Preischemic Vagal Efferent Stimulation Increases Infarct Size in Rabbits

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SUMMARY

It has been shown that vagal stimulation induces cardioprotective effects and the administration of acetylcholine mimics ischemic preconditioning. However, there are no conclusive data about the effects of in vivo vagal stimulation on myocardial infarction. The objective of this study was to evaluate the effects of vagal stimulation on experimental myocardial infarction induced in rabbits subjected to 45 min of regional myocardial ischemia by ligation of a branch of the left coronary artery, followed by 4 hours of reperfusion (G1, n=14). In group 2 (G2, n=9) G1 protocol was repeated and, before inducing ischemia, right efferent vagal stimulation was performed during 10 min to an intensity enough to reduce heart rate by 10-20% followed by a recovery period of 5 min. In group 3 (G3, n=5) the G2 protocol was repeated, but atropine was administered during vagal stimulation. In other experimental groups the G2 protocol was repeated and short-acting β1-adrenergic blocker (esmolol, G4, n=7) or long-acting beta blocker (atenolol, G5, n=5) were administered during the stimulation. Preischemic vagal stimulation increased the infarct size from 45.2%±2.4% to 62.9%±3.1% ($p < 0.05$). Atropine reverseed this effect reducing the infarct size to 44.8%±3.9% ($p < 0.05$ vs. G2). The administration of esmolol or atenolol attenuated the increase in infarct size to 50.1%±4.2% and 50.0%±2.9%, respectively ($p < 0,05$). Preischemic efferent vagal stimulation significantly increases the infarct size by a muscarinic cholinergic mechanism. This effect is reverseed by beta adrenergic blockade. Applying vagal stimulation to different clinical scenarios might cause deleterious secondary effects.

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Key words > Myocardial Infarction - Vagal Stimulation - Atropine - Esmolol - Atenolol

Abbreviations >

+dP/dt$_{max}$	Maximum derivatives of the ventricular pressure	**I/R**	Ischemia and reperfusion
ACh	Acetylcholine	**NPY**	Neuropeptide Y
Aten	Atenolol	**MAP**	Mean arterial pressure
Atr	Atropine	**LVEDP**	Left ventricular end-diastolic pressure
Esm	Esmolol	**EDP**	End-diastolic pressure
VS	Vagal stimulation	**LVP**	Left ventricular pressure
HR	Heart rate	**LV**	Left ventricle
		VIP	Vasoactive intestinal peptide

BACKGROUND

The autonomic imbalance, characterized by increased sympathetic tone and decreased parasympathetic tone, is detrimental to the myocardium and is associated with worse prognosis of cardiovascular diseases, particularly heart disease. This knowledge led to make efforts in recent decades and find therapies to reduce the sympathetic activity, as in the case of beta-andrenergic blockers, and to develop techniques to increase parasympathetic activity, such as vagal neurostimulation. (1)

In this regard, administration of acetylcholine (ACh) reduces the size of myocardial infarction in an isolated heart model with global ischemia and reperfusion. However, endogenously released ACh during an ischemic preconditioning protocol in vivo is unable to activate this protective mechanism, probably due to the lack of cholinergic innervation in ventricles compared with the atria. (2, 3) Therefore, vagal stimulation (VS) applied prior to ischemia may attenuate myocardial infarct size due to ACh release to threshold levels so as to activate preconditioning.

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However, innervation of the heart and autonomic regulation are highly complex. (4) Despite a great majority of the effects of the two arms –sympathetic and parasympathetic– of the autonomous nervous systems are antagonic; this is not the only way of interaction they have, since under certain experimental conditions, a branch may be activated as a result of the activation of the other. This is known as reciprocal action; the most common examples are the positive chronotropic or inotropic responses as a result of VS or ACh administration. (5, 6)

We have conducted this study because it is still unkown whether efferent stimulation of the vagus nerve prior to ischemia can activate protective mechanisms against ischemia and reperfusion similar to those in the in vitro studies, (2, 3), and if such stimulation can produce a reflex activation of the sympathetic nervous system which would be able to cause undesirable effects. The purpose of this work is to study the effects of efferent stimulation of the right vagus nerve over the size of the myocardial infarction in an in vivo model of regional ischemia with reperfusion, and the possible participation of the sympathetic nervous system.

MATERIAL AND METHODS

Male New Zealand rabbits were used (2.2-2.6 kg), which were randomly assigned to the different experimental groups.

