Renal sympathetic nerve activity during cardiac ischemia and reperfusion in rats

E. E. USTINOVA AND H. D. SCHULTZ
Department of Physiology and Biophysics, University of Nebraska College of Medicine, Omaha, Nebraska 68198

Ustinova, E. E., and H. D. Schultz. Renal sympathetic nerve activity during cardiac ischemia and reperfusion in rats. *Am. J. Physiol.* 271 (*Regulatory Integrative Comp. Physiol.* 40): R1033–R1040, 1996.—We studied the role played by prostaglandins and oxygen-derived free radicals in mediating reflex changes in renal sympathetic nerve activity (RSNA) during myocardial ischemia and reperfusion. Ligation of the left coronary artery for 20 min and reperfusion for 10 min were performed in anesthetized rats with sinoaortic denervation and with intact cardiac afferent nerves (control, $n = 7$), with cardiac sympathetic denervation (SD, $n = 6$), with vagal denervation (VD, $n = 7$), and with combined SD + VD ($n = 6$). In control rats, RSNA decreased by 10 ± 3% from baseline ($P < 0.05$) during the first minute of ischemia and increased above baseline after 5 min of ischemia, with the maximum increase at the first minute of reperfusion. In rats with SD, RSNA decreased by 19 ± 4% from baseline ($P < 0.05$) at the first minute of ischemia and remained depressed during the entire ischemic and reperfusion periods. In rats with VD, RSNA increased by 26 ± 5% from baseline ($P < 0.05$) at the first minute of ischemia, and the increase in RSNA at the end of the ischemic period and at reperfusion was greater than in control rats. No changes in RSNA during ischemia and reperfusion were observed with combined SD + VD. Reflex changes in RSNA that occurred at the onset of ischemia in both VD ($n = 7$) and SD ($n = 7$) rats were abolished by indomethacin (5 mg/kg iv, 20 min before ischemia). Reflex changes in RSNA after prolonged ischemia (>10 min) and during reperfusion in both VD ($n = 7$) and SD ($n = 7$) rats were abolished by the antioxidant deferoxamine (20 mg/kg iv, 20 min before ischemia). Deferoxamine also diminished the increase of RSNA at the onset of ischemia in VD rats. Thus, in rats, the vagal afferent reflex predominates during early ischemia and the sympathetic afferent reflex predominates during prolonged ischemia and reperfusion. Reflex changes in RSNA that occur at the onset of ischemia are mediated by activation of vagal and sympathetic afferent endings by prostaglandins. Reflex changes in RSNA after prolonged ischemia and during reperfusion are mediated by activation of vagal and sympathetic afferent endings by oxygen-derived free radicals.

myocardial ischemia; ventricular receptors; prostaglandins; oxygen-derived free radicals

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MYOCARDIAL ISCHEMIA activates cardiac sensory endings with either vagal or sympathetic afferent fibers that project to the central nervous system and mediate opposing reflexes. Activation of ventricular vagal afferents in ischemia and reperfusion causes reflex bradycardia, vasodilatation, and a decrease in efferent sympathetic activity (2, 14, 17, 21). An opposing reflex, mediated by sympathetic afferents, causes tachycardia, a rise in blood pressure, and activation of renal and cardiac sympathetic efferent nerve activity (9, 10, 13).

Several studies have demonstrated that the release of bradykinin, prostaglandins, and adenosine (15, 16) during myocardial ischemia can stimulate cardiac sensory endings and produce reflexes mediated by these endings. Our previous studies in the rat heart indicate that there are two important mechanisms that activate cardiac chemosensitive vagal afferent fibers during myocardial ischemia and reperfusion (19). The first causes activation of these endings within the ischemic zone at the onset of ischemia and is mediated by prostaglandin synthesis. The second mechanism leads to a more widespread activation of cardiac vagal chemosensitive afferents after 15–20 min of ischemia and at the moment of reperfusion and is mediated by oxygen-derived free radical formation. Subsequently, experiments performed by Huang et al. demonstrated that oxygen-derived free radicals can stimulate cardiac chemosensitive nerve endings with sympathetic C fiber afferents (5) and produce cardiovascular reflexes mediated by these afferents in cats (6).

The goal of this study was to determine the contribution of cardiac vagal and sympathetic afferents to reflex changes in renal sympathetic nerve activity (RSNA) during myocardial ischemia and reperfusion in rats and the role of prostaglandin synthesis and oxygen-derived free radical formation in evoking these reflex changes of RSNA.

METHODS

General. Young adult Sprague-Dawley rats (250–320 g, either sex) were anesthetized intraperitoneally with a mixture of 2% α-chloralose and 25% urethan in saline (5 ml/kg). The trachea was cannulated low in the neck, and the lungs were ventilated by a Harvard rat respirator (60 breaths/min) with air, supplemented with O_2. Body temperature was maintained at 37°C by a heating pad. Polyethylene catheters were inserted in a femoral artery and vein for measurement of arterial blood pressure (ABP) and administration of drugs, respectively. The chest was opened via a sternotomy, and a silk suture (5.0) was looped around the left coronary artery (LCA) at its origin to produce occlusion. Heart rate (HR) was measured by a cardiotachometer triggered by the arterial pressure pulse. ABP was measured by strain gauges. HR, ABP, and RSNA (see *Recording of sympathetic nerve activity*) were recorded by a thermal recorder (Astro-Med MT 95000). Estimated fluid loss was replaced with intravenous administration of physiological saline at a rate of 4–6 $ml \cdot kg^{-1} \cdot h^{-1}$.

Sinoaortic and cardiac denervations. Arterial baroreceptors were denervated by cutting the carotid sinus and aortic nerves. The carotid sinus was exposed and denervated bilaterally by cutting all the visible nerves between the internal and external carotid arteries and stripping these vessels. The aortic nerve was located in the cervical region between vagus and sympathetic trunk and transected bilaterally. Baroreceptor denervation was confirmed by the absence of changes in

RSNA and HR when ABP was increased by intravenous injections of phenylephrine (0.1–5 µg/kg). Vagal denervation (VD) was performed by cutting the cervical vagal nerves. Cardiac sympathetic denervation (SD) was performed by surgical transection of the stellate ganglia and T_1-T_4 sympathetic rami.

