Interactions of the Kallikrein-Kinin and Renin-Angiotensin Systems in Experimental Diabetes

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The kallikrein-kinin system (KKS) has been postulated to play a role in modulation of hemodynamic function in diabetes and to contribute to the hemodynamic effects of angiotensin-converting enzyme inhibition (CEI). To further explore the KKS and its interactions with the renin-angiotensin system (RAS), studies were conducted in nondiabetic control rats and in moderately hyperglycemic diabetic rats. In protocol 1, control and diabetic rats were studied before and after administration of one of two dissimilar B_2 kinin receptor antagonists (BK_2As), or vehicle. At a low dose (0.5 µg · kg^{-1} · min^{-1}), the first generation antagonist D-Arg,[Hyp^3,$Thi^{5,8}$,D-Phe^7]-bradykinin significantly reduced the glomerular filtration rate (GFR) and renal plasma flow rate in diabetic rats, despite variable effectiveness in blocking the hypotensive response to injected bradykinin. However, a similar hemodynamic effect occurred in nondiabetic rats, suggesting that the observed effect was not specific to diabetes. Higher doses (20 µg bolus, then 1 µg · kg^{-1} · min^{-1} infusion) did not affect hemodynamics in either group, perhaps because of partial agonist effect. The second BK_2A tested was the newer compound, icatibant (Hoe 140; D-Arg,[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin). Hoe 140 consistently blocked the vasodepressor action of injected bradykinin, but had no effect on systemic or renal hemodynamics in either control or diabetic rats. In protocol 2, control and diabetic rats were pretreated with the CEI ramipril for 1–2 weeks, after which renal function was studied before and after Hoe 140 (0.1 mg s.c. and i.v.) or vehicle. CEI lowered blood pressure in both groups. Hoe 140 did not affect renal function in control rats, but in diabetic rats pretreated with ramipril, it induced a modest but significant decline in GFR. Ramipril induced the predicted changes in the systemic and intrarenal RAS, while acute BK_2A had no consistent effect on RAS parameters. These studies suggest that the endogenous KKS has only a minor role in modulation of renal hemodynamics in the euvolemic diabetic rat, in the absence of KKS stimulation by CEI. However, angiotensin-converting enzyme is also kininase II, which serves to increase endogenous kinin activity. The increased kinin activity resulting from CEI treatment may participate, to a modest degree, in hemodynamic regulation of the diabetic kidney. ***Diabetes*** **46:107–112, 1997**

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BG, blood glucose; BK, bradykinin; BK_2A, B_2 kinin receptor antagonist; CEI, ACE inhibitor; ERPF, effective renal plasma flow; FF, filtration fraction; GFR, glomerular filtration rate; Hct, hematocrit; KKS, kallikrein-kinin system; MAP, mean arterial pressure; PAH, para-aminohippurate; P_{GC}, glomerular capillary pressure; PRC, plasma renin concentration; RAS, renin-angiotensin system; RIA, radioimmunoassay; RPF, renal plasma flow; $U_{alb}V$, urinary albumin excretion; UV, urinary volume.

Hemodynamic maladaptations of diabetes have been implicated in the pathogenesis of ultimate glomerular injury in this disease. Hormonal modulation of these hemodynamic changes is complex and, as yet, incompletely understood. Intervention studies implicate the renin-angiotensin system (RAS) in that several different angiotensin-converting enzyme (ACE) inhibitors (CEIs) have been shown to lower blood pressure, glomerular capillary pressure (PGC), urinary albumin excretion (UalbV), and glomerular injury in diabetic rats (1–3). Studies with specific angiotensin II (ANG II) receptor antagonists have generally tended to duplicate those findings (4,5), suggesting that suppression of ANG II is the predominant mode of action of CEI. However, lack of substrate specificity of the ACE enzyme still allows for the possibility of non-RAS actions to participate in CEI activity. In that regard, ACE inhibitors potentiate the action of endogenous bradykinin by virtue of their kininase activity (6). In addition, previous studies have found evidence of increased activity of the kallikrein-kinin system (KKS) in experimental (7,8) and clinical (9) diabetes. Accordingly, we sought to explore the role of the KKS in modulation of systemic and renal hemodynamics in the diabetic rat and to explore the interactions between the KKS and the RAS in this model.

