Intrapericardial infusion of endothelin-1 induces ventricular arrhythmias in dogs

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Received 14 April 1997; accepted 11 December 1997

Abstract

Objectives: Recently, extremely high levels of endothelin-1 ET-1 were detected in the pericardial fluid of patients with heart disease; however, the pathophysiological importance of this finding is not known. The present study was designed to characterize ET-1 levels in canine pericardial fluid and to investigate the effects of local high concentrations of exogenous ET-1 in vivo.

Methods: In anesthetized, open-chest dogs ET-1 Groups 1 and 2: 11 and 33 pmol kg⁻¹ min⁻¹; n = 6 and 6, respectively or physiological saline. Group 3, n = 5 were infused into the closed pericardial sac for 40 min. In serial pericardial fluid and aortic blood plasma samples, ET-1 levels were measured by radioimmunoassay, and analysed by high-performance liquid chromatography (HPLC). Systemic arterial blood pressure, heart rate, cardiac output (CO), standard ECG and right ventricular endocardial monophasic action potentials (MAPs) were recorded.

Results: Basal pericardial fluid ET-1 levels were significantly higher than respective plasma levels (342 ± 210 vs. 8.0 ± 5.2 pmol l⁻¹, n = 14, P < 0.001). In HPLC analysis pericardial fluid ET-1 was indistinguishable from ET-1. Infusion of exogenous ET-1 into the pericardial space induced ventricular arrhythmias in all instances, which were associated with 9.7-fold increase in pericardial fluid ET-1 levels. Ventricular tachycardias developed in 9 of 12 animals. The arrhythmogenic effect of ET-1 was more apparent in dogs with the larger dose. Before the onset of arrhythmias, intrapericardial infusion of ET-1 increased QT time (Group 1: 207 ± 18 to 230 ± 23 ms, P < 0.01; Group 2: 220 ± 12 to 277 ± 17 ms, P < 0.01) and MAP duration at 90% repolarization (at 300 ms cycle length) (Group 1: 192 ± 9 to 216 ± 9 ms, P < 0.01; Group 2: 205 ± 9 to 255 ± 9 ms, P < 0.001). Hemodynamic variables did not change significantly prior to the onset of ventricular tachyarrhythmias. In Group 3, arrhythmias were not observed and all electrophysiological and hemodynamic parameters remained unchanged. Conclusions: Administration of exogenous ET-1 into the pericardial space induces ventricular arrhythmias associated with prolongation of QT time and MAP duration. Whether pericardial fluid ET-1 under pathophysiological conditions can ever reach sufficiently high levels to induce ventricular arrhythmias remains to be elucidated. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Experimental heart electrophysiology; Endothelin; Pericardial fluid; Ventricular arrhythmia; Dog, heart

1. Introduction

Endothelin (ET), the most potent vasoconstrictor peptide known so far, was originally characterized from the supernatant of cultured porcine aortic endothelial cells [1]. ET-1, a 21-amino-acid peptide belonging to a family of highly homologous peptides, is constitutively secreted by endothelial cells. ET-2 and ET-3, which are expressed at a much lower rate, and the sarafotoxin peptides isolated from the venom of Actractaspis engaddensis are also in the family [2]. Secretion of ET-1 by endothelial cells is polar,
being directed toward the interstitial region rather than the vascular lumen [3], suggesting that ET-1 acts as a local autocrine and paracrine factor rather than as a circulating hormone. ET-1, produced by vascular endothelium, appears to be a significant factor in the regulation of smooth muscle tone [2]. Moreover, ET-1 released by the endocardial endothelium has recently been shown to influence myocardial contractile state of the subjacent cardiomyocytes [4,5]. In contrast to the vascular and endocardial endothelia, less attention has been paid to the physiological significance of the pericardium, which is lined with mesothelial cells, derived from the same stem cells as vascular endothelial cells [6,7]. In vitro, epicardial mesothelial cells synthesize and release ET-1 [8]. We have reported recently that pericardial fluid of human subjects with heart disease contains extremely high concentrations of ET-1, suggesting that epicardial secretion of the peptide into the pericardial fluid may occur in vivo [9]. However, no information is available whether or not high pericardial fluid ET-1 levels can influence cardiac function.

