British Journal of Pharmacology (1998) 125, 143–151
http://www.stockton-press.co.uk/bjp

Kinin B_2 receptor-mediated contraction of tail arteries from normal or streptozotocin-induced diabetic rats

[1]Zunzhe Wang, [2]Lingyun Wu & [1,3]Rui Wang

[1]Department of Physiology, University of Saskatchewan, 107 Wiggins Road, Saskatoon, Saskatchewan, Canada S7N 5E5; [2]Department of Physiology, Université de Montréal, Montréal, Québec, Canada H3C 3J7

1 The vasoactive effects of bradykinin (BK) are mediated by different subtypes of kinin receptors, of which the expression varies among different tissues. In rat tail artery tissues, BK elicited a concentration-dependent vasoconstriction (EC50, 25.9 ± 2.4 nM; Emax, 0.39 ± 0.01 g; $n=16$). This effect of BK was endothelium independent and indomethacin insensitive. The BK-induced contraction of tail artery tissues, however, depended on both membrane potential-sensitive extracellular Ca^{2+} entry and thapsigargin-sensitive intracellular Ca^{2+} release.

2 Kinin B_1 receptor antagonist or agonist did not affect the basal tension or the BK-induced contraction of tail artery tissues in the absence or presence of endothelium ($P>0.05$). In contrast, the BK-induced vasoconstriction was inhibited by kinin B_2 receptor antagonists. Pretreatment of vascular tissues with Hoe 140 (1 nM) significantly changed EC50 of the BK-induced vasoconstriction from 25.5 ± 7.4 nM to 82.6 ± 16.8 nM ($n=8$, $P<0.01$) and Emax from 0.43 ± 0.03 g to 0.16 ± 0.01 g ($n=8$, $P<0.01$).

3 In the tail artery tissues from streptozotocin-induced diabetic rats, the BK-elicited vasoconstriction was significantly reduced (EC50, 67.8 ± 11 nM; Emax, 0.19 ± 0.01 g) compared to their counterparts from normal rats. The decreased vasoconstrictive effects of BK on diabetic arteries were endothelium independent and indomethacin insensitive.

4 Our study demonstrated that the contraction of rat tail arteries induced by BK was mediated by B_2 receptors located on vascular smooth muscles. The altered B_2 receptor-mediated vascular activity may play an important role in the vascular complications of diabetes.

Keywords: Smooth muscle; contraction; bradykinin; tail artery; diabetes

Introduction

Biological effects of bradykinin (BK) and its active metabolites are mediated by at least two classes of receptors: kinin B_1 and kinin B_2 subtypes (Farmer & Burch, 1992; Regoli *et al*., 1990). B_2 receptors exhibit high affinities for intact BK and kallidin, and mediate a great number of kinin responses either in physiological or pathological states (Burch *et al*., 1990; Farmer & Burch, 1991). B_2 receptors are constitutively expressed in many different types of tissues. In contrast, B_1 receptors exhibit high affinities for kinin metabolites such as des-Arg^9-BK and des-Arg^9-kallidin, and are selectively antagonized by des-Arg^9,[Leu^8]-BK and des-Arg^9,[Leu^8]-kallidin. The constitutive expression of B_1 receptors is limited to certain types of tissues, such as dog coronary vessels (Nakhostine *et al*., 1993) and cat pulmonary vascular bed (DeWitt *et al*., 1994). B_1 receptors are also unique in that they can be up-regulated during certain pathophysiological situations, and a *de novo* synthesis of B_1 receptors occurs during *in vitro* incubation (Bouthillier *et al*., 1987; Deblois & Marceau, 1987).

The vasoactive effect is one of the important biological functions of BK. This nonapeptide has been shown to relax large blood vessels *via* the activation of B_2 receptors located on vascular endothelial cells, from which endothelium-derived nitric oxide, prostacyclin, and a hyperpolarizing factor are likely to be released (Groves *et al*., 1995). On the other hand, the activation of B_2 receptors, assuming to be located on vascular smooth muscle cells (Field *et al*., 1994), has been reported to contract vascular tissues (Fasciolo *et al*., 1990; Regoli & Barabe, 1980). The mechanisms by which the activation of B_2 receptors contracts vascular tissues, however, are unclear yet. Knowing that the activation of B_1 receptors located on vascular smooth muscle cells also leads to vasoconstriction (Churchill & Ward, 1987; Fasciolo *et al*., 1990; Levesque *et al*., 1993), the previously reported BK-induced vasoconstriction may result from the activation of B_1 receptors, instead of B_2 receptors as suggested (Fasciolo *et al*., 1990; Regoli & Barabe, 1980). This hypothesis is supported by the observation that both B_1 and B_2 receptors may co-exist in the same vascular tissues (Farmer *et al*., 1991). To elucidate the vascular responses to the activation of smooth muscle-located B_2 receptors will help to gain a better understanding of the role played by B_2 receptors in the regulation of vascular tone under physiological and pathophysiological conditions.

Vascular complications of diabetes mellitus are responsible for most of the morbidity and mortality of patients with diabetes. To date, the mechanisms responsible for the altered vascular contractility in diabetes, especially the involvement of kinin receptor activities, remain largely unknown. In our previous study, abnormal patterns of calcium mobilization in tail artery smooth muscle cells from streptozotocin (STZ)-induced diabetic rats have been shown (Wang *et al*., 1998). STZ-induced experimental diabetes is a well-established model of diabetes mellitus (Rossetti *et al*., 1987; Lisato *et al*., 1992), characterized with hyperglycemia, hypoinsulinemia, and insulin resistance. In this rat diabetes model, both the decreased activity of the kallikrein-kinin system and altered effects of BK on cell proliferation have been reported. However, the functionality of the B_2 receptor-mediated vasoconstriction in diabetes is not clear.

[3] Author for correspondence.

The present study was carried out to investigate the vasoactive effects of BK on isolated rat tail arteries as well as the underlying mechanisms. The expression and function of kinin B_1 and B_2 receptors in this vascular preparation were also determined in an attempt to search for an experimental system in which vasoconstriction may be solely mediated by the activation of smooth muscle-located B_2 receptors. Finally, whether the vascular effects of BK were altered in experimental diabetic rats was examined.