Recording of sympathetic nerve activity. The abdomen was opened at the midline to expose the renal nerves and kidneys. A branch of a sympathetic nerve running along the left renal artery was dissected free and its sheath removed. The nerve was cut distally and its central end was placed on a bipolar silver electrode. The nerve and electrode were covered with mineral oil. Nerve signals were amplified (Grass P511), displayed on an oscilloscope (Gould 450), and fed into a ratemeter (Frederick Haer). Impulses were counted by ratemeter in 0.1-s bins. Resting nerve discharge was normalized as 100% for each animal, and changes in nerve activity were expressed as a percent change from the resting (baseline) value.

Protocols. In anesthetized rats with sinoaortic denervation, capsaicin (10.0 µg in 10 µl) was applied on a circle of filter paper (3 mm in diameter) placed on the anterior surface of the left ventricle for 30 s and reflex responses of RSNA to the stimulation of cardiac afferents by capsaicin were recorded. Capsaicin was dissolved in saline containing 10% ethanol and 1% Tween 80. Applications of capsaicin vehicle alone had no effect on RSNA. After each application, the paper was removed and the surface of the heart was washed with warm saline. The response of RSNA to repeated applications of capsaicin was reproducible. No changes in RSNA, ABP, and HR were observed after capsaicin was washed from the surface of the heart.

Then, denervation of either sympathetic or vagal cardiac afferents was performed. Capsaicin application was repeated to confirm vagal or sympathetic denervation by the absence of its inhibitory or excitatory effect, respectively, on the RSNA. After a 15-min equilibration period, we ligated the LCA for 20 min followed by 10 min of reperfusion in four groups of rats: intact cardiac afferent nerves (n = 7), SD (n = 6), VD (n = 7), and SD + VD (n = 6). In two other groups of rats (n = 7 each) with either SD or VD, we studied the effects of the antioxidant deferoxamine (20 mg/kg iv 20 min before ischemia). In similar groups (n = 7 each), we studied the effect of the cyclooxygenase blocker indomethacin (5 mg/kg iv 20 min before ischemia). In these groups of rats, responses of RSNA to capsaicin applications were tested before and after administration of deferoxamine or indomethacin. In our previous study (19), we have shown that these drugs, deferoxamine and indomethacin, do not produce effects on cardiac afferents beyond their antioxidant or cyclooxygenase-blocking capacities, respectively.

In a separate group of rats (n = 5), we measured the ischemic zone using fluorescent latex microspheres (Fluoresbrite fluorescent carboxilated microspheres, diameter 10 µm) according to the method described by Hale et al. (3). One minute after the ligature around the left coronary artery was tightened, microspheres (0.31×10^6 in 0.12 ml) were injected into the left ventricle via needle puncture. The hearts were excised, washed with saline, and then frozen and cut into eight slices 0.5- to 1-mm thick perpendicular to the cardiac long axis. The sections were viewed by a video camera (Hitachi) through a dissecting microscope under the ultraviolet lamp (365 nm, Black Ray) to determine the ischemic area (absence of fluorescence). The images were captured and digitized by computer (Macintosh). With the use of analysis software (National Institutes of Health Image), the ischemic area and the total area of each section of left ventricle were measured. Area at risk was expressed as percent of total area of the left ventricle.

Analysis of data. Reported values are means ± SE. Resting RSNA, HR, and mean ABP were normalized as 100% for each animal, and changes were expressed as a percent change from the resting (baseline) value. Differences among groups were determined by analysis of variance for repeated measures, and differences between means were isolated by the Bonferroni correction for multiple t-tests. Student's t-tests were used for single comparisons. Statistical significance was accepted at $P < 0.05$.

RESULTS

Effects of cardiac denervations, deferoxamine, and indomethacin on resting RSNA, HR, and ABP. Table 1 presents resting values of RSNA, ABP, and HR for all experimental groups. SD did not change the resting RSNA and ABP, but significantly decreased HR from 368 ± 20 to 311 ± 16 beats/min (n = 6, $P < 0.05$). VD had no effect on the resting ABP and HR, but the resting RSNA increased after VD from 42 ± 2 to 51 ± 4 impulses/s (n = 7, $P < 0.05$). Administration of deferoxamine or indomethacin had no effect on the resting RSNA, HR, and ABP.

Effects of ischemia and reperfusion on RSNA, HR, and ABP. The ischemic area comprised 50.0 ± 3.2% of the total area of the left ventricle, encompassing a major transmural portion of the anterior and posterior free wall. The size and topography of the ischemic zone

Table 1. *Effect of denervations, deferoxamine, and indomethacin on the resting values of RSNA, HR, and ABP*

Experimental Groups	n	Baseline RSNA, impulses/s	Baseline HR, beats/min	Baseline Mean ABP, mmHg	Denervation RSNA, impulses/s	Denervation HR, beats/min	Denervation Mean ABP, mmHg	Drug Administration RSNA, impulses/s	Drug Administration HR, beats/min	Drug Administration Mean ABP, mmHg
Control	7	36 ± 4	368 ± 15	69 ± 3						
SD	6	41 ± 5	368 ± 20	65 ± 3	34 ± 4	311 ± 16*	67 ± 4			
SD + deferoxamine	7	46 ± 4	363 ± 19	68 ± 3	39 ± 4	318 ± 12*	61 ± 3	46 ± 5	283 ± 20	66 ± 3
SD + indomethacin	7	42 ± 3	359 ± 14	61 ± 3	35 ± 4	317 ± 12*	51 ± 2	37 ± 3	296 ± 13	57 ± 2
VD	7	42 ± 2	360 ± 20	64 ± 3	51 ± 4*	388 ± 14	68 ± 5			
VD + deferoxamine	7	43 ± 5	362 ± 18	67 ± 2	56 ± 3*	390 ± 18	65 ± 6	57 ± 6	380 ± 20	68 ± 5
VD + indomethacin	7	36 ± 6	360 ± 27	73 ± 6	55 ± 2*	398 ± 16	67 ± 4	53 ± 4	413 ± 13	64 ± 4
VD + SD	6	39 ± 4	370 ± 10	69 ± 4	35 ± 8	382 ± 14	67 ± 5			

Values are means ± SE; n = no. of animals in each group. RSNA, renal sympathetic nerve activity; HR, heart rate; ABP, arterial blood pressure; SD, sympathetic denervation; VD, vagal denervation. All variables were measured after sinoaortic denervation (baseline); after SD, VD, or SD + VD (denervation); and 10–15 min after administration of either deferoxamine or indomethacin (drug administration) for all groups where applicable. *$P < 0.05$ compared with baseline.

resulting from occlusion of the left anterior coronary artery were similar to that described by others in the rat (1, 7).