ET-1 has multiple actions in the heart. The peptide exerts potent coronary vasoconstrictor, and moderate inotropic and chronotropic effects [10–14], and induces hypertrophy of cardiomyocytes [15,16]. In addition, accumulating evidence suggests that ET-1 may have direct arrhythmogenic effect. In isolated ventricular myocytes, ET-1 increases the action potential duration and is capable of inducing early afterdepolarizations (EADs) [17]. In anesthetized dogs, intracoronary administration of ET-1 at low concentrations, which reduces coronary blood flow only slightly, induces severe ventricular arrhythmias accompanied by the prolongation of monophasic action potential duration and the development of EADs [18,19].

The aim of the present study was to characterize ET-1 levels in plasma and pericardial fluid and to study the potential pathophysiological effects of local high concentrations of the peptide by infusing ET-1 into the closed pericardial sac of anesthetized dogs.

2. Methods

2.1. Animal preparation

Acute experiments were conducted in mongrel dogs (n = 17) of either sex weighing 13–24 kg. Each animal was anesthetized with intravenous sodium pentobarbital (30 mg/kg) and supplemental doses were given throughout the experiment as needed to maintain constant level of anesthesia. An endotracheal tube was inserted, and the dogs were artificially ventilated with room air using a volume cycled respirator (Cape Eng., UK). Catheters were inserted into the abdominal aorta through both femoral arteries, one of them connected to a pressure transducer (model P23 ID, Gould Electronics, USA) for arterial blood pressure monitoring, and the other serving for arterial blood sampling. Transsternal thoracotomy was performed in the fourth intercostal space and a polyvinyl cannula was introduced into the pericardial sac for pericardial fluid sampling and drug administration. Surface electrocardiogram (leads I, II, III, aVR, aVL, aVF) and right ventricular endocardial monophasic action potential (MAP) was monitored. MAPs are extracellularly recorded waveforms that can represent the repolarization time course of intracellular action potentials [20]. MAPs were recorded by either quadrupolar silver–silver chloride (MAP-pacing combination catheter, EP Technologies, USA) or iridium coated endocardial catheters (AI Cath TWIN LP/X, Biotronik, Germany) inserted into the right ventricle through the left femoral vein and positioned against the anteroseptal endocardial surface (in three instances MAPs were also recorded from the anteroseptal endocardial surface of the left ventricle). MAPs were obtained after placement of the catheter electrode in a position providing continuous recordings with stable amplitude, smooth configuration and isopotential diastolic baseline that persisted for at least 10 min. Once the catheter was stabilized, MAPs could be recorded continuously from the same endocardial site for long periods without any additional catheter manipulation. Cardiac output was determined as an average of three measurements by standard thermodilution technique (Experimetria, Hungary). ECG, MAP and arterial blood pressure were recorded by means of a 12-channel direct writing recorder (model CU-12, Madaus Schwartzer, Germany). The analog MAP signals were digitized by a 12-bit analog to digital converter after being amplified and filtered (0.04 and 2000 Hz). The digitized signals were then stored on hard disk on an IBM-compatible computer system for later analysis.

