conditions associated with changes in hormonal
regulation of the atrial myocardium, as is probably the
case in hemodynamically overloaded atria (16) and
chronic atrial fibrillation (35). It is thus important to
know how ET-1 regulates the functional properties of
human atrial myocardium. It is already known that
both ETA and ET B receptors are expressed in this
tissue (18) and that they are coupled to distinct signal-
ing pathways (36). However, little is known on the
 cellular targets involved in the action of ET-1 on hu-
man atrial myocardium.

The aim of this study was to investigate the regula-
tion of \( I_{Ca} \) by ET-1 in myocytes isolated from surgical
samples of the human right atrial appendage, using
whole cell configuration of the patch-clamp technique
to record ionic currents. We found opposite effects of
the peptide on \( I_{Ca} \) depending on the baseline current
amplitude.

**MATERIALS AND METHODS**

Clinical data and cardiac myocyte preparation. With ap-
proval from our ethics committee, specimens of the right
atrial appendage were obtained from 72 patients (5 to 90 yr,
mean 56 ± 4 yr) undergoing heart surgery, mainly consisting
of coronary bypass surgery (\( n = 40 \)), mitral (\( n = 7 \)) or aortic
(\( n = 18 \)) valve repair, and congenital heart defect repair (\( n = 7 \)).
Except for four cases of atrial fibrillation, all the patients
were in sinus rhythm. Most patients were on pharma-
coli cal treatments that were stopped at least 8 h before surgery
(Ca\(^{2+}\) channel blockers, \( \beta \)-adrenergic antagonists, diuretics,
angiotensin-converting enzyme inhibitors, and nitric oxide
donors). Myocytes were enzymatically isolated as previously
described (2). Briefly, small pieces of atrial appendage were
cut up and washed in calcium-free Krebs-Ringer solution
containing (in mM) 35 NaCl, 4.75 KCl, 1.19 KH\(_2\)PO\(_4\), 1.6
Na\(_2\)HPO\(_4\), 10 HEPES, 10 glucose, 25 NaHCO\(_3\), 134 sucrose,
and 30 2,3-butanedione oxime (BDM) (pH 7.4 adjusted with
NaOH), gassed with 95% O\(_2\)-5% CO\(_2\), and maintained at
37°C. BDM was used transiently to prevent cutting injury
(20). Pieces were reincubated in the same solution with-
out BDM and containing bovine serum albumin (5 mg/ml,
Hoescht-Behring), 200 IU/ml collagenase (type IV, Sigma; St.
Louis, MO), and 6 IU/ml protease (type XXIV, Sigma). After
30 min of digestion, the enzyme solution was replaced by the
same solution containing only collagenase (400 IU/ml) for 15
min. Isolated myocytes were suspended in DMEM and incu-
bated at 37°C with continuous gassing with air supple-
mented with 5% CO\(_2\) for at least 1 h before use.

**Fig. 1.** Opposite effects of endothelin-1 (ET-1) on L-type Ca\(^{2+}\) current (\( I_{Ca} \)). A and B: current traces elicited by a
10-mV incremental test pulse protocol from a holding potential of −60 mV in control conditions (control) and at the
steady-state effect of 10 nM ET-1 in two distinct myocytes (numbers indicate the membrane potential at which
current was recorded). A: \( I_{Ca} \) was inhibited by ET-1 (membrane capacitance 109 pF). B: peptide stimulated the
current (membrane capacitance 80 pF). C and D: current density-voltage relationships of \( I_{Ca} \) in myocytes with
currents stimulated (C) or inhibited (D) by ET-1. Each point is the average current (mean ± SE) density in 10 cells.
*\( P < 0.05 \) and ** \( P < 0.01 \) compared with control.
Solutions and reagents. The composition of the standard external solutions was as follows (in mM): 136 NaCl, 5.4 KCl, 2 CaCl₂, 10 glucose, 1.06 MgCl₂, 0.33 NaH₂PO₄, and 10 HEPES; pH was adjusted to 7.4 with NaOH. In some experiments, NaCl was replaced by equimolar concentration tetraethylammonium chloride and 4 mM CaCl₂ was used instead of 2 mM CaCl₂. Experiments were carried out at room temperature (22–24°C). The pipette solution consisted of (in mM) 130 CsCl, 2 MgCl₂, 10 HEPES, 15 EGTA, 10 glucose, and 3 MgATP; pH was adjusted to 7.2 with CsOH. In some experiments, 0.42 mM Na₂-GTP was added to the pipette solution (26). Use of a multibarrel system allowed us to exchange the fluid solution bathing the myocyte within 2 s. ET-1 (porcine/human) and ANP (human) were dissolved in 1% acetic acid and stored as stock solutions at −20°C until use. BQ-123 and BQ-788 were dissolved in distilled water and stored as stock solutions at −20°C. Pertussis toxin (PTX) was diluted with DMEM at a final concentration of 0.1 mg/ml. Incubation with PTX took place at 37°C for 15 h in the incubator. All drugs were purchased from Sigma except for Iso (Sanofi; Winthrop, France).

