Bradykinin B_1 Receptor-Mediated Changes in Renal Hemodynamics during Endotoxin-Induced Inflammation

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Abstract. Kinins have been shown to influence renal hemodynamics and function. Under physiologic conditions, most kinin effects involve bradykinin B_2 receptors, but bradykinin B_1 receptors are often induced during inflammation. The purpose of this study was to examine *in vivo* the effects of bradykinin B_1 receptor activation on renal hemodynamics under normal and inflammatory conditions. In anesthetized rats, activation of bradykinin B_1 receptors by arterial infusion of bradykinin B_1 receptor agonist des-Arg^9-bradykinin reduced renal plasma flow and GFR. Prior administration (18 h) of lipopolysaccharide to induce inflammation resulted in a larger bradykinin B_1 receptor-induced reduction in renal plasma flow. Values of other parameters remained unchanged, thus resulting in an increased filtration fraction. The presence and the functionality of the bradykinin B_1 receptor at the level of glomerular afferent and efferent arterioles were studied by mRNA expression analysis and intracellular calcium ($[Ca^{2+}]_i$) mobilization studies. Stimulation with des-Arg^9-bradykinin of microdissected afferent arterioles from control and lipopolysaccharide-treated rats induced $[Ca^{2+}]_i$ mobilization without any significant difference in amplitude between control and lipopolysaccharide-treated rats. However, des-Arg^9-bradykinin only induced $[Ca^{2+}]_i$ mobilization in efferent arterioles from lipopolysaccharide-treated rats. It is suggested that activation of bradykinin B_1 receptors located along the efferent arteriole may participate in the modification of renal hemodynamics in inflammatory states.

The renal kinin-kallikrein system is involved in the control of renal hemodynamics and function (1). Activation of the kinin-kallikrein system produces biologically active peptides called kinins, which activate two distinct receptors, the bradykinin B_1 and B_2 receptor. Both receptors belong to the seven transmembrane G protein-coupled receptor family. Bradykinin is the natural agonist of the bradykinin B_2 receptor, which is constitutively expressed under physiologic conditions and is responsible for most known kinin effects. In the kidney, bradykinin (injected in the renal artery) increases renal blood flow with no change in GFR. Moreover, bradykinin affects directly tubular function by inducing diuresis and natriuresis (2).

In contrast to the bradykinin B_2 receptor, the bradykinin B_1 receptor is only weakly expressed under physiologic conditions. However, its expression is strongly induced under inflammatory conditions (3) and by its natural agonist (4). *In vitro*, inflammatory conditions to induce the bradykinin B_1 receptor can be mimicked by inflammatory mediators such as bacterial lipopolysaccharides and interleukin-1β (3). Inflammation induced by lipopolysaccharide also increases the production of the bradykinin B_1 receptor agonist in rabbits (5). This increase in bradykinin B_1 receptor agonist is most likely the result of an increased activity of the des-Arg^9-bradykinin-forming enzyme carboxypeptidase M, whose activity is increased during septic shock (lipopolysaccharide) and preexisting inflammatory disease in pigs (6). These data suggest that during inflammation the bradykinin B_1 receptor may replace the bradykinin B_2 receptor (3). Despite this potential importance, little is known about the *in vivo* effects of bradykinin B_1 receptor activation on renal hemodynamics and function under physiologic and, more importantly, inflammatory conditions.

In contrast to the vasoconstrictor response observed in isolated perfused rat kidney (7), bradykinin B_1 receptor activation in canine renal arteries induces vasodilation (8). Another study, using bradykinin B_1 and B_2 receptor antagonists in anesthetized dogs, showed that both bradykinin B_1 and B_2 receptors are necessary for a bradykinin-induced increase in renal plasma flow (9). These vasomotor effects induced by bradykinin B_1 receptor activation most likely influence renal hemodynamics by acting on the afferent arteriole, since the major resistance bed within the kidney lies on the preglomerular side. Indeed, Yu *et al.* (10) have recently shown that addition of des-Arg^9-bradykinin to the vascular lumen of isolated rabbit afferent arterioles induced a significant constriction. This suggests that the des-Arg^9-bradykinin-induced constriction of the afferent

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arteriole can contribute to the earlier observed increased renal vascular resistance (7), but does not explain the vasodilatory effect of bradykinin abolished by bradykinin B_1 and B_2 receptor antagonists in anesthetized dogs (9).

The above studies to determine the contribution of bradykinin B_1 receptor activation on renal hemodynamics are performed either on isolated tissues or with bradykinin in combination with a bradykinin B_1 receptor antagonist. We have recently reported that experimental inflammation induces the expression of functional bradykinin B_1 receptors along the rat nephron (11). The significant induction of the bradykinin B_1 receptor along the efferent arteriole observed in this study raises the question of whether the bradykinin B_1 receptor in this part of the nephron can contribute to the control of renal hemodynamics under inflammatory conditions. Moreover, it has been shown that the efferent arteriole participates in hemodynamic control (12). Therefore, we decided to analyze the contribution of bradykinin B_1 receptor activation *in vivo* on renal hemodynamics and function in anesthetized rats using arterial des-Arg^9-bradykinin infusion under control and inflammatory conditions induced by lipopolysaccharide treatment.

