Sympathetic activation triggers ventricular arrhythmias in rat heart with chronic infarction and failure

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Abstract

Objective: To seek direct evidence for a cause–effect relation between sympathetic activation and arrhythmogenesis. Methods: Rats underwent open-chest surgery with either coronary artery occlusion or sham operation, and were studied 8 weeks later using in situ heart perfusion and nerve stimulation methods. Results: Infarcted rats showed cardiac functional impairment and increased heart and lung weight. The extent of these changes correlated well with infarct size (IS). In in situ perfused hearts, sympathetic nerve stimulation (2 and 4 Hz, 45 s duration) induced a frequency-dependent release of norepinephrine (NE). NE release was lower in MI than that in control groups. In hearts with large IS ($\geq 40\%$, $n=19$) ventricular arrhythmias were rare at baseline, but nerve stimulation evoked the onset of ventricular premature beats (95%), tachycardia (37%) and fibrillation (26%). IS and stimulation frequency were key determinants for the inducibility of arrhythmias. Lower K+ concentration enhanced arrhythmia inducibility. β-blockade inhibited the frequency of arrhythmias produced by nerve stimulation. Conclusion: In infarcted rat hearts sympathetic activation is a potent trigger for the onset of ventricular tachyarrhythmias.

Keywords: Experimental; Heart; Pathophysiology; Myocardial infarction; Heart failure; Sympathetic nervous system; Ventricular arrhythmias; Rats

See Editorial of this article by Pugsley et al. (pages 830–831) in this issue.

1. Introduction

Onset of ventricular arrhythmias is common in patients with myocardial infarction (MI) and heart failure (HF) and bears prognostic significance. Approximately half of HF patients die suddenly as a consequence of lethal tachyarrhythmias [1] although bradyarrhythmias are another common reason in patients with advanced HF [2]. Thus, understanding and prevention of lethal arrhythmia in HF patients constitute important research and clinical objects.

The general consensus is that MI and HF form arrhythmogenic substrates but the onset of malignant arrhythmias requires a triggering mechanism [3]. It has been generally agreed that the sympathetic nervous system plays a key role in the pathogenesis of arrhythmias in the failing heart. However, this view is based on observations that are, to some degree, indirect and circumstantial. Higher levels of circulating norepinephrine (NE) or cardiac NE spillover and sympathetic nerve firing rates are associated with higher incidence of arrhythmias and mortality [4–9]. However, measurements of sympathetic activity failed in predicting the risk of sudden cardiac deaths only being predictive of overall HF mortality [4,6]. Although β-blockers are beneficial in reducing the total cardiac mortality in HF patients [10–12], there has been no convincing data showing a reduction in the risk of sudden deaths by β-blockade. It has been well documented that in HF patients baroreflex sensitivity (BRS) and heart rate variability (HRV) are suppressed and these changes are associated with higher risk for sudden death [13,14]. However, the importance of the sympathetic nervous system in the association of BRS or HRV with the risk of sudden cardiac deaths in HF subjects is unclear, con-
founded by simultaneous alterations in parasympathetic nervous activity.

Few experimental studies have been undertaken to generate direct evidence confirming the role of sympathetic activation in arrhythmogenesis in the failing heart, partly due to lack of suitable experimental models with sufficient clinical relevance. To overcome this difficulty and directly examine the possibility that sympathoadrenergic activation triggers arrhythmias under conditions of MI and HF, we induced MI and HF in rats and performed arrhythmia experiments using a perfused, innervated heart preparation [15]. The importance of infarct size, the intensity of sympathetic nerve stimulation, low K⁺, and gender was evaluated, as well as the effect of β-adrenergic blockade.

2. Methods

2.1. Animals and induction of MI

Sprague-Dawley rats of male (200–250 g) and female (180–200 g) gender were used. Animals were anesthetised with intraperitoneal methohexitone, pentobarbitone and atropine and a left thoracotomy was performed. The heart was exposed and a 4–0 silk suture placed around the proximal left coronary artery (2–3 mm from its origin), as previously described [16,17]. In rats randomized to infarction, the suture was tied securely and in sham-operated controls, the suture was pulled through. The thorax was closed and the rats allowed to recover. Procaainamide (10 mg/kg, iv) was given in all rats 7–8 min before coronary artery occlusion to reduce acute arrhythmic death. The surgical procedures were approved by the local Animal Experimentation and Ethics Committee and were in compliance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Animals were studied 8 weeks after surgery, the time when cardiac hypertrophy and failure are evident according to previous studies [16,17].

