Sympathetic Stimulation and Norepinephrine Infusion Modulate Extracellular Potassium Concentration During Acute Myocardial Ischemia

Margaret R. Warner, Timothy S. Kroeker, and Douglas P. Zipes

The purpose of this study was to investigate whether sympathetic stimulation modulated the rise in extracellular K⁺ concentration ([K⁺]ₑ) evoked by acute myocardial ischemia. In 35 α-chloralose-anesthetized dogs, we measured changes in [K⁺]ₑ during acute myocardial ischemia in the presence and absence of sympathetic stimulation or norepinephrine infusion. A series of four 5-minute occlusions of the distal left anterior descending coronary artery (LAD) was completed in 18 dogs. Thirty minutes of reperfusion separated each LAD occlusion. Four to five K⁺-sensitive electrodes were inserted into the left ventricular midmyocardium that was perfused by the distal LAD. Lead II of the electrocardiogram, arterial pressure, and [K⁺]ₑ were recorded, and the right atrium was paced at a constant cycle length. The first, second, and fourth LAD occlusions were done in the absence of sympathetic stimulation or norepinephrine infusion. The changes in [K⁺]ₑ evoked by the first LAD occlusion differed (p<0.05) from those elicited by the second and fourth occlusions. However, the changes in [K⁺]ₑ during the second and fourth LAD occlusions were similar (p>0.2) and served as controls for the responses obtained during the third occlusion. Two minutes before the third LAD occlusion, sympathetic stimulation (4 Hz) or norepinephrine infusion (0.25–0.5 μg/kg per minute i.v.) was begun and was continued until 2 minutes after reperfusion. We found that sympathetic stimulation and norepinephrine infusion increased (p<0.05) myocardial blood flow in both normal and ischemic tissue. The mean response recorded by 23 K⁺-sensitive electrodes in 11 dogs showed that sympathetic stimulation increased (p<0.001) the [K⁺]ₑ at 1, 2, 3, 4, and 5 minutes after the onset of LAD occlusion compared with the second and fourth occlusions. In contrast, the mean response recorded by 20 K⁺-sensitive electrodes in seven dogs showed that norepinephrine infusion reduced (p<0.02) the [K⁺]ₑ at 4 and 5 minutes after the onset of LAD occlusion. These data show that sympathetic stimulation increased the [K⁺]ₑ evoked by acute myocardial ischemia, an effect that was not mimicked by the intravenous administration of norepinephrine. (Circulation Research 1992;71:1078–1087)

KEY WORDS • sympathetic nervous system • norepinephrine • extracellular potassium concentration • myocardial ischemia • potassium

Acute myocardial ischemia evokes rapid changes in the metabolic, contractile, and electrical function of affected ventricular muscle and is often associated with life-threatening ventricular arrhythmias.1–5 The triggering event responsible for the ischemia-induced arrhythmias is not completely understood, but changes in intracellular and extracellular ion concentrations and alterations in autonomic neural activity influence the initiation and maintenance of these arrhythmias.1–14 The rapid rise in the extracellular K⁺ concentration ([K⁺]ₑ) occurring within a few seconds of ischemia depolarizes ventricular muscle cells and creates heterogeneous regions that provide a substrate for arrhythmia development.2–4,6–9 Indeed, intracoronary infusion of KCl elicits many of the electrophysiological changes evoked by acute myocardial ischemia.4,10 Inhomogeneities in the [K⁺]ₑ, between the endocardium and epicardium and between the center and border of the ischemic zone may also enhance arrhythmia development during acute myocardial ischemia.6–9

Alterations in cardiac autonomic neural activity also modulate ischemia-induced arrhythmias.1,11–14 Activation of the sympathetic nervous system during acute myocardial ischemia increases the likelihood for ventricular tachyarrhythmias,11–14 particularly reperfusion arrhythmias,13 and reduces the ventricular fibrillation threshold.12,14

Although increases in sympathetic activity and [K⁺]ₑ are both known to be arrhythmogenic during the early phase of myocardial ischemia, little is known about the interaction between these variables. Propranolol decreases the [K⁺]ₑ during acute myocardial ischemia,15,16 which suggests that β-adrenergic receptor stimulation...
would enhance ischemia-induced increases in \([K^+]_o\).

The purpose of this study was to test the hypothesis that sympathetic stimulation increases \([K^+]_o\) during acute myocardial ischemia. Thus, we measured ischemia-induced changes in \([K^+]_o\) in the presence and absence of sympathetic stimulation. To determine if norepinephrine was responsible for the sympathetically evoked changes in \([K^+]_o\), during ischemia, we also measured the changes in \([K^+]_o\) during left anterior descending coronary artery (LAD) occlusion in the presence and absence of an intravenous infusion of norepinephrine.