Figure 1*A* illustrates that, in control rats with intact cardiac afferents, RSNA decreased by 10 ± 3% ($P <$ 0.05 compared with baseline) during the first minute of ischemia and then returned to the basal level. RSNA began to increase gradually above baseline after 5 min of ischemia with the maximum activity (28 ± 7% increase from baseline, $P < 0.01$) occurring at the first minute of reperfusion.

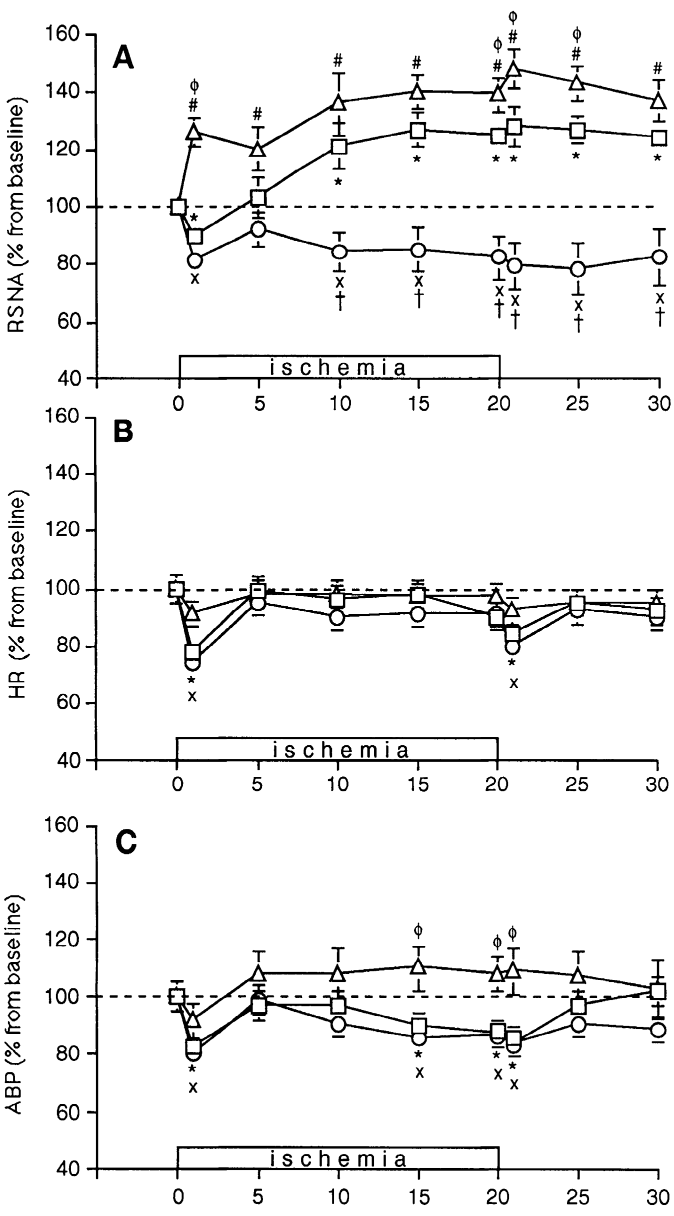

Fig. 1. Effect of 20-min left coronary artery (LCA) occlusion and 10-min reperfusion on renal sympathetic nerve activity (RSNA; *A*), heart rate (HR; *B*), and mean arterial blood pressure (ABP; *C*) in rats with intact cardiac innervation (control, □), cardiac sympathetic denervation (SD, ○), and vagal denervation (VD, △). Period of occlusion is marked by horizontal bar (ischemia). *Ordinates* show %change from baseline. $^{*x\#}P < 0.05$ compared with baseline; $^{\dagger}P <$ 0.05, SD vs. control; $^{\phi}P < 0.05$, VD vs. control.

In SD rats, RSNA decreased by 19 ± 4% from baseline ($P < 0.05$) at the first minute of ischemia and returned to the baseline by the fifth minute of ischemia. RSNA began to decrease gradually again after 5 min of ischemia and remained depressed ($P < 0.05$ compared with baseline) during the remaining ischemic and reperfusion periods. The decrease in RSNA was most pronounced at the first minute of reperfusion (21 ± 8% decrease from baseline, $P < 0.01$, Fig. 1*A*).

In VD rats, RSNA increased by 26 ± 5% from baseline ($P < 0.05$) at the first minute of ischemia and remained elevated above the baseline during the entire ischemic and reperfusion periods, with the peak activity (48 ± 7% increase from baseline, $P < 0.01$) at the first minute of reperfusion. The increase in RSNA at the first and twentieth minute of ischemia and at the first and fifth minutes of reperfusion in the VD group was significantly more pronounced than in the control group with intact cardiac afferents ($P < 0.05$, Fig. 1*A*).

In SD + VD rats, no changes in RSNA occurred during ischemia and reperfusion (not shown).

HR (Fig. 1*B*) in rats with intact cardiac afferents and in SD rats exhibited a transient decrease at the first minute of ischemia and at the first minute of reperfusion ($P < 0.05$ compared with baseline). In the group of VD rats, HR did not change from the baseline during the period of ischemia and reperfusion. No significant changes in HR were observed in the group of SD + VD rats (not shown).

