Endothelin-1-induced elevations in purine metabolite concentrations – autoregulatory protection in the canine pericardium?

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ABSTRACT

Pericardial fluid accumulates the cardioprotective purine metabolites, as well as the endogenous vasoconstrictor agent endothelin-1 (ET-1). The aim of the present study was to characterize the pericardial concentrations of the purine metabolites adenosine, inosine and hypoxanthine before and after intrapericardial administration of ET-1 to the in situ heart. The closed pericardial sac of anaesthetized dogs (n = 9) was cannulated for ET-1 administration and for obtaining native pericardial fluid and control pericardial infusate samples (C1 and C2), as well as consecutive pericardial infusate samples (samples I, II and III) obtained 15 min after intrapericardial administration of 150 pmol/kg ET-1. In an additional five dogs, using the same protocol, ventricular epicardial and endocardial monophasic action potential recordings were performed to assess local ischaemic electrophysiological changes. Significant elevations of pericardial purine metabolite concentrations (measured by HPLC) were found in sample II compared with sample C2: adenosine, 4.5 ± 1.7 compared with 0.5 ± 0.1 μM (P < 0.05); inosine, 18.3 ± 2.8 compared with 0.9 ± 0.2 μM (P < 0.001); hypoxanthine, 38.1 ± 8.0 compared with 13.4 ± 2.6 μM (P < 0.01). Systemic blood pressure, left ventricular pressure and contractility, and systemic plasma levels of the purine metabolites remained unchanged during the ET-1 effect, while significant ECG ST elevations (STmax 0.68 ± 0.01 mV; P < 0.001) were observed. In the five dogs analysed electrophysiologically, the left ventricular epicardial monophasic action potential duration and upstroke velocity decreased significantly at time point II compared with C2, while the endocardial monophasic action potential duration and upstroke velocity did not show ischaemia-related changes. The results suggest that intrapericardial administration of ET-1 induces subepicardial ischaemia, with parallel activation of coronary metabolic adaptive and cardiac self-protective mechanisms in the epimyocardial layer of the heart.

INTRODUCTION

Human and experimental animal studies have demonstrated that the pericardial fluid (PF), which is present in the intrapericardial space, not only serves a mechanical function but also accumulates several endogenous regulatory agents of cardiac origin [1–6]. The purine metabolites, such as adenosine and its degradative products inosine and hypoxanthine, play an important cardioprotective role as a part of an endogenous metabolic coronary

Key words: adenosine, autoregulation, endothelin-1, inosine, ischaemia, pericardium.

Abbreviations: ET-1, endothelin-1; MAPD90, duration of monophasic action potential evaluated at 90% of repolarization; PBSA, PBS containing 0.5% BSA, pH 7.4; PF, pericardial fluid; PI, pericardial infusate.

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adapative mechanism. The levels of these agents are coupled to the cardiac energy state, whereby coronary flow and myocardial oxygen consumption are kept in balance [7–9]. The intrapericardial concentrations of these nucleosides are several-fold higher than those measured in systemic and coronary sinus blood samples under normal physiological conditions [4,6]. Interestingly, the most potent endogenous vasoconstrictor peptide produced by the endothelial cells, endothelin-1 (ET-1) [10], also has a high intrapericardial concentration [3,5,9,11–13]. The intrapericardial levels of these agents may be augmented further in pathophysiological conditions. Moreover, these levels may be interrelated, if augmented intapericardial concentrations of ET-1 induce myocardial ischaemia [14].

The present study was designed to test this hypothesis and to analyse the intrapericardial concentrations of the purine metabolites before and after intrapericardial administration of 150 pmol/kg ET-1 to the canine heart in situ.

METHODS

General preparation

Acute experiments were performed on nine mongrel dogs (body weight 24 ± 1 kg) The investigation conformed with the Guide for the Care and Use of Laboratory Animals, published by the U.S. National Institutes of Health (NIH Publication no. 85–23; revised 1985).

Each animal was initially anaesthetized with intravenous pentobarbital sodium (Nembutal; CEVA; 30 mg/kg), and additional anaesthesia was given as needed to maintain a constant level. After tracheal intubation, the dogs were ventilated with room air by a Cape CV2424 ventilator (Cape Engineering Co. Ltd). One of the femoral arteries was cannulated for continuous arterial blood pressure monitoring (Experimetria HG 01 modem; Statham P23Db), and a carotid artery was prepared for left ventricular pressure and contractility measurements (Pigtail Catheter 4F; Cordis). Continuous ECG recording (Madaus Schwarzer CU12) was done simultaneously with haemodynamic monitoring throughout the experiment. Following trans-sternal thoracotomy in the fifth intercostal space, the intact pericardial sac was cannulated via a small incision. A soft rubber catheter was positioned below the heart in the oblique sinus (the lowest region of the pericardial sac with the animal in the supine position during the experiment) and the pericardium was tightened around the catheter entry site to prevent fluid escape. The pericardial catheter was used for obtaining PF and pericardial infusate (PI) samples, and for administration of ET-1.

