Role of the autonomic nervous system in cardioprotection by remote preconditioning in isoflurane-anaesthetized dogs

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Aims
Remote ischaemic preconditioning (rIPC) protects cardiac and non-cardiac tissues against ischaemic injury. Although there is increased demand to investigate its potential clinical applicability, fundamental mechanisms responsible for rIPC-mediated protection remain unresolved. We examined in isoflurane-anaesthetized dogs whether an intact cardiac nervous system was necessary to mediate rIPC protection against ischaemic injury.

Methods and results
Dogs were randomly allocated to six groups: 1, control (CON, no-rIPC); 2, rIPC (4 × 5 min renal artery occlusion/reperfusion); 3, autonomic ganglionic blockade with hexamethonium (HEX, no-rIPC; 20 mg/kg iv); 4, HEX + rIPC; 5, cardiac decentralization by surgical ablation of extracardiac nerves (DCN, no-rIPC); and 6, DCN + rIPC. All dogs underwent 60 min coronary occlusion and 180 min reperfusion; cardiac haemodynamic parameters were monitored. Regional blood flow (microspheres) in the heart and kidneys was assessed. Necrotic tissue was visualized using triphenyltetrazolium staining and related to anatomic risk zone size (area at risk; \( P = \text{NS between groups} \)) and coronary collateral blood flow. Infarct size (% AAR) was 29 ± 5 (mean ± 1 SD) in CON and 15 ± 4 in rIPC dogs (\( P = 0.001 \) vs. CON); 24 ± 3 in HEX vs. 12 ± 4 in HEX + rIPC (\( P = 0.001 \) vs. HEX); and 20 ± 2 in DCN vs. 12 ± 4 in DCN + rIPC (\( P = 0.001 \) vs. DCN). In CON dogs, infarct size was inversely related to coronary collateral flow; this relation was shifted downwards in all groups pre-treated with rIPC.

Conclusion
We report robust myocardial protection by rIPC against ischaemic injury in canines that was not abrogated by either pharmacological or surgical decentralization of cardiac nerves.

Keywords
Remote preconditioning • Ischaemia–reperfusion • Cardiac decentralization • Autonomic ganglionic blockade • Infarct size

1. Introduction
Neural and humoral pathways modulate endogenous cellular mechanisms responsible for myocardial adaptive responses to acute ischaemia–reperfusion injury. Cardioprotection by ischaemic preconditioning (IPC) was first described by Murry et al.\(^1\) in barbiturate-anaesthetized dogs subjected to repeated, sublethal coronary occlusion/reperfusion prior to a more prolonged period of acute ischaemia. IPC delays the development of cellular necrosis in cardiac and non-cardiac tissues in all mammalian species tested including humans;\(^2\) two distinct windows of cardioprotection by IPC have been described in the literature.\(^2\) To date, mechanisms responsible for classical IPC have not been clearly defined. The current paradigm proposes that the preconditioning stimulus generates endogenous ligands, such as adenosine, opioids, and catecholamines that trigger cellular transduction pathways (guanylate cyclase, kinases, and transcription factors) that mediate the protective signal from the cell surface to the mitochondria where end-effectors induce protection.\(^2,5\) A cross-tolerance phenomenon exists for different preconditioning stimuli,\(^6,7\) as evidenced by the similarity of mechanisms evoked for anaesthetic, pharmacological, and remote ischaemic preconditioning (rIPC).\(^7,8\) The present study focuses on rIPC where intermittent ischaemia produces a local preconditioning effect but also protects tissues of distant organs from ischaemic injury.\(^9,10\) Although the...
mechanisms responsible for rIPC are not established, signalling between tissues/organs probably occurs via humoral or neural pathways or an overlap between both. A key requirement for preconditioning is reperfusion; this implies that tissue in an organ subjected to ischaemia releases mediator(s) that can trigger cellular protection in distant tissues. Such a phenomenon has been documented in isolated non-preconditioned hearts perfused with effluent from preconditioned hearts. Neural pathways could transfer protective signals between distant organs; earlier findings using pharmacological ganglionic blockade document the abrogation of rIPC-induced cardioprotection in animals and patients. The overlap between humoral mediators and neural pathways has also been suggested; in rodents, adenosine released by the ischaemic tissues stimulates local afferent nerves to trigger myocardial protection. The requirement for functional cardiac nerves in protection of ischaemic myocardium by IPC is controversial. A recent classical preconditioning study showed that first window preconditioning protects ischaemic myocardium in cardiac-denervated pigs, whereas second window preconditioning required intact cardiac nerves. The goals of the current study were to examine whether (i) rIPC produces cardiac protection and (ii) pharmacological blockade of the autonomic nervous system or cardiac decentralization (surgical ablation of extracardiac nerves) influences rIPC-induced protection in canines exposed to acute ischaemia–reperfusion injury.

