Disruption of vagal efferent axon and nerve terminal function in the postischemic myocardium

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Kawada, Toru, Toji Yamazaki, Tsuyoshi Akiyama, Hidezo Mori, Kazunori Uemura, Tadayoshi Miyamoto, Masaru Sugimachi, and Kenji Sunagawa. Disruption of vagal efferent axon and nerve terminal function in the postischemic myocardium. Am J Physiol Heart Circ Physiol 283: H2687–H2691, 2002.—Despite the importance of vagal control over the ventricle, little is known regarding vagal efferent conduction and nerve terminal function in the postischemic myocardium. To elucidate postischemic changes in the cardiac vagal efferent neuronal function, we measured myocardial interstitial acetylcholine (ACh) levels by using in vivo cardiac microdialysis and examined the ACh responses to electrical stimulation of the vagi or local administration of ouabain in anesthetized cats. Sixty-minute occlusions of the left anterior descending coronary artery (LAD) followed by 60-min reperfusion abolished electrical stimulations of the left anterior descending coronary artery (LAD) and decreased to 20.4 ± 3.9 vs. 0.9 ± 0.4 nmol/l; means ± SE, P < 0.01). In different groups of animals, 60-min LAD occlusion followed by 60-min reperfusion decreased but did not completely abolish ouabain-induced release of ACh (9.2 ± 1.8 vs. 3.9 ± 0.7 nmol/l; P < 0.05). These results indicate that function of the vagal efferent axon was completely interrupted, whereas the local ACh release was partially suppressed in the postischemic myocardium. The postischemic disruption of vagal efferent neuronal function might exert deleterious effects on cardiac regulation.

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local ACh release from the vagal nerve terminals alone. Results indicate that vagal efferent axonal conduction was interrupted, and local ACh release was suppressed in the postischemic myocardium 60 min after reperfusion after 60-min LAD occlusion.

MATERIALS AND METHODS

Surgical preparations. Animal care was provided in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences approved by the Physiological Society of Japan. A total of 37 adult cats was anesthetized via an intraperitoneal injection of pentobarbital sodium (30–35 mg/kg) and ventilated mechanically with room air mixed with oxygen. The depth of anesthesia was maintained with a continuous intravenous infusion of pentobarbital sodium (1–2 mg·kg\(^{-1}\)·h\(^{-1}\)) through a catheter inserted from the right femoral vein. Systemic arterial pressure was measured from a catheter inserted into the right femoral artery. Heart rate (HR) was determined by using a cardiotachometer from an electrocardiogram. Esophageal temperature of the animal was measured by using a thermometer (model CTM-303; Terumo) and was maintained at around 37°C by using a heated pad and a lamp.

With the animal in the lateral position, the left fifth and sixth ribs were resected to expose the heart. A dialysis probe was implanted by inserting a fine guiding needle into the anterolateral free wall of the left ventricle perfused by the LAD (1, 2, 10–13, 20, 21). Heparin sodium (100 U/kg) was administered intravenously to prevent blood coagulation. When LAD occlusion was required, a 4-0 silk suture was passed around the LAD just distal to the first diagonal branch, and both of its ends were passed through a polyethylene tube to make a snare for occlusion. The discoloration area on the LAD occlusion was large enough to cover the full length of the implanted dialysis fiber macroscopically. When vagal stimulation was required, the vagi were exposed bilaterally in the neck. Bipolar platinum electrodes were then attached to the cardiac end of each sectioned vagal nerve. The nerves and electrodes were covered with warmed mineral oil for insulation. A pair of stainless steel wire electrodes were attached to the left ventricular apex removed from the implanted dialysis probe to pace the heart during electrical stimulation of the vagi.

At the end of the experiment, the experimental animals were killed with an overdose of pentobarbital sodium. Postmortem examination confirmed that the dialysis probe had been implanted within the left ventricular myocardium.

Dialysis technique. We measured the concentration of ACh in the dialysate sample as an index of myocardial interstitial ACh level (10–13). Materials and properties of the dialysis probe have been reported previously (2). Briefly, we designed a transverse dialysis probe. A dialysis fiber (13-mm length, 310 μm OD, 200 μm ID; 50,000 molecular weight cutoff) (model PAN-1200; Asahi Chemical, Japan) was glued at both ends to polyethylene tubes (25-cm length, 500 μm OD, 200 μm ID). The dialysis probe was perfused at a rate of 2 μl/min with Ringer solution containing a cholinesterase inhibitor, eserine (100 μM). Experimental protocols were initiated 2 h after implanting the dialysis probe. The dialysate sampling period was set at 15 min and was performed taking into account the dead space volume between the dialysis membrane and the sample tube. Concentration of ACh in the dialysate was measured by high performance liquid chromatography with electrochemical detection (Eicom).

