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Acute angiotensin-converting enzyme inhibition evokes bradykinin-induced sympathetic activation in diabetic rats

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Abstract

We have previously shown that acute intravenous injection of the angiotensin-converting enzyme (ACE) inhibitor enalapril in diabetic rats evokes a baroreflex-independent sympathoexcitatory effect that does not occur with angiotensin receptor blockade alone. As ACE inhibition also blocks bradykinin degradation, we sought to determine whether bradykinin mediated this effect.

Experiments were performed in conscious male Sprague-Dawley rats, chronically instrumented to measure mean arterial pressure (MAP), heart rate (HR), and renal sympathetic nerve activity (RSNA), 2 wk after streptozotocin (55 mg/kg iv, diabetic, $n = 11$) or citrate vehicle (normal, $n = 10$). Enalapril (2.5 mg/kg iv) decreased MAP in normal rats (-15 ± 3 mmHg), while a smaller response (-4 ± 1 mmHg) occurred in diabetic rats. Despite these different depressor responses to enalapril, HR ($+44 \pm 8$ vs. $+26 \pm 7$ bpm) and RSNA ($+90 \pm 21$ vs. $+71 \pm 8\%$ baseline) increased similarly between the groups ($P \geq 0.22$ for both). Pretreatment with the bradykinin B₂ receptor antagonist Hoe 140 (10 μ g/kg bolus followed by $0.8 \cdot \mu\text{g}^{-1}\text{kg} \cdot \text{min}^{-1}$ infusion) attenuated the decrease in MAP observed with enalapril in normal rats but had no effect in diabetic rats. Moreover, the normal group had smaller HR and RSNA responses (HR: $+13 \pm 8$ bpm; RSNA: $+32 \pm 13\%$ baseline) that were abolished in the diabetic group (HR: -4 ± 5 bpm; RSNA: $-5 \pm 9\%$ baseline; $P < 0.05$ vs. preenalapril values).

Additionally, bradykinin (20 μ g/kg iv) evoked a larger, more prolonged sympathoexcitatory effect in diabetic compared with normal rats that was further potentiated after treatment with enalapril. We conclude that enhanced bradykinin signaling mediates the baroreflex-independent sympathoexcitatory effect of enalapril in diabetic rats.

Keywords

angiotensin II; blood pressure; baroreflex

ALTERATIONS IN AUTONOMIC REGULATION of the cardiovascular system in diabetic patients, termed diabetic autonomic neuropathy, doubles the risk of mortality (1). Together, diabetic autonomic dysfunction and cardiovascular disease account for ~80% of all diabetic deaths (12). Parasympathetic tone to the heart is decreased in diabetic patients, as evidenced by reduced heart rate variability (2,15). This can lead to unopposed sympathetic stimulation of the heart. Together, these may contribute to silent myocardial ischemia, arrhythmia, and sudden cardiac

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death. Thus, alterations in diabetic autonomic function markedly contribute to mortality and decreased quality of life.

A recent study by Maliszewska-Scislo et al. (16) found that angiotensin AT₁ receptor blockade in both normal and diabetic rats decreased blood pressure and resulted in a typical baroreflex-mediated increase in heart rate (HR) and renal sympathetic nerve activity (RSNA). In contrast, acute administration of the angiotensin-converting enzyme (ACE) inhibitor enalapril resulted in an increase in HR and renal sympathetic nerve activity even at a dose that left blood pressure unchanged, consistent with a baroreflex-independent sympathoexcitatory response. The curves for the baroreflex responses of HR and RSNA were not changed by enalapril. Thus, in diabetic rats, enalapril evoked a baroreflex-independent sympathoexcitatory effect.

The cause for this baroreflex-independent increase in sympathetic outflow with ACE inhibition in diabetic rats was not elucidated in that study (16). However, ACE inhibition not only leads to reduced ANG II production but also to increased formation of the peptide bradykinin, because ACE catalyzes both the formation of ANG II and the degradation of kinins (3,4). The fact that this response occurred with ACE inhibition but not with AT₁ receptor blockade raises the possibility that bradykinin could be involved.

