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Endothelin stimulates hypertrophy and contractility of neonatal rat cardiac myocytes in a serum-free medium

Takahiko Suzuki[1], Hiroyoshi Hoshi[1] and Youji Mitsui[2]

[1]*Bio-Science Laboratory, Inc., 5-34-5, Shironishi, Yamagata 990, Japan* and [2]*Cell Science and Technology Division, Fermentation Research Institute, Agency of Industrial Science and Technology, Higashi 1-1-3, Tsukuba Science City, Ibaraki 305, Japan*

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The effect of endothelin (ET) on rat cardiac myocytes cultured in a serum-free, defined medium was determined. ET stimulated cardiac myocyte hypertrophy in a dose-dependent manner as determined by the protein synthesis and cell surface area. Since the myocyte hypertrophy was abolished by H-7, a protein kinase C inhibitor, ET-receptor mediated protein kinase C activation may be involved in cardiac myocyte hypertrophy. At the same time, ET also stimulated myocyte contractility in this medium, and this stimulatory effect was inhibited by nicardipine. This result indicates that the influx of extracellular calcium ion is necessary for the stimulation of contractility induced by ET.

Endothelin; Rat cardiac myocyte; Hypertrophy; Contractility; Protein kinase C

1. INTRODUCTION

Endothelin (ET), a novel vasoconstrictor peptide originally isolated from the culture supernatant of porcine aortic endothelial cells, is composed of 21 amino acid residues with two intramolecular disulfide linkages [1]. ET displays powerful vasoconstrictive [1] and cardiotonic properties [2]. ET possesses potent inotropic and chronotropic actions on isolated atria in humans [3,4], guinea pigs [3] and rats [2,4]. Furthermore, ET receptors in cardiac membrane [5] and in cultured heart cells [6,7] have been characterized. These reports suggest that ET exerts direct physiological role on cardiac myocytes. In this report, we have attempted to elucidate the stimulation of hypertrophy and contractility of ET on cardiac myocytes in a serum-free, defined culture medium.

2. MATERIALS AND METHODS

2.1. *Materials*

Nutrient medium MCDB 107 was a gift from Kyokuto Pharmaceutical Industrial Co. (Tokyo, Japan). Endothelin-1 was obtained from Peptide Institute, Inc. (Osaka, Japan). Collagenase was obtained from Wako Pure Chemical Co. (Tokyo, Japan). Insulin, human apo-transferrin, trypsin (Type XII, × 2 crystallized), nicardipine hydrochloride were purchased from Sigma. H-7 was obtained from Seikagaku Kogyo Inc. (Tokyo, Japan). [^{14}C]Leucine (12 GBq/mmol) was from Amersham. Fibronectin was prepared from bovine plasma according to the method of Vuento et al. [8].

Correspondence address: T. Suzuki, Bio-Science Laboratory, Inc., 5-34-5, Shironishi, Yamagata 990, Japan

2.2. *Cell isolation and primary cell cultures of neonatal rat cardiac myocytes*

Ventricular cardiac muscle cells (myocytes) were isolated from 1–3-day-old male Wistar rats as previously described [9]. The isolated cells were maintained in a serum-free, defined medium, MCDB 107 supplemented with 10 μg/ml of insulin and transferrin, on 24-well culture plates precoated with 10 μg/ml of fibronectin, and used within a week.

2.3. *Assay of protein synthesis*

There are increases in protein synthesis and in cell surface area of cardiac myocytes during myocyte hypertrophy [10,11]. To determine the increase of cardiac cell size (hypertrophy), protein synthesis was measured by monitoring the incorporation of [^{14}C]leucine into the trichloroacetic acid-insoluble materials as described previously [9].

2.4. *Measurements of rhythmic contraction of cardiac myocytes*

Beating rate and beating cell number of the cells were determined as described in a previous procedure [9].

3. RESULTS

ET increased the protein synthesis of cardiac myocytes in a dose-dependent manner, and the half-maximum dose was 170 pM (Fig. 1). The threshold concentration for ET-induced protein synthesis was approximately 100 pM whereas the maximum effect of protein synthesis occurred at 1 nM of the peptide. Following the stimulation of protein synthesis, the increase of cell surface area has been observed in the presence of ET (Fig. 2B). The cell surface area increased by 1 nM of ET was about 3–4-fold of the control cultures. While a high dose of nicardipine (1 μM) indicated a slightly inhibitory effect to ET-induced protein synthesis (Fig. 3), the inhibitory effect by nicardipine on ET-induced myocyte hypertrophy was