Protocols. Acute myocardial ischemia was induced by LAD occlusion. The study consisted of the following two different protocols (Fig. 1). In protocol 1, the myocardial interstitial ACh response to electrical stimulation of the vagi was examined (2, 13). Fifteen-minute vagal stimulation (20 Hz, 1 ms pulse duration, 10 V pulse amplitude) was performed under ventricular pacing at 200 beats/min in the following groups of vagotomized animals: control (n = 7), 15-min ischemia followed by 60-min reperfusion (CO\(_{15}\) group, n = 5), and 60-min ischemia followed by 60-min reperfusion (CO\(_{60}\) group, n = 5). Pacing was applied only during the vagal stimulation and not during the ischemic period.

In protocol 2, the myocardial interstitial ACh response to local ouabain administration was examined (13, 20). We collected a baseline dialysate sample while perfusing the dialysis probe with Ringer solution. We then replaced the perfusate with Ringer solution containing ouabain (100 μM),
thereby locally administering ouabain through the dialysis probe. Local ouabain administration was performed in control animals (n = 8), animals with vagotomy (VX group, n = 6), and animals with intact vagi subjected to 60-min ischemia followed by 60-min reperfusion (CO group, n = 6).

Statistical analysis. All data are presented as means ± SE. In protocol 1, differences in the electrical stimulation-induced ACh release among control, CO15, and CO60 groups were examined by using one-way ANOVA followed by Dunnett’s test (7). In protocol 2, changes in the ACh concentration in the CO group were examined by using a repeated-measures ANOVA. When there was significant difference, Dunnett’s test was applied to identify the difference relative to the baseline ACh concentration. We also examined differences in the baseline or maximum ACh responses among the control, VX, and CO groups by using one-way ANOVA, followed by Dunnett’s test. Differences were considered significant when P < 0.05.

RESULTS

Figure 2 shows ACh liberated into the myocardial interstitium in response to vagal stimulation in protocol 1. The electrical stimulation-induced ACh response was suppressed in the CO15 group compared with the control group and abolished in the CO60 group.

Figure 3 depicts changes in the myocardial interstitial ACh level in response to local ouabain administration through the dialysis probe obtained in protocol 2. The ACh level was increased significantly from the baseline value after 15–30 min of ouabain administration. †P < 0.05.

Table 1 summarizes mean arterial pressure (MAP) and HR obtained from the CO group in protocol 2. Changes in MAP and HR 60 min after LAD occlusion and 60 min after reperfusion were insignificant compared with preocclusion values.

DISCUSSION

To our knowledge, this is the first report on in vivo ACh release in the postischemic myocardium. The finding is in marked contrast to observations of ischemia-induced ACh release in our previous studies (10–13). The following discussion will focus on two aspects: postischemic deterioration of axonal conduction and nerve terminal function of the vagal efferent nerve.

Fig. 2. Myocardial interstitial ACh response to electrical vagal stimulation obtained from protocol 1. The ACh response was attenuated in the CO15 group and abolished in the CO60 group compared with the C (control) group.

Fig. 3. Myocardial interstitial ACh response to local ouabain administration through the dialysis probe obtained in the CO group in protocol 2. The ACh level was increased significantly from the baseline value after 15–30 min of ouabain administration. †P < 0.05.

Fig. 4. Effects of local ouabain administration on the myocardial interstitial ACh levels obtained from protocol 2. A: baseline ACh levels for control, VX, and CO groups. B: maximum ACh levels obtained after 15–30 min of ouabain administration for control, VX, and CO groups.

Table 1 Changes in MAP and HR in animals with intact vagi

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<th>Pre</th>
<th>CO</th>
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<tr>
<td>MAP, mmHg</td>
<td>112 ± 7</td>
<td>105 ± 8</td>
<td>100 ± 7</td>
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<tr>
<td>HR, beats/min</td>
<td>192 ± 11</td>
<td>168 ± 6</td>
<td>172 ± 9</td>
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Values are means ± SE. Data were obtained at preocclusion (Pre), 60-min after occlusion (CO), and 60-min after reperfusion (Rep) of the left anterior descending coronary artery. MAP, mean arterial pressure; HR, heart rate.
60 min after reperfusion after LAD occlusion in the presence of occlusions lasting 15 or 60 min (Fig. 2). The attenuation of the ACh response to electrical stimulation in the CO group was similar to that achieved by pretreatment with a voltage-sensitive Na⁺ channel inhibitor, tetrodotoxin (13), suggesting complete damage to vagal efferent neuronal function in the posts ischemic myocardium. Because ouabain-induced ACh release was not abolished in the CO group in protocol 2 (Fig. 3), the disruption of vagal efferent neuronal function can be attributed mainly to damage to vagal axons. Inoue and Zipes (8) demonstrated that the vagal efferent neuronal function, assessed by the lengthening of the effective refractory period in the nonischemic myocardium apical to the ischemic region, is impaired heterogenously 5–20 min after coronary artery occlusion. The present results indicate that the disruption of the vagal efferent axons in the posts ischemic myocardium persists for at least 60 min after LAD reperfusion began.