Within the peripheral vasculature, bradykinin stimulates endothelial nitric oxide synthase and promotes vasodilation; however, within the central nervous system, bradykinin is a potent stimulant of sympathetic outflow. Interestingly, in both humans (7) and animals (5) during diabetes, bradykinin receptors are upregulated in key brain stem nuclei that regulate autonomic control. It is currently thought that some of the beneficial effects of ACE inhibition therapy result from the promotion of nitric oxide release by bradykinin. However, potential increases in sympathetic nervous system activity mediated by bradykinin could contribute to the high cardiovascular mortality within this patient population and detract from the beneficial effects of ACE inhibition.

Thus, the first aim of the present study was to test whether the sympathoexcitatory effect of acute ACE inhibition in diabetic rats is mediated by bradykinin. Because bradykinin B₂ receptor expression levels are known to be upregulated during diabetes, the second aim was to ascertain whether the sympathoexcitatory effects of acute bradykinin infusion are potentiated after ACE inhibition.

METHODS

Adult male Sprague-Dawley rats weighing ~275–300 g were obtained from Harlan Sprague Dawley (Indianapolis, IN). They were housed under controlled conditions (21–23°C; lights on, 0700–1900) and had free access to water and standard rat chow. The rats were cared for in accordance with the principles of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All protocols were reviewed and approved by our Institutional Animal Investigation Committee.

Induction of Diabetes Mellitus

Rats were randomly assigned to one of two groups: 1) normal group and 2) diabetic group. Diabetes was induced by a single injection of streptozotocin (STZ) (55 mg/kg iv; Sigma) dissolved in 0.01 M citrate buffer, pH 4.5, and administered 15 days before the experiment. Normal rats received a similar volume of vehicle alone.

Surgical Procedures

Two days before surgery, each rat was conditioned to remain for 120 min within a custom-made Plexiglas study chamber that would be used during the experiment. The chamber allowed the rat to move forward and backward but not to turn around.

Rats were anesthetized with pentobarbital sodium (40 mg/kg ip). Catheters were inserted into the left carotid artery and jugular vein for arterial pressure monitoring and drug infusion, respectively. The catheters were filled with heparinized saline (100 U/ml), secured, tunneled subcutaneously, and exteriorized at the base of the neck. For recording RSNA, the left kidney was exposed through a retroperitoneal approach, and the renal nerve branch was isolated and carefully dissected free. The nerve was placed on electrodes constructed of Teflon-coated silver wire (0.0055-in. diameter, A-M Systems) with the exposed ends wound into single loops. The nerve and electrodes were covered with silicone gel (Kwik-Sil, World Precision Instruments), which was allowed to harden before closure. A ground wire was sewn into the surrounding tissue. The electrodes and ground wire were tunneled subcutaneously and exteriorized at the base of the neck. After animals had recovered from anesthesia, they were returned to their individual cages for a ~24-h recovery period before the start of any experimental procedures. By the end of the recovery period, the animals were grooming themselves normally and displayed normal cage activity.

Methods of measurement—Systemic arterial pressure and HR were measured on a beat-by-beat basis via a Gould P23 XL pressure transducer equipped with an analog-to-digital converter board (Biotech Products) and recorded on computer hard disk for off-line analysis. Data were sampled continuously at 6 Hz by using a DAP 3216a/415 data acquisition processor as the hardware platform.

Arterial pressure was measured by connecting the arterial catheter with a pressure transducer, which was coupled to an amplifier (Digi-Med BPA-200). The arterial pressure was digitized and recorded with a hemodynamic and neural data analyzer (Biotech Products). Mean arterial pressure (MAP) and HR were determined on-line from pulsatile pressure using the Biotech software and averaged over 1-s intervals. All data were stored on hard disk for subsequent analysis.

Renal nerve activity was amplified (5,000–20,000 times) and filtered (100–1,000 Hz) with a Grass P511 differential preamplifier and a high-impedance probe (HIP511GB). The probe and animals were located inside a shielded Faraday cage (Harvard). The amplified and filtered neurogram signal was channeled to an oscilloscope (Hameg Instruments, HM407) and Grass AM8 audiomonitor for visual and auditory evaluation, respectively. The amplified nerve activity was digitized, rectified, integrated, and averaged over 1-s intervals by the computer data acquisition system (Biotech Products). Background noise was determined at the end of experiment after administration of a bolus dose of trimethaphan camsylate, 15 mg/kg iv (Hoffman-La Roche). RSNA was defined as the amount of recorded nerve activity after subtraction of background noise.

