ELSEVIER

European Journal of Pharmacology 347 (1998) 329–335

ejp

Pericardial mesothelial cells produce endothelin-1 and possess functional endothelin ET_B receptors

Masayoshi Kuwahara [a,*], Maki Kuwahara [b]

[a] *Department of Comparative Pathophysiology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan*

[b] *Division of Toxicology, Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo 187-0011, Japan*

Received 25 August 1997; revised 2 February 1998; accepted 6 February 1998

Abstract

We investigated the endothelin production and endothelin receptor activity of pericardial mesothelial cells obtained from spontaneously hypertensive rats (SHR) and Wistar–Kyoto (WKY) rats. The pericardial mesothelial cells were maintained in vitro and the production of endothelin-1 by these cells was evaluated by using a sensitive sandwich-type enzyme immunoassay for endothelin-1 and big endothelin-1. Endothelin receptor subtypes were pharmacologically analyzed by measuring the changes of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in pericardial mesothelial cells. Mesothelial cells from both strains produced more immunoreactive endothelin-1 than big endothelin-1. The production of immunoreactive endothelin-1 progressively increased in a culture time-dependent manner. The amount of immunoreactive endothelin-1 detected after 72 h in pericardial mesothelial cells of SHR was significantly ($P < 0.05$) higher than that in the cells of WKY rats (SHR: 196.7 ± 19.1 pg/10^6 cells; WKY: 122.7 ± 10.6 pg/10^6 cells). Endothelin-1 and endothelin-3 induced elevation of $[Ca^{2+}]_i$ in pericardial mesothelial cells. The selective agonist of the endothelin ET_B receptor, sarafotoxin S6c, also induced elevation of $[Ca^{2+}]_i$. The endothelin- and sarafotoxin S6c-induced elevations of $[Ca^{2+}]_i$ in pericardial mesothelial cells from SHR were greater than those in mesothelial cells from WKY rats. The endothelin ET_B receptor antagonist, IRL 1038 ([Cys^{11},Cys^{15}]endothelin-1-(11-21)), significantly inhibited the endothelin-1- and endothelin-3-induced changes in $[Ca^{2+}]_i$. The endothelin ET_A receptor antagonist, FR1393171 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]ammino-4-methylpentanoyl]amino-3-[3-(1-methyl-1*H*-indoyl)]propionyl]amino-3-(2-pyridyl)propionic acid), did not affect these changes. From these results, pericardial mesothelial cells from both SHR and WKY rats shared the ability to produce endothelin-1 spontaneously in culture, although consistently greater production was detected in cultures of SHR origin. Moreover, pericardial mesothelial cells of SHR origin may have increased numbers of endothelin ET_B receptors and/or a more active signal transduction system compared with those of WKY rats. These results suggest that endothelin-1 may play an important role in the pericardium in an autocrine and/or paracrine fashion. © 1998 Elsevier Science B.V.

Keywords: Endothelin; Ca^{2+}, cytosolic; Mesothelial cell; Pericardium; Safarotoxin S6c; Spontaneously hypertensive rat (SHR)

1. Introduction

Endothelin-1 was originally isolated from the conditioned medium of cultured porcine aortic endothelial cells (Yanagisawa et al., 1988). Endothelin-1 induces a potent and sustained vasoconstrictive effect on a variety of blood vessels including coronary arteries. Endothelin-1 has been shown to have a positive inotropic as well as a positive chronotropic action on myocardium. Endothelin-1 also exerts long-term effects such as myocardial hypertrophy, and causes cellular injury in cardiac myocytes (Rubanyi and Polokoff, 1994). Because the production of endothelin-1 is markedly increased in the myocardium of rats with heart failure, and acute application of an endothelin receptor antagonist decreases myocardial contractility in such rats, myocardial endothelin-1 may help to support the contractility of the failing heart (Sakai et al., 1996). Epicardial application of prostaglandin I_2 alters the response of coronary arteries to bradykinin (Staszewska-Barczak et al., 1981). Epicardial mesothelial cells from adult rat heart have been shown to affect the differentiation process that occurs in long-term primary monocultures of adult ventricular cardiocytes (Eid et al., 1992). Therefore, endothelin-1 produced by pericardial mesothelial cells, which are in

* Corresponding author. Tel.: +81-3-3812-2111 ext. 5391; fax: +81-3-5800-6925; e-mail: akuwam@hongo.ecc.u-tokyo.ac.jp

direct access to the coronary arteries and myocardium, may also have a significant role in cardiac physiology and pathophysiology.

