Augmented Sympathetic Response to Bradykinin in the Diabetic Heart Before Autonomic Denervation

Zbigniew Pietrzyk, Stephen Vogel, Guenther J. Dietze, Sara F. Rabito

Abstract—We studied whether diabetes mellitus affects the bradykinin (BK)-induced release of norepinephrine (NE) from rat cardiac sympathetic endings in situ. Three groups were studied. Group A (n = 12) was rendered diabetic with streptozotocin (STZ), group B (n = 13) received STZ and insulin, and group C (n = 14) received citrate buffer only. NPH insulin was given to group B from day 7 after STZ. Atria were paced (3 Hz) with rectangular voltage pulses at mechanical threshold intensity (0.15 V/cm). The release of NE was assessed through its effects on contractile force in the presence of atropine (1 μmol/L). Intensifying the field stimulation above the neural threshold (≈0.4 V/cm) produced a graded positive inotropic effect that was due to the release of NE from sympathetic nerve endings. The additional effect of 0.1 μmol/L BK on the force of contraction was determined at half-maximal neural stimulation (ie, at ≈0.65 V/cm). Then, after washing out BK and lowering the stimulation intensity to mechanical threshold, a cumulative dose-response curve for added NE was generated, allowing the positive inotropic effects of neural stimulation (with or without BK) to be expressed in terms of an equivalent inotropic concentration of added NE ([NEeq]). Neural stimulation, in the absence of BK, gave an [NEeq] of 32 ± 3 nmol/L in group A, 44 ± 6 nmol/L in group B, and 37 ± 6 nmol/L in group C. BK increased [NEeq] by a factor of 6.2 ± 0.9 in group A, 4.5 ± 0.5 in group B, and 3.7 ± 0.3 in group C. This factor was greater in group A than in group C but indistinguishable in groups B and C. Atria from normal and diabetic rats were incubated in [3H]NE for 60 minutes. Excess tracer was removed, and atria were stimulated during a series of 1-minute episodes at half-maximal neural stimulation to cause exocytotic [3H]NE release. BK augmented [3H]NE release in normal (n = 4) and in diabetic (n = 4) atria. This BK-induced increase of [3H]NE overflow (expressed as a fraction of tissue [3H]NE radioactivity) was 4 times greater in diabetic than in normal preparations. The response to BK in releasing sympathetic neurotransmitter is augmented in diabetic rats, recovering in a manner dependent on insulin. (Hypertension. 2000;36:208-214.)

Key Words: bradykinin ■ diabetes mellitus ■ norepinephrine ■ insulin

In chronic diabetes, there is a cardiomyopathy involving cardiac contractile performance that is related to diabetic autonomic neuropathy (DAN).1 DAN is a common complication of diabetes and has been proposed as a cause of sudden cardiac death in diabetic patients.2,3 Cardiac denervation results in a fixed heart rate that is unresponsive to exercise or stress. This absence of heart rate variability secondary to DAN is predictive of left ventricular failure4,5 and increased mortality.6 Diabetic rats have defects in the intrinsic electrical and contractile properties of the heart7 and in adrenergic and cholinergic receptor populations,8 and they also exhibit reduced norepinephrine (NE) turnover.8 These alterations may lead to impaired cardiac function. Moreover, the streptozotocin (STZ)-induced diabetic rat model is known to develop DAN after 16 weeks of induction.8 We have shown that in isolated rat atria, bradykinin (BK) affects cardiac contractile force by its modulation of evoked NE release from cardiac sympathetic nerve endings.10 BK was without effect on atrial contractile force when stimulation intensity was set to low levels that excite heart muscle but not neurons.10

The present investigation was undertaken to determine whether (1) the development of diabetes mellitus affects the BK-induced NE release, as determined by the measurements of twitch contractile force and exocytotic [3H]NE release, and (2) insulin therapy reverses the contractile alterations in response to BK.