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

2.2. Experimental protocol

After a 30-min period of stabilization, exogenous ET-1 (Sigma Chemical, St. Louis, MO, USA) was administrered as a continuous infusion into the closed pericardial sac at a flow rate of 0.25 ml·min⁻¹ for 40 min, followed by 120 min recovery. In animals of Group 1 (n = 6) and Group 2 (n = 6), ET-1 was infused at a rate of 11 and 33 pmol·kg⁻¹·min⁻¹, respectively. Animals of time control group (n = 5) received vehicle (physiological saline) infusion according to the same protocol. Hemodynamic parameters including systemic arterial blood pressure, sinus heart rate and cardiac output were measured at baseline and repeated at 10-min intervals. Surface ECG (leads I, II, III, aVR, aVL, aVF) and endocardial MAPs were recorded continuously for arrhythmia analysis and for detection of EADs, respectively. The following electrophysiological para-
ters were determined at baseline and thereafter at 10-min intervals until the appearance of severe rhythm disturbances: cycle length, PQ interval for AV conduction time, QRS duration, and QT time. The QT interval was determined mainly from ECG lead II and was defined as the time between QRS onset and the point at which the line of maximal down-slope of the T wave crossed the baseline. Three consecutive beats were measured and averaged at all time points. For electrophysiological studies the programmed electrical stimulation of the right ventricle was performed at basal state and repeated at 10-min intervals by using an impulse generator (UHS-20, Biotronik, Germany). Electrical impulses (2.0 ms in duration) were delivered at twice the diastolic myocardial threshold. Ventricular effective refractory period (VERP) was determined by using ventricular pacing via the MAP electrode at cycle length (S1S1) of 300 ms for eight beats followed by an extrastimulus (S2) with decremental delay until the extrastimulus failed to produce response, which was taken as VERP. The duration of MAP was determined at 90% repolarization (MAPD90), which included EADs if present. MAPD90 was measured at 300 ms cycle length using only data from recordings with stable resting potentials. Three consecutive MAP signals were analyzed manually for MAPD90 and then averaged at all time points. EADs were defined according to Cranefield [21] and Damiano and Rosen [22] as depolarizing afterpotentials that interrupted or delayed repolarization of the action potential. The irritability of the ventricle was tested by using single or double early stimuli and bursts of rapid pacing at a rate of 300 per min (20 beats per burst). Arrhythmias were analysed by ECG recordings. Non-sustained ventricular tachycardia was defined as four or more consecutive ventricular premature beats lasting less than 30 s. Sustained ventricular tachycardia was defined as 100 or more consecutive ventricular extrasystoles, or tachycardia persisting for more than 30 s. Pericardial fluid and plasma samples were collected to estimate ET-1 levels at the peak of arrhythmia induction (ca. 50–60 min after the start of the experiments). All samples were immediately placed on dry ice and stored at −20 °C until assayed.

2.3. Extraction procedures

Plasma and pericardial fluid samples were extracted utilising SepPak C18 cartridges as described elsewhere [23]. Briefly, 1 ml plasma and 200–500 μl pericardial fluid samples were acidified to pH 4 with 10% trifluoroacetic acid and applied to SepPak C18 cartridges previously activated by methanol and triethanolamine acetate buffer (TEA, 20 mmol·l−1, pH 4). The peptide was eluted with 80% methanol in TEA buffer. Eluates were evaporated and reconstituted in RIA buffer containing Na2HPO4 (0.1 mol·l−1), NaCl (0.05 mol·l−1) and Triton X-100 (0.1% v/v). Recovery of added ET-1 was 80 ± 2%. Triplicates of 100-μl aliquot fractions from the above dilution were assayed for ET-1 using RIA.

2.4. Determination of ET-1-like immunoreactivity

Plasma and pericardial fluid ET-1 levels were measured utilizing antisera (RAS 6901N) purchased from Peninsula Laboratories (Mersyside, UK). Human ET-11–21 (Sigma Chemical, Germany) ranging from 0 to 80 pmol·l−1 per tube was used to construct the standard curve. Radiiodinated ET-1 was produced using the chloramine-T method. The sensitivity of the assay was 0.3 pmol·l−1 per tube. The 50% intercept of the standard curve was at 7.2 pmol·l−1 per tube, intra- and interassay coefficients of variation were less than 10% and 15%, respectively.

2.5. High-performance liquid chromatography (HPLC)

For HPLC analysis lyophillized pericardial fluid samples were redissolved in 40% acetonitrile/0.1% trifluoroacetic acid in water and applied onto a 7.8 × 300 mm ProteinPak 125 gel filtration HPLC column (Waters, Milford, MA, USA) and eluted with the same solvent. Fractions were collected every 30 s, evaporated and then dissolved in RIA buffer and measured in ET-1 RIA.

