Vagal Stimulation, Through its Nicotinic Action, Limits Infarct Size and the Inflammatory Response to Myocardial Ischemia and Reperfusion

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Abstract: Vagal activity has protective effects in ischemic heart disease. We tested whether vagal stimulation (VS) could modulate the inflammatory reaction, a major determinant of cardiac injury after ischemia/reperfusion. Four groups of male rats underwent myocardial ischemia (30 minutes) and reperfusion (24 hours). One group underwent VS (40 minutes), 1 VS plus atrial pacing (VS + Pacing), and 1 VS plus nicotinic inhibition by mecamylamine (VS + MEC). After 24 hours, the area at risk, infarct size, inflammation parameters, and apoptosis were quantified. Infarct size was reduced in all VS-treated rats (controls, 53 ± 18%; VS, 6.5 ± 3%; VS + Pacing, 23 ± 6%; VS + MEC, 33 ± 9%; P < 0.005 vs. controls). The infarct size in the VS + MEC group was larger than that in VS-treated animals, despite similar heart rate, suggesting partial loss of protection. The number of macrophages, neutrophils, and apoptotic cells in the area at risk and the plasma cytokines levels were significantly reduced in all VS-treated animals. In conclusion, VS decreases infarct size and inflammatory markers during ischemia/reperfusion independent of the heart rate. The anti-inflammatory and antiapoptotic properties of the nicotinic pathway are the primary underlying mechanism. The vagally mediated modulation of inflammatory responses may prove valuable in the clinical management of acute coronary syndromes and of heart failure.

Key Words: vagal activity, ischemia/reperfusion, myocardial infarction, autonomic nervous system, infarct size, inflammatory markers

INTRODUCTION

Recent clinical evidence suggests that chronic vagal stimulation (VS) is feasible in man and that it might be beneficial in the setting of chronic ischemic cardiomyopathy and heart failure.1,2 We are now advancing the hypothesis that VS might have protective effects also at a much earlier stage, when the initial ischemic insult occurs.

Ischemia and reperfusion have complex and deleterious effects on the myocardium. Inflammatory mediators are essential in the healing process of injured tissue, but when, after reperfusion, their response is excessive, they may worsen myocardial injury.3

Reperfusion is followed by rapid cellular infiltration of neutrophils and monocytes and by the release of several proinflammatory cytokines which orchestrate inflammation and tissue repair processes.3,4 Among them, lipopolysaccharide-inducible CXC chemokine (LIX) and monocyte chemokine-attraction protein-1 (MCP-1) play a pivotal role.5,6 There is a fine balance between the inflammatory reaction, which follows reperfusion with the potential of extending myocardial damage, and the mechanisms, which can positively affect tissue repair. A sound approach to effective management of this important clinical problem has been hampered by what seems to be a physiological catch 22: on one hand, inflammatory mediators are essential in the healing of injured tissue; on the other hand, an excessively intense inflammatory response after reperfusion may actually worsen myocardial injury.

A rationale solution might be provided by identifying means to physiologically modulate this inflammatory reaction. One of the areas, largely unexplored and of potential relevance in the modulation of the responses to ischemia and reperfusion, is the neural control of the heart. We consider the possibility that vagal activity might represent 1 such physiological modulator. We focused on the vagus nerve because there is evidence suggesting its significant role and because over the years, our experimental and clinical studies on the neural control of the heart have provided data relevant to the present study. We demonstrated the protective effect of VS in the prevention of ischemia-induced ventricular fibrillation,7 the association between depressed vagal reflexes, and increased cardiac mortality after a myocardial infarction,8,9 and more recently that chronic VS is not only feasible and safe in man but that it can effectively contribute to the management of patients with advanced heart failure.1,2
A protective role of 24-hour VS in experimental myocardial ischemia was suggested because of its association with decreased infarct size, cytokine expression, and neutrophil infiltration in reperfusion injury and represents an area of active research. To better understand the mechanisms underlying this apparent protection, we discriminated between the nicotinic and muscarinic actions of vagal activity and we also tested whether a more clinically realistic 40-minute stimulation would retain its efficacy.