Materials and Methods

Materials

Des-Arg^9-bradykinin, des-Arg^9-[Leu^8]bradykinin, D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]bradykinin, and lipopolysaccharide (serotype 0111:B4) were obtained from Sigma Chemical Co. Pentobarbital was from Sanofi (Montpellier, France). Para-aminohippurate and inulin were from Serb (Paris, France). Lithium was from Merck (Valence, France). Fura-2-acetoxymethyl (Fura-2 AM) was from Interchim (Montlucon, France).

Animal Treatment

Male Sprague Dawley rats (Iffa Credo, L'Arbresele, France) age 9 wk and weighing 219 to 265 g were used. The week before the experiments the rats had free access to standard feed containing 104 ± 3 μM sodium/g diet. The night before the experiments the rats were fasted but had free access to tap water. Lipopolysaccharide administration was performed by one intravenous injection in the jugular vein under slight anesthesia with ether of 50 μg of lipopolysaccharide in 0.9% saline solution 18 h before sacrifice. Eighty-six rats were randomly divided into two groups, of which 43 were treated with lipopolysaccharide and 43 with saline only (controls). Animals were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg). Eighty rats (40 lipopolysaccharide and 40 control) were used for *in vivo* hemodynamic measurements, and the remaining six rats were used to isolate the afferent and efferent arterioles. All experiments reported were conducted in accordance with guiding principles for the care and use of animals.

In Vivo Renal Hemodynamic Measurements

Body temperature was kept at 37°C by means of a warmed plate coupled to a rectal probe. The trachea was cannulated (Biotrol No. 12; Biotrol Pharma, Paris, France) to allow for unobstructed breathing. A cannula (Biotrol No. 3) was placed in the left carotid artery and used to collect blood samples (about 300 μl) and to monitor the mean arterial pressure (Statham P10 EZ and chart recorder TA4000; Gould, Paris, France). The jugular vein was cannulated with two catheters (Biotrol No. 3). The first was used to replace fluid lost during surgery by a Ringer-lactate solution with 3% gelatin (Plasmion®; Laboratoire Roger-Bellon, Neuilly, France). This solution was initially infused at a rate of 100 μl/min until 1.25 ml/100 g body wt had been administered, followed by 20 μl/min thereafter of the same solution diluted 50% with an isotonic glucose solution. The second jugular catheter was used to infuse a saline solution at 10 μl/min containing pentobarbital sodium (75 μg/min per kg), inulin (0.22 mg/min), para-aminohippurate (0.13 mg/min), and lithium (2.85 μg/min) to keep the animal anesthetized and maintain the plasma concentrations of inulin, para-aminohippurate, and lithium at 200 mg/L, 20 mg/L, and 0.3 mmol/L, respectively. A ventral midline incision was made and both ureters were cannulated (Biotrol No. 1) to collect separate urine samples. Under a stereomicroscope, the right suprarenal artery was located, liberated of surrounding tissue, and cannulated 5 to 6 mm before its connection to the renal artery (a catheter with an outer diameter of 0.2 mm pulled from a flame-heated PE 10). This cannula allows infusion at a rate of 10 μl/min into the renal circulation without changing the volume or blood flow in the renal right artery. All infusions were performed with precalibrated automated syringes. The surgery was followed by a 60-min equilibration period before starting the experiments. Right and left kidney hemodynamic parameters were evaluated separately for two successive 20-min periods. The first period, when only saline was infused, served as the control period for each rat. The second period was the test period, during which some rats continued to receive saline, whereas others received saline containing the appropriate agonists and antagonists. Inulin and para-aminohippurate concentrations were determined using colorimetric methods. Sodium and lithium concentrations were measured using a flame photometer. Clearances of para-aminohippurate, inulin, and lithium were calculated by use of standard formulas. GFR, renal plasma flow, filtration fraction, renal vascular resistance, fractional excretion of sodium, and fractional excretion of lithium were determined as follows: GFR = clearance of inulin; renal plasma flow = clearance of para-aminohippurate; renal vascular resistance = mean arterial pressure/renal blood flow (renal blood flow was calculated as the ratio of renal plasma flow/1-hematocrit); filtration fraction = clearance of inulin/clearance of para-aminohippurate; fractional excretion of sodium = ([urinary flow rate × urinary sodium excretion]/[plasma sodium concentration/GFR]) × 100; fractional excretion of lithium = (clearance of lithium/clearance of inulin) × 100.

Microdissection of Afferent and Efferent Arterioles

Animals were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg; Sanofi). Afferent and efferent arterioles were microdissected by a modified method described by Helou and Marchetti (13). Briefly, the abdominal aorta was cannulated with polyethylene tubing just below the left kidney. The left kidney was perfused with 10 ml of ice-cold solution-1 (135 mM NaCl, 1 mM Na_2SO_4, 1.2 mM Mg SO_4, 5 mM KCl, 2 mM $CaCl_2$, 5.5 mM glucose, and 5 mM Hepes, pH 7.4) followed by perfusion with 10 ml of solution-1 containing 1 mg/ml collagenase (0.38 U/mg collagenase A; Boehringer Mannheim) and 1 mg/ml bovine serum albumin (Sigma). The left kidney was removed, and several coronal sections over the entire cortico-papillary axis were made. These sections were cut into three pieces: cortex, outer medulla, and inner medulla. In the present study, only the cortex area was transferred into individual tubes containing solution-1 with 0.5 mg/ml collagenase and 1 mg/ml bovine serum albumin. The tubes were incubated for 8 to 15 min at 37°C in a shaking water bath, and their contents were continuously bubbled with 95% O_2/5% CO_2. The efferent and afferent arterioles from the cortex area were dissected on ice under a microscope and transferred

to solution-1 containing 10 mM of vanadyl ribonucleotide complex (Sigma), a potent RNase inhibitor, and were placed on ice until further use. Afferent and efferent arterioles came from different nephrons. Afferent arterioles were only isolated from glomeruli containing both the afferent and efferent arteriole. The efferent arteriole, having side branches, could be easily distinguished from the afferent arteriole (13). After isolation, the surface of each afferent and efferent arteriole was determined by repeated measurements (5 to 7 times), using an image morphometry system (BIOSTAT™; Toulouse, France) as described previously (11).

