

# Myocardial Protection by Vagal Stimulation in Ischemia-Reperfusion Injury in Mice

## *Protección miocárdica por estimulación vagal en la injuria por isquemia y reperusión en ratones*

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### ABSTRACT

**Background:** The beneficial effects of prolonged vagal stimulation (VS) applied during myocardial infarction have been previously demonstrated. However, the effects and mechanisms of protection are unknown when VS is applied selectively and briefly before ischemia or at the onset of reperfusion.

**Objective:** The aim of this study was to analyze whether VS applied during reperfusion is capable of reducing infarct size similarly to preischemic VS, and whether in both cases muscarinic or nicotinic receptors mediate the protection.

**Methods:** FVB mice were subjected to 30 minutes of regional myocardial ischemia and 2-hour reperfusion without VS (I/R); with 10 minutes preischemic VS (pVS), with pVS and muscarinic blockade by atropine and with pVS and  $\alpha$ -7 nicotinic blockade by methyllycaconitine. The effects of VS at the onset of reperfusion (rVS) were also studied with atropine and with methyllycaconitine. A left ventricular catheter was used to measure ventricular function. Area at risk was measured using Evans blue and infarct size was assessed with 2,3,5-triphenyltetrazolium.

**Results:** Vagal stimulation during reperfusion reduced infarct size similarly to pVS, albeit with different mechanisms of protection. Preischemic VS protected the heart through cholinergic activation of muscarinic receptors, while rVS protection was effected through an  $\alpha$ -7 cholinergic nicotinic pathway.

**Conclusion:** The present study demonstrated for the first time in an ischemia-reperfusion mice model that a brief 10-minute period of VS is able to similarly reduce infarct size when it is applied prior to ischemia or at the onset of reperfusion, mimicking ischemic preconditioning and postconditioning, respectively.

**Key words:** Myocardial Reperfusion - Infarction- Ischemia - Vagus Nerve - Receptors, Muscarinic - Nicotinic Acetylcholine Receptors

### RESUMEN

**Introducción:** Previamente se demostraron beneficios de la estimulación vagal (EV) prolongada en el infarto de miocardio. No obstante, se desconocen los efectos y los mecanismos de protección cuando se aplica en forma selectiva y brevemente antes de la isquemia o al inicio de la reperusión.

**Objetivo:** Estudiar si la EV en la reperusión reduce el tamaño del infarto de manera similar a la EV preisquémica y si en ambas la protección está mediada por receptores muscarínicos o nicotínicos.

**Material y métodos:** En ratones FVB se realizó una isquemia miocárdica regional de 30 minutos y 2 horas de reperusión sin EV (I/R), con EV preisquémica por 10 minutos (EVp), con EV preisquémica y bloqueo muscarínico con atropina y con EV preisquémica y bloqueo nicotínico  $\alpha$ -7 con metillicaconitina. También se estudiaron los efectos de la EV al inicio de la reperusión (EVr), con atropina y con metillicaconitina. Se cateterizó el ventrículo izquierdo para medir la función ventricular. Se midió el área de riesgo con azul de Evans y el área de infarto con cloruro de 2,3,5-trifeniltetrazolio.

**Resultados:** La EVr redujo el tamaño del infarto de forma similar a la EVp, aunque los mecanismos de protección fueron diferentes. La EVp protegió a través de la activación colinérgica de los receptores muscarínicos. La EVr, en cambio, protegió por una vía colinérgica nicotínica  $\alpha$ -7.

**Conclusión:** El presente estudio demuestra por primera vez en un modelo de isquemia y reperusión miocárdica en ratones que una EV breve de 10 minutos es capaz de reducir de manera similar el tamaño del infarto, tanto cuando se aplica previo a la isquemia como en el inicio de la reperusión, mimetizando de esta manera al preconditionamiento y al poscondicionamiento isquémicos, respectivamente.