MATERIALS AND METHODS

Our investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), it was approved by the Ethics Review Board of the Italian Ministry of Health, and all procedures were performed in accordance with the animal care guidelines of Federation of Laboratory Animal Science Associations. Ischemic injury, with or without VS, was made in male Sprague-Dawley rats (250–295 g).

Experimental Protocol

Sprague-Dawley rats were randomized into 5 groups, and results are reported for the 44 animals that completed the protocol. All treatment animals received a bolus of 5 mg/kg carprofen SC 30 minutes before surgery as analgesic. The protocol of each group is as follows:

- **Group 1 (n = 13):** controls; ischemia (30 minutes) and reperfusion (24 hours). The right vagus was exteriorized but not stimulated.
- **Group 2 (n = 6):** ischemia and reperfusion with VS (the right cervical vagus was stimulated from 5 minutes before ischemia to 5 minutes after reperfusion).
- **Group 3 (n = 9):** same as group 2 but with the addition of left atrial pacing to maintain heart rate at same level present in group 1.
- **Group 4 (n = 9):** same as group 2 but with the addition of mecamylamine (MEC) injection in the heart to inhibit nicotinic receptors.
- **Group 5 (n = 7):** sham-operated rats, no ischemia and reperfusion.

Ischemia and Reperfusion

All rats were anesthetized with isoflurane and ventilated through an endotracheal cannula. Anesthetized animals were continuous monitored for body temperature and electrocardiogram (ECG).

The left coronary artery was ligated with 4-0 silk suture (Ethicon), and a plain knot was tied over 2 pieces of suture that were pulled after 30 minutes to reperfuse the heart. The chest was closed under negative pressure. Ischemia was always confirmed by the appearance of discoloration of the cardiac surface and ST elevation on ECG, whereas reperfusion was verified by reddening of previously discolored area and by the presence of arrhythmias on ECG. Sham-operated rats underwent the same surgical procedures, without ligation of the left coronary artery.

Nicotinic Inhibition

MEC, the inhibitor of nicotinic receptors, was injected in the coronary arteries of the animals assigned to group 4. After clamping the ascending tract of the aortic arch, a bolus of MEC (2.1 mg/kg) was injected in the left ventricle. The clamp was removed after the injection; overall, the procedure lasted less than 30 seconds.

VS and Pacing

Electrical stimulation was performed by a digital stimulator Grass S88. The right vagus nerve was exteriorized and isolated at the cervical level through a single midline incision, and an electrode was placed around it. Stimulus parameters were 2.5 V, 0.5-ms pulse duration, and 8–10 Hz frequency. Atrial pacing was performed by means of a 29-gauge needle electrode placed in the left atrium, whereas the reference electrode was placed on the chest. In the VS + Pacing group, heart rate was maintained at 330 beats per minute (bpm), (the average value observed in the control rats).

Sample Collection and Determination of Area at Risk and Infarct Size

After 24 hours of reperfusion, the chest was reopened under anesthesia and ventilation. One milliliter of blood was withdrawn in heparin and centrifuged for 14 minutes at 3500 revolutions per minute at 4°C, and plasma was stored at −20°C. Then the ligature around the coronary artery was tightened again and 2 mL of 5% Evans Blue in saline solution was injected into the right ventricular chamber to distinguish the area at risk from the perfused area of the left ventricle. The heart was then excised and cut transversally into 3 main slices. In the first slice, the septum and the area at risk were excised and quickly frozen in liquid nitrogen. The second slice was fixed in formalin for histology and immunohistochemistry. The third slice was frozen at −20°C in optimal cutting temperature compound for tissue inclusion and cut transversally in 1-mm serial sections with the Mcllwain tissue chopper and incubated for 20 minutes at 37°C in triphenyltrazolium chloride. Photographs of the sections were taken, and the areas of perfused tissue (blue), viable ischemic tissue (red), and necrotic ischemic tissue (white) were measured with an image analyzer (Image Tool UTHSCSA for Windows) and was used to quantify the perfused left ventricle, the area at risk, and the necrotic area. (Fig. 1). The necrotic area was expressed as the percentage of the area at risk.