RESEARCH DESIGN AND METHODS

The diabetic model. Studies were conducted in adult male Munich-Wistar rats, with initial weights of 280–300 g. One group (C) served as nondiabetic control rats. The remainder (DM) were made diabetic by an intraperitoneal injection of streptozotocin (Sigma, St. Louis, MO), 65 mg/kg body weight. Two days later, induction of diabetes was confirmed by measurement of tail blood glucose (BG) level using a reflectance meter (One Touch II; Lifescan, Milpetas, CA). Diabetic rats received daily evening injections of ultralente insulin (Iletin II, Eli Lilly, Indianapolis, IN) in doses individually adjusted to maintain BG levels between 200 and 300 mg/dl (11–17 mmol/l). BG levels were monitored at least weekly in all diabetic rats. All rats were fed standard rat chow (Rodent Laboratory Chow 5001, Ralston Purina, Richmond, IN) ad libitum. These studies were approved by the Portland Veterans Affairs Institutional Animal Care and Use Subcommittee.

Protocol 1: effects of acute B_2 kinin receptor antagonist (BK_2A) in control and diabetic rats. To examine the acute hemodynamic consequences of bradykinin B_2 receptor antagonism, studies were performed in control and diabetic rats before and after administration of one of two distinct B_2 kinin receptor antagonists (BK_2As), or vehicle. The bradykinin (BK) antagonists stud-

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ied were the first generation drug D-Arg,[Hyp[3],Thi[5,8],D-Phe[7]]-bradykinin (Sigma), hereinafter designated BKA, and the newer agent icatibant (Hoe 140; D-Arg,[Hyp[3],Thi[5],D-Tic[7],Oic[8]]-bradykinin) (Upjohn, Kalamazoo, MI).

Acute renal function studies. At 6–8 weeks of diabetes, studies were performed in DM rats and in weight-matched nondiabetic control animals. After preparation for functional studies, as described below, and after equilibration, all rats underwent baseline measurements of BG, mean arterial pressure (MAP), glomerular filtration rate (GFR), effective renal plasma flow (ERPF), filtration fraction (FF), and urinary volume (UV). Thereafter, the systemic and renal functional responses to BK_2A were examined. For this purpose, rats received one of the following: BKA, low dose (0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ i.v.); BKA, high dose (20 µg bolus, then 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ i.v.); Hoe 140 (0.1 mg/kg, given simultaneously through subcutaneous and intravenous bolus) or saline vehicle. All infusions were given at a rate of 0.2 ml/h. After a 30-min equilibration period, all measurements were repeated to assess changes from baseline. To test the efficacy of bradykinin receptor blockade, some of the animals in each group received an intra-arterial injection of bradykinin (250 ng i.a., Sigma), and the maximum decrease in blood pressure was determined. This test was repeated after BK_2A administration to assess blockade of the depressor response to bradykinin.

After the experiment, blood and kidneys were collected for assessment for RAS activity. The left kidney was rapidly excised, weighed, and placed in cold methanol for subsequent measurement of ANG II levels. Cardiac blood was taken in a chilled syringe and then subdivided into tubes containing EDTA (for plasma renin concentration [PRC] determination), cold methanol (for ANG II), or heparin coating (for HbA_{1c}).

Protocol 2: effect of pretreatment with ACE inhibitor. Further studies were performed to assess whether pretreatment with the ACE inhibitor (CEI) ramipril, a kininase inhibitor, would influence the response to bradykinin receptor antagonism. Additional groups of control and diabetic rats received oral therapy with the CEI ramipril (Upjohn) at a dose of 5 mg/l (diabetic rats) or 20 mg/l (control rats) in the drinking water for 10–14 days before experimentation. Selected doses of the BK receptor antagonists were then studied, using the experimental protocol described above. In some of the rats, efficacy of ACE inhibition was tested by assessing the pressor response to an intravenous injection of ANG I, 200 ng i.v.