**Materials and Methods**

Dogs \((n=35)\) were premedicated with morphine sulfate \((2 \text{ mg/kg i.m.)}\) and anesthetized with sodium thiopental \((25 \text{ mg/kg i.v.)}\). Anesthesia was maintained with \(\alpha\)-chloralose \((60 \text{ mg/kg i.v. followed by 20 mg/kg per hour i.v.)}\). After intubation with a cuffed endotracheal tube, positive pressure ventilation was begun. The left femoral artery and vein were cannulated for arterial pressure measurement and drug infusions, respectively. Saline \((0.9\% \text{ NaCl})\) was infused to maintain fluid balance \((30 \text{ ml/hr i.v.)}\). The chest was opened via a median sternotomy, and the heart was suspended in a pericardial cradle. The right and left stellate ganglia were isolated, doubly ligated, and crushed. Bipolar stimulating electrodes were looped under the right and left ansae subclaviae, and the electrode wires were attached in parallel to a stimulator (model 6i, Haer). The right and left cervical vagi were isolated, doubly ligated, and transected.

The distal portion of the second or third diagonal branch of the LAD was dissected free, and a silk suture was positioned around the artery. The periadventitial tissue that contains many sympathetic fibers was left undisturbed as much as possible. The artery was totally occluded by lifting the suture and maintaining tension during the 5-minute occlusion. Releasing the suture allowed reperfusion. Before the occlusion, 250 units/kg heparin was infused intravenously, followed by 1,000 units/hr i.v.

A bipolar electrode catheter for atrial pacing was inserted into the right atrial appendage through a small incision. The right atrium was paced at a constant cycle length \((340–380 \text{ msec in individual dogs) throughout the experiment. The sinus node was crushed to attenuate the sinus tachycardia evoked by stimulation of the ansae subclaviae or norepinephrine infusion. Crushing the sinus node does not alter sympathetic effects on the ventricles.\[17\]

The \(K^+\)-sensitive electrodes were made according to the methods of Johnson et al.\[18\] The electrodes were fabricated by coating the tips of chlorided silver wire with a cellulose acetate–titantium dioxide sponge. A polyvinyl chloride–valinomycin coating provided the \(K^+\)-selective membrane. The electrodes were calibrated in vitro with solutions containing 2–20 mM KCl. The calibrations were done at 37°C in KCl–NaCl solutions with a total ionic strength of 0.15 M.\[19\] The NaCl concentration was adjusted to maintain constant ionic strength at the various KCl concentrations. Electrodes were used if they exhibited a 56–62-mV shift per 10-fold change in \([K^+]_o\).\[18\]

The wires from the reference and \(K^+\) electrodes were threaded retrograde into the tip of a 23-gauge needle. After inserting the electrode into the midmyocardium, the needle was withdrawn, leaving the \(K^+\) electrode in place. Four to five \(K^+\)-sensitive bipolar plunge electrodes were inserted into the midwall of the left ventricle 2–20 mm from the artery to be occluded. The integrity of the electrodes in vivo was assessed by responses to intravenous injections of 1.5 meq KCl in 3 ml saline.\[18\]

Lead II of the electrocardiogram, arterial blood pressure, and up to five \(K^+\) signals (in millivolts) were displayed on a strip-chart recorder (Gould, Inc., Cleveland, Ohio) and on a monitor (Bard Electrophysiology, Marcom, Inc., Minneapolis, Minn.). All signals were also recorded on videotape (VHS, Mitsubishi). Signals from the \(K^+\) electrodes were differentially amplified with filter settings of direct current to 1 Hz (Gould). The arterial pressure and \(K^+\) signals were fed to an analog-to-digital converter. These signals were stored on-line and displayed on a computer (Macintosh) monitor. The individual calibration curves obtained for each \(K^+\) electrode, in vitro, were also stored on computer. Results are presented as millimolar \(K^+\) concentrations, with the activity coefficient for \(K^+\) equal to 0.746.\[19\] To convert the millivolt changes recorded from the \(K^+\) electrodes, in vivo, to \([K^+]_o\), we used the Nernst equation (Weiss and Shine\[20\]). The reference \([K^+]_o\) was measured from an arterial blood sample withdrawn before each occlusion.

**Experimental Protocols**

Dogs were assigned randomly to either a sympathetic stimulation or norepinephrine infusion group. A series of four occlusions of the distal LAD was begun 45 minutes after insertion of the \(K^+\)-sensitive electrodes (Figure 1). Thirty minutes of reperfusion separated each 5-minute occlusion. Changes in \([K^+]_o\) evoked by the first LAD occlusion differed substantially from those elicited by the second and fourth occlusions (Figure 2). Thus, the data obtained during the first occlusion were not compared with data obtained during subsequent occlusions. Two minutes before the third occlusion, either sympathetic stimulation or norepinephrine infusion was begun and was continued until 2 minutes after reperfusion (Figure 1). The total stimulation or infusion time was 9 minutes.