Methods

After our Animal Care and Use Committee approved the protocol, 49 adult Sprague-Dawley rats (250 to 300 g) were randomly divided into 3 groups: (1) diabetic (n = 16), (2) insulin-treated diabetic (n = 13), and (3) control (n = 20). Diabetes was induced with a single injection of STZ (55 mg/kg) in 0.05 mol/L citrate buffer, pH 4.5. Control rats received citrate buffer (1 mL/kg). Injections were given intravenously through a tail vein while the animals were under light general anesthesia with 5% sevoflurane. Diabetic and control rats were caged separately but housed under similar conditions. Both
groups of animals were fed the same diet and water ad libitum until the day before the experiment. Rats were considered diabetic if fasting blood glucose exceeded 400 mg/dL 2 days after STZ. NPH human insulin was given to the second group at 3.3 U/d SC at 4:00 PM, from day 7 after STZ until the day before the experiment. Blood glucose, body weight, and water and food intake were measured after the injection of STZ and at weekly intervals thereafter. To measure blood glucose, rats were placed under light general anesthesia with 5% sevoflurane, and a drop of blood was collected from a tail vein with a 25-gauge needle. Blood glucose concentration was measured with an Accu-Chek III blood glucose monitor (model 766, Boehringer-Mannheim Corp).

**Measurement of Contractile Force in Isolated Heart Muscle**

Twelve to 16 weeks after the administration of STZ or citrate buffer, the rats were anesthetized with halothane. The hearts were removed and immediately perfused to remove the blood. The left atria were excised and attached between a force-displacement transducer (Grass FT 0.3) and a fixed point with use of a pair of stainless-steel hooks. The atria were immersed in a water-jacketed glass chamber (70-ml capacity) containing heated (33°C) and oxygenated (100% O2) Krebs-Henseleit solution. After a 30-minute equilibration period, the resting tension of the tissue was adjusted to give half-maximal twitch developed force. The muscle was then stimulated at a frequency of 3 Hz with rectangular current pulses delivered via a pair of platinum electrodes on either side of the preparation. The field strength (mV/cm) of current pulses was measured from a pair of silver wires on either side of the preparation at a distance of 1 cm; these recording electrodes were insulated except for their tips. Twitch contractions were recorded on a flatbed recorder (model 45, IITC) and simultaneously displayed on the video monitor of a computer (DELL 325P) after being digitized (Labmaster Board, Tecmar, Inc). Online automated measurements of the peak amplitude of twitch contractions were made and stored in a file for later analysis.

**Determination of Indirect Positive Inotropic Effect of BK**

Field stimulation was intensified for excitation of the sympathetic nerve endings embedded in atrial muscle. Atropine (1 μmol/L) was used to block possible cholinergic effects of intensified stimulation. To standardize excitation of sympathetic terminals, the electrical stimulus was set to the voltage giving half-maximal inotropy (V1/2) of the evoked sympathetic catecholamine. V1/2 was determined by increasing the stimulation intensity above the mechanical threshold in steps of 5 or 10 V and recording the resultant positive inotropic effects. V1/2 was estimated from a plot of effect versus voltage. Next, with stimulation intensity set at V1/2, the contractile force was measured until stable. BK (0.1 μmol/L) was added to the bath, and its positive inotropic effect was monitored until it developed fully. To prevent BK degradation by angiotensin I–converting enzyme (ACE)kininase II, 2.6 μmol/L enalaprilat was added to the bath 1 minute before BK administration.

**Inotropic Action of NE and Tyramine**

The bath liquid was replaced several times with drug-free solution to remove BK. After the recording of baseline contractile force, a cumulative concentration-effect curve for added NE (3 nmol/L to 6.7 μmol/L) was constructed (stimulation lowered to mechanical threshold). NE concentration was raised only after the twitch contractile force reached a steady state after the previous NE concentration. In some atria, once the concentration-effect curve for NE was completed, a baseline for contractility was reestablished, and the positive inotropic effect of tyramine (100 μmol/L) was determined.