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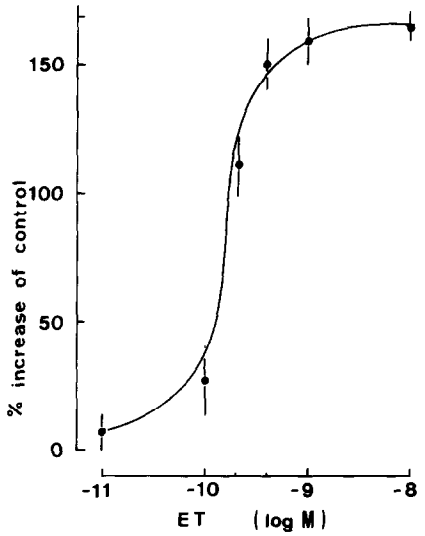

Fig. 1. Dose-dependent stimulation by ET on the incorporation of [^{14}C]leucine into myocardial cell protein. Data are expressed as the percent increase of the control cultures (1615 ± 70 dpm/10^5 cells). Values are the mean ± SE of triplicate cultures from 3 independent experiments.

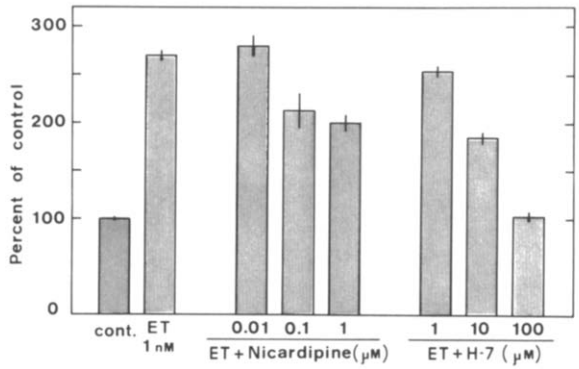

Fig. 3. Effect of nicardipine and H-7 on the incorporation of [^{14}C]leucine into myocardial cell protein stimulated by ET. Data are expressed as the percent of the control cultures (1538 ± 25/10^5 cells). Values are the mean ± SE of triplicate cultures from 3 independent experiments.

less effective (Fig. 2C). In contrast, H-7, a protein kinase C inhibitor, strongly attenuated the ET-induced protein synthesis in a dose-competitive manner (Fig. 3). Moreover, ET-induced myocyte hypertrophy was markedly attenuated in the presence of H-7 (100 μM) (Fig. 2D).

Cultured cardiac myocytes hardly elicited the rhythmic contraction in this serum-free medium (Table I). However, the addition of ET (1 nM) made the myocytes stable and elicited powerful rhythmic contraction. A dose-dependent inhibition of nicardipine was observed on ET-induced rhythmic contraction of myocytes and complete inhibition was shown at a dose of 0.1 μM.