We use the term “sympathetic stimulation” to indicate bilateral stimulation of the ansae subclaviae. The sympathetic stimulation was supramaximal \((8–10 \text{ V, 4-msec pulse width})\) and was applied at a frequency of 4 Hz. We chose a concentration of norepinephrine that increased the mean arterial pressure to a level similar to that evoked by sympathetic stimulation. The sympathetic stimulation increased mean arterial pressure by 43±8 mm Hg (range, 25–72 mm Hg). Thus, we infused norepinephrine at a concentration that increased mean arterial pressure by at least 30 mm Hg. The concentration of norepinephrine was 0.25 \(\mu\text{g/kg per minute}\) in five dogs and 0.5 \(\mu\text{g/kg per minute}\) in two dogs.

**Myocardial Blood Flow Measurement**

We measured myocardial blood flow during ischemia in the presence and absence of sympathetic stimulation \((n=3)\) or norepinephrine infusion \((n=4)\) with radioactive \((n=1)\) or colored microspheres \((n=6)\). Hale et al.\[21\] showed that measurement of myocardial blood flow...
with colored and radioactive microspheres produced comparable results. The microspheres were injected 2 minutes after the onset of the second and third LAD occlusions. The second and third occlusions were performed in the absence and presence of sympathetic stimulation or norepinephrine infusion, respectively (Figure 1). The microspheres were injected into a catheter positioned in the left atrium.

Radioactive microspheres. Approximately 2 million 15-µm carbonized plastic microspheres labeled with $^{57}$Co or $^{113}$Sn (DuPont de Nemours, Wilmington, Del.) were injected into the left atrium, followed by a 5-ml saline flush. The stock solutions (microspheres in 10 ml of 10% dextran with 0.1% Tween 80) were agitated to disperse the microspheres until immediately before injection. Reference blood samples were drawn from the left brachial and femoral arteries at 10 ml/min, starting 5 seconds before and ending 60 seconds after injection.

**Colored microspheres.** Approximately 5 million 15-µm colored polystyrene microspheres (E-Z Trac, Inc., Los Angeles) were injected into the left atrium, followed by a 5-ml saline flush. The stock solutions (microspheres in 0.025% Tween 80 and 1% thimerosal) were agitated until immediately before injection to disperse the microspheres. Reference blood samples were obtained from the femoral artery (20 ml/min) starting 5 seconds before and continuing until 60 seconds after the injection.

At the end of the experiment, the heart was fibrillated and immediately excised with the K+ electrodes in place. A block of tissue (0.66–1.15 g) containing each electrode was removed with a scalpel. The K+ electrodes were labeled so that the local blood flow could be compared with the changes in [K+] measured at each site. Samples (0.53–1.37 g) of midmyocardium were also obtained from normally perfused areas of the left ventricle. A thin layer (2 mm) of the endocardium and epicardium was removed from all tissue samples. Midmyocardial blood flow was measured in two to three tissue samples from the normally perfused myocardium and in two to four tissue samples from the ischemic zone.

The tissue samples were weighed, minced, and placed in scintillation vials (radioactive microspheres) or in 15-ml conical tubes (colored microspheres). The colored microspheres in tissue were isolated by digesting the tissue samples in alkaline solutions obtained from E-Z Trac.21 The reference blood samples containing colored microspheres were placed in 50-ml conical
Curves were compared by two-way repeated-measures analysis of variance (ANOVA). If the curves were significantly different, the differences at individual time points were analyzed by one-way repeated-measures ANOVA. The myocardial blood flows in the presence and absence of sympathetic stimulation or norepinephrine infusion were compared by paired t test. Differences were considered significant at \( p<0.05 \). Data are presented as mean±SEM.

**Results**

The summary data in Figure 2 show that the change in \([K^+]\), during the first 5-minute LAD occlusion differed from that measured during the second and fourth occlusions. The first, second, and fourth occlusions were done in the absence of sympathetic stimulation or norepinephrine infusion. \([K^+]\) was measured at 43 electrode sites in 18 dogs. At 21 sites (Figure 2A), \([K^+]\) was greater \((p<0.01)\) during the first compared with the second and fourth occlusions at 1, 2, 3, 4, and 5 minutes after LAD occlusion. In contrast, at 22 sites (Figure 2B), \([K^+]\) was less during the first than during the second and fourth occlusions, with the difference being significant \((p<0.05)\) at 4 and 5 minutes after occlusion. Importantly, the changes in \([K^+]\) were similar \((p>0.2)\) during the second and fourth control occlusions, independent of whether the first occlusion evoked significantly more (Figure 2A) or less (Figure 2B) extracellular \(K^+\) accumulation compared with subsequent occlusions.

Table 1 shows that occlusion of the LAD did not appreciably \((p>0.05)\) alter the mean arterial blood pressure.