**Palabras clave:** Reperusión miocárdica - Infarto - Isquemia - Nervio vago - Receptores muscarínicos - Receptores nicotínicos

REV ARGENT CARDIOL 2017;85:6-12. <http://dx.doi.org/10.7775/rac.v85.i1.10218>

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Received: 01/10/2017 – Accepted: 01/30/2017

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## Abbreviations

|                            |                                                           |                |                                                          |
|----------------------------|-----------------------------------------------------------|----------------|----------------------------------------------------------|
| <b>ACh</b>                 | Acetylcholine                                             | <b>pVS+Atr</b> | Preischemic vagal stimulation with atropine              |
| <b>dP/dt<sub>max</sub></b> | Maximum first derivative of pressure with respect to time | <b>pVS+MLA</b> | Preischemic vagal stimulation with methyllycaconitine    |
| <b>HR</b>                  | Heart rate                                                | <b>rVS</b>     | Vagal stimulation at reperfusion                         |
| <b>I/R</b>                 | Ischemia and reperfusion                                  | <b>rVS+Atr</b> | Vagal stimulation at reperfusion with atropine           |
| <b>LVEDP</b>               | Left ventricular end-diastolic pressure                   | <b>rVS+MLA</b> | Vagal stimulation at reperfusion with methyllycaconitine |
| <b>LVSP</b>                | Left ventricular systolic pressure                        | <b>TTC</b>     | 2,3,5-triphenyltetrazolium chloride                      |
| <b>MLA</b>                 | Methyllycaconitine                                        | <b>VS</b>      | Vagal stimulation                                        |
| <b>pVS</b>                 | Preischemic vagal stimulation                             |                |                                                          |

## INTRODUCTION

Ischemic heart disease is one of the main causes of morbidity and mortality in industrialized countries. (1, 2) Infarct size and sympathetic hyperactivity resulting from the autonomic imbalance involved in cardiovascular diseases are key determinants in the outcome of patients with ischemic cardiomyopathy. (3, 4) Even though the use of  $\beta$ -adrenergic receptor blockers to counterbalance sympathetic hyperactivity has been found to improve the outcome of these patients, mortality is still high. (5) Therefore, the search for protection strategies through increased parasympathetic tone has intensified in recent years. In this sense, vagal electro stimulation (VS) has provided promising results at the experimental level, both in acute myocardial infarction (6, 7) and post ischemic heart failure (8) models. This intervention is based on the release of acetylcholine (ACh) by postganglionic parasympathetic fibers of inherent cardiac nervous plexuses. (9) It is known that ACh can bind to both muscarinic and nicotinic receptors, generating intracellular signal transductions through different pathways involved in physiological or pathophysiological responses such as myocardial protection. (10) Muscarinic receptors are usually associated with the intracellular Akt and GSK-3 $\beta$  (glycogen synthase kinase-3 $\beta$ ) protection pathway. (11) It has been recently demonstrated in a myocardial ischemia-reperfusion (I/R) model in rabbits, that brief intermittent VS pulses prior to ischemia protected the myocardium, inhibiting the sympathetic system and activating the muscarinic Akt-GSK-3 $\beta$  pathway. Conversely, continuous VS increased infarct size through sympathetic coactivation, possibly through a species-dependent effect. (6) Moreover, it is well known that protective interventions at the onset of reperfusion have greater potential for clinical application. (12) However, the effects and possible mechanisms of VS protection selectively applied at the onset of reperfusion have not been clearly studied. Therefore, the aim of this study was to analyze whether brief periods of VS selectively applied before ischemia or at the onset of reperfusion can reduce infarct size, and to determine the participation of muscarinic and nicotinic  $\alpha$ -7 cholinergic receptors in the protective mechanism.

## METHODS

### Myocardial ischemia and reperfusion

Male FVB mice were anesthetized with an intraperitoneal induction dose of 90 mg/kg sodium pentobarbital and maintenance dose between 5-10 mg/kg/h as required, controlling superficial nervous reflexes. Then, the animals were intubated and ventilated with room air and oxygen. Temperature was controlled with a regulation system connected to a thermocouple (TCAT-2AC Controller-Physitemp) to maintain animal temperature at 37°C. After stabilization, an incision was performed at the level of fourth intercostal space and regional ischemia was produced occluding the anterior descending coronary artery with polypropylene 8-0 suture (Prolene).

### Vagal stimulation

In the animals receiving VS, the right vagus nerve was dissected at the cervical level and a bipolar electrode (MLA270 Stimulation Cable, AD Instruments) was placed connected to a neurostimulator (Grass S44 Stimulator). The stimulus was applied using constant stimulation parameters, with 0.1 ms electric rectangular pulses, at a frequency of 10 Hz and variable intensity to reduce baseline heart rate (HR) by approximately 10%. (6)

### Hemodynamic measurements

A medial cervical incision was performed to dissect the right carotid artery and a fluid-filled catheter was inserted to reach the left ventricle. Hemodynamic variables: left ventricular systolic pressure (LVSP),  $+dP/dt_{max}$ ,  $-dP/dt_{max}$ , left ventricular end-diastolic pressure (LVEDP) and HR were then measured using a preamplifier and a PowerLab system connected to a computer with specific software (LabChart).