Reverse Transcription-PCR/Southern mRNA Expression Analysis

The reverse transcription-PCR (RT-PCR) was performed on 480 ± 89 μm^2 of efferent and afferent arteriole, in which genomic DNA was digested by an RNase-free DNase as described (11). The program for bradykinin B_1 receptor cDNA amplification was: 2 min 30 s at 93°C; 1 min at 95°C; 1 min 30 s at 62°C; 1 min at 72°C (35 cycles); and 10 min at 72°C. The oligonucleotides used for the bradykinin B_1 receptor were: sense, 5′-CTACAGGCTCCTGGTATACC-3′ (bases 435 to 454, relative to B_1 start codon); antisense, 5′-CTCAGGGAGGC-CAGGATGTG-3′ (bases 700 to 719), which yields a PCR product of 285 bp. Glyceraldehyde-3-phosphate dehydrogenase (product 470 bp) was amplified in parallel on the same cDNA as described (14). Southern blots (15) using a nested ^{32}P-labeled bradykinin B_1 receptor probe (5′-CCTTGCAAAGTGCCAAGC-3′) were performed as described previously (16).

Intracellular Calcium Analysis

The intracellular calcium ($[Ca^{2+}]_i$) mobilization measurements were performed using Fura-2 AM as described by Bouby *et al.* (17). In brief, the microdissected arterioles were individually transferred onto a thin glass microscope coverslip in 1 ml of solution-1 containing 1% agarose (type IX; Sigma) at 37°C. Then the agarose was jelled by cooling the coverslip for 1 min on ice. The samples embedded in agarose were loaded with 5 μM Fura-2 AM at room temperature for 1 h. For fluorescence measurements, each sample was placed on the stage of an inverted microscope and was continuously perfused at a rate of 0.6 ml/min at 37°C with solution-1, which could be replaced at any time by the solutions to be tested. Fura-2 was alternatively excited at 340 and 380 nm using a 75W xenon light source, filters, and a chopper (PTI Photoscan II System; Kontron, Munich, Germany). Correction for autofluorescence was performed as described previously (18). $[Ca^{2+}]_i$ was calculated from the equation of Grynkiewicz *et al.* (19).

Statistical Analyses

Data are presented as means ± SEM. Statistical analyses were performed using SPSS software (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL). ANOVA (two-way ANOVA analysis, repeated measurements) with a *post hoc* Tukey alpha test were performed for comparison between the different groups. $P < 0.05$ was considered statistically significant.

Results

The right kidney was infused (10 μl/min) via the suprarenal artery for 20 min with saline (first period) followed by infusion for 20 min with saline with or without 150 ng/min of des-Arg^9-bradykinin (second period). The systemic parameters, mean arterial pressure (Table 1), and hematocrit (not shown) did not differ between these two periods or between saline with or without des-Arg^9-bradykinin. Furthermore, the functional parameters (urine flow rate, urine sodium excretion, and lithium clearance) and the renal hemodynamic parameters (renal plasma flow, renal vascular resistance, GFR, and filtration fraction) of the contralateral kidney did not change during the experiments (not shown). Preliminary experiments have shown that infusion of this low dose (150 ng/min) of des-Arg^9-bradykinin affected only the renal parameters of the right kidney. Moreover, the lack of change in BP supports an effect of des-Arg^9-bradykinin limited to the perfused kidney.

Arterial infusion of des-Arg^9-bradykinin in control rats produced a decrease in renal plasma flow (Figure 1) ($P < 0.05$) and in GFR ($P < 0.05$) of the right kidney (Figure 2). This was correlated with an increase in renal vascular resistance (Figure 3) ($P < 0.05$), which is consistent with the earlier observed increase in renal vascular resistance after infusion of des-Arg^9-bradykinin in isolated perfused rat kidney (7).

In lipopolysaccharide-treated rats, infusion of des-Arg^9-bradykinin also induced a significant decrease in renal plasma flow (Figure 1) ($P < 0.01$) and GFR (Figure 2) ($P < 0.05$) when compared with the value obtained in the lipopolysaccharide group receiving saline. Although des-Arg^9-bradykinin induced a significant ($P < 0.01$) greater decrease in renal plasma flow in lipopolysaccharide-treated rats compared to control rats, the decrease in GFR was not significantly different between lipopolysaccharide-treated and control rats (Figure 2), resulting in a significant increase in filtration fraction (Figure 4) ($P < 0.01$). As in control rats, des-Arg^9-bradykinin infusion in lipopolysaccharide-treated rats increased the renal vascular resistance ($P < 0.01$) and was significantly larger ($P < 0.01$) compared to control rats (Figure 3).

The effect of des-Arg^9-bradykinin was completely inhibited by simultaneous infusion of the specific bradykinin B_1 receptor antagonist des-Arg^9-[Leu^8]bradykinin (150 ng/μl) (Figures 1 through 4), but not by a bradykinin B_2 receptor antagonist (D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]bradykinin; not shown).

