International Journal of Cardiology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Intravenous electrical vagal nerve stimulation prior to coronary reperfusion in a canine ischemia-reperfusion model markedly reduces infarct size and prevents subsequent heart failure

Takahiro Arimura [a], Keita Saku [b,*], Takamori Kakino [a], Takuya Nishikawa [a], Takeshi Tohyama [a], Takafumi Sakamoto [a], Kazuo Sakamoto [a], Takuya Kishi [c], Tomomi Ide [a], Kenji Sunagawa [b]

[a] Department of Cardiovascular Medicine, Graduate School of Medical Sciences, 1-1-3 Maidashi Higashi-ku, Fukuoka 812-8582, Japan
[b] Department of Therapeutic Regulation of Cardiovascular Homeostasis, Center for Disruptive Cardiovascular Medicine, 1-1-3 Maidashi Higashi-ku, Fukuoka 812-8582, Japan
[c] Collaborative Research Institute of Innovative Therapeutics for Cardiovascular Diseases, Center for Disruptive Cardiovascular Medicine, Kyushu University, 1-1-3 Maidashi Higashi-ku, Fukuoka 812-8582, Japan

ARTICLE INFO

Article history:
Received 24 May 2016
Received in revised form 26 October 2016
Accepted 27 October 2016
Available online xxxx

Keywords:
Vagal nerve stimulation
Acute myocardial infarction
Chronic heart failure
Autonomic nervous system
Infarct size

ABSTRACT

Background: Reducing myocardial damage is a prerequisite to prevent chronic heart failure after acute myocardial infarction (AMI). Although vagal nerve stimulation (VNS) has been repeatedly demonstrated to have potent anti-infarct effect, technical difficulties have precluded its clinical application. We developed a novel therapeutic strategy of intravenous VNS (iVNS) and examined whether iVNS administered prior to coronary reperfusion in a canine AMI model reduces infarct size and prevents heart failure.

Methods and results: In 35 mongrel dogs, we induced ischemia by ligating the left anterior descending coronary artery and then reperfused 3 h later (I/R). We transvenously placed a catheter electrode in the superior vena cava and adjusted the stimulation intensity to a level that induced bradycardia but maintained stable hemodynamics (continuous, 5.1 ± 2.1 V, 10 Hz). We administered iVNS from onset (iVNS-0, n = 7) or 90 min after onset (iVNS-90, n = 7) of ischemia until one hour after reperfusion. Four weeks after ischemia–reperfusion, iVNS markedly reduced infarct size (iVNS-0: 2.4 ± 2.1%, $p < 0.05$ and iVNS-90: 4.5 ± 4.5%, $p < 0.05$) compared with I/R control (I/R: 13.3 ± 2.5%), and improved cardiac performance and hemodynamics. Atrial pacing (n = 7) to abolish iVNS-induced bradycardia significantly attenuated the beneficial effects of iVNS.

Conclusions: Short-term iVNS delivered prior to coronary reperfusion markedly reduced infarct size and preserved cardiac function one month after AMI. The bradycardic effect plays an important role in the beneficial effect of iVNS. How other mechanisms contribute to the reduction of infarct size remains to be studied.

Abbreviations: AAI, atrial pacing and sensing mode; AMI, acute myocardial infarction; ANOVA, one-way factorial analysis of variance; AP, arterial pressure; E_{es}, end-systolic elastance; ELISA, enzyme-linked immunosorbent assays; ESPVR, end-systolic pressure–volume relationship; HR, heart rate; I/R, ischemia–reperfusion; iVNS, intravenous vagal nerve stimulation; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; LAP, left atrial pressure; LV, left ventricle; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVEDP, left ventricular end-diastolic pressure; LVESV, left ventricular end-systolic volume; LVV, left ventricular volume; MAP, mean arterial pressure; Max +dP/dt, the maximum time derivative of left ventricular pressure; Min dP/dt, the negative peak derivative of left ventricular pressure; MVO_2, myocardial oxygen consumption; NT-proBNP, N-terminal pro-brain-type natriuretic peptide; PM, atrial pacing; RAP, right atrial pressure; SD, standard deviation; SVC, superior vena cava; TTC, 1% triphenyltetrazolium chloride; VNS, vagal nerve stimulation; V_0, volume axis intercept of left ventricular end-systolic pressure volume relationship.

* Corresponding author.
E-mail addresses: arimurat@cardiol.med.kyushu-u.ac.jp (T. Arimura), saku@cardiol.med.kyushu-u.ac.jp (K. Saku), kakino-t@cardiol.med.kyushu-u.ac.jp (T. Kakino), nisikawa@cardiol.med.kyushu-u.ac.jp (T. Nishikawa), tohyama@cardiol.med.kyushu-u.ac.jp (T. Tohyama), tsaka@cardiol.med.kyushu-u.ac.jp (T. Sakamoto), kazuos@cardiol.med.kyushu-u.ac.jp (K. Sakamoto), tkishi@cardiol.med.kyushu-u.ac.jp (T. Kishi), tomomi_i@cardiol.med.kyushu-u.ac.jp (T. Ide), sunagawa@cardiol.med.kyushu-u.ac.jp (K. Sunagawa).

http://dx.doi.org/10.1016/j.ijcard.2016.10.074
0167-5273/

1. Introduction

Although advances in early coronary reperfusion therapy have markedly reduced the short-term mortality of acute myocardial infarction (AMI) to less than 10% [1], 14.8–33.9% of AMI patients still develop heart failure in the long term [2], and AMI remains the leading cause of death in cardiovascular disease [3]. Since the size of the infarct after AMI is the major determinant of poor outcomes [4], therapies that reduce infarct size beyond early coronary reperfusion are needed to improve cardiac performance and prevent the subsequent development of heart failure in the long term.

Compelling preclinical evidence suggests that vagal nerve stimulation (VNS) may be of value in the treatment of AMI. Calvillo et al. [5]. reported that brief VNS during ischemia–reperfusion strikingly reduced infarct size in a rat model of ischemia–reperfusion. Uemura et al. [6] also reported that VNS during 3 days after coronary arterial occlusion reduced infarct size and attenuated left ventricular (LV) dysfunction in rabbits. Despite preclinical evidence showing the benefits of VNS for AMI, this therapy has not been introduced into clinical settings. A major reason is the lack of a technique that makes vagal nerve stimulation easier and less invasive under clinical conditions. Thus, the purpose of this investigation was to evaluate the use of an intravenous electrical catheter to stimulate the right vagal nerve in a canine model of AMI as a clinical applicable minimally invasive technique of intravenous VNS (iVNS), and examine the effect of iVNS prior to reperfusion on cardiac function, heart failure parameters, and infarct size at 4 weeks post-AMI.

