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Short communication

Antagonism of β-adrenergic stimulation of L-type Ca^{2+} current by endothelin in guinea-pig atrial cells

Nathalie Delpech, Hélène Soustre *, Daniel Potreau

Laboratoire de Physiologie Générale, CNRS URA 1869, Université de Poitiers, 86022 Poitiers Cedex, France

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Abstract

Experiments carried out with isolated guinea pig atrial cells, using the patch clamp technique, demonstrated that endothelin-1 reversed the increase in L-type Ca^{2+} current (I_{CaL}) induced by isoprenaline. Similar effects of endothelin-1 were observed when I_{CaL} was previously increased by forskolin or 8-bromo-cAMP. These results suggested that the endothelin antagonism of β-adrenergic stimulation of I_{CaL} was exerted independently of the cAMP-dependent phosphorylation pathway.

Keywords: Endothelin-1; Cardiac myocyte; L-type Ca^{2+} current; β-Adrenoceptor agonist; (Guinea-pig)

1. Introduction

Several vasodilating and vasoconstricting substances released by vascular endothelium, such as nitric oxide, angiotensin II, prostacyclin, and endothelin were shown to have direct myocardial effects. It has also been demonstrated that both coronary vascular and endocardial endothelium modulate the contractile characteristics of myocardium (for review see Li et al., 1994). Endothelin-1, a 21-amino-acid peptide isolated from cultured vascular endothelial cells, was reported to be the most potent vasoconstrictor agent. Three distinct isoforms of endothelin have been characterized. They elicit physiological responses by binding to specific membrane receptors coupling with G-proteins (Arai et al., 1990). Endothelin is now known to be synthesized by several mammalian cell types, including coronary and endocardial endothelial cells and cardiac myocytes. Besides coronary vasoconstrictive effects, endothelin-1 has been found to produce inotropic and chronotropic effects in various myocardial preparations and to stimulate the secretion of vasoactive agents from cardiomyocytes (Li et al., 1994). The underlying cellular mechanisms of endothelin cardiac effects, which are far from being completely elucidated, may involve elevation of intracellular Ca^{2+} (Vigne et al., 1990), stimulation of phosphoinositide hydrolysis by phospholipase C (Vigne et al., 1989) leading to the activation of protein kinase C (Hattori et al., 1993), inhibition of adenylate cyclase activity (Hilal-Dandan et al., 1992), and increase in contractile protein sensitivity to intracellular Ca^{2+} (Kelly et al., 1990). These observations suggested that endothelin receptors are coupled to multiple G-proteins, so that endothelin effects may be mediated by different complex signaling pathways and interfere in the cyclic nucleotide-dependent regulation of cardiac activity by neurotransmitters. The present study was undertaken to investigate the influence of endothelin on the cAMP-dependent β-adrenergic regulation of L-type Ca^{2+} channels in isolated guinea pig atrial cells.

2. Materials and methods

Guinea pig single atrial cells were enzymatically isolated, using collagenase (210 U/ml, Worthington, NJ, USA), and elastase (17 U/ml, ICN, Biomedicals, Costa Mesa, CA, USA). Membrane currents were recorded in the whole-cell voltage clamp mode of the patch clamp technique. These methods were described in detail by Petit-Jacques et al. (1993).

* Corresponding author. Laboratoire de Physiologie Générale, Université de Poitiers, 40, av. du Recteur Pineau, 86022 Poitiers Cedex, France.

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I_{CaL} was recorded in Na^+- and K^+-free solutions to suppress Na^+ and K^+ currents. The externally perfused standard cell solution contained (mM): tetraethylammonium chloride 140; CsCl 6; $MgCl_2$ 1; $CaCl_2$ or $BaCl_2$ 1.8; Hepes 10; pH 7.4 (with TEAOH). The pipette solution was (mM): CsCl 120; TEACl 10; $MgCl_2$ 1; Na_2ATP 3; Na_2GTP 0.3; EGTA 10; Hepes 10; pH 7.2 (with CsOH).

All chemicals were purchased from Sigma. Isoprenaline, endothelin and forskolin were prepared as stock solutions and stored at the appropriate temperature. When used they were added to the test solution at the desired final concentration. 8-Bromo-cAMP was dissolved in the standard internal solution at the final concentration of 200 μM.

Cells were stimulated by a double voltage pulse to suppress the T-type Ca^{2+} current and to reduce the possible run-down of I_{CaL}. I_{CaL} was then elicited by a depolarizing step to 0 mV for 200 ms, subsequent to a first depolarization 100 ms in duration from −80 mV to −40 mV.