In both the control and the lipopolysaccharide-treated group, the urinary flow rate tended to decrease after des-Arg^9-bradykinin infusion, but was not found to be statistically significant (Table 1). No effect of des-Arg^9-bradykinin was observed on the sodium content of the urine in control and lipopolysaccharide-treated rats (Table 1). Despite not being statistically significant, des-Arg^9-bradykinin seemed to induce a decrease in the lithium clearance in both lipopolysaccharide-treated and control rats (Table 1). However, the fractional excretion of lithium did not change, indicating that the possible decrease in the post-proximal flow is essentially caused by the decrease in GFR.

The expression of bradykinin B_1 receptor mRNA in microdissected afferent and efferent arterioles was studied by RT-PCR/Southern-blot analysis. Although with our method we were unable to detect bradykinin B_1 receptor mRNA in both arterioles of control rats (Figure 5, C and F), we observed a des-Arg^9-bradykinin-induced increase in $[Ca^{2+}]_i$ in the afferent arteriole (Figure 5A, trace a) (Table 2), which was prevented by the bradykinin B_1 receptor antagonist des-Arg^9-

Table 1. Effect of the des-Arg^9-bradykinin perfusion on MAP of the various experimental groups and the functional parameters of the right kidney of control and LPS-treated rats[a]

Parameter and Group	Treatment	1st Period	2nd Period
MAP (mmHg)			
control	Saline	113 ± 13	113 ± 12
	DBK	119 ± 8.3	120 ± 7.6
	DBK + B1-anta	116 ± 7.4	114 ± 10
LPS	Saline	108 ± 11	108 ± 11
	DBK	116 ± 15	113 ± 13
	DBK + B1-anta	112 ± 13	115 ± 11
UV (μl/min per g)			
control	Saline	4.0 ± 1.2	3.9 ± 1.1
	DBK	3.6 ± 1.1	2.7 ± 0.5
	DBK + B1-anta	3.9 ± 1.0	3.2 ± 0.7
LPS	Saline	3.6 ± 0.9	3.6 ± 1.1
	DBK	3.8 ± 2.0	2.8 ± 0.9
	DBK + B1-anta	3.8 ± 1.5	3.4 ± 1.2
$U_{Na}V$ (μmol/min per g)			
control	Saline	0.13 ± 0.12	0.13 ± 0.11
	DBK	0.07 ± 0.03	0.07 ± 0.04
	DBK + B1-anta	0.09 ± 0.07	0.08 ± 0.05
LPS	Saline	0.10 ± 0.09	0.10 ± 0.07
	DBK	0.12 ± 0.16	0.08 ± 0.06
	DBK + B1-anta	0.09 ± 0.07	0.08 ± 0.10
FE_{Na} (%)			
control	Saline	0.09 ± 0.09	0.10 ± 0.08
	DBK	0.06 ± 0.03	0.07 ± 0.05
	DBK + B1-anta	0.07 ± 0.05	0.09 ± 0.06
LPS	Saline	0.07 ± 0.06	0.08 ± 0.06
	DBK	0.08 ± 0.08	0.07 ± 0.05
	DBK + B1-anta	0.08 ± 0.06	0.07 ± 0.07
C_{Li} (μl/min per g)			
control	Saline	286 ± 69	292 ± 40
	DBK	256 ± 47	230 ± 53
	DBK + B1-anta	264 ± 62	287 ± 58
LPS	Saline	278 ± 77	270 ± 86
	DBK	251 ± 64	190 ± 44
	DBK + B1-anta	258 ± 72	245 ± 65
FE_{Li} (%)			
control	Saline	30.4 ± 6.3	31.9 ± 4.0
	DBK	30.0 ± 7.5	33.9 ± 6.3
	DBK + B1-anta	29.8 ± 6.5	31.3 ± 7.3
LPS	Saline	32.7 ± 5.4	29.2 ± 6.8
	DBK	27.0 ± 7.0	26.2 ± 6.0
	DBK + B1-anta	29.0 ± 7.4	27.5 ± 7.0

[a] All groups were infused with saline during the first period (20 min) and with saline, bradykinin B_1 receptor agonist, or antagonist during the second period (20 min) as indicated. MAP, mean arterial pressure; UV, urinary flow rate; $U_{Na}V$, urinary sodium excretion; FE_{Na}, fractional excretion of sodium; C_{Li}, lithium clearance; FE_{Li}, fractional excretion of lithium; control, nontreated rats; LPS, rats treated with lipopolysaccharide (18 h, 0.2 mg/kg); saline, saline infusion; DBK, des-Arg^9-bradykinin (150 ng/min) infusion; DBK + B1-anta, infusion of des-Arg^9-bradykinin (150 ng/min; bradykinin B_1 receptor agonist) in presence of des-Arg^9-[Leu^8]bradykinin (150 ng/min; bradykinin B_1 receptor antagonist).

[Leu^8]bradykinin (Figure 5A, trace b). No des-Arg^9-bradykinin-induced $[Ca^{2+}]_i$ mobilization was observed in the efferent arteriole of control rats (Figure 5D), which was not due to damaged tissue caused by the microdissection since stimulation with angiotensin II resulted in $[Ca^{2+}]_i$ mobilization (Figure 5D).