The animals were anesthetized with ketamine (75 mg/kg) and xylazine (0.75 mg/kg) induction into a muscle, and intubated for mechanical ventilation with a mixture of ambient air and oxygen. The anesthetic maintenance was performed with a 2 ml/kg attack dose of urethane (250 mg/ml) and chloralose (40 mg/ml), and then the same anesthetic combination was administered continuously with infusion pump through a catheter placed in the marginal ear vein (0.5-1.0 ml/kg/h). Blood pressure was measured with a catheter placed in one of the femoral arteries, and ventricular pressure, with a catheter placed in the left ventricle (LV) through the common right carotid artery.

Right vagus nerve was reached through a cervical incision; it was isolated and was dissected at the upper third of the neck. A silver bipolar electrode was hooked in the distal nerve stump for subsequent electrostimulation.

A left lateral thoracotomy was then peformed, followed by a pericardiectomy to expose the surface of the heart. Regional myocardial ischemia was induced by a ligation of a branch from the left coronary artery with a curved needle and synthetic suture 5-0. The suture was adjusted by interposing a small plastic tube so that, once completed the ischemic period, the tube could be easily released and reperfusion allowed. Myocardial ischemia was confirmed with the occurence of regional pallor in the VI surface.

Rectal temperature was continuously measured with a digital thermometer, which was kept between 37 and 38 ºC with a heating pad.

Vagal stimulation

VS was carried out with the right distal vagus nerve stump; thus, stimulation was purely efferent and afferences to central nervous system were avoided. A neurostimulator (model D7801, Hugo Sachs Elektronik) was used; constant electric parameters were utilized, delivering a train of rectangular pulses of 0.1 ms, 10 Hz, and a variable intensity that was adapted to each animal to obtain a 10-20% heart rate reduction prior to stimulation.

Cardiac catheterization

Left ventricular pressure (LVP) was recorded in real time through the catheter placed in the LV. Then, systolic function was analized on the basis of that record, considering the +dP/dtmáx (maximum derivatives of the ventricular pressure) and the diastolic function through the end-diastolic pressure (EDP). The catheter placed in the femoral artery registered the blood pressure and the analysis of systolic, diastolic and mean pressures. All the catheterization variables were assessed from the beginning of stabilization to the end of reperfusion.

Measuring risk area and size of myocardial infarction

After the reperfusion period, animals were sacrificed with an overdose of thiopental sodium intraveonusly. The chest was reopened and the coronary artery was performed a permanent ligation. The ascending thoracic aorta was immediately cannulated and a solution of Evans blue at 1% was infused in the coronary tree, so that the area irrigated by the ligated artery was not perfused by the dye. Then, the hearts were cut into 4 mm-thick cross sections from the tip to the base. The digital images obtained were analyzed using computerized planimetry (image analyzer Image Pro Plus, version 6.0) to determine the risk area (area not stained with Evans blue), which was expressed as percentage of total area of LV wall. Then the sections were incubated in 1% of 2,3,5-triphenyltetrazolium chloride solution (TTC), at pH 7.8, and at 37 °C for 20 minutes, and finally fixed in formaldehyde at 10% for 24 hours. Digital images were then analyzed, evaluating the areas dyed and nondyed by the TTC, considering the latter ones as nonviable. The myocardial infarction area was determined with these data. Finally, the infarction size was expressed as a percentage of the risk area.

Experimental protocols (Figure 1)

- Group 1 (ischemia and reperfusion [I/R]; n = 14): after a stablization period, the right vagus nerve was dissected and a catheterization follow-up was performed during 25 minutes. Once that period was over, myocardial ischemia was provoked through coronary ligation during 45 minutes, followed by reperfusion for 4 hours.
- Group 2 (vagal stimulation [VS]; n = 9): after stabilization and vagus nerve dissection, there was another stabilization period of 10 minutes. Then, nerve stimulation was performed for 10 minutes, followed by 5 minutes of recovery without stimulation. Ischemia and reperfusion then proceeded, in the same way as performed in group 1.
- Group 3 (vagal stimulation with atropine [VS + Atr]; n = 5): group 2 protocol was repeated, but a dose of atropine sulfate enough for each case (1.3-2.0 mg/kg) was administered during the VS period in order to block the fall in heart rate caused by VS.
- Group 4 (vagal stimulation with esmolol [VS + Esm]; n = 7): group 2 protocol was repeated, but esmolol (ultra-short acting β1 andrenergic blocking agent) was administered in an attack dose of 2-3 mg/kg in bolus, followed by 0.5 mg/kg/min.
- Group 5 (vagal stimulation with atenolol [VS + Aten]; n = 5): group 2 protocol was repeated, but atenolol (long