2. Methods

2.1. Experimental protocol

Protocols are shown in Fig. 1. We randomly allocated 35 dogs to 5 groups: Sham [thoracotomy only, no left anterior descending coronary artery (LAD) occlusion or iVNS; n = 7], ischemia-reperfusion (I/R) (3-h ischemia followed by reperfusion, no iVNS; n = 7), iVNS-0 (iVNS from time 0 of ischemia to 1 h after reperfusion; n = 7), iVNS-90 (iVNS from 90 min of ischemia to 1 h after reperfusion; n = 7), and iVNS-0 + PM [iVNS and atrial pacing (PM) from time 0 of ischemia to 1 h after reperfusion; n = 7]. Four weeks after ischemia–reperfusion, we evaluated cardiac function, parameters of heart failure and infarct size in each dog. All 35 dogs survived until the end of the experiments four weeks after sham operation or ischemia–reperfusion induction.

2.2. Animals and surgical preparations

Experiments and animal care were approved by the Committee on Ethics of Animal Experiments, Kyushu University Graduate School of Medical Sciences, and performed strictly in accordance with the 8th Edition of the Guide for the Care and Use of Laboratory Animals is published by National Academy Press, 2011. Mongrel dogs (n = 35, 15.6 ± 1.8 kg) were purchased from KBT Oriental, Co., Ltd. (Saga, Japan). Dogs were housed in individual cages in a room maintained at a temperature of 20 °C and a light/dark cycle of 12/12 h. The dogs were fed a commercial dog diet (PC 2; Oriental Yeast, Co., Ltd., Saga, Japan) once daily in the morning and had free access to tap water. All dogs were acclimatized to the laboratory environment for at least 7 days before being used in experiments.

After anesthesia induction by intravenous pentobarbital (25 mg/kg) and vecuronium bromide (0.08 mg/kg), the dogs were intubated and artificially ventilated with room air to maintain physiological pH and oxygen saturation. Anesthesia was maintained by continuous isoflurane (1–2%) inhalation during the experiment. Body temperature was maintained between 37 and 38 °C. A catheter (5-Fr) was placed in the left femoral vein for administering fluids. Arterial pressure (AP) was measured at the right femoral artery using a fluid-filled catheter-transducer system (Model DX-200; Nihon Kohden, Tokyo, Japan). The heart was exposed and the pericardium opened via a left lateral thoracotomy at the 5th intercostal space.

2.3. Intravenous vagal nerve stimulation (iVNS)

For iVNS, we inserted a catheter with electrodes (Ablaze Fantasista MMcurve 4-mm tip; Japan Lifeline Co., Ltd., Tokyo, Japan) from the right external jugular vein and advanced to the superior vena cava (SVC) (Fig. 2). We connected a stimulator (SEN3401; Nihon Kohden, Tokyo, Japan) to the catheter with electrodes and an electrical pad (Disposable Pad for human infants P-513; Nihon Kohden, Tokyo, Japan) attached to the dorsal thoracic region of the dog. We stimulated the vagal nerve continuously with rectangular pulses of 0.2-ms duration and 10 Hz. We up-titrated the intensity of iVNS to the maximum voltage that did not reduce AP (5.1 ± 2.1 V).

2.4. Ischemia–reperfusion model

To induce ischemia, we ligated the major branches and the first diagonal branch of the LAD with 3–0 silk suture. We also ligated the left circumflex coronary artery (LCX) branches that apparently perfused the LAD area to minimize the effect of collateral arteries. After inducing ischemia for 3 h, we reperfused the region by releasing all ligatures. One hour after reperfusion, we terminated the iVNS, removed the catheter electrode, and closed the chest by suturing muscles, subcutaneous tissues and skin. The animals were allowed to recover. Intravenous cefazolin (20 mg/kg, iv) was given twice prophylactically before and 1 h after reperfusion. Carprofen (4.4 mg/kg) was also administered subcutaneously 1 h after reperfusion for postoperative analgesia.

2.5. Atrial pacing

We sutured a bipolar electrode to the right atrial appendage for cardiac pacing (EDP 20; Nihon Kohden, Tokyo, Japan). The pacing condition was fixed at atrial pacing and sensing mode (AAI) with 0.9 ms pulse width and 3 V output. The pacing rate was determined as the level that totally abolished iVNS-induced bradycardia.

2.6. Assessment at 4 weeks after ischemia–reperfusion

2.6.1. Echocardiographic analysis

At 4 weeks after ischemia–reperfusion, we performed echocardiography in conscious dogs using a sector probe with 1–5 MHz extended operating frequency range (Phillips iU22/S5–1 Transducer; Philips NV, Amsterdam, Netherland). The conscious dog was

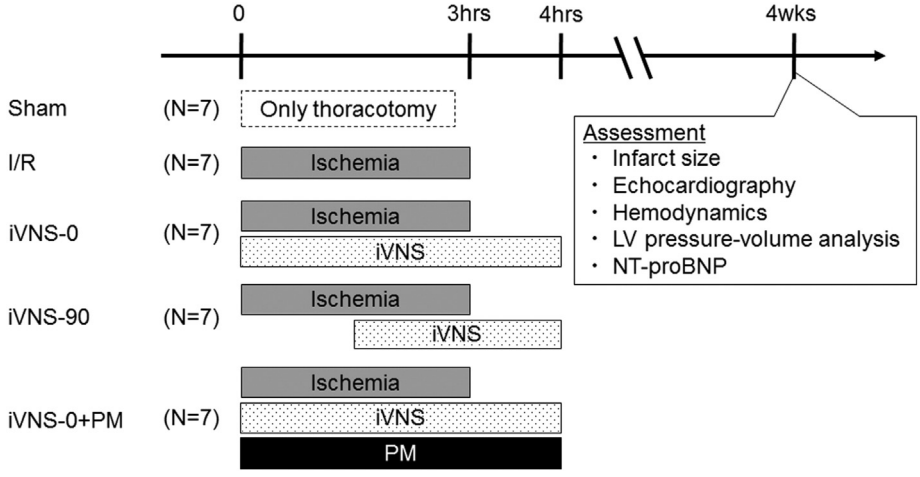

Fig. 1. Protocols of this study. Ischemia–reperfusion (I/R) model was produced by inducing ischemia for 3 h followed by reperfusion. Intravenous vagal nerve stimulation (iVNS) or atrial pacing (PM) was administered during ischemia until 1 h after reperfusion. Five groups were tested: Sham (thoracotomy only, no ischemia–reperfusion and no iVNS), I/R (3-h ischemia followed by reperfusion, no iVNS), iVNS-0 (iVNS from time 0 of ischemia to 1 h after reperfusion), iVNS-90 (iVNS from 90 min of ischemia to 1 h after reperfusion), and iVNS-0 + PM [iVNS and atrial pacing (PM) from time 0 of ischemia to 1 h after reperfusion]. At one month after ischemia–reperfusion, cardiac function, heart failure parameters and infarct size were evaluated in each dog.