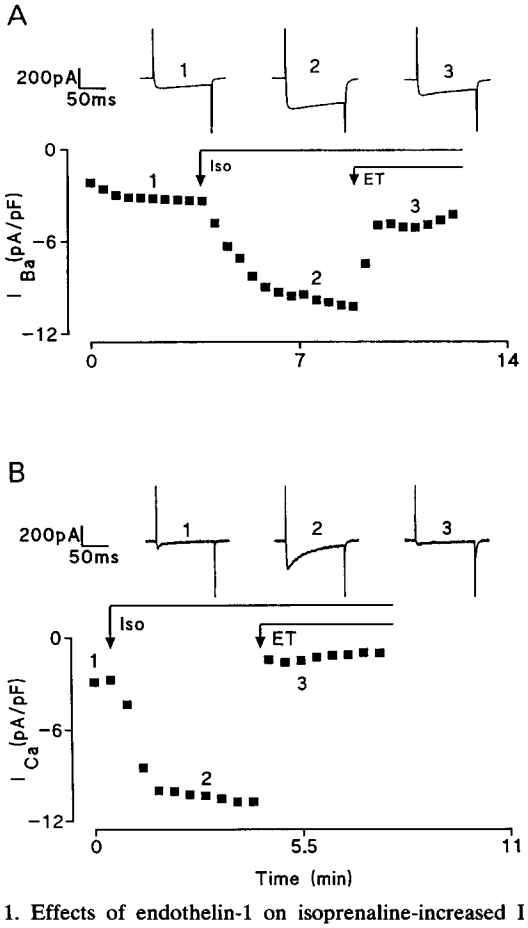

Fig. 1. Effects of endothelin-1 on isoprenaline-increased I_{BaL} (A) and I_{CaL} (B). Graphs represent time course of peak current density estimated from the cell membrane capacity. The cell was initially exposed to control solution, and then to 0.5 μM isoprenaline and finally to 20 nM endothelin-1 in the presence of isoprenaline. Insets: Individual current traces recorded at the times indicated by the corresponding numbers on the graph.

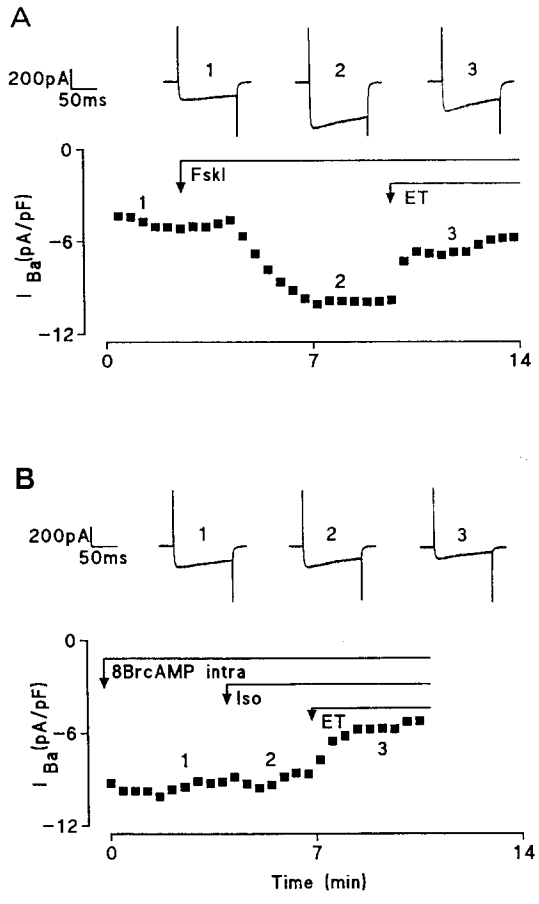

Fig. 2. Effects of endothelin-1 on the increase in I_{BaL} induced by forskolin (A) or 8-bromo-cAMP (B). A: The cell was successively perfused by control solution, 3 μM forskolin and 20 nM endothelin-1 in the presence of forskolin. B: I_{BaL} was recorded about 4 min after breaking patch to allow intracellular diffusion of 8-bromo-cAMP (200 μM). The cell was exposed to 0.5 μM isoprenaline and to 20 nM endothelin-1. Insets A and B: Individual current traces recorded at the times indicated by the corresponding numbers on the graph.

The current amplitude was measured as the difference between peak inward current and zero current. Its amplitude was expressed as a function of membrane capacity (Cm) (pA/pF) for plotting of I_{CaL} versus time. The Cm was estimated from the capacitance transient elicited by a 10 mV depolarizing step from a holding potential value of −80 mV.