2.2. Measurement of cardiac function in vivo

On the day of experiments, rats were anesthetized with pentobarbitone (60 mg/kg, ip) and a 3F micro-tipped transducer catheter (Millar Instruments) was placed, via the right carotid artery, in the aorta and then in the left ventricle (LV). The arterial blood pressure, LV pressure and the maximal rate of increase or decay of LV pressure, dP/dt, were recorded using a polygraph (Grass Instruments), and the average of ten sequential beats was used.

2.3. Heart perfusion in situ

After functional measurement, the experiment was carried out in an in situ perfused, innervated heart preparation described previously in detail [15] and illustrated in Fig. 1.

The perfusate was Krebs–Henseleit solution containing (in mmol/l) Na⁺ 148, K⁺ 4.0, Ca²⁺ 1.85, Mg²⁺ 1.05, HCO₃⁻ 25, PO₄⁻ 0.5, glucose 11 and EDTA 0.027, and was gassed with 95% O₂ and 5% CO₂ (pH 7.4). The chest was opened and the ascending aorta cannulated to start coronary perfusion in situ. The perfusion flow rate was controlled by a roller pump. To achieve a similar perfusion flow rate of 5 ml/min/g heart weight, perfusion flow rates were set at 4 to 8 ml/min, according to estimated heart weights, to maintain a perfusion pressure of 35 to 40 mm Hg. After ligation of the pulmonary vessels and the superior vena cava, the right atrium was cannulated to collect coronary effluent. Therefore, in this preparation, the LV was filled with effluent and sealed. This is different from the isolated Langendorff heart preparation in which the LV is open and empty. The Millar catheter was then inserted into the LV via the apex or non-infarcted region to record LV pressure (LVP) and dP/dt. Heart rate (HR) was derived from epicardial ECG signals. All parameters were continuously recorded on the polygraph.

2.4. Nerve stimulation

In the in situ perfused heart model, the cardiac efferent innervation is intact. The left cervicothoracic stellate ganglion, with cardiac sympathetic nerves attached, was dissected and placed on electrodes for subsequent electrical stimulation [15]. Nerve stimuli (2 ms and 0.8 mA) were generated by a Model SD-9 stimulator (Grass Instruments) and delivered at 2 and 4 Hz (45 s duration each), in random order, separated by a 10-min interval. Nerves were constantly superfused with warm perfusate except when stimulation was performed. Coronary effluent was collected for a period of 1 min before and during nerve stimulation. To test the effect of hypokalemia, in one experiment perfusate K⁺ was reduced from 4 to 3 mmol/l. To quantify evoked NE release from NE washout, free from confounding effects of NE reuptake, a neuronal uptake-1 inhibitor, desipramine (Sigma), was present at 0.1 μmol/l, unless specified elsewhere.

2.5. Analysis of ventricular arrhythmias

The epicardial ECG was recorded continuously for 5 min starting 1 min prior to each period of nerve stimulation. Arrhythmias that occurred within 2 min of the commencement of nerve stimulation were considered to be induced by nerve activation. Definition of VPB, VT (≥5 consecutive VPB) and VF were based on the criteria of the Lambeth Conventions [18].

Various systems for arrhythmia scoring have been used for clinical and experimental studies [18–20]. To analyze various forms of arrhythmias in an integrated and more quantitative manner, we designed an arrhythmia scoring system taking into consideration of the short duration of nerve stimulation and the spontaneous termination of
induced arrhythmias occurring within a few minutes in most hearts. The ranking scores are arbitrary numerical grades of different sorts of ventricular arrhythmias recorded in each preparation. The scaling applied was as follows: 0 = no arrhythmia, 1 = 1–5 VPB, 2 = 6–15 VPB, 3 = 16–30 VPB, 4 = more than 30 VPB, 5 = single episode of VT less than 5 s, 6 = combined VT duration of 5–20 s, 7 = VF less than 5 s, 8 = VT longer than 20 s or VF 5–20 s, and 9 = combined VF duration longer than 20 s. When multiple forms of arrhythmias occurred in one heart, only the highest single score was used.

