Coronary vasoconstriction to endothelin-1 increases with age before and after ischaemia and reperfusion

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Abstract

Objective: Ageing is known to be associated with changes within the heart. We investigated whether the coronary response to endothelin-1 (ET) and sarafotoxin S6c (S6c) is altered with increasing age, before and after cardioplegic arrest.

Methods: Using an isolated rat heart model, increasing concentrations of ET and S6c were administered to rats of different ages (group I=one month; group II=five months; group III=21 months). An identical series of experiments was performed following the addition of indomethacin and N^2-nitro-L-arginine methyl ester (L-NAME) to the Krebs perfusion fluid. In a third series of experiments, increasing doses of ET-1 were added to hearts following 4 h of cardioplegic arrest at 4°C.

Results: Coronary flows are expressed as a percentage of initial coronary flow±SEM. There was a greater decrease in coronary flow in the older rats for all doses of ET-1. ET-1 (10^-9 M) reduced coronary flows to 72.8±3.7, 53.2±6.7 and 56.5±10.7% for groups I–III respectively (P=0.01 I vs. II; P=0.1 I vs. III). A similar response to ET-1 was seen in hearts perfused with indomethacin and L-NAME when compared to those perfused without (P=NS). Perfusion with ET-1 (10^-9 M) following 4 h of cardioplegic arrest reduced coronary flows to 40.5±4.9, 26.8±4.8 and 24.1±3.9%, respectively (P=0.08 I vs. II; P=0.03 I vs. III). Perfusion with S6c (10^-10 M) produced coronary flows of 93.3±5.5, 77.0±3.5 and 73.9±3.9% for groups I–III, respectively (P=0.03 I vs. II; P=0.01 I vs. III). Perfusion with S6c (10^-7 M) in the presence of L-NAME and indomethacin reduced coronary flows to 85.7±3.0, 81.6±2.2 and 74.6±3.6% (P=NS I vs. II; P=0.03 I vs. III).

Conclusions: The coronary vasoconstrictor response to ET-1 and S6c increases with age. The increased vasoconstriction in response to ET-1 is independent of the decrease in NO release seen with ageing. © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: Endothelin; Sarafotoxin; Ageing; Rat hearts; Coronary artery

1. Introduction

Ageing is known to be associated with biochemical and functional changes in the heart [1,2]. These changes can affect one or more of the different cellular components of the heart, including the coronary endothelium. The endothelium constitutes a complex dynamic organ with diverse functions, which include the synthesis and release of vasoactive substances that modulate vascular tone [3].

The effects of β-adrenoceptor stimulation on vascular smooth muscle responses at different ages have been extensively investigated. The vasodilatation in response to β-adrenoceptor stimulation decreases with age [4,5], whereas the contractile response to α-adrenoceptor stimulation appears to be unaltered [6,7]. Endothelium-dependent relaxation is also reduced with age. We have previously demonstrated that the basal and stimulated release of nitric oxide (NO) by the coronary endothelium decreases with age in the rat [8].

Endothelins are a group of recently discovered peptides [9]. Endothelin-1 (ET-1) is the most potent endogenous vasoconstrictor substance discovered to date. ET levels are elevated in a number of cardiovascular disorders, e.g., following myocardial infarction, heart failure and following cardioplegic arrest and cardiac surgery [10–15]. The ageing heart is less tolerant of periods of ischaemia. Ischaemia and reperfusion result in a reduction in coronary
flow (so called ‘low coronary reflow’) [16]. It has been suggested that elevated levels of ET may be responsible, at least in part, for the low coronary reflow phenomenon [17]. One possible mechanism for this may be due to increased sensitivity to ET.

There have been a number of previous studies examining whether the response to ET alters with age. Some studies have shown a reduced response to ET-1 with age in the rat aorta, mesenteric and renal arteries [18–20]. However, it has also been reported that ET-1 has no effect with ageing in rat aorta [21]. It appears that there is considerable heterogeneity in response to ET in different vascular beds. In view of the important role that ET appears to play in the regulation of vascular tone, it is important to establish whether the coronary response to ET alters with ageing.

The effect of age on the contractile response of intact coronary arteries to ET has not been investigated previously. The aim of this study was to investigate if the response of rat coronary arteries to ET-1 and sarafotoxin S6c (ETB agonist) is altered with increasing age. In addition, the response to ET-1 following prolonged cardioplegic arrest, mimicking the conditions experienced during cardiac transplantation, was studied in rats of different ages.

