Vagosympathetic interactions in ischemia-induced myocardial norepinephrine and acetylcholine release

TORU KAWADA,1 TOJI YAMAZAKI,2 TSUYOSHI AKIYAMA,2 MASASHI INAGAKI,1 TOSHIKI SHISHIDO,1 CAN ZHENG,1 YUSUKE YANAGIYA,1 MASARU SUGIMACHI,1 AND KENJI SUNAGAWA1

1Department of Cardiovascular Dynamics and 2Department of Cardiac Physiology, National Cardiovascular Center Research Institute, Osaka 565-8565, Japan

Received 12 June 2000; accepted in final form 25 August 2000

Kawada, Toru, Toji Yamazaki, Tsuyoshi Akiyama, Masashi Inagaki, Toshiaki Shishido, Can Zheng, Yusuke Yanagiya, Masaru Sugimachi, and Kenji Sunagawa. Vagosympathetic interactions in ischemia-induced myocardial norepinephrine (NE) and acetylcholine (ACh) release. Am J Physiol Heart Circ Physiol 280: H216–H221, 2001.—To elucidate the pathophysiological roles of vagosympathetic interactions in ischemia-induced myocardial norepinephrine (NE) and acetylcholine (ACh) release, we measured myocardial interstitial NE and ACh levels in response to a left anterior descending coronary occlusion in the following groups of anesthetized cats: intact autonomic innervation (INT, n = 7); vagotomy (VX, n = 6); local administration of atropine (Atro, n = 6); transection of the stellate ganglia (TSG, n = 5); local administration of phentolamine (Phen, n = 6); and combined vagotomy and transection of the stellate ganglia (VX + TSG, n = 5). The maximum NE release was enhanced in the VX group (141 ± 30 nmol/l, means ± SE, P < 0.05) compared with the INT group (61 ± 12 nmol/l). Neither the Atro (50 ± 24 nmol/l) nor VX + TSG groups (84 ± 25 nmol/l) showed enhanced NE release. The maximum ACh release was unaltered in the TSG and Phen groups compared with the INT group (19 ± 4, 18 ± 4, and 13 ± 3 nmol/l, respectively). These findings indicate that the cardiac vagal afferent but not efferent activity reduced the ischemia-induced myocardial NE release. In contrast, the cardiac sympathetic afferent and efferent activities played little role in the ischemia-induced myocardial ACh release.

cardiac microdialysis; coronary artery occlusion; vagal nerve; sympathetic nerve; cats

REFLEXES FROM THE VENTRICLES can be classified into two groups depending on their afferent pathways (10). Activation of the cardiac sympathetic afferent fibers increases sympathetic efferent nerve activity but decreases vagal efferent nerve activity. On the other hand, activation of the cardiac vagal afferent fibers induces reflex responses generally opposite to those resulting from activation of the cardiac sympathetic afferent fibers. Although the normal physiological stimuli for the cardiac afferent fibers are still in dispute, mechanical and chemical stimuli during heart diseases such as myocardial ischemia and infarction are considered to activate the cardiac reflexes (10, 31). To elucidate the pathophysiological roles of the cardiac reflexes, reflex responses to acute coronary artery occlusion have been investigated in animal experiments (5, 18, 21). In these experiments, the effects of cardiac reflexes were evaluated by changes in heart rate (HR), blood pressure, and cardiac sympathetic and vagal efferent nerve activities. However, the cardiac efferent nerve activity and effective neurotransmitter concentration can dissociate in the ischemic myocardium due to a local neurotransmitter release mechanism in both the sympathetic and vagal systems (11, 15, 24). As a result, how the cardiac reflexes modulate myocardial norepinephrine (NE) and acetylcholine (ACh) release in the ischemic region remains unknown. Because NE and ACh directly act on the myocardium, quantification of myocardial NE and ACh levels would be a most reliable evaluation of the effects of cardiac reflexes on the heart. We therefore used a cardiac microdialysis technique (1–3, 11–13, 28–30) to measure myocardial interstitial NE and ACh levels from in situ cat hearts while performing coronary artery occlusion. We tested the hypothesis that cardiac vagal afferent and efferent pathways play an important role in the regulation of the ischemia-induced myocardial NE and ACh release by using vagotomy or local administration of atropine. We also tested the hypothesis that cardiac sympathetic afferent and efferent pathways play an important role in the regulation of the ischemia-induced myocardial NE and ACh release by using transection of the stellate ganglia or local administration of phentolamine. The results indicated that the cardiac vagal afferent but not efferent activity reduced the ischemia-induced myocardial NE release. In contrast, the cardiac sympathetic afferent and efferent activities played little role in the ischemia-induced myocardial ACh release.