2.6. Measurement of infarct size (IS)

MI was restricted to the LV and was transmural in all hearts. IS was quantified following the method reported previously [21]. At the end of experiments, hearts were excised and atria, the right ventricle and the LV were separated and weighed. Several incisions were then made such that the LV could be pressed flat. The epicardial and endocardial circumferences of the infarcted area and entire LV were outlined on a transparent sheet. IS (%) was calculated from the ratio of the surface area of the infarcted wall and the entire surface area of the LV.

2.7. NE assay

Coronary effluent samples were stored at −80°C until assay. NE was extracted with activated alumina, separated by reverse-phase HPLC and quantified using an electrochemical detector [22]. The interassay coefficient of variation was 3%.

2.8. Statistics

Two sets of results were obtained in this study, parametric and non-parametric. For parametric results, between-group differences were tested using analysis of variance.
incidences of arrhythmias and Mann–Whitney Rank-sum (r) and PdP
L V systolic pressure (mm Hg) 141.6
Mean arterial pressure (mm Hg) 123
HR (beats / min) 416.6
IS (% L V) ± 32.0
Lung weight (g) 1.41
Atria weight (g) 0.15
Right ventricle weight (g) 0.31
Heart weight (HW, g) 1.41
Baseline data from control and infarcted male and female rats 8 weeks after sham-operation or coronary occlusion (mean
Table 1

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th></th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham (n=19)</td>
<td>Moderate IS (n=17)</td>
<td>Large IS (n=19)</td>
</tr>
<tr>
<td>Body weight (BW, g)</td>
<td>465±7</td>
<td>450±7</td>
<td>454±10</td>
</tr>
<tr>
<td>Heart weight (HW, g)</td>
<td>1.41±0.03</td>
<td>1.66±0.04*</td>
<td>1.91±0.07*</td>
</tr>
<tr>
<td>HW/BW (mg/g)</td>
<td>3.03±0.07</td>
<td>3.69±0.11*</td>
<td>4.24±0.16*</td>
</tr>
<tr>
<td>LV weight (g)</td>
<td>0.94±0.04</td>
<td>1.05±0.03*</td>
<td>1.12±0.04*</td>
</tr>
<tr>
<td>Right ventricle weight (g)</td>
<td>0.31±0.03</td>
<td>0.43±0.02*</td>
<td>0.54±0.04*</td>
</tr>
<tr>
<td>Atria weight (g)</td>
<td>0.15±0.01</td>
<td>0.18±0.02</td>
<td>0.25±0.02*</td>
</tr>
<tr>
<td>Lung weight (g)</td>
<td>1.41±0.06</td>
<td>2.03±0.32*</td>
<td>3.01±0.22*</td>
</tr>
<tr>
<td>IS (% L V)</td>
<td>–</td>
<td>32.0±1.7</td>
<td>45.6±1.0</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>416±8</td>
<td>406±7</td>
<td>397±10</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>123±4</td>
<td>111±4*</td>
<td>101±3*</td>
</tr>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>141±3</td>
<td>127±4*</td>
<td>114±3*</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>0.8±0.3</td>
<td>5±1*</td>
<td>17±2*</td>
</tr>
<tr>
<td>dP/dt_{max} (mm Hg/s)</td>
<td>8445±260</td>
<td>6284±236*</td>
<td>5111±254*</td>
</tr>
<tr>
<td>dP/dt_{min} (mm Hg/s)</td>
<td>−7758±275</td>
<td>−5216±218*</td>
<td>−4094±239*</td>
</tr>
</tbody>
</table>

* Seven hearts tested at lower K⁺ are included in the male sham group. For the purpose of clarity, results from infarcted rats tested in the absence of desipramine (n=13), at 3 mmol/l K⁺ (n=8) or with β-antagonists (n=7) were not included.

*P<0.01 versus sham.

3. Results

3.1. Baseline results

Surgery was done on 184 rats, 160 males and 24 females. There was no death in the sham-operated rats (27 males and six females). In rats with coronary artery occlusion, the post-operative mortality was 46% for males (65 out of 141) and 22% for females (4 out of 18, P=0.09 by Chi-square test). About 90% of deaths occurred 6 to 24 h after surgery due to arrhythmias and acute HF. At the time of experiments, 12 infarcted hearts were discarded due to development of sustained arrhythmias at the beginning of heart perfusion (n=9) or lack of functional response to nerve stimulation (n=3). Thus, 104 preparations had complete data.

