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Vascular kinin B_1 and B_2 receptor-mediated effects in the rat isolated perfused kidney – differential regulations

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1 Bradykinin (BK) and analogs acting preferentially at kinin B_1 or B_2 receptors were tested on the rat isolated perfused kidney. Kidneys were perfused in an open circuit with Tyrode's solution. Kidneys preconstricted with prostaglandin $F_{2\alpha}$ were used for the analysis of vasodilator responses.

2 BK induced a concentration-dependent renal relaxation ($pD_2 = 8.9 \pm 0.4$); this vasodilator response was reproduced by a selective B_2 receptor agonist, Tyr(Me)^8-BK ($pD_2 = 9.0 \pm 0.1$) with a higher maximum effect ($E_{max} = 78.9 \pm 6.6$ and $55.8 \pm 4.3\%$ of ACh-induced relaxation respectively, $n = 6$ and 19, $P < 0.02$). Icatibant (10 nM), a selective B_2 receptor antagonist, abolished BK-elicited relaxation. Tachyphylaxis of kinin B_2 receptors appeared when repeatedly stimulated at 10 min intervals.

3 Des-Arg^9-BK, a selective B_1 receptor agonist, induced concentration-dependent vasoconstriction at micromolar concentration. Maximum response was enhanced in the presence of lisinopril (1 μM) and inhibited by R 715 (8 μM), a selective B_1 receptor antagonist. Des-Arg^9-[Leu^8]-BK behaved as an agonist.

4 A contractile response to des-Arg^9-BK occurred after 1 h of perfusion and increased with time by a factor of about three over a 3 h perfusion. This post-isolation sensitization to des-Arg^9-BK was abolished by dexamethasone (DEX, 30 mg kg^{-1} i.p., 3 h before the start of the experiment and 10 μM in perfusate) and actinomycin D (2 μM). Acute exposure to DEX (10 μM) had no effect on sensitized des-Arg^9-BK response, in contrast to indomethacin (30 μM) that abolished it. DEX pretreatment however had no effect on BK-induced renal vasodilation.

5 Present results indicate that the main renal vascular response to BK consists of relaxation linked to the activation of kinin B_2 receptors which rapidly desensitize. Renal B_1 receptors are also present and are time-dependently sensitized during the *in vitro* perfusion of the rat kidneys.

Keywords: Bradykinin; bradykinin receptors; vascular effects; rat isolated kidney; B_1 receptor sensitization; actinomycin D; R 715

Abbreviations: ACE, angiotensin I converting enzyme; ACh, acetylcholine; BK, bradykinin; des-Arg^9-BK, des-Arg^9-bradykinin; des-Arg^9-[Leu^8]-BK, des-Arg^9-[Leu^8]-bradykinin; DEX, dexamethasone; NA, noradrenaline; R 715, AcLys-[D-βNal^7,Ile^8]des-Arg^9-BK; SNP, sodium nitroprusside; Tyr(Me)^8-BK, Tyr(Me)^8-bradykinin

Introduction

The kinins, bradykinin (BK) and kallidin, are endogenous peptides locally generated by the proteolytic action of kallikreins on kininogen. In the kidney, all the components of the kallikrein-kinin system are present (Regoli & Barabé, 1980; Bhoola *et al*, 1992; Navar *et al*., 1996). Renal tissue levels of BK are much higher than circulating levels, being consistent with local synthesis (Campbell *et al*., 1993). Distal tubules are particularly rich in kallikrein and kininogen but both are also expressed in blood vessels (Bhoola *et al*., 1992; Nolly & Nolly, 1998). In the isolated rat kidney, active kallikrein is released in the venous effluent (Misumi *et al*., 1983; Bhoola *et al*., 1992) and vasoactive BK is formed when high-molecular-weight kininogen is added to the perfusate (Gardes *et al*., 1990). BK generated in the interstitial fluid may also diffuse to the vascular compartment and contribute to the paracrine regulation of renal haemodynamics, at least in low sodium states in which the kallikrein-kinin system is known to be activated (Navar *et al*., 1996; Siragy *et al*., 1994).