2. Methods

Experiments were performed in adult mongrel dogs of either sex (20–25 kg) in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication 85-23, revised 1996); Laval University is compliant with these guidelines (A5012-01). The experimental protocol was approved by the Laval University Animal Ethics Committee (#2007-001-2).

2.1 Surgical preparation

The anaesthetic procedure was the same for all dogs entered into these studies. Dogs were pre-medicated with acepromazine maleate (Atravet, 0.5 mg/kg im) and anaesthesia was induced with sodium pentothal (10 mg/kg iv). After endotracheal intubation, dogs were maintained on controlled ventilation with 1.0–1.3 minimum alveolar anaesthetic concentration (end-tidal concentration) isoflurane and oxygen-enriched room air; respiratory rate and tidal volume were adjusted to maintain blood gases within physiological values. Fentanyl (0.005 mg/kg iv bolus followed by constant infusion at 0.005 mg/kg/h) was administered for analgesia. Normothermia (38 ± 1 °C) was maintained with a water-jacketed Micro-Temp heating blanket (Zimmer, Dover, OH, USA) and body temperature was monitored with a temperature probe in the trachea. Saline was given iv (250 mL/h) to replace fluid loss.

In the supine position, vascular introducer sheaths (8 Fr, Terumo Medical Corp., USA) were placed in the left and right femoral arteries; a triple-lumen central venous catheter (7 Fr, Arrow-Howes, Arrow Intl. Inc., Reading, PA, USA) was placed in the right femoral vein for administration of drugs and fluids. Via a right flank incision, the main renal artery and renal vein of the right kidney were exposed; double ligatures were placed around each vessel and tied proximal to the segmental branches (the kidney was not extirpated) and the incision was closed. The main renal artery and vein of the contralateral left kidney were dissected free of perirenal tissues via a left flank incision. A transit time flow probe (Transonic Systems Inc., Ithaca, NY, USA) was positioned for the measurement of phasic kidney blood flow and a snare was positioned around this vessel for reversible occlusion and reperfusion (rIPC protocol). An arterial blood sample was withdrawn before manipulation of the kidneys and at the end of the experiment to determine serum creatinine and blood urea nitrogen levels.

A left lateral thoracotomy was performed through the third and fifth intercostal spaces (the fourth and fifth ribs were removed to facilitate access to extracardiac nerves); the right and left thoracic vagosympathetic complexes, left and right stellate ganglia and the anterior and posterior ansae subclaviae were dissected free of the surrounding tissues in all animals. In control (CON) dogs, no further actions were taken, whereas in cardiac decentralized (DCN) dogs, nerves were sectioned bilaterally as described previously. Connective and neural tissues around the ascending aorta, the left pulmonary vein, and the main pulmonary artery were not dissected (to preserve the function of intrinsic cardiac neurons). Reflex control of cardiac function is known to be mediated by complex interactions that occur among peripheral and central neuronal elements of the cardiac neuronal hierarchy. Transection of the ansae subclavia, vagosympathetic complexes, and stellate ganglia effectively eliminate potentiating effects of the central nervous system on intrinsic cardiac neural activity. Pharmacological decentralization (HEX) was achieved using hexamethonium bromide (20 mg/kg iv; Sigma-Aldrich Canada, Oakville, ON, USA); this agent blocks neuronal ganglionic nicotinic receptors.