Male rats with MI, except those used in experiments studying the effects of low K⁺, desipramine, or β-blockers (see below), were divided into two subgroups according to IS ranging from 17 to 57%: moderate IS group (M-IS, <40%, range 17–39.7%) and large IS group (L-IS, 40–57%). Table 1 shows the baseline data of control and MI groups. Functional impairment in infarcted rats was evidenced by the suppressed dP/dt_{max}, dP/dt_{min} and LV systolic pressure (LVSP), elevation in LV end-diastolic pressure (LVEDP) and greater lung wet weight. IS was significantly (all P<0.01) correlated with the extent of cardiac hypertrophy, estimated by heart weight/body weight ratio (r=0.649), lung weight (r=0.59), LVEDP (r=0.719), LVSP (r=−0.746), dP/dt_{max} (r=−0.771), and dP/dt_{min} (r=−0.815).

3.2. LV function and NE release in perfused hearts

There was no significant difference in the perfusion flow rates among groups. In control hearts from male (n=12) and female rats (n=6), all the functional and NE measurements were similar and therefore data were combined to form a single control group of 18 hearts.

Basal HR and NE overflow were similar but dP/dt_{max} was lower in L-IS and female MI groups compared with controls. Release of NE by nerve stimulation was frequency-dependent in quantity and 30% lower in L-IS and female MI groups than that in controls. In all three infarcted groups (male M-IS and L-IS, female MI), increase in HR in response to nerve stimulation was well maintained but the inotropic response was significantly attenuated (Table 2).

3.3. Nerve stimulation and arrhythmias

With perfusate K⁺ of 4 mmol/l, VPB and short episode of VT were seen in 14% preparations with MI at the beginning of heart perfusion. These hearts became stable a few minutes afterwards except in two hearts that developed...
Table 2
Coronary flow rate (CFR), functional enhancement and NE release in response to sympathetic nerve stimulation in perfused hearts

<table>
<thead>
<tr>
<th></th>
<th>CFR ml/min/g</th>
<th>HR beats/min</th>
<th>dP/dt max mm Hg/s</th>
<th>NE pmol/min/heart</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal 2 Hz</td>
<td>4 Hz</td>
<td>Basal 2 Hz</td>
<td>4 Hz</td>
</tr>
<tr>
<td>Sham (n=18)</td>
<td>4.8±0.1</td>
<td>182±14</td>
<td>244±18</td>
<td>266±15</td>
</tr>
<tr>
<td></td>
<td>1.68±0.38</td>
<td>151±2.0</td>
<td>34.9±3.8</td>
<td></td>
</tr>
<tr>
<td>M-IS (n=17)</td>
<td>4.7±0.1</td>
<td>165±13</td>
<td>231±14</td>
<td>264±12</td>
</tr>
<tr>
<td></td>
<td>1.04±0.24</td>
<td>12.2±1.3</td>
<td>25.7±2.7</td>
<td></td>
</tr>
<tr>
<td>L-IS (n=19)</td>
<td>4.5±0.2</td>
<td>170±10</td>
<td>228±9</td>
<td>260±9</td>
</tr>
<tr>
<td></td>
<td>1.23±0.20</td>
<td>10.6±1.4</td>
<td>21.7±2.5</td>
<td></td>
</tr>
<tr>
<td>Female (n=11)</td>
<td>4.7±0.2</td>
<td>177±13</td>
<td>245±14</td>
<td>253±16</td>
</tr>
<tr>
<td></td>
<td>0.93±0.13</td>
<td>9.36±1.3</td>
<td>23.5±2.9</td>
<td></td>
</tr>
</tbody>
</table>

Control group includes hearts from 12 male and six female rats. M-IS: moderate infarct size, male; L-IS: large infarct size, male; perfusate K⁺ concentration=4 mmol/l.

sustained VF. All control hearts were functionally stable throughout the experiment.

Under perfused conditions, nerve stimulation induced a few VPB in one (2 Hz) or two hearts (4 Hz) from control rats, but there was no VT and VF. In contrast, nerve stimulation induced VPB in almost all hearts with MI. In hearts with large IS, 30–40% developed VT and 26% developed VF (Table 3). Fig. 2 shows recording traces depicting the functional augmentation and the onset of arrhythmias during nerve stimulation in one control and three infarcted hearts. Inducibility of arrhythmias was dependent on the intensity of stimulation indicated by higher incidences of VT and VF with 4 Hz versus 2 Hz (Table 3). Such frequency-dependency is also seen in Fig. 3 in which arrhythmia scores from individual hearts are plotted against the frequency. All the arrhythmias were self-terminating within a few minutes, except for three hearts with large IS in which VT and VF were sustained.