2.6. Statistical analysis

The results are expressed as mean ± s.e.m. Since ET-1 values were not normally distributed, these are presented as mean ± s.d. and range. Statistical significance of the differences was tested with ANOVA, paired t-test, Wilcoxon signed-ranks test or independent-groups t-test, when appropriate. To assess correlation between data

<table>
<thead>
<tr>
<th>Arhythmia</th>
<th>Group 1</th>
<th></th>
<th>Group 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevalence</td>
<td>Episodes</td>
<td>Prevalence</td>
<td>Episodes</td>
</tr>
<tr>
<td>VES</td>
<td>6/6</td>
<td>185 (29–408)</td>
<td>6/6</td>
<td>287 (32–711)</td>
</tr>
<tr>
<td>Couplet and triplet</td>
<td>6/6</td>
<td>66 (2–237)</td>
<td>6/6</td>
<td>129 (20–363)</td>
</tr>
<tr>
<td>nsVT</td>
<td>3/6</td>
<td>32 (0–140)</td>
<td>6/6</td>
<td>63 (14–158)</td>
</tr>
<tr>
<td>sVT</td>
<td>0/6</td>
<td>0</td>
<td>4/6</td>
<td>2 (0–4)</td>
</tr>
<tr>
<td>VF</td>
<td>0/6</td>
<td>2/6</td>
<td>0.3 (0–1)</td>
<td></td>
</tr>
</tbody>
</table>

Arrhythmia analysis was performed in animals receiving intrapericardial infusion of ET-1 (Group 1: 11 pmol·kg−1·min−1, n = 6; Group 2: 33 pmol·kg−1·min−1, n = 6). Prevalence indicates the number of affected animals per total number of animals. VES = ventricular extrasystole; nsVT = non-sustained ventricular tachycardia; sVT = sustained ventricular tachycardia; VF = ventricular fibrillation. Values for arrhythmia episodes are expressed as mean (range).
Spearman’s rank correlation was used. Differences were considered statistically significant at the level of \( P < 0.05 \).

### 3. Results

#### 3.1. Effects of intrapericardial administration of ET-1

**3.1.1. Arrhythmia induction**

Infusion of ET-1 (11 and 33 pmol·kg\(^{-1}\)·min\(^{-1}\)) into the pericardial space for 40 min induced ventricular arrhythmias in all instances \((n = 12)\), whereas upon saline infusions \((\text{Group } 3, n = 5)\) arrhythmias could not be detected. The arrhythmogenic effect of ET-1 is summarized in Table 1. In Groups 1 and 2, ET-1 infusion \((n = 6 \text{ and } 6, \text{ respectively})\) induced substantial number of ventricular extrasystoles, couplets and triplets with onset at 10 to 20 min (Fig. 1). The increasing number of ventricular extrasystoles were followed by non-sustained ventricular tachycardias in three instances in Group 1, whereas in all instances in Group 2. At 30 to 50 min, ET-1 induced non-sustained ventricular tachycardias regularly followed each other in an incessant manner. In Group 2, ET-1 induced sustained ventricular tachycardias in four of six animals (Table 1, Fig. 2). Ventricular tachycardia degenerates into ventricular fibrillation in an animal receiving intrapericardial infusion of ET-1 at a rate of 33 pmol·kg\(^{-1}\)·min\(^{-1}\).
Table 2
Effect of intrapericardial administration of ET-1 on electrophysiological parameters in anesthetized dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 Baseline 20 min</th>
<th>Group 2 Baseline 20 min</th>
<th>Group 3 Baseline 20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL, ms</td>
<td>337 ± 18 343 ± 18</td>
<td>392 ± 24 413 ± 19</td>
<td>348 ± 15 348 ± 14</td>
</tr>
<tr>
<td>PQ, ms</td>
<td>98 ± 7 99 ± 8</td>
<td>110 ± 10 110 ± 8</td>
<td>96 ± 4 96 ± 3</td>
</tr>
<tr>
<td>QRS, ms</td>
<td>53 ± 6 54 ± 6</td>
<td>56 ± 7 56 ± 6</td>
<td>48 ± 4 48 ± 4</td>
</tr>
<tr>
<td>QT, ms</td>
<td>207 ± 18 230 ± 23</td>
<td>220 ± 12 227 ± 17</td>
<td>202 ± 7 198 ± 7</td>
</tr>
<tr>
<td>VERP, ms</td>
<td>132 ± 8 138 ± 9</td>
<td>167 ± 7 167 ± 4</td>
<td>138 ± 12 142 ± 10</td>
</tr>
<tr>
<td>MAPD&lt;sub&gt;90&lt;/sub&gt;, ms</td>
<td>192 ± 9 216 ± 9</td>
<td>205 ± 9 255 ± 9</td>
<td>217 ± 11 216 ± 10</td>
</tr>
</tbody>
</table>