The heart was exposed and suspended in a pericardial cradle. A suture was placed around the inferior vena cava for construction of left ventricular (LV) pressure–volume relations. A segment of the left anterior descending artery was dissected distal to the first diagonal branch to allow positioning of a vascular clamp for acute coronary occlusion. Polyethylene catheters, filled with heparinized saline, were inserted into the internal thoracic artery (for withdrawal of reference blood samples) and the left atrium (injection of microspheres); catheters (5 Fr) were inserted into the coronary sinus and the pulmonary artery. A 5 Fr micro-tipped pressure transducer (MP5000, Millar Instruments Inc., Houston, TX, USA) was placed in the LV cavity through the apex to measure LV pressure and its first derivative; a 7 Fr Pigtail catheter was advanced to the aortic root via the left femoral artery to measure arterial pressures. A 7 Fr, 12-electrode conductance catheter (CD Leycom, Zoetermeer, The Netherlands) was advanced via the femoral artery sheath and positioned at the LV apex along the longitudinal axis of the ventricle. After all catheters were positioned, dogs were given 500 IU of heparin sodium and were allowed to stabilize for 30 min prior to data collection.

Left atrial, ascending aorta, and coronary sinus catheters were connected to Statham P23BD strain gauge manometers; zero was set at mid-chest level. The Millar microanometer transducer was cross-calibrated with systolic aortic and diastolic left atrial pressures. The conductance catheter was connected to a Sigma SDF signal conditioning and processing unit; signals were acquired using ConductNT software (version 3.8). All haemodynamic data were continuously recorded and stored on a computer hard drive for later analysis using AxiScope data acquisition software. These parameters include heart rate, LV systolic and diastolic pressures, systemic arterial pressure, maximum first derivative of LV systolic pressure (+dP/dt), LV volume, and mean renal artery blood flow.

2.2 Experimental protocol

The experimental design is depicted schematically in Figure 1. Dogs were randomly assigned to one of the six experimental groups. CON, DCN, and HEX dogs underwent a 40 min wait period (equivalent to the time of rIPC); rIPC, DCN + rIPC, and HEX + rIPC dogs were subjected to four cycles of intermittent renal artery occlusion (5 min) and reperfusion (5 min). All dogs underwent 60 min regional coronary occlusion and 180 min reperfusion. The completeness of cardiac decentralization was confirmed by direct electrical stimulation of the left and right ansae subclaviae (10 Hz, 5 ms, 5–7 V) and the left and right thoracic vagi (20 Hz, 5 ms, 5–7 V) as described previously. The absence of change in
heart rate and LV dynamics with electrical stimulation of these nerves confirmed total cardiac denervation.

Hearts that developed ventricular fibrillation or sustained ventricular tachycardia were cardioverted (1 J/kg) using internal paddles; if normal sinus rhythm was not restored after two attempts, the experiment was terminated.

2.3 Regional myocardial blood flow measurements

Regional myocardial blood flow was determined at baseline (5 min prior to 40 min wait period or rIPC protocol), 30 min coronary occlusion, and 30 min reperfusion using neutron-activated microspheres (± 15 μm; BioPAL Inc., Worcester, MA, USA) as described previously. Microspheres were also injected under steady-state baseline conditions 20 min after administration of hexamethonium bromide to evaluate the possible drug-induced effects on blood flow distribution compared with baseline [no differences were discerned between HEX (1.4 ± 0.6 mL/min/g) and HEX + rIPC (1.4 ± 0.9 mL/min/g) compared with baseline blood flow; cf. Table 4].

2.4 LV pressure–volume relations

The conductance catheter technique used for the determination of LV pressure–volume relations has been described. LV pressure–volume loops were constructed (during baseline and at the end of the ischaemia–reperfusion protocol) by a brief constriction of the inferior vena cava for 10–15 beats; the slope of the end-systolic pressure–volume relationship (ESV) can be used as an index of ventricular contractility (insensitive to loading conditions). The relation between end-systolic pressure and LV volume is preload defined, afterload independent, and sensitive to changes in inotropic status of the heart. Parallel conductance (due to conductance of structures outside the ventricular blood pool) was determined by injection of hypertonic saline into the pulmonary artery; blood resistivity (μ) was measured using a rhometer. Conductance catheter data were recorded continuously during the experiment and analysed off-line using ConductNT software (CD Leycom).