IS was also related to the severity of triggered arrhythmias. The incidences of VPB and VT and arrhythmia scores were all higher in L-IS than M-IS groups (see Table 3). There was no significant difference in VPB numbers between the M-IS and L-IS groups (2 Hz:5±3 versus 12±4 beats; 4 Hz: 9±5 versus 18±4 beats).

In this preparation, presence of desipramine is known to increase the quantity of NE overflow by nerve stimulation [23]. To test whether inhibition of neuronal uptake of NE contributes to nerve stimulation induced arrhythmogenesis, a separate group of 13 infarcted hearts (IS 40.5±2.3%, range 23–50%) were studied in the absence of desipramine. The sympathetic nerves were stimulated twice at 2 and 4 Hz (45 s each), respectively. Increased in HR by nerve stimulation (2 Hz: from 162±10 to 243±8 beats/min; 4 Hz: 168±11 to 269±8 beats/min) was similar to that observed in the presence of desipramine (Table 2). As shown in Table 3, tachyarrhythmias were induced by nerve stimulation in the absence of desipramine.

3.4. Effect of low perfusate K⁺

Hypokalemia is proarrhythmic in patients with HF [24–26]. The effect of hypokalemia was examined separately in seven control and 15 infarcted hearts by lowering the perfusate K⁺ from 4 to 3 mmol/l. In control hearts

Table 3
Incidence of arrhythmias induced by sympathetic nerve stimulation and arrhythmia scores in rats with sham-operation or myocardial infarction (MI)

<table>
<thead>
<tr>
<th></th>
<th>IS%LV</th>
<th>Hz</th>
<th>VPB%</th>
<th>VT%</th>
<th>VF%</th>
<th>Arhythmia score</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 mmol/l K⁺</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>2</td>
<td>5.6</td>
<td>0</td>
<td>0</td>
<td>0.06±0.05</td>
</tr>
<tr>
<td>(n=18)</td>
<td></td>
<td>4</td>
<td>11.1</td>
<td>0</td>
<td>0</td>
<td>0.1±0.1</td>
</tr>
<tr>
<td>Male M-IS*</td>
<td>32.0</td>
<td>2</td>
<td>47.1*</td>
<td>0</td>
<td>0</td>
<td>0.9±0.3*</td>
</tr>
<tr>
<td>(n=17)</td>
<td>±1.7</td>
<td>4</td>
<td>58.8*</td>
<td>23.5</td>
<td>5.9</td>
<td>2.0±0.6*</td>
</tr>
<tr>
<td>Male L-IS†</td>
<td>45.6</td>
<td>2</td>
<td>89.5*</td>
<td>26.3*</td>
<td>0</td>
<td>2.2±0.5*</td>
</tr>
<tr>
<td>(n=19)</td>
<td>±1.0</td>
<td>4</td>
<td>94.7*</td>
<td>36.8*</td>
<td>26.3*</td>
<td>4.2±0.7*</td>
</tr>
<tr>
<td>Female MI</td>
<td>46.8</td>
<td>2</td>
<td>81.8*</td>
<td>18.2</td>
<td>0</td>
<td>2.3±0.6*</td>
</tr>
<tr>
<td>(n=11)</td>
<td>±1.8</td>
<td>4</td>
<td>54.5*</td>
<td>18.2</td>
<td>0</td>
<td>2.1±0.7†</td>
</tr>
<tr>
<td>Male MI, no DMI</td>
<td>40.5</td>
<td>2</td>
<td>61.5*</td>
<td>7.7</td>
<td>0</td>
<td>1.7±0.4*</td>
</tr>
<tr>
<td>(n=13)</td>
<td>±2.3</td>
<td>4</td>
<td>92.3*</td>
<td>46.1*</td>
<td>0</td>
<td>3.6±0.6*</td>
</tr>
<tr>
<td>3 mmol/l K⁺</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham (n=7)</td>
<td>–</td>
<td>2</td>
<td>14.2</td>
<td>0</td>
<td>0</td>
<td>0.3±0.2</td>
</tr>
<tr>
<td>(n=8)</td>
<td></td>
<td>4</td>
<td>28.6</td>
<td>0</td>
<td>0</td>
<td>0.4±0.2</td>
</tr>
<tr>
<td>MI</td>
<td>45.3</td>
<td>2</td>
<td>100*</td>
<td>37.5</td>
<td>0</td>
<td>4.3±1.0*</td>
</tr>
<tr>
<td>(n=8)</td>
<td>±2.1</td>
<td>4</td>
<td>100*</td>
<td>75.0*</td>
<td>0</td>
<td>6.6±0.5*</td>
</tr>
</tbody>
</table>

*DMI: desipramine. M-IS and L-IS refer to moderate (<40%) or large (≥40%) infarct size.