ABP (Fig. 1*C*) in rats with intact cardiac afferents and in SD rats was significantly decreased compared with baseline at the first minute of ischemia ($P < 0.05$) and then at 15–20 min of ischemia and the first minute of reperfusion ($P < 0.05$). ABP in the group of VD rats was not significantly different from baseline during the entire ischemic and reperfusion periods. At *minutes 15–20* of ischemia and at the first minute of reperfusion, it was significantly higher than in control rats ($P < 0.05$). No significant changes in ABP were observed in the group of SD + VD rats (not shown).

Effect of deferoxamine on RSNA, HR, and ABP during ischemia and reperfusion. Administration of deferoxamine to the SD rats did not affect the decrease in RSNA at the first minute of ischemia; however, the subsequent continuous decrease in RSNA during the remaining ischemic period and during reperfusion was totally abolished by the antioxidant (Fig. 2*A*). Deferoxamine had no effect on HR during the ischemic and reperfusion periods (Fig. 2*B*). In the deferoxamine-treated SD rats, ABP at the first minute of ischemia significantly decreased compared with baseline ($P <$ 0.05) and was not different from the ABP in untreated SD rats. However, by the fifth minute of ischemia, ABP in the deferoxamine-treated SD rats returned to the baseline level, and, at 20 min of ischemia and at 1 min of reperfusion, ABP was significantly higher compared with untreated SD rats ($P < 0.05$, Fig. 2*C*).

In VD rats, deferoxamine abolished the initial increase in RSNA at the first minute of ischemia as well as its increase during late ischemia and reperfusion ($P > 0.05$ compared with baseline) (Fig. 3*A*). HR and

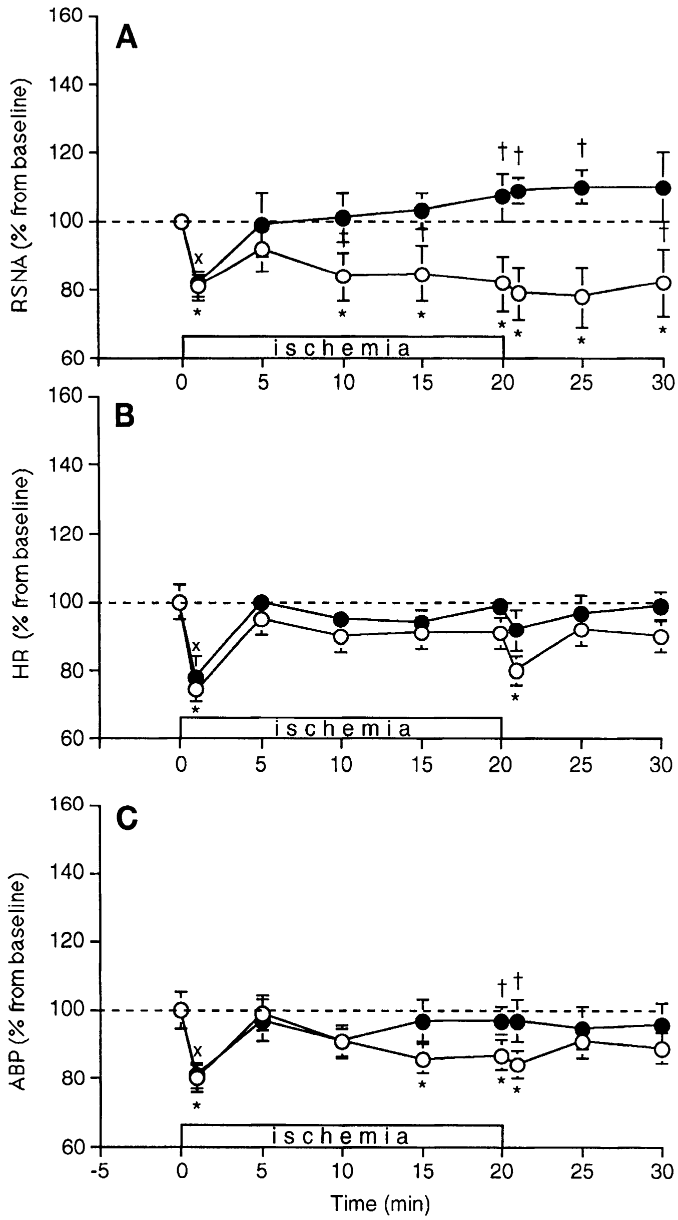

Fig. 2. Effect of deferoxamine on RSNA (*A*), HR (*B*), and mean ABP (*C*) in SD rats during 20-min LCA occlusion and 10-min reperfusion. ○, Untreated SD rats; ●, SD rats treated with deferoxamine (20 mg/kg, 20 min before ischemia). Period of occlusion is marked by horizontal bar (ischemia). *Ordinates* show %change from baseline. $^{*x}P < 0.05$ compared with baseline; †$P < 0.05$, SD vs. SD + deferoxamine.

ABP in the deferoxamine-treated VD rats were not different from those in untreated VD rats (Fig. 3, *B* and *C*).

Effect of indomethacin on RSNA, HR, and ABP during ischemia and reperfusion. Administration of indomethacin to the SD rats abolished the decrease in RSNA at the first minute of ischemia ($P > 0.05$ compared with baseline); however, indomethacin had no effect on the subsequent sustained decrease in RSNA during the remaining ischemic period and during reperfusion (Fig. 4*A*). Indomethacin also abolished the decrease in HR and ABP in SD rats at the first minute of the ischemic period ($P > 0.05$ compared with baseline) (Fig. 4, *B* and *C*). After 5 min of ischemia, the HR and ABP in the indomethacin-treated SD rats were not significantly different from those in untreated SD rats (Fig. 4, *B* and *C*).

In VD rats, indomethacin abolished the initial increase in RSNA at the first minute of ischemia ($P > 0.05$ compared with baseline) but not the increase during late ischemia and reperfusion (Fig. 5*A*). HR and ABP in indomethacin-treated VD rats during ischemia and reperfusion were not different from those in untreated VD rats (Fig. 5, *B* and *C*).