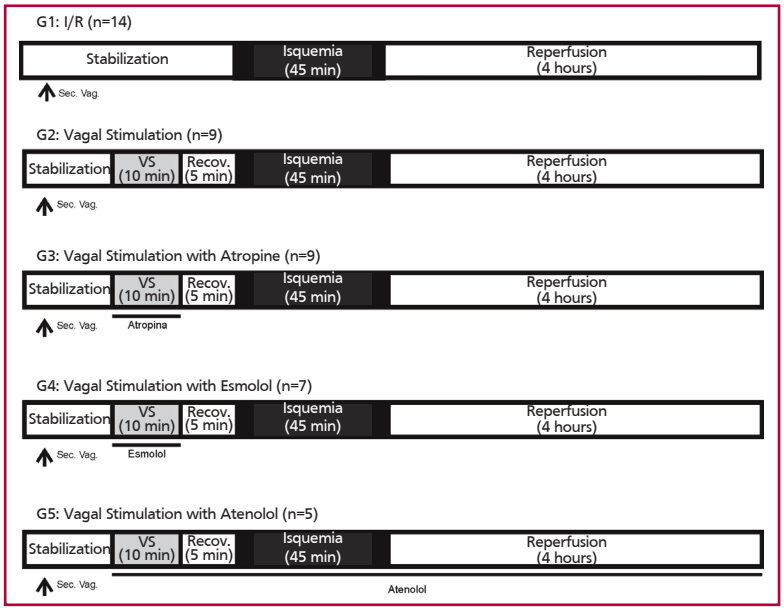

Fig. 1. Experimental protocols. Vag. Sec.: Vagus nerve section. VS: Vagal stimulation. Recov.: Recovery with no stimulation.

acting β1 andrenergic blocking agent) was administered in doses of 0.06 mg/kg/min from the beginning of stimulation to the end of reperfusion.

Statistical Analysis

Results are expressed as the mean ± standard error (SE). Catheterization values were analized comparing the different times within each group using ANOVA test for repeated measures followed by the Student-Newman-Keuls test. Data corresponding to risk area and size of myocardial infarction were analyzed using one-way ANOVA test followed by the Student-Newman-Keuls test. A difference was considered significant when the p value was < 0.05.

RESULTS

Table 1 shows the detailed values of heart rate (HR), maximum derivatives of the ventricular pressure (+dP/dtmáx), left ventricular end-diastolic pressure (LVEDP), and mean arterial pressure (MAP) at baseline (prior to vagus nerve dissection), at 45 minutes of ischemia, and at different times of reperfusion. An increase in LVEDP can be observed at 45 minutes of ischemia and at the end of reperfusion in groups I/R, VS, VS + Atr, and VS + Aten with respect to the corresponding baseline. The values of the other studied variables did not show significant changes and were stable throughout the experiment, with the exception of heart rate, which increased substantially both in ischemia and in reperfusion in the VS + Esm group.

Figure 2 shows the fall of heart rate caused by VS prior to ischemia. A 20% fall of heart rate was observed with respect to baseline within 5 minutes of VS. Five minutes after the end of the stimulus (beginning of ischemia), heart rate had recovered values close to control. No changes in heart rate of animals from the VS + Atr group were observed.

Figure 3 A charts the values of the risk area size corresponding to group I/R (46.3% ± 2.8%), to group SV (42.0% ± 3.5%), and to group SV + Atr (48.8% ± 53%), with no significant differences among the groups. Figure 3 B shows the size of myocardial infarction in the mentioned groups, which was 45.2% ± 2.4% in the group I/R. Right efferent vagus nerve stimulation significantly increased the size of the infarction to 62.9% ± 3.1%. Atropine administration reversed the effect of VS, and a size of infarction of 44.8% ± 3.9% was observed.

Figure 4 shows the fall of heart rate caused by SV in groups VS + Esm, and SV + Aten. In both groups, heart rate was about 20% below the value at the beginning of stimulation, and it recovered baseline value five minutes after the end of VS.