### Experimental protocols (Figure 1)

- 1) *Ischemia and reperfusion (I/R)*: After a stabilization period, myocardial ischemia was performed for 30 minutes followed by a 2-hour reperfusion period (control group).
- 2) *Preischemic vagal stimulation (pVS)*: The right vagus nerve was stimulated for 10 minutes, followed by a 5-minute recovery period without VS before performing I/R.
- 3) *Preischemic vagal stimulation with atropine (pVS+Atr)*: The right vagus nerve was stimulated for 10 minutes similarly to the previous group, but with atropine during VS, administered intravenously through the cannulated jugular vein at a dose sufficient to block the HR reduction. Five minutes after ending VS, ischemia was performed similarly to the other groups.

- 4) *Preischemic vagal stimulation with methyllycaconitine (pVS+MLA)*: Intraperitoneal methyllycaconitine (MLA) was administered 20 minutes before VS. Then, the right vagus nerve was stimulated for 10 minutes followed by a 5-minute recovery period without VS before I/R.
- 5) *Vagal stimulation at reperfusion (rVS)*: Ischemia and reperfusion periods were similar to the I/R group. In this case, the right vagus nerve was stimulated for 10 minutes starting at the onset of reperfusion.
- 6) *Vagal stimulation at reperfusion with atropine (rVS+Atr)*: Group 5 protocol was repeated but atropine was intravenously administered during VS.
- 7) *Vagal stimulation at reperfusion with methyllycaconitine (rVS+MLA)*: Group 6 protocol was repeated except that MLA was administered 20 minutes before the onset of VS.

### Measurement of myocardial infarction

At the end of reperfusion, the animals were euthanized with an overdose of anesthesia to sequentially measure the area at risk and infarct size. To this end, the anterior descending coronary artery was reoccluded and the ascending segment of the aortic arch was cannulated to infuse 1% Evans blue solution. Then, the hearts were excised, frozen and cut in transverse 2 mm thick sections from the apex to the base. Immediately, the sections were incubated in 1% 2,3,5 triphenyltetrazolium chloride solution (TTC) during 20 minutes and then fixed in 10% formalin during 24 hours. Finally, digital images of the stained hearts with Evans blue and TTC were obtained to measure by planimetry the area at risk and the infarcted area with Image-Pro Plus software. The area at risk was expressed as a percentage of the left ventricular area and infarct size as a percentage of the area at risk. (6)

### Statistical analysis

Results were expressed as mean and standard error of the mean. Hemodynamic values were analyzed comparing the different values within each group using ANOVA for repeated measures followed by the Bonferroni test. The data corresponding to the area at risk and infarct size were analyzed using one-way ANOVA followed by the Bonferroni test. A  $p$  value  $<0.05$  was considered statistically significant.

### Ethical considerations

The experimental protocol for the present project was approved by the Institutional Committee for the Care and Use of Laboratory Animals of the School of Medicine of Buenos Aires University (Provision # 1857/15).

