Oxidative stress impairs cardiac chemoreflexes in diabetic rats

ELENA E. USTINOVA,1 CAROLYN J. BARRETT,2 SHU-YU SUN,1 AND HAROLD D. SCHULTZ1
1Department of Physiology and Biophysics, University of Nebraska College of Medicine, Omaha, Nebraska 68198-4575; and 2Department of Physiology, University of Auckland, Private Bag 92019, Auckland, New Zealand

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Ustinova, Elena E., Carolyn J. Barrett, Shu-Yu Sun, and Harold D. Schultz. Oxidative stress impairs cardiac chemoreflexes in diabetic rats. Am J Physiol Heart Circ Physiol 279: H2176–H2187, 2000.—We investigated the effects of diabetes mellitus and antioxidant treatment on the sensory and reflex function of cardiac chemosensory nerves in rats. Diabetes was induced by streptozotocin (STZ; 85 mg/kg ip). Subgroups of sham- and STZ-treated rats were chronically treated with an antioxidant, vitamin E (60 mg/kg per os daily, started 2 days before STZ). Animals were studied 6–8 wk after STZ injection. We measured renal sympathetic nerve activity (RSNA), mean arterial blood pressure (MABP), and cardiac vagal and sympathetic afferent activities in response to stimulation of chemosensitive sensory nerves in the heart by epicardial application of capsaicin (Caps) and bradykinin (BK). In cardiac sympathetic-denervated rats, Caps and BK (1–10.0 μg) evoked a vagal afferent mediated reflex depression of RSNA and MABP, which was significantly blunted in STZ-treated rats (P < 0.05). In vagal-denervated rats, Caps and BK (1–10.0 μg) evoked a sympathetic afferent-mediated reflex elevation of RSNA and MABP, which also was significantly blunted in STZ-treated rats (P < 0.05). Chronic vitamin E treatment effectively prevented these cardiac chemoreflex defects in STZ-treated rats without altering resting blood glucose or hemodynamics. STZ-treated rats with insulin replacement did not exhibit impaired cardiac chemoreflexes. In afferent studies, Caps and BK (0.1 g–10.0 μg) increased cardiac vagal and sympathetic afferent nerve activity in a dose-dependent manner in sham-treated rats. These responses were significantly blunted in STZ-treated rats. Vitamin E prevented the impairment of afferent discharge to chemical stimulation in STZ rats. The following were concluded: STZ-induced, insulindependent diabetes in rats extensively impairs the sensory and reflex properties of cardiac chemosensitive nerve endings, and these disturbances can be prevented by chronic treatment with vitamin E. These results suggest that oxidative stress plays an important role in the neuropathy of this autonomic reflex in diabetes.

autonomic nervous system; diabetes; free radicals; antioxidants; angina

SILENT MYOCARDIAL ISCHEMIA and cardiac arrhythmias are frequent and major complications of diabetes mel-
sistent with the generalized peripheral autonomic neuropathy characteristic of chronic diabetes (1, 7). Nevertheless, no direct evidence exists to describe how the sensory innervation of the heart is altered in diabetes. One of the factors known to contribute to cardiomyopathy and peripheral neuropathy in diabetes is oxidative stress (5). Because cardiac afferent endings are known to be sensitive to oxygen radicals (9, 23–25), it is possible that the elevated presence of oxygen radicals in cardiac tissue during diabetes may lead to alterations in cardiac afferent function.

The objectives of this study were to determine whether chemosensory function from the heart is altered in diabetes and whether antioxidant treatment can influence these alterations in cardiac afferent function.

METHODS

Methods used in this study conform with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and were approved by the Institutional Animal Care and Use Committee at the University of Nebraska Medical Center.

Induction of diabetes. Male Sprague-Dawley rats (180–200 g) were divided randomly into sham, diabetic (streptozotocin [STZ] rats), and insulin (Ins) replacement (STZ + Ins rats) groups. The rats were housed individually in cages and provided access to food and water ad libitum. Diabetes was maintained at 37°C by a heating pad. Polyethylene tubing in the lungs was ventilated by a Harvard rat respirator (60 breaths/min) with air supplemented with O₂. Body temperature (37°C) was maintained by a heating pad. Polyethylene catheters were inserted into a femoral artery and vein for measurement of mean arterial blood pressure (MABP) and administration of drugs, respectively. The neck was opened in the midline to expose the cervical vagus and aortic nerves. The chest was opened via a sternotomy to allow application of chemicals on the surface of the heart. Heart rate (HR) was measured by a cardiotachometer triggered by the arterial pressure pulse. MABP were measured by strain gauges. Estimated fluid loss was replaced with intravenous administration of physiological saline at a rate of 4–6 mL·kg⁻¹·h⁻¹.