Please cite this article as: T. Arimura, et al., Intravenous electrical vagal nerve stimulation prior to coronary reperfusion in a canine ischemia-reperfusion model markedly reduc..., Int J Cardiol (2016), http://dx.doi.org/10.1016/j.ijcard.2016.10.074

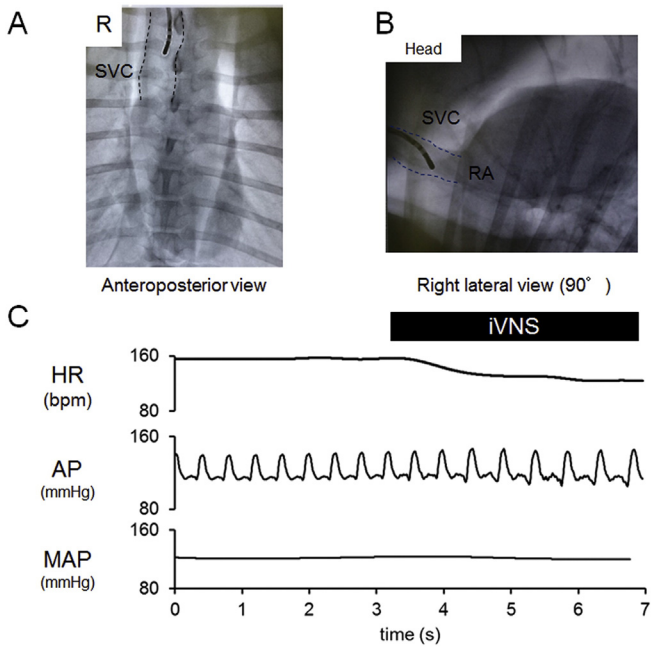

Fig. 2. Cine images of intravenous vagal nerve stimulation (iVNS), and hemodynamic changes in response to iVNS. (A) (B) Cine images show an effective vagal nerve stimulation site in supra vena cava (SVC) from anteroposterior view (A) and right lateral view (B). (C) Hemodynamic response to iVNS. HR decreases immediately after initiating iVNS, while arterial pressure does not change significantly. HR, heart rate; AP, arterial pressure; MAP, mean arterial pressure.

positioned in dorsal recombency on a sling frame (DFH1PU and DFC1PU, Bio Research Center Inc., Nagoya, Japan). Left ventricular end-systolic volume (LVESV), end-diastolic volume (LVEDV) and ejection fraction (LVEF) were measured on two-dimensional echocardiograms using the modified Simpson's rule [7].

2.6.2. Hemodynamic analysis

Four weeks after ischemia–reperfusion, all dogs were anesthetized by pentobarbital sodium (6.25 mg/kg) and vecuronium bromide (0.08 mg/kg), and artificially ventilated. 5-Fr sheaths were placed into the right femoral artery to measure AP and bilateral femoral veins to administer pentobarbital sodium (2.5 mg/kg/h) and saline (1–2 ml/kg/h as needed). A 6-Fr Millar catheter was inserted from the right common carotid artery into the LV to measure LV pressure, and fluid-filled catheters were placed directly into both atria to measure right (RAP) and left atrial pressures (LAP).

2.6.3. The pressure–volume relationship of LV

Two pairs of 2-mm ultrasonic microtransducer crystals (Sonometrics, London, Ont., Canada) were implanted in the endocardium of the LV to measure the anterior–posterior (short axis, D_{SA}) and base–apex (long axis, D_{LA}) dimensions with Sonomicrometer system and Sono Lab (Sonometrics, London, Ont., Canada) as described previously [8,9]. Briefly, the anterior crystal was implanted at the bifurcation between the major LAD and the second diagonal branch, and the posterior crystal at the opposite LV wall. The basal crystal was implanted just under the left atrial appendage, and the apical crystal at the LV apical cap. The left ventricular volume (LVV) was calculated by the modified ellipsoid formula: $LVV = (\pi/6) \times D_{SA} \times D_{SA} \times D_{LA}$ [8,9,10]. Left ventricular pressure-volume relationship was plotted. We obtained multiple pressure-volume loops by inferior vena cava occlusion [8,10] and determined the slope of the end-systolic pressure-volume relationship (end-systolic elastance; E_{es}) [11].

2.6.4. N-terminal-pro brain-type natriuretic peptide analysis

We collected serum samples before induction of anesthesia, and performed enzyme-linked immunosorbent assays (ELISA) for N-terminal pro-brain-type natriuretic peptide (NT-proBNP) (Cardiopet® proBNP; IDEXX Laboratory, Inc., Tokyo, Japan).

2.6.5. Infarct size assessment and LV weight

At the conclusion of the experiment, we arrested the heart by injecting potassium chloride. We excised the heart and dissected the left atrium, right atrium and right ventricle from the LV. We measured LV weight with an electric balance (UX220H, Shimadzu Corporation, Kyoto, Japan). Then, we cut the LV into 4 slices perpendicular to the long axis and incubated the slices in 1% triphenyltetrazolium chloride (TTC) at 37 °C for 15 min. We photographed each slice, and traced and measured both the infarct area and the whole LV area using an image processing program (Image J®; NIH, Bethesda, MD, USA) [12]. We presented infarct size (%) as the sum of the infarct areas in 4 slices normalized by the sum of whole LV areas in the same 4 slices.

2.7. Data analysis

We digitized time-series data at 200 Hz with a 16-bit analog-to-digital converter (Power Lab 16/35; ADInstruments, USA) and stored on a dedicated laboratory computer system. Data are presented as mean ± standard deviation (SD). All dates were evaluated by one-way factorial analysis of variance (ANOVA). Tukey–Kramer test was used for post-hoc comparisons. Data were analyzed using statistical software (Ekuseru-Toukei 2013; Social Survey Research Information Co. Ltd., Tokyo, Japan). Differences were considered significant when $p < 0.05$.

3. Results

3.1. Changes in heart rate and arterial pressure before, during and after iVNS

In instrumented dogs, iVNS immediately reduced heart rate (HR) without changing mean AP (MAP) (Fig. 2). Table 1 shows the changes in HR and MAP before, during and at the end of iVNS. As might be expected, iVNS produced an approximately 30% reduction in HR during the ischemia period in the iVNS-0 and iVNS-90 groups compared to I/R group ($p < 0.05$). Whereas, HR was significantly increased above baseline during ischemia in the I/R and iVNS-0 + PM groups. By 1 h after reperfusion, HR was not different among the five groups. MAP was not significantly different among the experimental groups at any time point.

Table 1
Changes in heart rate (HR) and mean arterial pressure (MAP) before, during and after intravenous vagal nerve stimulation (iVNS).