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Kinins exert their biological activity through the activation of two receptor types, B_1 and B_2, which both have been cloned and belong to the seven transmembrane G-proteins coupled receptor family (Regoli & Barabé, 1980; Regoli *et al*., 1998; Marceau *et al*., 1998). The kinin B_2 receptor is constitutively expressed and widely distributed in blood vessels in which activation provokes vasodilation and plasma extravasation. The naturally occurring peptide, BK, and its synthetic analogue, Tyr(Me)^8-BK, are high affinity B_2 receptor agonists whose effects are competitively antagonized by icatibant, a potent and selective B_2 receptor antagonist (Hock *et al*., 1991).

The kinin B_1 receptor displays high affinity and selectivity for kinin metabolites lacking the C-terminal arginine residue, such as des-Arg^9-BK. The B_1 receptor is seldomly expressed in normal tissues but seems to be upregulated in pathological states associated with tissue injury (Marceau, 1995; Marceau *et al*., 1998). Moreover, isolated smooth muscle preparations, which are initially not sensitive to des-Arg^9-BK, also become responsive to B_1 receptor activation in a time-dependent fashion. For instance, in the isolated rabbit aorta, sensitization to the vasoconstrictor response of des-Arg^9-BK appeared within a period of 6 h of incubation. This required *de novo* B_1

receptor synthesis, while sensitization was prevented by glucocorticoids and mimicked by the application of bacterial lipopolysaccharide or interleukines (Marceau, 1995). Taken together, these data suggest that the vascular sensitization to des-Arg^9-BK may occur in tissues injured by isolation and *in vitro* incubation.

BK elicits variable effects on the renal circulation. In the dog, renal vasodilation was the unique effect exerted by the kinin (Lahera *et al*., 1991; Rhaleb *et al*., 1989) whereas only vasoconstriction occurred in the rabbit kidney (Barabé *et al*., 1979). In the rat, vasodilation and vasoconstriction have both been described *in vivo* (Hofbauer *et al*., 1983). *In vitro*, Guimaraes and coworkers (1986) using an isolated kidney perfused in a closed-circuit system, reported short-lived renal vasodilation induced by BK together with vasoconstriction mediated by kinin B_1 receptors. Desensitization or sensitization of these effects however was not addressed. The aim of the present study was to characterize the kinin receptor(s) present in the rat renal vasculature by their functional response and to evaluate their up- or down-regulation with time or exposure to agonist. For this purpose we used a rat isolated kidney perfused in an open circuit, which enables the testing of several concentrations of kinins on the same kidney, limits their local catabolism, avoids inflammatory injury due to recirculation and allows the study of renal vascular reactivity over several hours.

Methods

Animals

Male Wistar rats (220–280 g, Janvier breeding, Le Genest St Isle, France) were used. Animals were housed in a room at 20°C with a 12 h light/dark cycle (light on at 06.00 h) and allowed free access to tap water and standard food (AO4 pellets, 0.2% sodium, UAR, Villemoisson/Orge, France). The rats stayed in our animal facility for at least 1 week before the start of the experiments. Experiments were performed in accordance with guidelines of the European Community and the French Government concerning the use of animals.

Preparation of the isolated perfused rat kidney

After sodium pentobarbital anaesthesia (45 mg kg^{-1} i.p.), the right kidney was prepared with special care to avoid ischaemia, and perfused *via* the mesenteric artery, in an open circuit, as described previously (Schmidt & Imbs, 1981; Barthelmebs *et al*., 1996). The perfusion medium was a prewarmed (37°C), oxygenated (95% O_2/5% CO_2), colloid free Tyrode's solution of the following composition (mM): NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.1; NaH_2PO_4 0.42; $NaHCO_3$ 12; glucose 10; pH was adjusted at 7.4. After a 45 min equilibration period, perfusion flow was adjusted at 8 ml min^{-1} and kept constant thereafter (Gilson Minipuls 3, Bioblock, Illkirch, France). Perfusion pressure was continuously monitored (Statham P23 Db tranducer, Statham Intruments, Hato Rey, Porto Rico) and recorded (Philips PM 8222, Philips, Bobigny, France) throughout the experiment.

