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The inotropic effect of endothelin-1 on rat atria involves hydrolysis of phosphatidylinositol

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Endothelin-1 induces a positive inotropic response in isolated left atria of the rat with an IC_{50} value of 20 nM. The contractile effect of endothelin is larger than that of other inotropic hormones such as phenylephrine and epinephrine and smaller than that of Bay K8644. In the spontaneously active right atria, endothelin induces a positive inotropic effect with no chronotropic effect. Endothelin does not modify intracellular levels of cAMP under basal conditions or after stimulation with isoproterenol but stimulates the formation of inositol phosphates. Mobilization of inositol phospholipids is observed in the same range of concentrations as for the contractile action of endothelin. The contractile action of endothelin is not mediated by protein kinase C. It is antagonized by blockers of L-type Ca^{2+} channels, low external Ca^{2+} concentrations and drugs such as caffeine and ryanodine that interfere with Ca^{2+} release by the sarcoplasmic reticulum.

Endothelin; Phosphatidylinositol; (Heart)

1. INTRODUCTION

Endothelin is a newly discovered 21-residue polypeptide that is synthesized by the vascular endothelium and displays powerful vasoconstrictive [1] and cardiotonic properties [2]. A previous paper [3] from this laboratory describes an investigation of the action of endothelin on rat vascular smooth muscle cells and demonstrates that endothelin (i) stimulates the production of inositol phosphates, (ii) mobilizes Ca^{2+} from intracellular stores (see also [4,5]) and (iii) activates a non-selective cationic channel that is permeable to Ca^{2+} with the result that the plasma membrane depolarizes and that voltage-dependent L-type Ca^{2+} channels are activated. It is for that reason that the vasoconstricting action of endothelin is partially prevented by Ca^{2+} channel antagonists [3].

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Abbreviation: DiC8, 1,2-dioctanoyldiacylglycerol

The purpose of the present paper is to analyze the mechanism of endothelin action on the contractility of isolated atria of rat heart.

2. MATERIALS AND METHODS

2.1. *Materials*

Synthetic endothelin-1 was purchased from Scientific Marketing Associates (UK); *myo*-[2-^{3}H]inositol (19 Ci/mmol) from Amersham; (−)-epinephrine, prazosin, caffeine, isoproterenol, phorbol myristate acetate and 1,2-dioctanoyl-rac-diacylglycerol (DiC8) from Sigma; Bay K8644 from Bayer; ryanodine from Calbiochem and (−)-D888 from Knoll.

2.2. *Contraction measurements*

Atria were isolated from 2-month-old male Sprague-Dawley rats and suspended in 3 ml organ chambers filled with Tyrode solution (127 mM NaCl, 4 mM KCl, 1 mM $MgSO_4$, 1.8 mM $CaCl_2$, 0.5 mM NaH_2PO_4, 12 mM $NaHCO_3$, 5 mM glucose) maintained at 37°C and gassed with 95% O_2/5% CO_2. Following a 30 min equilibration period with a 1 g load, contractions were measured using force displacement transducers coupled to a Gould-Brush 2600 polygraph. Left atria were paced at 1 Hz. In experiments using phenylephrine, atria were pretreated with 1 μM propranolol to favor the occupancy of α_1-adrenoceptors by phenylephrine.

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2.3. *Preparation of isolated atrial cells*

Atria were dissected from 1–2-day-old rats and dissociated into single cells under sterile conditions at 37°C using 0.1% trypsin. Cells were centrifuged and plated into culture dishes at a density of 2×10^5 cells/cm^2. The culture medium was Ham's F-12 supplemented with 10% fetal bovine serum, 10% horse serum, 50 U/ml of penicillin and 200 μg/ml of streptomycin. Cultures were maintained at 37°C in a humidified atmosphere consisting of 95% air/5% CO_2. After 2 days, the culture medium was changed and cells used 2 days later for biochemical experiments. All cultures used showed spontaneous contractile activity.

2.4. *Biochemical assays*

Monolayers of rat atrial cells were labelled for 48 h with 2 μCi/ml of *myo*-[2-^{3}H]inositol and then incubated in an Earle's salt solution (140 mM NaCl, 5 mM KCl, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 5 mM glucose buffered at pH 7.4 with 25 mM Hepes-Tris) supplemented with 40 mM LiCl at 37°C for 10 min. Endothelin was then added to the cells for 10 min and the radioactivity incorporated into inositol phosphates determined as described [6]. For cAMP assays, cells were treated with 100 nM endothelin in the absence or presence of 10 μM isoproterenol for 5 min and then extracted with an ice-cold solution consisting of ethanol/5 mM EDTA (2:1). The extracts were centrifuged and assayed for cAMP content using a radioimmunoassay (Amersham). Means ± SD are indicated.

