

Left Ventricular Function after Acute Ischemia-Reperfusion Injury in Cardiac Decentralized Dogs Subject to Ischemic Conditioning

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ABSTRACT

Objective: Ischemic conditioning (IC) mediated protection against ischemic injury continues independent of extrinsic cardiac nerve status; however, controversy persists regarding its ability to limit ischemia-mediated LV contractile dysfunction. Here, we investigate the acute cardiac consequences of loss of sympathetic and parasympathetic inputs to the local cardiac neuronal hierarchy on post-ischemic LV function. Additionally, we examined whether IC could affect potential recovery of LV function.

Methods: Anesthetized, open-chest dogs were randomly distributed to surgical nerve ablation or ganglionic blockade (hexamethonium bromide) groups; each group comprised dogs subject to IC. All dogs were exposed to 60-min acute coronary occlusion (CO) followed by 180-min reperfusion (REP). IC consisted of 4 cycles of 5-min CO and 5-min REP of the left main coronary artery. LVP-V relations were constructed by inferior vena cava occlusion at different times before and during CO-REP. The load insensitive conductance catheter method was used to evaluate LV contractility, diastolic function and ventriculoarterial arterial coupling.

Results: We found robust protection against necrosis in all IC treated dogs that was not abrogated by nerve ablation or ganglionic blockade; however, LV contractile functional status did not improve. Parameters of LV function were not markedly different between groups; however, statistical significance was observed during CO-REP between nIC and IC groups. These changes were similar regardless of the method used for cardiac decentralization.

Conclusions: Diminished LV contractile function produced by CO-REP did not recover with IC pre-treatment; cardiac decentralization (surgical or pharmacologic) did not markedly affect outcomes.

Keywords

Decentralized heart, Nerve ablation, Ganglionic blockade, Ischemia, Reperfusion, Ischemic conditioning, Ventricular function, Pressure-volume relations.

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Introduction

Interactions between peripheral and central neuronal elements of the cardiac neuronal hierarchy match cardiac output to regional blood flow demands [1]. Restoration of blood perfusion to the

ischemic vascular bed is essential to limit progression of tissue necrosis; it may also be required to transfer, yet unidentified, endogenous cytoprotective compounds between distant organs. Some studies have examined the role of cardiac nerves on

pathogenesis of ischemia-induced myocardial injury with variable results [2-4]. Neural pathways could also transfer humoral mediators or other signals that trigger organ protection [5,6].

In heart transplant patients sympathetic and parasympathetic fibers of the autonomic nervous system are excised; disruption of connections between supraspinal structures and preganglionic neurons that control the heart and systemic vasculature can result in marked cardiac inotropic dysfunction [7], alterations in cardiac loading [8,9] and changes in cardio-autonomic function. Proper functioning of the allograft requires provision of catecholamines from noncardiac sites [10]. Consequences of cardiac denervation include both chronotropic and inotropic supersensitivity to exogenous catecholamines. Stressors (hypovolemia, hypoxia, anemia etc.) markedly delay transfer of circulating catecholamines that exert positive chronotropic cardiac effects [11]. Sympathetic dysinnervation that occurs secondary to myocardial infarction may also affect overall function of cardiac neurons.

We previously examined development of post-ischemic cardiac injury in cardiac decentralized dogs [12,13] to verify if loss of sympathetic and parasympathetic inputs to the local neuronal hierarchy would influence pathogenesis of myocyte necrosis. Additionally, we examined the potential cardioprotective effects of ischemic conditioning (IC). Our findings showed that a reduction of ischemic injury was still possible even after cardiac decentralization. However, changes in left ventricle (LV) contractile function produced by cardiac decentralization were not addressed. In the present study we used combined pressure and conductance transducers to evaluate dynamic LV contractile function changes [14-17] in cardiac decentralized (surgical ablation of cardiac nerves or ganglionic blockade with hexamethonium bromide) dogs subject to acute coronary occlusion and reperfusion (with or without IC).