Effect of deferoxamine and indomethacin on RSNA responses to epicardial capsaicin. Figure 6 illustrates

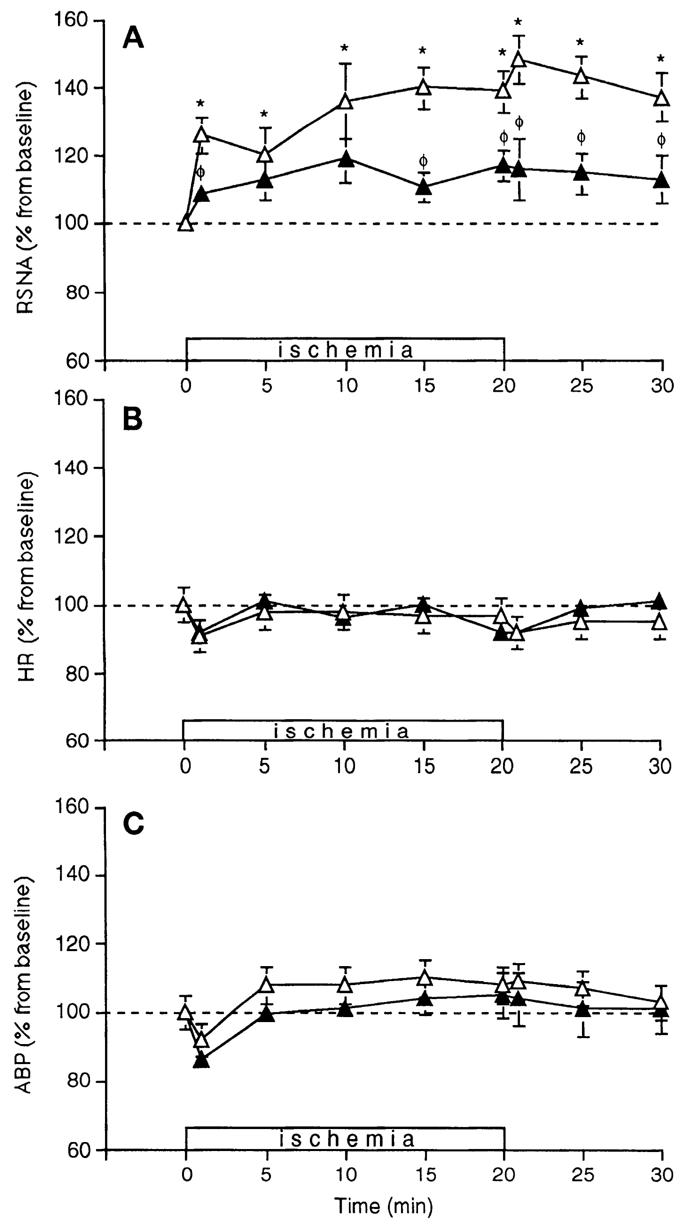

Fig. 3. Effect of deferoxamine on RSNA (*A*), HR (*B*), and mean ABP (*C*) in VD rats during 20-min LCA occlusion and 10-min reperfusion. △, Untreated VD rats; ▲, VD rats treated with deferoxamine (20 mg/kg, 20 min before ischemia). Period of occlusion is marked by horizontal bar (ischemia). *Ordinates* show %change from baseline. $^{*}P < 0.05$ compared with baseline; $^{\phi}P < 0.05$, VD vs. VD + deferoxamine.

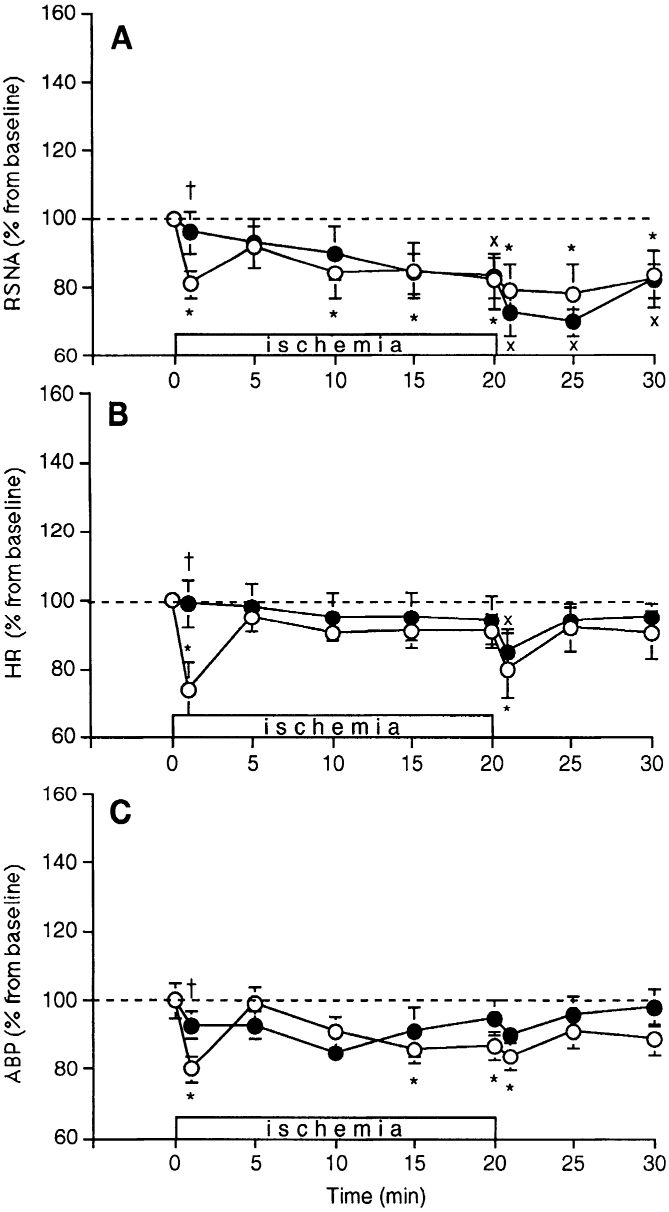

Fig. 4. Effect of indomethacin on RSNA (*A*), HR (*B*), and mean ABP (*C*) in SD rats during 20-min LCA occlusion and 10-min reperfusion. ○, Untreated SD rats; ●, SD rats treated with indomethacin (5 mg/kg, 20 min before ischemia). Period of occlusion is marked by horizontal bar (ischemia). *Ordinates* show %change from baseline. $^{*x}P < 0.05$ compared with baseline; $^{\dagger}P < 0.05$, SD vs. SD + indomethacin.