## RESULTS

### Effects of vagal stimulation on infarct size

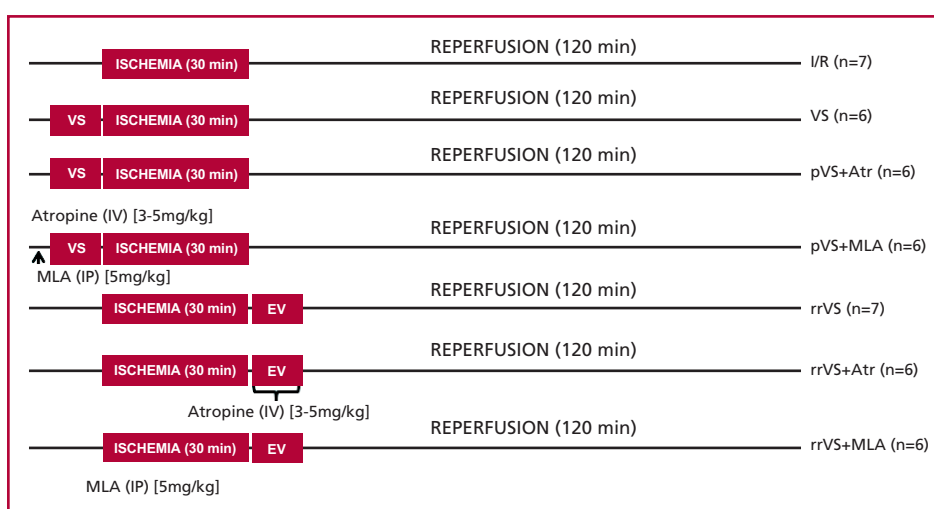
Figure 2 shows the area at risk and the infarct size of the groups with VS prior to ischemia. As expected, no differences were observed in the areas at risk among the groups studied (*Panel A*) (I/R:  $42.29\% \pm 2.66\%$ ; pVS:  $45.00\% \pm 2.91$ ; pVS+Atr:  $45.67\% \pm 1.50\%$  and pVS+MLA:  $47.33\% \pm 3.13\%$ ) ( $p=NS$ ). Preischemic VS reduced infarct size compared with the I/R group ( $41.00\% \pm 3.14\%$  vs.  $58.29\% \pm 3.20\%$ , respectively) ( $p<0.001$ ) (*Panel B*). The protective effect of VS was blocked by atropine administration ( $57.17\% \pm 1.74\%$ ) ( $p<0.01$  vs. pVS), but not with MLA administration ( $41.33\% \pm 1.76\%$ ) ( $p<0.001$  vs. I/R;  $p=NS$  vs. pVS).

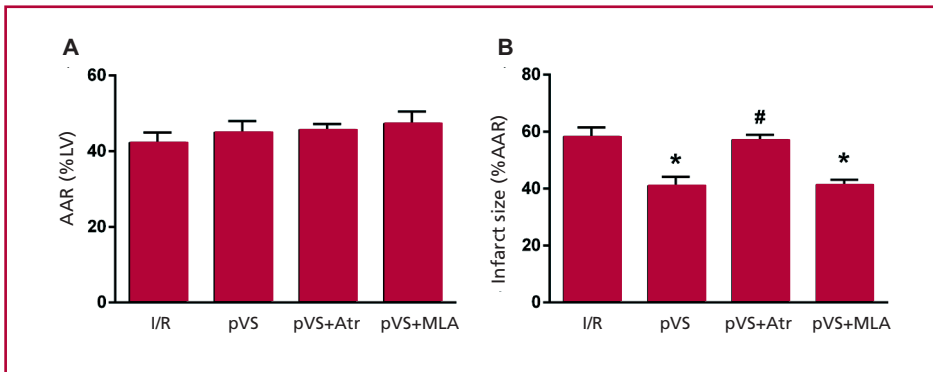
Figure 3 shows the areas at risk and infarct size of groups with VS at reperfusion. No differences were either observed in the areas at risk among these groups (*Panel A*) (I/R:  $42.29\% \pm 2.66\%$ ; rVS:  $47.13\% \pm 2.77\%$ ; rVS+Atr:  $43.50\% \pm 2.17\%$ ;  $42.33\% \pm 3.66\%$  and rVS+MLA:  $41.40\% \pm 3.09\%$ ) ( $p=NS$ ). Vagal stimulation at reperfusion reduced infarct size compared with the I/R group ( $39.29\% \pm 2.79\%$  vs.  $58.29\% \pm 3.20\%$ , respectively) ( $p<0.001$  vs. I/R) (*Panel B*). The protective effect of VS was blocked by MLA administration ( $62.00\% \pm 2.53\%$ ) ( $p<0.001$  vs. rVS), but not with atropine administration ( $37.17\% \pm 1.82\%$ ) ( $p<0.001$  vs. I/R;  $p=NS$  vs. rVS).