Materials and Methods

Dogs were acquired through the Division of Laboratory Animal Services at Laval University; they were housed in individual cages under conditions of constant temperature and humidity and kept on a strict 12:12 h dark light cycle. Dogs had free access to food and water. This study was approved (#2007-001-2) by the institutional animal welfare committee at Laval University (A5012-01) and was carried out in compliance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (publication 85-23; revised 1996). The experiments were carried out, and results reported as described in the ARRIVE guidelines [18].

Surgical Preparation

Anesthesia protocols have been described in earlier studies from our laboratory [19-21]. Briefly, dogs (both male and female; 20-25 Kg) were intubated and anesthesia was maintained with isoflurane (1-2%) and oxygen-enriched room air. Fentanyl (0.005 mg/Kg IV bolus followed by constant infusion at 0.005 mg/Kg/h) was administered for analgesia. Normothermia was maintained with a

water-jacketed Micro-Temp heating blanket (Zimmer, Dover, OH, USA); saline was given (250 mL/h IV) to replace fluid loss.

Dogs were placed in the supine position and vascular introducer sheaths (8Fr, Terumo Medical Corp. USA) were placed in the left and right femoral arteries; a triple lumen central venous catheter (7Fr, Arrow-Howes™, Arrow Intl. Inc., Reading, PA, USA) was positioned in the right femoral vein.

In this open-chest study a left lateral thoracotomy was done through the third and fifth intercostal spaces (ribs four and five 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 surrounding tissues in each dog [12,22]. In order to preserve intrinsic cardiac neuron function, connective tissues around the ascending aorta, the left pulmonary vein, and the main pulmonary artery were not dissected. The heart was exposed and suspended in a pericardial cradle. A section of the left anterior descending artery branch (distal to the first diagonal branch) was dissected to allow placement of a vascular clamp for regional coronary artery occlusion (CO). Umbilical tape was placed around the inferior vena cava (IVC) cranial to the diaphragm. This allowed occlusion of the IVC later in the experiment to reduce cardiac preload for construction of LV pressure-volume (LVP-V) relations. A catheter (7Fr) was advanced into the main pulmonary artery (for determination of parallel conductance calibration factors using hypertonic saline) [23]. A solid-state pressure transducer (5Fr, MPC500, Millar Instruments Inc., Houston, TX, USA) was placed in the LV cavity via an apical approach. A 12-electrode conductance catheter (7Fr, CD Leycom, Zoetermeer, The Netherlands) was advanced (via femoral artery) to the LV apex along the longitudinal axis of the ventricle as previously described [24]. LVP-V loops were recorded during apnea; blood resistivity was assumed to be constant (150 Ω cm). Heparin sodium (500 IU, IV) was given as a bolus and then hourly (100 IU, IV) to limit blood clotting. After all surgical procedures were completed the preparation was allowed to stabilize for 30-min before initiation of the experimental protocol.

The Millar solid-state pressure transducer was cross-calibrated with both systolic aortic and diastolic left atrial pressure; the conductance catheter was connected to a Sigma 5DF signal conditioning and processing unit. All data were recorded continuously and stored on computer hard drive for later analysis.

Experimental Protocol

The completeness of cardiac decentralization in all dogs was confirmed by direct electrical stimulation of the left and right ansae subclavia (10 Hz, 5 ms, 5-7 V) and the left and right thoracic vagi (20 Hz, 5 ms, 5-7 V) [2,25]. Cardiac decentralized dogs – by surgery (bilateral nerve ablation [26]) or ganglionic blockade (hexamethonium bromide), were randomly assigned to no-IC (nIC) or IC groups (Figure 1). In the nIC group, a 40-min wait period was instituted to allow comparisons between treatments. In the IC group, dogs were subject to 4 cycles of 5-min CO and 5-min

coronary reperfusion (REP) prior to acute CO as described [13]. Prior to onset of ischemia, and at end of REP, LV P-V loops were recorded under steady-state conditions (during apnea); preload was reduced by temporary occlusion of the inferior vena cava [27,28]. Dogs were then subject to 60-min regional CO followed by 180-min REP; xylocaine was administered (10 mg IV bolus; Astra Pharma, Inc., Mississauga, ONT, CAN) after 30-min of CO and just prior to REP to limit ischemia- or reperfusion-induced arrhythmias. Hearts that fibrillated were cardioverted (DC shock ≤ 50 Joules) with a cardiac defibrillator (General Electric); if defibrillation was not successful after two attempts, the animal was euthanized and not entered into the data analysis.