HR (bpm)		Baseline	I-180 min	R-60 min
Sham	(n = 7)	129 ± 9	126 ± 5	132 ± 10
I/R	(n = 7)	122 ± 23	160 ± 6 *	159 ± 15
iVNS-0	(n = 7)	132 ± 19	108 ± 17 †	155 ± 15
iVNS-90	(n = 7)	135 ± 11	114 ± 18 †	161 ± 20
iVNS-0 + PM	(n = 7)	135 ± 15	154 ± 4 *‡	158 ± 15
MAP (mmHg)		**Baseline**	**I-180 min**	**R-60 min**
Sham	(n = 7)	111 ± 10	111 ± 4	113 ± 14
I/R	(n = 7)	114 ± 13	114 ± 13	108 ± 11
iVNS-0	(n = 7)	98 ± 16	93 ± 18	103 ± 24
iVNS-90	(n = 7)	115 ± 10	112 ± 12	108 ± 10
iVNS-0 + PM	(n = 7)	106 ± 8	101 ± 14	105 ± 13

Baseline, before induction of ischemia; I-180 min, 180 min after onset of ischemia; R-60 min, 60 min after start of reperfusion. Sham (thoracotomy only, no ischemia–reperfusion and no iVNS); I/R (3-h ischemia followed by reperfusion, no iVNS), iVNS-0 (iVNS from time 0 of ischemia to 1 h after reperfusion), iVNS-90 (iVNS from 90 min of ischemia to 1 h after reperfusion) and iVNS-0 + PM [iVNS and atrial pacing (PM) from time 0 of ischemia to 1 h after reperfusion]. Data are expressed as mean ± SD.
* $p < 0.05$ versus Sham, † $p < 0.05$ versus I/R, ‡ $p < 0.05$ versus iVNS-0.

3.2. Echocardiogram at 4 weeks after ischemia–reperfusion

Four weeks after ischemia–reperfusion, we performed echocardiography in the conscious state (Fig. 3). Compared with Sham, I/R increased LVEDV but not significantly. Because I/R significantly increased LVESV compared to Sham, I/R decreased LVEF. LVEF was significantly higher in both iVNS-0 and iVNS-90 compared with I/R (Sham: 63 ± 3%, iVNS-0: 64 ± 6%, iVNS-90: 60 ± 5%, vs. I/R: 48 ± 6%; p = 0.0007), indicating improvement of LV systolic function by iVNS. On the other hand, iVNS-0 + PM did not change LVEF compared with I/R (iVNS-0 + PM: 53 ± 5% vs. I/R: 48 ± 6%; $p = 0.45$). Moreover, iVNS-0 + PM also did not change LVEDV or LVESV, compared with I/R.

3.3. Hemodynamics and NT-proBNP at 4 weeks after ischemia–reperfusion

Table 2 shows the hemodynamics at 4 weeks after ischemia–reperfusion. All data were acquired under anesthesia. HR and MAP were not significantly different among groups. Intravenous VNS reduced LV end-diastolic pressure (LVEDP) and LAP significantly compared with I/R, indicating improvement of pulmonary congestion. The maximum

Please cite this article as: T. Arimura, et al., Intravenous electrical vagal nerve stimulation prior to coronary reperfusion in a canine ischemia-reperfusion model markedly reduc..., Int J Cardiol (2016), http://dx.doi.org/10.1016/j.ijcard.2016.10.074

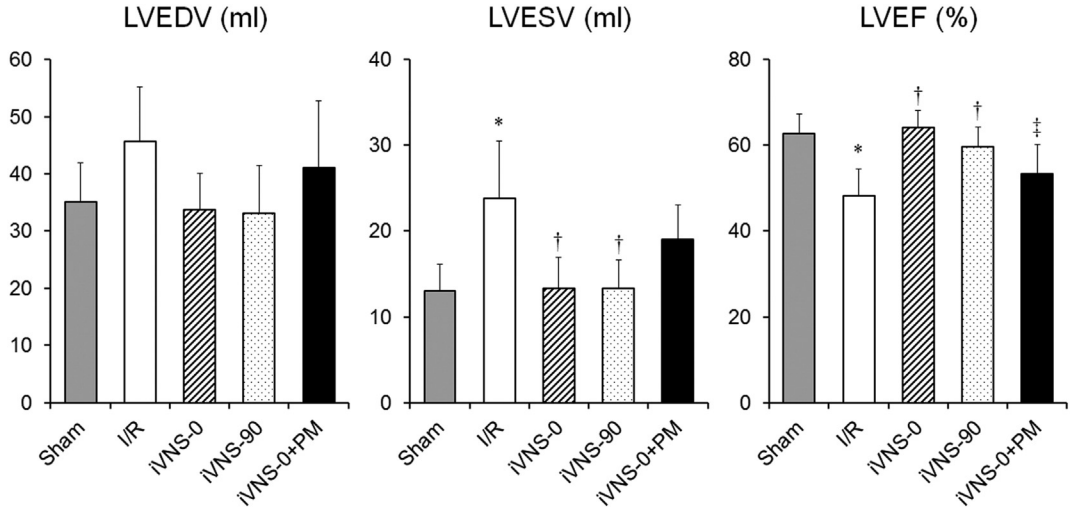

Fig. 3. Echocardiographic measurements at 4 weeks after ischemia–reperfusion. Left ventricular end diastolic volume (LVEDV), left ventricular end systolic volume (LVESV) and left ventricular ejection fraction (LVEF) were measured on two-dimensional echocardiograms using modified Simpson's method. Experimental groups are as described in Fig. 1. In both iVNS-0 and iVNS-90, LVEF was higher than that in I/R. LVEF in iVNS-0 + PM was lower than that in iVNS-0. Data are expressed as mean ± SD. *$p < 0.05$ versus Sham, †$p < 0.05$ versus I/R, ‡$p < 0.05$ versus iVNS-0 group.

time derivative of LV pressure (Max +dP/dt) tended to decrease in iVNS-0 and iVNS-90 compared with I/R, but the differences were not significant. The negative peak derivative of LV pressure (Min −dP/dt) was higher in I/R than in Sham, and lower in iVNS-0 than in I/R.

Fig. 4 presents the pressure-volume analysis. Compared with I/R, iVNS-0 and -90 significantly increased LV E_{es} which is a load insensitive index of LV contractility, while iVNS-0 + PM did not (Sham: 13.5 ± 3.7, I/R: 4.0 ± 0.7, iVNS-0: 15.4 ± 5.9, iVNS-90: 10.7 ± 3.3 and iVNS-0 + PM: 7.3 ± 1.6 mmHg/ml; $p < 0.05$ for iVNS-0 and iVNS-90 vs. I/R).

Serum levels of NT-proBNP were measured as an index of severity of heart failure (Fig. 5). The levels were significantly higher in I/R than in Sham (I/R: 3712 ± 1142 vs. Sham: 945 ± 618 pmol/L, $p < 0.05$). Intravenous VNS groups (iVNS-0, −90 and iVNS-0 + PM) significantly decreased NT-proBNP compared with I/R (iVNS-0: 1330 ± 471, iVNS-90: 1377 ± 444, iVNS-0 + PM: 2000 ± 1076 pmol/L vs. I/R: 3712 ± 1142 pmol/L; $p < 0.05$).

3.4. Infarct size and LV weight analysis

As shown in Fig. 6, iVNS-0 markedly reduced infarct size by more than 80% compared with I/R, and iVNS-90 also significantly reduced infarct size even though the treatment started 90 min after ischemia (infarct size: iVNS-0: 2.4 ± 2.1%, iVNS-90: 4.5 ± 4.5% vs. I/R: 13.3 ± 2.5%; $p < 0.05$). In contrast, iVNS-0 + PM significantly attenuated the anti-infarct effect of iVNS (iVNS-0 + PM: 9.8 ± 4.4% vs. iVNS-0: 2.4 ± 2.1%; $p < 0.05$), suggesting that the bradycardic effect of VNS may play an important role in protection against ischemic injury. LV weights were not significantly different among groups (Sham: 87.6 ± 7.3g, I/R: 77.3 ± 10.9g, iVNS-0: 85.4 ± 10.0g iVNS-90: 78.3 ± 4.5g, and iVNS-0 + PM: 72.0 ± 9.2g; $p = 0.05$).