Mesothelial cells of both the pleura and pericardium have been shown to be metabolically active and to be involved in growth factor synthesis and extracellular matrix production (Baumann et al., 1993; Gerwin et al., 1987; Honda et al., 1991; Lee et al., 1993; Owens and Milligan, 1994; Satoh and Prescott, 1987). There are a few studies on endothelin production in pleural and epicardial mesothelial cells including our previous study (Eid et al., 1994; Kuwahara et al., 1992; Waters et al., 1997). Although endothelin has multiple physiological and pathophysiological effects (Masaki et al., 1992), including the chronotropic and inotropic response of the heart as well as significant coronary arterial vasoconstriction, to our knowledge investigators have not focused on endothelin production by pericardial mesothelial cells. Therefore, we investigated the endothelin production and endothelin receptor activity of pericardial mesothelial cells. For this purpose, the pericardial mesothelial cells obtained from rats were maintained in vitro. The production of endothelin was confirmed by a sensitive sandwich-type enzyme immunoassay for endothelin-1 and big endothelin-1 (Suzuki et al., 1989) and endothelin receptor subtypes were pharmacologically analyzed by the changes in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in pericardial mesothelial cells. Furthermore, recent studies have documented that the responses of endothelial cells and smooth muscle cells mediated by the endothelin ET_B receptor are greater in spontaneously hypertensive rats (SHR) than in the Wistar–Kyoto (WKY) rats (Batra et al., 1993; Kuwahara et al., 1996; Yokokawa et al., 1994). Therefore, endothelin-1 production and the response to endothelins of pericardial mesothelial cells from SHR were compared with those of cells from WKY rats.

2. Materials and methods

2.1. Animals

Nine-week-old male SHR and WKY rats were obtained from Charles River Japan (Kanagawa, Japan).

2.2. Reagents, media, and buffers

For the Ca^{2+} measurements we used a *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer containing (in mM) 10 HEPES, 136.9 NaCl, 5.4 KCl, 1.0 $MgCl_2$, 1.5 $CaCl_2$, 0.001 EDTA and 5.5 glucose (HEPES-buffered solution). In Ca^{2+}-free experiments, the same solution was used except that 1.5 $CaCl_2$ was omitted and 0.5 ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid (EGTA) was added. Dulbecco's modified Eagles medium (DMEM), Hanks' balanced salt solution (HBSS), fetal bovine serum, and trypsin–EDTA were purchased from Gibco, Grand Island, NY. Ionomycin was purchased from Sigma Chemical, St. Louis, MO. Endothelin-1, endothelin-3, and Sarafotoxin S6c were purchased from Peptide Institute, Osaka, Japan. Fura 2-acetoxymethyl ester (fura 2-AM) was obtained from Dojindo Laboratories, Kumamoto, Japan. FR1393171 ((*R*) 2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)] carbonyl]ammino-4-methylpentanoyl]amino-3-[3-(1-methyl-1*H*-indoyl)]propionyl]amino-3-(2-pyridyl)propionic acid) (Sogabe et al., 1993) and IRL 1038 ([Cys^{11},Cys^{15}]endothelin-1-(11-21)) (Urade et al., 1992) were generous gifts. Other materials and chemicals were obtained from commercial sources.

2.3. Rat pericardial mesothelial cell cultures

Rat pericardial mesothelial cells were obtained and established in culture, as described previously (Kuwahara et al., 1991). Briefly, rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and were immediately killed by exsanguination from a severed abdominal aorta. The pericardium was removed under sterile conditions and immersed in petri dishes for 20 min in HBSS. The pericardial surfaces were scraped repeatedly with cell scrapers. The cells were then seeded into culture dishes. The cultures were maintained for up to 10 passages in DMEM with 10% fetal bovine serum, 10^5 U/l penicillin, and 100 mg/l streptomycin, at 37°C in humidified environment containing 5% CO_2. Confluent cultures of pericardial mesothelial cells from SHR and WKY rats displayed essentially similar morphologic characteristics. Cells from both strains formed a cobblestone mosaic pattern composed of polyhedral cells. The mesothelial origin of the cells was confirmed by immunoreactivity for keratin and vimentin (Kuwahara et al., 1991). Essentially, all of the cells from both strains stained positively with keratin as well as vimentin, indicating high purity of the cultures.

2.4. Preparation of pericardial mesothelial cell supernatant-conditioned medium

Rat pericardial mesothelial cells were routinely maintained in complete culture medium, which consists of DMEM with 10% fetal bovine serum, 10^5 U/l penicillin, and 100 mg/l streptomycin, at 37°C in a humidified environment containing 5% CO_2 as previously described (Kuwahara et al., 1991). Cells of passage 8–10 were used in this experiment. When confluence was reached (9 cm^2), the cells were washed three times with HBSS and incubated with 2 ml of serum-free medium for up to 72 h. Conditioned medium was collected at defined culture times and frozen in aliquots at −70°C. After collection of conditioned medium, the cells were detached by trypsin–

EDTA from each dish and viable cell numbers were confirmed by trypan blue dye exclusion.