Determination of Macrophage and Neutrophil Infiltration, α-7 Subunit of the Nicotinic Acetylcholine Receptor Expression on Cardiac Macrophages, and Apoptosis

One coronal slice of the heart was formalin-fixed and paraffin-embedded; then, 4 serial sections were cut from each block and the following immunostaining reactions were performed.

Macrophages

After deparaffinization in xylene and endogenous peroxidase blockade with 3% hydrogen peroxide for 20 minutes, antigen retrieval was carried out by microwave treatment in 0.01
M EDTA buffer. Slides were incubated for 2 hours at room temperature with a mouse antirat CD68 monoclonal antibody (1:100; AbD Serotec, Oxford, United Kingdom). After washing with Tris buffer solution, slides were incubated with a “mouse on rat” HRP-polymer kit (Biocare Medical, Concord, CA) and Super Sensitive IHC detection system (BioGenex, San Ramon, CA), respectively. Peroxidase activity was visualized with diaminobenzidine. Slides were counterstained with hematoxylin.

**Double Immunofluorescence Staining for Macrophages and α-7nAChR**

After incubation with the primary antibodies (mouse antirat CD68 at 1:50 and a rabbit antirat polyclonal antibody α-7nAChR 1:10 from Abcam, Cambridge, United Kingdom), slides were incubated with secondary antibodies (Alexa Fluor 488 antirabbit and IgG and Alexa Fluor 594 antimouse IgG; Invitrogen, Carlsbad, CA) both at 1:500 dilutions. Nuclei were counterstained with Hoechst (1:500).

**Polymorphonuclear Leukocytes**

Naphthol AS-D chloroacetate esterase kit (Sigma-Aldrich, Inc, St Louis, MO) was used to detect polymorphonuclear leukocytes (PMN).

**Apoptosis**

In situ detection of apoptosis was performed using CardioTacs kit (Trevigen, Inc, Gaithersburg, MD) according to the manufacturer’s instructions. Optimized conditions in our laboratory were permeabilization with proteinase K at 37°C for 15 minutes and labeling procedure with TdT enzyme for 90 minutes.

**Plasma Cytokines Expression**

**Cytokine Array**

To detect LIX expression, we used a Rat Cytokine Array Panel A kit (R&D Systems) following the kit instructions. Briefly, the membranes were blocked with a blocking buffer and then plasma was incubated for 1 hour at room temperature. The blocking buffer was then removed from the membranes, and the sample–antibody mix was added for the overnight incubation at 4°C. After washing, the membranes were incubated at room temperature for 30 minutes with 1.5 mL of horseradish peroxidase–conjugated streptavidin and then thoroughly washed. The membranes were then developed by using enhanced chemiluminescence technique (GE Healthcare, formerly Amersham Biosciences), exposed to x-ray film, and processed by autoradiography.

To detect MCP-1 expression, we used a Rat Cytokine Antibody Array kit (RayBiotech) following the kit instructions. Briefly, the membranes were blocked with a blocking buffer and then incubated with the plasma samples for 2 hours at room temperature. The membranes were washed and then incubated with 1 mL of primary biotin-conjugated antibody at 4°C overnight. After washing, the membranes were incubated at room temperature for 2 hours with 2 mL of horseradish peroxidase–conjugated streptavidin and then thoroughly washed. The membranes were then developed by using enhanced chemiluminescence technique, exposed to X-ray film and processed by autoradiography. For data analysis, autoradiograms of the arrays were scanned to determine the density of the protein array positions and pixel intensity was measured with the image analyzer NIH ImageJ 1.41 software for Windows. The values from scans were adjusted based on the intensity of control spots on the filter corners.