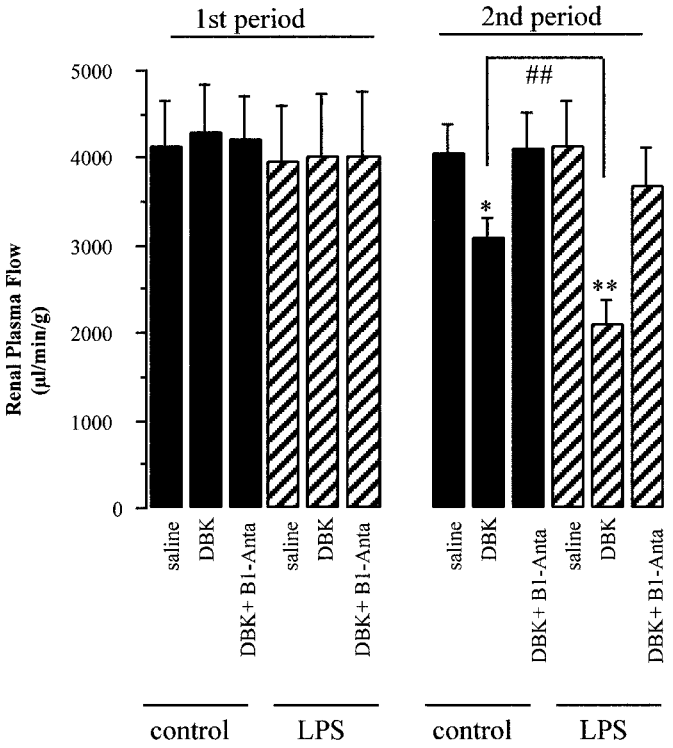

Figure 1. Renal plasma flow of the right kidney of control and lipopolysaccharide-treated rats. All groups were infused with saline during the first period (20 min) and with saline or bradykinin B_1 receptor agonist or antagonist during the second period (20 min). control, nontreated rats; LPS, rats treated with lipopolysaccharide (18 h, 0.2 mg/kg); saline, saline infusion; DBK, des-Arg^9-bradykinin (150 ng/min) infusion; DBK + B1-Anta, infusion of des-Arg^9-bradykinin (150 ng/min) in the presence of des-Arg^9-[Leu^8] bradykinin (150 ng/min). Data are presented as means ± SEM ($n = 10$) for each group. $^*P < 0.05$; $^{**}P < 0.01$ when compared to the respective saline-infused rats during the second period; $^{\#\#}P < 0.01$ when compared to DBK infusion in the control group during the second period.

Lipopolysaccharide clearly induced bradykinin B_1 receptor mRNA expression in both afferent and efferent arterioles (Figure 5, C and F). Controls, in which the reverse transcription was performed in the absence of the reverse transcriptase enzyme, produced no amplification products. The observed expression is thus not due to DNA contamination of the samples. The expression of a housekeeping gene, in this case glyceraldehyde-3-phosphate-dehydrogenase, amplified in parallel, was not different between the afferent and efferent arteriole (Figure 5, C and F). The lipopolysaccharide-induced bradykinin B_1 receptors in the efferent arteriole were functional, as demonstrated by the des-Arg^9-bradykinin-induced $[Ca^{2+}]_i$ mobilization in the microdissected segment (Figure 5E, trace a) (Table 2), which was abolished by the bradykinin B_1 receptor antagonist des-Arg^9-[Leu^8]bradykinin (Figure 5E, trace b). Surprisingly, in the afferent arterioles from lipopolysaccharide-treated rats, the observed amplitudes of the des-Arg^9-bradykinin-induced $[Ca^{2+}]_i$ mobilization were similar to those observed in control rats (Figure 5, A and B [traces a]) (Table 2).

Discussion

Induction of bradykinin B_1 receptors during inflammation strikingly differentiates them from bradykinin B_2 receptors. However, little information is available on how bradykinin B_1 receptor induction is translated into functional changes. The clear link between bradykinin B_1 receptor induction and the cytokine network (3) indicates that this receptor might be induced in renal diseases related to inflammation (although not yet demonstrated), including glomerulonephritis, HIV nephropathy, renal transplant rejection, lupus nephritis, ischemia, and acute hypertensive nephritis (20,21). We have recently reported that experimental inflammation induces functional bradykinin B_1 receptors along the rat efferent arteriole (11). Because it is known that this renal structure can participate in renal hemodynamic control (12), we studied, *in vivo* in anesthetized rats, the functional significance of bradykinin B_1 receptor induction along the efferent arteriole. Experimental inflammation was induced by 18 h of lipopolysaccharide treatment, which is usually sufficiently long to induce bradykinin B_1 receptor expression in the kidney (11) and other organs (3).

The most important observation of our study is that *in vivo* intrarenal des-Arg^9-bradykinin infusion alters renal hemodynamics and that the alteration is significantly enhanced under experimental inflammatory conditions induced by lipopolysaccharide.