Sinoaortic and cardiac denervation. Arterial baroreceptors were denervated by cutting the carotid sinus and aortic nerves. Baroreceptor denervation was confirmed by the absence of changes in renal sympathetic nerve activity (RSNA) and HR when MABP was increased by intravenous injections of phenylephrine (0.1–5 μg/kg). Cardiac vagal denervation was performed by cutting the cervical vagal nerves. Cardiac sympathetic denervation was performed by surgical transection of the stellate ganglia and T1–T4 sympathetic rami. Confirmation of vagal or sympathetic denervation was assessed by the absence of an inhibitory or excitatory effect, respectively, of epicardial capsaicin (Caps) on RSNA.

Recording of RSNA. 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 the sheath was removed. The nerve was cut distally, and the 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 rate meter (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.

Recording of cardiac afferent impulses. Vagal afferent impulses were recorded from “single fibers” split from fine slips dissected from the distal cut end of the left cervical vagus (3). Sympathetic afferent impulses were recorded similarly from nerve fibers split from fine slips dissected from the distal cut end of the left stellate nerve (9). Nerves were covered in a pool of mineral oil, and the nerve fibers were placed on a silver electrode. Impulses were amplified (Grass P511), displayed on an oscilloscope (Gould 450), and fed into a rate meter (Frederick Haer) whose window discriminators were set to accept potentials of a particular amplitude. Impulses were counted by rate meter in 1-s bins. Nerve fibers that had one, or at most two, easily distinguishable action potentials were used. We studied only spontaneously active fibers that had receptive fields in the heart that could be located precisely.

Measurement of lipid peroxides in plasma. Lipid peroxides in the plasma of the animals of all experimental groups were measured at the time of the experiment by the method described by Ohkawa et al. (14). Venous blood (0.05 ml) was withdrawn and put into 1 ml of physiological saline. After centrifugation at 4,000 rpm for 10 min, we transferred 0.5 ml of supernatant to another centrifuge tube, and plasma lipids were isolated by precipitating with phosphotungstic acid, followed by the reaction with thiobarbituric acid and fluorometric measurement of thiobarbituric acid-reactive substances (TBARS), which was made at 515 nm excitation and 553 nm emission. The results were expressed in terms of malonaldehyde (in nmol/ml of blood), with the use of the fluorescence intensity of the solution obtained by reacting 0.5 nmol of tetraethoxypropane with thiobarbituric acid as a standard. This method is well established for estimation of...
the intensity of free radical reactions in experimental and clinical conditions (19).

**Experimental protocol for reflex studies.** To test cardiac chemoreflexes in anesthetized rats with sinoaortic denervation, Caps (1.0, 5.0, and 10.0 μg in 10 μl of saline) and BK (1.0, 5.0, and 10.0 μg in 10 μl of saline) were applied on a circle of filter paper (3 mm in diameter) and placed on the anterior surface of the left ventricle for 30 s. Reflex responses of RSNA and hemodynamics to the stimulation of cardiac afferents by these chemicals were recorded. Arterial baroreceptors were denervated to eliminate any potential influence on reflex responses secondary to changes in hemodynamics. After each application, we removed the paper, and the surface of the heart was washed with warm saline. A recovery period of at least 10–15 min was observed between applications. After constructing the three-point dose-response relationship for each test chemical, we transected either the cervical vagi or cardiac sympathetic nerves, and the epicardial applications of the test chemicals were repeated. At the end of the experiment, the remaining afferent nerves (either cervical vagi or cardiac sympathetic nerves) were transected, and epicardial applications of the test chemicals were repeated to verify the reflex nature of the evoked responses.

The response of RSNA to repeated applications of the same dose of tested chemicals was reproducible (25). Caps (1 mg/ml) was dissolved in saline containing 10% ethanol and 1% Tween 80 and then diluted to the final concentrations with saline. Applications of Caps vehicle alone or saline had no effect on RSNA.

**Experimental protocol for cardiac afferent studies.** To prevent reflex changes in hemodynamics in response to epicardial application of test substances from potentially influencing afferent responses, the experiments were performed after vagotomy and cardiac sympathectomy. We recorded impulses from vagal and sympathetic afferent fibers with chemosensitive endings in the heart. The chemosensitivity of a cardiac afferent ending was affirmed by its response to topical application of Caps to the surface of the heart (10 μg). We considered the fiber to be activated when its activity increased by >25% above the basal level. Caps was chosen as a test chemical because it is known to directly stimulate only chemosensitive C fiber endings and not cardiac mechanoreceptors (3).