MATERIALS AND METHODS

Surgical preparation. Animal care was conducted in accordance with the “Guiding Principles for the Care and Use of

Address for reprint requests and other correspondence: T. Kawada, Dept. of Cardiovascular Dynamics, National Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan (E-mail: torukawa@res.nevc.go.jp).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Animals in the Field of Physiological Sciences” approved by the Physiological Society of Japan. Adult cats weighing 2.6–5.0 kg were 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. Mean systemic arterial pressure (MAP) was measured from a catheter inserted from the right femoral artery. HR was determined from an electrocardiogram.

With the animal in the lateral position, the left fifth and sixth ribs were resected to expose the heart. A 4–0 silk suture was passed around the left anterior descending coronary artery (LAD) just distal to the first diagonal branch for later LAD occlusion. With a fine guiding needle, a dialysis probe was implanted into the left ventricular free wall perfused by the LAD to measure myocardial interstitial NE and ACh levels in the ischemic region. Heparin sodium (100 U/kg) was administered intravenously to prevent blood coagulation before implanting the dialysis probe. A maintenance dose of heparin sodium (50 U/kg) was given every hour throughout the experiment.

At the end of the experiment, the experimental animals were killed by increasing the depth of anesthesia with an overdose of pentobarbital sodium. We confirmed that the dialysis probe had been implanted within the left ventricular myocardium.

**Dialysis technique.** We measured dialysate NE and ACh concentrations as indexes of myocardial interstitial NE and ACh levels, respectively. The materials and properties of the dialysis probe have been described elsewhere (2, 3). Briefly, we designed a transverse dialysis probe. A dialysis fiber [length 13 mm, outer diameter (OD) 310 µm, inner diameter (ID) 200 µm; PAN-1200, 50,000 molecular weight cutoff, Asahi Chemical, Japan] was glued at both ends to polyethylene tubes (length 20 cm, OD 500 µm, ID 200 µm). The dialysis probe was perfused at a rate of 2 µl/min with Ringer solution containing the cholinesterase inhibitor eserine (10⁻⁴ M). Dialysate samples were collected 2 h after implanting the dialysis probe, when the dialysate NE concentrations reached a steady state (2). Within this time period, the dialysate ACh concentrations had reached a steady state as well (3). One sampling period was set at 15 min, which yielded a sample volume of 30 µl. The actual dialysate sampling lagged by 5 min behind a given collection period, taking into account the dead space volume between the dialysis membrane and the sample tube. Each sample was collected in a microtube containing 3 µl of phosphate buffer (0.1 M; pH 3.5) to prevent amine oxidation.

Two-thirds of the dialysate sample was used for the ACh measurement, and the remaining one-third was used for the NE measurement. The dialysate ACh concentration was measured directly by high performance liquid chromatography (HPLC-ECD) (Eicom, Japan). The dialysate NE concentration was measured by another HPLC-ECD after removing interfering compounds by an alumina procedure. Details of HPLC-ECD for the NE and ACh measurements have been described elsewhere (2, 3).

**Protocols.** We occluded the LAD in animals with intact autonomic innervation (INT group, n = 7) for 60 min and collected four consecutive 15-min dialysate samples to measure ischemia-induced changes in myocardial interstitial NE and ACh levels. To examine the role of vagal innervation in the ischemia-induced NE and ACh responses, we performed the LAD occlusion protocol in animals undergoing transection of the bilaterally stellate ganglia (TSG group, n = 5). To evaluate the extent of presynaptic interactions via the sympathetic efferent activity while preserving the sympathetic afferent activity, we locally administered the α-adrenergic blocker phentolamine (10 µM) and performed the LAD occlusion protocol (Phen group, n = 6). We also examined the influences of combined vagotomy and transection of the stellate ganglia on the ischemia-induced NE and ACh responses (VX+TSG group, n = 5). The doses of local atropine and phentolamine administrations were determined based on the doses used in the previous experiment (1 µM each) (6) and the in vitro recovery of the dialysis probe (~10%). The local administration of pharmacological agent was started 60 min before the LAD occlusion and continued throughout the protocol.

**Statistical analysis.** All data are presented as means ± SE values. To examine the differences in the myocardial interstitial NE and ACh levels in each collection period among the INT, VX, Atro, TSG, Phen, and VX+TSG groups, we used one-way analysis of variance (9). When there was a significant difference among groups, we used Dunnett's test to determine the difference of each group against the INT group. Differences were considered significant when P < 0.05. To facilitate an intuitive comparison, data related to the vagal effects and those related to the sympathetic effects are separately presented despite the simultaneous multiple comparison among all groups. Furthermore, data obtained from the INT group are repeated in three illustrations for convenience. Differences in MAP and HR among groups were examined using the same statistical procedure.