**Calculation of Equivalent Inotropic Concentration of NE**

The effects of intensified field stimulation on contractile force, with or without BK, were compared with the effect of added NE to determine the equivalent inotropic concentration of NE ([NEeq]). [NEeq] was calculated from \( \frac{K_{d,NE} \times E}{E_{max,NE} - E} \), where \( K_{d,NE} \) is the NE concentration producing the half-maximal effect, \( E_{max,NE} \) is the experimentally determined maximal inotropic effect of NE, and E is the observed stimulation effect (at \( V_{1/2} \)) with or without the addition of BK (Figure 1). \( K_{d,NE} \) was calculated by fitting a sigmoidal function to the log NE concentration versus effect data. This calculation relies on the fact that the entire inotropic effect of field stimulation and BK are due to NE released from sympathetic nerve endings.\(^{10}\)

**Measurement of Exocytotic \( ^{3} \text{H} \text{NE} \) Release**

Isolated atria were prepared for simultaneous measurements of twitch contractile force and \( ^{3} \text{H} \text{NE} \) release. Preparations were attached to a force displacement transducer and paced at mechanical threshold (0.4 Hz, 0.2 V/cm). A smaller experimental vessel (10-ml capacity) was used. Attria were labeled for 60 minutes with 27 Ci/mmol \( ^{3} \text{H} \)NE and immediately perfused to remove the blood. The left atria were determined. A1 and A2, Tracings of twitch contractions recorded from an isolated rat left atrial preparation (atropine concentration 1.0 μmol/L). A1, Positive inotropic effect due to evoked release of sympathetic agonist. Field strength was intensified in small (5- to 10-V) steps, starting at 10 V; positive inotropic effect of stimulation was graded >30 V (ie, the neural threshold value), and maximal was >70 V. \( V_{1/2} \) (45 V) was estimated from the voltage-effect plot of the data (Figure 2). The inotropic effect was reversed by lowering the stimulation intensity below the neural threshold. A2, Augmentation by BK (0.1 μmol/L) of the positive inotropic effect of stimulation at \( V_{1/2} \) intensity. A3, Positive inotropic effect of NE (cumulative addition) at indicated bath concentrations (stimulation intensity 10 V). Records A1 to A3 were obtained for each preparation studied. Time between 2 vertical lines is 2 minutes. Force between 2 horizontal lines is 100 mg. B, Calculation of the apparent concentration of neurally evoked NE. B1, Concentration-effect curve for added NE. \( K_{d,NE} \) and \( E_{max,NE} \) were obtained by fitting the sigmoidal function shown to log (NE) versus effect data; NE effect has been plotted relative to \( E_{max,NE} \). B2, Double-reciprocal plot of function shown in panel B1 (1/effect versus 1/[NE]). The observed effect of evoked NE release (as illustrated in panel A2) was converted to [NEeq] by using the analytic expression \( K_{d,NE} \times E/(E_{max,NE} - E) \), where E represents the inotropic effect of stimulation at voltage \( V_{1/2} \) in the presence or absence of BK; \( K_{d,NE} \), if \( E = 0.25 \), then [NEeq] = 3.3 × 10^{-8} \text{ mol/L} \ (K_{d,NE} \text{ and } E_{max,NE} = 10^{-7} \text{ mol/L and 1.0, respectively).
Protocol for Stimulation-Evoked Release of $^3$H]NE
Preparations were bathed in Krebs’ solution supplemented with 3 μmol/L cocaine for the remainder of the experiment. This portion of the experiment was divided into seven 10-minute periods (S0 to S6). For S0, no stimulation was applied; for S1 to S6, the preparations received (during the first minute only) field stimulation above neural threshold (0.7 V/cm, 2 Hz; see Figure 2A legend) to evoke exocytotic NE release. At the end of each 10-minute period, a 100-μL sample was withdrawn from the bath for later determination of tritium, and the bath liquid was replaced with fresh cocaine-containing Krebs’ solution. BK (0.1 μmol/L) was administered immediately before S5. At the conclusion of the experiment, the atria were blotted, weighed, and placed overnight in a glass vial containing 1 mL of 2% (vol/vol) perchloric acid. Radioactivity in tissue extracts and collected fractions was determined by mixing them with a biodegradable counting cocktail (Econo-Safe) by liquid scintillation counting (Packard 2000 Tri-Carb, Packard Instrument Co).

Tissue Uptake of $^3$H]NE
By use of the tracer washout samples (see above), the number of $^3$H]NE counts remaining in unstimulated tissue was plotted against time. A double-exponential function was fitted to the data. The fast component (time constant 5 to 10 minutes) accounted for 90% of the tissue uptake; extrapolation of this exponential component to 0 minutes gave an estimate of initial tissue uptake of tracer (see Figure 5 legend).