### Effects of vagal stimulation on ventricular function

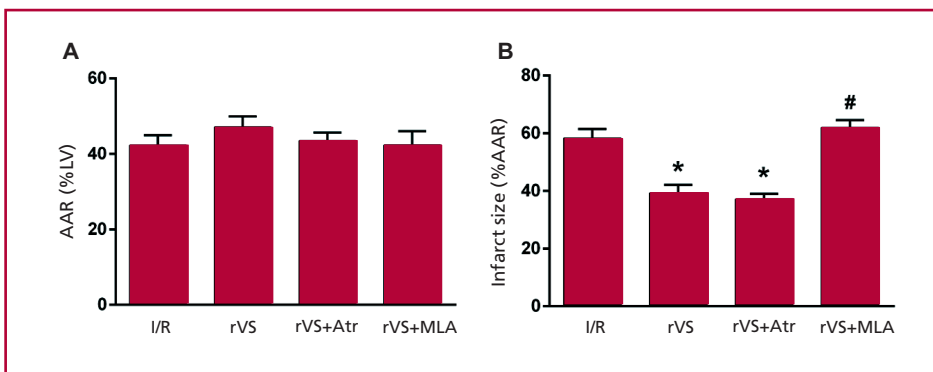
Table 1 shows left ventricular function data in I/R, pVS, pVS+Atr and pVS+MLA groups. No significant differences were observed in HR, LVSP, LVEDP,  $+dP/dt_{max}$  and  $-dP/dt_{max}$  among groups in baseline conditions. A 10 percent reduction in HR during VS was found in

**Table 1.** Schematic representation of experimental protocols. VS: Vagal stimulation. IV: Intravenous. MLA: Methyllycaconitine. IP: Intraperitoneal. I/R: Ischemia and reperfusion. pVS: Preischemic vagal stimulation. pVS+Atr: Preischemic vagal stimulation with atropine. pVS+MLA: Preischemic vagal stimulation with methyllycaconitine. rVS: Vagal stimulation at reperfusion. rVS+Atr: Vagal stimulation at reperfusion with atropine. rVS+MLA: Vagal stimulation at reperfusion with methyllycaconitine.





**Fig. 2.** Area at risk (Panel A) and infarct size (Panel B) of groups receiving preischemic vagal stimulation. LV: Left ventricle. AAR: Area at risk. I/R: Ischemia and reperfusion. pVS: Preischemic vagal stimulation. pVS+Atr: Preischemic vagal stimulation with atropine. pVS+MLA: Preischemic vagal stimulation with methyllycaconitine. Mean±standard error. (\* $p<0.001$  vs. I/R; # $p<0.01$  vs. pVS).



**Fig. 3.** Area at risk (Panel A) and infarct size (Panel B) of groups receiving vagal stimulation at reperfusion. LV: Left ventricle. AAR: Area at risk. I/R: Ischemia and reperfusion. rVS: Vagal stimulation at reperfusion. rVS+Atr: Vagal stimulation at reperfusion with atropine. rVS+MLA: Vagal stimulation at reperfusion with methyllycaconitine. Mean±standard error (\* $p<0.001$  vs. I/R; # $p<0.01$  vs. rVS).

the pVS group and a 9% reduction in the pVS+MLA group. Atropine blocked the effect of VS on HR. In all the groups, LVEDP increased during ischemia, together with a decrease in LVSP,  $+dP/dt_{max}$  and  $-dP/dt_{max}$ . These values did not recover significantly during reperfusion, even in the groups with smaller infarct size.

Left ventricular function data in I/R, rVS, rVS+Atr and rVS+MLA groups are depicted in Table 2. Similarly to results shown in Table 1, no significant differences were observed in HR, LVSP, LVEDP,  $+dP/dt_{max}$  and  $-dP/dt_{max}$  among groups in baseline conditions. A 9 percent reduction in HR relative to that before onset of VS (30 minutes of ischemia) was observed in rVS and rVS+MLA. Atropine blocked the HR descent elicited by VS. In all the groups, LVEDP increased during ischemia, together with a decrease in LVSP,  $+dP/dt_{max}$  and  $-dP/dt_{max}$ . These values did not recover significantly during reperfusion in any of the groups.

## DISCUSSION

The present study shows for the first time in a myocardial I/R model in mice, that a brief, 10-minute VS is similarly able to reduce infarct size, both when applied prior to ischemia as at the onset of reperfusion, thus mimicking ischemic preconditioning and post-conditioning, respectively. However, despite attaining the same protective effect, the involved mechanisms are different. In preischemic VS, the protective effect is lost with the administration of the muscarinic receptor blocker atropine. Conversely, VS protection

at reperfusion is completely lost with the administration of the nicotinic receptor blocker  $\alpha$ -7 MLA.

The ability of ACh to protect the myocardium through the activation of ischemic preconditioning pathways was well documented by Downey et al. (13) in isolated rabbit hearts, by our laboratory with intermittent VS in *in vivo* rabbits, (6) and now in rodents by continuous VS. Interestingly, continuous VS increased infarct size in rabbits (6) but reduced it in mice. These controversial results might be probably explained by a different innervation of the heart and vagal autonomic tone between rabbits and mice.