**Statistical Analysis**

Continuous variables are presented as mean ± SD. Their comparisons between groups were performed by 1-way analysis of variance. All variables, with the exception of heart rate and area at risk, for which the analysis of variance showed significant differences were further analyzed by post hoc comparisons using the Bonferroni method. Whenever the assumption of homogeneity of variance, evaluated by the Levene test, was questionable, the Games–Howell test for multiple comparisons was used. $P < 0.05$ was considered significant.

**RESULTS**

**Effect of VS on Infarct Size and Heart Rate**

At baseline, heart rate was similar in all groups (Table 1). Similarly, the area at risk, calculated as percentage of the left ventricle, was not significantly different (61 ± 16% in the control group, 52 ± 8% in the VS group, 60 ± 10% in the VS + Pacing group, and 48 ± 8% in the VS + MEC group; $P = NS$). VS markedly decreased infarct size, compared with control, from 53 ± 18% to 6.5 ± 3% ($P < 0.001$). Keeping heart rate controlled by atrial pacing blunted, but did not eliminate, the
protective effect of VS, as infarct size was still significantly reduced to 23 ± 6% in the VS + Pacing group (P < 0.01; Fig. 1). Inhibition of cardiac nicotinic receptors by MEC reduced the protective effect of VS because the infarct size in group 4 (VS + MEC) was significantly larger (33 ± 9% vs. 6.5 ± 3%, P < 0.001) despite a similar reduction in heart rate during ischemia (234 ± 12 bpm in the VS group, 251 ± 17 bpm in the VS + MEC group; P = NS).

**VS and Inflammatory Response**

**Effects on Infiltrating Cells**

Compared to control animals, VS significantly reduced the number of infiltrated macrophages in all treatment groups with the exception of VS + MEC (Figs. 2A, B and Table 1, P < 0.001 for all comparisons). Pacing did not affect the result of VS, as the number of infiltrating macrophages in the area at risk remained essentially the same in these 2 groups (491 ± 240 and 600 ± 215 in the VS and the VS + Pacing groups, respectively). Cardiac nicotinic receptor inhibition associated with VS produced a nonsignificantly greater number of macrophages compared with VS (962 ± 381 vs. 491 ± 240, NS). In the sham-operated animals, the number of infiltrated macrophages in the left ventricle free wall was extremely low (33 ± 28) and significantly lower compared with all the other groups (Table 1). The macrophages infiltrating the infarcted and reperfused myocardial tissue expressed immunoreactivity for the α-7nAChR (Fig. 3).

VS had a similar effect in reducing the number of PMN in myocardial tissue 24 hours after ischemia and reperfusion.

### Table 1. Effect of VS and Nicotinic Receptor Inhibition on Heart Rate, Area at Risk, Infarct Size, Apoptosis, Macrophages, and PMN After Ischemia and Reperfusion