2.5 Risk area and infarct size

At the end of each study, the left anterior descending artery was re-ligated at the original site of occlusion; the area at risk (AAR) was outlined by perfusion of the coronary ostium with Monastral blue dye. Under deep pentobarbital anaesthesia, cardiac arrest was induced by intra-atrial injection of saturated potassium chloride. A 1.3% solution of warmed (37 °C) 2,3,5-triphenyltetrazolium chloride was infused into the ischaemic region via a cannula in the coronary artery (distal to the snare occluder) over 30 min to identify necrotic myocardium. The heart was then rapidly excised, rinsed in saline, and fixed in 10% buffered formaldehyde for later determination of infarct size and distribution of myocardial blood flow; the left kidney was also removed, rinsed in saline, and fixed in buffered formaldehyde for later determination of blood flow distribution. The LV was cut from the apex to the base and the outline of each slice, the necrotic area (AN), and the AAR were traced onto acetates. The LV area, risk area (AR), and AN were determined using a digitizing tablet (Summagraphics II Plus) interfaced with a personal computer and analysed with Sigma Scan software (SPSS Inc., CA, USA). Results are expressed as the AR indexed to total LV mass and the area of necrosis indexed to either AR or total LV mass.

Tissue samples were taken from the AAR and non-ischaemic LV and subdivided into endocardial and epicardial pieces for standard histology (haematoxylin–eosin and Masson’s trichrome staining) and blood flow analyses; in addition, tissue samples were also taken from the left kidney. Blood and tissue samples were dried for 48 h at 50 °C and sent to a core-processing facility (BioPAL Inc.) as described in an earlier report from our laboratory; blood flow is expressed as mL/min/g.

2.6 Statistical analysis

Intergroup comparisons of cardiac haemodynamic and regional blood flow data were performed by two-way ANOVA; post hoc comparisons were performed using the Student–Newman–Keuls multiple range test. A probability (P) level of ≤0.05 was considered statistically significant. The incidence of arrhythmias such as ventricular fibrillation and survival due to the combined ischaemia–reperfusion insult were compared using the Fisher exact test and by χ² analysis; all statistical analyses...
were carried out using SAS software (SAS Institute Inc., Cary, NC, USA). Sample size determination for these studies was based on the provision of a 90% power to detect at a $P \leq 0.05$ significance level a minimum 20% reduction/ augmentation [expected standard deviation (SD) of ±8%] in infarct size. Because of the importance of anatomic risk zone size, coronary collateral flow, and cardiac metabolic demand for the development of tissue necrosis in the canine, these parameters were incorporated into the statistical analysis. Treatment interactions for variables measured once (i.e. area of necrosis, AAR, infarct size) were analysed by ANOVA. An analysis of covariance (ANCOVA) was also done to assess differences between experimental groups when variability due to coronary collateral flow (independent variable) was considered. The regression between infarct size and endocardial blood flow within the risk zone was determined by a linear least-squares fit method.

### 3. Results

Forty-seven dogs were entered into the study. Ventricular fibrillation did not occur in any subject during the ischaemic period but occurred at the onset of reperfusion in two of seven CON, three of seven rIPC (29 vs. 43%; $P = NS$), two of eight HEX, five of 10 HEX + rIPC (25 vs. 50%; $P = NS$), two of seven DCN, and five of eight DCN + rIPC (29 vs. 63%; $P = NS$) dogs; non-convertible ventricular fibrillation resulting in mortality and exclusion from the experiment occurred in one of eight HEX (13%), three of 10 HEX + rIPC (30%), and one of eight DCN + rIPC (13%) dogs ($P = NS$ between groups). Results reported here are based on 42 dogs ($n = 7$ per group) that completed these studies.