Figure 5 A shows the risk area sizes corresponding to group I/R (46.3% ± 2.8%), group VS (42.0% ± 3.5%), and to gruops VS + Esm (43.4% ± 3.6%) and VS + Aten (48.2% ± 2.3%), with no significant differences. In Figure 5 B we can observe the sizes of myocardial infarction corresponding to group I/R (44.2% ± 2.5%), group VS (60.5% ± 3.7%), group VS + Esm (50.1% ± 4.2%), and group VS + Aten (50.0% ± 2.9%). As already pointed out, efferent VS significantly increases the size of myocardial infarction. However, administering an either short-acting –as esmolol– or long-acting –as atenolol– β1-adrenergic blocking agent reverses the increase in infarct size caused by the SV.

DISCUSSION

This work demonstrates for the first time that SV applied before ischemia increases the size of myocardial infarction in a model of regional ischemia and in vivo reperfusion. In addition, it shows that this harmful effect is reversed when atropine is administered, which suggests that efferent VS causes injury mechanisms activated by the stimulation of cholinergic muscarinic receptors.

These in vitro models in which Ach is administered

Table 1. Catheterization values of the different groups studied and assessed at baseline, at the end of ischemia, and at different times of reperfusion.

Parameter	Group	Basal	45' isq	30' rep	60' rep	120' rep
	I/R (n = 11)	206 ± 10	207 ± 8	206 ± 9	206 ± 9	182 ± 18
HR	VS (n = 4)	221 ± 8	232 ± 7	226 ± 10	223 ± 9	232 ± 13
(bpm)	VS + Atr (n = 5)	218 ± 19	231 ± 5	264 ± 14	266 ± 24	258 ± 13
	VS + Esm (n = 7)	191 ± 3	210 ± 6*	217 ± 9*	221 ± 7*	228 ± 7*
	VS + Aten (n = 5)	195 ± 9	195 ± 10	200 ± 6	203 ± 7	201 ± 6
	I/R	3.3 ± 0.6	7.7 ± 1.1*	5.3 ± 1.1	5.3 ± 1.3	6.2 ± 1.3*
LVEDP	VS	3.2 ± 1.2	6.6 ± 1.4*	3.9 ± 1.0	3.5 ± 1.0	3.6 ± 0.7
(mm Hg)	VS + Atr	2.1 ± 0.8	5.2 ± 1.1*	3.6 ± 0.4	4.2 ± 0.5	2.9 ± 0.5
	VS + Esm	2.7 ± 0.4	5.3 ± 1.5	2.9 ± 0.3	2.9 ± 0.8	2.2 ± 0.5#
	VS + Aten	1.8 ± 0.3	6.5 ± 1.2*	5.3 ± 1.7	5.6 ± 1.7	7.0 ± 1.8*
	I/R	2,494 ± 188	2,494 ± 217	2,466 ± 309	2,496 ± 362	2,723 ± 411
$+dP/dt_{max}$	VS	3,464 ± 373	3,267 ± 309	3,157 ± 350	2,928 ± 276	3,385 ± 373
(mm Hg/msec)	VS + Atr	3,255 ± 469	2,862 ± 874	3,037 ± 933	3,241 ± 102	3,595 ± 745
	VS + Esm	2,495 ± 161	2,732 ± 393	2,555 ± 440	2,540 ± 446	2,986 ± 512
	VS + Aten	2,653 ± 143	2,705 ± 189	2,669 ± 254	2,806 ± 243	2,957 ± 203
	I/R	54 ± 3	49 ± 4	47 ± 5	46 ± 6	48 ± 5
MAP	VS	63 ± 7	67 ± 6	59 ± 5	50 ± 5	64 ± 7
(mm Hg)	VS + Atr	67 ± 4	51 ± 16	48 ± 7	53 ± 10	60 ± 4
	VS + Esm	53 ± 2	60 ± 8	58 ± 8	56 ± 9	63 ± 8
	VS + Aten	54 ± 3	60 ± 9	60 ± 1	78 ± 7	79 ± 4