<table>
<thead>
<tr>
<th>Groups</th>
<th>Basal HR (bpm)</th>
<th>HR After Ischemia or Sham (bpm)</th>
<th>AAR/LV (%)</th>
<th>IS/AAR (%)</th>
<th>No. Macrophages in AAR</th>
<th>No. PMN in AAR</th>
<th>No. Apoptotic Cell in AAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>332 ± 43</td>
<td>334 ± 39</td>
<td>61 ± 16</td>
<td>53 ± 18</td>
<td>1406 ± 343</td>
<td>174 ± 59</td>
<td>190 ± 88</td>
</tr>
<tr>
<td>VS</td>
<td>303 ± 38</td>
<td>234 ± 15†</td>
<td>52 ± 8</td>
<td>6.5 ± 3†</td>
<td>491 ± 240†</td>
<td>48 ± 39**</td>
<td>49 ± 27*</td>
</tr>
<tr>
<td>VS + Pacing</td>
<td>301 ± 41</td>
<td>330 ± 0¶</td>
<td>60 ± 10</td>
<td>23 ± 6**†</td>
<td>600 ± 215†</td>
<td>60 ± 41**</td>
<td>59 ± 10*</td>
</tr>
<tr>
<td>VS + MEC</td>
<td>327 ± 25</td>
<td>251 ± 17†</td>
<td>48 ± 8</td>
<td>33 ± 9¶</td>
<td>962 ± 381</td>
<td>142 ± 85•</td>
<td>153 ± 67•</td>
</tr>
<tr>
<td>Sham</td>
<td>344 ± 54</td>
<td>303 ± 49</td>
<td>NA</td>
<td>NA</td>
<td>33 ± 28†</td>
<td>42 ± 27†</td>
<td>45 ± 9*</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, †P < 0.001 vs. control. ‡P < 0.05, §P < 0.01 VS + Pacing or VS + MEC vs. VS.

†P < 0.05 vs. sham; •P < 0.01 vs. sham.

AAR, area at risk; HR, heart rate; IS, infarct size; L V, left ventricle; NA, not applicable.

![FIGURE 2](image-url). Macrophage and PMN infiltration in the area at risk. Immunohistochemical staining shows macrophage and PMN infiltration in control animals (A and C, respectively) and in animals treated with VS (B and D) (magnification, ×20).
Compared with control animals, the number of PMN was reduced significantly \((P < 0.01)\) in both VS and VS + Pacing groups (Figs. 2C, D and Table 1). In contrast, the number of PMN in the VS + MEC group was not significantly different from the control group and from the VS and VS + Pacing groups.

In the sham-operated animals, the number of PMN was 42 ± 27, markedly lower versus controls \((P < 0.001)\), lower versus VS + MEC group \((P < 0.05)\), but similar to that of VS and VS + Pacing groups.

### Effects on Cytokines

A rat cytokine-array approach, to detect the presence of proteins in the plasma, was used to identify specific cytokines released after myocardial ischemia and reperfusion injury. Data from a representative array are shown in Figure 4. The signal intensity for the 2 cytokines involved in the recruitment of neutrophils (LIX) and macrophages (MCP-1) was 2-fold higher in the heart of control rats when compared with that in sham-operated rats. VS, even in presence of controlled HR, decreased the signal intensity by almost 2-fold, thus reversing

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**FIGURE 3.** Immunostaining for the \(\alpha-7nAChR\) and CD68. A, Staining for \(\alpha-7nAChR\) in green. B, CD68 staining in red; the microphotograph in (C) represents the merge of (A) and (B). D, Another representative double staining for \(\alpha-7nAChR\) and CD64, clearly demonstrating that the infiltrating macrophages coexpress \(\alpha-7nAChR\) receptor.

**FIGURE 4.** Rat protein array analysis was used to determine the differences in the level of rat plasma cytokines during ischemia and reperfusion injury (control). Cytokines levels were decreased by VS + Pacing (VS + PAC) treatment. Autoradiographs of the arrays were scanned to determine the density of the protein array positions. The values from scans were adjusted based on the intensity of control spots on the filter corners, and the level decreases for specific cytokines are shown.
the consequences of ischemia and reperfusion on cytokine plasma levels. Local inhibition of nicotinic receptors in VS + MEC–treated rats did not affect the plasma levels of LIX and MCP-1 with respect to VS-treated animals.