3. Results

Fig. 1A shows that 0.5 μM isoprenaline strongly increased L-type current density when Ba^{2+} ions were the charge carriers. This classical increase was rapid and the maximal steady state effect was obtained with about 4 min of isoprenaline superfusion. At this stage, further application of 20 nM endothelin-1 rapidly (25–

50 s) and completely reversed the effect of isoprenaline with a typical initial jump. This result, which was obtained in 12 other cells with Ba^{2+}, was also observed when Ca^{2+} ions were the charge carriers of the current (Fig. 1B). When applied without prestimulation by isoprenaline, endothelin-1 had no effect on I_{CaL} or I_{BaL} (result not shown).

To elucidate the mechanism of action of endothelin-1 on the isoprenaline-induced increase in I_{CaL} amplitude, the endothelin-1 effect was investigated when Ca^{2+} channels were phosphorylated through direct activation of adenylate cyclase by forskolin or activation of protein kinase A by internal application of the non-hydrolysable nucleotidic compound, 8-bromo-cAMP. Fig. 2A demonstrates that the steady state increase of I_{BaL} by 3 μM forskolin was reversed by subsequent addition of 20 nM endothelin-1. This result, similar to the effect of endothelin-1 after β-adrenergic activation of Ca^{2+} channels by isoprenaline, was observed in 4 cells. When I_{BaL} was increased by intracellular application of 8-bromo-cAMP (200 μM in the patch pipette solution) (Fig. 2B), I_{BaL} density was not further affected by an extracellular application of isoprenaline, demonstrating that Ca^{2+} channels were maximally phosphorylated. Under these conditions, application of 20 nM endothelin-1 was still able to diminish I_{BaL} in a way similar to that observed with isoprenaline or forskolin.

4. Discussion

The present study with guinea pig atrial myocytes demonstrated that endothelin-1 had no direct effect on the L-type Ca^{2+} current but reversed its increase induced by β-adrenoceptor agonist stimulation. Endothelin-1 has already been found to have no effect on basal I_{CaL} in guinea pig cardiac cells (Tohse et al., 1990), while other reports have shown that endothelin-1 either increased (Ishikawa et al., 1988) or decreased I_{CaL} (Ono et al., 1994). Such conflicting results thus cannot explain the positive inotropic effect of endothelin-1 consistently observed in guinea pig atrium (Ishikawa et al., 1988; Tohse et al., 1990; Hattori et al., 1993). The increase in I_{CaL} induced by isoprenaline classicaly resulted from the well known activation of the cAMP-dependent phosphorylation pathway. The use of forskolin and 8-bromo-cAMP allowed us to determine whether endothelin-1 could bypass this pathway. Forskolin is known to be a direct activator of adenylate cyclase without a requirement of G-protein; 8-bromo-cAMP, a non-hydrolysable cAMP derivate induced maximal phosphorylation of Ca^{2+} channels, consecutively to a sustained protein kinase A activity. When I_{CaL} was enhanced by either forskolin or 8-bromo-cAMP, endothelin-1 was able to antagonize this increase. Therefore, the endothelin-1 antagonism of β-adrenergic stimulation of I_{CaL} seemed unlikely to be mediated by adenylate cyclase-coupled G-proteins and by protein kinase A inhibition. These results contrasted with observations made with a similar preparation by Ono et al. (1994), who demonstrated that the antagonism of isoprenaline effects on I_{CaL} by endothelin-1 was mediated through cAMP production inhibition. In their experimental conditions, endothelin-1 failed to inhibit the I_{CaL} increased by perfusing cAMP intracellularly; only a transient decrease in I_{CaL} was described consequently to an activation of I_{KAch}. In the present work, activation of I_{KAch} could not account for endothelin-1 inhibition of I_{CaL} enhanced by intracellular 8-bromo-cAMP, because of the presence of Ba^{2+} ions as charge carriers.

Endothelin-1 has also been reported to stimulate phospholipase C and then phosphoinositide hydrolysis, resulting in increased production of inositol trisphosphate and diacylglycerol (Hattori et al., 1993). Consequently, it may be assumed that endothelin-1-induced elevation of the intracellular free Ca^{2+} pool (Vigne et al., 1989) could lead to Ca^{2+}-dependent inhibition of I_{CaL}. In our experiments the presence of EGTA in the pipette solution made such a mechanism unlikely. A more suitable experimental procedure including intracellular free Ca^{2+} measurement might contribute to specify the role of the inositol trisphosphate-sensitive Ca^{2+} pool in endothelin-1 action.

The above data indicating that the endothelin-1-induced decrease in I_{CaL} was only expressed when Ca^{2+} channels were phosphorylated make it interesting to consider the involvement of phosphatases in the endothelin-1 action. Further study is required to determine whether the antagonism of the isoprenaline effect on I_{CaL} by endothelin-1 might involve a diacylglycerol-dependent activation of protein kinase C, which was shown to inhibit I_{CaL} amplitude and the opening of L-type Ca^{2+} channels in guinea pig cardiomyocytes (Satoh, 1992).

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