### 3.1 Systemic haemodynamics and LV function

Changes in cardiac haemodynamic data are summarized in the tables: Table 1, CON vs. rIPC; Table 2, HEX vs. HEX + rIPC; and Table 3, DCN vs. DCN + rIPC. Heart rate was stable for all experimental groups except in CON dogs where it was higher ($P = 0.01$) compared with rIPC. LV systolic pressure and mean arterial pressure were similar among groups with the exception of the DCN + rIPC group (compared with DCN; $P = 0.001$). The rate–pressure index (product of heart rate and LV systolic pressure) was higher in CON (vs. rIPC; $P = 0.02$) and DCN (vs. DCN + rIPC; $P = 0.02$) dogs.

The rIPC protocol did not affect LV function parameters. During acute coronary occlusion, LV ejection fraction (stroke volume/$V_{\text{max}} \times 100$) decreased and remained lower than baseline values in all dogs. LV ejection fraction during reperfusion returned to near baseline values in all dogs with the exception of the HEX and HEX + rIPC groups. LV end-systolic elastance ($LVE_n = \text{mmHg/s}$) obtained at the onset/end of the experimental protocol was similar for CON (2.6 ± 1.9/2.9 ± 1.7; data are mean ± 1 SD) and rIPC (2.4 ± 1.0/ 2.4 ± 0.8) dogs. $LVE_n$ in HEX + rIPC (3.1 ± 1.1/3.8 ± 1.4) was significantly different ($P = 0.02$) compared with HEX (2.2 ± 1.1/2.9 ± 1.3) animals. Similar findings were obtained ($P = 0.05$) for DCN + rIPC (3.0 ± 1.0/5.0 ± 1.5) compared with DCN (2.7 ± 0.6/2.8 ± 1.0) dogs; however, in addition, $LVE_n$ at the end of the experiment (5.0 ± 1.5) was significantly elevated ($P = 0.02$) compared with baseline values (3.0 ± 1.0) in DCN + rIPC dogs.

#### 3.2 Myocardial infarct size and blood flow

The LV AAR was similar between groups (data are mean ± 1 SD: CON, 30 ± 8%; rIPC, 37 ± 5%; HEX, 36 ± 8%; HEX + rIPC, 36 ± 6%; DCN, 35 ± 10%; DCN + rIPC, 35 ± 6% of LV area). Myocardial infarct size (expressed as per cent of the AAR) was 29 ± 5% in CON dogs. Infarct size was reduced in HEX (24 ± 3%; $P = 0.05$ vs. CON) and DCN (20 ± 2%; $P = 0.05$ vs. CON) (Figure 2). rIPC significantly diminished infarct size (15 ± 4%; $P = 0.001$ vs. CON); similar reductions in infarct size were observed in HEX + rIPC (12 ± 2%; $P = 0.001$ vs. HEX) and DCN + rIPC (12 ± 4%; $P = 0.001$ vs. DCN). No significant differences were observed between either of the experimental groups subjected to the rIPC protocol. Although direct statistical comparisons of infarct size (ratio of area of necrosis vs. AAR) by ANOVA indicated a significant reduction of tissue injury by rIPC and also by pharmacological or surgical decentralization (compared with control animals), this statistical method does not take into account the influence of coronary collateral flow within

### Table 1 Cardiac haemodynamic data for control rIPC studies

<table>
<thead>
<tr>
<th></th>
<th>HR (b.p.m.)</th>
<th>LVSP (mmHg)</th>
<th>MAP (mmHg)</th>
<th>RPI (b.p.m./mmHg)</th>
<th>LVEF (%)</th>
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<tr>
<td>CON ($n = 7$)</td>
<td></td>
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<td>102 ± 21</td>
<td>82 ± 24</td>
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<td>122 ± 18</td>
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<td>11.5 ± 2.7</td>
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<td>70 ± 15</td>
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<td>69 ± 10</td>
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<tr>
<td>30 min REP</td>
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Data are mean ± 1 SD. CO, coronary occlusion; REP, reperfusion; HR, heart rate (b.p.m.); LVSP (mmHg), LV systolic pressure; MAP (mmHg), mean arterial pressure; RPI (b.p.m. × mmHg/1000), rate–pressure index; LVEF (%), LV ejection fraction.
the AAR. Consequently, any real effect on tissue necrosis can only be determined if the statistical method considers the relationship between infarct size and coronary collateral blood flow (independent covariate) for each experimental group. In all dogs that were subjected to rIPC, and in HEX and DCN dogs, a downward shift of covariate) for each experimental group. In all dogs that were sub-