Experimental Protocols

In normal and diabetic rats, hemodynamic and sympathetic neural responses to both enalapril and bradykinin were studied. Experiments were performed at least 24 h after completion of surgery. On the day of the study, the animal was placed in the study chamber and connected to the recording equipment. MAP, HR, and RSNA were recorded continuously during a 30-min stabilization period, which was followed by ~10 min of baseline data collection.

Protocol 1—After a stable baseline was achieved, the rat was randomly assigned to receive either enalapril (2.5 mg/kg iv) alone or pretreatment with the bradykinin B₂ receptor antagonist Hoe 140 (10 µg/kg bolus plus 0.8 µg·kg⁻¹·min⁻¹ infusion) followed 5 min later by enalapril (2.5 mg/kg iv). On the following day, the rats underwent the alternate protocol. MAP, HR, and RSNA were continuously recorded for 60 min after the injection of enalapril. This dose of Hoe 140 evoked a transient (<60 s) change in all parameters, which returned to baseline; the response to exogenous bradykinin (10–30 µg/kg) was abolished (22).

Protocol 2—On the next experimental day, bradykinin (20 µg/kg iv) was injected. At least 15 min were allowed for all parameters to return to baseline, and then each rat was injected with enalapril (2.5 mg/kg iv), followed by bradykinin (20 µg/kg iv) 10 min later. MAP, HR, and RSNA were continuously recorded for 10 min after bradykinin was injected.

Data Analysis

A direct comparison of the absolute level of nerve activity either between experiments or across animals is not possible because of nonphysiological factors (nerve electrode contact, size of nerve bundle). Thus, resting nerve activity was normalized to 100%. Responses were calculated as the percent of baseline.

Baseline values before enalapril or bradykinin were calculated as the mean of a 1-min period before the injection. For *protocol 1*, data were averaged across 1-min intervals for 3 min before injection and 10 min afterward. MAP and HR data were quantified as the change in absolute value from baseline. For *protocol 2*, because the hemodynamic and sympathetic neural responses to bradykinin are much more rapid than to enalapril, data were averaged across 5-s intervals for 1 min before injection and 2 min afterward. MAP and HR were expressed in absolute values and change in absolute value from baseline. In addition, the area under the curve was calculated between 30 and 120 s after bradykinin as the difference of the delta response in the absence and presence of enalapril. The first 30 s after bradykinin were omitted to avoid any artifact, as immediately after bradykinin injection, the rats moved for ~10 s.

Statistical Analysis

All data are presented as means ± SE. A one-way ANOVA was used to compare baseline parameters. Time course data were compared using a two-way ANOVA followed by a Newman-Keuls post hoc analysis. A Student paired *t*-test was used to compare the differences in area under the curve data between normal and diabetic rats. A value of *P* < 0.05 was considered statistically significant.

RESULTS

The baseline characteristics of normal and diabetic rats are shown in Table 1. Two weeks after the injection of streptozotocin, diabetic rats had significantly higher glucose levels than normal rats, which were injected with vehicle. Resting MAP and HR values were significantly lower in the diabetic rats compared with the normal rats. Body weight tended to be lower in the diabetic rats, but it did not reach statistical significance (*P* = 0.095).

Time Course of Changes in MAP, HR, and RSNA in Response to Enalapril Before and After Pretreatment with Hoe 140

Diabetic rats exhibited a markedly different hemodynamic response to acute enalapril compared with normal rats, as shown previously (16). Enalapril in normal rats (Fig. 1) evoked a significant depressor response (-15 ± 3 mmHg at 10 min, *P* < 0.05 vs. baseline MAP), while in the diabetic rats, the magnitude of this decrease was significantly attenuated (-4 ± 1 mmHg at 10 min, *P* < 0.05 vs. baseline). Despite having a reduced effect upon MAP in diabetic rats,

enalapril-evoked increases in HR and RSNA (Fig. 1) that were of similar magnitude between normal and diabetic rats. Thus, in the diabetic rats, a significant component of the rise in HR and RSNA occurred independently of a change in blood pressure. This is further supported by the fact that the ratio of RSNA to MAP (Table 2) in diabetic rats was significantly greater compared with that in normal rats.