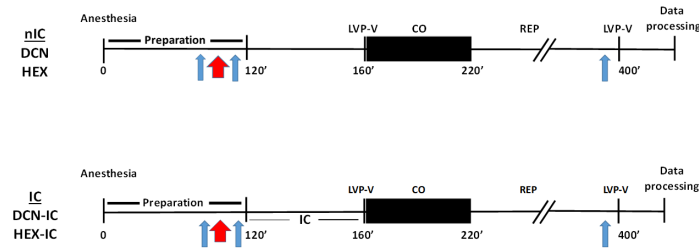


Figure 1: Timeline of the experimental protocol. LVP-V loops were recorded prior to acute CO and just before the end of REP. The time at which nerve stimulation (thin arrows) and decentralization by nerve ablation or ganglionic blockade (red arrow) as outlined in Methods is shown; nerve stimulation was also carried out prior to sacrifice.

Statistical Analysis

Data are expressed as means \pm 1SD. Data normality was verified by the Shapiro-Wilk test (after Cholesky factorization); a linear mixed effects model was used to identify changes in cardiac variables. A repeated-measure ANOVA (linear mixed model) allowed determination of statistical differences; selection of a covariance structure was based on the Akaike information criterion. Comparisons were done by Tukey's test when no interaction was significant. A p value <0.05 was considered statistically significant for all analyses. Statistical analyses were performed using the statistical packages R v3.0.2 (R Foundation for Statistical Computing, Vienna, and Austria.) and SAS v9.4 (SAS Institute Inc., Cary, NC, U.S.A.).

Results

Thirty-two dogs (n=8 per group) were randomly allocated to the study groups and all completed the experimental protocol. Incidence of ventricular tachycardia/fibrillation caused by CO or REP was not statistically different between experimental groups. Arterial blood gas and hematocrit values (data not shown) were stable and remained within physiological levels.

Surgical Decentralization

Table 1 summarizes changes in cardiac dynamics for these experiments. Heart rate (HR) was maintained throughout the experimental protocol in both nIC and IC groups. During CO and REP, maximum derivative change in LV systolic pressure over

time (dPdtmax) and minimum derivative change in LV diastolic pressure over time (dPdtmin) decreased significantly; end-systolic LV pressure (ESP) was unchanged but a significant decline in end-diastolic LV pressure (EDP) was observed during REP in both nIC groups. Stroke volume (SV) remained constant in both experimental groups (Table 2); however, a significant decrease in LV ejection fraction (LVEF) was observed during CO and REP. Stroke work (SW) also decreased markedly during CO and REP. The time constant of LV relaxation (Tau) and time to peak filling rate (TPFR) decreased significantly during REP (compared to baseline) in the IC group. End-systolic and end-diastolic volume (ESV, EDV) increased substantially during CO and REP as expected.

Elastance at end-systole (E_{es} ; mm Hg/mL) of the LVP-V relation after surgical decentralization of the heart was unchanged between nIC and IC groups (Figure 2). The rightward shift of LVP-V loops after acute CO was principally due to increases in ESV and EDV (Figure 3).