Evaluation of vasodilator responses

Vascular tone of the isolated kidney was increased by the continuous perfusion of prostaglandin $F_{2\alpha}$ at a concentration (0.5–2 μM) sufficient to induce a steady increase in perfusion pressure of about 20 mmHg. BK or Tyr(Me)^8-BK, a selective B_2 receptor agonist, were tested on preconstricted kidney preparations. In a first set of experiments, a sequential concentration-relaxation curve to BK was constructed in each kidney (increasing concentrations 0.1 nM–0.3 μM, perfused at 10 min intervals). Since desensitization occurred with such a protocol, only two or three concentrations were subsequently tested at 30 min intervals. Therefore, full range concentration response curves to BK or Tyr(Me)^8-BK (0.1 nM–0.3 μM) were obtained from several kidney preparations. Acetylcholine (ACh) and sodium nitroprusside (SNP) were included as markers for endothelium-dependent and -independent vasodilator reactivity, respectively (Barthelmebs *et al*., 1996). Thus, a supramaximal concentration of ACh (30 nM) was tested after each concentration of agonist while a supramaximal concentration of SNP (10 μM) was administered at the end of the experiments. In order to investigate the dependence of the responses from interaction with the kinin B_2 receptor, icatibant (10 nM) was added to the perfusate 30 min before the administration of BK. The vasodilator responses to BK or Tyr(Me)^8-BK were expressed as percentage of the relaxation induced by ACh (30 nM) in the same kidney to take into account variations in endothelium-dependent vasodilator reactivity between kidney preparations. Other vasodilator responses (ACh, SNP) were expressed as percentage reversal of the prostaglandin $F_{2\alpha}$-induced preconstriction.

Evaluation of vasoconstrictor responses

The vasoconstriction induced by des-Arg^9-BK (0.3–8 μM), a selective B_1 receptor agonist, was compared to that elicited by a supramaximal concentration of noradrenaline (NA, 10 μM) in the same kidney. Since high concentrations of des-Arg^9-BK were required to induce vasoconstriction, the same study was performed in the presence of lisinopril (1 μM) in order to inhibit the catabolism of the kinin by angiotensin I converting enzyme (ACE). AcLys-[D-βNal^7,Ile^8]des-Arg^9-BK (R 715, 0.1–8 μM), the selective B_1 receptor antagonist (Gobeil *et al*., 1996), was used to ascertain interaction with the B_1 receptor. Experiments with R 715 were performed in the presence of lisinopril (1 μM) since R 715 is only partially resistant to ACE (Marceau *et al*., 1998). Des-Arg^9-[Leu^8]-BK, another classic B_1 receptor antagonist, induced vasoconstriction in our kidney preparation and was evaluated for agonist activity (0.01–1 μM). The vasoconstrictor responses were expressed as increases in renal vascular resistance calculated as the ratio of perfusion pressure to perfusion flow.

Time-dependent sensitization to des-Arg^{9}BK

To investigate the possibility of B_1 receptor sensitization during the *in vitro* perfusion of the isolated kidney, the responses to des-Arg^9-BK (3 and 8 μM) were evaluated 1, 2 and 4 h after the onset of perfusion. Des-Arg^9-BK was also tested in kidneys obtained from rats pretreated with dexamethasone (DEX, 30 mg kg^{-1} i.p., 3 h before the kidney preparation) and perfused from the onset with DEX (10 μM). The acute effects of DEX (10 μM) on the renal response to des-Arg^9-BK after a 4 h perfusion were also compared to those of acute indomethacin (30 μM). In order to investigate the contribution of *de novo* B_1 receptor synthesis, other kidneys were perfused from the onset with actinomycin D (2 μM), an inhibitor of RNA synthesis, before the evaluation of des-Arg^9-BK elicited response after a 2 h perfusion period. Finally, the effects of DEX (10 μM, with pretreatment of the donor rats) were evaluated on BK-elicited vasorelaxation after a 2 h perfusion period.

Drugs

The following drugs were used: ACh hydrochloride, actinomycin D, BK, des-Arg^9-BK, des-Arg^9-[leu^8]-BK, DEX, indomethacin, lisinopril, NA hydrochloride, SNP (all from Sigma, St Quentin Fallavier, France); icatibant (HOE 140, Hoechst-Marion-Roussel, Frankfurt, Germany); prostaglandin $F_{2\alpha}$ tromethamine salt (Dinolytic®, Upjohn Laboratories, Paris, France); sodium pentobarbital (Nembutal®, Sanofi Santé, Libourne, France); sodium heparinate (Léo, St Quentin Yvelines, France); Tyr(Me)^8-BK and R 715 (Dr Regoli, Sherbrooke, Canada). All other chemicals were of pro-analysis quality from Merck (Darmstadt, Germany).