Caps (0.1–10.0 μg in 10 μl), BK (0.1–10.0 μg in 10 μl), and veratridine (0.001–0.1 μg in 10 μl) were applied, as in the reflex study above, directly to the area of the receptive field of the fiber on the surface of the heart. These test chemicals were chosen because of their diverse influence on membrane receptors. Caps activates membrane receptors directly linked to nonspecific ion channels in the afferent endings (18). BK activates membrane receptors indirectly linked to ion channels through a second-messenger system, protein kinase C (18). Veratridine stimulates afferent endings by direct activation of voltage-gated Na⁺ channels in the spike-initiating region of the afferent axon (2).

Applications of Caps vehicle or saline alone had no effect on fiber activity. After each application, we removed the paper, and the surface of the heart was washed with warm saline. Intervals of at least 10–15 min were allowed between applications. We have verified in previous studies (23–25) that cardiac afferent responses to repeated chemical activation are reproducible using these procedures.

**Analysis of data.** HR, MABP, RSNA (reflex study), and cardiac afferent activity (afferent study) were recorded by a thermal recorder (Astro-Med MT 95000) and captured by a data-processing computer (PowerLab, ADInstruments). In the reflex study, resting RSNA was normalized as 100% for each animal, and changes were expressed as a percent change from the resting (baseline) value. In the afferent study, fiber activity was calculated as the average number of impulses per second (imp/s) over the 10 s of maximal activity occurring during the final 30 s of the control period and during the first 30 s of the experimental intervention (peak afferent responses always occurred within the first 30 s). Because of the individual variability in the control activity of different fibers, the neural responses were also expressed as a percent change from the baseline.

Reported values are means ± SE. 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-test was used for single comparisons. Statistical significance was accepted at P < 0.05.

**RESULTS**

Body weights, blood glucose, and baseline hemodynamics are listed in Table 1 for each group of rats at the time of the experiments (6–8 wk post-STZ injection). STZ and STZ + VE rats gained less weight and exhibited higher blood glucose than sham rats at the time of the experiments. The STZ + Ins rats exhibited weight gain, blood glucose, and hemodynamics similar to sham rats. Chronic VE administration was without effect on any of these variables independently of STZ, although STZ + VE rats tended to gain more weight than those with STZ alone (not significant). Body weight and blood glucose were similar among all of the groups before STZ injection.

**Cardiac chemoreflex responses in diabetic rats with intact cardiac innervation.** Figure 1, A and D, illustrates representative tracings of the changes in RSNA in sham and STZ rats with intact cardiac innervation, respectively, in response to application of Caps (10

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**Table 1. The change in body weight (pre-post-STZ), blood glucose, and baseline hemodynamics in all groups of rats 6–8 wk after STZ or vehicle treatment**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Change in Body Weight, g</th>
<th>Glucose, mg/dl</th>
<th>HR, beats/min</th>
<th>MABP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>24</td>
<td>152 ± 21</td>
<td>115 ± 7</td>
<td>339 ± 10</td>
<td>92 ± 3</td>
</tr>
<tr>
<td>STZ</td>
<td>24</td>
<td>15 ± 17*</td>
<td>451 ± 15*</td>
<td>327 ± 8</td>
<td>91 ± 4</td>
</tr>
<tr>
<td>STZ + Ins</td>
<td>6</td>
<td>139 ± 34</td>
<td>114 ± 8</td>
<td>335 ± 15</td>
<td>95 ± 8</td>
</tr>
<tr>
<td>Sham + VE</td>
<td>24</td>
<td>151 ± 22</td>
<td>125 ± 5</td>
<td>346 ± 17</td>
<td>90 ± 6</td>
</tr>
<tr>
<td>STZ + VE</td>
<td>24</td>
<td>21 ± 21*</td>
<td>421 ± 35*</td>
<td>358 ± 12</td>
<td>89 ± 4</td>
</tr>
</tbody>
</table>

Values are reported as means ± SE; n represents the number of rats. STZ, streptozotocin; Ins, insulin; VE, vitamin E; HR, heart rate; MABP, mean arterial blood pressure. *P < 0.05 compared with sham group.
\(10 \mu\text{g}/10 \mu\text{l}\) onto the anterior surface of the left ventricle. In sham rats, both Caps and BK (data not shown) caused a biphasic response: initial inhibition of RSNA and hypotension for 3–5 s followed by a more prolonged activation and hypertension. Changes in HR were small and inconsistent. Cardiac sympathetic denervation and vagotomy studies confirmed that the initial sympathoinhibitory phase was mediated by cardiac vagal afferents and the secondary sympathoexcitatory phase was mediated by cardiac sympathetic afferents (results described below).

As exemplified in Fig. 1D, the baseline RSNA was higher in STZ rats than in sham rats (66 ± 10 vs. 27 ± 7 imp/s, respectively, \(P < 0.05\)), and changes in RSNA and MABP in response to the cardiac chemoreflexes were depressed (results described below). Reflex changes in RSNA and MABP in STZ + Ins rats with intact cardiac innervation did not differ from sham rats (Tables 2 and 3).