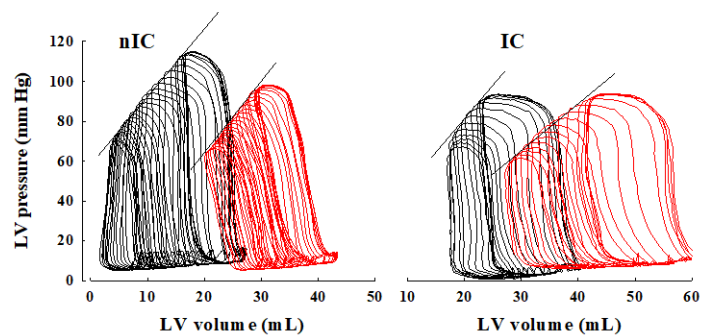


Figure 2: Representative LVP-V relations obtained by transient IVC occlusion in surgically decentralized dogs in nIC (left panel) and IC (right panel) groups at baseline (black line) and after 180-min REP (red line). In nIC dogs, E_{es} was 3.31 mm Hg/mL ($r^2 = 0.94$) before acute CO and 3.41 mm Hg/mL ($r^2 = 0.91$) at the end of REP; in IC dogs, E_{es} was 3.71 mm Hg/mL ($r^2 = 0.93$) before acute CO and 2.18 mm Hg/mL ($r^2 = 0.92$) at the end of REP. The rightward shift of the LVP-V relation produced by CO was not diminished by IC.

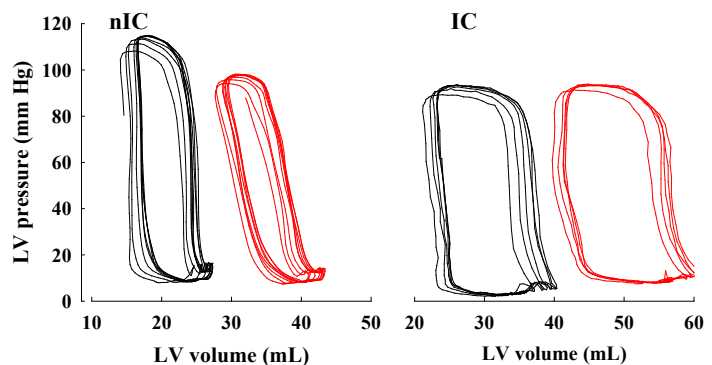


Figure 3.

Figure 3: Representative LVP-V loops in surgically decentralized dogs at baseline (blackline) and 180-min REP (red line) for nIC (left panel) and IC (right panel) groups. No difference between groups was afforded by IC.

Table 1: Summary of Cardiac Hemodynamics for Cardiac Decentralized (Nerve Ablation) Dogs.

	nIC			IC			ANOVA		
	Base	CO	REP	Base	CO	REP	Grp	Int	Grp*Int
HR	113 ± 15	109 ± 13	115 ± 11	100 ± 15	104 ± 15	108 ± 15	0.22	0.20	0.66
dPdtmax	1400 ± 270	1225 ± 145	1087 ± 210	1342 ± 167	1141 ± 110	1047 ± 139	0.57	0.001	0.90
dPdtmin	1148 ± 237	1040 ± 133	1067 ± 277	1189 ± 178	1052 ± 181	1127 ± 193	0.90	0.002	0.85
ESP	82 ± 12	78 ± 4	76 ± 15	96 ± 16	82 ± 9	82 ± 13	0.36	0.09	0.40
EDP	6 ± 2	7 ± 1	5 ± 1	9 ± 3	8 ± 4	5 ± 2	0.59	0.001	0.52

Data are means ± 1SD. nIC, IC: no ischemic conditioning, ischemic conditioning; HR: heart rate (beats per minute); dPdtmax: rate of pressure change over time during systole (mmHg/sec); dPdtmin: rate of pressure change over time during diastole (mmHg/sec); ESP, EDP: end-systolic and end-diastolic pressure (mm Hg). Grp, Int, Grp*Int; group, intervention, group*intervention statistical analyses.

Table 2: Summary of LV Pressure-Volume Loop Data for Cardiac Decentralized (Nerve Ablation) Dogs.