It is unlikely that the LAD occlusion exerted mechanical damage to the intracardiac vagal efferent axons. The reasons are as follows. First, most vagal efferent fibers cross the atrioventricular groove diverging intramurally and are located in the subendocardium in the ventricle (22). Therefore, the vagal efferent fibers run apart from the LAD ligation snare. Second, the suppression of electrical stimulation-induced ACh release depended on the duration of LAD occlusion (Fig. 2). If the vagal efferent fibers were mechanically damaged by the ligation procedure, an abrupt interruption of electrical stimulation-induced ACh release should have resulted. The fact that 5-min brief LAD occlusion followed by 20-min reperfusion did not suppress the ACh release in response to subsequent 5-min brief LAD occlusion in a previous study (11) is also in opposition to the possible mechanical damage to the intracardiac vagal efferent axons by the ligation procedure.

Impaired local ACh release. Our previous studies indicated that acute myocardial ischemia increases myocardial interstitial ACh levels in the ischemic region (10, 12). In those studies, vagotomy did not abolish ischemia-induced ACh release, suggesting that a local release mechanism independent of the centrally mediated vagal control plays a significant role in the ischemia-induced ACh release. Whereas no significant differences were observed in MAP and HR 60 min after ischemia and reperfusion compared with preischemic baseline values (Table 1), marked ACh release evoked by acute myocardial ischemia might have caused depletion of ACh stores or structural membrane abnormalities in the vagal efferent nerve terminals. Although the baseline ACh levels were similar between the control and CO groups (Fig. 4), this does not guarantee functional recovery of the vagal nerve terminals in the posts ischemic myocardium. To assess the functional recovery of the vagal nerve terminals more precisely, we examined the effect of local ouabain administration on the myocardial interstitial ACh levels.

Integrity of the axoplasmic membrane may be judged by normal Na⁺-K⁺-ATPase activity. Ouabain inhibits the membrane Na⁺-K⁺-ATPase and induces intracellular Na⁺ accumulation, which leads to exocytotic ACh release via the reversal of Na⁺/Ca²⁺ exchanger, activation of voltage-sensitive Ca²⁺ channels, and/or intracellular Ca²⁺ mobilization (16, 18). Because Na⁺-K⁺-ATPase is the major target for ATP at the nerve terminal, ischemia induces a progressive failure of Na⁺-K⁺-ATPase activity by depletion of ATP. If the integrity of the axoplasmic membrane had been lost due to ischemia and the Na⁺-K⁺-ATPase remained inactive after reperfusion, ouabain would not be able to evoke ACh release in the posts ischemic myocardium. Similarly, if the ACh stores were depleted by ischemia-induced ACh release, ouabain would also fail to induce ACh release. However, ouabain increased ACh levels in CO group (Fig. 3), indicating that membrane function was restored within 60 min after reperfusion. The observed result is consistent with the fact that the Na⁺-K⁺-ATPase becomes active on reperfusion, leading to restoration of the Na⁺ gradient across the axoplasmic membrane in isolated rat hearts (19).

Whereas ouabain administration evoked myocardial interstitial ACh release, the ACh levels so induced were lower in the CO than in the control group (Fig. 4). To examine whether the difference in ouabain-induced ACh release is associated with the absence of baseline vagal activity, we performed local ouabain administration in vagotomized animals. As shown in Fig. 4B, the VX group showed that ACh levels were in between the control and CO groups. Therefore, absence of the baseline vagal activity due to the interruption of axonal conduction, on top of the impaired local ACh release, could contribute to the suppression of ouabain-induced ACh release in the CO group. According to a study by Schmid et al. (17), changes in the activity of choline acetyltransferase in ischemic myocardial tissue are insignificant 2.5 and 5 h after coronary artery occlusion. The discrepancy between ACh synthesis and ouabain-induced ACh release suggests that nerve terminal function depends not only on axoplasmic enzyme activity but also on extraneuronal circumstances, such as extracellular ion content.

Limitations. Methodological limitations associated with anesthesia and eserine administration have been described previously (10–13). Other limitations to the present study are as follows. First, we did not examine whether vagal efferent function after LAD occlusion was reversible in the long term. To answer this question, further studies focusing on the recovery of vagal efferent function by using chronic experimental models are required. Second, we did not assess the impact of dispersion of the vagal efferent neuronal function on the arrhythmogenesis. Vagal nerve activity is known to exert both antiarrhythmic and proarrhythmic effects on the heart (6). Further studies are clearly needed to elucidate the functional significance of the disruption of the vagal control on the posts ischemic cardiac events.

In conclusion, we found interruption of the myocardial ACh release in response to vagal stimulation as well as suppression of local ACh release in the posts ischemic myocardium 60 min after reperfusion after
60-min LAD occlusion. The disruption of vagal control in the postischemic myocardium might have deleterious effects on the heart.

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