2.5. Sandwich-enzyme immunoassay

The amount of immunoreactive endothelin-1 and big endothelin-1 in the conditioned medium was determined by using a sandwich-enzyme immunoassay kit purchased from International Reagents, Tokyo, Japan. Briefly, AwETN40-coated microtest plates were incubated at 4°C for 24 h with 100 μl/well of standard endothelin-1, human big endothelin-1, or the sample to be tested in buffer E (0.02 M phosphate buffer, pH 7.0, containing 10% Block Ace, 2 mg/ml of bovine serum albumin, 0.4 M NaCl, and 2 mM EDTA). After being washed with PBS, the plates were incubated with 100 μl/well of horseradish peroxidase-labeled anti-endothelin-1 (15-21) Fab′ or horseradish peroxidase-labeled anti-human big endothelin-1 (22–38) Fab′ in buffer C (0.02 M phosphate buffer, pH 7.0, containing 10 mg/ml of bovine serum albumin, 0.4 M NaCl, and 2 mM EDTA) for 24 h at 4°C. The plates were washed with PBS, and the bound enzyme activity was measured by using a microwell peroxidase system.

2.6. Measurement of $[Ca^{2+}]_i$

Changes in $[Ca^{2+}]_i$ were determined as previously reported (Ito et al., 1995). Pericardial mesothelial cells were incubated on 25-mm glass coverslips (Matsunami, Tokyo, Japan) in DMEM with 10% fetal bovine serum. After reaching confluence, the culture medium was depleted of fetal bovine serum for 12 h, and then the mesothelial cell monolayers were loaded with fura 2 by incubating them with 2 μM fura 2-AM for 30 min at 37°C in HEPES-buffered solution. Loaded cells were washed in HEPES-buffered solution and maintained in this solution for 20 min at room temperature to allow for complete hydrolysis of fura 2 to the acid form.

The glass coverslip was placed horizontally in a temperature-controlled (37°C) bath that was mounted on an apparatus for fluorescence measurements (CAF-100, Japan Spectroscopic, Tokyo, Japan). Fluorescence excitation was obtained from a xenon high-pressure lamp (150 W). Ultraviolet light of alternating 340 and 380 nm (10 nm bandwidth) was obtained with a monochromator equipped with a chopping wheel (400 Hz) placed in front of the monochromator. The dichroic mirror was used as a beam splitter to transmit emitted fluorescence (500 nm) into the photomultiplier. The fluorescence signals (340 and 380 nm) and their ratio (340:380 nm) were continuously recorded on a chart recorder. At the end of an experimental run, background autofluorescence (the inherent fluorescence emitted from cells, coverslip, and bath at 340 and 380 nm) was obtained by the method of Hallam et al. (1988).

After autofluorescence was subtracted, the changes in $[Ca^{2+}]_i$ were determined quantitatively by using the following equation: $[Ca^{2+}]_i = K_d[(R - R_{min})/(R_{max} - R)](Sf_2/Sb_2)$ where the dissociation constant K_d has a value of 224 nM (Grynkiewicz et al., 1985), R is the fluorescence ratio within the cells, R_{max} is the maximal fluorescence ratio after addition of 40 μM ionomycin in the presence of 1.5 mM $CaCl_2$, R_{min} is the minimal ratio determined by the subsequent addition of 5 mM EGTA, and Sf_2/Sb_2 is the ratio of fluorescence values at 380 nm excitation determined at R_{min} and R_{max}, respectively.

2.7. Statistics

Results were expressed as the means ± S.E.M. of five separate experiments for each category. Statistical comparisons were made by using a one-way analysis of variance (ANOVA).

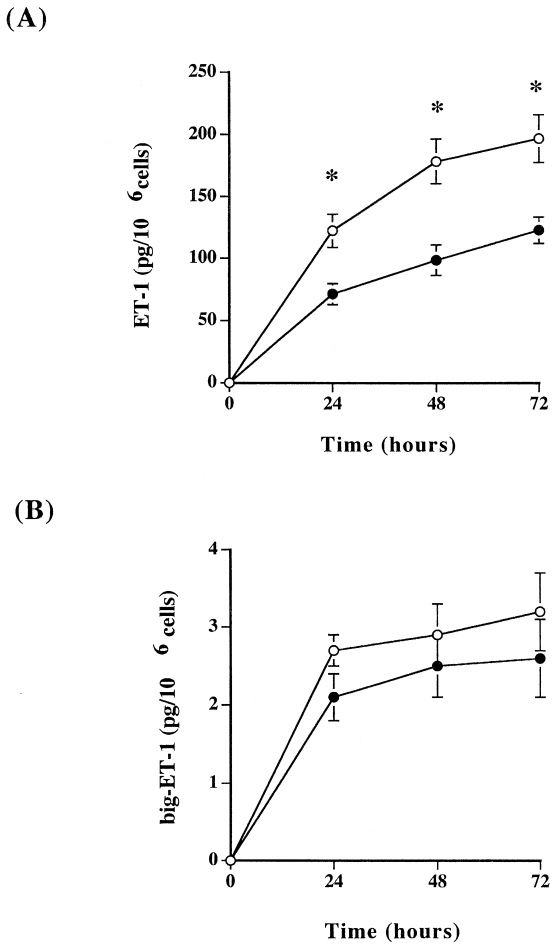

Fig. 1. The progressive production of immunoreactive endothelin-1 (A) and big endothelin-1 (B) in conditioned medium obtained from pericardial mesothelial cell cultures. Open circles = spontaneously hypertensive rats; solid circles = Wistar–Kyoto rats. Each point represents mean ± S.E.M. of five separate experiments. * = $P < 0.05$, compared with respective values of Wistar–Kyoto rats.