VS and Apoptosis

Compared with control myocardial tissue, which contained 190 ± 88 apoptotic cells per area at risk, the myocardium of rats undergoing VS showed (Fig. 5) a marked and significant reduction in their number (49 ± 27), which was not modified by pacing (59 ± 10) (P < 0.05 for both comparisons). Cardiac nicotinic receptor inhibition resulted in an increased number of apoptotic cells with respect to VS group (153 ± 67 in VS + MEC group vs. 49 ± 27 in VS-treated rats, P ≤ 0.05). In the sham-operated animals, this number was 45 ± 9, almost identical to that observed with VS and similar to that of VS + Pacing (NS); in contrast, it was significantly (P < 0.05) lower compared with controls and also with VS + MEC, thus showing that nicotinic receptor inhibition had largely interfered with the protective effect of VS.

DISCUSSION

The main finding of the present study is that a brief period of VS, independent of its muscarinic action, drastically limits infarct size and favorably attenuates the physiological response to acute myocardial ischemia and reperfusion by its nicotinic pathway. This protective effect is independent from heart rate changes, is demonstrated by a significant reduction in the number of macrophages, PMN, and apoptotic cells, and is paralleled by decreased levels of circulating proinflammatory cytokines.

These results provide new insights on how the neural control of the heart may modulate cardiac responses to life-threatening events and limit, or worsen, the damage produced by ischemia and reperfusion. Also, they may offer a clue to understand, at least in part, some recent results obtained by chronic VS in patients with heart failure, which are not entirely explained by heart rate changes.1,2

Cardiac Responses During Ischemia–Reperfusion and the Role of VS

At the time of reperfusion, blood containing leukocytes enters an area rich in chemotactic factors and inflammatory mediators.19,20 VS inhibits not only 2 of the key players in this process, LIX (IL-8 analogue in the rat) and MCP-1, but also the infiltration by PMN and macrophages, which is essential in the entire innate immune process after myocardial reperfusion injury.21

LIX is a murine chemokine with almost the same function as IL-8, responsible for 80% of PMN infiltration into an ischemic myocardium.5 MCP-1 (CCL2) is a small cytokine with profibrotic properties that recruits monocytes, memory T cells, and dendritic cells to sites of tissue injury and seems to regulate fibrous tissue deposition in the injured heart, critically regulating also mononuclear cell recruitment and activation in healing myocardial infarcts.5,22 All these cytokines have proinflammatory properties that seem to be clinically relevant. LIX attracts PMN in the site of injury, thus allowing the intense reaction triggered by myeloperoxidase (MPO). MPO is a peroxidase present in PMN that damages cardiomyocytes during reperfusion. MPO serum levels in patients with acute coronary syndrome, monitored during a 6-month follow-up, correlate significantly with increased risk for subsequent cardiovascular events.23

MCP-1, responsible for the recruitment of monocytes to sites of inflammation, seems to play a critical role in atherosclerosis and in remodeling after myocardial infarction. In a large cohort of patients with acute coronary syndromes, an elevated baseline level of MCP-1 was associated both with traditional risk factors for atherosclerosis and with an increased risk for death or myocardial infarction.24 Moreover, in patients with congestive heart failure, MCP-1 levels were significantly inversely correlated with left ventricular ejection fraction and were particularly high in those with heart failure of ischemic origin.25

VS inhibits also the apoptotic process in the ischemic and reperfused myocardium, a possible explanation being a PI3K/Akt/HIF-1alpha pathway activation inside the target cells. This would be supported by the evidence that acetylcholine had protective effects on rat cardiomyocytes subjected to normoxia/hypoxia by increasing Akt phosphorylation and preventing hypoxia-induced apoptosis and mitochondrial membrane potential collapse.26 Activation of the same pathway by VS could explain our results.