Cardiac vagal afferent chemoreflex in diabetic rats. In this series, the cardiac sympathetic nerves were cut to confine reflex responses solely to activation of cardiac vagal afferent fibers (Bezold-Jarisch reflex). Transection of cardiac sympathetic afferent fibers did not affect baseline RSNA in either sham or STZ rats.

Figure 2 illustrates dose-related reflex changes in RSNA in response to epicardial application of Caps and BK in sham and STZ rats before and after cardiac sympathetic denervation. The sympathoinhibitory phase of the RSNA response to both Caps (Fig. 2A) and BK (Fig. 2C) in STZ rats was depressed compared with sham rats before cardiac sympathetic denervation.

In sham rats, cardiac sympathetic denervation augmented the inhibitory phase of the reflex RSNA re-

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Fig. 1. Chart recordings illustrating changes in renal sympathetic nerve activity (RSNA), arterial blood pressure (ABP), and heart rate in response to epicardial application of capsaicin (Caps) to the left ventricle in sham and streptozotocin (STZ)-induced diabetic rats with intact cardiac innervation and those after either vagal (VD) or cardiac sympathetic denervation (SD). A: sham rat with intact cardiac innervation. B: sham rat after VD. C: sham rat after SD. D: diabetic rat with intact cardiac innervation. E: diabetic rat after VD. F: diabetic rat after SD. Recordings after VD and cardiac SD were obtained from separate animals.
Table 2. Average gains (slopes) of RSNA and MABP dose-response curves to epicardial application of Caps and BK in sham, STZ, and STZ + Ins groups before and after cardiac SD

<table>
<thead>
<tr>
<th>Group</th>
<th>Caps, % change/µg</th>
<th>BK</th>
<th>MABP, mmHg/µg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>SD</td>
<td>Intact</td>
</tr>
<tr>
<td>Sympathoinhibitory phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>−2.9 ± 0.7</td>
<td>−4.3 ± 0.7†</td>
<td>−2.5 ± 0.6</td>
</tr>
<tr>
<td>STZ</td>
<td>−1.2 ± 0.5*</td>
<td>−1.2 ± 0.4*</td>
<td>−0.7 ± 0.3*</td>
</tr>
<tr>
<td>STZ + Ins</td>
<td>−2.5 ± 0.6</td>
<td>−4.0 ± 0.6†</td>
<td>−2.1 ± 0.4</td>
</tr>
</tbody>
</table>

| Sympathoexcitatory phase |
| Sham      | 4.8 ± 0.7         | 0.2 ± 0.2†  | 7.0 ± 1.0      | 0.4 ± 0.4†   | 2.0 ± 0.3 | 0.0 ± 0.1†  | 1.2 ± 0.3 | 0.1 ± 0.1†   |
| STZ       | 4.3 ± 0.7         | 0.1 ± 0.2†  | 2.0 ± 0.8*     | 0.1 ± 0.3†   | 1.2 ± 0.2* | −0.1 ± 0.2† | 0.4 ± 0.2* | 0.0 ± 0.1†   |
| STZ + Ins | 4.5 ± 0.6         | 0.1 ± 0.2†  | 6.5 ± 0.9      | 0.1 ± 0.2†   | 2.1 ± 0.3 | 0.1 ± 0.2†  | 1.1 ± 0.2 | 0.1 ± 0.1†   |

Values reported as means ± SE; n = 6 rats in each group. RSNA, renal sympathetic nerve activity; Caps, capsaicin; BK, bradykinin; SD, sympathetic denervation. †P < 0.05 compared with sham group; ‡P < 0.05 compared with intact state.

Table 3. Average gains (slopes) of RSNA and MABP dose-response curves to epicardial application of Caps and BK in sham, STZ, and STZ + Ins groups before and after cervical SD

<table>
<thead>
<tr>
<th>Group</th>
<th>Caps, % change/µg</th>
<th>BK</th>
<th>MABP, mmHg/µg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>VD</td>
<td>Intact</td>
</tr>
<tr>
<td>Sympathoinhibitory phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>−3.2 ± 0.8</td>
<td>0.2 ± 0.2†</td>
<td>−2.4 ± 0.7</td>
</tr>
<tr>
<td>STZ</td>
<td>−1.2 ± 0.7*</td>
<td>0.1 ± 0.3†</td>
<td>−0.5 ± 0.2*</td>
</tr>
<tr>
<td>STZ + Ins</td>
<td>−3.0 ± 0.5</td>
<td>0.1 ± 0.3†</td>
<td>−2.5 ± 0.6</td>
</tr>
</tbody>
</table>