3. Results

3.1. Production of endothelin-1 and big endothelin-1 by pericardial mesothelial cells

We detected immunoreactive endothelin-1 and big endothelin-1 in conditioned medium from cultured pericardial mesothelial cells from SHR and WKY rats whose viability, based on trypan blue dye exclusion, always exceeded 95%. These mesothelial cells from both strains produced more immunoreactive endothelin-1 than big endothelin-1. The production of immunoreactive endothelin-1 progressively increased in a culture time-dependent manner. The amount of immunoreactive endothelin-1 detected after 72 h in pericardial mesothelial cells from SHR was significantly ($P < 0.05$) higher than that in the cells from WKY rats (SHR: 196.7 ± 19.1 pg/10^6 cells; WKY: 122.7 ± 10.6 pg/10^6 cells) as shown in Fig. 1A. There was no significant difference in the amount of immunoreactive big endothelin-1 between the two strains (Fig. 1B).

Table 1
The half-maximal effective concentration (EC_{50}) and maximum effect (E_{max}) of endothelin-1, endothelin-3, and sarafotoxin S6c in pericardial mesothelial cells from SHR and WKY rats

	Endothelin-1	Endothelin-3	Sarafotoxin S6c
EC_{50} (−log M)			
WKY	6.60 ± 0.05	6.38 ± 0.06	7.19 ± 0.05
SHR	7.00 ± 0.04[a]	6.55 ± 0.05	7.82 ± 0.04[a]
E_{max} (nM)			
WKY	623 ± 24	552 ± 35	650 ± 57
SHR	686 ± 54	585 ± 57	710 ± 56

Each value represents mean ± S.E.M. of five experiments.
[a] $P < 0.05$, compared with respective values of Wistar–Kyoto rats.

3.2. Effects of endothelin-1 and endothelin-3 on $[Ca^{2+}]_i$

Both endothelin-1 and endothelin-3 induced in pericardial mesothelial cells a biphasic elevation of $[Ca^{2+}]_i$ that consisted of a transient initial component, followed by a sustained component in the presence of 1.5 mM extracellular Ca^{2+}. The initial component peaked within 30 s after stimulation with endothelin-1 and endothelin-3. The characteristics of the endothelin-1- and endothelin-3-induced response were similar for pericardial mesothelial cells from both SHR and WKY rats. Both endothelin-1 and endothelin-3 elicited an elevation of $[Ca^{2+}]_i$ in a dose-dependent manner. Concentration–response curves were obtained for the peak values of each initial component of the $[Ca^{2+}]_i$ response as shown in Fig. 2. The endothelin-1-induced (0.03–0.3 μM) elevation of $[Ca^{2+}]_i$ in pericardial mesothelial cells from SHR was significantly greater than that in mesothelial cells from WKY rats (Fig. 2A). However, the endothelin-3-induced elevation of $[Ca^{2+}]_i$ in SHR

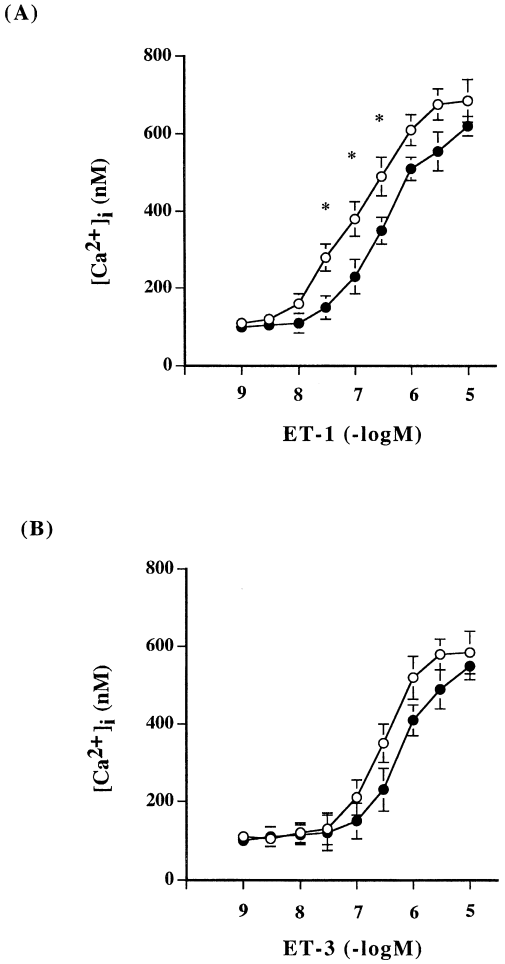

Fig. 2. Dose–response curves for endothelin-1 (A) and endothelin-3 (B) showing the magnitude of the initial transient increase in $[Ca^{2+}]_i$ in pericardial mesothelial cells. Open circles = spontaneously hypertensive rats; solid circles = Wistar–Kyoto rats. Each point represents mean ± S.E.M. of five separate experiments. * = $P < 0.05$, compared with respective values of Wistar–Kyoto rats.