**Effects of Sympathetic Stimulation on \([K^+]\), During Ischemia**

Figure 3 shows representative examples of the changes in \([K^+]\), measured during 5-minute LAD occlusions in the presence and absence of sympathetic stimulation. The data in Figures 3B and 3C were recorded from separate \(K^+\) recording sites in the same dog, whereas the data in Figure 3A are from a different dog. Figures 3A and 3B show that during the third occlusion with sympathetic stimulation the increase in \([K^+]\) was substantially greater compared with that during the second and fourth occlusions. In Figure 3A, \([K^+]\) at 5 minutes into the second LAD occlusion was 5.4 mM, but during the third occlusion in the presence of sympathetic stimulation, \([K^+]\) was increased to 7.4 mM. Similarly, Figure 3B shows that at 5 minutes into the

**TABLE 1. Mean Arterial Pressure Before and During Left Anterior Descending Coronary Artery Occlusions**

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Occlusion 1 Before</th>
<th>Occlusion 1 During</th>
<th>Occlusion 2 Before</th>
<th>Occlusion 2 During</th>
<th>Occlusion 3 Before</th>
<th>Occlusion 3 During</th>
<th>Occlusion 4 Before</th>
<th>Occlusion 4 During</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS ((n=11))</td>
<td>99±6</td>
<td>100±6</td>
<td>105±6</td>
<td>107±5</td>
<td>108±6</td>
<td>151±9*</td>
<td>152±8*</td>
<td>109±6</td>
</tr>
<tr>
<td>NE ((n=7))</td>
<td>102±9</td>
<td>104±10</td>
<td>99±7</td>
<td>101±8</td>
<td>94±7</td>
<td>153±10*</td>
<td>153±10*</td>
<td>97±4</td>
</tr>
</tbody>
</table>

SS, sympathetic stimulation; \(n\), number of dogs; NE, norepinephrine. Values are mean±SEM.

Mean arterial pressure was measured 10 seconds before and 2.5 minutes after occlusion of the left anterior descending coronary artery. Two minutes before occlusion 3, SS or NE infusion was begun. Left anterior descending coronary artery occlusion did not significantly affect the mean arterial pressure during occlusions 1, 2, or 4 or during occlusion 3 in the presence of SS or NE infusion \((p>0.05)\). The SS and NE infusion significantly increased mean arterial pressure.

\(^*p<0.05\) vs. before the occlusion.

tubes. The blood cells were hemolyzed, and the colored microspheres were isolated by centrifugation.\(^21\) Once isolated, the colored microspheres were diluted to 300 \(\mu\)l, and an aliquot was placed on a hemocytometer for counting. For the radioactive microspheres, radioactivity in the blood and tissue samples was measured for 1 minute in a gamma counter (model 5530, Packard Instrument Co., Inc., Downers Grove, Ill.). Data on net counts per minute for each isotope were provided after corrections of raw-count data for interisotope interference, background, and decay. Myocardial blood flow (MBF, in milliliters per minute per gram) was calculated as follows: MBF = \((CT\times R)/(CR\times WT))\), where CT is counts in the tissue, \(R\) is the reference flow rate (in milliliters per minute), \(CR\) is counts in the reference blood sample, and \(WT\) is the weight of the tissue (in grams).

**Data Analysis**

Electrode responses were separated into two categories based on whether the \([K^+]\), reached a level that was greater or less than 7 mM during the second and fourth occlusions. The electrode responses were separated into these two groups to show the effects of sympathetic stimulation and norepinephrine infusion on extracellular \(K^+\) accumulation in areas of myocardium that were near the margin (i.e., less than 7 mM) or center (i.e., greater than or equal to 7 mM) of the ischemic zone. If \([K^+]\), during the second and fourth occlusions differed by more than 1 mM at an individual \(K^+\) electrode site, then the data from that electrode site were excluded from analysis. The number of acceptable electrode sites in each dog ranged from one to four with an average of 2.4±0.3 sites \((n=18)\) dogs.

Data from 11 of 23 dogs in the sympathetic stimulation group and data from seven of 12 dogs in the norepinephrine infusion group were accepted for analysis. Data from 12 dogs in the sympathetic stimulation group were excluded from analysis for the following reasons: ventricular fibrillation occurred during the first occlusion (either during occlusion or at the onset of reperfusion) \((n=2)\), clot formation prevented reperfusion \((n=1)\), all \(K^+\) electrodes failed before the protocol was completed \((n=3)\), and changes in \([K^+]\), during the second and fourth occlusions differed by more than 1 mM at all electrode sites \((n=6)\). Data were excluded from five dogs in the norepinephrine infusion group for the following reasons: ventricular fibrillation on reperfusion \((n=1)\), \(K^+\) electrode failure \((n=2)\), and inconsistent responses to control occlusions \((n=2)\).
second occlusion \([K^+]_o\) was 6.8 mM, but during the third occlusion in the presence of sympathetic stimulation, \([K^+]_o\) increased to 8.5 mM. In contrast, Figure 3C shows that, compared with the second and fourth occlusions, sympathetic stimulation had little effect on extracellular \(K^+\) accumulation during ischemia at this electrode site. Note in Figure 3 that, before the occlusion, sympathetic stimulation alone evoked a slight increase in \([K^+]_o\).