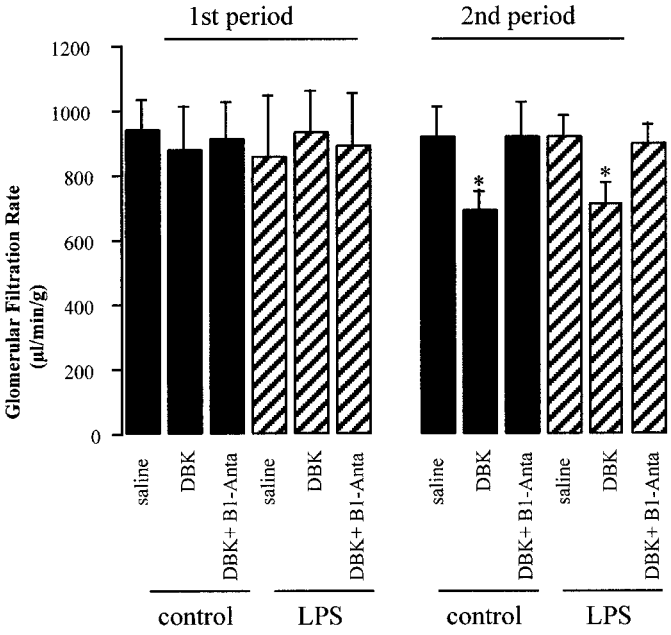

Figure 2. GFR of the right kidney of control and lipopolysaccharide-treated rats. All groups were infused with saline during the first period (20 min) and with saline or bradykinin B_1 receptor agonist or antagonist during the second period (20 min). control, nontreated rats; LPS, rats treated with lipopolysaccharide (18 h, 0.2 mg/kg); saline, saline infusion; DBK, des-Arg^9-bradykinin (150 ng/min) infusion; DBK + B1-Anta, infusion of des-Arg^9-bradykinin (150 ng/min) in the presence of des-Arg^9-[Leu^8] bradykinin (150 ng/min). Data are presented as means ± SEM ($n = 10$) for each group. $^*P < 0.05$ when compared to the respective saline-infused rats during the second period.

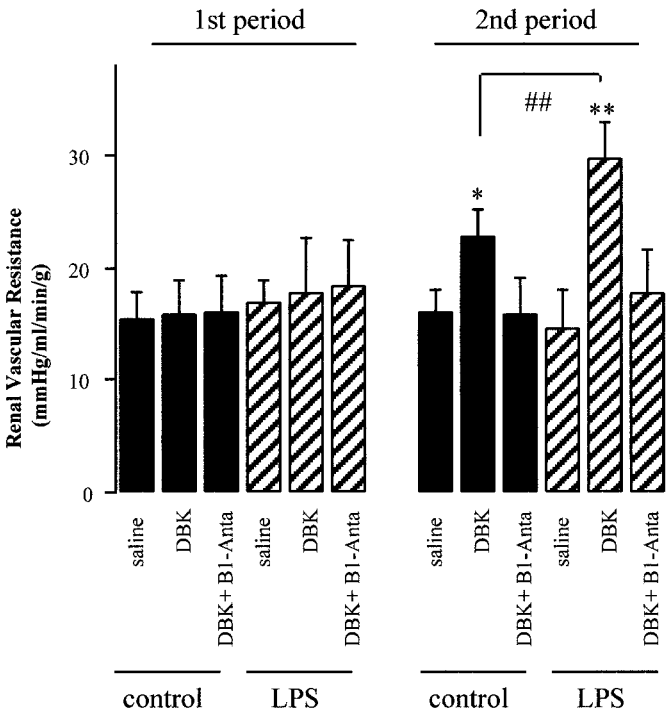

Figure 3. Renal vascular resistance of the right kidney of control and lipopolysaccharide-treated rats. All groups were infused with saline during the first period (20 min) and with saline or bradykinin B_1 receptor agonist or antagonist during the second period (20 min). control, nontreated rats; LPS, rats treated with lipopolysaccharide (18 h, 0.2 mg/kg); saline, saline infusion; DBK, des-Arg^9-bradykinin (150 ng/min) infusion; DBK + B1-Anta, infusion of des-Arg^9-bradykinin (150 ng/min) in the presence of des-Arg^9-[Leu^8] bradykinin (150 ng/min). Data are presented as means ± SEM ($n = 10$) for each group. *$P < 0.05$; **$P < 0.01$ when compared to the respective saline-infused rats during the second period; $^{\#\#}P < 0.01$ when compared to DBK infusion in the control group during the second period.

Under control conditions, we have observed equal falls in renal plasma flow and GFR upon bradykinin B_1 receptor agonist infusion with no consequent change in filtration fraction. Under these conditions, using calcium mobilization studies, no functional bradykinin B_1 receptors were found in microdissected efferent arterioles, whereas functional bradykinin B_1 receptors were identified in microdissected afferent arterioles. Although no corresponding bradykinin B_1 receptor mRNA was detected in afferent arterioles from control rats, it is possible that a small basal number of B_1 receptors, undetectable by our methods, are efficiently coupled to the phospholipase C transduction pathway that was not changed by the induction of new bradykinin B_1 receptor mRNA after lipopolysaccharide treatment. We obtained similar results for the bradykinin B_1 receptor in microdissected rat medullary thick ascending limb and cortical collecting duct (11). These observations, together with the observation of Yu *et al.* (10) that des-Arg^9-bradykinin induced vasoconstriction of isolated rabbit afferent arterioles, suggest that under control conditions, des-Arg^9-bradykinin can induce vasoconstriction of the afferent arteriole resulting in the observed decrease in renal plasma flow and in GFR.