Exocytotic Release of $^3$H]NE
From samples S1 to S6, stimulated $^3$H]NE release (overflow) was expressed as a fraction of tissue radioactivity with correction for fractional background (unstimulated) release of tritium. The latter was obtained as the $^3$H]NE release during S0 (expressed as a fraction of tissue radioactivity). The BK effect for each preparation was taken as the difference between $^3$H]NE overflow in S6 and S4.

Drugs and Solutions

**Drugs**
Atropine, NE, BK, STZ, ascorbic acid, and all salts used in the Krebs-Henseleit solution were obtained from Sigma Chemical Co. Enalaprilat was a gift from Merck Research Laboratories (Rahway, NJ). Sevoflurane was obtained from Abbott Laboratories, and halothane was from Ayerst Laboratories Inc. NPH human insulin (recombinant DNA origin) was acquired from Novo Nordisk Pharmaceuticals Inc. dL-NE hydrochloride ($^3$H-N) was purchased from American Radiolabeled Chemicals, Inc, and Econo-Safe was from Research Products International Corp. Cocaine was obtained from the University of Illinois Hospital Pharmacy.

**Solutions**
The composition of the Krebs-Henseleit solution was as follows (mmol/L): NaCl 118, KCl 4.7, CaCl2 0.2, H2O 1, HEPES (acid) 5.55, Na+ HEPES 4.45, MgCl2 0.25, glucose 10, and Na+ -EDTA 0.025. A stock solution of BK (0.1 mmol/L) was prepared in advance in 154 mmol/L NaCl and kept at −20°C until used. A stock solution of NE (1.0 mmol/L) was prepared before each experiment in Krebs-Henseleit solution containing equimolar ascorbic acid.

Statistical Analysis
Results are expressed as mean±SEM. Statistical comparisons among group means were made by 1-way ANOVA with the Newman-Keuls multiple comparison post hoc test. Differences were considered statistically significant at *P<0.05.*

Results

**General Observations**
After STZ administration, the animals exhibited the common characteristics of diabetes: polyuria, polydipsia, and polyphagia. As shown in the Table, at the time of the experiment, STZ-treated rats had significantly lower body weight and elevated blood glucose levels compared with control rats, which received citrate buffer only, indicating that the STZ-treated rats became diabetic. Insulin treatment partially reversed the changes observed in diabetic rats. Although diabetic and insulin-treated diabetic rats had smaller hearts, the heart weight/body weight ratio was significantly increased in diabetic rats, indicative of myocardial hypertrophy, but the ratio was not different from control in diabetic rats receiving insulin.

**Indirect Inotropic Action of BK in Isolated Atria From Normal and Diabetic Rats**

**Effect of Increasing Stimulation Intensity on Atrial Contractility**
Increasing the intensity of field stimulation produced graded positive inotropic effects in all groups. Atropine prevented muscarinic cholinergic effects on the atrial preparation from released parasympathetic agonists (see Methods). The relationship between stimulation intensity and force of contraction for each group was sigmoidal (Figure 2). Positive inotropy due to the release of NE from sympathetic nerve endings was evident at intensities >≈30 V (Figure 2A), corresponding to a neural threshold value of ≈0.4 V/cm (Figure 2B). The maximal inotropic effect of stimulation, which occurred at a field strength of >0.8 V/cm, was markedly depressed in diabetic animals (Figure 2A). Insulin treatment did not reverse this change. The $V_{1/2}$ values were statistically identical among the 3 treatment groups (see Figure 2 legend).

**Effect of NE on Atrial Contractility**
To determine whether the diabetic state causes a reduction in NE sensitivity, the inotropic responses to cumulative doses of added NE were obtained. As shown in Figure 3, atria from control animals gave the largest increase in contractile force in response to increasing concentrations of NE. The most depressed response was observed in atria from diabetic animals. Insulin treatment produced only a minimal improvement in NE responsiveness. The average EC50 values were 94±14 nmol/L in the control group, 108±15 nmol/L in the diabetic group, and 119±15 nmol/L in the insulin-treated diabetic group. Because these values were not significantly different from one another, the reduced sensitivity to NE in left atrial preparations from diabetic rats is attributed to a smaller NE maximal effect.