Figure 4 summarizes the changes in \([K^+]_o\) evoked by 5-minute LAD occlusions in the presence and absence of sympathetic stimulation at 23 electrode sites in 11 dogs. \([K^+]_o\) was greater \((p<0.001)\) during the third occlusion in the presence of sympathetic stimulation compared with that during the second and fourth occlusions at 1, 2, 3, 4, and 5 minutes after the occlusion. The effect of sympathetic stimulation to increase \([K^+]_o\) was similar at sites where the maximum \([K^+]_o\) during the second and fourth occlusions was less than 7 mM (Figure 4A) or greater than 7 mM (Figure 4B). The changes in \([K^+]_o\) during the second and fourth occlusions were not significantly different \((p=0.6)\). Note that, before the occlusion, sympathetic stimulation alone slightly increased the \([K^+]_o\), but this effect was not statistically significant. In response to sympathetic stimulation alone, \([K^+]_o\) increased at 17 sites (0.1–1.3 mM), decreased at three sites (0.1–0.3 mM), and was unchanged at three sites.

Although mean values are shown in Figure 4, at individual electrode sites, sympathetic stimulation increased \([K^+]_o\) during ischemia at 12 of 12 sites where the maximum \([K^+]_o\) was less than 7 mM during the second and fourth occlusions (Figure 4A) and at seven of 11 sites where the maximum \([K^+]_o\) was greater than 7 mM (Figure 4B). At the remaining four sites, sympathetic stimulation slightly decreased or did not appreciably affect \([K^+]_o\) during ischemia (e.g., see Figure 3C). At these four sites, the maximum \([K^+]_o\) was 8.1±0.6 mM during the second and fourth occlusions and 7.7±0.3 mM \((p>0.05)\) during the third occlusion in the presence of sympathetic stimulation.

When the data in Figures 4A and 4B are combined, or if the electrode responses from each dog are averaged before repeated-measures ANOVA, \([K^+]_o\), is greater \((p<0.01)\) during the third occlusion with sympathetic stimulation compared with that during the second and fourth occlusions at 1, 2, 3, 4, and 5 minutes after the occlusion.

Figure 4. Graphs showing the effects of sympathetic stimulation (SS) at sites where the extracellular \(K^+\) concentration increased to less than 7 mM (panel A) or greater than 7 mM (panel B) during the second and fourth occlusions. The SS was begun 2 minutes before the third occlusion only. Data are from 23 \(K^+\) electrodes in 11 dogs. Values are mean±SEM (*p<0.03).
Effects of Norepinephrine Infusion on \([K^+]_o\) During Ischemia

Figure 5 shows representative examples of increases in \([K^+]_o\) during 5-minute occlusions of the LAD in the presence and absence of an intravenous infusion of norepinephrine. The data in Figures 5A and 5B were recorded from separate \(K^+\) electrode sites in the same dog, and data in Figure 5C are from a different dog. Figures 5A and 5B show that \([K^+]_o\), during the third occlusion with norepinephrine infusion was substantially less than that during the second and fourth occlusions. In contrast, Figure 5C shows an example in which \([K^+]_o\) during ischemia was greater in the presence than in the absence of the norepinephrine infusion. Note that before the occlusion norepinephrine infusion alone did not substantially affect \([K^+]_o\).

The summary data in Figure 6 show the changes in \([K^+]_o\), measured at 20 \(K^+\) electrode sites during 5-minute occlusions of the LAD in the presence and absence of norepinephrine infusion in seven dogs. Figure 6A shows that norepinephrine did not significantly \((p<0.9)\) alter \([K^+]_o\), during ischemia at electrode sites where the maximum \([K^+]_o\) during the second and fourth occlusions was less than 7 mM. At nine of the 11 sites represented in Figure 6A, norepinephrine decreased \([K^+]_o\), and at two sites, norepinephrine increased \([K^+]_o\), during ischemia compared with the second and fourth occlusions. Figure 6B shows that at sites where \([K^+]_o\) rose above 7 mM during the second and fourth occlusions, the norepinephrine infusion decreased \((p<0.01)\) \([K^+]_o\), at 3, 4, and 5 minutes after the onset of the occlusion. At all nine sites represented in Figure 6B, norepinephrine infusion reduced \([K^+]_o\), during the third occlusion compared with the second and fourth occlusions. The changes in \([K^+]_o\) during the second and fourth control occlusions were similar \((p=0.4)\), and before occlusion, norepinephrine alone did not significantly affect \([K^+]_o\).