| Sympathoexcitatory phase |
| Sham      | 4.6 ± 1.0         | 10.2 ± 1.6† | 6.5 ± 0.6      | 10.5 ± 1.1† | 2.0 ± 0.2 | 3.3 ± 0.2†  | 1.1 ± 0.2 | 2.1 ± 0.4†   |
| STZ       | 3.0 ± 0.9         | 5.4 ± 1.1* | 1.6 ± 0.4*     | 2.2 ± 0.4*  | 1.2 ± 0.1* | 2.0 ± 0.3†  | 0.5 ± 0.3* | 0.8 ± 0.5*   |
| STZ + Ins | 4.2 ± 0.9         | 9.5 ± 2.0* | 4.0 ± 0.8      | 9.4 ± 1.0*  | 1.8 ± 0.3 | 3.0 ± 0.3†  | 1.0 ± 0.2 | 1.9 ± 0.3†   |

Values reported as means ± SE; n = 6 rats in each group. VD, vagal denervation. *P < 0.05 compared with sham group; †P < 0.05 compared with intact state.
response to BK was significantly depressed at all doses in STZ rats (Fig. 3D).

Transection of the vagus in sham rats increased the baseline RSNA from 27 ± 6 to 53 ± 13 imp/s (P < 0.05) and augmented the excitatory phase of the reflex RSNA response to epicardial Caps (Figs. 1C and 3B). The average gain (slope) of the dose-response relationship for the sympathoexcitatory phase of the reflex RSNA response doubled after vagal denervation in both sham and STZ Ins rats (Table 3).

Vagotomy in STZ rats caused neither a significant change in baseline RSNA (66 ± 8 vs. 77 ± 6 imp/s; not significant) nor did it alter the excitatory effect of epicardial Caps or BK on RSNA (Fig. 3, B and D, and Table 3). Consequently, the sympathoexcitatory (sympathetic afferent) component of the cardiac chemoreflex was markedly depressed in diabetic rats compared with sham and Ins-replacement rats after vagotomy.

Vagotomy abolished the inhibitory phase of the reflex RSNA response to epicardial Caps and BK (Fig. 3A and C, and Table 3) in all groups of rats (sham, STZ, STZ + Ins). This result confirms that the sympathoinhibitory phase of the chemoreflex was mediated by cardiac vagal afferents.

The increase in RSNA evoked by epicardial chemicals was accompanied by a significant increase in MABP in all groups, but this pressor response was smaller in STZ rats compared with sham rats (Tables 2 and 3). The increase in arterial pressure evoked by epicardial chemicals was augmented after vagotomy in sham and STZ + Ins rats but not in STZ rats (Table 3). HR during the sympathoexcitatory phase was not affected by the epicardial applications in any group (Table 4).

Table 4. MABP and HR responses to epicardial application of Caps and BK in sham, STZ, and vitamin-treated (sham + VE and STZ + VE) groups after either VD or cardiac SD

<table>
<thead>
<tr>
<th>Group</th>
<th>Caps (10 μg)</th>
<th>BK (10 μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ΔMABP, mmHg</td>
<td>ΔHR, beats/min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vagal afferent reflex (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>-18 ± 3*</td>
<td>-30 ± 7*</td>
</tr>
<tr>
<td>STZ</td>
<td>-9 ± 3†</td>
<td>-3 ± 3†</td>
</tr>
<tr>
<td>Sham + VE</td>
<td>-20 ± 2*</td>
<td>-31 ± 14*</td>
</tr>
<tr>
<td>STZ + VE</td>
<td>-16 ± 3*</td>
<td>-38 ± 10*</td>
</tr>
<tr>
<td>Sympathetic afferent reflex (VD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>38 ± 2*</td>
<td>8 ± 4</td>
</tr>
<tr>
<td>STZ</td>
<td>22 ± 4†</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>Sham + VE</td>
<td>33 ± 3*</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>STZ + VE</td>
<td>29 ± 2*</td>
<td>6 ± 3</td>
</tr>
</tbody>
</table>

Values reported as means ± SE; n = 6 rats in each group. ΔMABP, change in MABP; ΔHR, change in HR. *P < 0.05 compared with control; †P < 0.05 compared with sham group.
The increases in RSNA and MABP in response to epicardial chemicals in vagotomized rats were abolished after subsequent cardiac sympathetic denervation, confirming the evoked responses were mediated by cardiac sympathetic afferent fibers (data not shown).

Cardiac chemoreflex responses in diabetic rats treated with VE. Baseline RSNA in sham + VE and STZ + VE rats did not differ from its respective controls (sham, STZ rats) and was not affected by cardiac sympathetic denervation. In cardiac sympathectomized rats, reflex decreases in RSNA, MABP, and HR in response to both epicardial Caps and BK were significantly greater in STZ + VE rats compared with STZ rats and were not different from reflex decreases observed in either sham or sham + VE rats (Fig. 4 and Table 4). Chronic VE had no effect on reflex responses in sham rats (sham + VE vs. sham rats: Fig. 4 and Table 4). These results indicate that chronic VE treatment prevented the impairment of the vagal afferent cardiac chemoreflex in diabetic rats.