Functional studies. After BG measurement, rats were anesthetized with Inactin (100 mg/kg i.p.) and placed on a temperature-regulated table. The left femoral artery was catheterized, and a baseline blood collection was obtained for measurement of hematocrit (Hct) and inulin and para-aminohippurate (PAH) "blanks." This arterial catheter was used for subsequent periodic blood sampling and measurement of MAP via an electronic transducer connected to a direct-writing recorder. After tracheostomy, venous catheters were inserted for infusions of inulin and plasma. Intravenous infusions of rat plasma, and 10% inulin solution in 0.9% NaCl, were started at rates of 6.0 and 1.2 ml/h, respectively. PAH was added to the inulin solution at a concentration of 0.8%. The left ureter was catheterized for urine collection. Euvolemia was maintained by infusing isoncotic rat serum at 6 ml/h in a total amount equal to 1% of the body weight, followed by a reduction in infusion rate to 1.6 $ml \cdot kg^{-1} \cdot h^{-1}$ to maintain the Hct constant. Diabetic rats received extra saline to match the excessive urinary losses during the procedure. Timed samples of urine were collected for determination of flow rate and inulin concentration. Arterial blood was taken for determination of Hct and plasma concentrations of inulin and PAH, and 20- to 30-min urine collections were obtained for determination of flow rate and inulin and PAH concentrations. These measurements permitted calculation of GFR (inulin clearance) and renal plasma flow (RPF) (PAH clearance) by standard formulas. Continued hyperglycemia was confirmed by repeat BG measurement at the end of the procedure, at which time blood was taken for measurement of HbA_{1c}.

Analytical analysis. Inulin concentrations in plasma and urine were measured using a macro-anthrone method. HbA_{1c} was determined by affinity column chromatography (Glyco-Gel B, Pierce Chemical, Rockford, IL) (10). Plasma renin concentration was measured by incubating 100 µl of rat plasma with 100 µl of rat anephric plasma and 400 µl of 0.2 mol/l maleate buffer, pH 6.0, at 37°C for 1 h. Appropriate dilutions of rat plasma samples were made using Tris buffer. The generation of ANG I was then determined by radioimmunoassay using commercially available reagents (New England Nuclear, Boston, MA).

Angiotensin II measurements were performed using the methods of Fox, Navar, and colleagues (11–13). Blood was collected by cardiac puncture into a prechilled syringe, with 1 ml being rapidly placed into 9 ml of cold methanol. Kidneys were rapidly homogenized in 10 ml of cold methanol. After centrifugation at 4°C, supernatant was stored at –20°C until extraction. For extraction, supernatants were dried overnight in a vacuum centrifuge, and then the dried residue was reconstituted in phosphate buffer and applied to a phenyl-bonded SPE column (Bond-Elut, Varian, Harbor City, CA), which had been prewashed with methanol and then H_2O. Each SPE column is then washed sequentially with H_2O, hexane, and chloroform. Angiotensin peptides were eluted from the SPE column with 90% methanol in H_2O. The eluants were collected and stored at –20°C. Before radioimmunoassay (RIA), the eluants were evaporated to dryness under vacuum and reconstituted in assay diluent. ANG II levels were quantitated by RIA, using rabbit anti–ANG II antisera (Arnel, New York, NY), monoiodinated ^{125}I-labeled ANG II (Amersham, Arlington Heights, IL), and ANG II standards (Sigma). Each lyophilized antiserum was diluted sufficiently to yield a specific binding of 30–35% after incubation with ~3,000 counts/min label. After an incubation period of 2 days at 4°C, bound and free angiotensin peptides were separated with Dextran-coated charcoal. The supernatant was then decanted and counted, and B/B_0 determined.

Statistical analysis. Statistical analysis was performed by paired *t* test (for studies before and after an intervention) or by one-way analysis of variance followed by computation of modified *t* values according to the method of Bonferroni (for multiple groups), as appropriate. Values that were not normally distributed were analyzed using nonparametric methods. Statistical significance was defined as $P < 0.05$. All values represent means ± SE.

TABLE 1
Baseline systemic and renal parameters

	Control rats	Diabetic rats
n	31	28
Body weight (g)	313 ± 2	301 ± 2*
Left kidney weight (g)	1.16 ± 0.02	1.58 ± 0.04*
LKW/100 g body wt	0.37 ± 0.01	0.52 ± 0.01*
BG (mmol/l)	3.8 ± 0.1	14.3 ± 0.4*
HBA_{1c} (%)	5.2 ± 0.4	12.5 ± 0.6*
MAP (mmHg)	121 ± 2	107 ± 2*
Hct (%)	0.47 ± 0.004	0.48 ± 0.005
GFR (ml/min)	1.20 ± 0.04	1.54 ± 0.07*
ERPF (ml/min)	4.89 ± 0.19	4.69 ± 0.15
Filtration fraction	0.25 ± 0.01	0.33 ± 0.01*
UV (µl/min)	5.6 ± 0.3	15.0 ± 1.3*

Data are means ± SE. *$P < 0.05$ vs. controls.