Under inflammatory conditions, des-Arg^9-bradykinin infusion induced a significant larger fall in renal plasma flow without an accompanied larger fall in GFR, resulting in an increased filtration fraction. This suggests a predominant des-Arg^9-bradykinin-induced vasoconstriction at the level of the efferent arteriole under inflammatory conditions and correlates with the induction of functional bradykinin B_1 receptors along the efferent arteriole in lipopolysaccharide-treated rats without an increased functional expression of bradykinin B_1 receptors along the afferent arteriole. Therefore, these data suggest a preferential des-Arg^9-bradykinin-induced vasoconstriction of the efferent arteriole under inflammatory conditions. However, one cannot exclude des-Arg^9-bradykinin-induced changes in the ultrafiltration coefficient. The latter is determined by the surface area available for filtration and the hydraulic properties of the membrane. In this context, we have shown that bradykinin results in contraction of cultured mesangial cells (22), which correlates with a decreased ultrafiltration coefficient upon renal bradykinin infusion (23). However, we have not yet studied the effect of des-Arg^9-bradykinin addition on mesangial cell contraction in cultured cells pretreated with inflammatory agents such as lipopolysaccharide or the proinflammatory cyto-

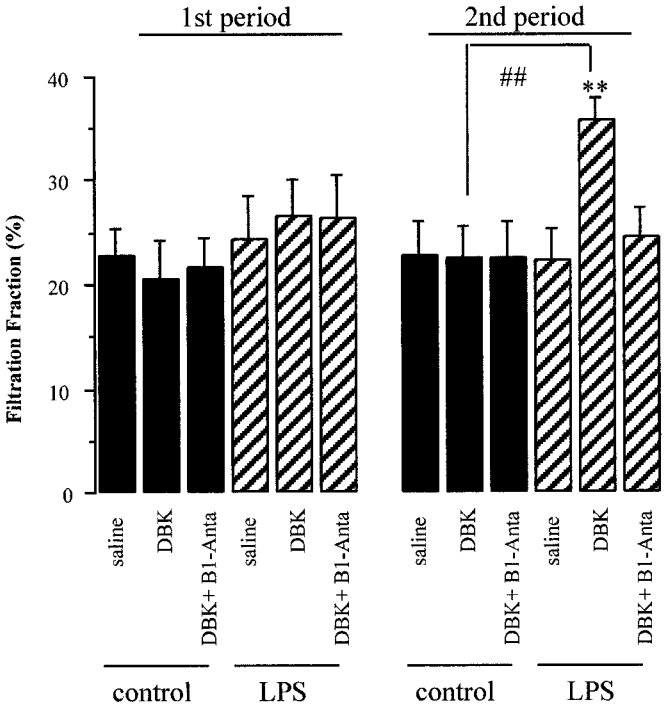

Figure 4. Filtration fraction of the right kidney of control and lipopolysaccharide-treated rats. All groups were infused with saline during the first period (20 min) and with saline or bradykinin B_1 receptor agonist or antagonist during the second period (20 min). control, nontreated rats; LPS, rats treated with lipopolysaccharide (18 h, 0.2 mg/kg); saline, saline infusion; DBK, des-Arg^9-bradykinin (150 ng/min) infusion; DBK + B1-Anta, infusion of des-Arg^9-bradykinin (150 ng/min) in the presence of des-Arg^9-[Leu^8] bradykinin (150 ng/min). Data are presented as means ± SEM ($n = 10$) for each group. **$P < 0.01$ when compared to the respective saline-infused rats during the second period; $^{\#\#}P < 0.01$ when compared to DBK infusion in the control group during the second period.

