Preischemic efferent vagal stimulation increases the size of myocardial infarction in rabbits. Role of the sympathetic nervous system

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The vagal nerve stimulation (VNS) has been tested for the treatment of chronic heart failure in humans [1]. However, many of the effects of parasympathetic stimulation in ischemic heart disease are still unknown. Kawada et al. have shown a significant increase in acetylcholine (Ach) levels in the ventricular myocardium subjected to VNS [2]. Since, Ach has been implicated in the mechanism of ischemic preconditioning [3], we can hypothesize that VNS applied before ischemia may reduce myocardial infarct size by a release of Ach. However, under certain experimental conditions, the two divisions of the autonomic nervous system (sympathetic and parasympathetic) can be activated. Therefore, co-activation of the sympathetic nervous system could generate a situation adverse and contrary to the possible beneficial effect expected for VNS.

Thus, it is still unknown whether acute VNS, prior to ischemia, is capable of activating protective mechanisms against ischemia and reperfusion injury, or whether such stimulation is capable of producing a reflex activation of the sympathetic nervous system that can cause unwanted effects. Keeping in mind the above, the aim was to study the effects of efferent stimulation of the right vagus nerve on myocardial infarct size. A second aim was to evaluate the possible co-activation of the sympathetic nervous system as a result of vagal stimulation.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [4]. Male New Zealand rabbits (2.2 to 2.6 kg) were anesthetized and subjected to 45 min of coronary occlusion followed by 4 h of reperfusion. The right vagus nerve was sectioned at the mid-cervical level and its distal end was efferently stimulated to obtain a reduction between 10 and 20% of heart rate (HR). The animals were catheterized and ventricular function was analyzed from the beginning of stabilization until the end of reperfusion. The triple product (beats.mm $Hg^2/s^2 \times 10^5$), an indirect index of oxygen consumption, was calculated as: $HR \times LV$ Systolic Pressure $\times LV + dP/dt_{max}$. Triple product was expressed as an average of the different times (baseline, 5 and 10 min of vagal stimulation, pre-ischemia, 5, 15 and 45 min of ischemia and 15, 60 and 120 min of reperfusion) for each group.

After the end of the reperfusion period, the infarct size was measured using 2, 3, 5-triphenyltetrazolium chloride (TTC). Plasma noradrenaline and adrenaline were measured by HPLC.

In control group ($n = 14$), myocardial ischemia (45 min) was induced by coronary ligation followed by 4 h of reperfusion. In a second group (VNS, $n = 9$), vagus nerve was sectioned and stimulated for 10 min. Then, ischemia and reperfusion were applied in the same way as in control group. In a third group (VNS + Atropine, $n = 5$) the same protocol as VNS group was performed, but during VNS, atropine sulfate (Atr) was administered. In a fourth group (VNS + Atenolol, $n = 6$), the same protocol as VNS group was performed, but from the VNS to the end of reperfusion Atenolol (Aten) was administered. In a fifth group (Aten, $n = 4$) atenolol was administered without VNS. In a sixth group

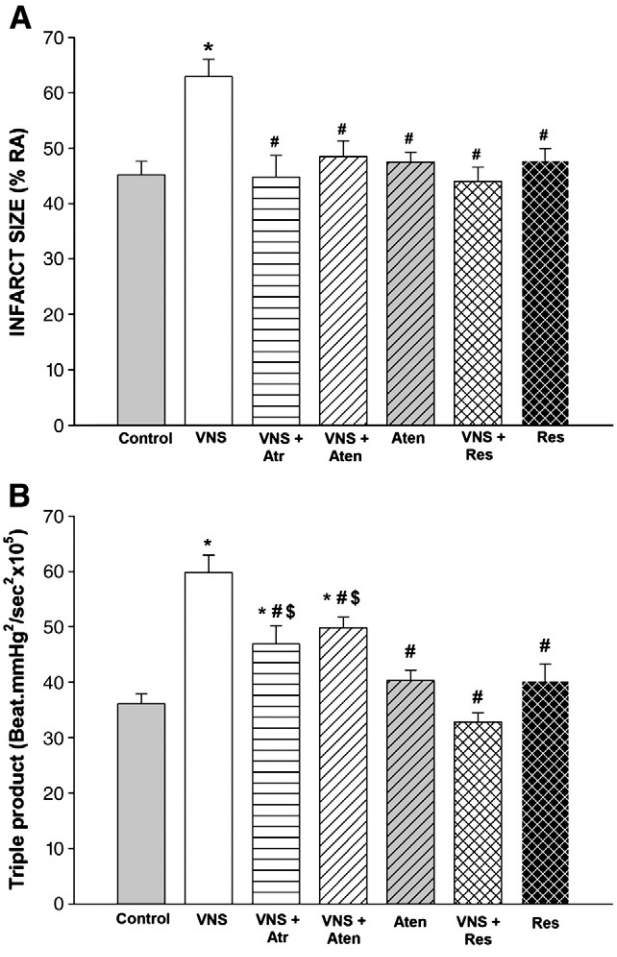

Fig. 1. Panel A: Shows the infarct sizes of the studied groups. VNS significantly increased infarct size and this effect was reversed by atropine, atenolol and reserpine. Panel B: Shows the changes in the triple product. The VNS group presented a significant increase in the triple product. This increase was attenuated by the atropine and atenolol administration. Reserpine completely abolished the triple product increment. LV: left ventricle; RA: risk area; VNS: vagus nerve stimulation; Atr: atropine; Aten: atenolol; Res: reserpine. *$p < 0.05$ vs. control; #$p < 0.05$ vs. VNS; $$p < 0.05$ vs. VNS + Res.