Current measurements. Macroscopic calcium currents were recorded using the patch-clamp technique in the whole cell configuration. Borosilicate glass pipettes with a tip resistance of 1–2 MΩ were connected to the input stage of a patch-clamp amplifier (Axoclamp 200A, Axon Instruments). Currents filtered at 5 kHz were digitized by a Labmaster (Lab Rac, Scientific Solution) and stored on the hard disk of a personal computer. Data were acquired and analyzed using a program written for our laboratory (Acquis, G. Sadock, CNRS, Gif/Yvettes). Resistance in series, but not the capacitive or leakage current, was compensated for to obtain the fastest capacity transient current. Membrane capacitance was calculated by using the fit of the capacity transient decay. Depolarizing voltage pulses were delivered at 0.2 Hz. The amplitude of the peak ICa was calculated as the difference between the amplitude of the inward current at its peak and at the end of the 350-ms test pulses.

Measurement of cAMP concentration. Myocytes were washed twice with 1× PBS and stimulated for 20 min with test compounds. Cells were scraped in 250 μl of 0.01 N HCl and frozen in liquid nitrogen until use. Cell extracts were then thawed and sonicated. The lysates were separated by centrifugation (10,000 g, 10 min), and cAMP was measured in the supernatant using a radioimmunoassay kit (Biotrak, Amersham Pharmacia Biotech).

Statistical analysis. Values are expressed as means ± SE. Differences between values were tested for statistical significance by using Student’s paired and unpaired t-tests. Fisher exact test was used to compare the distribution of the effect.
RESULTS

Heterogeneous effects of ET-1 on \( I_{\text{Ca}} \) in human atrial myocytes. Figure 1 shows examples of the effects of external application of 10 nM ET-1 on \( I_{\text{Ca}} \) elicited by 350-ms depolarizing test pulses from −60 to 0 mV. No sodium current was recorded, as illustrated in Fig. 1, which also shows that a sizable current was only observed at potentials more positive than −50 mV; there was no evidence for a T-type \( Ca^{2+} \) current. In 68 of 154 myocytes studied, ET-1 decreased \( I_{\text{Ca}} \) (33 ± 2%, \( n = 68\), \( P < 0.0001 \)), whereas in the remaining myocytes it increased the current (99 ± 7%, \( n = 60\), \( P < 0.001 \)) (Fig. 1, A and B) or had no effect (i.e., the ET-1 effect did not exceed the classic rundown of \( I_{\text{Ca}} \) or the run-up phenomenon sometimes observed at the beginning of the current recording) (\( n = 26 \)). The inhibitory effect of ET-1 on \( I_{\text{Ca}} \) was associated with a 10-mV shift of the current-voltage relationship toward positive potentials (Fig. 1C), whereas the stimulatory effect shifted the current-voltage relationship leftward (Fig. 1D). Neither the inhibitory nor the stimulatory effect of ET-1 was associated with a change in the apparent reversal potential of the current, ruling out major changes in its ionic selectivity. To further eliminate the possibility that part of the ET-1 effects was due to modulation of a contaminating sodium current, in some experiments NaCl was replaced by an equimolar concentration of tetraethylammonium chloride in the external solution. In these conditions, ET-1 still stimulated or inhibited \( I_{\text{Ca}} \) depending on the baseline current amplitude (not shown). Plots of the percent change in \( I_{\text{Ca}} \) in the presence of ET-1 against the current density measured before peptide application (Fig. 2A) showed that the stimulatory effect was observed in cells characterized by an \( I_{\text{Ca}} \) with a relatively low density (2.3 ± 0.1 pA/pF, \( n = 60 \)) and was never observed for a current density of more than 5 pA/pF. The inhibitory effect predominated in cells with relatively high current densities (5.8 ± 0.3 pA/pF, \( n = 68 \)) (Fig. 2B). ET-1 had no effect in 26 myocytes whatever the baseline current density. There was no relationship between the cell size and the response of \( I_{\text{Ca}} \) to ET-1 (Fig. 2C). Similar heterogeneous effects of ET-1 were observed using an external solution containing 2 or 4 mM \( Ca^{2+} \) (\( n = 8 \)). As shown in Table 1, there was no clear relationship between the type of ET-1 effect on \( I_{\text{Ca}} \) and the patient’s clinical history, including \( \beta \)-adrenergic antagonists treatment. This probably reflects the fact that currents of small or high density were not associated with a given clinical parameter, in keeping with published observations that a number of factors can alter \( I_{\text{Ca}} \) including hemodynamic overload of the atria, which is associated with a number of cardiopathies (16, 35).