**Effect of STZ and Insulin on Sympathetically Mediated Contractile Response to BK**
Figure 4 summarizes the data for the effect of diabetes on the response to BK. Diabetes did not significantly affect the baseline inotropic response ([NEeq]0) to stimulation of atria at $V_{1/2}$ intensity (32±3 mmol/L in diabetic rats, 44±6 mmol/L in insulin-treated diabetic rats, and 37±6 mmol/L in control rats). The addition of enalaprilat before BK production had no measurable inotropic action. However, the BK-induced increase in [NEeq] above baseline values differed significantly in the 3 groups. [NEeq] was increased by a factor of 6.2±0.9 in the diabetic group, 4.5±0.5 in the diabetic group receiving insulin.
Pietrzyk et al.  Augmented Response to BK in Diabetic Heart

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**administration of STZ or diluent. V1/2 was 45.8 ± 6.3 V in diabetic (DM, n = 12) and insulin-treated diabetic (DM+Ins, n = 13) rats. Preparations were isolated 12 to 16 weeks after the administration of STZ or diluent. V1/2 was 42.5 ± 6.6 V in control rats, and 43.9 ± 6.6 V in DM rats, and 42.5 ± 6.6 V in DM+Ins rats.**

**Figure 1.** Relationship between contractile force (% increase) and stimulation intensity (V) in atria from control (●, n = 14), DM (○, n = 12), and DM+Ins (▲, n = 13) rats. Inotropic effect is expressed as percentage of basal contractile force (see legend of Figure 2).

**Figure 2.** Positive inotropic effect of intensified stimulation in isolated rat left atria. A, Relationship between contractile force and stimulation intensity in atria from control (●, n = 14), diabetic (DM, ○; n = 12), and insulin-treated diabetic (DM+Ins, ▲; n = 13) rats. Preparations were isolated 12 to 16 weeks after the administration of STZ or diluent. V1/2 was 45.8 ± 2.3 V in control rats, 43.9 ± 2.3 V in DM rats, and 42.9 ± 2.2 V in DM+Ins rats. B, Relationship between field strength (V/cm) and stimulation intensity (V). The regression line through the data points has a slope of 0.0145. In general, the slope obtained depends on bath volume and geometry of stimulating electrodes. Inotropic effect is expressed as percentage of basal contractile force that was 334 ± 53 mg in the control group, 298 ± 27 mg in the DM+Ins group, and 427 ± 75 mg in the DM group. Values are mean ± SEM.

**Figure 3.** Relationship between the logarithm of the concentration of added NE and inotropic effect in isolated atria from control (●, n = 14), DM (○, n = 12), and DM+Ins (▲, n = 13) rats. Inotropic effect is expressed as percentage of basal contractile force (see legend of Figure 2).

**Effect of Tyramine on Atrial Contractility**

We measured the indirect positive inotropic effect of tyramine as a functional test for possible differences in the extent of sympathetic innervation in the atrial preparations. Tyramine causes the release of NE from sympathetic nerve endings. At a concentration of 100 µmol/L, the relative inotropic response to tyramine was virtually identical in atria from diabetic and control rats. The results indicate that sympathetic denervation had not occurred in the diabetic preparations used.

**BK-Induced [3]H[NE] Overflow in Atria Isolated From Normal and Diabetic Rats**

**Tissue Uptake of [3]H[NE]**

In unstimulated atria, the tissue uptake of [3]H[NE] was estimated by extrapolation of the tracer washout curve to time 0 (Figure 5). In all preparations, the washout was adequately described as a double-exponential decaying function, which was essentially the same in normal and diabetic groups. The fast and slow time constants were 9 ± 0.8 and 102 ± 8 minutes, respectively, for the normal group and 8 ± 0.9 and 93 ± 2 minutes, respectively, for the diabetic group. The fast and slow time constants were statistically indistinguishable in the 2 groups. The tissue uptake of [3]H[NE] averaged 40.359 ± 4350 and 27.647 ± 5717 counts per milligram wet atrial weight for normal and diabetic rats, respectively, and the difference between the mean values was statistically significant (P < 0.05). This difference, however, was attributable to the higher wet weight of atria of diabetic origin (49.3 ± 9.4 mg) versus control atria (29.7 ± 2.4 mg). The difference in tissue uptake was scaled by the same factor as for the difference in weight. This indicated that the magnitude of tissue loading of tracer was equivalent in both groups.