Peptides were prepared as stock solutions (1 mg ml^{-1} in distilled water), stored in aliquots at $-20°C$ and diluted extemporaly to the desired final concentration with 0.9% saline. To avoid adsorption of peptides, perfusion material was coated with a 1% silicone solution (Aquasil, Interchim, Monluçon, France). DEX was dissolved in ethanol and actinomycin D in DMSO (protected from light), before further dilution with saline. Indomethacin was prepared as a N-methyl-D-glucamine salt (final pH of the solution = 6). Other solutions were freshly prepared.

Statistical analysis

Results are expressed as mean ± s.e.mean. Differences were tested for statistical significance by unpaired or paired Student's *t*-test, one-way variance analysis (ANOVA), or two-way ANOVA with repeated measurements when appropriate. Multiple comparisons between groups were performed by a modified *t*-test according to Bonferroni. A *P*-value less than 0.05 was considered significant. Concentration response curves were analysed by linear regression for the determination of the 50% effective concentration (EC_{50}). All statistics were run in GraphPad Prism (GraphPad, San Diego, U.S.A.).

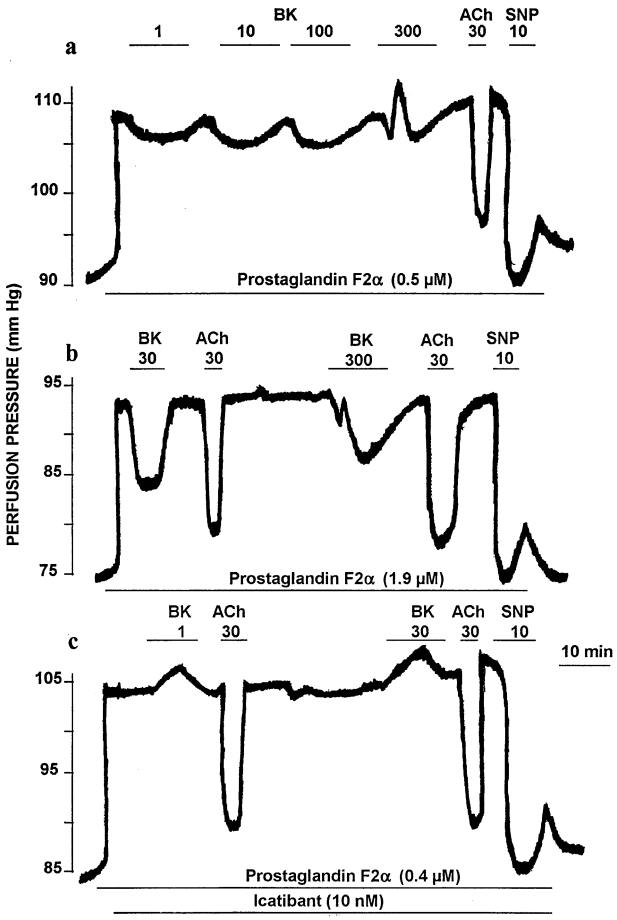

Figure 1 Typical recordings obtained in rat isolated perfused kidneys preconstricted by prostaglandin $F_{2\alpha}$ showing the vascular response induced by BK administered at sequential increasing concentrations (a) or at single concentrations separated by a 30 min interval, in the absence (b) or in the presence of icatibant (10 nM) (c). BK and ACh concentrations are given in nM while SNP one is given in μM.