2. Methods

2.1. Animals

Male Sprague-Dawley rats were used in all experiments. The age groups studied were as follows: Group I, one month (± one week); group II, four–five months and Group III, 21 months (± two months). In all studies, animals received humane care in compliance with the ‘Principles of Laboratory Animal Care’ formulated by the National Society for Medical Research and the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Institutes of Health (NIH Publication No. 85-23, revised 1985).

2.2. Experimental preparation

The Langendorff isolated rat heart preparation was used in this study, as has already been described in detail elsewhere [17,22]. Briefly, the animals were sacrificed by cervical dislocation. The femoral vein was immediately exposed and heparin (200 IU) was injected. The heart was then excised and immediately placed in ice-cold (4°C) Krebs solution. The aorta was rapidly cannulated (within approximately 30 s) and Langendorff perfusion was initiated. The hearts were perfused with Krebs-Henseleit bicarbonate buffer (pH 7.4) consisting of (mmol/l): NaCl 118.5, NaHCO3 25.0, KCl 4.8, MgSO4 1.2, KH2PO4 1.2, CaCl2 2.25 and glucose 11.0, which was continuously gassed with 95% O2 and 5% CO2, and maintained at 37°C. The Krebs buffer flowed from a reservoir that was 100 cm above the heart. The heart was suspended in a water jacketed chamber maintained at 37°C.

Coronary flow was monitored by an in-line electromagnetic flow probe (ECM2 20 ml, Scalar, Delft, Netherlands) connected to its compatible flowmeter (MLD 1401, Scalar). This provided an accurate (0.0–40.0 ml/min) digital read-out of mean coronary flow, with a simultaneous hard copy recording through a connection with a chart recorder (RS3400, Gould Electronics, Hainault, UK). This allowed accurate monitoring of steady-state conditions (less than 0.1 ml/min change in coronary flow over 3 min). Heart rate was measured using a pressure transducer within the aortic flow-line, attached to the same chart recorder. The resultant waveform was analysed by an in-built module (Gould) that allowed continuous recording of heart rate.

In the experiments looking at the effects of ET-1 following ischaemia, a model of cardioplegic arrest, mimicking the conditions of the donor heart undergoing cardiac transplantation, was used. This was achieved by infusing 10 ml of St Thomas’ Hospital No. 1 Solution (made up in 1 l Ringer’s lactate solution) at 4°C via the side-arm of the aortic cannula from a reservoir that was 60 cm above the heart. The heart was then maintained immersed in cardioplegic solution at 4°C for 4 h.

2.3. Drugs and chemicals

The drugs used in this experiment were all made up to the required concentration in the Krebs buffer. The drugs used were endothelin-1 (Peninsula Labs, Merseyside, UK) (initially dissolved in 0.1% ethanol and then further diluted to the correct concentration in Krebs) and sarafotoxin S6c (Peninsula Labs). In some of the experiments (see experimental time course below), N'G-nitro-L-arginine methyl ester (10⁻⁴ M; L-NAME) (Sigma, Poole, UK), an inhibitor of NO synthase, and indomethacin (10⁻⁶ M) (Sigma), an inhibitor of prostacyclin synthesis, were added to the Krebs buffer. Previous experiments in our laboratory have confirmed that these doses totally inhibit the release of NO and prostacyclin [23].

2.4. Experimental time course

2.4.1. Effect of endothelin-1 on coronary flow at different ages

Each heart was perfused in the Langendorff mode with plain Krebs until steady coronary flow was achieved. This took approximately 15 min. Perfusion was then switched to Krebs containing 10⁻¹⁰ M ET-1 for 10 min and coronary flow was again recorded. Following this, perfusion was changed to Krebs containing 10⁻¹⁸ M ET-1 for a
further 10 min, followed by 10⁻⁸ M ET-1 for a final 10 min. Coronary flow was recorded at each time point.

2.4.2. Effect of endothelin-1 on coronary flow at different ages following cardioplegic arrest

In a separate set of experiments, hearts were perfused in the Langendorff mode with plain Krebs until steady coronary flow was achieved. Following this, each heart was perfused with 10 ml of St Thomas' Hospital No.1 cardioplegic solution at 4°C. Each heart was then maintained ischaemic at this temperature for 4 h. Each heart was then reperfused with plain Krebs for 15 min and a baseline coronary flow was recorded. A period of 15 min was chosen as previous experiments had shown that this time is required for steady state coronary flow to be achieved following cardioplegic arrest in this model. Following this, each heart was perfused for 10-min periods with increasing doses of ET-1 (10⁻¹⁰ to 10⁻⁸ M) in the same manner as for the experiments performed pre-ischaemia. Coronary flow was recorded at each time point.