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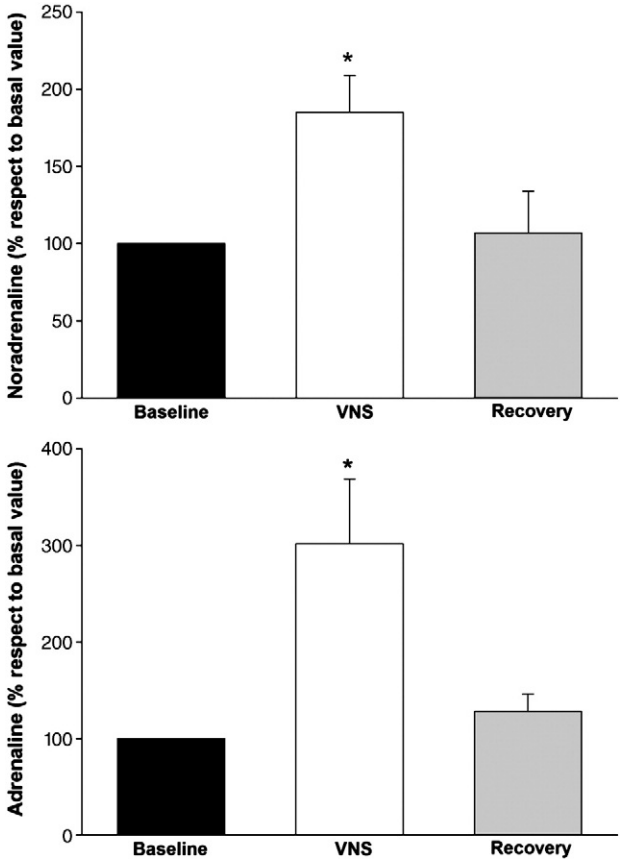

Fig. 2. Shows noradrenaline and adrenaline plasma concentration before, during VNS and at 5 min recovery expressed as percentage of baseline value. VNS significantly increased catecholamines plasma concentration. *$p<0.05$ vs. baseline.

(VNS + Reserpine, $n=5$) the same protocol as VNS group was performed, but reserpine (Res) was administered 24 h before experiment (5 mg/kg). Finally in a seventh group (Res, $n=4$), the same protocol as control group was performed, but reserpine was administered 24 h before experiment.

The hemodynamic and left ventricular function variables were similar between groups at the beginning of the protocols. As expected, VNS induced a significant decrease in HR.

There were no significant differences between groups in risk area values. Infarct size (Fig. 1, panel A) in the control group was $45.2 \pm 2.4\%$; the VNS significantly increased infarct size to $62.9 \pm 3.1\%$. Atropine reversed the effect of VNS ($44.8 \pm 3.9\%$). Infarct size was $48.5 \pm 2.8\%$ in the VNS + Aten group, and $47.5 \pm 1.9\%$ in the Aten group. Finally, the administration of reserpine during VNS also reversed the deleterious effects of the latter on the infarct size ($44.0 \pm 2.6\%$). Reserpine without VNS did not change infarct size ($47.7 \pm 2.2\%$).

On the other hand, vagal stimulation increased the triple product and atropine and atenolol attenuated the increase of this variable, while the administration of reserpine completely abolished the increment (Fig. 1, panel B). VNS increased plasma concentration of noradrenaline by $185 \pm 24\%$ ($p<0.05$ vs. baseline value) and adrenaline by $302 \pm 67\%$ ($p<0.05$ vs. baseline value). Interestingly, the increase of noradrenaline and adrenaline returned to baseline values after VNS Fig. 2.

In summary, the present study demonstrates that, at least in our experimental conditions, vagal stimulation applied before ischemia increases infarct size. This deleterious effect is reversed by atropine suggesting that efferent vagal stimulation triggers injury mechanisms activated by muscarinic cholinergic receptors. One possible explanation for this finding is the fact that electrical stimulation of the vagus nerve may cause unwanted effects when the reflex activation of the sympathetic nervous system takes place. In accordance with this concept, we found an abolition of the deleterious effect of vagal stimulation with reserpine, indicating the involvement of the sympathetic nervous system. Consistent with the last finding, catecholamines clearly increased during vagal stimulation. It is known that the cardiac response to the activation of an autonomic system division depends on the activity levels of the other division. Generally, the sympathetic-parasympathetic interaction occurs in an antagonistic manner. However, under certain circumstances, it is possible to generate a sympathetic co-activation during a parasympathetic stimulation [5].

The higher triple product in VNS group suggests an increase in myocardial oxygen consumption, which could be the cause, at least in part, of the larger infarct size [6]. The administration of atenolol attenuated this increase and particularly reserpine completely reversed this effect. Both findings strongly support this hypothesis highlighting the relationship between infarct size and an increase in oxygen consumption caused by the co-activation of the sympathetic pathway.

Other studies have shown beneficial effects of vagal stimulation on infarct size [7]. However, in this study, reperfusion was allowed for 8 weeks and vagal stimulation was applied throughout ischemia and during the first three days of reperfusion. Our results strongly suggest that the co-activation of the autonomic pathways would result in greater damage; and that in order to obtain favorable results—as suggested by other authors [8], an antagonist action between pathways would be required. Since in our study oxygen consumption persisted elevated in VNS group, we cannot rule out the existence of another mechanism responsible for the findings.

Furthermore, since vagal stimulation is used to treat human diseases, these findings should be taken into consideration for the potential side effects of this therapeutic technique.

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