**RESULTS**

Figure 1A shows the effects of vagotomy or the local administration of atropine on the ischemia-induced myocardial interstitial NE response. Figure 1A, inset, is an enlarged ordinate for the data at 0–15 min. The NE levels increased progressively as the ischemic period was prolonged in the INT group. The VX group showed significantly higher NE levels compared with the INT group at all collection periods. The Atro group did not show an enhanced NE response compared with the INT group. Figure 1B illustrates the ischemia-induced myocardial interstitial ACh responses obtained from the INT, VX, and Atro groups. In the INT group, ACh was elevated to a level comparable with the INT group at all collection periods. The Atro group showed significantly higher NE levels compared with the INT group. The VX+TSG group, which received combined vagotomy and transection of the stellate ganglia, showed significantly lower NE levels compared with the INT group.
period. The NE levels in the Phen group were similar to those in the INT group within 30 min of the LAD occlusion. The NE levels in the Phen group increased from 30 min after the LAD occlusion and were significantly higher than the INT group at 45–60 min. Figure 2B illustrates the ischemia-induced myocardial interstitial ACh responses obtained from the INT, TSG, and Phen groups. Although the ACh level seemed to be more elevated in the TSG group than in the INT group at 0–15 min, the difference was not statistically significant. Neither the TSG nor Phen group showed significant differences in the ACh levels when compared with the INT group at any collection period.

Figure 3 shows the effects of combined vagotomy and transection of the stellate ganglia on the ischemia-induced myocardial interstitial NE and ACh responses. The NE levels did not differ between the VX+TSG and INT groups at any collection periods (Fig. 3A). Although the ACh level seemed to be lower in the VX+TSG group than in the INT group at 0–15 min, there were no significant differences in the ACh levels at any collection period (Fig. 3B).

Table 1 summarized changes in MAP in response to the LAD occlusion. The Atro and Phen groups showed similar changes in MAP compared with the INT group throughout the experimental run. The baseline preischemic MAP was significantly higher in the VX and VX+TSG groups and was significantly lower in the TSG group compared with the INT group. MAP remained increased in the VX and VX+TSG groups compared with the INT group during the LAD occlusion. Differences in MAP between the TSG and INT group during the LAD occlusion were statistically insignificant.

Table 2 summarized changes in HR in response to the LAD occlusion. The Atro and Phen groups showed similar changes in HR compared with the INT group throughout the experimental run. The baseline preischemic HR was significantly lower in the TSG and VX+TSG groups than in the INT group. HR in the TSG group but not in the VX+TSG group remained decreased compared with the INT group during the LAD occlusion. There were no significant differences in HR between the VX and INT groups.

DISCUSSION

The present study demonstrated that the myocardial NE release in response to LAD occlusion was enhanced in the VX group compared with the INT group. No enhanced NE release was observed in the Atro or VX+TSG group. The ischemia-induced myocardial ACh release was hardly affected by any of the interventions in the present study.
Regulation of ischemia-induced myocardial NE release. The LAD occlusion progressively increased myocardial interstitial NE level in the ischemic region. The maximum NE level was more than 100 times the baseline preischemic NE level (0.5 ± 0.1 nmol/l) in the INT group (11). The ischemia-induced myocardial NE release was enhanced in the VX group compared with the INT group (Fig. 1A). Two mechanisms can be put forward to explain the suppression of the ischemia-induced NE release by the intact vagal innervation. One is a reflex inhibition of the cardiac sympathetic efferent activity through the vagal afferent activity (10). The other is a presynaptic inhibition of the NE release from the cardiac sympathetic nerve terminals caused by muscarinic receptor activation through the vagal efferent activity (17, 19). Because atropine did not affect the ischemia-induced NE release (Fig. 1A), the reflex inhibition rather than the presynaptic inhibition would account for the suppression of the ischemia-induced NE release by the intact vagal innervation. When the reflex inhibition was interrupted by vagotomy, the cardiac sympathetic efferent activity increased, resulting in enhanced NE release in response to acute myocardial ischemia. This interpretation is supported by the finding that vagotomy failed to enhance the ischemia-induced NE release when performed in combination with transection of the stellate ganglia (Fig. 3A). Higher MAP in the VX group than in the INT group during the LAD occlusion would reflect increased systemic sympathetic nerve activity (Table 1).