P<0.05 versus respective sham-operated controls.

P<0.05 versus male L-IS group by Mann–Whitney rank sum test.

P<0.05 versus 2 Hz within the group by Signed rank test for paired data.
Fig. 2. Recording traces showing LV dP/dt, LV pressure (LVP) and epicardial ECG in in situ perfused rat hearts and the responses to electrical stimulation of the left sympathetic ganglion at 4 Hz (arrows). The functional enhancement in response to nerve stimulation was indicated by increases in dP/dt, LVSP and heart rate. Note that in the heart from a sham rat (panel A), no arrhythmia occurred during nerve stimulation. In hearts with MI (panels B–D), various forms of ventricular arrhythmias were recorded during or soon after nerve stimulation. The heart in panel D subsequently developed sustained VF. In all the experiments showed, the perfusate K⁺ was 4 mmol/l. Bar=5 s.
perfused with 3 mmol/l K⁺, there was no spontaneous arrhythmias and nerve stimulation evoked a few VPB in one (2 Hz) or two (4 Hz) out of seven hearts. In 15 infarcted hearts (IS 40.5±2.2%), seven hearts (47%, P< 0.01 versus 11% at 4 mmol/l K⁺) developed sustained VT or VF spontaneously during early perfusion and nerve stimulation was performed in eight remaining hearts (IS 37–51%, average 45±2%). When nerves were stimulated at 2 and 4 Hz, all eight hearts developed VPB with VPB frequencies higher than that in the L-IS group tested at 4 mmol/l K⁺ (2 Hz: 27±7 versus 12±4 beats, P=N.S; 4 Hz: 52±17 versus 18±4 beats, P<0.05). The incidence of VT at 4 Hz and the arrhythmia scores at both 2 and 4 Hz were all significantly higher than that in L-IS with perfusate K⁺ of 4 mmol/l (Table 3).

3.5. Gender differences in the severity of arrhythmias

In order to compare with the male large IS group, of 14 infarcted rats, results from three female rats with IS<40% (34.4–35.6%) were excluded. In 11 infarcted hearts from female rats, IS (46.1±1.8%, ranging 40–58%), extent of cardiac dysfunction and quantities of NE release by nerve stimulation were similar to that in the male L-IS group (Tables 1 and 2). Although the frequency of evoked arrhythmias by stimulus at 2 Hz recorded over the 2-min period was similar between the two groups, at 4 Hz incidences of VPB and arrhythmia score were lower in female than that in L-IS male groups (both P<0.05, Table 3).

3.6. Pacing and exogenous NE infusion

To examine whether nerve-stimulation-induced arrhythmias was due to a rise in HR, in 12 infarcted hearts (IS 39.6±1.3%), HR was increased by either atrial pacing or administration with NE (Sigma). NE was given at 30 nmol/l, a dose increasing cardiac function to an extent similar to that induced by nerve stimulation at 4 Hz. NE infusion was for a period of 1 min followed by 10 min recovery period. Hearts were then paced, using atrial electrodes, at 300 beats/min for a period of 2 min. ECG was monitored during and 2 min after these interventions.

Nerve stimulation at 4 Hz increased HR (from 153±13 to 240±12 beats/min) and dP/dt max (from 889±37 to 1228±69 mm Hg/s). Infusion with NE similarly increased HR (144±9 to 224±14 beats/min) and dP/dt max (856±41 to 1233±57 mm Hg/s). Nerve stimulation induced 75% VPB and 42% VT with arrhythmia score of 3.8±0.9. In contrast, NE infusion and pacing were both less effective in inducing arrhythmias. NE infusion induced VPB in two hearts (group score 0.4±0.3, P<0.01 versus nerve stimulation). During pacing two hearts developed VPB and one of them had a short episode of VT (arrhythmia score 0.6±0.4, P<0.01 versus nerve stimulation).