**ET-1 inhibits \( I_{\text{Ca}} \) prestimulated by Iso.** The aim of the following experiments was to determine the nature of the relationship between the \( I_{\text{Ca}} \) amplitude and its modulation by ET-1. First, we tested the effect of ET-1 in myocytes pretreated with the \( \beta \)-adrenergic agonist Iso (1 \( \mu \)M). As illustrated in Fig. 3, A and B, when \( I_{\text{Ca}} \) was stimulated by Iso (150 ± 13%, \( n = 7\), \( P < 0.01 \)) ET-1 always decreased \( I_{\text{Ca}} \) by 32 ± 7% (5.9 ± 0.7 vs. 9.1 ± 1.1 pA/pF, \( n = 7\), \( P < 0.05 \)). When the current was stimulated by a mechanism not involving \( \beta \)-adrenergic regulation, such as that induced by the dihydropyridine agonist BAY K 8644 (10 \( \mu \)M), which increases \( I_{\text{Ca}} \) to the same extent as Iso, ET-1 only slightly inhibited \( I_{\text{Ca}} \) (Fig. 3A), whereas it suppressed \( I_{\text{Ca}} \) after Iso application to current pretreated with BAY K 8644 (not shown). A similar inhibitory effect of ET-1 was observed when the current was stimulated with the phosphodiesterase inhibitor IBMX (10 \( \mu \)M, \( n = 6 \), Fig. 3C). The effects of ET-1 on \( I_{\text{Ca}} \) prestimulated by Iso were suppressed by pretreating myocytes with 0.1 \( \mu \)g/l PTX (−8 ± 2%, \( n = 7\), \( P < 0.05 \), Fig. 3D). In this experiment, the muscarinic agonist acetylcholine was used to check that G proteins were effectively inhibited by the toxin. Moreover, in the absence of Iso, a stimulatory effect of ET-1 on \( I_{\text{Ca}} \) was observed in 7 of 10 PTX-treated myocytes studied (3.6 ± 1.0 pA/pF in PTX-treated cells). Taken together, these results indi-

### Table 1. Characteristics of patients

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cated that the inhibitory effect of ET-1 occurred when $I_{Ca}$ was stimulated by the β1-adrenergic signaling pathway, an effect mediated at least in part by coupling of the ET receptor to adenylyl cyclase via a G$_i$ protein.