Electrophysiological parameters were measured at baseline and 20 min after the start of ET-1 (Group 1: 11 pmol·kg<sup>-1</sup>·min<sup>-1</sup>, n = 6; Group 2: 33 pmol·kg<sup>-1</sup>·min<sup>-1</sup>, n = 6) or vehicle infusions (Group 3, n = 5). In ET-1-treated animals, 20 min represents a time point prior to the onset of severe arrhythmias. CL = cycle length; PQ, QRS and QT = electrocardiographic PQ, QRS and QT intervals; VERP = ventricular effective refractory period; MAPD<sub>90</sub> = monophasic action potential duration at 90% of repolarization measured at 300 ms cycle length. Values are expressed as mean ± s.e.m. and depict absolute changes vs. baseline values. P < 0.01 increased, and EADs trigger incessant non-sustained ventricular salves.

3.1.2. Electrophysiological and hemodynamic parameters

Table 2 summarizes the effects of pericardial infusion of ET-1 on electrophysiological parameters. In Group 1, ET-1 infusion significantly (P < 0.01) increased QT interval compared with respective baseline values (Table 2). In Group 2, prolongation of QT interval was even more pronounced (Table 2, Fig. 3). No effects on either atrioventricular or intraventricular conduction time, or ventricular refractory period were noted (Table 2). Moderate ischemic ECG signs (ST elevation 0.25 ± 0.08 mV) prior to the onset of ventricular arrhythmias could be observed only with the higher dose of ET-1.

Monophasic action potential duration at 90% repolarization (MAPD<sub>90</sub>) (at 300 ms cycle length), as revealed by endocardial MAP recordings of the right ventricle, significantly increased at both ET-1 doses when compared to respective baseline values (Table 2, Fig. 3). Moreover, the prolongation of MAPD<sub>90</sub> was significantly greater (P < 0.01) in Group 2 compared with Group 1 (Fig. 3). The prolongation of QT interval and MAPD<sub>90</sub> always preceded the appearance of arrhythmias. In 7 of 12 dogs, using endocardial MAP recordings of the right ventricle, EADs could be detected during phase 3 of MAP in association with the development of arrhythmias. When MAPs were obtained simultaneously from the right and left ventricle,
in two of three instances EADs developed in both recordings (Fig. 4).

As shown in Table 3, hemodynamic parameters remained unchanged when saline was infused into the closed pericardial sac at a low infusion rate (0.25 ml·min⁻¹). Intrapericardial infusion of ET-1 also did not induce significant change in blood pressure, cardiac output, systemic vascular resistance and heart rate prior to the onset of ventricular arrhythmias (Table 3). The hemodynamic changes were also insignificant when compared with the saline time control group. At 50–60 min, development of ventricular tachyarrhythmias was followed by significant decrease of cardiac output and increase of systemic vascular resistance (data not shown).

3.1.3. Plasma and pericardial fluid ET-1 levels

As shown in Fig. 5A, basal pericardial fluid ET-1 levels (342 ± 210 pmol·1⁻¹, range: 139–958, n = 14) were significantly higher (P < 0.001) than respective plasma levels (8.0 ± 5.2 pmol·1⁻¹, range: 1.9–22). Of note is that in one animal the concentration of pericardial fluid ET-1 was extremely high exceeding 3 s.d., therefore this value has been excluded. The mean pericardial fluid to plasma ratio of ir-ET-1 was 79 ± 115 (range: 9–436). No significant correlation was found between pericardial fluid and plasma ir-ET-1 levels (r = −0.24, P = n.s.). In HPLC analysis the total ET-1-like immunoreactivity of the pericardial fluid co-eluted with ET-1 standard (Fig. 5B).