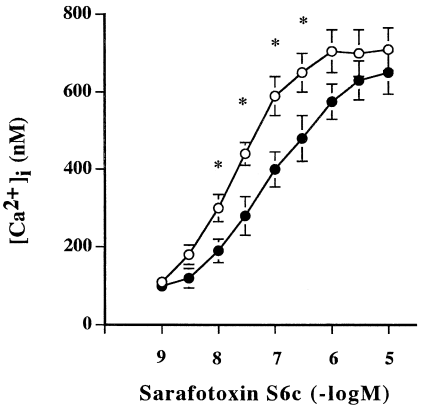

Fig. 3. Dose–response curves for sarafotoxin S6c showing the magnitude of the initial transient increase in $[Ca^{2+}]_i$ in pericardial mesothelial cells. Open circles = spontaneously hypertensive rats; solid circles = Wistar–Kyoto rats. Each point represents mean ± S.E.M. of five separate experiments. * = $P < 0.05$, compared with respective values of Wistar–Kyoto rats.

was greater (but not significant) than that in WKY rats (Fig. 2B). The half-maximal effective concentration (EC_{50}; in $-\log M$) and maximum effect (E_{max}) values for endothelin-1 and endothelin-3 are listed in Table 1. EC_{50} values for endothelin-1 were significantly different for SHR and WKY rats (7.00 ± 0.04 vs. 6.60 ± 0.05), but the values for endothelin-3 were not significantly different (6.55 ± 0.05 vs. 6.38 ± 0.06). Furthermore, E_{max} values for endothelin-1 and endothelin-3 were not significantly different.

3.3. Effects of sarafotoxin S6c on $[Ca^{2+}]_i$

The effect of sarafotoxin S6c, an endothelin ET_B receptor agonist, was also tested to determine the endothelin receptor subtype in pericardial mesothelial cells. Sarafotoxin S6c elicited an elevation of $[Ca^{2+}]_i$ in a dose-dependent manner (Fig. 3). The sarafotoxin S6c-induced (0.01–0.3 μM) elevation of $[Ca^{2+}]_i$ in pericardial mesothelial cells from SHR was significantly greater than that in mesothelial cells from WKY rats. The EC_{50} and E_{max} values for sarafotoxin S6c are also listed in Table 1.

3.4. Effects of the endothelin ET_A and ET_B receptor antagonist

An endothelin ET_A (FR139317, 1 μM) or an endothelin ET_B (IRL 1038, 1 μM) receptor antagonist was used to identify the endothelin receptor subtype mediating the increased $[Ca^{2+}]_i$. IRL 1038 significantly inhibited the endothelin-1- (Fig. 4A) and endothelin-3-induced (Fig. 4B) changes in $[Ca^{2+}]_i$. However, FR139317 treatment did not affect these changes (Fig. 4). These observations provide further evidence that pericardial mesothelial cells have the endothelin ET_B receptor.

4. Discussion

This study has shown that cultured pericardial mesothelial cells obtained from SHR and WKY rats produce an endothelin-1 and possess functional endothelin ET_B receptors. Pericardial mesothelial cells from both SHR and WKY rats shared the ability to produce endothelin-1 spontaneously in culture, although a consistently greater production was detected in cultures of SHR origin. The endothelin- and sarafotoxin S6c-induced elevations of $[Ca^{2+}]_i$ in pericardial mesothelial cells from SHR were greater than those in mesothelial cells from WKY rats. These results suggest that endothelin-1 may play an important role in the pericardium in an autocrine and/or paracrine fashion. Moreover, pericardial mesothelial cells of SHR may have an increased number of endothelin ET_B receptors and/or a more active signal transduction system than those of WKY rats.

Plasma endothelin-1 levels of SHR and WKY rats have been measured by many investigators. These reports showed higher (Khraibi et al., 1993), lower (Ohno et al., 1992) or unchanged (Hirata et al., 1994; Vemulapalli et al., 1991) plasma endothelin-1 levels in adult SHR, and in some of these studies, slightly higher but not significantly higher levels were seen. However, plasma endothelin-1 levels may not be a good index of endothelin secretion because this peptide probably acts in a paracrine fashion, and plasma endothelin-1 may merely represent spillover of endothelin secretion in the intercellular space (Masaki et al., 1992). Our result is consistent with an earlier report in which the perfused mesenteric territory in SHR released more endothelin-1 than the same territory in WKY rats (Miyamori et al., 1991). However, the kidneys and the brain of SHR contain less endothelin-1 than the same tissues of WKY rats (Bolger et al., 1991; Hughes et al., 1992; Yoshimi et al., 1991). Therefore, it seems that these regional differences between the two strains may reflect potential sites of endothelin-1 action.