2.4.3. Comparison of effects of endothelin-1 pre- vs. post cardioplegic arrest

Due to the detrimental effects of high doses of ET-1 on coronary flow, it was not possible to compare the effects of ET-1 pre- and post ischaemia in the same hearts. In order to compare the effects, the results of the two previous experiments were compared.

2.4.4. Comparison of the effects of endothelin-1 with and without inhibition of endogenous vasodilator release

In this series of experiments, we studied the effects of ET-1 on coronary flow during the inhibition of NO and prostacyclin release. Hearts were perfused throughout the experiment with Krebs to which 10⁻⁶ M L-NAME and 10⁻⁶ M indomethacin had been added. A period of perfusion was allowed until steady coronary flow was achieved (approximately 15 min). Hearts were then perfused with increasing doses of ET-1 (10⁻¹⁰ to 10⁻⁸ M) in the same manner as for the experiments performed above.

2.4.5. Comparison of the effects of sarafotoxin S6c on coronary flow at different ages with and without inhibition of endogenous vasodilator release

In this series of experiments, hearts were perfused with Krebs until steady coronary flow was achieved. Following this, increasing doses of sarafotoxin S6c (10⁻¹⁰ to 10⁻⁸ M) were added (in the same manner as for the ET-1 experiments) for 10-min periods and coronary flow was recorded. An identical series of experiments was performed following the addition of 10⁻⁴ M L-NAME and 10⁻⁶ M indomethacin to the Krebs perfusion buffer.

2.5. Expression of results

The coronary flows are expressed as a percentage of the initial steady state coronary flow. Coronary flow data were compared by analysis of variance; whenever significance was indicated, Scheffé’s test for multiple comparison was used to compare between groups. Significance was assumed when the $P$ value was less than 0.05. Values are given as mean±standard error of the mean (SEM).

3. Results

The baseline characteristics and coronary flows for each experimental group are shown in Table 1.

3.1. Effect of endothelin-1 on coronary flow at different ages (Fig. 1)

As expected, there was a dose-dependent decrease in coronary flow in response to increasing doses of ET-1. At each of the concentrations studied, the vasoconstriction seen was greater in the older groups of rats (groups II and III) when compared to the young rats (group I). The effect of ET-1 on heart rate is shown in Table 2. In all three age groups, there was no significant difference in heart rate between baseline and perfusion with either 10⁻¹⁰ or 10⁻⁹ M ET-1. At a concentration of 10⁻⁸ M, hearts became dys-rhythmic due to ischaemia, with a resultant significant reduction in heart rate.

Table 1
Baseline coronary flows (CF) in the different experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Rat weight (g)</th>
<th>Heart weight (g)</th>
<th>Effect of ET-1 (Fig. 1)</th>
<th>Effect of S6c (Fig. 5)</th>
<th>Effect of ET-1 post-ischaemia L-NAME and indomethacin (Fig. 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>CF (ml/min)</td>
<td>n</td>
<td>CF (ml/min)</td>
<td>CF (ml/min)</td>
</tr>
<tr>
<td>I</td>
<td>119±4</td>
<td>0.96±0.10</td>
<td>10</td>
<td>8.4±0.5</td>
<td>6</td>
</tr>
<tr>
<td>II</td>
<td>482±12</td>
<td>2.2±0.07</td>
<td>10</td>
<td>14.2±0.9</td>
<td>6</td>
</tr>
<tr>
<td>III</td>
<td>876±39</td>
<td>2.76±0.15</td>
<td>6</td>
<td>18.1±0.9</td>
<td>6</td>
</tr>
</tbody>
</table>
3.2. Effect of endothelin-1 on coronary flow at different ages following cardioplegic arrest (Fig. 2)

In a similar manner to pre-ischaemia, there was a dose-dependent decrease in coronary flow in response to ET-1. Likewise, the vasoconstriction seen was greater in the older rats (groups II and III) when compared to the young rats (group I).