The study of protective mechanisms applied before ischemia is scientifically interesting but of limited significance for a potential application in patients due to the difficulty in predicting the exact moment at which a coronary event will occur. In this sense, an intervention performed at the time of reperfusion has greater clinical relevance. However, attempts to demonstrate the protective effect of ACh administration *in vitro* to the isolated heart were not conclusive and *in vivo* studies are scarce. Recently, Uitterdijk et al. demonstrated that 20-minute VS starting 5 minutes before reperfusion and ending 15 minutes after its onset reduces infarct size through an anti-inflammatory mechanism in an ischemia-reperfusion model in pigs. (14) Conversely, Shinlapawittayatorn et al. reported that intermittent VS applied during reperfusion was unable to reduce infarct size in the same animal species. (15) We have now shown that continuous VS se-

**Table 1.** Ventricular function and heart rate of groups receiving preischemic vagal stimulation

|                                   | GROUPS  | Baseline  | 5 min FU/VS | Pre-Isch             | 30 min Isch             | 5 min Rep              | 15 min Rep             | 120 min Rep            |
|-----------------------------------|---------|-----------|-------------|----------------------|-------------------------|------------------------|------------------------|------------------------|
| HR<br>(beats/min)                 | I/R     | 436±16    | 440±20      | 440±17               | 458±14                  | 464±10                 | 458±11                 | 443±26                 |
|                                   | pVS     | 471±10    | 419±8       | 481±6                | 476±17                  | 479±18                 | 486±23 <sup>§</sup>    | 523±30 <sup>§</sup>    |
|                                   | pVS+Atr | 458±10    | 500±35      | 473±17               | 489±16                  | 490±17                 | 486±16                 | 493±30                 |
|                                   | pVS+MLA | 477±20    | 437±20      | 522±24* <sup>§</sup> | 530±30 <sup>§</sup>     | 541±24* <sup>§</sup>   | 535±11* <sup>§</sup>   | 554±22 <sup>§</sup>    |
| LVSP<br>(mmHg)                    | I/R     | 93±3      | 86±2        | 89±3                 | 77±5 <sup>#</sup>       | 81±4                   | 86±5                   | 87±5                   |
|                                   | pVS     | 92±5      | 91±6        | 96±8                 | 80±3                    | 82±3                   | 89±4                   | 82±7                   |
|                                   | pVS+Atr | 93±6      | 81±6        | 87±4                 | 79±4                    | 82±4                   | 85±5                   | 72±7 <sup>#</sup>      |
|                                   | pVS+MLA | 107±2     | 105±5       | 97±3                 | 92±4                    | 93±3                   | 86±4 <sup>#</sup>      | 90±2 <sup>#</sup>      |
| LVEDP<br>(mmHg)                   | I/R     | 3.6±0.4   | 3.3±0.4     | 3.4±0.4              | 9.1±1.3 <sup>§</sup>    | 8.4±1.5                | 7.6±0.8                | 7.7±1.7                |
|                                   | pVS     | 3.6±0.5   | 5.4±0.4*    | 4.5±0.9              | 11.2±1.6 <sup>#</sup>   | 8.8±1.3 <sup>#</sup>   | 9.3±1.8                | 7.7±2                  |
|                                   | pVS+Atr | 3.1±0.4   | 3.4±0.3     | 3.1±0.2              | 9.7±1 <sup>#</sup>      | 8.2±0.8 <sup>#</sup>   | 7.3±0.4 <sup>#</sup>   | 5.2±0.8                |
|                                   | pVS+MLA | 4±0.6     | 5.8±0.7*    | 4.6±0.3              | 9.3±1.1 <sup>#</sup>    | 8.5±0.9 <sup>#</sup>   | 6.9±0.4                | 8±1.1 <sup>#</sup>     |
| +dP/dt <sub>máx</sub><br>(mmHg/s) | I/R     | 5530±175  | 5139±206    | 5532±204             | 4608±489                | 4437±321               | 5136±462               | 4760±525               |
|                                   | pVS     | 5155±495  | 5058±534    | 5755±539             | 4423±415                | 4419±344               | 5049±380               | 4477±596               |
|                                   | pVS+Atr | 5168±503  | 4535±525    | 4895±519             | 4553±413                | 4555±444               | 4832±449               | 3784±582 <sup>#</sup>  |
|                                   | pVS+MLA | 7637±388  | 7357±508*   | 7150±283             | 6669±374*               | 6151±342*              | 5975±378               | 5742±283 <sup>#</sup>  |
| -dP/dt <sub>máx</sub><br>(mmHg/s) | I/R     | -5281±192 | -4572±303   | -4864±361            | -3735±398 <sup>#</sup>  | -4044±298 <sup>#</sup> | -4311±381              | -4052±405 <sup>#</sup> |
|                                   | pVS     | -4791±472 | -4720±461   | -4823±448            | -3710±314               | -3935±340              | -4401±326              | -3845±573              |
|                                   | pVS+Atr | -4913±600 | -3905±588   | -4437±467            | -3739±344               | -3984±418              | -4118±428              | -3212±543 <sup>#</sup> |
|                                   | pVS+MLA | -7368±253 | -7003±426*  | -6579±327*           | -5701±424* <sup>#</sup> | -5916±364*             | -5352±370 <sup>#</sup> | -5270±336 <sup>#</sup> |