	nIC			IC			ANOVA		
	Base	CO	REP	Base	CO	REP	Grp	Int	Grp*Int
SV	15 ± 4	16 ± 4	14 ± 3	14 ± 5	14 ± 5	13 ± 5	0.86	0.11	0.29
LVEF	36 ± 12	30 ± 9	23 ± 7	33 ± 5	26 ± 6	23 ± 5	0.66	0.001	0.58
Tau	34 ± 6	39 ± 10	33 ± 1	40 ± 5	38 ± 4	32 ± 2	0.57	0.001	0.07
SW	971 ± 156	830 ± 193	700 ± 116	1025 ± 116	870 ± 129	731 ± 97	0.96	0.001	0.41
tPFR	406 ± 66	424 ± 108	373 ± 63	414 ± 32	407 ± 37	379 ± 40	0.84	0.02	0.34
ESV	25 ± 14	31 ± 14	37 ± 17	24 ± 9	32 ± 13	34 ± 12	0.99	0.001	0.77
EDV	38 ± 15	46 ± 18	49 ± 19	37 ± 12	45 ± 16	46 ± 16	0.99	0.001	0.90

Data are means ± 1SD. nIC, IC: no ischemic conditioning, ischemic conditioning; SV: stroke volume (mL); LVEF: LV ejection fraction (%); Tau: LV isovolumetric relaxation time constant (ms); SW: stroke work (mm Hg/mL); tPFR: time to peak filling rate (ms); ESV, EDV: end-systolic and end-diastolic volume (mL). Grp, Int, Grp*Int; group, intervention, group*intervention statistical analyses.

Table 3: Summary of Cardiac Hemodynamics for Cardiac Decentralized (Ganglionic Blockade) Dogs.

	nIC			IC			ANOVA		
	Base	CO	REP	Base	CO	REP	Grp	Int	Grp*Int
HR	107 ± 20	111 ± 25	109 ± 24	104 ± 13	120 ± 14	134 ± 11	0.35	0.002	0.03
dPdtmax	1263 ± 273	1656 ± 421	1533 ± 184	1711 ± 741	2195 ± 691	2359 ± 627	0.06	0.001	0.50
dPdtmin	1400 ± 390	1600 ± 743	1501 ± 505	1647 ± 301	1896 ± 408	1884 ± 310	0.21	0.005	0.60
ESP	73 ± 8	78 ± 11	68 ± 6	78 ± 9	84 ± 11	73 ± 3	0.11	0.001	0.72
EDP	11 ± 5	14 ± 5	7 ± 3	11 ± 2	12 ± 4	7 ± 6	0.66	0.001	0.87

Data are means ± 1SD. nIC, IC: no ischemic conditioning, ischemic conditioning; HR: heart rate (beats per minute); dPdtmax: rate of pressure change over time during systole (mmHg/sec); dPdtmin: rate of pressure change over time during diastole (mmHg/sec); ESP, EDP: end-systolic and end-diastolic pressure (mm Hg). Grp, Int, Grp*Int; group, intervention, group*intervention statistical analyses.

Table 4: Summary of LV Pressure-Volume Loop Data for Cardiac Decentralized (Ganglionic Blockade) Dogs.

	nIC			IC			ANOVA		
	Base	CO	REP	Base	CO	REP	Grp	Int	Grp*Int
HR	107 ± 20	111 ± 25	109 ± 24	104 ± 13	120 ± 14	134 ± 11	0.35	0.002	0.03
dPdtmax	1263 ± 273	1656 ± 421	1533 ± 184	1711 ± 741	2195 ± 691	2359 ± 627	0.06	0.001	0.50
dPdtmin	1400 ± 390	1600 ± 743	1501 ± 505	1647 ± 301	1896 ± 408	1884 ± 310	0.21	0.005	0.60
ESP	73 ± 8	78 ± 11	68 ± 6	78 ± 9	84 ± 11	73 ± 3	0.11	0.001	0.72
EDP	11 ± 5	14 ± 5	7 ± 3	11 ± 2	12 ± 4	7 ± 6	0.66	0.001	0.87

Data are means ± 1SD. nIC, IC: no ischemic conditioning, ischemic conditioning; SV: stroke volume (mL); LVEF: LV ejection fraction (%); Tau: LV isovolumetric relaxation time constant (ms); SW: stroke work (mm Hg/mL); tPFR: time to peak filling rate (ms); ESV, EDV: end-systolic and end-diastolic volume (mL). Grp, Int, Grp*Int; group, intervention, group*intervention statistical analyses.