NE is released from the sympathetic nerve terminals via exocytotic and nonexocytotic release mechanisms (12, 22, 24, 28, 29). The marked increase in myocardial interstitial NE levels noted during acute myocardial ischemia has been mainly attributed to the nonexocytotic release mechanism (1, 24). The fact that the ischemia-induced NE response did not differ between the TSG and INT groups (Fig. 3A) supports the nonexocytotic NE release mechanism. Moreover, the present study indicates that when the cardiac sympathetic efferent activity was increased by vagotomy, the exocytotic NE release could occur on top of the nonexocytotic NE release, resulting in the enhanced NE response (Fig. 1A). Du et al. (7) demonstrated that electrical sympathetic nerve stimulation can evoke exocytotic NE release during myocardial ischemia in the innervated perfused rat heart. Taken together, although the exocytotic release mechanism was not impaired, the nonexocytotic release mechanism represented the ischemia-induced NE release in the INT group, because the sympathetic efferent activity was suppressed by the reflex inhibition through the vagal afferent activity. Although vagotomy would exacerbate ischemia thereby affecting myocardial NE release via

Table 1. Changes in MAP in response to left anterior descending coronary artery occlusion

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre</th>
<th>5</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>INT</td>
<td>117 ± 12</td>
<td>101 ± 6</td>
<td>103 ± 8</td>
<td>104 ± 7</td>
</tr>
<tr>
<td>VX</td>
<td>140 ± 9*</td>
<td>137 ± 8*</td>
<td>138 ± 8*</td>
<td>133 ± 7*</td>
</tr>
<tr>
<td>Atro</td>
<td>125 ± 5</td>
<td>101 ± 6</td>
<td>99 ± 6</td>
<td>102 ± 6</td>
</tr>
<tr>
<td>TSG</td>
<td>92 ± 5*</td>
<td>79 ± 10</td>
<td>84 ± 2</td>
<td>81 ± 2</td>
</tr>
<tr>
<td>Phen</td>
<td>110 ± 9</td>
<td>95 ± 3</td>
<td>96 ± 5</td>
<td>107 ± 6</td>
</tr>
<tr>
<td>VX + TSG</td>
<td>143 ± 9*</td>
<td>135 ± 6*</td>
<td>128 ± 3*</td>
<td>125 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Mean arterial pressure (MAP) (in mmHg) obtained during baseline, preischemic period (Pre), and after 5, 30, and 60 min of coronary occlusion. The following groups of animals were studied: INT, intact autonomic innervation; VX, bilateral vagotomy; Atro, locally administered atropine (10 μM); TSG, transection of the bilateral stellate ganglia; Phen, locally administered phentolamine (10 μM); and VX + TSG, combined vagotomy and transection of the stellate ganglia. *P < 0.05 against INT group.
the nonexocytotic release mechanism, because transection of the stellate ganglia abolished the enhanced NE release (the VX + TSG group), the enhanced NE release in the VX group would mainly depend on the exocytotic release mechanism.

Local administration of atropine did not affect changes in hemodynamics in response to the LAD occlusion compared with the INT group (Tables 1 and 2). The Atro group did not show a significant difference in the NE levels compared with the INT group. Cholinergic modulation of the exocytotic NE release is reduced during myocardial ischemia in the innervated perfused rat heart (7). Furthermore, because the exocytotic NE release is suppressed due to the reflex inhibition of the cardiac sympathetic efferent activity regardless of atropine administration, the presynaptic inhibition is thought to exert little effect on the myocardial NE release. Nevertheless, atropine administration reduces the antifibrillatory effect of electrical vagal stimulation against coronary occlusion-induced lethal arrhythmias in anesthetized cats (23). The present results imply that the antifibrillatory effect of electrical vagal stimulation is a direct effect of ACh on the myocardium rather than the presynaptic inhibition of NE release by ACh.

Local administration of phentolamine did not affect changes in hemodynamics in response to the LAD occlusion compared with the INT group (Tables 1 and 2). The ischemia-induced myocardial NE release was enhanced in the Phen group compared with the INT group at 45–60 min of the LAD occlusion (Fig. 2A). Because activation of presynaptic α2-adrenergic receptors on the sympathetic nerve terminals suppresses the NE release via a negative feedback mechanism (16), inhibition of the presynaptic α2-adrenergic receptors by phentolamine enhances the myocardial NE release (8). However, because the exocytotic NE release is suppressed by the reflex inhibition of the sympathetic efferent activity regardless of phentolamine administration, inhibition of presynaptic α2-adrenergic receptors alone could not account for the enhanced NE release by phentolamine. According to Kitakaze et al. (14), activation of α2-adrenergic receptors ameliorates myocardial ischemia through adenosine release from the ischemic myocardium. Therefore, inhibition of α2-adrenergic receptors by phentolamine likely aggravates myocardial ischemia, thereby increasing the nonexocytotic NE release.