When the data from all electrode sites are combined (i.e., data in Figures 6A and 6B) or when the data from individual electrode sites from each dog are averaged before repeated-measures ANOVA, \([K^+]_o\) is less \((p<0.02)\) during the third occlusion with norepinephrine infusion compared with the second and fourth occlusions at 4 and 5 minutes after the onset of LAD occlusion.

![Figure 5](http://circres.ahajournals.org/)

**Figure 5.** Graphs showing representative changes in extracellular \(K^+\) concentration during 5-minute occlusions of the distal left anterior descending coronary artery in the presence (third occlusion) and absence (second and fourth occlusions) of an intravenous infusion of norepinephrine (NE). Rep, reperfusion. Panels A and B: NE infusion reduced the extracellular \(K^+\) concentration during the third occlusion compared with the second and fourth occlusions. Panel C: At this \(K^+\) electrode site, NE slightly increased the extracellular \(K^+\) concentration. Data in panels A and B are from separate \(K^+\) electrodes in the same dog, and data in panel C are from a different dog. NE infusion was begun 2 minutes before the third occlusion only.

![Figure 6](http://circres.ahajournals.org/)

**Figure 6.** Graphs showing the effects of intravenous norepinephrine (NE) infusion at sites where the maximum extracellular \(K^+\) concentration was less than 7 mM (panel A) or greater than 7 mM (panel B) during the second and fourth occlusions. The NE infusion was begun 2 minutes before the third occlusion only. Data are from 20 \(K^+\) electrodes in seven dogs. Values are mean±SEM \(*p<0.01\).
Effects of Sympathetic Stimulation and Norepinephrine Infusion on Myocardial Blood Flow

Table 2 summarizes the myocardial blood flows (in milliliters per minute per gram) measured in normal and ischemic myocardium from three dogs in the sympathetic stimulation group and from four dogs in the norepinephrine infusion group. Both sympathetic stimulation and norepinephrine infusion increased \( [K^+] \) myocardial blood flow in the ischemic and nonischemic myocardium.

The changes in \( [K^+] \), and local myocardial blood flow at individual \( K^+ \) electrode sites are shown in Figure 7. The change in \( [K^+] \), was calculated by subtracting the \( [K^+] \), measured at 5 minutes into the second occlusion from that measured at 5 minutes into the third occlusion in the presence of sympathetic stimulation (Figure 7A) or norepinephrine infusion (Figure 7B). Similarly, the change in blood flow was calculated by subtracting the blood flow measured during the second occlusion from that measured during the third occlusion. The brackets in Figure 7 demarcate measurements from a single animal. At seven of 10 sites in the sympathetic stimulation group (Figure 7A; sites 1, 2, 5, 6, 8, 9, and 10), there was an increase in \( [K^+] \), as well as an increase in myocardial blood flow. At two sites (sites 4 and 7) in the sympathetic stimulation group, there was an increase in blood flow and a decrease in \( [K^+] \), and at the remaining site (site 3), there was a substantial increase in \( [K^+] \), but no change in blood flow. In the norepinephrine infusion group (Figure 7B), at 11 of 12 sites there was an increase in blood flow and a decrease in \( [K^+] \). At one site (site 10) in the norepinephrine group, there were slight increases in both \( [K^+] \), and myocardial blood flow.

Discussion

Major Observations

Our data show that sympathetic neural stimulation increased \( [K^+] \), evoked by 5-minute LAD occlusions at most sites in the ischemic myocardium. The effect of sympathetic stimulation to increase \( [K^+] \), during ischemia was not mimicked by an intravenous infusion of norepinephrine. Indeed, intravenously infused norepinephrine decreased \( [K^+] \), during 5-minute occlusions of the LAD. Both sympathetic neural stimulation and norepinephrine infusion increased myocardial blood flow in ischemic and normal myocardium. Thus, it is unlikely that alterations in blood flow alone are responsible for the disparate effects of sympathetic stimulation and norepinephrine infusion on ischemia-induced increases in \( [K^+] \). The norepinephrine released from sympathetic nerve endings may reach different populations of receptors than the intravenously infused norepinephrine. Further, substances other than norepinephrine (e.g., neuropeptide Y) that are released from...
sympathetic nerve terminals may modify cardiac function globally and at the cellular level to enhance $[K^+]_o$ during acute myocardial ischemia.