RESULTS

Protocol 1: effects of bradykinin receptor antagonism. Baseline characteristics of control and diabetic rats did not differ among subgroups; results were pooled and are summarized in Table 1. Body weights were slightly, though significantly, lower in the diabetic rats. Despite the lower body weights, renal hypertrophy was apparent in the diabetic animals, whether expressed as absolute kidney weight or relative to body weight. As expected, values for both blood glucose and HBA_{1c} were higher in the diabetic animals. Values for Hct did not differ between the groups. MAP was somewhat lower in the diabetic animals. The diabetic rats exhibited hyperfiltration, with baseline GFR values significantly higher than those in the control animals, and values for filtration fraction as well as urinary flow rates in the diabetic animals exceeded those in the normal rats.

The hemodynamic consequences of acute BK receptor antagonism are summarized in Table 2 and Fig. 1. In control rats, infusion of BKA at the low dose did not affect blood pressure (Table 2), but resulted in a significant reduction in both GFR and ERPF (Fig. 1) and a slight increase in urine flow rate. These effects occurred despite an inconsistent ability to demonstrate blockade of the acute vasodepressor response to bradykinin. In contrast, the higher dose of the same antagonist was without effect on any systemic or renal functional parameter. The newer antagonist, Hoe 140, was

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TABLE 2
Effects of BKA in control and diabetic rats

	MAP (mmHg)	FF	UV (μl/min)
Control rats			
Baseline	121 ± 2	0.25± 0.01	5.6 ± 0.3
+ LD BKA (8)	−2 ± 1	−0.01 ± 0.01	+2.6 ± 0.7†
+ HD BKA (7)	−1 ± 1	−0.00 ± 0.01	+1.3 ± 0.7
+ Hoe 140 (7)	−3 ± 2	+0.01 ± 0.01	+0.9 ± 0.4
+ Vehicle (9)	+0 ± 2	−0.02 ± 0.02	+ 3.1 ± 1.0†‡
Diabetic rats			
Baseline	107 ± 2*	0.33 ± 0.01*	15.0 ± 1.3*
+ LD BKA (5)	−0 ± 2	−0.02 ± 0.01†	−2.7 ± 0.7†‡
+ HD BKA (8)	+0 ± 1	+0.01 ± 0.01	−1.3 ± 1.9
+ Hoe 140 (8)	−2 ± 2	+0.00 ± 0.02	+1.9 ± 2.1
+ Vehicle (7)	+1 ± 1	−0.01 ± 0.01	+0.4 ± 0.5

Data are means ± SE. * $P < 0.05$ vs. control rats; † $P < 0.05$ vs. baseline; ‡ $P < 0.05$ vs. change in nondiabetic control rats.

more consistent in its ability to block the vasodepressor response to bradykinin. In the baseline period, BK injection lowered blood pressure by 23 ± 3 mmHg ($n = 8$, $P < 0.001$), whereas after Hoe 140, the same dose of BK elicited only a 0.5 ± 0.9 mmHg (NS) fall in MAP. However, despite this evidence of BK receptor blockade, Hoe 140 had no significant effect on any hemodynamic parameter. In the control rats receiving saline vehicle, there was a slight increase in urine flow rate over time, but no significant change in any other hemodynamic parameter.

Results were qualitatively and directionally similar in the diabetic rats. In these animals as well, the lower dose of the BK antagonist did not consistently block the vasodepressor response to BK injection, but nevertheless resulted in significant reductions in both GFR and ERPF (Fig. 1). MAP was unchanged, while filtration fraction and urinary flow rates fell slightly. At the higher dose, there was no significant change in any hemodynamic parameter. Hoe 140 was again effective in blocking the vasodepressor response to BK (pre, −17 ± 2 mmHg, $n = 8$, $P < 0.001$; post, 2 ± 1 mmHg, NS). Saline vehicle had no significant effect on any parameter in the diabetic rats.