Pretreatment with the B₂ receptor antagonist Hoe 140, markedly altered the response pattern to enalapril in both the normal and the diabetic rats (Fig. 1). The depressor response to enalapril was smaller after Hoe 140 in the normal rats (-5 ± 2 mmHg at 10 min; $P < 0.05$ vs. baseline) and differed significantly compared with rats treated only with enalapril ($P < 0.05$). In the diabetic group, the depressor response to enalapril after Hoe 140 (-3 ± 3 mmHg at 10 min; $P > 0.05$ vs. baseline) was similar to that with enalapril alone. Enalapril still increased HR and RSNA in the normal group following Hoe 140, although these responses were significantly smaller compared with those without B₂ receptor blockade. The effect of Hoe 140 in the diabetic group was even more profound, as it abolished the rise in HR and RSNA that occurred with enalapril alone (Fig. 1). Hoe 140 significantly decreased the ratio of HR to MAP in both groups, and the ratio of RSNA to MAP in the diabetic group (Table 2).

Time Course of Changes in MAP, HR, and RSNA in Response to Bradykinin, Before and After Pretreatment With Enalapril

Figure 2 shows the summary data of the time course of hemodynamic and renal sympathetic nerve responses to bradykinin injection, before and after enalapril. Baseline MAP and HR were significantly lower in the diabetic group compared with the normal group. In both normal and diabetic rats, bradykinin evoked a transient depressor response. The reduction in MAP was significantly less in the diabetic group compared with the normal group. In both groups, the depressor response was accompanied by significant increases in HR and RSNA ($P < 0.05$ vs. baseline). Despite the attenuated depressor response in the diabetic group, the concomitant increases in HR and RSNA were significantly greater compared with normal rats.

After pretreatment with enalapril, bradykinin still evoked a depressor effect; however, blood pressure remained below baseline. Notably, the increases in HR and RSNA in response to bradykinin in the diabetic group were approximately twice as great as those observed in the normal group.

Figure 3 depicts the differences in the area under the curves (from Fig. 2) between bradykinin alone and bradykinin with enalapril in normal and diabetic groups. The timeframe included the time immediately after the initial transient response (defined as 30 s in the bradykinin alone group) to 120 s. Although the difference in MAP was similar in both normal and diabetic rats, HR and RSNA were three- to four-fold greater in the diabetic group.

DISCUSSION

ACE inhibitors are highly recommended for treatment of the cardiovascular and renal complications that commonly occur in diabetics. ACE inhibitors reduce ANG II production but also lead to bradykinin accumulation due to blockade of bradykinin degradation (3,4). Although it is known that increased bradykinin levels during ACE inhibition can act presynaptically on sympathetic nerve terminals to augment norepinephrine release (11,18,19), no previous studies have investigated a potential sympathoexcitatory effect of bradykinin in the diabetic setting. This is surprising, considering that bradykinin is a potent central sympathetic stimulant and that bradykinin receptor expression levels during diabetes mellitus in both animals (5) and humans (7) are elevated in key regions of the brain stem known to regulate cardiovascular function. The purpose of this study was to provide evidence for proof of concept that increased bradykinin signaling with acute ACE inhibition in conscious diabetic

rats can lead to sympathetic activation. The major findings after 2 wk of STZ-induced diabetes are 1) enalapril evokes baroreflex-independent increases in HR and RSNA that are abolished with bradykinin B₂ receptor blockade; 2) bradykinin-injected intravenously leads to increases in HR and RSNA that are of greater magnitude and prolonged in diabetic rats compared with normal rats; and 3) the sympathoexcitatory effect of bradykinin is potentiated after pretreatment with enalapril to a significantly greater extent in diabetic rats compared with normal rats.