Experimental protocol

After a 20 min stabilization period, the native PF was removed quantitatively. Thereafter, the pericardial space was washed repeatedly with 5 × 5 ml of PBS containing 0.5% BSA, pH 7.4 (PBSA) to minimize the contamination of a sample with small quantities of the previous sample possibly retained in the pericardial sac. Pericardial washing was repeated after each sampling. The PI samples, i.e. PBSA solutions of 0.75 ml/kg, were each incubated for 15 min in the pericardial sac. Then the total volume of the PI sample and an arterial blood sample were collected simultaneously.

All samples were collected into syringes containing ice-cold stop solution prepared with 400 μmol/l dipyridamole and 100 μmol/l EHNA (erythro-4-(2-hydroxy-3-nonil) adenine hydrochloride) to prevent further degradation of the purine metabolites, since a high degree of degradation of adenosine is expected on the basis of earlier studies [4,6,15,16].

In the first phase of the experiments, the control incubation was repeated twice (samples C1 and C2). For sample I, PBSA containing 150 pmol/kg ET-1 (Sigma-Aldrich) was incubated for 15 min in the pericardial space. The incubation was then repeated with ET-1-free PBSA two more times to obtain samples II and III.

Purine metabolite levels were corrected to the native PF volume of each animal and to the total volume of the PI and the stop solution. The concentrations of adenosine and its derivatives in the serial pericardial and arterial blood samples were determined by isocratic HPLC with UV detection, as described previously [17].

Registration of the monophasic action potential

For recording of the epicardial monophasic action potential, a fractally coated screw-in monophasic action potential catheter (V177; Biotronik G.m.b.H.) was fixed on to the anterolateral epicardial surface of the right and left ventricles. For recording of the endocardial monophasic action potential, fractally coated electrophysiology catheters (AlCath Blue; Biotronik G.m.b.H.) were inserted through the right femoral vein into the apex of the right ventricle and through the femoral artery into the left ventricular apex. The duration of monophasic action potential was evaluated at 90% of repolarization (MAPD90). Upstroke velocity was defined as the quotient of the depolarization amplitude and the depolarization duration of the monophasic action potential curve. Monophasic action potential parameters were digitized by a 12-bit analogue-to-digital converter after being amplified and filtered. The analogue signals were recorded on a 12-channel direct chart recorder (Madaus Schwarzer CU 12), and the digitized signals were stored on a PC.
**Statistical analysis**

Data are presented as means ± S.E.M. Unpaired Student’s \( t \) tests were performed for comparison of the data. \( P \) values of < 0.05 were considered significant.

**RESULTS**

The native PF contained a higher concentration of adenosine than the plasma. The concentration of adenosine in PI sample C1 was lower than in the native PF sample. However, no significant difference was found between PI samples C1 and C2 taken consecutively, thus confirming the reproducibility of the incubation method (Table 1).

Intrapericardial administration of ET-1 induced myocardial ischaemia, as signalled by significant ST segment elevations in the ECG (\( ST_{\text{max}} = 0.68 ± 0.01 \text{ mV}; P < 0.001 \) at the sample I time point), while overall haemodynamic parameters (Table 2) and systemic plasma levels of the purine metabolites did not change significantly. A positive inotropic effect of ET-1 was not observed.

The plasma levels of the purine metabolites remained unchanged during the myocardial ET-1 effect, but the concentrations of all three purine metabolites increased in the PF. The adenosine concentration showed a 9-fold elevation and that of inosine a 20-fold elevation following ET-1 administration. The most marked change was measured in sample II after ET-1 administration (Figure 1).

In the five dogs that were analysed electrophysiologically, the left ventricular epicardial \( \text{MAPD}_{90} \) and the upstroke velocity of the monophasic action potential had decreased significantly at time point II compared with time point C2. The endocardial \( \text{MAPD}_{90} \) and upstroke velocity did not exhibit significant changes. Considering that ischaemia and ET-1 have opposite effects on \( \text{MAPD}_{90} \) and upstroke velocity, (i.e. ischaemia decreases while ET-1 increases the values of these variables), an unequivocal ischaemic change was not proven in the endomyocardial layer of the heart (Table 3).

**DISCUSSION**

Increased intrapericardial concentrations of the endogenous vasoconstrictor peptide ET-1 elicit significant elevations in intrapericardial purine metabolite levels. We found that under normal physiological conditions the nucleoside concentrations of the PI samples, corrected to the native and stop solution volumes, were obviously lower than those of the native PF, apparently due to the limited time of incubation (15 min) of the samples.

### Table 1 Reproducibility of basal concentrations of purine metabolites in pericardial and plasma samples

C1 and C2 represent controls. Values are means ± S.E.M. \((n = 9); * P < 0.05\) compared with native PF.