Methods

Measurement of isometric tension development of isolated rat tail artery tissues

The method for measurement of isometric tension development of isolated rat tail artery tissues has been described previously (Wang *et al*., 1996; 1997). Briefly, tail arteries were isolated from male Sprague-Dawley (SD) rats (150–200 g). After cleaning connective tissues, the arteries were cut into helical strips (approximately 1.5 cm in length). The vascular tissues were mounted in 10 ml organ baths filled with Krebs' bicarbonate solution (bubbled with 95% O_2 and 5% CO_2) which was composed of (in mM): NaCl 115, KCl 5.4, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, and $CaCl_2$ 1.8. A Ca^{2+} -free Krebs solution contained 0 mM $CaCl_2$ and 10 mM EGTA. When the concentration of KCl of Krebs' solution was increased to 20 mM in some experiments, the concentration of NaCl of Krebs solution was decreased to 100 mM. When the concentrations of Ca^{2+} of Krebs' solution were purposely altered, NaCl concentrations of Krebs solution were equimolarly changed to maintain the original osmolality level. The pH and osmolality of Krebs' solution were adjusted to 7.4 and 290 mOsm, respectively. The tail artery strip was mounted with one end immobilized and another end tied to a transducer. These strips were mechanically stretched to achieve a basal tension of approximately 0.7 g, and were allowed to equilibrate for 1 h before the start of experiments. Endothelium was either kept functionally undamaged or removed from vascular strips by a rubbing procedure. The presence or absence of a functional endothelium was verified by the acetylcholine (1 μM)-induced relaxation of tail artery tissues as shown in our previous study (Wang *et al*., 1997). Unless otherwise specified, the time from mounting vascular tissues in organ baths to application of BK or other agents was kept constant (1.5 h) to avoid discrepancies in results caused by possible up-regulation of B_1 receptors. Concentration-response curves to BK were obtained by non-cumulative addition of BK to the organ bath with an equilibrium period of 40 min between each concentrations.

The isometric tension development was measured with FT 03 force displacement transducers (Grass Ins. Co., Quincy). Data acquisition and analysis were accomplished using a Biopac system (Biopac System, Inc., Golata), including MP 100 WS acquisition units, TCI 100 amplifiers, an Acknowledge software (3.01), universal modules, and a Macintosh computer.

Diabetic model of rats

Male SD rats (150–200 g) were maintained on standard rat chow and tap water *ad libitum* with 12 h light/dark cycles in a quiet environment. Diabetes was induced by a single injection *via* the lateral tail vein or penis vein of streptozotocin (STZ, 60 mg kg^{-1} body weight), dissolved in sodium citrate buffer (pH 4.5). Age-matched control rats were treated with injection of equal volume of buffer solution. The STZ-treated rats continuously lost their weights, accompanied by severe glycosuria and hyperuresis. The diabetic rats after STZ injection for 4 weeks were used in the present study because at this time diabetes had been fully established (Ramanadham *et al*., 1984; Wang *et al*., 1998). The mean blood glucose level of rats 4 weeks after receiving STZ injection was 28 mM ($n=16$), but that of control rats was 7.5 mM ($n=18$), measured with an Accu-check III system (Boehringer Mannheim, Germany).

Chemicals and data analysis

Phenylephrine (PHE), acetylcholine, bradykinin (BK), des-Arg^9-BK, des-Arg^9,[Leu^8]-BK, Nα-adamantaneacetyl-D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK (ADA-BK), indomethacin, streptozotocin (STZ), and other chemicals were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). Thapsigargin (TSG) was from Calbiochem-Novobiochem Inc. (La Jolla, California, U.S.A.). Hoe 140, D-Arginyl-L-arginlyl-L-polyl-*trans*-4-hydroxy-L-prolylglycyl-3-(2-thienyl)-L-alanyl-L-seryl-D-1,2,3,4-tetrahydro-3-isoquinolinecarbonyl-L-(2α,3β,7aβ)-octahydro-1H-indole-2-carbonyl-L-arginine, was a gift from Hoechst (Frankfurt, Germany). Stock solutions (1 mL) of peptides and other agents were made in bidistilled water and kept at -20°C. The data were expressed as means $\pm$ s.e.mean unless otherwise specified. The number of tissue preparations was denoted by '*n*'. Each rat tail artery usually generated two to four helical strips. Concentration-response curves were analysed using a computerized curve fitting software (Microcal Origin, version 4.1, Microcal Software Inc., Northampton, U.S.A.) to obtain EC50 and the maximal development of tension (Emax). Emax values obtained under different conditions were used as 100% responses, respectively, to construct the relative concentration-response curves in some cases. EC50 and Emax values were compared by analysis of variance (ANOVA) followed by Student's *t*-test in conjunction with the Newman-Keuls test where applicable. The significant difference between treatments was defined at a level of $P<0.05$.

Results

The BK-induced contraction of vascular tissues from normal rats

In the first set of experiments composed of 33 endothelium-free tail artery strips obtained from 12 rats, BK consistently induced a transient vasoconstriction. A desensitization of rat tail artery tissues to BK was revealed when the same tissue was repeatedly excited by BK at the same concentration (100 nM) with different inter-stimulation equilibrium time (Figure 1A). When the repeated BK stimulations were given at an interval of 40 min, a comparable run of contractions was obtained (Figure 1B). The BK-induced contraction of endothelium-free tail artery tissues was concentration dependent with Emax achieved at a BK concentration of 300 nM (Figure 2). EC50 of BK effect was 25.9 ± 2.4 nM and Emax 0.39 ± 0.01 g. The presence of indomethacin (1 μM) in Krebs', solution did not interfere with the BK-induced vasoconstriction (Figure 2B). For example, the tension development of tail artery strips induced by BK (100 nM) was 0.35 ± 0.05 g ($n=8$) or 0.32 ± 0.07 g ($n=8$) in the presence or absence of indomethacin (1 μM), respectively ($P>0.05$). In endothelium-intact tail artery tissues, BK also

elicited transient and reversible contractions. EC50 (27.6 ± 3.5 nM) and Emax (0.40 ± 0.01 g) of the BK-induced contraction of the endothelium-intact tail artery tissues ($n=8$) were not different from those of endothelium-free tail artery tissues ($P>0.05$), indicating that the BK-induced contraction of rat tail artery tissues was due to a direct effect on vascular smooth muscles. The presence of indomethacin (1 μM) in the bath solution did not affect the concentration-dependent vasoconstriction of endothelium-intact tail arteries induced by BK ($n=8$) (Figure 2C). When calcium concentrations of the bath solution were increased from 0 mM (in the presence of EGTA) to 10 mM (in the absence of EGTA), a calcium dependence of the BK-induced contraction of rat tail artery tissues was clearly demonstrated (Figure 3). In order to elucidate the nature of the extracellular calcium influx pathway stimulated by BK, KCl concentration of the bath solution was increased from 5.4 mM to 20 mM. The vascular tissues incubated with the modified Krebs' solution containing 20 mM KCl had a greater contractile responses to BK (Figure 4A and B). Furthermore, nifedipine (10 μM) significantly inhibited the BK-induced contraction of endothelium-free tail artery tissues incubated in normal Krebs' solution (Figure 4C). In the absence of extracellular calcium BK still elicited a transient tension development (0.07 ± 0.06 g, $n=12$, $P<0.05$ when compared to the basal tension) (Figure 3B). However, this extracellular calcium-independent vasoconstrictive effect of BK was abolished after the vascular tissues were pretreated with thapsigargin (TSG, 10 μM) for 10 min (Figure 4C).