3.3. Comparison of effects of endothelin-1 pre vs. post cardioplegic arrest (Fig. 3)

The effects of ET-1 were compared pre vs. post 4 h of cold ischaemia in the different age groups. There were no significant differences in baseline coronary flows when experiments performed prior to ischaemia were compared to post-ischaemic experiments ($P=\text{NS}$; for values, see Table 1). Cardioplegic arrest decreased post-ischaemic coronary flow to 79±6% of pre-ischaemic flow in group I, 75±13% in group II and 84±6% in group III ($P=\text{NS}$ between groups). In the group I animals, there was a greater decrease in coronary flow following ischaemia at all the doses of ET-1 studied, which was highly significant. A similar trend was seen in the group II and III animals, however, this only reached statistical significance at concentrations of ET-1 of $10^{-9}$ M.

3.4. Effect of endothelin-1 on coronary flow following inhibition of endogenous vasodilator release (Fig. 4)

A dose-dependent decrease in coronary flow was seen in response to ET-1 following the blockade of NO synthase and prostacyclin release. The vasoconstrictor response seen in rats in groups I and II was similar. The increased vasoconstriction seen in the older rats (group III) during perfusion with normal Krebs, when compared to rats in group I, was also seen during blockade of endogenous vasodilator release.

3.5. Effect of sarafotoxin S6c on coronary flow at different ages (Fig. 5)

Sarafotoxin (S6c) acts on ET$_A$ receptors. In this experiment, we observed no significant effects on coronary flow when it was administered in increasing doses to the hearts in group I. However, it produced a significant vasoconstrictor response in the group II and III rats. This response appeared not to be dose-dependent, as a similar degree of vasoconstriction was seen for the different concentrations studied.

Table 2
Effect of increasing concentrations of ET-1 on heart rate (±SEM) at different ages

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal</th>
<th>$10^{-10}$ M ET-1</th>
<th>$10^{-9}$ M ET-1</th>
<th>$10^{-8}$ M ET-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>295±13</td>
<td>291±16</td>
<td>288±14</td>
<td>178±15</td>
</tr>
<tr>
<td>II</td>
<td>263±6</td>
<td>249±9</td>
<td>237±12</td>
<td>98±48</td>
</tr>
<tr>
<td>III</td>
<td>229±8</td>
<td>213±8</td>
<td>199±14</td>
<td>113±52</td>
</tr>
</tbody>
</table>

$P<0.05$ vs. basal heart rate.
3.6. Effect of sarafotoxin S6c on coronary flow following inhibition of endogenous vasodilator release (Fig. 6)

Following the blockade of NO and prostacyclin release, there was a significant vasoconstrictor response to S6c in rats of all ages, unlike in the experiments with plain Krebs in which a significant response was only seen in the older animals (groups II and III). There was a trend towards an increased constrictor response with age. However, this was only statistically significant between rats in groups I and III (P<0.03). Likewise to experiments with plain Krebs, the constriction seen did not appear to be dose-dependent, with similar responses to the different concentrations studied.

4. Discussion

This study has shown that the coronary vasoconstrictor effect of ET-1 increases with age. A similar finding was seen following 4 h of ischaemia at 4°C. The amount of coronary vasoconstriction seen following ischaemia was greater in rats of all ages. Following the inhibition of endogenous vasodilator release, the effect of ET-1 was still greatest in the senescent rats compared to the younger age groups. Perfusion with an ET<sub>B</sub> agonist produced no effect on coronary flow in the young rats, however, it produced a vasoconstrictor response in the adult and senescent rats. However, following inhibition of endogenous vasodilator release, perfusion with the ET<sub>B</sub> agonist revealed a vasoconstrictor response in rats of all ages, with an increased response in the older rats.

Resting coronary tone depends on a variety of factors. This includes the balance between the endogenous release of vasodilator (i.e. NO, prostacyclin) and vasoconstrictor substances (i.e. ET) [23,24]. We have previously demonstrated that both basal and stimulated release of NO by the coronary endothelium decreases with age in a rat model [8].

There appears to be considerable heterogeneity in response to ET in different vascular beds. Some studies have shown a reduced response to ET-1 with age in the rat aorta, mesenteric and renal arteries [18–20]. However, it has also been reported that ET-1 has no effect with ageing in rat aorta [21]. A study using rings of isolated rat coronary artery showed an increased response to ET with age [25]. To date, there are no studies looking at the response to ET and S6c in the intact coronary circulation of the rat, or in other species.