<table>
<thead>
<tr>
<th>Purine metabolite</th>
<th>Native PF</th>
<th>Arterial plasma</th>
<th>PI</th>
<th>Arterial plasma</th>
<th>PI</th>
<th>Arterial plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>3.19 ± 0.53</td>
<td>0.04 ± 0.01</td>
<td>0.51 ± 0.1*</td>
<td>0.04 ± 0.01</td>
<td>0.54 ± 0.1*</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>Inosine</td>
<td>1.73 ± 0.70</td>
<td>0.19 ± 0.04</td>
<td>1.26 ± 0.20</td>
<td>0.19 ± 0.06</td>
<td>0.87 ± 0.15</td>
<td>0.25 ± 0.06</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>7.26 ± 1.81</td>
<td>3.55 ± 0.62</td>
<td>14.10 ± 3.16</td>
<td>4.13 ± 0.94</td>
<td>13.43 ± 2.44</td>
<td>4.51 ± 0.66</td>
</tr>
</tbody>
</table>

### Table 2 Changes in haemodynamic variables following pericardial administration of ET-1

MBP, mean blood pressure; HR, heart rate; \( \text{dP/dt} \), rate of change of pressure. Control data are from the C2 sample time point. Values are means ± S.E.M. \((n = 9); * P < 0.05\) compared with control.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Sample I</th>
<th>Sample II</th>
<th>Sample III</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP (mmHg)</td>
<td>116 ± 1</td>
<td>112 ± 1</td>
<td>114 ± 1</td>
<td>110 ± 1</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>157 ± 1</td>
<td>155 ± 1</td>
<td>161 ± 1</td>
<td>161 ± 2</td>
</tr>
<tr>
<td>( \text{dP/dt} ) (mmHg/s)</td>
<td>2827 ± 65</td>
<td>2600 ± 36</td>
<td>2012 ± 79</td>
<td>2037 ± 69</td>
</tr>
<tr>
<td>ST elevation (mV)</td>
<td>-0.01 ± 0.00</td>
<td>0.68 ± 0.01*</td>
<td>0.44 ± 0.01*</td>
<td>0.26 ± 0.01*</td>
</tr>
</tbody>
</table>

### Figure 1 Concentrations of purine metabolites in PI samples following intrapericardial administration of ET-1

Control data represent purine metabolite concentrations in sample C2. Values are means ± S.E.M. \((n = 9); * P < 0.05, ** P < 0.01\) compared with control.
Table 3  Changes in electrophysiological data following pericardial administration of ET-1

<table>
<thead>
<tr>
<th>Time point</th>
<th>Endocardial MAPD90 (ms)</th>
<th>Epicardial MAPD90 (ms)</th>
<th>Endocardial Upstroke velocity (V/s)</th>
<th>Epicardial Upstroke velocity (V/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>215.7 ± 2.4</td>
<td>214.6 ± 2.9</td>
<td>1.43 ± 0.25</td>
<td>1.76 ± 0.1</td>
</tr>
<tr>
<td>Sample I</td>
<td>217.8 ± 3.0</td>
<td>196.5 ± 2.7*</td>
<td>1.44 ± 0.22</td>
<td>1.22 ± 0.08*</td>
</tr>
<tr>
<td>Sample II</td>
<td>214.1 ± 3.1</td>
<td>193.0 ± 3.2*</td>
<td>1.66 ± 0.29</td>
<td>0.83 ± 0.06**</td>
</tr>
<tr>
<td>Sample III</td>
<td>218.6 ± 1.7</td>
<td>186.0 ± 5.6*</td>
<td>1.45 ± 0.26</td>
<td>1.13 ± 0.08</td>
</tr>
</tbody>
</table>

However, when applying this incubation time and sampling technique, repetitive intrapericardial sampling ensured adequate reproducibility (Table 1).

While detectable ischaemia was indicated by the surface ECG, haemodynamic parameters were preserved, due to the apparently not transmural ischaemic effect of the intrapericardial ET-1, as suggested by the different endocardial and epicardial monophasic action potential recordings. Moreover, intrapericardial ET-1 showed no arrhythmogenic effect at a dosage of 150 pmol/kg.

A continuous increase in cardioprotective purine metabolite release was revealed by the nucleoside levels in samples I and II. At the time point of sample III a moderate recovery was observed in purine metabolite changes, suggesting slow elimination of the action of ET-1.

In conclusion, significant elevations in the intrapericardial concentrations of the purine metabolites following intrapericardial ET-1 administration suggest that the vasoconstrictor ET-1 may induce subepicardial ischaemia and markedly enhance myocardial purine metabolite release. The rapid local appearance of endogenous cardioprotective regulatory products in the PF suggests that the entire pericardial space may serve as the milieu of cardiac self-defence mechanisms against myocardial ischaemia. Given that the PF accumulates endogenous regulatory agents from the adjoining myocardial interstitial space, it seems that coronary metabolic adaptive and cardiac self-protective mechanisms could be activated effectively under ischaemic conditions induced by increased intrapericardial ET-1 levels.

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