representative tracings of the changes in RSNA in intact, VD, and SD rats in response to application of capsaicin (10 µg/10 µl) onto the anterior surface of the left ventricle. In rats with intact cardiac innervation, capsaicin caused a biphasic response: initial inhibition of RSNA for 3–5 s followed by a more prolonged activation (Figs. 6*A* and 7). Cardiac sympathetic denervation augmented the inhibitory effect on RSNA ($P < 0.05$) and abolished the subsequent increase in RSNA ($P < 0.05$, Figs. 6*B* and 7). Elimination of vagal afferents abolished the initial inhibitory phase of the response ($P < 0.05$) and enhanced the increase in RSNA ($P < 0.05$, Figs. 6*C* and 7).

Figure 7 illustrates the effect of intravenous administration of deferoxamine and indomethacin on changes in RSNA in intact, VD, and SD rats in response to epicardial application of capsaicin. Although deferoxamine tended to decrease the reflex response to capsaicin mediated by vagal afferents (inhibition of RSNA), only its inhibitory effect on the sympathetic afferent reflex (increase in RSNA) was statistically significant ($P < 0.05$). Indomethacin had no effect on the sympathetic component of the reflex; the increase in RSNA in VD rats was the same before and after indomethacin. Indomethacin significantly decreased reflex inhibition of RSNA mediated by vagal afferents in SD rats in response to capsaicin application ($P < 0.05$).

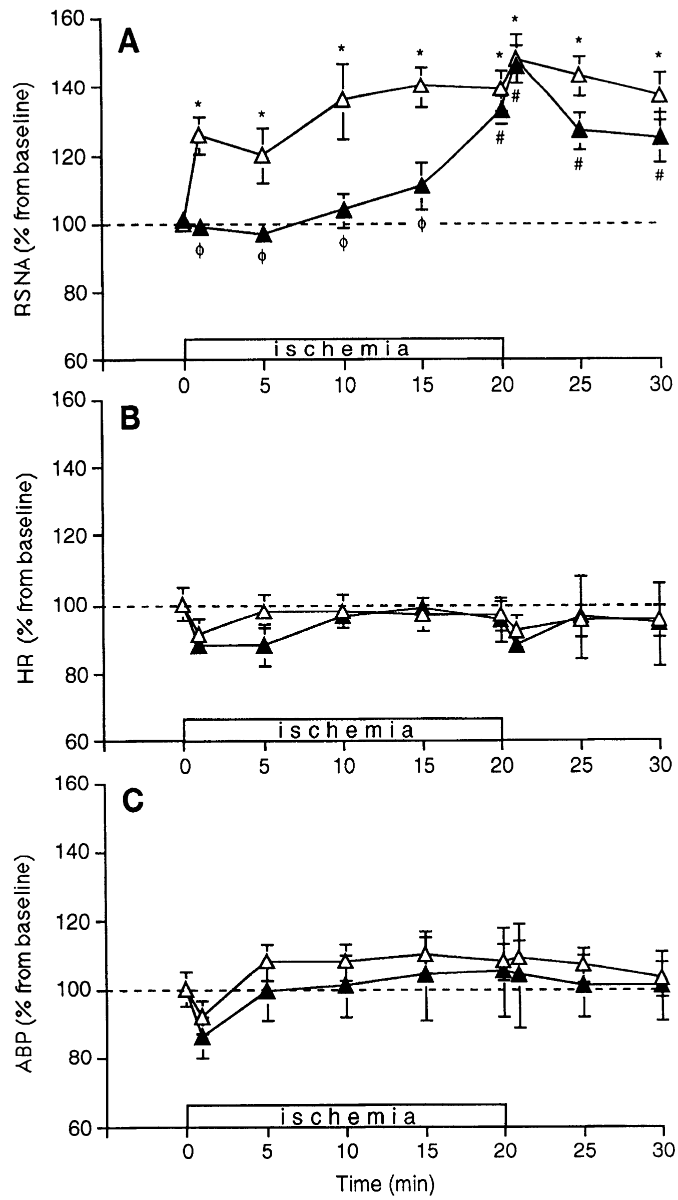

Fig. 5. Effect of indomethacin on RSNA (*A*), HR (*B*), and mean ABP (*C*) in VD rats during 20-min LCA occlusion and 10-min reperfusion. △, Untreated VD rats; ▲, VD rats treated with indomethacin (5 mg/kg, 20 min before ischemia). Period of occlusion is marked by horizontal bar (ischemia). *Ordinates* show %change from baseline. $^{*\#}P < 0.05$ compared with baseline; $^{\phi}P < 0.05$, VD vs. VD + indomethacin.