Effect of Enalapril on HR and RSNA in Conscious Diabetic Rats

A recent study by Maliszewska-Scislo et al. (16) demonstrated in diabetic rats that acute treatment with enalapril at a dose that did not decrease blood pressure resulted in increased HR and RSNA. This response was not seen with the angiotensin AT₁ receptor antagonist losartan. Importantly, after 2 wk of STZ-induced diabetes, baroreflex curves for the HR and RSNA responses were similar in normal and diabetic rats. Furthermore, neither enalapril nor losartan altered these responses. That HR and RSNA increased independent of any change in blood pressure at a time when baroreflex function was normal and that this effect was seen with enalapril but not with losartan suggest an effect that is a specific property to ACE inhibitors.

The current data confirm and extend our previous findings (16), inasmuch as enalapril in normal rats decreased blood pressure, while the same dose in diabetic rats had no effect upon blood pressure. Yet the magnitude of the increase in HR and RSNA was similar between the groups. That this is a real phenomenon whereby enalapril has a blood pressure-independent sympathoexcitatory effect in diabetic rats that does not exist with losartan led us to hypothesize that bradykinin could be responsible. Our results show that in normal rats, pretreatment with Hoe 140 attenuated the decrease in blood pressure and the concomitant rise in HR and RSNA that occurred with enalapril alone. This indicates that a significant fraction of the depressor effect of acute enalapril is, in fact, mediated by bradykinin in normal rats. This most likely occurs through stimulation of peripheral bradykinin B₂ receptors. These are G protein-coupled receptors that lead to the production of nitric oxide and ensuing vasodilation (i.e., endothelial-dependent vasodilation). As diabetic rats are known to have endothelial dysfunction (17), it is then not surprising that enalapril did not change blood pressure in diabetic rats, regardless of whether Hoe 140 was present or not. The lack of change in blood pressure with enalapril rules out that the increase in HR and RSNA being mediated by a baroreflex-dependent mechanism. On the contrary, the importance of bradykinin in mediating this increase in HR and RSNA in the diabetic rats was very clear, as pretreatment with Hoe 140 followed by acute enalapril abolished this effect. Similar findings have been shown in both normal and hypertensive humans (10).

Cardiovascular Effects of Intravenous Bradykinin in Normal and Diabetic Rats

Although ACE inhibition leads to bradykinin accumulation and appears to have differential effects upon normal and diabetic rats (at least at the dose we used), we sought to test the effect of acute exogenous bradykinin directly. In normal rats, bradykinin injection was accompanied by a transient decrease in blood pressure, whereas there was an equally transient rise in HR and RSNA. As expected, in the diabetic rats, the same dose of bradykinin evoked a smaller transient decrease in blood pressure. However, unexpectedly, pressure then increased above baseline. In spite of this, HR and RSNA also increased above baseline and to a greater extent compared with normal rats. After pretreatment with enalapril, bradykinin led to a greater and more sustained reduction in blood pressure and greater increases in HR and RSNA in the normal rats. This would be consistent with blockade of the generation of ANG II by the ACE inhibitor. In the diabetic rats, the initial depressor response during enalapril + bradykinin was smaller than in the normal group; however, similar to the normal rat, it was also sustained. Nonetheless, the magnitude of the increases in HR and RSNA was at least twofold greater than those observed in the normal rat. Thus, the effect of bradykinin is augmented by enalapril and

substantially greater in diabetic rats. Recently, Sivieri et al. (20) found that the vasodilator effect of bradykinin was potentiated with ACE inhibition in an isolated mesenteric arterial bed preparation in both normotensive and hypertensive (one-kidney, one-clip) rats. If ACE inhibition can potentiate the effect of bradykinin on vascular smooth muscle (20), it is logical to suggest that ACE inhibition could also potentiate bradykinin action on receptors that mediate its sympathoexcitatory effects.