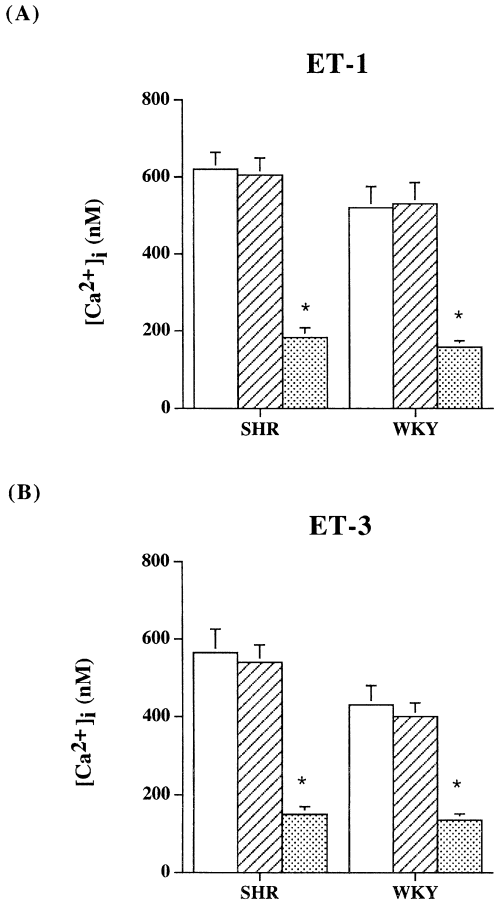

Fig. 4. Effects of endothelin ET_A (FR139317; 1 μM) and ET_B (IRL 1038; 1 μM) receptor antagonist on 1 μM endothelin-1- (A) and endothelin-3- (B) induced elevation of $[Ca^{2+}]_i$ in pericardial mesothelial cells. Histogram showing the magnitude of the initial transient increase in $[Ca^{2+}]_i$. Open bars = control; hatched bars = treated with FR139317; dotted bars = treated with IRL 1038. Each bar represents mean ± S.E.M. of five separate experiments. $* = P < 0.05$, significant difference from each control.

Although we found that pericardial mesothelial cells of SHR produced much more endothelin-1 than those of WKY rats, big endothelin-1 production was almost the same in pericardial mesothelial cells of SHR and WKY rats. A possible explanation is that the activity of endothelin-converting enzyme is different in these strains of rats. However, this possibility is not likely because treatment with phosphoramidon, a putative endothelin-converting enzyme inhibitor, increased the amount of big endothelin-1 in the conditioned medium of pericardial mesothelial cells from SHR much more than it did the amount big endothelin-1 in the medium of mesothelial cells from WKY rats (unpublished observation). Therefore, there is no obvious explanation for the greater propensity of pericardial mesothelial cells of SHR to produce endothelin-1, and this finding may simply reflect the strain differences in endothelin-1 synthesis by pericardial mesothelial cells.

Endothelin receptors have been classified into the endothelin ET_A and endothelin ET_B receptor subtypes. The endothelin ET_A receptor is activated relatively selectively by endothelin-1 whereas the endothelin ET_B receptor is non-selectively activated by endothelin-1 and endothelin-3 (Masaki et al., 1992). Both endothelin-1 and endothelin-3 induced elevation of $[Ca^{2+}]_i$ in a similar concentration range in the pericardial mesothelial cells. We thus considered that these responses may be mediated by the endothelin ET_B receptor. This speculation was supported by the use of sarafotoxin S6c, FR139317, and IRL 1038. The selective agonist of the endothelin ET_B receptor, sarafotoxin S6c, also induced elevation of $[Ca^{2+}]_i$. Moreover, IRL 1038 significantly inhibited the endothelin-1- and endothelin-3-induced changes in $[Ca^{2+}]_i$. However, FR139317 treatment did not affect these changes. These results suggest that pericardial mesothelial cells have endothelin ET_B receptors. Moreover, stimulation of the endothelin ET_B receptor in endothelial cells may release nitric oxide (NO) and prostacyclin. Because mesothelial cells are also capable of synthesizing and/or releasing NO and prostacyclin (Owens and Grisham, 1993; Satoh and Prescott, 1987), endothelins may be of relevance in the regulation of these reactions in mesothelial cells.

The endothelin- and sarafotoxin S6c-induced elevations of $[Ca^{2+}]_i$ in pericardial mesothelial cells from SHR were greater than those in mesothelial cells from WKY rats. The EC_{50} values of endothelins in pericardial mesothelial cells were 10–100-fold higher than those in endothelial cells and vascular smooth muscle, the EC_{50} values in endothelial cells and vascular smooth muscle being 1–10 nM. Batra et al. (1993) recorded EC_{50} values of 5.3 and 4.8 nM for the effects of endothelin-1 on $[Ca^{2+}]_i$ in aortic smooth muscle cells from SHR and WKY rats respectively. Furthermore, we estimated the EC_{50} value of sarafotoxin S6c from their data and arrived at a value of $\sim 0.02\ \mu M$, which was nearly identical to our results for the EC_{50} value for sarafotoxin S6c in pericardial mesothelial cells. However, a relatively high concentration of endothelin might be needed to induce physiological responses in the pericardial surface, because sarafotoxin S6c is not the endogenous agonist at the endothelin ET_B receptors.