Determination of kinin receptor subtypes

The selective B_1 receptor agonist, des-Arg^9-BK, had no effect on the basal tension of tail artery tissues in the presence or absence of endothelium ($n=8$ for each group, data not shown). If B_1 receptors were constitutively expressed in rat tail artery tissues, the specific stimulation of B_1 receptors would elicit either vasoconstriction (Bouthillier *et al.*, 1987) or vasodilation (Deblois & Marceau, 1987), and the BK-induced vasoconstriction would be subsequently potentiated or suppressed. The stimulation of existed B_1 receptors may also lead to homologous or heterologous desensitization of tail artery tissues to BK (Wohlfart *et al.*, 1997). However, it was found that at concentrations of 0.1, 0.2, and 10 μM des-Arg^9-BK did not affect the BK-induced contractions of tail artery tissues in the presence or absence of endothelium (Figure 5). Similarly, the selective B_1 receptor antagonist, des-Arg^9,[Leu^8]-BK, had no effect on the basal tension of tail artery tissues ($n=12$, $P>0.05$). This antagonist, at concentrations of 1, 3, and

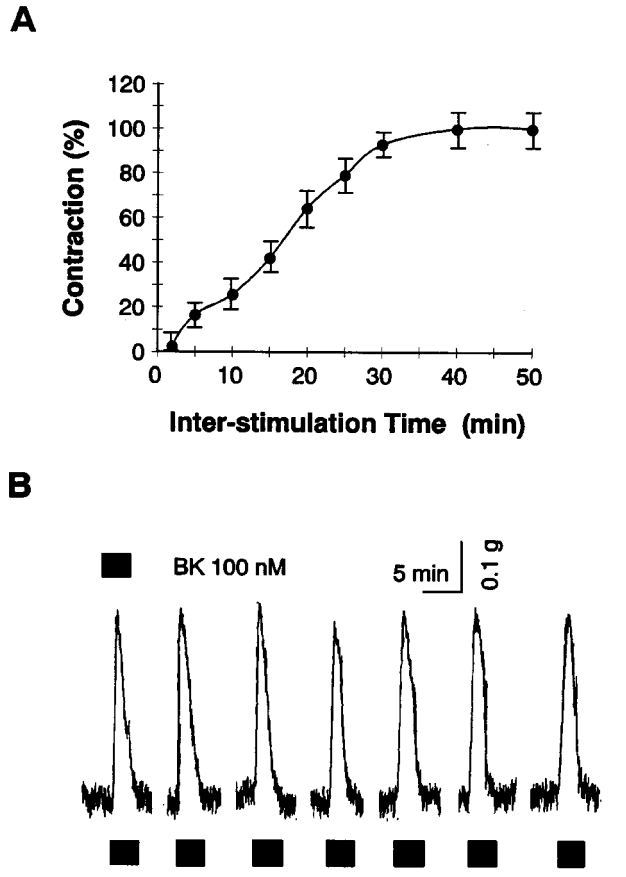

Figure 1 The BK-induced contraction of endothelium-free rat tail artery strips. (A) The BK-induced vasoconstriction as a function of inter-stimulation equilibrium time ($n=8$). The maximum vasoconstriction induced by BK (100 nM) was taken as 100%. As the equilibrium period between BK (100 nM) stimulations was shortened, the amplitudes of tension development induced by BK were reduced. (B) Comparable vasoconstrictions induced by BK with a fixed inter-stimulation time of 40 min.

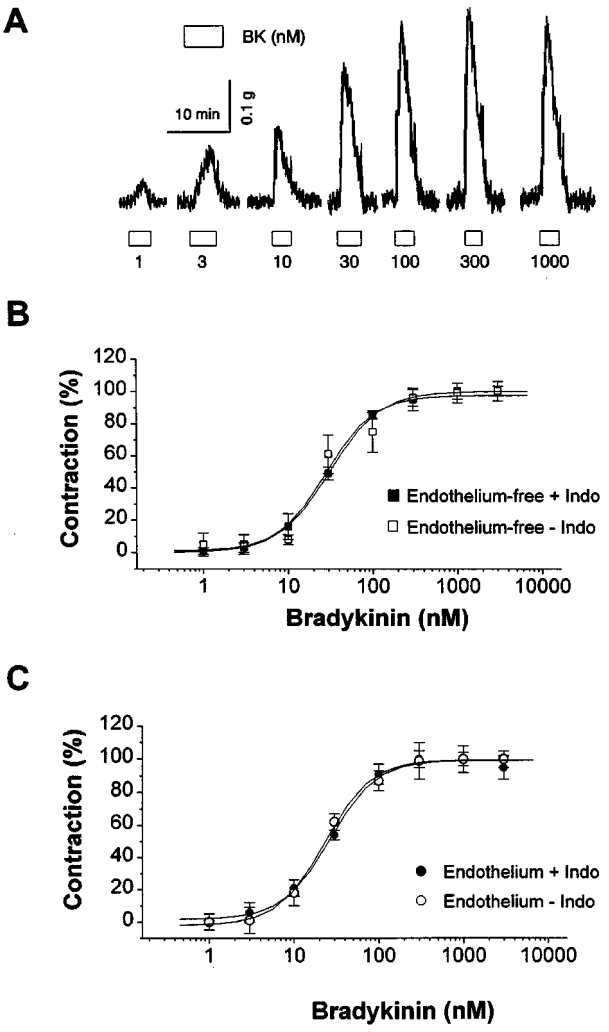

Figure 2 The BK-induced concentration-dependent contraction of rat tail artery strips. (A) Representative records of the BK-induced concentration-dependent contraction of endothelium-free tail artery tissues. (B) Concentration-response curves of BK effect on endothelium-free tail artery strips ($n=16$ for each group). (C) Concentration-response curves of BK effect on endothelium-intact tail artery strips ($n=16$ for each group). Indo, indomethacin (1 μM).