In this study, we have demonstrated that the coronary vasoconstriction seen in response to ET-1 is increased with advancing age. This result is in contrast to studies in other vascular beds in which the effect of ET-1 is unaltered or reduced with ageing [18–21]. This difference may be due to increased ET receptor density, or increased receptor density.
Fig. 3. Comparison of effects of increasing doses of endothelin-1 (10^{-10} to 10^{-8} M) on coronary flow pre- vs. post cardioplegic arrest (4 h at 4°C) in hearts of different ages.

affinity, with ageing of the coronary circulation. Although the vasoconstriction to ET tended to increase with age, it did not increase significantly between the adult and senescent rats. It appears that the coronary circulation in the rat becomes more sensitive to ET, particularly during maturation from one month old to adulthood. It is unclear what role this increased sensitivity to ET may play in the adult circulation.

Ischaemia and reperfusion result in a reduction in coronary flow (so called 'low coronary reflow') [16]. It has
Fig. 4. Effect of endothelin-1 (10^{-10} to 10^{-8} M) on coronary flow following inhibition of endogenous vasodilator release with L-NAME and indomethacin in hearts of different ages.

Fig. 5. Effect of increasing doses of sarafotoxin S6c (10^{-10} to 10^{-8} M), when compared to controls, on coronary flow at different ages.
been suggested that elevated levels of ET may be responsible, at least in part, for the low coronary reflow phenomenon [17]. ET-1 levels in plasma are raised with increasing age [26,27]. In this study, we have shown that there is an enhanced response to ET-1 with age following prolonged cardioplegic arrest. In addition, the degree of vasoconstriction was greater for all age groups when compared to the experiments performed prior to ischaemia. This is in keeping with the findings of other groups who have demonstrated increased sensitivity to ET-1 following periods of warm (37°C) ischaemia [28,29]. These findings may have implications for cardiac surgery and cardiac transplantation, especially when older hearts undergo surgery, or are being selected for potential donors. It is possible that older hearts may be more prone to low coronary reflow following prolonged periods of hypothermic storage. There is some experimental evidence that inhibition of ET-1 during ischaemia may improve coronary flow following prolonged cardioplegic arrest [17].

In mammalian tissue, ET-1 acts at two receptors (ET_A and ET_B). The predominant receptor type is ET_A, which mediates vasoconstriction of smooth muscle cells [30]. ET_B receptors are present on both endothelial cells (where they mediate vasodilatation through the release of NO and prostacyclin) and smooth muscle cells (where they mediate vasoconstriction) [31,32]. The result of this is that ET-1 may partially regulate the amount of vasoconstriction it causes through a negative feedback loop via ET_B receptors (producing NO and prostacyclin). One possible explanation for the increased vasoconstriction seen with ageing may be through a reduced ability to secrete NO in ageing coronary arteries [8]. This has been shown not to be the case in these studies. In the presence of L-NAME and indomethacin, there was still a significantly greater vasoconstrictor response in the older animals. This would suggest that the difference seen with ageing is due to changes in ET receptor binding or density, and not due to the previously observed reduction in endogenous vasodilator release.

In this study, we have also shown that the response to the ET_B agonist sarafotoxin S6c increases with ageing. There was no response seen in the young rats in response to S6c, whereas a significant vasoconstriction was seen in the adult and senescent animals. ET_B receptor stimulation can cause both vasoconstriction or vasodilatation [31–33]. It is possible that, in the younger animals, these effects cancelled each other out, whereas in the older animals, the vasoconstrictor response predominated. Indeed, in the series of experiments where endogenous vasodilator release was blocked, S6c produced a constrictor response in rats of all ages. Similarly to the experiments with ET-1, the vasoconstrictor response to S6c increased with age. The observed response to S6c was less than that to ET-1. It appears therefore that the constrictor responses to both ET_A and ET_B are altered with ageing.

There are a number of limitations to this study. Firstly, a crystalloid perfused isolated heart does not accurately represent the normal conditions experienced by the heart in...
vivo. However, the benefits of an isolated system are that it reduces any confounding factors, such as circulating levels of catecholamines, neuronal control and any effects due to neutrophil/endothelial cell interactions. Secondly, this study was performed on rats. Further studies are required to see whether similar effects are seen in man and other animals.

In conclusion, the coronary vasconstrictor response to ET-1 and S6c increases with age in this rat model. These findings are independent of the decrease in NO release seen with ageing. In addition, coronary vasconstriction to ET-1 is increased following prolonged hypothermic cardioplegic arrest. These findings may have important implications for clinical practice.

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References