Potential Mechanisms of Bradykinin-Induced Sympathetic Activation During Diabetes

Bradykinin is known to act on bradykinin B₂ receptors to exert potent sympathoexcitatory effects in several regions of the brain stem known to play critical roles in cardiovascular regulation (6,9,13,14). The sensitivity of the pressor effect of bradykinin when injected into the fourth cerebral ventricle, which is on the dorsal surface of the medulla oblongata, is 20–100 times greater than when injected into either the third or lateral ventricles (14). This suggests that this is a key site of bradykinin action. The area postrema, which lies on the floor of the fourth ventricle outside the blood-brain barrier, contains neurons that appear to mediate this effect. That is, the rise in blood pressure evoked by bradykinin is abolished with lesioning of the area postrema (21). The cardiovascular effects of bradykinin within the brain stem during diabetes mellitus have never been studied. Interestingly, receptor binding studies in both diabetic rats (5) and humans (7) clearly show upregulation of bradykinin B₂ receptors within these brain stem regions. Thus, this provides the conceptual framework for a cause-and-effect relationship between ACE inhibition during diabetes mellitus and bradykinin-mediated sympathetic activation.

Conclusion

In summary, the present study indicated that after 2 wk of streptozotocin-induced diabetes, acute enalapril evokes a blood pressure-independent increase in HR and RSNA that is mediated by bradykinin acting upon B₂ receptors. In addition, bradykinin injection into diabetic rats evokes significantly greater increases in HR and RSNA compared with normal rats, both in terms of magnitude and duration. It is important to note that our study only investigated the acute effects of ACE inhibition in diabetic rats; therefore, further studies are warranted to determine whether this also occurs with chronic ACE inhibition in diabetic rats.

Perspectives

The presence of impaired autonomic function during diabetes mellitus contributes to the high risk of cardiovascular mortality among these patients. While ACE inhibition treatment is clearly a beneficial therapy for diabetic subjects, bradykinin accumulation could stimulate increases in sympathetic outflow. Theoretically, adding a central sympatholytic agent to ACE inhibition therapy could maximize the beneficial effects of ACE inhibition. In this regard, Ebbehøj et al. (8) have shown that adding β -blocker treatment to concurrent ACE inhibition in patients with Type I diabetes improved autonomic function.

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Fig. 1.

Time course of the change in mean arterial pressure (MAP), heart rate (HR), and renal sympathetic nerve activity (RSNA) from baseline to either enalapril alone (●) or enalapril following pretreatment with the bradykinin B₂ receptor antagonist Hoe 140 (○) in normal ($n = 7$) and diabetic rats ($n = 6$); enalapril was injected at time 0. * $P < 0.05$ vs. enalapril alone.

Fig. 2. Time course of absolute MAP, HR, and RSNA responses to bradykinin alone (injected at *time 0*; *left*) or to bradykinin following pretreatment with enalapril (*right*) in normal (•, *n* = 9) and diabetic (○, *n* = 9) rats. **P* < 0.05 vs. normal.

Fig. 3.

The differences in the area under the curves (from Fig. 2) between bradykinin alone and bradykinin with enalapril in normal (■) and diabetic (□) groups for MAP, HR, and RSNA between 30 and 120 s after bradykinin. * $P < 0.05$ vs. normal.

Table 1

Baseline values in normal and diabetic rats

	<i>n</i>	Weight, g	Glucose, mg/dl	Mean Arterial Pressure, mmHg	Heart Rate, bpm
Normal	10	281±7	134±3	129±3	405±11
Diabetic	11	265±10	507±18*	110±2*	310±13*

Values are expressed as means ± SE; *n*, number of rats.

* *P* < 0.05 vs. normal.

Table 2
Effect of Enalapril or Enalapril + Hoe 140 on the ratio of heart rate or RSNA to MAP in normal and diabetic rats

	Normal	Diabetic
HR, bpm		
Enalapril	-3.37 ± 0.76	-5.10 ± 1.29
Enalapril + Hoe 140	$-1.02 \pm 0.85^*$	$-0.49 \pm 0.99^*$
RSNA, %baseline/mmHg		
Enalapril	-6.76 ± 1.45	$-17.39 \pm 4.25^\dagger$
Enalapril + Hoe 140	-2.09 ± 3.23	$-2.65 \pm 2.07^*$

Values are expressed as means $\pm$ SE

HR, heart rate; RSNA, renal sympathetic nerve activity; MAP, mean arterial pressure.

* $P < 0.05$ vs. enalapril

† $P < 0.05$ vs. normal.