3.7. Effects of β-blockers

A separate experiment was performed to test whether the functional and arrhythmic response to nerve stimulation were mediated by β-adrenergic receptors. In perfused hearts of normal rats the combination of β1- and β2-
antagonists, atenolol and ICI-118551 (both from Sigma), at 0.03 and 0.3 μmol/l had mild inhibitory effects on functional response (data not shown) and micromolar concentrations were required to inhibit the responses to both nerve stimulation by 80%. In perfused hearts from sham-operated (n=8) and infarcted rats (n=7, IS=38.5±2.9% ranging from 26.5–46.2%), sympathetic stimuli were performed three times (S1 to S3, 4 Hz each, 45 s in duration) in the absence (S1) and presence of both atenolol and ICI-118551 (both at 1 μmol/l for S2 and 3 μmol/l for S3). Drugs were present 10 min before till 2 min after the start of the nerve stimulation. HR and dP/dtmax were increased by S1 and combined treatment with atenolol and ICI-118551 largely inhibited the functional responses in control (data not shown) and in infarcted hearts (Fig. 4). All seven infarcted hearts developed VPB and two hearts had short episodes of VT by nerve stimulation at 4 Hz (r=0.358, P<0.01, n=56, Fig. 5), but not at 2 Hz (r=0.213, P=NS, n=49).

3.8. Correlation between IS and arrhythmia score

After combining results from four groups (see Table 3 and Fig. 3) of male rats with infarct (moderate IS, n=17; large IS, n=19; the experiment without desipramine, n=13; the experiment with β-blockers, n=7), there was a moderate and significant correlation between IS and arrhythmia scores by nerve stimulation at 4 Hz (r=0.358, P<0.01, n=56, Fig. 5), but not at 2 Hz (r=0.213, P=NS, n=49).

4. Discussion

Induction of ventricular arrhythmias in hearts with chronic MI and failure by activation of sympathetic nerves has not been reported. The present study demonstrated that brief sympathetic nerve stimulation, but not cardiac pacing or administration with NE, is a potent trigger for ventricular tachyarrhythmias in rat hearts with MI and failure. In this model, intensity of nerve activation, IS, hypokalemia, and probably male gender, are significant determinants of the inducibility of arrhythmias. This proarrhythmic action appears to be mediated by β-adrenergic receptors. Thus, this is the first study to provide direct evidence for a cause–effect relation between activation of sympathetic nerves and onset of ventricular arrhythmias in infarcted and failing heart.
The in situ perfused and innervated heart model allows us to study the role of activation of cardiac sympathetic nerves alone and minimize confounding factors such as induction of regional ischemia and systemic neurohormonal changes seen with MI and HF. Activation of the left stellate sympathetic ganglion has long been known to be proarrhythmic [27–29]. Previous studies in vivo have shown that in canine hearts with chronic MI, sympathetic activation by nerve stimulation or exercise, together with the simultaneous induction of acute ischemia, is proarrhythmic [28]. However, the animals studied did not have signs of HF and acute ischemia was necessary for the initiation of arrhythmias [28]. Although active ischemic events do occur in HF patients, at any point it is absent in most. Furthermore, the mechanism of arrhythmogenesis under ischemic conditions may differ substantially from that without ischemia [30].

Arrhythmia inducibility by electrophysiological programmed stimulation (EPS) is widely used in patients [31–33] or laboratory animals [20,34–40]. Arrhythmia inducibility provides insight into electrophysiological instability and proarrhythmic substrates of the failing heart. In these studies, factors associated with high arrhythmia inducibility are large IS [35,38,39], ventricular remodeling [20,35,36], severe ventricular dysfunction [35], higher work-loading status [36], hypokalemia [37] and heterogeneous infarct morphology [33,35,40]. Findings from these studies clearly help to characterize the proarrhythmic substrates in the diseased heart. However, the mechanisms triggering the onset of arrhythmias are not necessarily uncovered by EPS studies.

The observations from this study support the view that the combination of a pro-arrhythmic substrate and a trigger is necessary to initiate arrhythmias under conditions of HF since nerve stimulation per se is not arrhythmogenic in control hearts [44]. Thus, a proarrhythmic substrate is a prerequisite for the demonstration of sympathetic activation and arrhythmogenesis. The severity of arrhythmia is proportional to the stimulatory intensity and quantities of NE overflow. Whereas the amount of NE release evoked by nerve activation was significantly lower in infarcted than in control hearts, the development of arrhythmias was observed only in infarcted hearts.