Vagotomy in STZ + VE rats increased the baseline RSNA from 22 ± 8 to 44 ± 10 imp/s (P < 0.05). After vagotomy, reflex increases in RSNA and MABP in

Fig. 3. Changes in peak RSNA in response to epicardial application of either Caps (A and B) or BK (C and D) in sham and STZ-induced diabetic rats before (open symbols) and after (closed symbols) VD. A and C: initial sympathoinhibitory phase of biphasic response as illustrated in Fig. 1. B and D: secondary sympathoexcitatory phase of the response. *P < 0.05, sham vs. STZ; ‡P < 0.05, intact vs. VD.

Fig. 4. Effect of vitamin E treatment (VE) on the reflex inhibition of RSNA in response to epicardial application of Caps (left) and BK (right) in sham and STZ-induced diabetic rats with cardiac SD (cardiac vagal afferent reflex). *P < 0.05 compared with sham.
response to both epicardial Caps and BK were signifi-
cantly greater in STZ + VE rats compared with STZ rats and were not different from reflex increases ob-
served in either sham or sham + VE rats (Fig. 5 and Table 4). Chronic VE had no effect on reflex responses in sham rats (sham + VE vs. sham rats: Fig. 5 and Table 4). These results indicate that chronic VE treatment prevented the impairment of the sympathetic afferent cardiac chemoreflex in diabetic rats.

Cardiac vagal afferent activity in diabetic rats. We studied impulse activity from single vagal fibers arising from chemosensitive endings in the heart in response to epicardial application of Caps and BK. Examples of the cardiac vagal afferent recordings are illustrated in Fig. 6. All of the fibers had receptive fields in the left ventricle. Baseline activity of the vagal afferent fibers was not different in sham and STZ rats (0.6 ± 0.2 vs. 0.9 ± 0.6 imp/s, respectively; not significant). Cardiac vagal afferent responses to Caps and BK were depressed in STZ rats compared with sham rats (Fig. 7). Vagal afferent responses to epicardial Caps and BK were significantly greater in STZ + VE rats compared with STZ rats and were not different from afferent responses observed in either sham or sham + VE rats (Fig. 7). These results indicate that chronic VE prevented the impairment of cardiac vagal afferent discharge to chemical stimulation in the diabetic state. Chronic VE had no effect on the responses of cardiac vagal afferents to Caps and BK in sham rats (sham + VE vs. sham rats: Fig. 7).

Cardiac sympathetic afferent activity in diabetic rats. Fig. 8 illustrates responses of cardiac sympathetic afferent fibers to epicardial Caps and BK. All of the fibers had receptive fields in the left ventricle. Baseline activity of the sympathetic afferent fibers was not different in sham and STZ rats (2.1 ± 1.4 vs. 2.3 ± 0.8 imp/s; not significant). Cardiac sympathetic afferent responses to Caps and BK were depressed in STZ rats compared with sham rats (Fig. 9). Sympathetic afferent responses to both epicardial Caps and BK were significantly greater in STZ + VE rats compared with STZ rats and were not different from afferent responses observed in either sham or sham + VE rats (Fig. 9). Chronic VE had no effect on the responses of sympathetic afferents to Caps and BK in sham rats (sham + VE vs. sham rats: Fig. 9). These results indicate that chronic VE prevented the impairment of cardiac sympathetic afferent discharge to chemical stimulation in the diabetic state.

Comparison of cardiac vagal and sympathetic afferent responses. As exemplified in Figs. 6 and 8, there were no differences between cardiac vagal and sympathetic afferents with respect to the latency, time to peak activity, and duration of the afferent responses to the chemical mediators in any of the experimental groups. In general, both populations of afferent endings were activated within a few seconds of application of the chemical, followed by a rapid crescendo in activity that peaked and waned during the period of exposure. Differences in afferent responses between sham and STZ rats were confined to the frequency of impulse activity (Figs. 6–9).

Afferent responses to veratridine. Figure 10 illus-

Fig. 5. Effect of VE on the reflex activation of RSNA in response to epicardial application of Caps (left) and BK (right) in sham and STZ-induced diabetic rats with VD (cardiac sympathetic afferent reflex). *P < 0.05 compared with sham.
different fibers to epicardial veratridine. Unlike afferent responses to Caps and BK, afferent responses to veratridine were not altered in the STZ rats compared with sham rats.