Yokokawa et al. (1994) have shown that the endothelin-3-induced increases in $[Ca^{2+}]_i$ and inositol 1,4,5-trisphosphate level in endothelial cells of SHR are greater than those of endothelial cells of WKY rats. Furthermore, Batra et al. (1993) have shown that sarafotoxin S6c evokes much larger increases in $[Ca^{2+}]_i$ in aortic smooth muscle cells isolated from SHR than in the same cells isolated from WKY rats. Our results for the increase in $[Ca^{2+}]_i$ in pericardial mesothelial cells in response to endothelins are consistent with these observations for SHR and WKY rats. Therefore, these results suggest that many cell types in SHR may have a more active signal transduction system compared to that of the same cells in WKY rats.

5. Conclusion

We have shown that cultured pericardial mesothelial cells obtained from SHR and WKY rats produce endothelin-1 and possess endothelin ET_B receptors. Pericardial mesothelial cells from both SHR and WKY rats shared the ability to produce endothelin-1 spontaneously in culture, although a consistently greater production was detected in cultures of SHR origin. Moreover, pericardial mesothelial cells from SHR may have increased numbers of endothelin ET_B receptors and/or a more active signal transduction system compared with those of cells from WKY rats. These results suggest that endothelin-1 may play an important role in the pericardium in an autocrine and/or paracrine fashion.

Acknowledgements

We are grateful to Ciba-Geigy Japan and to Fujisawa Pharmaceutical, for the generous gifts of IRL 1038 and FR139317, respectively. This work was supported in part by Grant-in-Aid from the Scientific Research Fund on the Ministry of Education, Science, Sports and Culture, Japan.

References

Batra, V.K., McNeill, J.R., Xu, Y., Wilson, T.W., Gopalakrishnan, V., 1993. ET_B receptors on aortic smooth muscle cells of spontaneously hypertensive rats. Am. J. Physiol. 264, C479–C484.

Baumann, M.H., Heinrich, K., Sahn, S.A., Strange, C., 1993. Pleural macrophages differentially alter mesothelial cell growth and collagen production. Inflammation 17, 1–12.

Bolger, G.T., Liard, F., Jodoin, A., Jaramillo, J., 1991. Vascular reactivity, tissue levels, and binding sites for endothelin: a comparison in the spontaneously hypertensive and Wistar–Kyoto rats. Can. J. Physiol. Pharmacol. 69, 406–413.

Eid, H., Larson, D.M., Springhorn, J.P., Attawia, M.A., Nayak, R.C., Smith, T.W., Kelly, R.A., 1992. Role of epicardial mesothelial cells in the modification of phenotype and function of adult rat ventricular myocytes in primary coculture. Circ. Res. 71, 40–50.

Eid, H., Kuroski de Bold, M.L., Chen, J.H., de Bold, A.J., 1994. Epicardial mesothelial cells synthesize and release endothelin. J. Cardiovasc. Pharmacol. 24, 715–720.

Gerwin, B.I., Lechner, J.F., Reddel, R.R., Roberts, A.B., Robbins, K.C., Gabrielson, E.W., Harris, C.C., 1987. Comparison of production of transforming growth factor-β and platelet-derived growth factor by normal human mesothelial cells and mesothelioma cell lines. Cancer Res. 47, 6180–6184.

Grynkiewicz, G., Poenie, M., Tsien, R.Y., 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J. Biol. Chem. 260, 3440–3450.

Hallam, T.J., Pearson, J.D., Needham, L.A., 1988. Thrombin stimulated elevation of endothelial cell cytoplasmic free calcium concentration causes prostacyclin production. Biochem. J. 251, 243–249.

Hirata, Y., Kayakawa, H., Sazuki, E., Omota, M., 1994. Does endothelin work as an intrarenal mechanism to alter pressure natriuresis in spontaneously hypertensive rats?. J. Hypertens. 12, 251–257.

Honda, A., Noguchi, N., Takehara, H., Ohashi, Y., Asuwa, N., Mori, T., 1991. Cooperative enhancement of hyaluronic acid synthesis by combined use of IGF-I and EGF, and inhibition by tyrosine kinase inhibitor genistein, in cultured mesothelial cells from rabbit pericardial cavity. J. Cell. Sci. 98, 91–98.

Hughes, A.K., Cline, R.C., Kohan, D.E., 1992. Alterations in renal endothelin-1 production in the spontaneously hypertensive rat. Hypertension 20, 666–673.

Ito, K., Kuwahara, M., Sugano, S., Kuwahara, M., 1995. Role of intra- and extracellular calcium stores in mesothelial cell response to histamine. Am. J. Physiol. 268, L63–L70.

Khraibi, A.A., Heublein, D.M., Knox, E.G., Burnett Jr, J.C., 1993. Increased plasma level of endothelin-1 in the Okamoto spontaneously hypertensive rat. Mayo Clin. Proc. 68, 42–46.