One interesting finding in this study is the ineffectiveness of rapid pacing or NE infusion in inducing arrhythmias compared with nerve stimulation. The onset of sudden death in HF patients peaks in the morning hours [3,41], matching closely the morning peaks of HR [42,43]. It is known that sinus tachycardia can immediately proceed the onset of VF and other tachyarrhythmias [44]. However, compared with nerve stimulation, a short period of increasing HR by pacing to the same level achieved by nerve stimulation was far less effective as an arrhythmic trigger. Infusion with NE was also less effective in inducing arrhythmias. It is likely that compared with exogenous NE infusion, adrenergic activation by nerve stimulation is relatively heterogeneous in the heart, which would be more proarrhythmic. It is also unknown whether co-transmitters released with NE from sympathetic nerves in the failing heart, such as neuropeptide Y and epinephrine, play a role in triggering arrhythmias.

Except for the intensity of nerve activation, several other determinants of arrhythmia inducibility by nerve activation have been demonstrated in this study. We observed that a large infarct size is associated with more severe arrhythmias induced by nerve stimulation. IS is the most important determinant of the structural and functional consequences of MI. IS determines the extent of hypertrophy, ventricular remodeling and cardiac dysfunction [16,17,45], changes which all might synergistically contribute to increased susceptibility to arrhythmias. Asynchrony of conduction and impulse generation occurred in the infarct border zone would increase with IS. A large scar forms stable reentry pathways and hyperactivity of non-infarcted myocardium creates slow conduction, marked dispersion in the duration of repolarization and early or later afterdepolarizations [34,46]. These changes favor reentry and triggered activity and importantly, can be exacerbated by adrenergic activation [29,44]. However, it needs to be pointed out that under conditions of heterogeneous infarct, i.e. the presence of viable myocardial fibers within the infarcted zone, arrhythmia inducibility might not be a function of IS, as shown by others [30,40], since the slow conduction with unidirectional conduction block, key ingredients for reentrant arrhythmias [33,47], become a major arrhythmic substrate.

In the rat infarct model, the infarct is always transmural with relatively clear lateral border. This anatomic feature probably contributes to the correlation between IS and severity of induced tachyarrhythmias seen in our study.

Hypokalemia is a common complication in HF subjects [24–26] and is clearly accompanied by higher risk of arrhythmias. In perfused and acutely dilated rabbit hearts, Reiter et al. showed that VF% by EPS was higher with reduced perfusate K⁺ [37]. Our study confirmed that perfusion with a low K⁺ substantially increases the probability of both ‘spontaneous’ and nerve stimulation induced arrhythmias. The later observation suggests a synergism of adrenergic activation and hypokalemia. Low K⁺ is known to initiate afterdepolarizations and triggered activity and reduce conduction velocity [34], allowing arrhythmias to be triggered more frequently by adrenergic activation.

Clinical and experimental studies indicate that female gender may confer a lower risk of arrhythmic death [48,49]. In the Framingham cohort, presence of cardiac hypertrophy, HF and VPBs are risk factors of sudden death for men, but not for women [50,51]. In survivors of cardiac arrest or patients with recent VT or VF, male gender is one of the strongest and independent predictors for the inducibility of VT and VF by EPS [31]. In rats who underwent coronary artery occlusion, females have a lower
severity of arrhythmias and a lower 24-h mortality rate than that in males [49]. In this study, the post-infarct mortality tended to be lower in female than male rats. The finding that female rats with MI were less likely to develop arrhythmias in response to sympathetic nerve stimulation may partly explain the relative protection afforded by the female gender in vivo.

Some limitations of our study need to be addressed. In this study hearts were tested without performing external work. Since a higher workload facilitates the development of arrhythmias initiated by EPS [36], it is possible that sympathetic activation would be more arrhythmogenic in the failing heart performing external work. A major limitation in this study is the lack of experiments on the electrophysiological mechanisms for the initiation and maintenance of arrhythmias triggered by nerve activation. Our findings, however, indicate that the combination of chronic MI and perfused, innervated heart preparation leads to a useful model for further studies on the mechanism of arrhythmogenesis in HF and therapeutic interventions.

In conclusion, this study provides evidence that transient activation of cardiac sympathetic nerves triggers arrhythmias in rat hearts with MI and failure. Thus, preventing the occurrence of such trigger of sympathoadrenal activation might limit arrhythmias and related death.

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