FU: Follow-up of ventricular function; Isch: Ischemia; Rep: Reperfusion; VS: Vagal stimulation; min: Minutes; s: Seconds; HR: Heart rate; bpm: beats per minute. LVSP: Left ventricular systolic pressure; LVEDP: Left ventricular end-diastolic pressure; dP/dt: First derivative of the left ventricular pressure curve. I/R: Ischemia and reperfusion. pVS: Preischemic vagal stimulation. pVS+Atr: Preischemic vagal stimulation with atropine. pVS+MLA: Preischemic vagal stimulation with methyllycaconitine. Mean±standard error (\*p<0.05 vs. I/R; #p<0.05 vs. baseline; §p<0.05 vs. 5 min VS).

lectively applied at the onset of reperfusion reduces infarct size similarly to VS prior to ischemia.

Surprisingly, the mechanisms of protection of the two moments of VS are different. Although these data may seem striking at first, their rationale could be explained by the different neuroendocrine cardiovascular conditions found after myocardial ischemia. In this sense, it is known that after 30-minute ischemia the vagus nerve endings are injured, leading to lower Ach release. (9, 16) Moreover, there is an increase of catecholamine levels in the ischemic heart, which in turn inhibit the release of this parasympathetic cholinergic neurotransmitter. (17) It is thus possible, that the decrease in cardiac Ach bioavailability, at least in the ischemic zone, does not reach the activation threshold of muscarinic receptors during VS at reperfusion.

Recently, Dvorakova et al. and Mazloom et al. demonstrated the existence of  $\alpha$ -7 nicotinic receptors at the sarcolemmal level of both atrial and ventricular rat cardiomyocytes. (18, 19) In addition, a piece of research by Li et al. showed a compensatory expression of  $\alpha$ -7 nicotinic receptors over that of muscarinic receptors after 30-minute ischemia and 60-minute

reperfusion in rat ventricles. (20) Greater expression of  $\alpha$ -7 nicotinic receptors was also observed by Kong et al. after 3 hours of ischemia in an infarct model in rats. (21) This increase of  $\alpha$ -7 nicotinic receptors with myocardial injury might explain their participation in VS protection at reperfusion and not in preischemic VS.  $\alpha$ -7 nicotinic receptors have been associated with I/R injury protection in other tissues such as the brain (22), but their local participation in myocardial protection had not been demonstrated.

Although other authors showed beneficial effects of VS on myocardial function (23, 24) we could not find differences in left ventricular function recovery at reperfusion in our experimental model, even in the groups with less infarct size. Although this was not the main purpose of our study, it is possible that areas of myocardial stunning might hamper ventricular function recovery during the time interval studied. (25)

## CONCLUSIONS

Brief, continuous VS applied before ischemia or at the onset of reperfusion reduces myocardial infarct size, mimicking ischemic preconditioning and postcondi-