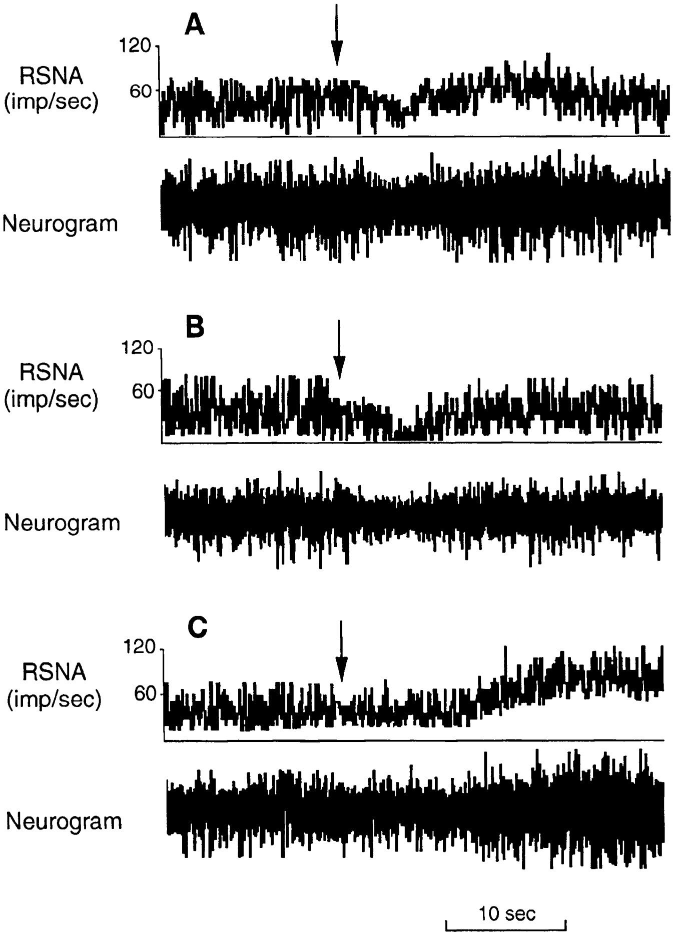

Fig. 6. Example of chart recordings of RSNA response to the application of capsaicin (10 µg/10 µl) on the surface of the left ventricle in an intact rat (*A*), a VD rat (*B*), and a SD (*C*) rat. *Upper traces* in *A-C*: ratemeter; *lower traces*: neurogram. Arrows indicate moment of capsaicin application. Capsaicin was applied on the surface of the heart for 30 s. imp, Impulses.

DISCUSSION

The present data from the rat confirm previous findings in other species that sympathetic and vagal afferent nerves in the heart mediate opposing reflex changes in RSNA during myocardial ischemia and reperfusion (2, 9, 10, 13–17, 20, 21). With cardiac afferent innervation intact, LCA occlusion in rats with sinoaortic denervation caused a transient decrease in RSNA, HR, and ABP at the first minute during occlusion. This reflex was abolished by VD, which allowed us to conclude that it was mediated by activation of vagal afferents. Similar effects have been described in baroreceptor-denervated cats (17) and dogs (14) during short-lasting occlusion of the LCA. However, in our experiments after 10 min of occlusion, the cardiac sympathetic reflex appeared to predominate and resulted in an increase of RSNA during the rest of the ischemic period and during reperfusion; VD made this increase in RSNA even more pronounced by abolishing the inhibitory effect of vagal afferents. Conversely, SD resulted in a progressive decrease in RSNA during the entire ischemic and reperfusion periods. These results provide good evidence that the reflex increase in RSNA in intact animals during late ischemia and at reperfusion was mediated by cardiac sympathetic afferents.

In general, these data indicate that both cardiac vagal and sympathetic afferent fibers are activated throughout ischemia and reperfusion; however, the reflex changes in RSNA that occur depend on a balance between these two opposing systems. In the anesthetized rat, the cardiac vagal afferent reflex predominates at the onset of ischemia and the sympathetic afferent reflex predominates after prolonged ischemia and during reperfusion.

Unlike the vagal reflex, activation of cardiac sympathetic afferents during prolonged ischemia and during reperfusion did not cause a corresponding reflex change in HR and ABP. These results suggest that, in rats, cardiac sympathetic afferents have a more profound effect on reflex changes in RSNA than reflex regulation of ABP and HR, whereas cardiac vagal afferent fibers can more effectively mediate reflex changes in HR and ABP as well as in renal nerve activity. However, our interpretation of the reflex changes in cardiac function is limited by the effect of denervation procedures on efferent neural pathways to the heart. Thus the contribution of cardiac sympathetic afferents to vagal efferent control and cardiac vagal afferents on sympathetic efferent control of heart rate cannot be determined from our study. In addition, it is important to keep in mind that these data were obtained in urethan/chloralose-anesthetized animals. It is well accepted that anesthesia can alter autonomic nervous system responses. However, it is not likely that anesthesia affected our results in terms of the types of mediators

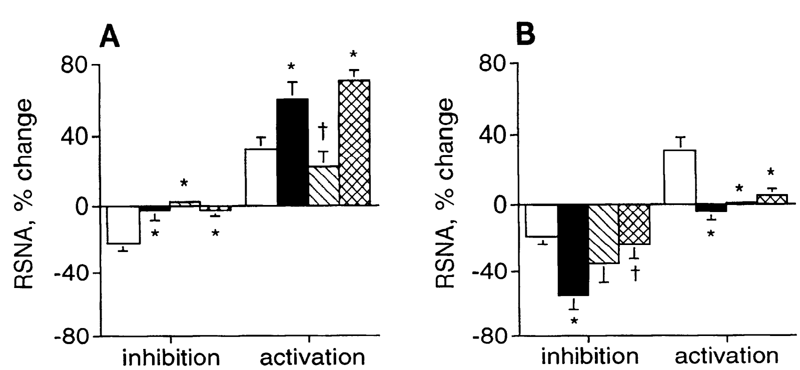

Fig. 7. Effect of deferoxamine (20 mg/kg iv) and indomethacin (5 mg/kg iv) on the RSNA biphasic response (initial inhibition and subsequent activation) to the application of capsaicin (10 µg/10 µl) on the surface of the left ventricle in intact, VD (*A*), and SD (*B*) rats. RSNA expressed as %change from baseline. Open bars, intact; filled bars, denervation; hatched bars, denervation + deferoxamine; crosshatched bars, denervation + indomethacin. $^{*}P < 0.05$ compared with intact group; $^{\dagger}P < 0.05$ compared with denervated group.

that act to stimulate afferent endings at various phases of ischemia and reperfusion.

The activation of both sympathetic and vagal reflexes at an early and late phase of ischemia and at reperfusion suggests that multiple mechanisms may be involved in the activation of the afferent endings. Thames and Minisi (15) demonstrated that inhibition of RSNA during short-term ischemia in dogs can be abolished by the inhibition of prostaglandin synthesis. In our previous experiments, we performed direct recordings of the activity from cardiac vagal chemosensitive afferent fibers that mediate this inhibition of RSNA. These experiments demonstrated that blockade of prostaglandin synthesis abolished the stimulation of the afferents in the ischemic zone of the left ventricle at the onset of ischemia and had no effect on their activation at the end of ischemic period and at reperfusion (19). In remarkable correlation with the results of our afferent studies, the present data reveal that in rats with SD, i.e., in the absence of sympathetic afferent input, blockade of prostaglandin synthesis with indomethacin abolished the decrease in RSNA, HR, and ABP only at the onset of ischemia and had no effect on the decrease in RSNA at the end of the ischemic period and at reperfusion.