4. Discussion

4.1. Vagal nerve stimulation using intravenous catheter with electrodes

Although there is preclinical evidence that VNS has beneficial effects on myocardial infarction and subsequent cardiac remodeling, effective implementation of this method as a treatment modality in acute AMI setting has been hampered by technical difficulties. Thus, the goal of this study is to develop a transvenous delivery system to stimulate vagal nerve electrically as a clinically feasible approach.

In this report, we investigated a less invasive method to stimulate the vagal nerve running behind the SVC using an intravenous electrical catheter. This concept of implementing VNS using a transvenous approach has been proposed by several investigators. Schauerte et al. [13] stimulated the vagal nerve with a multipolar electrode catheter in the SVC or coronary sinus, and showed the negative chronotropic and dromotropic effects of VNS for the diagnosis and treatment of supraventricular tachycardia. In addition, Kox et al. [14] examined the anti-inflammatory effect of VNS in humans using an eight-electrode catheter placed in the internal jugular vein at the C5-C7 spinal level. Although the study failed to demonstrate the anti-inflammatory effect of VNS,

Table 2
Hemodynamics at 4 weeks after ischemia–reperfusion.

	Sham (n = 7)	I/R (n = 7)	iVNS-0 (n = 7)	iVNS-90 (n = 7)	iVNS-0 + PM (n = 7)
HR, bpm	149 ± 14	133 ± 14	141 ± 31	145 ± 12	146 ± 13
MAP, mmHg	126 ± 27	108 ± 13	108 ± 7	109 ± 40	114 ± 22
LVEDP, mmHg	4.6 ± 2.4	16.8 ± 6.4*	4.2 ± 1.6†	5 ± 2.8†	7.6 ± 1.5†
Max +dP/dt, mmHg/s	3108 ± 1121	1778 ± 195*	2968 ± 1234	2710 ± 571	2045 ± 482
Min −dP/dt, mmHg/s	−4460 ± 946	−2382 ± 349*	−3966 ± 1630†	−3201 ± 571	−2505 ± 398*
LAP, mmHg	5.7 ± 1.4	15.3 ± 4.9*	4.8 ± 1.1†	6.5 ± 1.8†	7.1 ± 1†
RAP, mmHg	2.9 ± 1.3	5.5 ± 1.2	1.8 ± 1.1†	3.2 ± 2.3	3.7 ± 2.5

HR, heart rate; MAP, mean arterial pressure; LVEDP, left ventricular end-diastolic pressure; Max +dP/dt, the maximum time derivative of left ventricular pressure; Min −dP/dt, the negative peak derivative of left ventricular pressure; LAP, left atrial pressure; RAP, right atrial pressure. Experimental groups are as described in Table 1. Data are expressed as mean ± SD. *$p < 0.05$ versus Sham, †$p < 0.05$ versus I/R.

Please cite this article as: T. Arimura, et al., Intravenous electrical vagal nerve stimulation prior to coronary reperfusion in a canine ischemia-reperfusion model markedly reduc..., Int J Cardiol (2016), http://dx.doi.org/10.1016/j.ijcard.2016.10.074

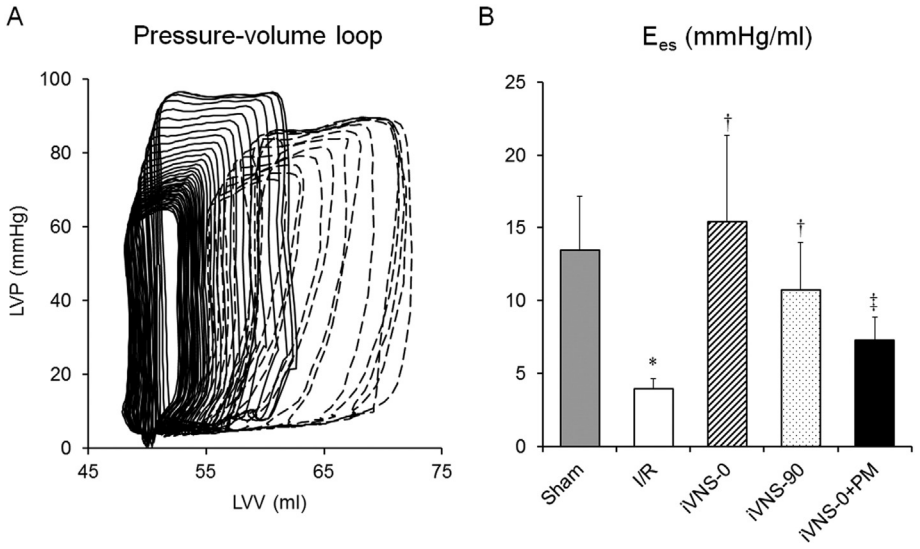

Fig. 4. Pressure-volume analysis at 4 weeks after ischemia–reperfusion. Experimental groups are as described in Fig. 1. (A) Representative pressure-volume relationship of left ventricle (LV) in I/R (dotted line) and iVNS-0 (solid line). In iVNS-0, the pressure-volume loops shifted leftward compared with I/R. (B) The end-systolic pressure–volume relationship (ESPVR) and the volume axis intercept (V_0) were determined by the inferior vena cava occlusion method. The end systolic elastance (E_{es}) was defined as the slope of ESPVR. Intravenous vagal nerve stimulation (iVNS-0 and iVNS-90) significantly increased E_{es} compared with I/R. E_{es} in iVNS-0 + PM was lower than that in iVNS-0. Data are expressed as mean ± SD. *$p < 0.05$ versus Sham, †$p < 0.05$ versus I/R, ‡$p < 0.05$ versus iVNS-0 group.

the results showed the feasibility and safety of transvenous VNS and its substantial effect of reducing HR. As shown in Fig. 2C and Table 1, iVNS rapidly decreased HR as previously reported [15] with a sustained reduction in HR (−30%) between 2.5 (iVNS-90) and 4 h (iVNS-0).

In addition, alternative methods of VNS have been reported. Implantable VNS devices have been developed for patients with epilepsy [16] and heart failure [17–22]. Although these devices provide vagal nerve stimulation, they are impractical, if not unfeasible, for AMI because they require prior implantation of electrodes and devices. Pharmacological parasympathetic stimulation has also been reported. Li et al. [23] reported that donepezil, a choline esterase inhibitor, activated the parasympathetic system and improved long-term survival in rats with chronic heart failure. However, the impact of pharmacological stimulation on AMI in reducing infarct size remains to be studied. Taken together, we believe that iVNS is one of the most practical and safe methods to stimulate vagal nerve in AMI settings.