ET-1 infusion into the pericardial space increased basal pericardial fluid ET-1 levels 9.7-fold (from 387 ± 242 to 3772 ± 2639 pmol·1⁻¹, P < 0.01, n = 9), measured at the peak of arrhythmia induction, whereas, plasma ET-1 levels remained practically unaltered (from 8.7 ± 6.4 to 8.7 ± 4.7 pmol·1⁻¹, P = n.s., n = 9). In the saline time control group neither pericardial fluid (from 261 ± 118 to 155 ± 28 pmol·1⁻¹, P = n.s., n = 5), nor plasma ET-1 levels were affected significantly (from 7.6 ± 1.2 to 7.8 ± 2.8 pmol·1⁻¹, P = n.s., n = 5).

4. Discussion

In the present study we were able to show the presence of immunoreactive ET-1 in the pericardial fluid of dogs. In HPLC, pericardial fluid ir-ET-1 was indistinguishable from ET-1, as concentrations of ET-1 in the pericardial fluid are far higher than in any biological fluids tested thus far, including plasma, urine [24], saliva [25], and cerebrospinal fluid [26]. Previously we have reported that high ET-1 levels can be found in the pericardial fluid of patients undergoing cardiac surgery [9]. The pericardial fluid to plasma ratio of ET-1 was comparable in humans and dogs (36 ± 12 versus 79 ± 31), suggesting that the presence of high ET-1 levels in the pericardial fluid is a physiological phenomenon, at least in some species, and can not be related exclusively to disease state.

Analysis of protein and ion composition of pericardial fluid indicates that it is mainly formed as a passive ultrafiltrate of blood plasma [27], however, the high pericardial fluid to plasma ratio of ET-1 seems to suggest that the major site of origin of this peptide in the pericardial fluid cannot be the blood plasma. Since epicardial mesothelial cells have been shown to secrete ET-1 in vitro [8], it is tempting to speculate that pericardial fluid ET-1 originates from the epicardium itself.
Pericardial fluid partially originates as an overflow of myocardial interstitial fluid [27], suggesting that substances released by cardiac myocytes into the interstitial space can be translocated into the pericardial compartment with the flow of interstitial fluid. Several experimental data suggest that substances administered at the external surface of the epicardium can be translocated into the myocardial interstitium. Page et al. demonstrated that distension of isolated rat atrial preparation at pressures prevailing in vivo, renders atrial epicardium and myocardium permeable even to large molecules (40 to 500 kDa) [28]. Polypeptides such as substance P have been reported to be rapidly absorbed through the epicardium [29]. In perfused rabbit atria, radio-labelled inulin, which has similar molecular weight to ET-1, introduced into the pericardial space is translocated with the flow of interstitial fluid through the myocardium into the atrial lumen upon stretch and release [30]. Prostaglandins have been reported to modulate cardiac autonomic neurotransmission when applied in a canine epicardial superfusion model [31]. Intrapericardial injection of basic fibroblast growth factor and heparin sulfate induced angiogenesis and myocardial salvage in a canine model of myocardial infarction [29]. Previously, epicardial mesothelial cells have been shown to modify the phenotype and function of adult rat ventricular cardiomyocytes in primary coculture [32]. Later, it was proposed that ET-1 may take part in epicardial mesothelial cell–cardiomyocyte interaction [8]. In vivo, ET-1 released by mesothelial cells may accumulate in the pericardial fluid and may be transported into the myocardial interstitial space. Upon binding to specific endothelin receptors located on the surface of cardiomyocytes [33], ET-1 can influence cardiac function and myocyte phenotype.

An accumulating body of evidence suggests that ET-1 may have a direct arrhythmogenic effect. In anesthetized dogs, intracoronary administration of ET-1 at low concentrations, which reduces coronary blood flow only slightly, induces severe ventricular arrhythmias that commonly degenerate into ventricular fibrillation and death [34,18]. Our present results showed that ET-1, when applied intrapericardially, could also evoke major ventricular arrhythmias. The tachyarrhythmias were always preceded by prolongation of QT interval and monophasic action potential duration, in a similar manner as was reported previously upon intracoronary administration of ET-1 [18,19]. Development of EADs could be detected in more than 50% of dogs, suggesting that the arrhythmias could be of triggered origin [35,36]; however, the involvement of other mechanisms cannot be ruled out.