### Table 2 Cardiac haemodynamic data for pharmacological decentralization studies

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<tr>
<th></th>
<th>HR</th>
<th>LVSP</th>
<th>MAP</th>
<th>RPI</th>
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<td>62 ± 5</td>
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<td>67 ± 6</td>
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<td>30 min REP</td>
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<td>180 min REP</td>
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Data are mean ± 1 SD. CO, coronary occlusion; REP, reperfusion; HR, heart rate (b.p.m.); LVSP (mmHg), LV systolic pressure; MAP (mmHg), mean arterial pressure; RPI (b.p.m. × mmHg/1000), rate–pressure index; LVEF (%), LV ejection fraction; HEX, cardiac decentralization by hexamethonium bromide.

### Table 3 Cardiac haemodynamic data for surgical cardiac decentralization studies

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<td>Baseline</td>
<td>105 ± 11</td>
<td>82 ± 2</td>
<td>64 ± 2</td>
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<td>30 min CO</td>
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<td>44 ± 16</td>
</tr>
<tr>
<td>60 min CO</td>
<td>107 ± 11</td>
<td>80 ± 6</td>
<td>63 ± 5</td>
<td>8.6 ± 1.3</td>
<td>44 ± 16</td>
</tr>
<tr>
<td>30 min REP</td>
<td>113 ± 17</td>
<td>83 ± 4</td>
<td>65 ± 4</td>
<td>9.5 ± 1.8</td>
<td>53 ± 19</td>
</tr>
<tr>
<td>180 min REP</td>
<td>113 ± 15</td>
<td>85 ± 4</td>
<td>67 ± 6</td>
<td>9.6 ± 1.3</td>
<td>52 ± 19</td>
</tr>
<tr>
<td>P-value (groups)</td>
<td>NS</td>
<td>0.001</td>
<td>0.001</td>
<td>0.02</td>
<td>NS</td>
</tr>
<tr>
<td>P-value (inter)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are mean ± 1 SD. CO, coronary occlusion; REP, reperfusion; HR, heart rate (b.p.m.); LVSP (mmHg), LV systolic pressure; MAP (mmHg), mean arterial pressure; RPI (b.p.m. × mmHg/1000), rate–pressure index; LVEF (%), LV ejection fraction; DCN, cardiac decentralization by nerve transection.

### 3.3 Kidney blood flow, biochemistry, and pathology

Mean renal blood flow (measured with flow probe) at baseline was similar for all experimental groups (range: 160–218 mL/min). No significant differences were observed between groups during coronary occlusion and reperfusion, but there was a trend to lower renal blood flows (range: 130–170 mL/min) at the end of each study; the AAR. Consequently, any real effect on tissue necrosis can only be determined if the statistical method considers the relationship between infarct size and coronary collateral blood flow (independent covariate) for each experimental group. In all dogs that were subjected to rIPC, and in HEX and DCN dogs, a downward shift of the infarct size/endocardial blood flow ratio was observed (Figure 3). Our findings confirm that the limitation of infarct size was independent of endocardial blood flow within the AAR during acute ischaemia. The overall change in myocardial blood flow in endocardial and epicardial tissue layers within ischaemic and non-ischaemic regions for all experimental groups at baseline, during coronary occlusion, and during coronary reperfusion is summarized in Table 4. Although endocardial/epicardial blood flow ratios in the ischaemic region tended to be higher in rIPC dogs (2.2 ± 0.8 vs. 1.5 ± 0.5 in CON; mean ± 1 SD) at 30 min reperfusion, no significant change was observed for this variable in either of the cardiac decentralized groups.
these results were confirmed with microsphere studies (data not shown). Since pre- and post-rIPC serum creatinine (range: 50–90 μmol/L) and blood urea nitrogen (range: 5–9 μmol/L) were within physiological levels for canines and no tubular injury (microscopic examination) was detected in renal biopsies (taken at the end of the experiment), we concluded that kidney function was normal during these experiments.