**ANP inhibits $I_{Ca}$ by decreasing intracellular cAMP.** In the following experiment, we examined whether ANP could be used to antagonize the β1-adrenergic effect on $I_{Ca}$ to study the effects of ET-1 on a reduced current. In the absence of GTP in the internal solution, ANP first transiently increased the current and then suppressed it, indicating the GTP dependency of its effect. Thus to optimize the effect of ANP, myocytes were dialyzed with an internal solution containing 0.42 mM GTP (15). As shown in Fig. 4, A and B, in GTP-loaded myocytes, ANP decreased the current by 41 ± 3% (2.2 ± 0.1 pA/pF vs. 4.5 ± 0.3 pA/pF, $n = 48, P < 0.0001$). Because one likely mechanism of the GTP-dependent inhibitory effect of ANP on $I_{Ca}$ involves stimulation of PDE (6), these enzymes were inhibited by using IBMX. Figure 4C shows that IBMX stimulated $I_{Ca}$ (195 ± 30%, $n = 6, P < 0.01$) and prevented the inhibition of $I_{Ca}$ by ANP in GTP-loaded myocytes ($n = 6$). Moreover, ANP totally reversed the increase in $I_{Ca}$ caused by Iso ($n = 5$, Fig. 4D). At the steady state of the ANP effect on $I_{Ca}$ in GTP-loaded myocytes and after the peptide washout, Iso stimulated $I_{Ca}$ with a magnitude significantly higher than that obtained in control myocytes (263 ± 49 vs. 116 ± 24%, $n = 6, P < 0.05$), but $I_{Ca}$ density after Iso application was not different between control and ANP-treated myocytes (10.8 ± 1.5 vs. 8.2 ± 1.5 pA/pF, $n = 6$, not significant) (data not shown). These results indicated that ANP and Iso had opposite effects on cAMP production in human atrial myocytes. Additional evidence that the inhibitory effect of ANP on $I_{Ca}$ resulted in large part...
from the degradation of cAMP was obtained by measuring the concentration of the nucleotide in control myocytes, after 20 min of incubation with ANP and Iso. Figure 5 shows that in control conditions myocytes contained a significant amount of cAMP that varied from 0.06 ± 0.01 to 0.24 ± 0.01 fmol·mg⁻¹·l⁻¹ depending on the atrial specimen studied. Treating myocytes with ANP caused a marked reduction in cAMP content (0.06 ± 0.01 fmol·mg⁻¹·l⁻¹ (n = 11) vs. 0.16 ± 0.01 fmol·mg⁻¹·l⁻¹ (n = 11), P < 0.001), whereas treating myocytes with Iso caused a major accumulation of the nucleotide [0.37 ± 0.06 fmol·mg⁻¹·l⁻¹ (n = 4) vs. 0.16 ± 0.01 fmol·mg⁻¹·l⁻¹ (n = 11), P < 0.05].

**ET-1 stimulates I_{Ca} inhibited by ANP.** Having established that the main effect of ANP in human atrial myocytes is to reduce intracellular cAMP concentration via the stimulation of cGMP-dependent PDE, the effects of ET-1 were tested on I_{Ca} recorded in myocytes pretreated with ANP. ET-1 sometimes decreased (27 ± 3%, n = 13) but most often increased (52 ± 7%, n = 28) I_{Ca} in these conditions (Fig. 6). A typical example of a
stimulatory effect of ET-1 on $I_{\text{Ca}}$ pretreated by ANP is shown in Fig. 6B. Figure 7 summarizes the distribution of the dual effects of ET-1 on $I_{\text{Ca}}$ in the different conditions tested. The stimulatory effect of ET-1 predominated in ANP-treated myocytes relative to control myocytes and Iso-treated myocytes. However, when the distribution of the two effects of ET-1 in ANP-treated myocytes was compared with that in control myocytes characterized by a low $I_{\text{Ca}}$ density (<5 pA/pF) there was no longer a significant difference between the two experimental conditions.

Both $ET_A$ and $ET_B$ receptors mediate the effects of ET-1 on $I_{\text{Ca}}$. BQ-123, a specific $ET_A$ receptor antagonist, was used to study the specificity of the effects of ET-1 on $I_{\text{Ca}}$ and the type of receptor involved. We first examined the inhibitory effect of ET-1 on Iso-prestimulated $I_{\text{Ca}}$ in presence of BQ-123. As illustrated Fig. 8A, on the top of the stimulation of $I_{\text{Ca}}$ by 1 μM Iso, application of BQ-123 (1 μM) prevented its inhibition by ET-1 ($n = 8$). To test the involvement of $ET_A$ receptors in the stimulatory effect of ET-1, we studied the effect of BQ-123 on current inhibited by ANP to enhance the incidence of the stimulatory effect. In these conditions, BQ-123 did not block the stimulatory effect of ET-1 (Fig. 8B), which, in contrast, was suppressed by application of the $ET_B$ receptor antagonist BQ-788 (1 μM, $n = 6$, Fig. 8C). For control experiments, we checked that $I_{\text{Ca}}$ recorded in myocytes isolated from the same sample responded positively to ET-1 after prolonged ANP exposure ($n = 6$). Taken together, these results indicated that $ET_A$ receptors were responsible for the inhibitory effect of ET-1, whereas the stimulatory effect was mediated by the $ET_B$ receptors.