Kuwahara, M., Kuwahara, M., Bijwaard, K.E., Gersten, D.M., Diglio, C.A., Kagan, E., 1991. Mesothelial cells produce a chemoattractant for lung fibroblasts: role of fibronectin. Am. J. Respir. Cell Mol. Biol. 5, 256–264.

Kuwahara, M., Kuwahara, M., Suzuki, N., 1992. Production of endothelin-1 and big endothelin-1 by pleural mesothelial cells. FEBS Lett. 298, 21–24.

Kuwahara, M., Masuda, T., Tsubone, H., Sugano, S., Karaki, H., 1996. Cardiovascular responses mediated by two types of endothelin ET_B receptor in spontaneously hypertensive and Wistar–Kyoto rats. Eur. J. Pharmacol. 296, 55–63.

Lee, T.C., Zhang, Y., Aston, C., Hintz, R., Jagirdar, J., Perle, M.A., 1993. Normal human mesothelial cells and mesothelioma cell lines express insulin-like growth factor 1 and associated molecules. Cancer Res. 53, 2858–2864.

Masaki, T., Yanagisawa, M., Goto, K., 1992. Physiology and pharmacology of endothelins. Med. Res. Rev. 12, 391–421.

Miyamori, I., Takeda, T., Yoneda, T., Takaeda, R., 1991. Endothelin-1 release from mesenteric arteries of spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 17, S408–S410.

Ohno, A., Naruse, M., Kato, S., Hosaka, M., Naruse, K., Demura, H., Sugino, N., 1992. Endothelin-specific anti-bodies decrease blood pressure and increase glomerular filtration rate and renal plasma flow in spontaneously hypertensive rats. J. Hypertension 10, 781–785.

Owens, M.W., Grisham, M.B., 1993. Nitric oxide synthesis by rat pleural mesothelial cells: induction by cytokines and lipopolysaccharide. Am. J. Physiol. 265, L110–L116.

Owens, M.W., Milligan, S.A., 1994. Growth factor modulation of rat pleural mesothelial cell mitogenesis and collagen synthesis. Effects of epidermal growth factor and platelet-derived growth factor. Inflammation 18, 77–87.

Rubanyi, G.M., Polokoff, M.A., 1994. Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. Pharmacol. Rev. 45, 325–415.

Sakai, S., Miyauchi, T., Sakurai, T., Kasuya, Y., Ihara, M., Yamaguchi, I., Goto, K., Sugishita, Y., 1996. Endogenous endothelin-1 participates in the maintenance of cardiac function in rats with congestive heart failure, marked increase in endothelin-1 production in the failing heart. Circulation 93, 1214–1222.

Satoh, K., Prescott, S.M., 1987. Culture of mesothelial cells from bovine pericardium and characterization of their arachidonate metabolism. Biochim. Biophys. Acta 930, 283–296.

Sogabe, K., Nirei, H., Shoubo, M., Nomoto, A., Ao, S., Notsu, Y., Ono, T., 1993. Pharmacological profile of FR139317, a novel, potent endothelin ET_A receptor antagonist. J. Pharmacol. Exp. Ther. 264, 1040–1046.

Staszewska-Barczak, J., Dusting, G.J., May, D.E., Nolan, P.N., 1981. Effects of prostacyclin on cardiovascular reflexes from the ventricular epicardium of the dog: comparison with the effects of prostaglandin E_2. Prostaglandins 21, 905–915.

Suzuki, N., Matsumoto, H., Kitada, C., Masaki, T., Fujino, M., 1989. A sensitive sandwich-enzyme immunoassay for human endothelin. J. Immunol. Methods 118, 245–250.

Urade, Y., Fujitani, Y., Oda, K., Watakabe, T., Umemura, I., Takai, M., Okada, T., Sakata, K., Karaki, H., 1992. [Cys^{11}–Cys^{15}]-endothelin-1 (11-21), IRL 1038, is an antagonist specific for endothelin B receptors. FEBS Lett. 311, 12–16.

Vemulapalli, S., Chiu, P.J., Rivelli, M., Foster, G.J., Sybertz, E.J., 1991. Modulation of circulating endothelin levels in hypertension and endotoxemia in rats. J. Cardiovasc. Pharmacol. 18, 895–903.

Waters, C.J., Chang, J.Y., Glucksberg, M.R., DePaola, N., Grotberg, J.G., 1997. Mechanical forces alter growth factor release by pleural mesothelial cells. Am. J. Physiol. 272, L552–L557.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., Masaki, T., 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332, 411–415.

Yokokawa, K., Johnson, J., Kohno, M., Mandal, A.K., Yanagisawa, M., Horio, T., Yasunari, K., Takeda, T., 1994. Phosphoinositide turnover signaling stimulated by ET-3 in endothelial cells from spontaneously hypertensive rats. Am. J. Physiol. 267, R635–R644.

Yoshimi, H., Kawano, Y., Akabane, S., Ashida, T., Yoshida, K., Kinoshita, O., Kuramachi, M., Omae, T., 1991. Immunoreactive endothelin-1 contents in brain regions from spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 17, S417–S419.