* p < 0.05 vs basal. # p < 0.05 vs 45' isq

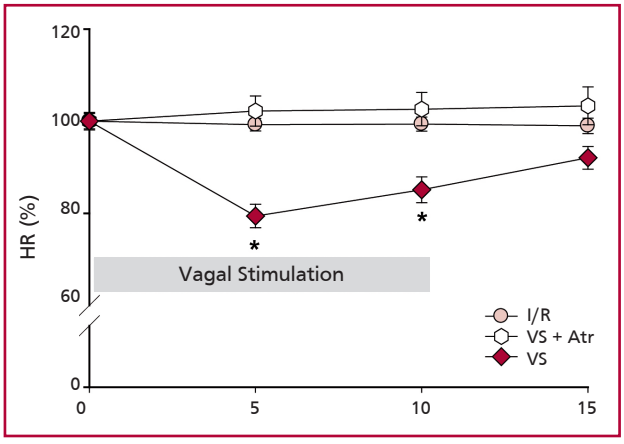

Fig. 2. Changes in heart rate during the 15 minutes prior to ischemia. Vagal stimulation significantly reduces heart rate. * p < 0.05 vs I/R.

exogenously, myocardial infarction size is reduced through preconditioning activation. (2, 3) Moreover, studies conducted on isolated myocytes show that ACh causes protective effects, as increased production of nitric oxide and Akt phospholyration, decreased apoptosis, and activation of induced factor by hypoxia HIF-1α through a non-hypoxic pathway; this leades the myocyte to a state of increased tolerance to low oxygen concentrations. (7)

Still, when ischemia and reperfusion cycles are used in vivo to activate preconditioning pathways (ischemic preconditioning), endogenous ACh release does not reach the threshold necessary to provide protection. (2, 3, 8) This fact is consistent with the concept that ventricles have little parasympathetic innervation, demonstrated both in the distribution of nerve fibers and receptors, as well as in the functional response of ventricles to selective stimulation of both arms of the autonomic nervous system. (9)

In the in vivo model of our work, the SV applied before the ischemia tries to achieve an induced release of ACh of such intensity that it can reach the threshold necessary to activate preconditioning. Surprisingly, that stimulation significantly increases the size of the myocardial infarction. A possible explanation could be that the autonomic regulation of the heart would occur in a hypothetical model of neural hierarchies, in which the intrinsic cardiac nerve plexus would be able to generate regional cardiodynamic regulations as subtle but very effective modulations, which would not necessarily involve systemic reflexes. (4) Thus, electrical stimulation of the vagus nerve might cause undesirable efects when reflexively activating the sympathetic nervous system.

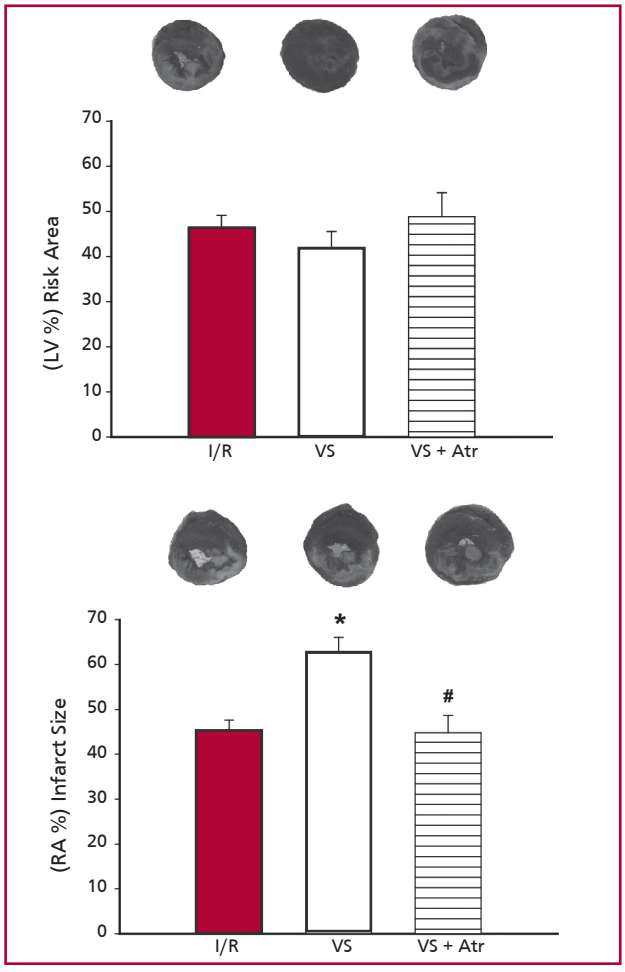

Fig. 3. A. Sizes of the risk areas expressed as percentage of the left ventricular areas, with no significant differences among groups. Representative pictures corresponding to the delimitation of the risk area in each group are shown on the bars. **B.** Infarct sizes of the three studied groups are represented on the chart. Vagal stimulation prior to ischemia significantly increases infarct size. This is reversed with atropine administration. Representative pictures of each of the groups are shown on the bars. * $p < 0.05$ vs I/R. # $p < 0.05$ vs VS.