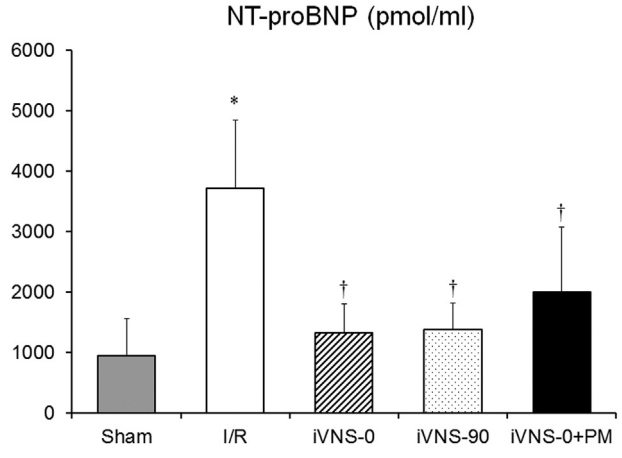

Fig. 5. N-terminal pro-brain-type natriuretic peptide (NT-proBNP) levels at 4 weeks after ischemia–reperfusion. Experimental groups are as described in Fig. 1. NT-proBNP level was significantly higher in I/R than in Sham, while the levels were significantly lower in iVNS-0, iVNS-90 and iVNS-0 + PM than that in I/R. Data are expressed as mean ± SD. *$p < 0.05$ versus Sham group, †$p < 0.05$ versus I/R group.

4.2. Effect of short-term iVNS during acute phase of AMI on infarct size and prevention of heart failure

This study was designed to evaluate the effectiveness and feasibility of short-term iVNS administered during acute AMI in mitigating subsequent development of heart failure. As shown in Figs. 3 to 6, iVNS administered from the onset of ischemia and continued until one hour after reperfusion (iVNS-0) markedly reduced infarct size by more than 80% compared with untreated ischemia–reperfusion models, and consequently preserved normal LV function, thereby preventing the development of heart failure 4 weeks later. Several preclinical investigations have already reported that direct cervical VNS markedly reduced infarct size in rat [5] and swine [24,25] models of ischemia–reperfusion. The efficacy of iVNS in our findings are consistent with the effects of VNS in previous reports.

We also examined the effect of iVNS administered from 90 min of ischemia and continued until one hour after reperfusion (iVNS-90) to simulate clinical conditions. Although the efficacy tended to be inferior to that of iVNS-0, iVNS-90 significantly reduced infarct size by 66% compared with I/R and prevented the aggravation of cardiac function. The optimal timing of VNS is an important issue to address for clinical application. Shinlapawittayatorn et al. [25] reported that VNS provides significant cardio-protective effects even when initiated in the later phase of ischemia, but not after reperfusion in swine I/R model. Recently, Uitterdijk et al. [24] reported that VNS just prior to reperfusion (5 min) limited reperfusion injury and infarct size in a swine model of I/R. These findings suggest that earlier administration of VNS before reperfusion therapy may maximize its efficacy.

4.3. Contribution of HR reduction to the efficacy of iVNS in myocardial infarction

The mechanisms of the beneficial effects of VNS have not been fully elucidated. Reduction of HR by VNS decreases myocardial oxygen consumption (MVO_2) [26] and presumably contributes favorably to limit infarct size. Meanwhile, many animal studies have suggested other favorable effects of VNS beyond HR reduction on ischemic and reperfusion injuries, such as anti-inflammatory response through the efferent

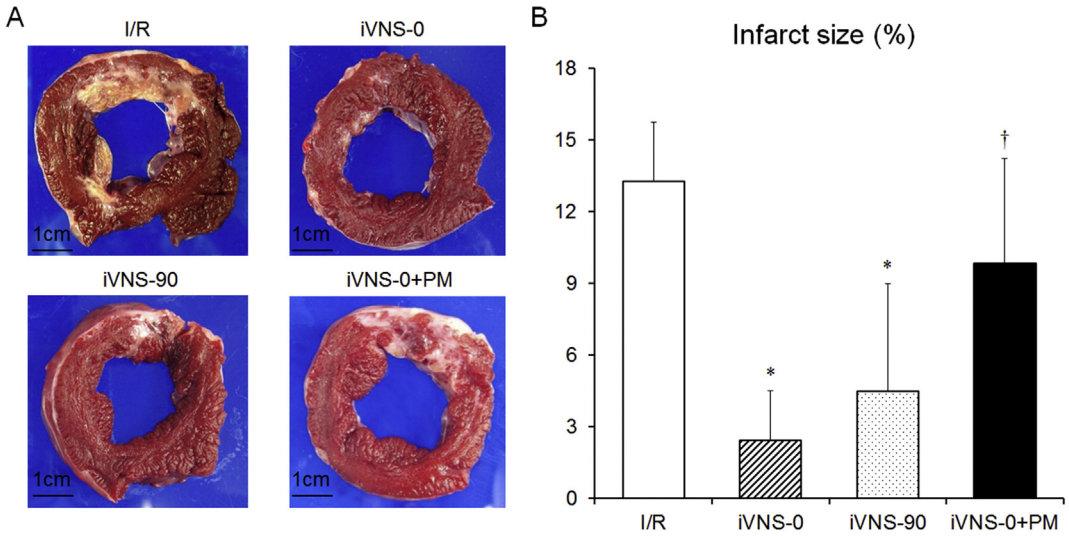

Fig. 6. Infarct size assessment. Experimental groups are as described in Fig. 1. (A) Representative mid-level slices of the left ventricle (LV) in 4 groups, which were stained with triphenyltetrazolium chloride. (B) Comparison of infarct sizes in 4 groups. Whole left ventricular (LV) area and the infarct area were traced and measured using an image analyzer. Each infarct area was normalized by whole LV areas and expressed as percentage. Intravenous VNS significantly decreased infarct size compared with I/R, while iVNS-0 + PM did not show any positive impact on infarct size. Data are expressed as mean ± SD. * $p < 0.05$ versus I/R group, † $p < 0.05$ versus iVNS-0 group.

arm [6,27] and sympatho-inhibition via the afferent arm [28]. Among these HR reduction is the most prominent physiological response of VNS. Hence investigating HR reduction as the principal beneficial mechanism of VNS on AMI is important, particularly in optimizing the stimulation conditions of VNS. However, the contribution of HR reduction to the efficacy of VNS in large animals remains unknown. To address this issue, we verified the HR-dependent effect of iVNS using atrial pacing that cancels the bradycardic effect (iVNS-0 + PM). HR in iVNS-0 + PM was the same as that in I/R (Table 1). As shown in Figs. 3 to 6 and Table 2, iVNS-0 + PM attenuated the significant reduction in infarct size and other beneficial effects of iVNS, including the improved cardiac function and prevention of heart failure. We speculate that the decrease in MVO_2 by iVNS-induced HR reduction may greatly affect infarct size. The imbalance of oxygen supply and demand in the myocardium is fundamental to the pathophysiology of AMI [29], which would explain why a decrease in MVO_2 reduces infarct size [30,31]. Consistent with this being the underlying mechanism, Suga et al. [26] reported a linear relationship between reduction in HR and decrease in MVO_2. In the present study, we chose the maximal intensity of iVNS that did not affect AP. At this intensity, iVNS reduced HR by approximately 30% compared with no treatment. We also confirmed that iVNS at the same setting as in this experiment reduced MVO_2 by more than 40% in normal dogs (see Supplemental materials). These results indicate that HR reduction plays an important role in the beneficial mechanism of iVNS. How other mechanisms contribute to the reduction of infarct size remains to be studied.