**Table 2.** Ventricular function and heart rate of groups receiving vagal stimulation at reperfusion

|                       | GROUPS  | Baseline  | 30 min Isch            | 5 min Rep              | 15 min Rep               | 120 min Rep             |
|-----------------------|---------|-----------|------------------------|------------------------|--------------------------|-------------------------|
| HR<br>(beats/min)     | I/R     | 436±16    | 458±14                 | 464±10                 | 458±11                   | 443±26 <sup>δ</sup>     |
|                       | pVS     | 467±13    | 463±27                 | 420±18                 | 457±19                   | 508±31                  |
|                       | pVS+Atr | 478±21    | 447±32                 | 460±24                 | 500±13                   | 510±25                  |
|                       | pVS+MLA | 425±14    | 477±17                 | 437±13                 | 481±12                   | 515±30 <sup>δδ</sup>    |
| LVSP<br>(mmHg)        | I/R     | 93±3      | 77±5 <sup>#</sup>      | 81±4                   | 86±5                     | 87±5                    |
|                       | pVS     | 98±5      | 80±3 <sup>#</sup>      | 77±2 <sup>#</sup>      | 84±3 <sup>#</sup>        | 90±4                    |
|                       | pVS+Atr | 102±4     | 70±6 <sup>#</sup>      | 71±5 <sup>#</sup>      | 87±5 <sup>#δΔ</sup>      | 82±5 <sup>#</sup>       |
|                       | pVS+MLA | 98±6      | 83±5                   | 83±3                   | 90±4                     | 85±7                    |
| LVEDP<br>(mmHg)       | I/R     | 3.6±0.4   | 9.1±1.3 <sup>#</sup>   | 8.4±1.5                | 7.6±0.8                  | 7.7±1.7                 |
|                       | pVS     | 4.5±0.8   | 12.8±1.8 <sup>#</sup>  | 9.3±1.4                | 9.7±1.9                  | 7.6±1.7                 |
|                       | pVS+Atr | 3.3±0.1   | 8.5±0.5 <sup>#</sup>   | 5.3±0.7                | 8.8±0.9 <sup>#δ</sup>    | 5.8±1.3                 |
|                       | pVS+MLA | 5.1±1     | 11.9±1.2 <sup>#</sup>  | 9.4±1.1 <sup>#</sup>   | 8.7±1                    | 6.3±0.8 <sup>Δ</sup>    |
| +dP/dtmax<br>(mmHg/s) | I/R     | 5530±175  | 4608±489               | 4437±321               | 5136±462                 | 4760±525                |
|                       | pVS     | 5714±183  | 4434±353 <sup>#</sup>  | 3836±318 <sup>#</sup>  | 4798±268                 | 5074±277 <sup>δ</sup>   |
|                       | pVS+Atr | 6722±275  | 4121±680 <sup>#</sup>  | 4037±476 <sup>#</sup>  | 5341±564 <sup>#</sup>    | 4896±583 <sup>#</sup>   |
|                       | pVS+MLA | 6183±601  | 5599±547               | 5206±340               | 6149±439                 | 5276±627                |
| -dP/dtmax<br>(mmHg/s) | I/R     | -5281±192 | -3735±398 <sup>#</sup> | -4044±298 <sup>#</sup> | -4311±381 <sup>#</sup>   | -4052±405 <sup>#</sup>  |
|                       | pVS     | -5215±241 | -3643±240 <sup>#</sup> | -3443±129 <sup>#</sup> | -4075±207 <sup>#</sup>   | -4283±223 <sup>#δ</sup> |
|                       | pVS+Atr | -6026±371 | -3285±574 <sup>#</sup> | -3463±434 <sup>#</sup> | -4604±416 <sup>#δΔ</sup> | -4220±562 <sup>#</sup>  |
|                       | pVS+MLA | -5986±596 | 4529±448 <sup>#</sup>  | -4543±336 <sup>#</sup> | 5305±387                 | 4642±595                |

Isch: Ischemia; Rep: Reperfusion; VS: Vagal stimulation; min: Minutes; s: Seconds; HR: Heart rate; LVSP: Left ventricular systolic pressure; LVEDP: Left ventricular end-diastolic pressure; dP/dt: First derivative of the left ventricular pressure curve. I/R: Ischemia and reperfusion. rVS: Vagal stimulation at reperfusion. rVS+Atr: Vagal stimulation at reperfusion with atropine. rVS+MLA: Vagal stimulation at reperfusion with methyllycaconitine. Mean±standard error (\*p<0.05 vs. I/R; #p<0.05 vs. baseline; Δp<0.05 vs. 30 min Isch; δ<0.05 vs. 5 min Rep/VS).

tioning. However, the mechanisms of protection differ according to the moment in which the intervention is applied. Preischemic VS protects through cholinergic activation of muscarinic receptors, while VS at reperfusion reduces infarct size through a mechanism involving cholinergic activation of α-7 nicotinic receptors.

### Conflicts of interest

None declared. (See authors' conflicts of interest forms on the website/Supplementary material).

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