3. RESULTS AND DISCUSSION

Fig.1 compares the contractile effects of endothelin on isolated left atria from rat heart with those of epinephrine, phenylephrine and the L-type Ca^{2+} channel activator, Bay K8644. Endothelin (100 nM) has a biphasic action on contractility. Within the first minute of its application, a slight negative inotropic effect (<10% of initial tension) develops. This is followed by a large positive inotropic effect that takes 7–8 min to stabilize (fig.1A). Phenylephrine (acting via α_1-adrenoceptors) also produces a biphasic effect on contraction similar to that of endothelin (fig.1C). The action of endothelin, however, is not mediated by α_1-adrenoceptors since their blockade with prazosin does not prevent this effect (fig.1A). No evidence for an early transient increase in tension as described for phenylephrine action on rat ventricular papillary muscle [7] was observed with either phenylephrine or endothelin. The increase in tension produced by 100 nM endothelin was 2.25 ± 0.16-fold (n = 32). This is greater than that produced by 1 μM epinephrine (1.67 ± 0.34-fold, n = 5) or 10 μM phenylephrine (1.90 ± 0.33-fold, n = 3) and smaller than the effect of 1 μM Bay K8644 (2.79 ± 0.61-fold, n = 7).

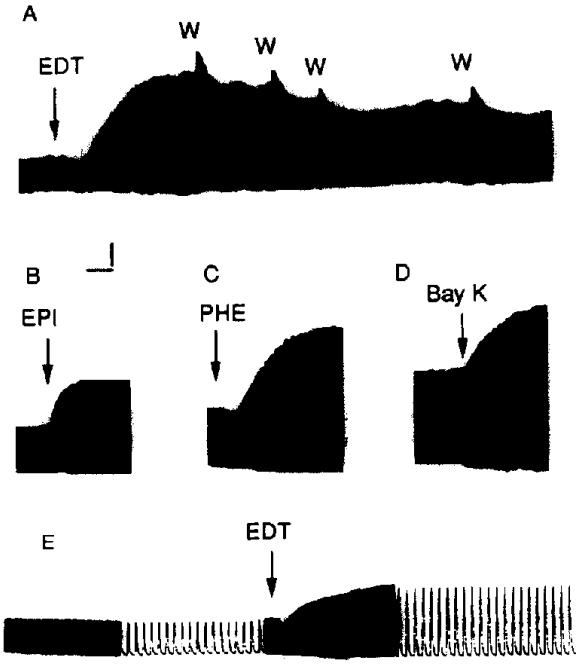

Fig.1. Kinetics of contractile responses to endothelin, phenylephrine, epinephrine and Bay K8644 of isolated rat atria. Concentrations: 100 nM endothelin (EDT), 1 μM epinephrine (EPI), 10 mM phenylephrine (PHE), 1 μM Bay K8644. (A) Atria were treated with 1 μM prazosin before addition of endothelin; (C) atria treated with 1 μM propranol before the addition of phenylephrine. (A–D) Left atria paced at 1 Hz, (E) right atria showing spontaneous activity. Time scale is 6 min for all records. (E) Chart speed was increased during the experiment (time scale: 1.30 s) to show that endothelin had no chronotropic effect. Arrows indicate time of addition of the different effectors. Tension scales: 50 mg (C–E) or 100 mg (A,B). W, washes.

The inotropic effect of endothelin is stable for at least 45 min and decreases slowly upon repetitive washings (fig.1A). After recovery of the initial tension, atria remain unaffected by a second application of endothelin. Endothelin is inactive at <1 nM. The IC_{50} value for contractile action was determined as 20 nM (fig.2C). This is at least two orders of magnitude higher than the IC_{50} value for the vasoconstricting effect of the peptide [1]. It could be that atrial and vascular smooth muscle cells express different types of receptor for endothelin. Endothelin-1 would recognize its binding sites with a high affinity in aortic smooth muscle cells and with a lower affinity in atrial cells.

Endothelin also produces a biphasic change in contractility in the spontaneously active right atria (fig.1E). The time course of its action is similar to

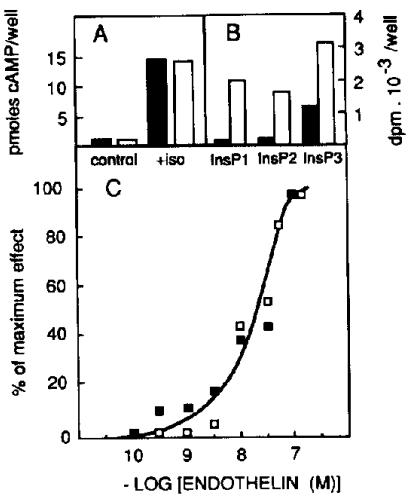

Fig.2. Endothelin increases the production of inositol phosphates by rat atrial cells. (A) Intracellular levels of cAMP in control (open bars) and endothelin (100 nM) treated atrial cells (filled bars). Experiments were performed in the absence or presence of 10 μM isoproterenol as indicated. (B) Influence of endothelin on intracellular levels of inositol polyphosphates. Open bars, controls; filled bars, endothelin (100 nM) treated cells. (C) Dose-response curves for endothelin action on contractions of the isolated left atria (□) and on production of total inositol phosphates (■). Data are expressed as % of the maximum response observed at 100 nM endothelin. For inositol phosphates the background level of radioactivity (5000 dpm) was subtracted from all data. Maximum counts incorporated into total inositol phosphates was 15000 dpm.