Regulation of ischemia-induced myocardial ACh release. The LAD occlusion increased myocardial interstitial ACh level in the ischemic region. The maximum ACh level was about 20 times the baseline preischemic ACh level (0.7 ± 0.1 nmol/l) in the INT group (11). The intact sympathetic innervation can modulate ACh release from the vagal nerve terminals via two mechanisms. One is a reflex inhibition of the vagal efferent activity through the cardiac sympathetic afferent activity (10, 25). The other is a presynaptic inhibition of the ACh release from the vagal nerve terminals by the stimulation of α1-adrenergic receptors (27). Neuropeptide Y released from the sympathetic nerve terminals also suppresses the ACh release from the vagal nerve terminals (20). Regardless of these possible sympathovagal interactions, the ACh levels did not differ between the TSG and INT groups (Fig. 2B), suggesting that the intact sympathetic innervation contributed little to the modulation of the ischemia-induced ACh release. The phentolamine administration also failed to modulate ischemia-induced ACh release, indicating that the presynaptic inhibition of ACh release was insignificant. Although the negative feedback regulation of ACh release via the muscarinic receptors on the vagal nerve terminals has been demonstrated (26), the effect of atropine on the ischemia-induced ACh release was not significant (Fig. 1B). Taken together, the ischemia-induced ACh release in the ischemic myocardium was mainly attributable to the local release mechanism (11) and hardly affected by cardiac reflexes or presynaptic modulations.

There were several limitations to the present study. First, we investigated the myocardial interstitial NE and ACh levels in cats anesthetized with pentobarbital sodium. Because the barbiturate anesthesia affects both the autonomic afferent and efferent nerve activities, the results might have differed had the experiment been performed using a nonbarbiturate anesthesia or in the absence of anesthesia.

Second, because no proper method was available to inhibit the cardiac afferent activity while preserving neurotransmitter release from the corresponding cardiac efferent nerve terminals in both sympathetic and vagal pathways, we could not assess the extent of sympatho-sympathetic or vago-vagal reflexes (10). In addition, it was not possible to dissect out the relative importance of intrinsic cardiac ganglia in the modulation of cardiac NE and ACh release during myocardial ischemia (4). The effects of these interactions on the ischemia-induced myocardial NE and ACh release await further characterization.

Third, we administered eserine through the dialysis probe. Because ACh released from the vagal nerve terminal is immediately degenerated by acetylcholinesterase, cholinesterase inhibition was necessary to detect changes in the myocardial interstitial ACh levels. Although the results of electrical vagal stimulation suggest that changes in the myocardial interstitial ACh levels reflect ACh release from the postganglionic vagal nerve terminals (3), the possibility cannot be ruled out that eserine modified the ACh release from the vagal nerve terminals.

In conclusion, acute myocardial ischemia induced by LAD occlusion increased myocardial interstitial NE levels in the ischemic region mainly through the nonexocytotic release mechanism, because the sympathetic efferent nerve activity was suppressed by the reflex inhibition through the vagal afferent activity. When the reflex inhibition of the sympathetic efferent nerve activity was interrupted by vagotomy, NE can be released via the exocytotic release mechanism, resulting in enhanced NE release in the ischemic myocardium (Fig. 1A). Because the presynaptic inhibition of the NE release by ACh was insignificant, the reflex
inhibition of the NE release by the vagal afferent activity is considered to play an important role in modulating cardiac events during the early phase of myocardial ischemia. In contrast to the vagal modulation of the ischemia-induced myocardial NE release, there was no significant sympathetic modulation of the ischemia-induced myocardial ACh release, suggesting the predominance of the local release mechanism for the ACh release.

This study was supported by Research Grants for Cardiovascular Diseases (9C-1, 11C-3, and 11C-7) from the Ministry of Health and Welfare of Japan, by a Health Sciences Research Grant for Advanced Medical Technology from the Ministry of Health and Welfare of Japan, by the Special Funds for Encourage System of Center of Excellence from the Science and Technology Agency of Japan, by a Grant-In-Aid for Young Scientists (11770391) from Ministry of Education, Science, Sports and Culture of Japan, and by a grant provided by the Ichiro Kanehara Foundation.

REFERENCES