4. Discussion

Our findings show that rIPC-induced cardioprotection was not reversed by pharmacological or surgical decentralization of the intrinsic cardiac nervous system. Although a mechanism responsible for rIPC-mediated protection has not been clearly established, the release of humoral factors12,31 and the activation of neural pathways5,17 are believed to be involved. Animal studies using either surgical or chemical sympathectomy document that the activation of the sympathetic nervous system may not be obligatory for first window preconditioning.23,32,33 However, second window preconditioning requires intact cardiac nerves and α-adrenergic receptors.21 We also show that both pharmacological and surgical decentralization without rIPC limits tissue necrosis but to a lesser extent than rIPC. Earlier studies have demonstrated that acute or chronic cardiac decentralization increases tolerance to ischaemic injury and decreases ventricular fibrillation threshold.27,34,35 Potential mechanisms include reduced myocardial oxygen demand and improved myocardial perfusion.26,36–38 An earlier study from our laboratory reported preserved coronary autoregulation and myocardial blood flow distribution following cardiac decentralization in canines.23

Although the present findings suggest that neural pathways may not be directly involved in cardioprotection produced by rIPC, local circuit neurons are known to process information from the intrathoracic nervous system and transduce afferent neuronal inputs to peripheral autonomic ganglia, even when such ganglia have been disconnected from the central neurons.23,24 As such, attenuated neuronal responses due to ischaemic stress could delay the development of cellular injury.24 Endogenous compounds released into the bloodstream or extracellular milieu during and/or after ischaemia could also stimulate intercellular pathways involved in transduction of the preconditioning effect.39 Even though our findings appear to favour the humoral hypothesis for rIPC protection, stimulation of cardiac neurons by an unknown humoral cytoprotective compound remains plausible.

4.1 Study limitations

The effects of each of the major determinants known to influence infarct size development such as anatomic risk zone size, core body temperature, and the level of coronary collateral circulation within
that myocardium might be preconditioned by the use of a volatile myocardial injury, vascular dysfunction, and renal impairment. \(^{42,43}\) arteries or limb ischaemia has been used to reduce post-operative kidney. In humans, intermittent cross-clamping of unilateral iliac porter of humoral cytoprotective compound) is distinct for each expect this to be the case as venous blood flow (i.e. potential trans-
tive in the presence of an intact right kidney; however, we would not
whether renal rIPC protection in canines would be more or less effec-
tive in the presence of an intact right kidney; however, we would not expect this to be the case as venous blood flow (i.e. potential trans-
porter of humoral cytoprotective compound) is distinct for each kidney. In humans, intermittent cross-clamping of unilateral iliac arteries or limb ischaemia has been used to reduce post-operative myocardial injury, vascular dysfunction, and renal impairment.\(^ {42,43}\)

Cardiac decentralization used for the present studies is relevant to study the potential role for the autonomic nervous system in inter-
organ protection. Surgical ablation of sympathetic and parasympa-
thetic efferent neuronal inputs to the heart results in decentralization or autonomic ganglionic blockade. As a result, remote preconditioning could be useful for organ protection in patients, regardless of the nervous system status. Further investigation is necessary to identify compounds that modulate or activate intrinsic neuronal populations to induce cellular protection.

4.2 Perspectives

The present study reports that inter-organ crosstalk does not appear to require an intact autonomic nervous system since rIPC-induced myocardial protection was not reversed by either cardiac decentralization or autonomic ganglionic blockade. As a result, remote preconditioning could be useful for organ protection in patients, regardless of the nervous system status. Further investigation is necessary to identify compounds that modulate or activate intrinsic neuronal populations to induce cellular protection.

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