Protocol 2: effects of BK receptor antagonism in CEI-treated rats. Results of studies in ramipril-treated animals are presented in Tables 3 and 4. Versus their respective groups not pretreated with CEI (described above), values for body and kidney weights were comparable (Table 3). Mean arterial pressure was lower in CEI-treated control rats, though not in CEI-treated diabetic rats. Both GFR and ERPF were higher in diabetic rats than in control rats treated with CEI, and in diabetic/CEI rats as compared with diabetic rats not receiving the drug.

Verification of inhibition of ACE was obtained by measuring the pressor response to intravenous injection of ANG I

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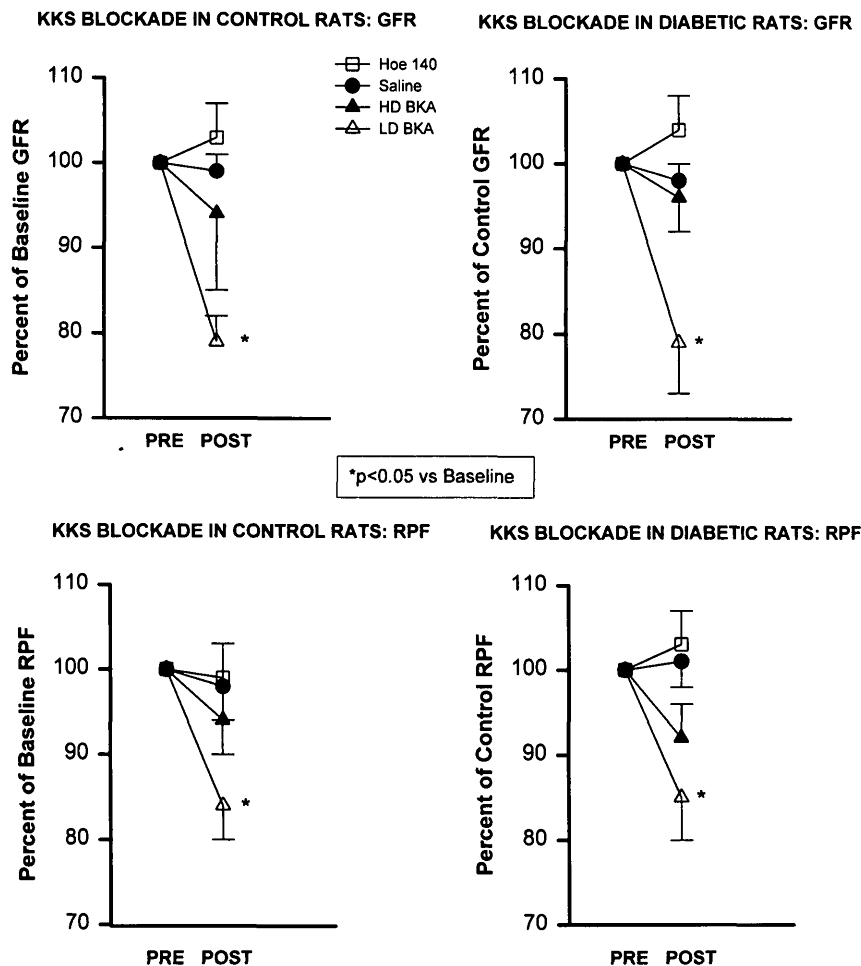

FIG. 1. Effects of KKS blockade on GFR and RPF in control and diabetic rats. Low-dose (LD) bradykinin receptor antagonist (BKA) lowered GFR and RPF in both groups. Infusions of high-dose (HD) BKA, Hoe 140, and saline vehicle were without effect. * $P < 0.05$ vs. pre.

TABLE 3
Effects of Hoe 140 in CEI-treated rats

	Control rats (n = 8)		Diabetic rats (n = 7)	
	Pre	Post	Pre	Post
BW (g)	314 ± 6	—	301 ± 2	—
LKW (g)	1.13 ± 0.05	—	1.71 ± 0.14†	—
LKW/100 g body wt	0.36 ± 0.01	—	0.57 ± 0.05†	—
BG (mmol/l)	3.6 ± 0.1	—	14.1 ± 1.3 †	
HBA_{1c} (%)	6.2 ± 0.4	—	16.0 ± 2.0 †	
MAP (mmHg)	102 ± 1*	98 ± 3	105 ± 3	104 ± 4
GFR (ml/min)	1.29 ± 0.07	1.23 ± 0.08	1.88 ± 0.12*†	1.71 ± 0.15*†‡
ERPF (ml/min)	5.33 ± 0.37	5.35 ± 0.23	6.37 ± 0.12	6.09 ± 0.36
FF	0.25 ± 0.01	0.23 ± 0.01	0.30 ± 0.02 †	0.29 ± 0.03 †
UV (μl/min)	6.7 ± 7.2	7.2 ± 0.8	14.8 ± 2.8 †	14.4 ± 1.7 †