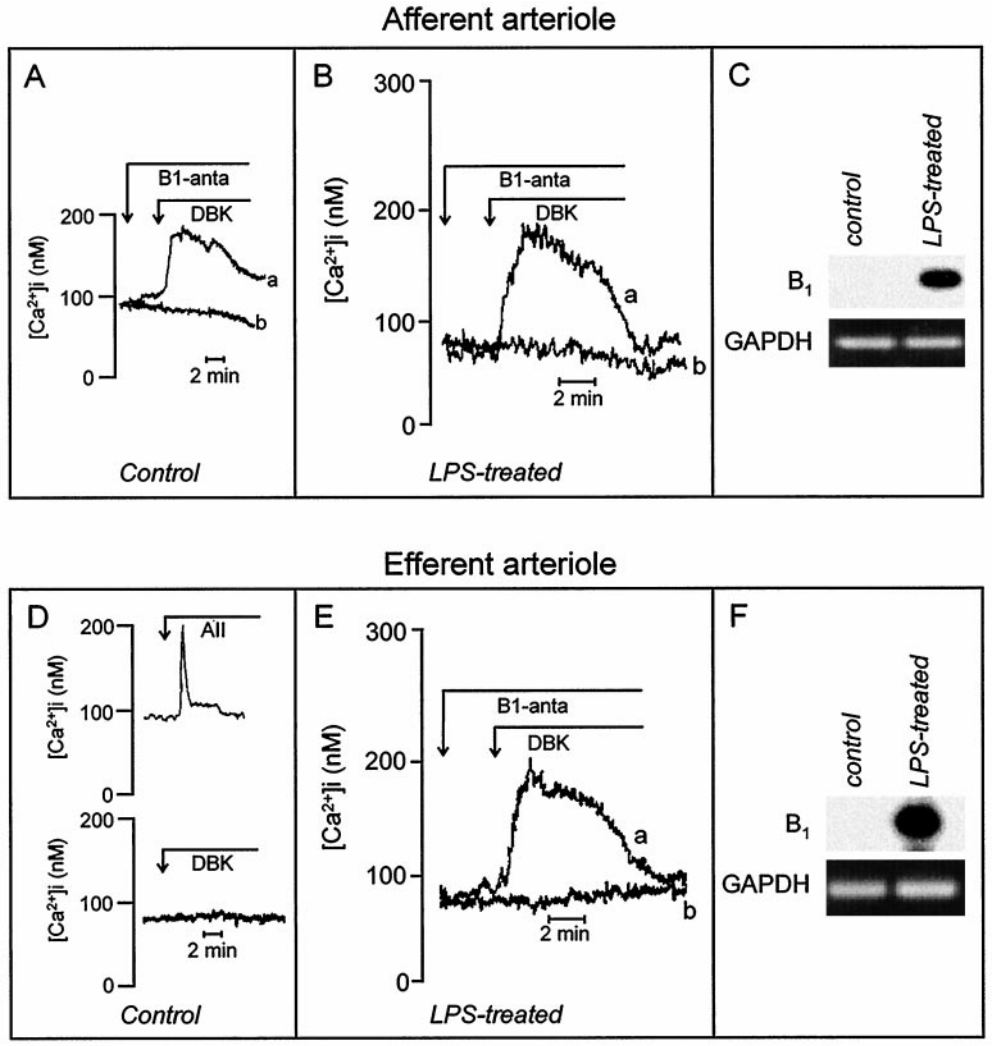

Figure 5. Bradykinin B_1 receptor mRNA expression and des-Arg^9-bradykinin-induced intracellular calcium ($[Ca^{2+}]_i$) mobilization in microdissected afferent and efferent arterioles of control and lipopolysaccharide-treated (LPS-treated) rats. Profiles are representative of 12 different experiments. (Three rats were used for each group, and from each rat four afferent and four efferent arterioles were microdissected.) Des-Arg^9-bradykinin (0.1 μM) induced $[Ca^{2+}]_i$ mobilization in afferent arterioles of control (Panel A, trace a) and lipopolysaccharide-treated (Panel B, trace a) rats, which was prevented by a bradykinin B_1 receptor antagonist (des-Arg^9-[Leu^8]bradykinin [1 μM], Panels A and B, trace b) perfused 2 min before addition of the bradykinin B_1 receptor agonist. In control efferent arterioles, des-Arg^9-bradykinin (0.1 μM) did not result in $[Ca^{2+}]_i$ mobilization (Panel D, bottom panel), while angiotensin II (0.1 μM) was able to induce $[Ca^{2+}]_i$ mobilization (Panel D, top panel). Des-Arg^9-bradykinin (0.1 μM) induced $[Ca^{2+}]_i$ mobilization in efferent arterioles isolated from LPS-treated rats (Panel E, trace a), which was prevented by a bradykinin B_1 receptor antagonist (des-Arg^9-[Leu^8]bradykinin [1 μM], Panel E, trace b). The autoradiograms show representative reverse transcription-PCR/Southern blot analyses of bradykinin B_1 receptor mRNA expression (B_1 in Panels C and F). The expression of the housekeeping gene glyceraldehyde-3-phosphate-dehydrogenase is shown on photographs of ethidium bromide-stained gels (GAPDH in Panels C and F). The initiation and duration of the perfusion is indicated by the length of the arrows above the calcium profile. DBK, des-Arg^9-bradykinin; B1-anta, des-Arg^9-[Leu^8]bradykinin; AII, angiotensin II.

kine interleukin-1β. These studies and micropuncture studies to analyze single-nephron GFR and glomerular capillary pressure should be undertaken to better define the observed effects.

Infusion of des-Arg^9-bradykinin in control rats did not modify diuresis or natriuresis under control conditions, or after lipopolysaccharide treatment. Urine flow rate tended to decrease upon des-Arg^9-bradykinin infusion, but was not found to be statistically significant. This putative antidiuretic effect of des-Arg^9-bradykinin is in contrast to the well known diuretic action of bradykinin, which probably counteracts the effects of the antidiuretic hormone in the collecting duct (24). No such interaction between des-Arg^9-bradykinin and antidiuretic hormone has been reported thus far. In contrast to our results, Lortie *et al.* (9) concluded that activation of the bradykinin B_1 receptor is natriuretic. This difference might be explained by species differences (dog *versus* rat) and/or by the fact that a bradykinin B_1 receptor antagonist was used to block the effect of bradykinin infusion, which is different from the direct infusion of bradykinin B_1 receptor agonist in our study.

Under physiologic conditions, des-Arg^9-bradykinin is the

Table 2. Effect of des-Arg^9-bradykinin (0.1 μM) on the $[Ca^{2+}]_i$ concentration in afferent and efferent arterioles from control and LPS-treated rats[a]

Arteriole	Afferent	Efferent
Control		
basal	83 ± 9	75 ± 8
peak	181 ± 18	75 ± 8
LPS-treated		
basal	82 ± 11	81 ± 7
peak	183 ± 13	193 ± 21

[a] Values are expressed in nM. Each value is the mean ± SEM of 12 different arterioles. For each group, three rats were used and from each rat four afferent and four efferent arterioles were dissected and stimulated by des-Arg^9-bradykinin. $[Ca^{2+}]_i$, intracellular calcium; LPS, lipopolysaccharide.

minor kinin peptide in the rat kidney (25). Therefore, under physiologic conditions, the observed effects of exogenous des-Arg^9-bradykinin infusion may be absent or minimal among the action of various other vasoactive agents such as bradykinin and angiotensin II. The present results of an enhanced des-Arg^9-bradykinin-induced hemodynamic response after lipopolysaccharide treatment, along with the general observation that des-Arg^9-bradykinin expression is increased during inflammation (5,6), show that bradykinin B_1 receptor activation in the kidney might be of importance under chronic inflammatory conditions.

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