**Measurement of Ischemia-Induced Changes in $[K^+]_o$**

The reproducibility of changes in $[K^+]_o$ during serial occlusions of coronary arteries has been demonstrated in various animal models.\(^7,8,20\) Invariably, the first ischemic episode evokes changes in $[K^+]_o$ that differ substantially from subsequent occlusions in the series. In the anesthetized pig, the maximum increase in $[K^+]_o$ during an initial 10-minute occlusion of the LAD was substantially less than that elicited by subsequent occlusions.\(^7\) In the anesthetized dog, however, we found that at about half of the $K^+$ electrode sites the $[K^+]_o$ was significantly greater during the first than during subsequent 5-minute LAD occlusions (Figure 2A). Similar to the data obtained in the pig, at the remaining $K^+$ electrode sites the ischemia-evoked changes in $[K^+]_o$ were less during the first than during subsequent occlusions (Figure 2B). It is unclear why the rise in $[K^+]_o$ during the first occlusion in a series markedly differs from that during subsequent occlusions. If the variation in $[K^+]_o$, between the first and subsequent ischemic episodes was related to the phenomenon of ischemic preconditioning,\(^22\) it might be expected that the first occlusion would always evoke a greater increase in $[K^+]_o$ than subsequent occlusions. This would also be consistent with the observation in anesthetized dogs that ischemia-induced changes in cardiac electrical activity are more marked during the first than during subsequent periods of myocardial ischemia.\(^23\) Similarly, in humans undergoing coronary angioplasty, the first coronary artery occlusion evokes greater ST segment shifts and lactate production than the second period of coronary artery occlusion during angioplasty.\(^24\) Importantly, although the rise in $[K^+]_o$ during the first occlusion differed from that during subsequent occlusions, reproducible changes in $[K^+]_o$ were obtained after the first occlusion. The reproducibility of the ischemia-induced changes in $[K^+]_o$, allowed us to determine the effects of sympathetic stimulation and norepinephrine infusion on changes in $[K^+]_o$, during ischemia.

**Adrenergic Stimulation and $[K^+]_o$**

Previous studies have shown that propranolol reduces the rise in $[K^+]_o$ evoked by 10 minutes of acute myocardial ischemia compared with the control condition.\(^15,16\) Although these data do not provide direct evidence, they suggest that sympathetic activity, via $\beta$-adrenergic receptor activation, modulates the rise in $[K^+]_o$, during acute myocardial ischemia. Our data directly support this hypothesis by showing that sympathetic stimulation significantly increased $[K^+]_o$, during 5 minutes of ischemia at sites near the margin (Figure 4A) and center (Figure 4B) of the ischemic zone. The norepinephrine infusion data show, however, that $\beta$-adrenergic receptor stimulation reduced extracellular $K^+$ accumulation during acute myocardial ischemia (Figures 5 and 6). Gettes et al\(^16\) have also shown that intravenous infusions of isoproterenol decreased the maximum $[K^+]_o$, obtained in the center of the ischemic zone during 10-minute occlusions of the LAD in anesthetized pigs. Similarly, in isolated rat hearts, infusion of norepinephrine substantially reduced $K^+$ release during global ischemia.\(^25\)

**Mechanisms of Adrenergic Modulation of $[K^+]_o$**

Several mechanisms may account for the disparate effects of sympathetic stimulation and norepinephrine infusion on the changes in $[K^+]_o$, evoked by 5-minute occlusions of the LAD, including differences in the concentration of norepinephrine in ischemic tissue. Adrenergic-induced effects on the heart depend on the concentration of norepinephrine at receptor sites. During an intravenous infusion of norepinephrine, the amount of norepinephrine perfusing ischemic tissue would be reduced on total occlusion of the LAD. In contrast, during sympathetic stimulation, norepinephrine may continue to be released and, thereby, modulate cellular function throughout the ischemic area. It may be, however, that the concentration of norepinephrine in ischemic tissue is higher during norepinephrine infusion compared with sympathetic stimulation. Although norepinephrine overflow into veins draining ischemic myocardium was not attenuated during 10 minutes of ischemia in anesthetized dogs,\(^26,27\) it is clear that ischemic metabolites can alter sympathetic nerve function and inhibit norepinephrine release during the first few minutes of ischemia.\(^28,29\) Variations in the concentration and distribution of norepinephrine at neuroeffector junctions during sympathetic stimulation compared with norepinephrine infusion could be responsible for the disparate effects of these interventions on $[K^+]_o$, during ischemia.

It is well established that catecholamines increase the activity of Na$^+$,K$^+$-ATPase and thereby enhance the cellular uptake of K$^+$.\(^30,31\) In the anesthetized dog, intravenously infused norepinephrine at concentrations that did not increase arterial pressure enhanced K$^+$ uptake in normal and ischemic myocardium.\(^32\) This mechanism may have been operative during norepinephrine infusion as well as during sympathetic stimulation in our study. In the latter case, however, increased metabolic demand or the effects of other neurally released substances on $[K^+]_o$, may have masked the effects of norepinephrine on Na$^+$,K$^+$-ATPase activity.