**[3]H[NE] Overflow**

Figure 6 illustrates our method for determining the effects of BK on [3]H[NE] overflow. BK increased both [3]H[NE] overflow

| Effects of STZ-Induced Diabetes and Insulin Treatment on Blood Glucose Level and Body and Heart Weights |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Group                                           | Blood Glucose, mg/dL | Body Weight, g  | Heart Weight, g | Heart Weight/Body Weight, ×100 |
| Control (n = 14)                                | 137 ± 10          | 390 ± 22        | 1.71 ± 0.07     | 0.44 ± 0.025     |
| Diabetic (n = 12)                               | 442 ± 7*†         | 303 ± 15*†      | 1.51 ± 0.08*    | 0.50 ± 0.012†    |
| Diabetic + insulin (n = 13)                     | 257 ± 17*         | 354 ± 11*       | 1.52 ± 0.04*    | 0.43 ± 0.010     |

Values are mean ± SEM. Measurements were taken 12–18 weeks after STZ injection.

*P < 0.05 vs control group; †P < 0.05 vs insulin-treated diabetic group; and ‡P < 0.05 vs control or insulin-treated diabetic group.
and the twitch contractile force more in the diabetic rats. Figure 7 summarizes the data for BK-induced [3H]NE overflow in atria from normal and diabetic rats. In the diabetic group, the BK effect was 4 times greater than that observed in the control group (0.025 ± 0.005 versus 0.006 ± 0.002).

Discussion

The present study indicates that in diabetic rats the response to BK in releasing sympathetic neurotransmitters is augmented and that this high BK sensitivity is an early functional marker of autonomic neuropathy before the occurrence of sympathetic neuronal degeneration.

Because the diabetic heart muscle has an altered baseline contractility and a reduced sensitivity to catecholamines, we assessed evoked NE release in our myocardial preparation as [NEeq], thus facilitating comparison of BK responses among the different study groups. [NEeq] refers to the exact concentration of added NE that would produce the observed effects of intensified electric stimulation in the absence and presence of BK. This analysis makes the assumption that the positive inotropic effect of intensified stimulation and the further contractile effect of BK are due to NE release from sympathetic nerve endings, provided that atropine is present to block cholinergic effects. We previously demonstrated this to be the case in isolated rat left atrial preparations. In the present study, only minor variations in basal NE liberation at V1/2 stimulation occurred in the 3 treatment groups, whereas the action of BK to augment the evoked release of NE was decidedly more pronounced in diabetic preparations compared with normal preparations. Moreover, insulin treatment produced partial recovery of the enhanced BK sensitivity.

Because we performed an NE concentration-effect curve in each preparation in which we tested BK, our analysis was able to take into account possible differences in NE sensitivity among preparations and study groups. We indeed found, as reported, marked differences in sensitivity to added NE among the 3 treatment groups. Dose-response analysis of NE demonstrated that atria from diabetic animals have a drastically reduced maximal NE effect compared with atria from control animals. Insulin treatment gave a partial recovery of the NE sensitivity toward the control response. Interestingly, the values obtained for NE EC50 (Kd) were not significantly different among the 3 groups. These basal values did not differ significantly among the 3 groups. In contrast, atria from DM rats had a significantly greater response to BK than atria from control or DM+Ins rats. Values are mean ± SEM. *P < 0.05 vs control or DM+Ins groups.
changed in any group, indicating that changes in receptor affinity to NE do not account for the differences in overall response to NE in rat atria.

On the basis of the results of the inotropic response to BK, we predicted that the atria from diabetic animals would show a greater induced release of NE from sympathetic nerve endings in response to BK. The fact that (unstimulated) tracer uptake and efflux were equivalent in both groups indicates that the difference in the BK response observed cannot be attributed to differences in tracer loading or rate of background release of tracer. In addition, because cocaine was present during all stimulation periods, differences in NE uptake cannot explain the observed differences in BK action. Although tritiated metabolites of NE could have been a contaminant in the measured tracer release, the fact that 3H]NE overflow was correlated with simultaneously recorded twitch contractile force suggests that the tracer released actually reflected a physiologically relevant NE pool. These findings independently confirm the conclusions drawn from the [NEeq] data.