Use of a β-blocker is a therapeutic option that is capable of modulating MVO_2 by reducing HR and cardiac contractility [32]. In the METOCARD-CNIC trial, early intravenous metoprolol before coronary reperfusion in AMI patients with stable hemodynamics reduced infarct size as measured by serum creatinine kinase and magnetic resonance imaging [33]. Subsequently, metoprolol treatment resulted in higher long-term LVEF and less severe LV systolic dysfunction at 6 months after AMI, and fewer heart failure admissions during a median follow-up of 2 years [34]. On the other hand, Chen et al. [35] reported that intravenous metoprolol significantly increased the risk of cardiogenic shock especially in the acute phase of AMI. Since changing the intensity of the stimulus makes VNS capable of producing variable and reversible modulation of HR depending on an individual's hemodynamic state, it may be a superior method to drug therapy in terms of safe hemodynamic management in the acute setting of AMI.

Although further device development and validation in animal models are required, the present results are important basic findings for further evaluation of the feasibility of applying iVNS to clinical use.

4.4. Limitations

This study has several limitations. First, we used a commercially available electrical catheter intended for catheter ablation. Although we were able to initiate iVNS within 15 min after venous puncture and stimulate the vagal nerve continuously for 2.5 to 4 h in anesthetized dogs, there is a need to develop a special device that allows easy detection of effective vagal nerve stimulation site, stable placement of electrode in the SVC, and more prompt initiation of iVNS without delaying the reperfusion therapy for clinical application.

Second, several cardio-protective effects of VNS beyond HR reduction have been reported, such as cholinergic anti-inflammatory effect [27], inhibition of norepinephrine spill-over [36,37], inhibition of free radical production [38,39], and inhibition of sympathetic nerve activity via vagal afferent pathway [28]. Since these factors may contribute at least in part to infarct size reduction and cardiac function improvement, the particular role of each cardio-protective effect should be studied.

Third, the infarct size in this study was smaller compared to previous reports. Since our focus was to assess longer term cardiac function and heart failure parameters, it was not appropriate to create extensive myocardial ischemia in dogs because a large myocardial infarction frequently evokes lethal ventricular arrhythmia and pumping failure leading to sudden cardiac death. With our protocol, all the dogs, including those in I/R group, survived at the end of four weeks after AMI induction. The I/R group showed significantly reduced LV function and increased NT-proBNP at 4 weeks compared with Sham, and iVNS improved these parameters. Therefore, the general trend that iVNS reduces infarct size and prevents subsequent heart failure may remain valid in clinical situations.

Fourth, in clinical settings, iVNS cannot be initiated at the onset of ischemic insult. Furthermore, although the iVNS procedure may take a short time (10 min in our study), it inevitably delays reperfusion. Whether the negative impacts of delayed iVNS delivery and delayed reperfusion can be overcome by iVNS remains to be studied. To address these issues, further investigations of iVNS with clinically acceptable protocols and insertion method are needed.

Please cite this article as: T. Arimura, et al., Intravenous electrical vagal nerve stimulation prior to coronary reperfusion in a canine ischemia-reperfusion model markedly reduc..., Int J Cardiol (2016), http://dx.doi.org/10.1016/j.ijcard.2016.10.074

5. Conclusions

In a canine model of ischemia–reperfusion, short-term iVNS delivered prior to coronary reperfusion produced a bradycardic effect during the period of ischemia which markedly reduced infarct size and preserved LV function one month after ischemia–reperfusion. Transvascularly delivered VNS may be a new non-pharmacological therapeutic strategy and contribute to improve the long-term survival in patients with AMI.

Funding sources

This work was supported by Grandin-Aid from Translational Research Network Program [grant number B07], Grant-in-Aid for Scientific Research (S) from the Japan Society for the Promotion of Science [grant number 23220013], Research and development of supportive device technology for medicine using ICT, Grant-in-Aid for Young Scientists (B) from the Japan Society for the Promotion of Science [grant number 15K19383], and Actelion Academia Prize 2015.

Conflict of interest

All authors declare no conflict of interest.

Acknowledgements

The authors thank Mr. Takuya Akashi, Mr. Yoshinori Murayama and staff of the Department of Cardiovascular Medicine, Kyushu University for technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.ijcard.2016.10.074.