Data are means ± SE. LKW, left kidney weight; Pre, baseline; post, after Hoe 140. *$P < 0.05$ vs. non-CEI treated; †$P < 0.05$ vs. control rats in same period; ‡$P < 0.05$ vs. baseline.

(200 ng). In rats not pretreated with CEI, ANG I induced increases in MAP of 48 ± 6 mmHg in control rats (n = 11) and 50 ± 3 mmHg in diabetic rats (n = 11). In the rats pretreated with ramipril, the same dose of ANG I increased MAP by only 2 ± 1 and 1 ± 0.4 mmHg, respectively (both $P < 0.001$ vs. changes in respective untreated groups). Blockade of BK receptors was verified by measuring the vasodepressor response to injected BK before and after Hoe 140 administration. Before Hoe 140, BK lowered blood pressure by 35 ± 2 mmHg in control rats and by 27 ± 3 mmHg in diabetic rats. In the same animals, after Hoe 140, BK lowered blood pressure by only 2 ± 1 mmHg in control animals (n = 8) and 1 ± 0.4 mmHg in diabetic rats (n = 7).

Effects of Hoe 140 on renal function are also depicted in Table 3. BK receptor antagonism had no effect on functional parameters in the CEI-treated nondiabetic rats. In contrast, and in contrast to the findings with the first generation BK antagonist, Hoe 140 produced a modest but statistically significant decrease in GFR in diabetic rats pretreated with ramipril. There were no significant effects on plasma flow rate, filtration fraction, or urinary volume with Hoe 140.

Effects of diabetes and of ramipril on the systemic and intrarenal renin-angiotensin systems are depicted in Table 4. In control and diabetic rats (studied after saline vehicle infusion), there were no significant differences in PRC, or plasma or renal ANG II levels. Administration of ramipril produced the expected changes in the RAS, i.e., elevation of PRC and reduction in plasma (numerically) and renal (significantly) ANG II levels. There were no apparent differences in RAS parameters between control and diabetic rats treated with short-term ramipril.

DISCUSSION

The mediators of diabetic glomerular hyperfiltration have been the subject of extensive study, with a panoply of vasoactive substances proposed to participate in hemodynamic regulation. A contributory role of the RAS has been well established, since ACE inhibitors (1–3) and specific ANG II receptor antagonists (4) reliably lower efferent arteriolar resistance and glomerular pressure and increase the ultrafiltration coefficient in diabetic models. As with all actions of ACE inhibitors, observations of effects consistent with those of ANG II blockade do not necessarily prove that the given effect was RAS mediated, given the lack of substrate specificity of the ACE enzyme. ACE is known to participate in a number of enzymatic processes, including breakdown of kinins, and therefore, accentuation of kinin effect has often been proposed as a mechanism of action of ACE inhibitors (6,14,15).