DISCUSSION

The complex effects of ET-1 on the $I_{\text{Ca}}$ of cardiac myocytes of various species is well established and has been attributed to the coupling of ET-1 receptors to different signaling pathways. The new contributions of our study to this field are as follows: 1) this is the first observation of complex and opposite effects of ET-1 on $I_{\text{Ca}}$ in human atrial myocytes; 2) both ET-1 effects appear to depend on regulation of basal $I_{\text{Ca}}$ by β-adrenergic pathways; and 3) whereas the $ET_A$ receptor mediates the inhibitory effect of ET-1 on $I_{\text{Ca}}$ via negative coupling with cAMP-dependent signaling pathway, $ET_B$ mediates the stimulatory effect of the peptide.

In various species and cell types, including human atrial myocytes, application of nanomolar concentration of ET-1 decreases the intracellular cAMP concentration via $G_\text{i}$ protein-mediated inhibition of adenyl cyclase (8, 22). A similar regulatory mechanism prob-
ably accounts for the inhibition of $I_{Ca}$ by ET-1 in human atrial myocytes, because this effect was consistently observed when adenyl cyclase was stimulated by the $\beta_1$-agonist Iso and was blunted in PTX-treated myocytes. Because the incidence of the inhibitory effect of ET-1 on $I_{Ca}$ was not enhanced by pretreating the current with the dihydropyridine agonist BAY K 8644, a direct blocking effect of the peptide on the channel is very unlikely. An antiadrenergic effect of ET-1 on $I_{Ca}$ has already been reported in rabbit (39) and in dog (38) ventricular myocytes. However, because ET-1 inhibits the calcium current stimulated by IBMX, it is possible that its antiadrenergic effect also involves mechanisms distinct from the modulation of cAMP generation, such as protein kinase C (PKC) activation (43), interaction at the level of phosphatase, and/or direct modulation of the channels (39).

The stimulation of $I_{Ca}$ by ET-1 appears to depend on the species (34), the cell type (21, 41), or the presence of intracellular factors easily dialyzed by the patch pipette (11). Although no clear mechanism has been identified to explain the stimulation of $I_{Ca}$ by ET-1, a number of studies point to a role of phospholipase-dependent signaling pathway activation (31). Phospholipases are coupled to ET-1 receptors via PTX-insensitive G proteins and, in response to ET-1 application, activate a number of major second messengers, including diacylglycerol, $\alpha$-myo-inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$], arachidonic acid (5), and PKC, which can directly or indirectly regulate L-type Ca$^{2+}$ channels. For instance, it has been proposed that ET-1 stimulates the Na$^+$/H$^+$ exchanger via a phospholipase C-PKC signaling pathway and, in turn, alkalinizes the intracellular medium (41). In human atrial myocytes too, stimulation of $I_{Ca}$ by ET-1 appears to result from activation of a signaling pathway distinct from that involved in current suppression, mainly because the effect is PTX insensitive. In addition, there is biochemical evidence that, in human right atrial myocardium, ET-1 is coupled to at least two distinct signal transduction pathways leading to adenyl cyclase inhibition and Ins(1,4,5)P$_3$ formation via the stimulation of phospholipase C (36). The lack of convenient pharmacological tools hinders studies of the contribution of these phospholipase-dependent pathways to the effect of ET-1 on $I_{Ca}$ in human atrial myocytes. The main information that our study adds to the characterization of the stimulatory effect of ET-1 on $I_{Ca}$ is that this effect depends indirectly on cAMP-dependent regulation of the Ca$^{2+}$ current. This was first suggested by