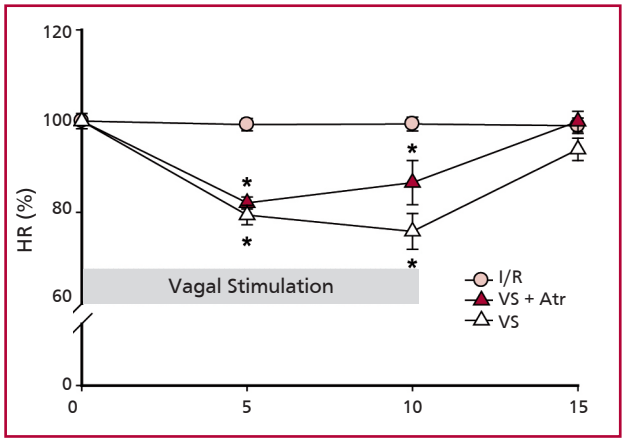

Fig. 4. Changes in heart rate during the 15 minutes prior to ischemia. It does not change in the group I/R, but it is reduced significantly when the vagus nerve is stimulated, both in the group with esmolol and the group with atenolol. * $p < 0.05$ vs I/R.

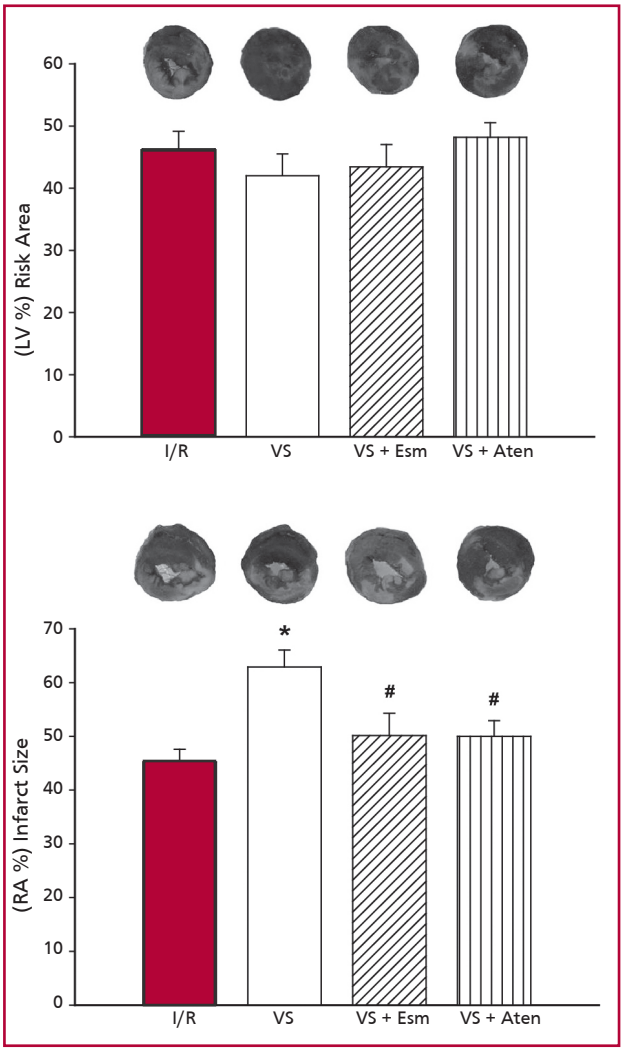

Fig. 5. A. Sizes of the risk areas expressed as percentage of the left ventricular areas, with no significant differences among groups. Representative pictures corresponding to the delimitation of the risk area in each group are shown on the bars. **B.** Infarct sizes of the three studied groups are represented on the chart. Vagal stimulation significantly increases the infarct size. This increase does not occur when beta andrenergic receptors are blocked with esmolol or atenolol. * $p < 0.05$ vs I/R. # $p < 0.05$ vs VS.