In canine cardiac tissues, it has been shown that ET-1 increases the action potential duration and is capable of inducing EADs [17]. Nicardipine, a Ca\(^{2+}\) channel blocker, abolished the development of EADs and shortened the ET-induced action potential prolongation, suggesting the involvement of dihydropyridine-sensitive Ca\(^{2+}\) channels [17]. Since patch-clamp studies have established that ET-1 inhibits the ATP-sensitive K\(^+\) current [37] and the delayed rectifier K\(^+\) current in ventricular myocytes [38,39], it is conceivable that in addition to Ca\(^{2+}\) channels K\(^+\) channels may also be involved in the arrhythmogenic effect of ET-1. However, the cellular mechanisms underlying the arrhythmias induced by intrapericardial application of ET-1 remain to be established.

In the present study, underlying myocardial ischemia cannot be ruled out; however, several lines of evidence suggest that ET-1 may have direct arrhythmogenic effect upon intrapericardial administration. First, modest if any ischemic signs could be detected on ECG; however, severe ventricular arrhythmias were observed. Previously, Hori et al. have reported that epicardial application of ET-1 induces subepicardial microischemia formation, which is accompanied by sustained epicardial ST-segment elevation [40]. Second, prolongation of QT interval and MAPD\(_{90}\) occurred in every instance, whereas ischemia per se leads to the shortening of MAP duration [41]. Third, in dogs, upon intracoronary administration of ET-1, significant coronary vasoconstriction occurred in association with substantial increase in coronary sinus and aortic blood plasma arterial ET-1 concentrations. Finally, in the absence of significant hemodynamic changes prior to the onset of arrhythmias, severe myocardial ischemia as the sole mechanism of ET-1-induced arrhythmias is unlikely.

Clinical conditions such as cardiac hypertrophy and failure are associated with high risk of sudden death, believed in most cases secondary to ventricular tachyarrhythmias [43]. Myocardial hypertrophy has been shown to lengthen transmembrane action potential duration and to increase their dispersion [44], producing an electrophysiological milieu which may facilitate the development of arrhythmias [45]. In the hypertrophied and failing myocardium the production of ET-1 is markedly increased and the density of myocardial ET receptors is elevated [46–48]. ET expression in cardiac hypertrophy may be induced by several stimuli, including mechanical stretch [49,50] produced by cardiac overload, increased activity of the intracardiac renin–angiotensin system [51] and the induction of myocardial expression of transforming growth factor-\(\beta\) [52]. It is feasible that the factors responsible for the upregulation of the myocardial ET system may also induce the synthesis of ET-1 in epicardial mesothelial cells and increase pericardial fluid ET-1 concentrations. In support of this hypothesis, angiotensin II has been shown to stimulate ET-1 release of epicardial mesothelial cells in vitro [8]. Taken together, we propose that in cardiac hypertrophy and failure in the presence of multiple arrhythmogenic factors, including structural abnormalities, elevated catecholamine levels, electrolyte abnormalities and prolongation of the cardiac action potential, high concentrations of pericardial fluid ET-1, in conjunction with the upregulated myocardial ET system, may take part in the initiation
of ventricular tachyarrhythmias leading to sudden cardiac death.

In summary, our results show for the first time that administration of ET-1 into the pericardial space, increasing basal pericardial fluid ET-1 levels 9.7-fold, induces severe ventricular arrhythmias. Our results suggest that the arrhythmogenic effect of ET-1 may be based on prolongation of QT interval and monophasic action potential duration. Previously, high ET-1 levels have been reported in the pericardial fluid of patients with heart failure [9], a clinical condition associated with high risk of occurrence of ventricular tachyarrhythmias [43]. Under pathophysiological states such as cardiac hypertrophy and failure accompanied by an electrophysiological milieu that may facilitate the development of rhythm disturbances [45], high concentrations of pericardial fluid ET-1 may be involved in the induction of ventricular tachyarrhythmias and sudden cardiac death. Whether pericardial fluid ET-1 in these clinical conditions can ever reach sufficiently high levels to induce ventricular arrhythmias remains to be elucidated.

Acknowledgements

This study was supported by the Hungarian Research Foundation (OTKA) F020258, F016331, T016327, T023506, and the Ministry of Social Welfare. The authors wish to thank Marta Rajczi and Eszter Szendrei for expert technical assistance.

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