Similarly, in the rats with VD, indomethacin abolished the increase of RSNA at the onset of ischemia with no effect on the RSNA at the end of the ischemic period and at reperfusion. These results suggest that inhibition of prostaglandin synthesis prevents stimulation of both vagal and sympathetic sensory endings in the heart at the onset of ischemia, resulting in the absence of the corresponding reflex changes of RSNA. It seems that, at least in the rat model, prostaglandins are more important for stimulating of cardiac sensory endings and corresponding reflex changes at the onset of ischemia and have less effect after 15–20 min of ischemia and at reperfusion.

The excitatory effect of oxygen-derived free radicals on cardiac sensory endings was demonstrated recently (5, 6, 18, 19). Formation of oxygen-derived free radicals is enhanced in the rat heart ~10 min after the onset of ischemia and most prominently at reperfusion (11, 12). Our previous experiments with afferent recordings demonstrated that activation of cardiac chemosensitive vagal afferents after 15–20 min of ischemia and at the onset of reperfusion can be abolished with antioxidants (19).

In the present experiments in SD rats, deferoxamine had no effect on the inhibition of RSNA and HR at the onset of ischemia, although the fall of the ABP in deferoxamine-treated SD rats was less than in those that did not receive antioxidant. However, deferoxamine completely abolished the decrease in RSNA, HR, and ABP at the end of ischemic period and at the beginning of reperfusion, suggesting that these effects were mediated by free oxygen-derived radical-induced activation of vagal afferents. This is in agreement with our afferent recordings (19).

Similarly, in VD rats, deferoxamine limited the increase of RSNA at the end of the ischemic period and at reperfusion, suggesting that the sympathetic afferent reflex also was due to increased free oxygen-derived radical formation. However, deferoxamine in VD rats also diminished the increase in RSNA at the onset of ischemia. Similar results were reported by Huang et al. (5), who demonstrated that both deferoxamine and dimethylthiourea abolished sympathetic afferent excitation after 2 min of myocardial ischemia in cats. Iron-loaded deferoxamine, devoid of antioxidant abilities, was without effect. Because such short periods of ischemia have been reported to be without appreciable activation of free radical formation (4, 11, 12), another explanation of the inhibitory effect of these antioxidants may exist.

One possibility is that antioxidants affect prostaglandin release in the ischemic myocardium during ischemia and thus limit prostaglandin-induced activation of sympathetic afferents in the heart at the onset of ischemia. Data exist suggesting that deferoxamine can interfere with prostaglandin synthesis (8), but, in this case, deferoxamine should affect activation of both vagal and sympathetic afferents and their corresponding changes in RSNA at the onset of ischemia, which did not happen in our experiments.

Another, more likely, explanation of this effect of antioxidants is that free radical production, even under basal conditions, affects the sensitivity of cardiac sympathetic afferent endings in the heart to other chemical stimuli. In the present study, we found that deferoxamine inhibited the reflex increase in RSNA caused by stimulation of cardiac sympathetic afferent endings by capsaicin. This inhibitory effect of deferoxamine on the capsaicin-evoked chemoreflex was much more pronounced for sympathetic afferents than for vagal afferents. On the basis of this observation, we speculate that deferoxamine limits the reflex increase of RSNA at the onset of ischemia in VD rats by lowering the sensitivity of sympathetic nerve endings to mediators released at the onset of ischemia, probably to prostaglandins.

Perspectives

The hypothesis that oxygen radicals play an important role in modulating the sensitivity of cardiac sympathetic afferent endings to chemical mediators may be a key factor in determining the balance between cardiac vagal and sympathetic afferent reflexes during myocardial ischemia and reperfusion. In early ischemia, when free radical production is not appreciably elevated, cardiac sympathetic afferents cannot respond maximally to prostaglandins released from the ischemic myocardium, thus allowing the reflex consequences of activation of cardiac vagal afferents to predominate. Then, as enhanced free radical production increases the sensitivity of cardiac sympathetic afferents in late ischemia and reperfusion, the sympathetic afferent reflexes are likely to exert a progressively larger influence. Our observation that the cardiac vagal afferent reflex predominated only during the onset of ischemia, whereas the sympathetic afferent reflex predominated during late ischemia and reperfusion, is consistent with this hypothesis. This hypothesis may provide a new approach to the study of disturbances in autonomic regulation in numerous pathological states associated with the increased formation of oxygen-derived free radicals.

In summary, activation of cardiac chemosensitive nerve endings with vagal and sympathetic afferents mediates opposing reflex changes in RSNA during ischemia and reperfusion in the rat heart: activation of vagal afferents decreases RSNA, whereas activation of sympathetic afferents increases it. The cardiac vagal afferent reflex predominates during early ischemia, and the sympathetic afferent reflex predominates during prolonged ischemia and reperfusion. Reflex changes in RSNA that occur at the onset of ischemia are mediated by activation of vagal and sympathetic afferent endings by prostaglandins and can be abolished with indomethacin. Reflex changes in RSNA after prolonged ischemia and during reperfusion are mediated by activation of vagal and sympathetic afferent endings by oxygen-derived free radicals and can be abolished with the antioxidant deferoxamine. Inhibition of free radical production reduces the sensitivity of sympathetic nerve endings to the chemical mediator, capsaicin. Modulation of afferent sensitivity to chemical mediators by oxygen-derived free radicals may play an important role in the expression of the cardiac sympathetic afferent reflex during ischemia and reperfusion.

Address for reprint requests: H. D. Schultz, Dept. of Physiology and Biophysics, Univ. of Nebraska Medical Center, 600 S. 42nd St., Omaha, NE 68198–4575.

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