Both sympathetic stimulation and norepinephrine infusion increased the blood flow in normal and ischemic myocardium (Table 2, Figure 7). Martins et al\(^33\) also showed that these interventions increased blood flow in ischemic and nonischemic myocardium in anesthetized dogs. The increase in myocardial blood flow could enhance the washout of extracellular K$^+$ and reduce $[K^+]_o$, in ischemic tissue. However, neurally released and exogenous norepinephrine will also increase metabolic demands on the tissue by enhancing contractile function. Sympathetic stimulation and intravenous isoproterenol infusion\(^23,34,35\) improve the mechanical function of ischemic tissue, but the increase in contractility intensifies the myocardial ischemia. If $[K^+]_o$, is used as a measure of myocardial ischemia, our data suggest that the increased blood flow to ischemic tissue did not match the increase in metabolic demand evoked by sympathetic stimulation, and thus, extracellular K$^+$ accumulation during ischemia was enhanced. In contrast, during norepinephrine infusion, the increase in blood flow was more than adequate to meet the metabolic demand and thereby enhanced the washout of extracellular K$^+$ during ischemia. It may be, however, that
sympathetic stimulation and norepinephrine infusion differentially affect blood flow very near the K⁺ electrodes. Because relatively large tissue samples (0.66–1.15 g) were taken, the myocardial blood flows measured may not adequately reflect subtle variations in blood flow near the tips of the individual K⁺ electrodes.⁶⁶

Although the mean effect of sympathetic stimulation was to significantly increase [K⁺], during LAD occlusion (Figure 4), at four of 23 K⁺ electrode sites, sympathetic stimulation did not appreciably alter the rise in [K⁺], (e.g., see Figure 3C). The density of sympathetic innervation varies in different areas of the left ventricular myocardium.⁶⁷ Therefore, sites at which sympathetic stimulation did not affect the ischemia-induced [K⁺], may have contained fewer sympathetic nerve endings than sites at which sympathetic stimulation increased the rise in [K⁺]. The ischemia at these sites may also have attenuated the myocyte response to substances released from the sympathetic nerve terminals or may have attenuated the ability of the sympathetic nerves to respond to electrical stimulation; i.e., sympathetic denervation may have occurred at these sites.⁶⁸ Alternatively, because the increase in [K⁺], was above 7 mM at the four electrode sites unaffected by sympathetic stimulation, the rise in [K⁺], may already have been maximal, and sympathetic activity could not further increase the extracellular K⁺ accumulation at these sites.

Mean arterial blood pressure rose to similar levels during sympathetic stimulation and norepinephrine infusion (Table 1). Thus, the differences in extracellular K⁺ accumulation during ischemia cannot be explained by differential increases in coronary perfusion pressure during sympathetic stimulation compared with norepinephrine infusion.

Norepinephrine-induced changes in cardiac electrophysiology and metabolism may influence [K⁺], during ischemia. β-Adrenergic stimulation increases the slow inward current and augments intracellular stores of Ca²+⁶⁷.⁶⁹ The enhanced intracellular Ca²⁺ concentration augments myocardial contractility, leading to a higher rate of ATP utilization.⁶⁹ In ischemic tissue, in which the rate of aerobic metabolism is limited,⁴¹ the fall in ATP levels might be expected to enhance the opening of ATP-regulated K⁺ channels. The ATP-regulated K⁺ channels appear to contribute to the rise in [K⁺], during acute myocardial ischemia, because blockade of these channels with sulfonylurea compounds reduced [K⁺], during ischemia.⁴²,⁴³ Thus, by increasing energy utilization, sympathetic stimulation may enhance the opening of the ATP-regulated K⁺ channels and increase K⁺ efflux during ischemia.

During sympathetic stimulation, various substances, including ATP⁴⁴ and neuropeptide Y⁴⁵–⁴⁷ are released together with norepinephrine from sympathetic nerve endings. These substances would be expected to accumulate in the ischemic myocardium along with other ischemic metabolites. Neuropeptide Y evokes coronary vasoconstriction⁶⁶,⁴⁷ and could thereby intensify ischemia. These neurally released substances may also alter the ATP-regulated or other classes of K⁺ channels to enhance K⁺ efflux during ischemia. The role of these sympathetic cotransmitters in ischemia-induced changes in [K⁺], remains to be determined.

Acute myocardial ischemia can evoke reflex changes in cardiac effenter sympathetic activity.¹,⁴⁸ Our data indicate that increases in sympathetic activity can enhance the rise in extracellular K⁺ accumulation during the first 5 minutes of ischemia. The increased rate of K⁺ accumulation evoked by sympathetic stimulation during ischemia may create conditions favorable for the initiation and maintenance of ventricular arrhythmias, including ventricular fibrillation. Indeed, Pelleg et al⁴⁹ showed that increases in the rate of rise of [K⁺], and the absolute level of [K⁺], enhance the development of ventricular arrhythmias.

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M R Warner, T S Kroeker and D P Zipes

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