Ganglionic Blockade

Table 3 summarizes changes in cardiac dynamics for these experiments. Heart rate in the IC group increased significantly in IC treated dogs; dPdtmax and dPdtmin was significantly higher in both groups during CO and REP. ESP and EDP were markedly lower during REP in nIC and IC groups. As shown in Table 4, SV was constant for both experimental groups during the experiment. A significant decline in LVEF produced by CO was observed and was maintained during REP. On the other hand, SW was not markedly affected. Tau decreased during REP in nIC and IC groups but tPFR was not affected. ESV was significantly increased during REP in the IC group; however, EDV was stable.

Elastance at end-systole (E_{es} ; mm Hg/mL) of the LVP-V relation after administration of hexamethonium was unchanged between nIC and IC groups (Figure 4). The rightward shift of LVP-V loops after acute CO was similar to that observed in surgically decentralized studies (Figure 5).

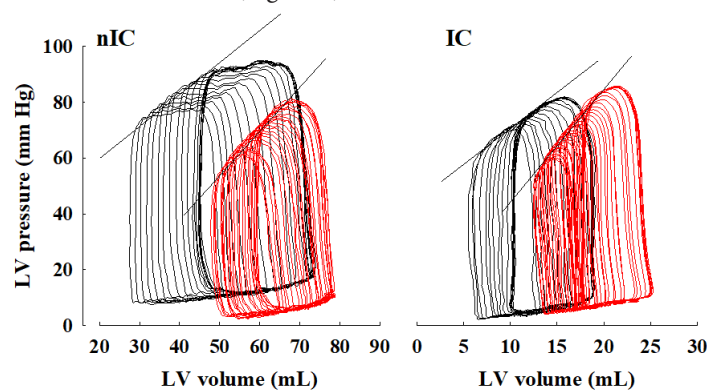


Figure 4: Representative LVP-V relations obtained by transient IVC occlusion for after ganglionic blockade in nIC (left panel) and IC (right panel) dogs at baseline (black line) and after 180-min REP (red line). In nIC dogs, E_{es} was 3.43 mm Hg/mL ($r^2 = 0.91$) before acute CO and 2.81 mm Hg/mL ($r^2 = 0.97$) at the end of REP; in IC dogs, E_{es} was 4.71 mm Hg/mL ($r^2 = 0.96$) before acute CO and 2.66 mm Hg/mL ($r^2 = 0.94$) at the end of REP. The rightward shift of the LVPV relation after CO was mitigated by IC.

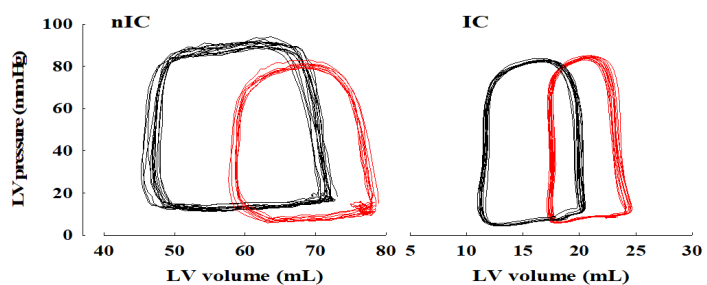


Figure 5: Representative LVP-V loops after ganglionic blockade at baseline (black line) and 180-min REP (red line) in nIC (left panel) and IC (right panel) groups. No difference between groups was observed with IC treatment.

Discussion

Ischemia-reperfusion injury produces marked reductions in LV contractile function that may, or may not be mitigated by

pharmacologic or non-pharmacologic interventions. Earlier findings from our laboratory showed that protection by IC against ischemic injury was possible in dogs that underwent cardiac decentralization [13]; however, that study did not focus on potential changes in LV contractile dysfunction. The present findings, in the canine, indicate that global LV contractile dysfunction is not reversed by IC. Furthermore, cardiac decentralization (i.e. by surgical ablation of extracardiac nerves or ganglionic blockade) did not affect the negative effects of acute CO on LV contractile function.