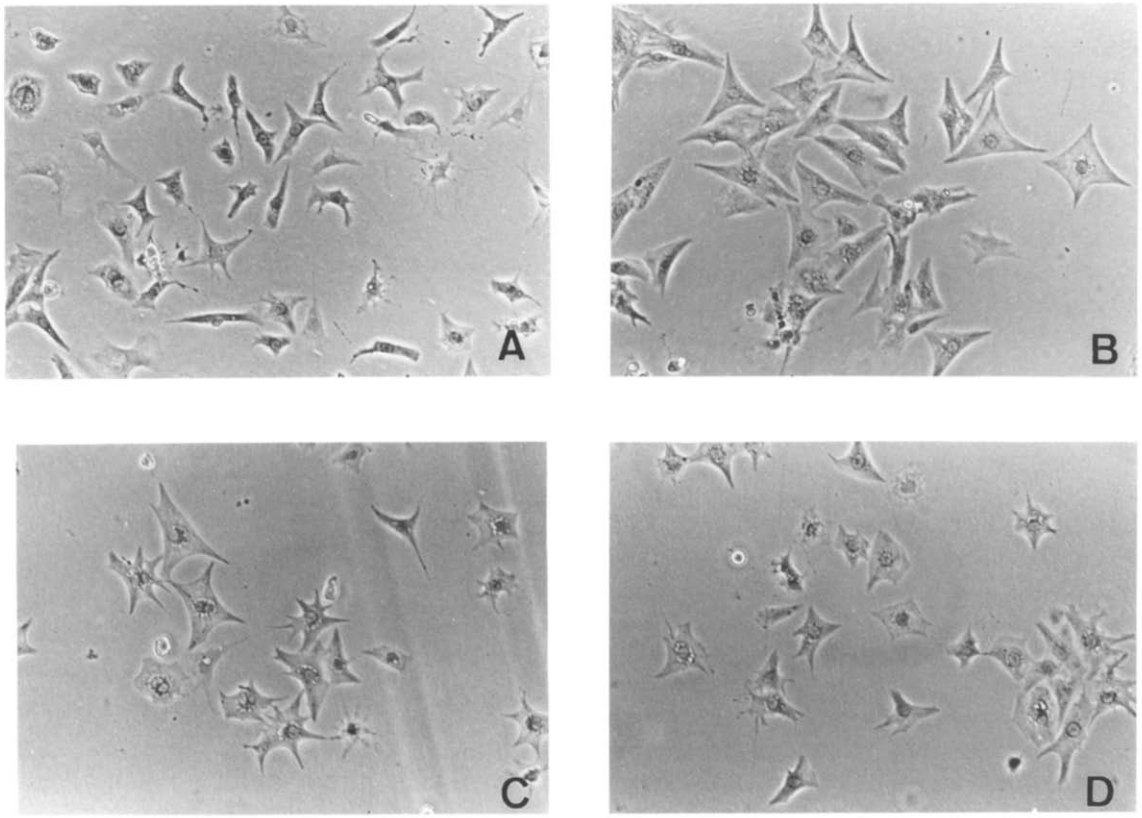

Fig. 2. Hypertrophic stimulation of ET on cultured cardiac myocytes and the effect of nicardipine and H-7 in the presence of maximal dose (1 nM) of ET. Cells were photographed 48 h after incubation with different test samples. (A) Control; (B) ET (1 nM); (C) ET (1 nM) + nicardipine (1 μM); (D) ET (1 nM) + H-7 (100 μM). Magnification × 52.5.

Table I

Dose-dependent inhibition of nicardipine (NP) on the rhythmic contraction of cardiac myocytes stimulated by endothelin (ET)

Factor added	Beating	
	Cells (%)	Rate (min^{-1})
No supplement	2.7 ± 1.3	15.4 ± 4.8
ET 1 nM	91.3 ± 5.2	136.9 ± 10.5
ET (1 nM) + NP (0.01 μM)	63.0 ± 7.5	96.6 ± 7.5
ET (1 nM) + NP (0.1 μM)	7.5 ± 2.8	19.4 ± 6.7
ET (1 nM) + NP (1 μM)	0.9 ± 0.5	9.1 ± 2.8

Values are the mean ± SE

4. DISCUSSION

Myocyte hypertrophy results in an increase in myocardial cell size, sarcomere number, and myocardial protein content [10,12]. This is the first report that ET stimulated cardiac myocyte hypertrophy as qualified by the protein synthesis and cell surface area of cultured cardiac myocytes in a serum-free medium. The ET-induced myocyte hypertrophy was only partially attenuated by a voltage-dependent calcium channel blocker, nicardipine. This may suggest that voltage-dependent calcium channel activation is not directly involved in the development of cardiac myocyte hypertrophy. Simpson and co-workers [10,12] demonstrated that cardiac myocyte hypertrophy was induced by norepinephrine and serum in neonatal rat heart cells in a serum-free medium, and the hypertrophy induced by norepinephrine was mediated through an α_1-adrenergic receptor [11,12]. Stimulation of α_1-adrenergic receptors results in the accumulation of inositol phosphates and diacylglycerol in cardiac myocytes [13]. Furthermore, increased level of c-*myc* protooncogene expression was associated with cardiac myocyte hypertrophy caused by norepinephrine and serum [14] as well as a physical load [15]. There are reports that ET stimulates c-*myc* and c-*fos* gene expression [16] and diacylglycerol accumulation activating protein kinase C [17] in cultured vascular smooth muscle cells. In the present study, H-7, a protein kinase C inhibitor, completely abolished the ET-induced increase of protein synthesis and myocyte hypertrophy. This suggests that receptor-mediated protein kinase C activation may be involved in the ET-induced cardiac myocyte hypertrophy. At the same time, the rhythmic contraction was elicited by ET in the cultured cardiac myocytes. The molecular mechanism of the stimulation of contractility induced by ET has not been clearly understood. Recent study has demonstrated that ET induced the phospholipase C-mediated hydrolysis of phosphoinositides followed by the release of calcium from calcium ion stores in rabbit aortic smooth muscle cells [18]. Furthermore, Vigne et al. [19] reported that the inotropic effect of ET involves hydrolysis of phosphatidylinositol. And they also reported that since the effect of ET on rat atrium was antagonized by a calcium channel blocker, low external calcium ion concentration and also drugs that interfere with calcium ion release by the sarcoplasmic reticulum, the inotropic effect of ET involved both calcium ion entry through L-type calcium ion channels and calcium ion release from the sarcoplasmic reticulum [19]. We also found that ET-induced rhythmic contraction of cardiac myocytes was strongly attenuated by nicardipine. This finding suggests that influx of extracellular calcium is required for the contractile action of ET.

In summary, receptor-mediated phospholipase C activation followed by protein kinase C activation may be involved in the development of cardiac myocyte hypertrophy. At the same time, the receptor-mediated phospholipase C activation followed by phosphatidylinositol hydrolysis and calcium mobilization may be involved in the contractile activation of ET in cultured cardiac myocytes.

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