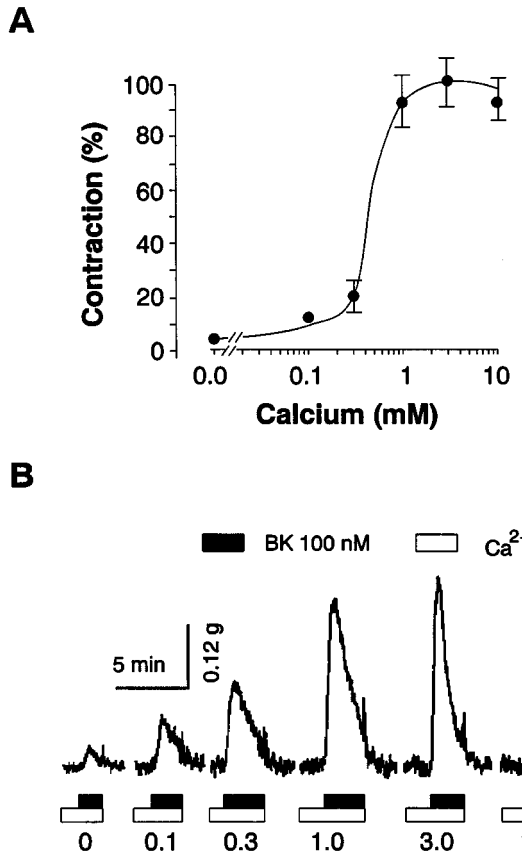

Figure 3 Calcium dependence of the BK-induced contraction of endothelium-free tail artery tissues. (A) The BK (100 nM)-induced calcium-dependent contraction of rat tail artery strips ($n=8$). (B) Representative records showing the BK-induced calcium-dependent vascular contraction.

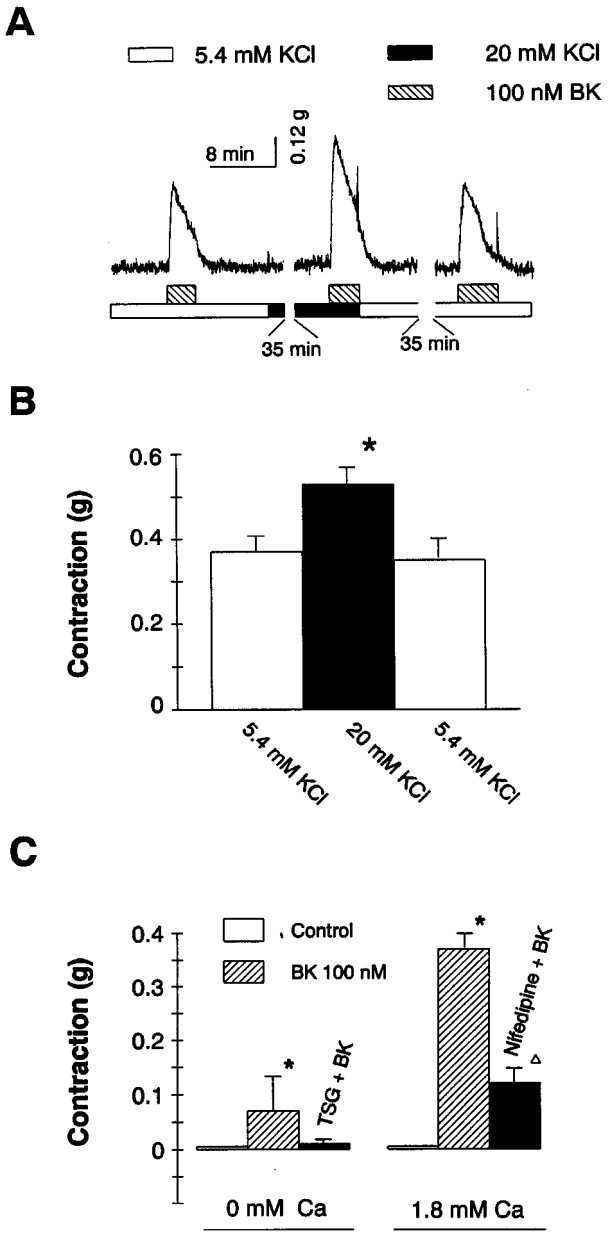

Figure 4 The contributions of potential-dependent extracellular calcium entry and thapsigargin (TSG)-sensitive intracellular calcium release to the BK-induced contraction of endothelium-free tail artery tissues. (A) Membrane depolarization with 20 mM KCl enhanced the BK (100 nM)-induced contraction of rat tail artery tissues ($n=8$). (B) Summary of the membrane potential-dependent vasoconstrictions induced by BK (100 nM) ($n=8$, $^*P<0.01$). (C) TSG (10 μM) incubation for 10 min completely abolished the BK-induced vasoconstriction in the absence of calcium in the bath solution ($n=8$, $^*P<0.01$). With the vascular tissues incubated in a normal Krebs' solution nifedipine (10 μM) significantly inhibited the BK-induced vasoconstriction ($n=8$, $^*P<0.01$; $\Delta P<0.01$ vs control values).

10 μM, also failed to modulate the BK-induced contraction of rat tail artery tissues ($n=6-8$, $P>0.05$) (Figure 6). To investigate whether B_1 receptors could be up-regulated under our experimental conditions, in another group of experiments the isolated tail artery tissues were incubated for 6 h at 37°C in Krebs' solution and under tension in the organ bath. With this prolonged incubation, des-Arg^9-BK (10 μM) still failed to affect either the basal tension or the BK-induced contraction of tail artery tissues in the presence or absence of endothelium ($n=6-8$ for each group, data not shown). When indomethacin (1 μM) was included in the bath solution, des-Arg^9-BK also had no effect on either the basal tension or the BK-induced contraction of endothelium-free or endothelium-intact tail artery tissues ($n=8$ for each group, data not shown).

ADA-BK (Banerjee *et al*., 1994; Grider *et al*., 1995) and Hoe 140 (Feletou *et al*., 1994), two selective B_2 receptor antagonists, had no effect on the basal tension of tail artery tissues. Interestingly, 5 min of incubation of vascular tissues with ADA-BK at 3 μM ($n=6$), or Hoe 140 at 10 nM ($n=4$) or 100 nM ($n=6$), completely blocked the BK-induced contraction of rat tail artery tissues ($P<0.01$) (Figure 7).