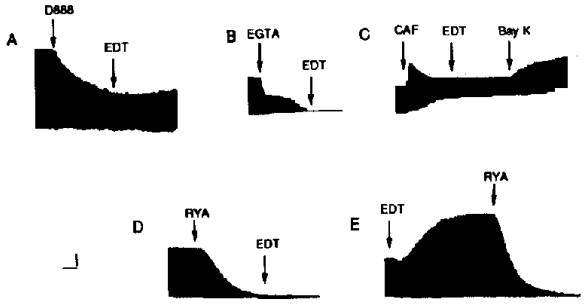

Fig.3. The contractile effect of endothelin requires external Ca^{2+} and a functional sarcoplasmic reticulum. Left atria, paced at 1 Hz, were treated with different effectors as indicated. Concentrations: endothelin (EDT, 100 nM), (−)-D888 (2 μM), EGTA (2 mM), caffeine (CAF, 5 mM), Bay K8644 (1 μM), ryanodine (RYA, 1 μM). Tension scales: 25 mg (A), 50 mg (C,E) and 100 mg (B,E). Time scale is 6 min for all records.

that observed in the left atria. However, the increase in tension produced by 100 nM endothelin (1.53 ± 0.08-fold, n = 11) is lower than for the left atria. Fig.1E further shows that endothelin has no effect on the beating frequency. In the guinea pig, endothelin is less potent than phenylephrine in increasing the force of contractions of the left atria [2] and produces a positive chronotropic effect in the right atria [3]. The reason for these differences in the action of endothelin between rat and guinea pig atria is not known.

The atrial action of endothelin is different from that of β-adrenergic agonists. It develops slowly as compared to that of epinephrine (fig.1B) and does not modify the time course of contractions in both guinea pig [2] and rat atria (not shown). Endothelin does not change intracellular levels of cAMP both under basal conditions and after stimulation of β-adrenergic receptors with 10 μM isoproterenol (fig.2A). These results rule out a possible involvement of cAMP in endothelin action.

Fig.2B shows that endothelin increases intracellular levels of inositol monophosphate, bisphosphate and trisphosphate in atrial cells. The dose-response curve for endothelin action on the production of inositol phosphates (fig.2C) shows an IC_{50} value of 20 nM and is superimposable on that for contractions. This suggests that hydrolysis of phosphatidylinositol is involved in the contractile action of endothelin. Phenylephrine, acting via α_1-adrenoceptors [7,9,10], and histamine, acting via H1 receptors [11], have also been suggested to increase myocardial contractility via activation of phospholipase C and the production of inositol phosphates.

Endothelin action could involve activation of protein kinase C by diacylglycerol which is released from phosphatidylinositol in addition to inositol phosphates [12]. In order to check this possibility, we compared the contractile actions of endothelin, phorbol myristate acetate and DiC8. DiC8 (300 μM) produces a transient inotropic effect in isolated left atria. However, the mean increase in tension (1.37± 0.12-fold, n = 7) is lower than for endothelin. We also observed that the action of DiC8 does not desensitize the cells nor does it prevent endothelin action. Similarly, phorbol myristate acetate (3 μM) produces only a weak inotropic response (1.15 ± 0.07-fold, n = 4) and does not desensitize the cells to the action of endothelin. These experiments strongly suggest that activation

of protein kinase C cannot account for the contractile effect of endothelin.

Blockade of Ca^{2+} channels with (−)-D888 decreases the amplitude of contractions of the isolated left atria and completely prevents endothelin action (fig.3A). Conversely, a reduction in the external free Ca^{2+} concentration by addition of EGTA to the bath medium abolishes contractions (fig.3B). Addition of endothelin after complete cessation of beating does not restore contractions (fig.3B). These results suggest that Ca^{2+} influx via Ca^{2+} channels is probably important for endothelin action. A possible role of the sarcoplasmic reticulum in endothelin action was investigated using caffeine and ryanodine. Caffeine produces a transient positive inotropic effect in the isolated left atria which is followed by a sustained negative inotropic effect (fig.3C). After treatment with caffeine, atria no longer respond to endothelin whereas they still do so to the Ca^{2+} channel activator Bay K8644 (fig.3C). Ryanodine also reduces contractility and prevents the contractile effect of endothelin (fig.3D). It also abolished the inotropic action of endothelin once it had developed (fig.3E). Taken together, these results suggest that the sarcoplasmic reticulum is an important site for endothelin action.

In summary, the inotropic effect of endothelin involves both Ca^{2+} entry through L-type Ca^{2+} channels and Ca^{2+} release from the sarcoplasmic reticulum. Ca^{2+} channels may be important for the maintenance of high Ca^{2+} stores in the sarcoplasmic reticulum [13].

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