**Lipid peroxide levels in plasma.** Figure 11 illustrates the plasma levels of TBARS in each of the experimental groups. The level of lipid peroxides in STZ rats was significantly greater than in sham rats. Chronic treatment with VE had no significant effect on the level of TBARS in sham animals but prevented the accumulation of TBARS in diabetic animals.

**DISCUSSION**

Results from the present study indicate that both the vagal- and sympathetic-afferent limbs of the cardiac chemoreflex are markedly depressed in STZ-induced diabetic rats. The results also indicate that this functional impairment in the diabetic state can be prevented by chronic treatment with VE. From these results, we believe that oxidative stress is the major underlying mechanism causing impairment of the chemosensitive and reflex properties of cardiac sensory nerve endings in diabetes.

It is well documented that diabetes is associated with increased oxidative stress, as evidenced by the increased accumulation of lipid peroxides in plasma of rats (10) and humans with diabetes mellitus (15). Oxidative stress is thought to be a major contributor to cardiovascular disease in diabetes mellitus (8). Treatment of diabetic animals with antioxidants diminishes indexes of the oxidative stress and improves endothelium-dependent relaxation of coronary vessels (21). A morphological study (21) has also shown that chronic treatment with VE largely prevents the degeneration of autonomic nerve fibers in the hearts of diabetic rats. However, to our knowledge, this is the first study to document changes in the functional properties of cardiac afferent nerve endings in diabetes and the important role of oxidative stress in these changes.

STZ is a glucose molecule with a highly reactive nitrosourea side chain. The mechanism of the cytotoxic action of the drug and its selectivity for pancreatic β-cells is thought to involve binding to a glucose transporter on the plasma membrane of the β-cell and subsequent methylation, free radical generation, and nitric oxide production (20). It is unlikely that STZ could have exerted a toxic influence directly on cardiac vagal afferent endings. We found that cardiac chemoreflex function was not impaired in STZ rats with insulin implants to maintain normoglycemia. This result links cardiac afferent dysfunction to insulin depletion and hyperglycemia rather than to the drug itself. Hyperglycemia-driven metabolic changes in diabetes increase generation of free radicals through several mechanisms, including glucose autoxidation, protein glycation, increased substrate flux through the polyol pathway, and depression of natural antioxidant systems (5). One or more of these potential mechanisms is likely to be involved in the impairment of cardiac afferent function in the diabetic rats.

In the present study we administered VE before STZ injection to minimize oxidative stress from the onset of hyperglycemia. Because the cytotoxic effect of STZ is thought to involve free radical generation, it is possible...
that the efficacy of VE could be related to a potential protective effect of the antioxidant on pancreatic β-cells to prevent STZ-induced cell death. Although we did not assess β-cell viability or plasma insulin levels directly in our study, we found that STZ rats treated with VE became hyperglycemic to the same degree as rats treated with STZ alone and exhibited similar signs of diabetes, such as polydipsia, polyuria, and impaired weight gain. Furthermore, in other unpublished studies (4), we have found that administration of VE several weeks after the induction of diabetes also is effective in reversing the impairment in cardiac chemoreflex function.

An important observation from the present study is that epicardial application of mediator substances, such as Caps and BK, to the heart of normal, anesthetized rats evokes a biphasic sympathetic reflex (with respect to RSNA) consisting of an initial, short-lasting, sympathoinhibitory phase mediated by cardiac vagal afferents and a secondary, longer-lasting, sympathoexcitatory phase mediated by cardiac sympathetic afferents. The afferent pathways and reflex effects of these two opposing reflexes from the heart have been recognized for many years (6, 11–12, 22). However, epicardial application of chemical mediators in other species, such as the dog or cat, generally evokes a prominent sympathetic afferent reflex with little or no opposing input from cardiac vagal afferents (11). On the basis of these observations, it is generally assumed that cardiac sympathetic afferent endings are located primarily in the epicardial regions of the heart, whereas the vagal afferent endings more extensively innervate the subendocardial regions, not accessible to epicardial applications.

In the present study in rats, epicardial application of these test chemicals was capable of stimulating both populations of afferents to a degree sufficient to cause mutual antagonism of the reflex renal sympathetic nerve responses. This mutual antagonism was evidenced by an enhancement of the cardiac sympathetic afferent reflex after vagotomy and similar enhancement of the cardiac vagal afferent reflex after cardiac sympathectomy. The biphasic nature of the integrated reflex response appeared to be mediated principally by differences in the latency and duration of central integration of the two reflex pathways. We could find no evidence of distinct differences in the two pathways with respect to the dynamics of the afferent discharge to the chemical mediators. Our results may suggest that cardiac sympathetic and vagal afferent endings are distributed more uniformly in the epicardium of the rat heart than those of larger species. It is also possible that the small size of the rat heart allows chemicals to diffuse rapidly to endings below the epicardial surface, allowing more uniform activation of vagal and sympathetic afferent endings.