It usually took more than 4 h to construct a complete concentration-dependent response measurement for the vasoactive effect of BK with an equilibrium period of 40 min between the application of BK at different concentrations. Therefore, the concentration-dependent effects of BK in the absence or presence of Hoe-140 were examined in paired vascular tissues. To assure the reproducibility of the concentration-dependent effects of BK in paired tissues, the vascular tissues were discarded if the tension development induced by a single dose of BK (100 nM) in the absence of Hoe-140 were not comparable either between the pair or before and after the construction of a complete concentration-response curve of BK. It was found that EC50 of the BK-induced vasoconstriction was changed from 25.5 ± 7.4 nM in untreated tissues to 82.6 ± 16.8 nM in Hoe 140 (1 nM) treated tissues ($n=8$ for each group, $P<0.01$, ANOVA) (Figure 8A). Emax of the BK-induced contraction of untreated vascular tissues was 0.43 ± 0.03 g ($n=8$) whereas in paired tissues

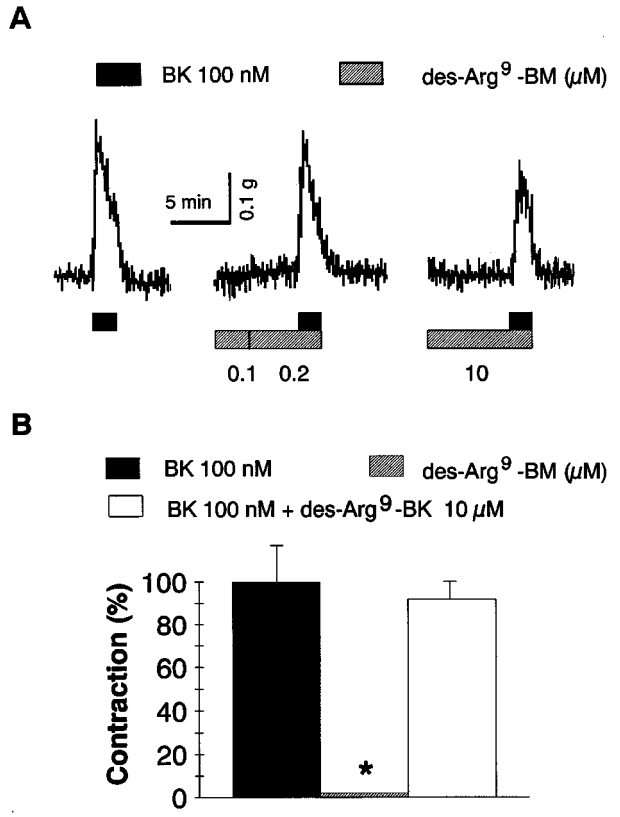

Figure 5 The effect of des-Arg^9-BK on the tension development of endothelium-free tail artery strips. (A) Representative records showing that des-Arg^9-BK had no effect on the basal tension as well as on the BK-evoked tension development of rat tail artery strips. (B) Summary of the effect of des-Arg^9-BK on the tension development of rat tail artery strips. $n=4$, $^*P<0.01$ (ANOVA).

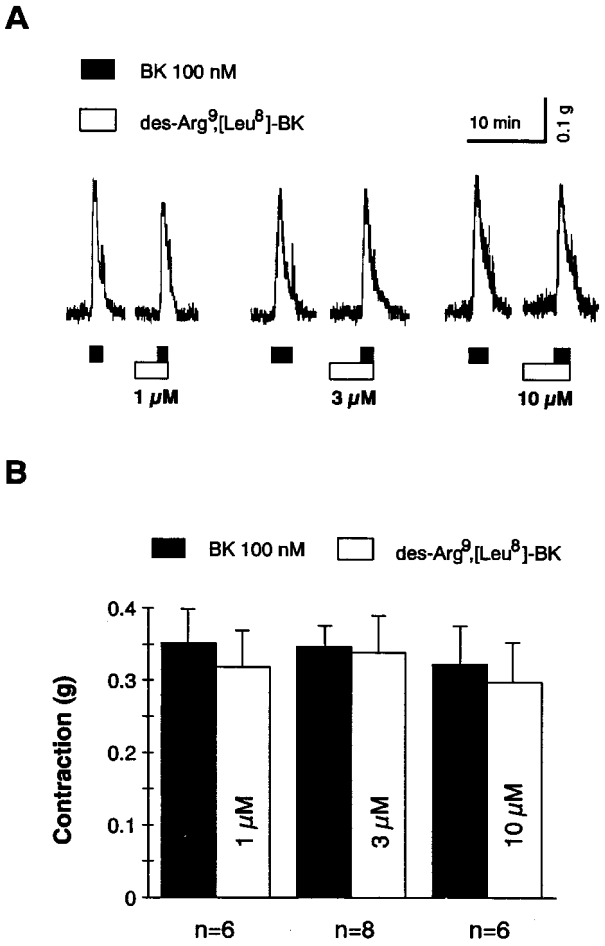

Figure 6 The effect of des-Arg^9,[Leu^8]-BK on the basal tension as well as on the BK-evoked tension development of endothelium-free tail artery strips. (A) Representative records showing the interaction of BK and des-Arg^9,[Leu^8]-BK on the tension development of rat tail artery strips. (B) Summary of the interaction of BK and des-Arg^9,[Leu^8]-BK on the tension development of rat tail artery strips.

treated with Hoe 140 (1 nM) it decreased to 0.16 ± 0.01 g ($n=8$, $P<0.01$) (Figure 8B). The concentration-response curve of BK was shifted to the right in the presence of Hoe 140. At any concentrations tested the full BK effect was not achieved after pretreatment of tissues with Hoe 140. These results, together with the observation that at concentrations higher than 10 nM Hoe 140 completely abolished the BK effect, indicated a non-competitive antagonism of Hoe 140 for BK effect on rat tail artery tissues.

The BK-induced contraction of vascular tissues from diabetic rats

BK also induced a concentration-dependent contraction of endothelium-free tail artery tissues from STZ-induced diabetic rats. The vasoconstrictive effect of BK on these diabetic arteries, however, was significantly reduced compared to their counterparts from normal rats (Figure 9). The altered concentration-dependent vasoconstrictive effect of BK on diabetic rat tail arteries without an intact endothelium was shown in Figure 9A with EC50 changed from a value of 25.9 ± 2.4 nM in non-diabetic rats to 67.8 ± 11 nM ($P<0.01$, ANOVA). Emax of the BK-induced contraction of diabetic tissues ($n=12$) was also significantly smaller than that of non-diabetic tissues ($n=16$) (0.19 ± 0.1 g vs 0.39 ± 0.01 g, $P<0.01$, ANOVA) (Figure 9B). Since dysfunction of vascular endothelium in diabetes has been reported, we further examined the BK effect on endothelium-intact tissues from STZ-treated rats. It was found that in the presence of endothelium the BK-induced vasoconstric-