When taken together, these data strongly suggest that the immune reactions occurring during acute myocardial ischemia can worsen prognosis and that the molecules analyzed in the present study and modified by VS are likely to play a major role in the harmful side effects of innate immune responses.
VS and Nicotinic Inhibition

The specific functional role of the nicotinic pathway in our results was assessed by inhibiting nicotinic receptors with MEC during VS. The myocardial nicotinic receptors were inhibited by a local injection in the heart without affecting the systemic cholinergic preganglionic neurons. Despite a similar heart rate–lowering effect, cardiac nicotinic receptor inhibition by MEC largely prevented the reduction of both the infarct size and the number of macrophages, PMN and apoptotic cells produced by VS alone. Furthermore, local inhibition of the nicotinic receptors did not affect the plasma content of LIX and MCP-1 with respect to VS-treated animals, suggesting that the nicotinic pathway importantly contributed to the vagally mediated cardioprotection.

It is true that 77% of the infarct size reduction produced by VS was lost after inhibition of the nicotinic receptors, but this also means that 23% is contributed by the muscarinic component. We have elected not to block muscarinic receptors because the ensuing large increase in heart rate would have introduced a significant confounder.

The Cholinergic Anti-inflammatory Pathway

We explored whether it might be reasonable to suggest an involvement of the cholinergic anti-inflammatory pathway in myocardial ischemia and reperfusion injury. This pathway is a bidirectional communication between the brain and the immune system that seems to play a critical role in the control of inflammation as cholinergic neurons inhibit acute inflammatory response and, conversely, inflammation in peripheral tissue alters neural signaling in hypothalamus. It seems that one of the main cell types involved in this process is the macrophage, through the α7nAChR on its surface.

We tested whether the macrophages infiltrated in the heart would express α7nAChR on their surface after ischemia and reperfusion, which would suggest a possible involvement of the cholinergic anti-inflammatory pathway in myocardial reperfusion injury. By performing a double staining with immunofluorescence on macrophages infiltrated in the myocardial injured tissue, we did indeed observe that they exhibited immunoreactivity for the subunit α7 on their surface. The macrophages expressing α7nAChR were detected in several zones of the area at risk.

The lack of a commercial antibody able to neutralize bioactivity in vivo hampered our possibility of inhibiting α7nAChR receptors on infiltrated macrophages or in other cells in the rat. As a consequence, we regard the α7nAChR staining on macrophages surface just as a further step suggesting the involvement of the cholinergic anti-inflammatory pathway in myocardial reperfusion injury. However, despite the evidence of an anti-inflammatory and cardioprotective property of the nicotinic component of VS, we could not discriminate between the different subunits of the nicotinic receptor. It is reasonable to posit that nicotinic protection could actually be mediated by the α7 receptor subunit, but the evidence for the real contribution of the cholinergic anti-inflammatory pathway to the myocardial response to reperfusion injury must await further proof.

Relationship With Heart Failure of Ischemic Origin and Clinical Implications

The present data are clinically relevant, given that the strong evidence, experimental and clinical, linking depressed vagal activity to poor outcome in patients with ischemic heart disease and heart failure, has already prompted a first-in-man assessment of the feasibility and safety of chronic VS with encouraging results. Our data show that even a brief VS, lasting only 40 minutes during ischemia and reperfusion can protect the myocardium from inflammatory and ischemic injury. Uemura et al observed cardioprotection after 24 to 72 hours of continuous VS after ischemia and reperfusion. A shorter (<1 hour) VS would be clinically implementable and might be considered in conjunction with angioplasty or other clinical conditions with a potential for reperfusion injury, such as bypass grafting or cardiac transplant.

VS shows a remarkable ability to modulate innate immune responses, cellular networks, and organ homeostasis. A major difference between a simple pharmacological inhibition of cytokines or leukocytes and VS is that the latter represents an already existing physiological anti-inflammatory mechanism, which just needs reinforcement. The possibility of fine-tuning, physiologically a complex process involving cytokines, PMN, and neurohormones, is especially attractive in light of the current failure of pharmacological interventions aimed to modulate the inflammatory responses to ischemic heart disease and heart failure. The fact that all these responses can be modulated by VS points to unexpected cross talk between pathways present in the myocardium and raises the possibility of novel therapeutic interactions.

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