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Fig. 8. Two types of ET receptors mediate the effects of ET-1 on $I_{Ca}$. A: time course of $I_{Ca}$ after initial stimulation by Iso (1 μM) and exposure to BQ-123 (1 μM) and ET-1 in the continuous presence of Iso and BQ-123 (membrane capacitance 40 pF). B: time course of changes in $I_{Ca}$ on exposure to ANP plus BQ-123 and then to ET-1 (membrane capacitance 150 pF). C: time course of changes in $I_{Ca}$ on exposure to ANP plus the ETB receptor antagonist BQ-788 (1 μM) and then to ET-1 (membrane capacitance 150 pF).
our observation that ET-1 stimulated currents of low density, an effect associated with a leftward shift of the current-voltage relationship similar to that induced by Iso. It was further supported by the finding that an experimental reduction in the intracellular cAMP concentration by myocyte treatment with ANP markedly increased the frequency of the stimulatory effect relative to the inhibitory effect. Our results indicate that, in human atrial myocytes, ANP decreases intracellular cAMP by activating phosphodiesterase stimulated by cGMP (13). However, it is possible that part of the inhibitory effect of ANP on $I_{Ca}$ also results from coupling of the ANP receptor to a G protein or from activation of cGMP-dependent protein kinases, as previously suggested (15). ANP modulation of the effects of ET-1 did not seem to result from a direct synergistic or cooperative action on a signal transduction pathway but rather from the ability of ANP to decrease intracellular cAMP and, in turn, to maintain the current in a status appropriate for its stimulation by ET-1. This is also consistent with the lack of significant difference in the distribution of the dual effects of ET-1 between ANP-treated myocytes and myocytes with a low density of baseline $I_{Ca}$, i.e., myocytes that were probably already dephosphorylated. When myocytes were phosphorylated by Iso, ANP always decreased $I_{Ca}$, but failed to restore the sensitivity of Ca$^{2+}$ channels to the stimulatory effects of ET-1, suggesting that, when adenyl cyclase is stimulated, ET-1 preferentially inhibits $I_{Ca}$.

This may indicate preferential coupling of ET-1 receptors to PTX-sensitive G protein-dependent regulatory pathways. Our assumption that the variation among myocytes in the density of baseline $I_{Ca}$ could be due to changes in the current phosphorylation status is also supported by previous reports of a basal production of cAMP in human atrial myocytes, unstimulated by peptides and hormones (13, 26). However, in addition to cAMP-dependent regulation of $I_{Ca}$, other mechanisms may contribute to the heterogeneous effects of ET-1 including PKC- (3) or tyrosine kinase-dependent (37, 2) regulatory cascades or alterations in the composition of channel subunits (17, 24), which are also good substrates for intracellular second messengers (25).

In human atrial myocytes, as in most other species (22, 12), the inhibitory effect of ET-1 on $I_{Ca}$ is mainly mediated by type A receptors. In contrast, the increase in $I_{Ca}$ produced by ET-1 appears to be mediated by the ET$_B$ receptor subtype in human atrial myocytes. Previous studies have shown that part of the effects of endothelin on the heart are due to activation of the ET$_B$ receptor subtype. In rabbit ventricular myocytes, ET$_B$ receptors are involved in the anti-adrenergic effect of ET-1 on $I_{Ca}$ (39), whereas they mediate the stimulatory effect of ET-3 on $I_{Ca}$ (12). There is another recent report that, in contrast to rabbit ventricular myocytes (39), ET$_B$ receptor mediated in part the positive inotropic effect of ET-1 in human atria trabeculae (28). Finally, the fact that ET$_A$ receptor mediates the inhibitory effects of ET-1 and that, in human atrial myocytes, this receptor subtype constitutes the majority of endothelin receptors (18) could explain the predominantly inhibitory effect of ET-1 on $I_{Ca}$ observed here.

Conflicting results have been reported on the regulation of cardiac contractility by ET-1, with both positive and negative inotropic effects. These discrepancies are attributed to differences in the species, tissue, or development stage, and in experimental procedures. Our results provide an additional explanation, namely, that the type of Ca$^{2+}$ current response to ET-1 depends on the phosphorylation status of channels and/or the various proteins that regulate $I_{Ca}$ in human atrial myocytes. Opposite inotropic effects of ET-1 have also been observed among rat ventricular myocytes, a finding interpreted as indicating variations in receptors and regulatory pathway activity. In situ, the phosphorylation status of the atrial myocardium is continuously regulated by a subtle balance among neuromediators, peptides and hormones, which may largely determine the effect of ET-1. For instance, in pathological setting characterized by a degree of Ca$^{2+}$ channel dephosphorylation (15, 35), ET-1 may predominantly stimulate $I_{Ca}$. ANP and ET-1 are two major regulators of cardiovascular function and often counteract each other at various levels. Thus it is possible that by increasing $I_{Ca}$ ET-1 prevents excessive suppression of the current caused by prolonged ANP exposure. Further studies of animal models and tissue culture systems should help to determine the significance of these dual effects of ET-1 on atrial myocardial function, including the regulation of ANP secretion by ET-1.

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