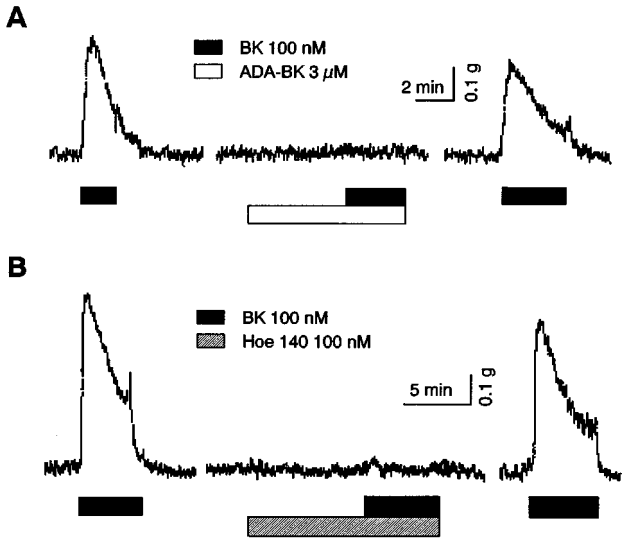

Figure 7 Representative records showing that ADA-BK (A) and Hoe 140 (B) completely blocked the BK-induced contraction of endothelium-free tail artery strips. One-hour after washing out ADA-BK or Hoe 140 from the bath solution the vascular contracting effect of BK was fully recovered.

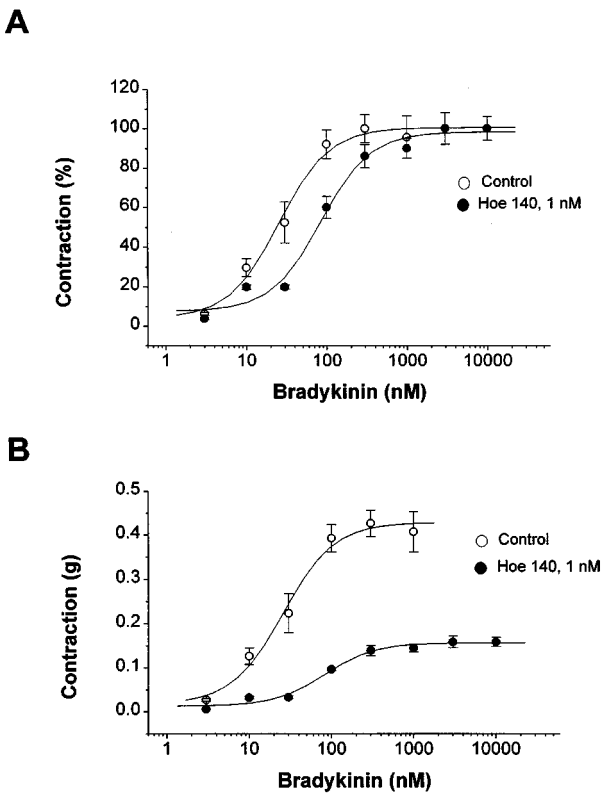

Figure 8 Influence of Hoe 140 (1 nM) on the concentration-dependent vasoconstrictive effects of BK on endothelium-free tail artery tissues. (A) Changes in the relative contraction (%) of rat tail artery strips ($n=8$). (B) Changes in the absolute contraction (g) of rat tail artery strips ($n=8$).

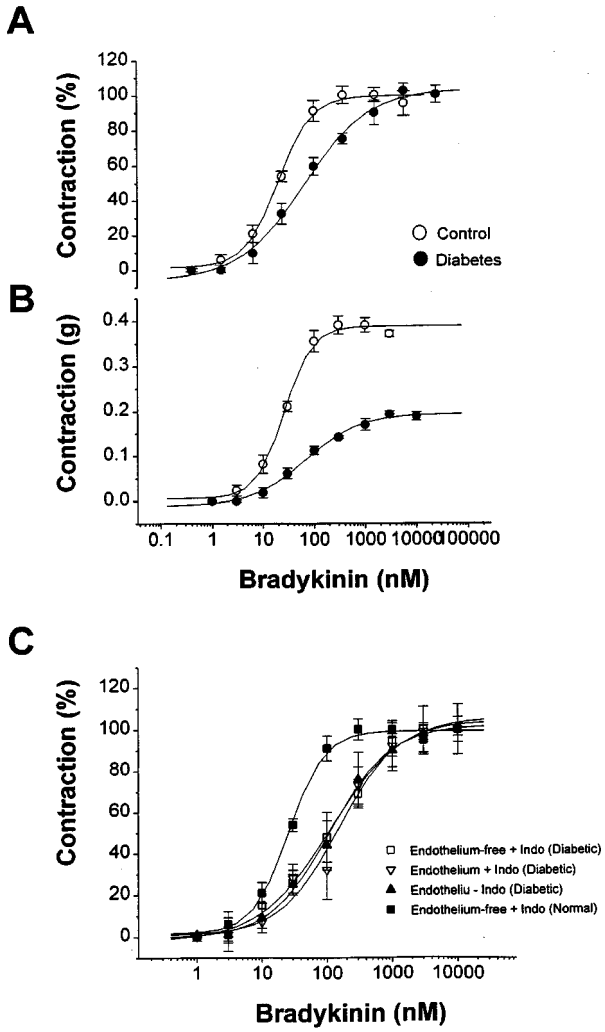

Figure 9 Comparison of the BK-induced vasoconstrictions between tail artery tissues from normal or STZ-diabetic rats. (A) Altered vascular effect of BK on the relative isometric tension development of endothelium-free tail artery tissues ($n=8$ for normal and diabetic tissues, respectively). (B) Altered vascular effect of BK on the absolute isometric tension development of endothelium-free tail artery tissues ($n=8$ for normal and diabetic tissues, respectively). (C) Contributions of an intact endothelium as well as the presence of indomethacin (Indo, 1 μM) in the bath solution to the diabetes-related altered vasoconstrictive effects of BK. $n=8$ for each group.

tion also significantly decreased with an EC50 of 59.2 ± 9 nM and an Emax of 0.22 ± 0.01 g ($n=8$, $P<0.01$, compared to the contractile responses of non-diabetic tail artery tissues with an intact endothelium) (see Figure 2C). The BK-induced contractions of endothelium-free or endothelium-intact rat tail artery tissues from diabetic rats were not significantly different from each other (Figure 9C). Moreover, des-Arg^9-BK at concentrations of 0.1, 3, and 10 μM had no effect on the basal tension or the precontracted endothelium-free tail artery tissues from diabetic rats ($n=4-8$ for each group, not shown). The diabetes-induced functional expression of B_1 receptors in tail artery endothelium was unlikely since des-Arg^9-BK (10 μM) also had no effect on the basal tension or the precontracted endothelium-intact tail artery tissues from diabetic rats ($n=8$, not shown). A prolonged incubation (6 h) did not render the endothelium-intact tail artery tissues from diabetic rats reactive to des-Arg^9-BK (10 μM) ($n=8$, not shown). Similar to what had been found in non-diabetic tail artery tissues, the effects of BK or des-Arg^9-BK on diabetic vascular tissues were not affected by indomethacin (1 μM) ($n=4-8$ for each group, Figure 9C). Taken together, these data suggested that the reduced BK-evoked vasoconstriction in diabetic rat tail arteries was also mediated by smooth muscle-located kinin B_2 receptors.