A fair amount of evidence has also been put forth invoking a role for the KKS in diabetic hyperfiltration. A role for this system is intuitively attractive in that bradykinin is felt to be a dilator of the glomerular afferent and efferent arterioles (16,17). Though direct micropuncture studies with BK receptor antagonists have not been performed in the setting of diabetes, Hoe 140 has been shown to increase efferent arteriolar resistance in volume-depleted CEI-treated rats (18). Urinary kallikrein excretion is increased in poorly controlled diabetic patients (9). In untreated (vasoconstricted) diabetic rats, renal prokallikrein synthesis is impaired, and kallikrein and kallikrein mRNA levels are low but normalized with insulin therapy (7); in some studies, renal kallikrein is increased above normal in insulin-treated diabetic rats (19). Kallikrein inhibitors reduce GFR and renal blood flow (RBF) in diabetic rats (19), and a recent study found that administration of the first generation BK_2A used in the present studies resulted in significant reductions in GFR and RPF in diabetic rats but not in control rats (8). The reason for the disparity in results between their study and ours is not readily apparent, though there were some differences in the experimental designs. Those studies used a different rat strain, a slightly earlier time point in the course of diabetes, and a bolus injection of the antagonist preceding the low-dose infusion. Nevertheless, our findings in diabetic rats were in general agreement with that study. The outstanding question relates to the issue of whether the inhibitor has consistent effects in the absence of diabetes. Our results with Hoe 140 (in the absence of ACE inhibition) are in accord with a recently published study (20), which also found no effect of Hoe 140 alone on renal hemodynamics in diabetic rats. That study also found a slight (though not significant) effect of Hoe 140 on GFR in diabetic rats pretreated with acute intravenous ramiprilat, whereas there had been no effect with Hoe 140 alone. Thus, despite different experimental designs (acute versus chronic CEI), our results are in accord. However, in neither study was the effect of Hoe 140 on GFR in diabetic CEI-treated rats very substantial. This varies with previous stud-

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TABLE 4
Effects of ramipril on the renin-ANG system

	n	PRC (ng ANG I · ml^{-1} · h^{-1})	Plasma ANG II (fmol/ml)	Renal ANG II (fmol/g)
Controls	7	11 ± 3	82 ± 11	431 ± 58
Controls + ramipril	8	42 ± 8*	32 ± 11	124 ± 36*
Diabetes	13	18 ± 4	69 ± 32	277 ± 55
Diabetes + ramipril	6	43 ± 12*	32 ± 13	97 ± 22*

Data are means ± SE. *$P < 0.05$ vs. respective group not receiving ramipril.

ies of other putative mediators, such as atrial natriuretic peptide (21), where virtual complete normalization of diabetic hyperfiltration has been reported. Thus, while the existing data is consistent with a role for kinins in the mediation of diabetic hyperfiltration, their participation appears to be relatively modest, at least in the euvolemic state. Whether diabetic rats would respond differently from their nondiabetic counterparts under conditions of KKS stimulation, such as volume depletion or sodium restriction, remains to be explored.

There is also evidence of significant KKS/RAS interactions in various conditions. Kallikrein-containing cells are most prominent in the connecting tubule, where their close proximity to both the afferent arteriole and the macula densa has prompted suggestion of a paracrine function of the KKS in regulation of both renal hemodynamics and renin release (22,23). One possible scenario is that compensatory KKS hyperactivity may represent a response to a primary vasoconstrictor stimulus (elevation of ANG II). Indeed, stimuli to ANG II production also stimulate the KKS because a low-sodium diet increases interstitial BK in dogs (24), and infusing ANG II into the isolated perfused kidney increases urinary kallikrein (25).

Whether changes in KKS activity modulate RAS activity is not yet well studied, though it has been noted that renal arterial infusion of bradykinin stimulates renin release (26), whereas aprotinin suppresses it (27). While our studies did not find a pronounced effect of acute Hoe 140 on RAS parameters, others (28) found that with chronic administration, Hoe 140 significantly increased plasma ANG II levels in nondiabetic female Wistar rats. Thus, the literature suggests an effect of the KKS on the RAS, but the direction of the effect seems to vary (stimulation versus suppression), a discrepancy no doubt due to differing experimental conditions, but also possibly due to differences among BK_2As. Whether the renin-stimulating effect of BK_2A occurs only with the first generation antagonists (such as the one used in our studies), but not with the newer and more specific antagonists such as Hoe 140, awaits further clarification.

In summary, these studies found that acute administration of a first generation bradykinin receptor antagonist lowered GFR and RPF in euvolemic diabetic rats. However, similar changes occurred in nondiabetic control rats. Furthermore, infusion of higher doses of the first generation agent, or of potent doses of the newer agent Hoe 140, failed to induce any hemodynamic changes. In diabetic rats pretreated with the ACE inhibitor ramipril, Hoe 140 induced a modest but significant decrease in GFR. Taken together, these findings suggest that the endogenous KKS has only a minor role in modulation of renal hemodynamics in the euvolemic diabetic rat in the absence of KKS stimulation by CEI. However, since angiotensin-converting enzyme is also kininase II, the subsequent increase in endogenous kinin activity with CEI treatment may participate, to a modest degree, in diabetic hemodynamic regulation.

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