The significance of the enhanced sympathetic response to BK in atria from diabetic rats is difficult to assess at the present time. It could be suggested that an increased formation of kinins, as observed in the diabetic state, is a compensatory mechanism to improve autonomic function. In fact, ACE inhibitors, which are known to potentiate BK actions, exert a beneficial effect on peripheral neuropathy in STZ-induced diabetic rats. Nevertheless, the results of the present study indicate that the duration of diabetes is particularly important. During the early stages, cardiac NE levels are increased, whereas in long-term diabetes, cardiac NE levels either return to normal or are decreased compared with control levels. No changes in the pattern of noradrenergic innervation were noted in the heart of diabetic rats at any age of onset or duration of STZ-induced diabetes, although the hearts of rats with short-term (1-month) diabetes appeared to be more densely innervated and to have more branching fibers than did the hearts of control rats. The fact that in the present study both stimulation (V1/2)-induced and tyramine-induced NE release were statistically indistinguishable in the 3 groups examined indicates that sympathetic neurons in diabetic tissue had a normal capacity to release NE. This is in accord with the assessment that overt DAN morphological changes after STZ do not take place before 6 months. However, because of the abnormal response to BK, it is now clear that at an early stage in the development of DAN, there are functional alterations of sympathetic nerve endings in the heart. Thus, our method could be a valuable tool to investigate incipient cardiac functional changes of diabetes.

We also found that the enhanced sympathetic response to BK reverses in a manner dependent on insulin. Although many studies have confirmed the beneficial effects of improved glycemic control on the progression of peripheral somatic nerve deficit, including neuropathic symptoms, definitive evidence that good glycemic control prevents or delays the progression of DAN is still lacking. So far, tests in humans have given variable results. Improved metabolic control was reported to slow the progression of DAN, reflected as deficits in heart rate variability in insulin-dependent diabetic patients in some studies but not in others. Because very little, if any, is known about the reversibility of the altered atrial response to BK observed in diabetic animals, a secondary purpose of the present study was to examine the effect of insulin therapy on atrial contractile responses in diabetes. We found that at the dose used, 3.3 U/kg per day administered at ~4:00 pm, insulin produced only a partial recovery of the metabolic and mechanical changes induced by STZ. As reflected by the blood glucose level measured at ~10:00 am, this dose of insulin was probably inadequate to produce optimal metabolic control. Nevertheless, the results of the present study (Figure 4) give a direct indication that improved metabolic control with insulin indeed reverses some of the functional changes induced by diabetes. This finding indicates that insulin plays a role in the regulation of the BK B1 receptor on sympathetic nerve endings. HOE 140 completely blocks the effect of BK on atria from normal and diabetic rats (S. Vogel and S.F. Rabito, unpublished data, 1999). In addition, neither Lys-BK nor Des-Arg9-BK, agonists on the BK B1 receptor, modify the twitch contractile force in atria from diabetic rats (S. Vogel and S.F. Rabito, unpublished data, 1999). These findings are in agreement with a recent study that demonstrated that the expression of the BK B1 receptor was not detectable in the myocardium of normal or diabetic rats.

We cannot exclude the possibility that differences in localized degradation of BK could account for the enhanced NE-releasing effect of BK in the diabetic group. Though we...
used a high concentration of BK in the conducted experiments and treated the preparations with enalaprilat to block the degradation of BK by ACE, the myocardium contains at least a minimal amount of neutral endopeptidase \(^2^8\) and kininase I activity \(^2^9\) that were not blocked with the ACE inhibitor.

In summary, our results indicate that the response to BK in releasing sympathetic neurotransmitters is augmented in diabetic rats and that BK responsiveness recovers toward control values with insulin treatment. Because the adult rat myocardium lacks inotropically functional postsynaptic BK receptors, these results can be interpreted as resulting from an increased number of BK receptors per nerve ending or an improved efficiency of the BK \(B_2\) receptor, as a consequenc(e(s) of diabetes. In addition, because these changes were partially prevented in animals treated with insulin, it could be inferred that insulin plays a role in the regulation of the BK \(B_2\) receptor on sympathetic nerve endings.

References

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