To address the concern of a non-specific decrease in vascular contractility in diabetes, we calibrated the BK-induced contraction against the KCl-induced vascular contraction in both normal and STZ-induced diabetic rats. In normal rat tail arteries the BK (100 nM)-evoked tension development was 0.35 ± 0.03 g, being approximately $57.4\pm5\%$ relative to the tension development (0.61 ± 0.03 g) elicited by KCl (60 mM) in the same tissues ($n=18$). However, in diabetic rat tail arteries the BK (100 nM)-evoked tension development was 0.21 ± 0.03 g, being approximately $31.8\pm4.5\%$ relative to the tension development (0.66 ± 0.07 g) elicited by KCl (60 mM) in the same tissues ($n=18$). This relative decrease in the BK-induced contraction of diabetic rat tail arteries was significant ($P<0.01$). The vascular contractions induced by phenylephrine (PHE) were further compared between normal and diabetic rats. As shown in Figure 10, EC50 and Emax of the PHE-elicited contraction of STZ-induced diabetic tail artery tissues ($n=8$) did not differ from those of normal tissues ($n=8$) ($P>0.05$, ANOVA). Thus, a decreased vasoconstrictive effect of BK on diabetic tail artery tissues could not be ascribed to a general down-regulation of a signal transduction system which might affect many receptor-mediated vascular contractions.

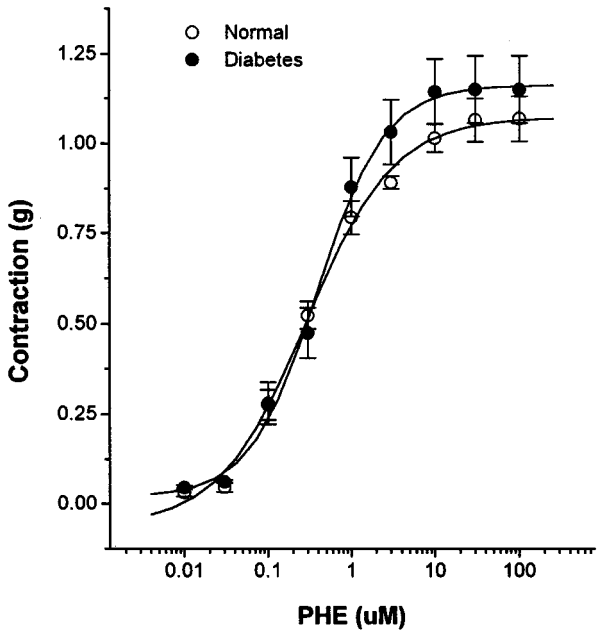

Figure 10 The phenylephrine (PHE)-induced contractions of endothelium-free tail artery strips from normal ($n=8$) or STZ-diabetic ($n=8$) rats. EC50 for BK effects on normal tissues or diabetic tissues were $0.29\pm0.05\,\mu$M or $0.39\pm0.04\,\mu$M, respectively.

Discussion

It is generally acknowledged that the activation of B_1 receptors located on smooth muscle cells leads to the contraction of various types of vascular tissues, including rat mesenteric artery (Fasciolo *et al*., 1990) and rabbit mesenteric artery and aorta (Churchill & Ward, 1987; Levesque *et al*., 1993). The B_1 receptor-mediated vasoconstriction is endothelium independent and not mediated by prostaglandins (Regoli *et al*., 1982). The vascular responses to B_2 receptor activation, however, have been controversial. A wealth of experimental evidence demonstrates that B_2 receptor activation leads to vasorelaxation either directly or through the release of mediators, such as endothelium-derived relaxing factor or prostaglandins (Cherry *et al*., 1982; Fostermann *et al*., 1986; Toda, 1977). An equally substantive body of data, in contrast, claims that the activation of B_2 receptors leads to vasoconstriction (sheep femoral artery without endothelium, Feletou *et al*., 1994; rabbit jugular vein and aorta with or without endothelium, Calixto & Medeiros, 1992; Rhaleb *et al*., 1991; perfused rat mesenteric arteries with endothelium, Fasciolo *et al*., 1990). A previous study on rabbit jugular vein showed that the BK-induced vasoconstriction was potentiated 1000 fold by the removal of endothelium (Calixto & Medeiros, 1992). Our present study demonstrated that BK contracted rat tail artery tissues *via* the activation of B_2 receptors. The absence of endothelium did not significantly affect the contracting effect of BK on rat tail artery tissues. The discrepancy in these results emphasizes the difference in the distribution of B_2 receptors among different tissues and species, and recalls the exercise in searching for a reliable experimental model to study vascular responses to the activation of B_2 receptors localized on vascular smooth muscles.

Rat tail artery is a peripheral vascular preparation, often used in physiology and pharmacology studies. The distribution of B_1 and/or B_2 receptors in this vessel as well as the effect of BK on the contractility of rat tail artery have been unknown although the information is important for broadening and refining our understanding of the vascular effects of BK. It was found in the present study that neither the agonist, nor the antagonist, of B_1 receptors affected the basal tension or the BK-elicited tension development of isolated rat tail artery tissues in the presence or absence of endothelium. As such, the possible activation of constitutive B_1 receptors in this vascular tissue is excluded. Our study further disapproved a *de novo* synthesis of B_1 receptors in this tissue preparation under our experimental conditions. It may be concerned that the kinin receptor-mediated release of prostaglandins (Churchill & Ward, 1986) might be involved in the effect of BK on endothelium-intact tail artery tissues. In our study, the BK-induced vasoconstriction was not sensitive to indomethacin treatment either in the presence or absence of endothelium (Figure 2 and 9). Therefore, the observed vasoconstriction induced by BK was unlikely affected by prostaglandin generation from rat tail artery tissues. Furthermore, the vasoconstrictive effect of BK was antagonized by ADA-BK or Hoe 140, indicating that the BK-induced contraction of endothelium-free or endothelium-intact tail artery tissues was mediated by B_2 receptors. The observed non-competitive antagonistic effect of Hoe 140 on B_2 receptors in rat tail artery is not surprising as the action of this selective B_2 receptor antagonist has been reported to be competitive as well as non-competitive. For example, a potent non-competitive antagonistic effect of Hoe 140 has been shown in blood vessels isolated from rabbit or sheep (Feletou *et al*., 1994). The acting mechanism of Hoe 140, being competitive or non-competitive, is largely related to B_2 receptor subtypes that vary among tissues and species (Regoli *et al*., 1994) and to the differences in bioassay methods used in experiments (Cuthbert *et al*., 1992). Taken together, our results indicate that the vasoconstriction induced by BK is due to the stimulation of one type of kinin receptors, B_2 receptors, which were located on the smooth muscle cells of rat tail artery.