Transection of sympathetic and parasympathetic fibers of the autonomic nervous is necessary for heart transplantation in patients [10]; however, cardiac denervation can produce troublesome consequences. Additionally, acute myocardial infarction can result in significant sympathetic dysinnervation but the injury threshold of sympathetic and parasympathetic neurons is less clear [29,30]. In another scenario, spinal cord injury immediately affects supraspinal structures and sympathetic preganglionic neuron networks. In all of these situations, control of the heart and systemic vasculature is disrupted and can lead to critical reductions in cardiac inotropic function and changes in cardiac loading [8,9,31]. Modifications of cardio-autonomic function along with other cardio-metabolic sequelae (i.e. metabolism, hemodynamics, arterial stiffness, etc.) promote acute cardiovascular events; however, the underlying pathogenetic mechanisms responsible for untoward clinical outcomes are not established. Efficacy of clinical interventions after an acute event is in all probability dependent on the level of recovery of neurological function.

Ischemic conditioning (all types) increases tolerance to myocardial necrosis produced by ischemia-reperfusion injury and limits severe ventricular dysrhythmias and vascular dysfunction [32,33]. The benefits of IC to restore post-ischemic LV contractile performance are pending; however, in our laboratory we have not been able to demonstrate significant restoration of LV function with varying durations of CO. A variety of methods with different levels of accuracy have been used to assess LV function; the present studies used the conductance catheter method (gold standard) which enables continuous LVP-V loop analysis for the assessment of in vivo LV systolic contractile performance independent of preload, afterload and within limits heart rate [34]. Comparison of cardiac function indices between intact innervated and cardiac decentralized hearts showed almost no differences between surgical or chemical decentralization methods (cf. results in [32]); however, in both groups, HR was markedly lower and both ESV and EDV were higher. Reduced pressure-generating capacity and LV contractility after cardiac decentralization may be due to loss of bulbospinal sympathetic inputs to sympathetic preganglionic neurons [7,35]. It is interesting to note that long-term effects of cardiac decentralization could profoundly change the intrinsic structure of cardiomyocytes and have a direct effect on LV contractile function. Whether IC may play, a protective role in this situation has not been determined. As mentioned earlier, protection against myocardial necrosis by IC did not produce the expected positive outcome with regard to recovery of LV function

(in the short term). Our results concur with recent findings in rodents where sustained reductions in LV contractile function occur moments after onset of spinal cord injury [7].

This study has some limitations; we used the anesthetized open-chest canine model with a health profile distinctly different from humans with either heart disease or other comorbidities. A large and diverse data bank of cardiovascular studies using canine experimental models exists and favors comparisons with human data. While anesthesia may influence development of ischemic injury [36,37] suitable alternatives are not presently available. We use the terminology 'decentralized' rather than 'denervated' for this experimental model; acute bilateral ablation of extracardiac nerves results in a complete disconnect from central command peripheral nerve networks [38,39] but parasympathetic involvement is not completely eliminated [40]. Transection of the ansae subclavia, vagosympathetic complexes and stellate ganglia eliminates potentiating effects of the central nervous system on intrinsic cardiac neural activity. To assess whether changes in cardiac function were neurally mediated dogs we administered hexamethonium bromide at a dosage that blocks nicotinic acetylcholine receptors located within ganglia [7,41,42]. Finally, longer reperfusion or post-ischemic recovery periods may be warranted and could provide results that could add to clinical relevancy.

Conclusion

Reduced LV function capacity after acute myocardial infarction has important implications with regard to quality of life and clinical outcomes. While IC can markedly reduce ischemic injury in the heart and other organs, the expected improvement of LV function afforded by this protection was not manifest. Whether this is related to experimental design of these studies is a possibility; however, future studies should tackle the question of recovery of LV function over the long term. The role of sympathetic and parasympathetic pathways in the potential for recovery of LV contractile function also needs to be established as these pathways surely affect responses to different physiological stressors post-ischemia. Herein we report that cardiac decentralization did not exacerbate post-ischemic LV contractile function. Further understanding how the loss of nervous system control affects cardiac structure and function are warranted.

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