References

[1] H.D. White, D.P. Chew, Acute myocardial infarction, Lancet 372 (2008) 570–584.
[2] R.S. Velagaleti, M.J. Pencina, J.M. Murabito, et al., Long-term trends in the incidence of heart failure after myocardial infarction, Circulation 118 (2008) 2057–2062.
[3] Mendis S, Puska P and Norrving B. Global Atlas on Cardiovascular Disease Prevention and Control. Published by the World Health Organization in collaboration with the World Heart Federation and the World Stroke Organization.
[4] E. Larose, J. Rodés-Cabau, P. Pibarot, et al., Predicting late myocardial recovery and outcomes in the early hours of ST-segment elevation myocardial infarction, J. Am. Coll. Cardiol. 55 (2010) 2459–2469.
[5] L. Calvillo, E. Vanoli, E. Andreoli, et al., Vagal stimulation, through its nicotinic action, limits infarct size and the inflammatory response to myocardial ischemia and reperfusion, J. Cardiovasc. Pharmacol. 58 (2011) 500–507.
[6] K. Uemura, C. Zheng, M. Li, T. Kawada, M. Sugimachi, Early short-term vagal nerve stimulation attenuates cardiac remodeling after reperfused myocardial infarction, J. Card. Fail. 16 (2010) 689–699.
[7] R.M. Lang, M. Bierig, R.B. Devereux, et al., Recommendations for chamber quantification, Eur. J. Echocardiogr. 7 (2) (2006) 79–108.
[8] K. Saku, T. Kakino, T. Arimura, et al., Total mechanical unloading minimizes metabolic demand of left ventricle and dramatically reduces infarct size in myocardial infarction, PLoS One 11 (4) (2016), e0152911.
[9] J.S. Rankin, P.A. McHale, C.E. Arentzen, et al., The three-dimensional dynamic geometry of the left ventricle in the conscious dog, Circ. Res. 39 (3) (1976) 304–313.
[10] T. Sakamoto, T. Kakino, K. Sakamoto, et al., Changes in vascular properties, not ventricular properties, predominantly contribute to baroreflex regulation of arterial pressure, Am. J. Physiol. Heart Circ. Physiol. 308 (2015) H49–H58.
[11] A. Kono, W.L. Maughan, K. Sunagawa, K. Hamilton, K. Sagawa, M.L. Weisfeldt, The use of left ventricular end-ejection pressure and peak pressure in the estimation of the end-systolic pressure-volume relationship, Circulation 70 (1984) 1057–1065.
[12] C.A. Schneider, W.S. Rasband, K.W. Eliceiri, NIH image to ImageJ: 25 years of image analysis, Nat. Methods 9 (2012) 671–675.
[13] P. Schauerte, K. Mischke, J. Plisiene, et al., Catheter stimulation of cardiac parasympathetic nerves in humans. A novel approach to the cardiac autonomic nervous system, Circulation 104 (2001) 2430–2435.
[14] M. Kox, L.T. van Eijk, T. Verhaak, et al., Transvenous vagus nerve stimulation does not modulate the innate immune response during experimental human endotoxemia: a randomized controlled study, Arthritis Res. Ther. 17 (2015) 150.
[15] G.A. Ng, K.E. Brack, J.H. Coote, Effects of direct sympathetic and vagus nerve stimulation on the physiology of the whole heart – a novel model of isolated Langendorff perfused rabbit heart with intact dual autonomic innervation, Exp. Physiol. 86 (2001) 319–329.
[16] C.D. Binnie, Vagus nerve stimulation for epilepsy: a review, Seizure 9 (3) (2000) 161–169.
[17] F. Zannad, G.M. De Ferrari, A.E. Tuinenburg, et al., Chronic vagal stimulation for the treatment of low ejection fraction heart failure: results of the NEural Cardiac TherApy foR Heart Failure (NECTAR-HF) randomized controlled trial, Eur. Heart J. 36 (2015) 425–433.
[18] P.J. Schwartz, G.M. De Ferrari, A. Sanzo, et al., Long term vagal stimulation in patients with advanced heart failure first experience in man, Eur. J. Heart Fail. 10 (2008) 884–891.
[19] G.M. De Ferrari, H.J. Crijns, M. Borggrefe, et al., Chronic vagus nerve stimulation: a new and promising therapeutic approach for chronic heart failure, Eur. Heart J. 32 (2011) 847–855.
[20] M.R. Gold, D.J. van Veldhuisen, D.L. Mann, Vagal nerve stimulation for heart failure: new pieces to the puzzle? Eur. J. Heart Fail. 17 (2) (2015) 125–127.
[21] R.K. Premchand, K. Sharma, S. Mittal, et al., Autonomic regulation therapy via left or right cervical vagus nerve stimulation in patients with chronic heart failure: results of the ANTHEM-HF trial, J. Card. Fail. 20 (11) (2014) 808–816.
[22] P.J. Hauptman, P.J. Schwartz, M.R. Gold, et al., Rationale and study design of the increase of vagal tone in heart failure study: INOVATE-HF, Am. Heart J. 163 (6) (2012) (954-962.e1).
[23] M. Li, C. Zheng, T. Kawada, et al., Adding the acetylcholinesterase inhibitor, donepezil, to losartan treatment markedly improves long-term survival in rats with chronic heart failure, Eur. J. Heart Fail. 16 (10) (2014) 1056–1065.
[24] A. Uitterdijk, T. Yetgin, H.M. te Lintel, et al., Vagal nerve stimulation started just prior to reperfusion limits infarct size and no-reflow, Basic Res. Cardiol. 110 (2015) 508.
[25] K. Shinlapawittayatorn, K. Chinda, S. Palee, et al., Vagus nerve stimulation initiated late during ischemia, but not reperfusion, exerts cardioprotection via amelioration of cardiac mitochondrial dysfunction, Heart Rhythm. 11 (2014) 2278–2287.
[26] H. Suga, R. Hisano, S. Hirata, T. Hayashi, O. Yamada, I. Ninomiya, Heart rate-independent energetics and systolic pressure-volume area in dog heart, Am. J. Phys. 244 (1983) H206–H214.
[27] L.V. Borovikova, S. Ivanova, M. Zhang, et al., Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin, Nature 405 (2000) 458–462.
[28] K. Saku, T. Kishi, K. Sakamoto, et al., Afferent vagal nerve stimulation resets baroreflex neural arc and inhibits sympathetic nerve activity, Physiol. Rep. 2 (9) (2014), e12136.
[29] V. Fuster, A. Lewis, Conner Memorial Lecture. Mechanisms leading to myocardial infarction: insights from studies of vascular biology, Circulation 90 (1994) 2126–2146.
[30] W.E. Shell, B.E. Sobel, Deleterious effects of increased heart rate on infarct size in the conscious dog, Am. J. Cardiol. 31 (1973) 474–479.
[31] K.D. Müller, S. Sass, M.G. Gottwik, W. Schaper, Effect of myocardial oxygen consumption on infarct size in experimental coronary artery occlusion, Basic Res. Cardiol. 77 (1982) 170–181.
[32] R.M. Knabb, S.W. Ely, A.N. Bacchus, R. Rubio, R.M. Berne, Consistent parallel relationships among myocardial oxygen consumption, coronary blood flow, and pericardial infusate adenosine concentration with various interventions and beta-blockade in the dog, Circ. Res. 53 (1983) 33–41.
[33] B. Ibanez, C. Macaya, V. Sánchez-Brunete, et al., Effect of early metoprolol on infarct size in ST-segment-elevation myocardial infarction patients undergoing primary percutaneous coronary intervention: the Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction (METOCARD-CNIC) trial, Circulation 128 (2013) 1495–1503.
[34] G. Pizarro, L. Fernández-Friera, V. Fuster, et al., Long-term benefit of early pre-reperfusion metoprolol administration in patients with acute myocardial infarction: results from the METOCARD-CNIC trial (Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction), J. Am. Coll. Cardiol. 63 (2014) 2356–2362.
[35] Z.M. Chen, H.C. Pan, Y.P. Chen, et al., Early intravenous then oral metoprolol in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial, Lancet 366 (2005) 1622–1632.
[36] M.N. Levy, B. Blattberg, Effect of vagal stimulation on the overflow of norepinephrine into the coronary sinus during cardiac sympathetic nerve stimulation in the dog, Circ. Res. 38 (1976) 81–84.
[37] Y. Zhang, Z.B. Popovic, S. Bibevski, et al., Chronic vagus nerve stimulation improves autonomic control and attenuates systemic inflammation and heart failure progression in a canine high-rate pacing model, Circ. Heart Fail. 2 (2009) 692–699.
[38] S.S. Kong, J.J. Liu, X.J. Yu, Y. Lu, W.J. Zang, Protection against ischemia-induced oxidative stress conferred by vagal stimulation in the rat heart: involvement of the AMPK-PKC pathway, Int. J. Mol. Sci. 5 (13(11)) (2012) 14311–14325.
[39] T. Tsutsumi, T. Ide, M. Yamato, et al., Modulation of the myocardial redox state by vagal nerve stimulation after experimental myocardial infarction, Cardiovasc. Res. 1 (77(4)) (2008) 713–721.

Please cite this article as: T. Arimura, et al., Intravenous electrical vagal nerve stimulation prior to coronary reperfusion in a canine ischemia-reperfusion model markedly reduc..., Int J Cardiol (2016), http://dx.doi.org/10.1016/j.ijcard.2016.10.074