![Fig. 9. Effect of VE treatment on the IF of cardiac sympathetic afferent fibers in response to epicardial application of Caps (left) and BK (right) in sham and STZ-induced diabetic rats. *P < 0.05 compared with sham.](image)

![Fig. 10. IF of cardiac vagal afferent fibers (left) and cardiac sympathetic afferent fibers (right) in response to epicardial application of veratridine in sham and STZ-induced diabetic rats.](image)
The effect of diabetes on the chemosensory properties of both cardiac vagal and sympathetic afferent fibers to rat cardiac, and efferent. Because cardiac chemosensitive afferents are stimulated acutely by oxygen radicals (9, 23), chronic exposure to oxygen radicals may desensitize or injure the afferent endings and diminish the reflexes that arise from their activation. We tested this possibility by directly recording action potentials from cardiac vagal and sympathetic chemosensitive afferents in response to stimulation by Caps, BK, and veratridine. Our data demonstrate that the responses of both cardiac vagal and sympathetic afferent fibers to Caps and BK were depressed in diabetic rats. Chronic treatment with VE completely abolished the detrimental effect of diabetes on the chemosensory properties of the cardiac afferent nerves. At the same time, VE prevented the accumulation of free radical oxidation products, lipid peroxides, in the plasma of the diabetic animals. This defect of the sensory properties of the cardiac afferent nerve endings appears to be mediated by oxidative stress and provides a primary explanation for the depressed cardiac chemoreflexes in our diabetic animals.

One aspect of our afferent and reflex results that is discrepant is the correlation between resting RSNA and resting cardiac chemosensitive afferent activity in sham and diabetic rats. Baseline nerve activities from both cardiac vagal and sympathetic afferent fibers did not differ between sham and diabetic rats, whereas resting RSNA was elevated and vagal restraint of resting sympathetic outflow was blunted in diabetic animals. The functional significance of this paradox cannot be resolved in the present study, but several possible explanations can be considered. An impairment in central integration of the vagal afferent limb of the cardiac chemoreflex may also occur in the diabetic state, causing a reduction in tonic restraint of sympathetic outflow in the face of normal resting afferent activity. In addition, other vagal reflexes that may play a comparatively larger role in the tonic control of sympathetic outflow also may be impaired in the diabetic state. It is well documented that the neural component of the volume reflex is blunted in STZ diabetic rats (16). It is possible that cardiopulmonary mechanoreceptors are similarly blunted in the diabetic state and that a reduction in afferent input from this vagal reflex pathway can account for the alterations in resting renal sympathetic outflow observed in diabetic rats (17).

We found that the responses of cardiac vagal and sympathetic afferent chemosensitive endings to veratridine, unlike Caps or BK, were not attenuated in diabetic rats. This observation may provide some insight into the mechanism of the oxidative impairment of the afferent endings. Most chemical mediators of chemosensitive endings, including Caps and BK, evoke depolarization of the afferent neuron by binding to membrane receptors or ligand-gated ion channels in the nerve terminal, leading to depolarization (18). Unlike Caps and BK, veratridine bypasses the receptor-mediated complex and evokes action potentials by direct activation of the voltage-gated Na\(^+\) channel (2) at the level of the spike-initiating zone. As we have shown, the impairment of cardiac vagal and sympathetic afferent discharge in diabetic rats did not extend to the general ability of the afferent fibers to propagate action potentials, because veratridine responses were normal. Thus the impairment in cardiac afferent responses to Caps and BK in the diabetic rats appears to involve a dysfunction in the chemical transduction process before spike initiation. However, we cannot deduce from the present study whether the duration or severity of the diabetic state can influence the degree and mechanism(s) of oxidative impairment of afferent function. It seems reasonable to suggest that a more prolonged exposure to oxidative injury may lead to axonal injury and more generalized neuropathy.
Chemosensitive nerve endings in the heart play an important role in mediating cardiovascular adjustments to myocardial ischemia and the perception of cardiac pain (6, 11–12, 22–25). The conclusions of this study are that cardiac chemoreflexes are markedly depressed in diabetic rats and, correspondingly, that chronic antioxidant treatment can effectively prevent this impairment. Our results show that cardiac chemosensory nerves are desensitized in diabetes as a result of a dysfunction in the chemical transduction process in the sensory terminal. The efficacy of VE in preventing this afferent dysfunction is consistent with a mechanism mediated by free radicals. An impairment in cardiac chemosensory function may play an important role in exacerbating diabetic cardiac complications, such as silent ischemia and arrhythmias. In addition, these results support evidence that chronic antioxidant therapy may be effective in reducing the risk of these complications in diabetic patients. Given the uncertainties in extrapolation of our results from diabetic rats to the human condition, further studies are needed to assess their clinical implications.

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