Consistent with this concept, two types of β receptor blockers were administered in this study; an ultra-short acting one, as esmolol, and a long-acting one, as atenolol. In both cases, we observed a reduced size of myocardial infarction with respect to the group VS, which indicates the participation of the sympathetic nervous system in the injury mechanisms activated by efferent SV. However, we cannot disregard the possible participation of α receptors or coronary vessels, and of the coreleased neuromodulators in the autonomic nervous system, particularly the neuropeptide Y (NPY). (10) Thus, local sympathetic activation secondary to SV could stimulate the β1 pathway, but it could also cause a coronary vascular response due to α and NPY participation. Both are vasoconstriction mechanisms;

NPY is particularly known because it causes intense and sustained coronary vasoconstriction. Finally, participation of secondary neurotransmitters such as the vasoactive intestinal peptide (VIP) would also be possible, of which it has been demonstrated that it may be released by vagus nerve electrostimulation. (11) This way, direct b1 pathway, and α and NPY pathways through the production of ischemia could be able to generate or worsen injury mechanisms set forth during ischemia and/or reperfusion.

The results of our work may also seem clashing when considering that other studies showed benefits from the vagus nerve stimulation in the context of myocardial infarction (12-14), and they have reported that chronic SV significantly attenuates left ventricular remodeling, and improves survival of rats with post-infarction chronic heart failure. (15) However, stimulation protocols applied in these studies are chronic and, on the contrary, the purpose of our study was to assess the effect of SV on acute infarct size.

Although the mechanisms involved have not been studied directly, we consider that the findings of this work may be of practical use in clinics, since the SV technique is used in different places in the world for the treatment of some neurological diseases, such as epilepsy and depression. More recently, it is being evaluated as a possible alternative to treat chronic heart failure. (16) In those contexts, our findings might explain the occurrence of undesirable collateral effects.

In conclusion, in an in vivo model with ischemia and reperfusion, SV applied prior to ischemia increases the size of myocardial infarction due to the activation of chollinergic muscarinic receptors; this harmful effect reverses with beta-andrenergic blocking, and this also suggests the participation of the sympathetic nervous system.

RESUMEN

La estimulación vagal eferente preisquémica aumenta el tamaño del infarto de miocardio en conejos

La estimulación vagal induce efectos cardioprotectores y la administración de acetilcolina mimetiza el efecto del precondicionamiento isquémico. No obstante, no existen datos concluyentes con respecto a los efectos de la estimulación vagal en el infarto de miocardio in vivo. Con el objetivo de evaluar los efectos de la estimulación vagal sobre el infarto de miocardio experimental en conejos, se provocó isquemia miocárdica regional por ligadura de una rama coronaria izquierda durante 45 min seguida de 4 horas de reperfusión (G1, n = 14). En el grupo 2 (G2, n = 9) se repitió el protocolo de G1 aplicándose, antes de la isquemia, estimulación vagal eferente derecha durante 10 min a una intensidad tal que produjo una reducción de la frecuencia cardíaca de entre el 10% y el 20%, seguida de 5 min de recuperación. En el grupo 3 (G3, n = 5) se repitió el protocolo de G2, pero se administró atropina durante la estimulación vagal. En otros grupos experimentales se repitió el protocolo de G2, pero administrando un bloqueante adrenérgico 1 de acción corta (esmolol) durante la estimulación (G4, n = 7) o uno de acción prolongada (atenolol) (G5, n = 5). La estimulación vagal preisquemia aumentó el tamaño del infarto desde el 45,2% ± 2,4% al 62,9% ± 3,1% (p < 0,05). La atropina revirtió este efecto, reduciéndolo al 44,8% ± 3,9% (p < 0,05 vs. G2). La administración de esmolol o de atenolol atenuó el incremento del tamaño del infarto al 50,1% ± 4,2% y al 50,0% ± 2,9%, respectivamente (p < 0,05). La estimulación vagal eferente preisquémica incrementa significativamente el tamaño del infarto por un mecanismo colinérgico muscarínico, efecto que es reverseido por bloqueo betaadrenérgico. La estimulación vagal, aplicada en diversas situaciones clínicas, podría causar efectos secundarios perjudiciales.

Palabras clave > Infarto de miocardio - Estimulación vagal Atropina - Esmolol - Atenolol

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