Dixon *et al*. (1990) reported that the exposure of cultured arterial smooth muscle cells from rat mesenteric arteries to BK evoked a rapid release of calcium from intracellular stores. This observation could be echoed by our finding that BK still induced vasoconstriction even in the absence of extracellular calcium (Figure 3 and 4). The major contractile response of rat tail artery tissues to BK, however, relied on the presence of calcium in the bath solution. This result is somehow different from the observation made by Levesque *et al*. (1993). In their studies, the des-Arg^9-BK-evoked contraction of rabbit aortic tissues exhibited a lesser dependence on extracellular calcium. This difference is expected since in our experiments the activation of B_1 receptors did not elicit any vasoconstriction whereas the activation of B_2 receptors did. It seems that different calcium mobilization pathways are coupled to B_1 and B_2 receptors. In rat tail artery tissues, BK might *via* B_2 receptor activation release intracellular calcium as well as promote extracellular calcium entry. Thapsigargin (TSG), a tumor-promoting sesquiterpene lactone, is well known for its inhibitory effect on sarcoplasmic reticulum Ca-ATPase. The inhibitory effect of TSG on the BK-induced vasoconstriction in the absence of extracellular calcium suggested the mobilization of calcium by BK from the IP_3-sensitive intracellular Ca^{2+} stores. To investigate whether BK stimulated the voltage-dependent extracellular calcium entry, we incubated rat tail artery tissues with the modified Krebs' solution containing 20 mM KCl. KCl is a depolarizing agent to stimulate extracellular calcium entry (Cauvin *et al*., 1983). With 20 mM KCl in the extracellular space, smooth muscles would be

depolarized to certain levels. This level of depolarization was not great enough to induce a visible vasoconstriction, as shown in Figure 4A, but would significantly activate voltage-dependent calcium channels. We have used the similar protocol to study membrane potential-dependent effect of parathyroid hormone on voltage-dependent extracellular calcium entry into neurons (Wang *et al*., 1994). Our present results showed that the vasoconstriction induced by BK was significantly potentiated by KCl-induced membrane depolarization. After the concentration of KCl in the bath solution returned from 20 mM to 5.4 mM, the membrane depolarization-related enhancement of the BK effect was abolished (Figure 4A and B). The involvement of voltage-dependent extracellular calcium entry in the vasoconstriction induced by BK was further indicated by the effect of nifedipine, a classical blocker of voltage-dependent calcium channels. It has to be noticed that voltage-dependent calcium channels may not be the only target of BK action since nifedipine did not completely inhibit the BK-induced vasoconstriction (Figure 4C). Further studies need to be carried out for a better understanding of the mechanisms responsible for the calcium-dependency of the vasoconstrictive effect of BK.

A decreased activity of the kallikrein-kinin system in the STZ-treated diabetic rats has been shown previously (Margolius, 1989). In mesangial cells isolated from normal rats, both des-Arg^9-BK and BK stimulated cell proliferation and collagen synthesis, indicating the involvements of B_1 and B_2 receptors in those processes. In mesangial cells harvested from STZ-induced diabetic rats, however, BK and des-Arg^9-BK no longer had any effects on cell proliferation and collagen synthesis. Insulin blunted cell responses to BK and des-Arg^9-BK in normal cells but did not restore the responses to BK and des-Arg^9-BK in cells from STZ-induced diabetic rats. These results suggested that the B_1 and B_2 receptor activities were decreased in insulin-dependent diabetes (Girolami *et al*., 1995). In the present study, the involvement of the kallikrein-kinin system in diabetes-related cardiovascular complications was studied by examining the contractility of the peripheral vascular tissues in response to B_2 receptor stimulation. It was found that the BK-induced vasoconstriction was significantly decreased in diabetic rats. This altered BK effect may not result from a general down regulation of vascular responsiveness in diabetes. Ramanadham *et al*. (1984) have found that tail artery strips, obtained from 4-week STZ-treated rats, were actually supersensitive to α-adrenergic agonists relative to control tissues. Our present study showed that the vascular contractions induced by phenylephrine were not altered in diabetic rat tail arteries (Figure 10). Moreover, when the contraction induced by BK was normalized to the contraction of the same tissues induced by KCl, the relative effectiveness of BK in evoking vasoconstriction was found to be significantly reduced in diabetes. It is possible that this decreased BK effect may result from a decreased density of B_2 receptors on vascular smooth muscles in diabetic rats. Alternatively, the sensitivity of B_2 receptors to BK or the signal transduction pathway downstream to B_2 receptors was altered in diabetes such that the same stimulation provided by BK only evoked a weaker response. Unfortunately, the densities of kinin receptors have not been determined in tail artery tissues from normal rats or diabetic rats. This warrants further investigation. In any case, the discovery of a decreased B_2 receptor-mediated vasoconstriction in diabetes by itself is important for our understanding of the vascular complications of diabetes.

In conclusion, the vasorelaxing effects of BK have been extensively investigated, which contribute to the systemic vasodilation and a fall in blood pressure. Also important for the regulation of vascular tone, if not equally, is the vasoconstrictive effect of BK, especially those mediated by B_2 receptors. The nature and role of the B_2 receptor-mediated vasoconstriction, however, have been unclear to date. Our results provide evidence that BK contracts rat tail arteries via the activation of B_2 receptors located on vascular smooth muscle cells. The B_2 receptor-mediated tail artery contraction relied both on intracellular thapsigargin-sensitive calcium release and on membrane potential-dependent extracellular calcium entry. Furthermore, we demonstrated for the first time that the vascular contractile response to the stimulation of smooth muscle-located B_2 receptors was reduced in diabetes. In addition, our results suggest that rat tail artery can be used as a reliable bioassay by which the potency and specificity of B_2 receptor modulators can be adequately evaluated, and as an experimental model to study the B_2 receptor-mediated vasoconstriction under physiological and pathophysiological conditions.

This study has been partially supported by Juvenile Diabetes Foundation International and Natural Sciences and Engineering Research Council of Canada. R. Wang is a McDonald Scholar of the Heart and Stroke Foundation of Canada.

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(Received February 18, 1998
Revised May 19, 1998