Results

Characteristics of the isolated perfused rat kidneys

Overall mean arterial blood pressure of the rats before starting kidney perfusion amounted to 89.9 ± 1.2 mmHg ($n = 114$). Kidneys were perfused at a fixed flow rate of 8 ml min^{-1}, allowing a basal perfusion pressure between 70 and 90 mmHg. Accordingly, calculated renal vascular resistance averaged 10.1 ± 0.13 mmHg min ml^{-1}. Renal vascular tone was increased by 21.6 ± 0.2 mmHg at a mean prostaglandin $F_{2\alpha}$ concentration of 1.10 ± 0.14 μM ($n = 59$). SNP (10 μM) elicited an overall complete relaxation (104.8 ± 1.7% reversion of prostaglandin $F_{2\alpha}$-induced preconstriction, $n = 59$), while the response elicited by ACh (30 nM) averaged 65.3 ± 1.6% relaxation. NA (10 μM) increased renal vascular resistance by 29.2 ± 0.6 mmHg min ml^{-1} ($n = 55$). No significant difference in basal parameters or in prostaglandin $F_{2\alpha}$, ACh, SNP or NA-induced responses was observed over the different experimental groups.

Renal response elicited by BK

When perfused at sequential increasing concentrations, BK elicited only weak renal vascular responses as shown on a typical recording in Figure 1a. Relaxation was evident at a concentration of 1 nM, reached a maximum at 10 or 30 nM and became complex at a higher concentration (300 nM) when a vasoconstrictor component partially or completely prevented the vasodilator response.

To explain the weakness of the vasodilator response to BK at sequential increasing concentrations, we considered the possibility of BK receptor desensitization. This hypothesis was confirmed by perfusing BK at a single concentration of 30 nM, three times on the same kidney, at 30 min intervals. In this case, BK-induced relaxation was markedly enhanced (40.8 ± 4.4% ACh-induced relaxation *vs* 16.9 ± 4.4% in sequential perfusion, $P < 0.001$). Moreover, the response remained stable over time (Table 1). This protocol was therefore used for further investigations, two or three concentrations of BK being tested on the same kidney preparation at 30 min intervals. Figure 1b shows a typical recording.

A complete concentration response curve for BK was thus constructed from the data obtained from 28 kidney preparations (Figure 2). BK produced renal vasorelaxation with a low threshold concentration (0.1 nM), and E_{max} of 55.8 ± 4.3% of ACh-induced relaxation ($n = 19$) at 30 nM and a calculated pD_2 value of 8.9 ± 0.4. As above, a vasoconstrictor component appeared at 300 nM BK (Figure 1b).

Renal vasodilation elicited by Tyr(Me)^8-BK

Tyr(Me)^8-BK produced a concentration-dependent renal vasorelaxation in the same concentration range as BK (Figure

2). The E_{max} obtained at 10 nM (78.9±6.6% of ACh-induced relaxation, $n=6$) was higher than that elicited by BK ($P<0.02$). There was no evidence for any vasoconstrictor component in Tyr(Me)^8-BK-induced renal response, even at a concentration of 300 nM. A pD_2 value of 9.0±0.1 was determined.

Effects of icatibant on the renal vasodilation elicited by BK

Icatibant (10 nM) completely abolished the vasorelaxation elicited by BK (1–300 nM). Moreover, the antagonist unmasked a weak vasoconstriction in response to a concentration of BK as low as 1 nM (Figure 1c). The antagonism by icatibant was selective for BK since the vasodilator responses elicited by 30 nM ACh and 10 μM SNP on the same kidneys remained unchanged (respectively 74.4±5.0 and 114.9±0.8% reversion of prostaglandin $F_{2\alpha}$-induced preconstriction, $n=5$). Icatibant *per se* failed to modify the renal vascular resistance whether the kidney had been preconstricted ($n=5$) or not ($n=1$).

Renal vasoconstriction elicited by des-Arg^9-BK and des-Arg^9-[Leu^8]-BK

Des-Arg^9-BK caused no renal vascular response up to 1 μM. At higher concentrations, des-Arg^9-BK induced a concentration-dependent vasoconstriction (Figure 3). The response developed rapidly (within 3 min) and disappeared promptly at the end of drug infusion. At the maximum concentration tested (8 μM), renal vascular resistance increased by 13.6±1.5 mmHg min ml^{-1} ($n=11$), which corresponded to about half of the response elicited by 10 μM NA (29.8±1.0 mmHg min ml^{-1}). This response was obtained roughly 2 h after the onset of kidney perfusion. No tachyphylaxis occurred when successive concentrations were tested at 10 min intervals.

Before using des-Arg^9-[Leu^8]-BK to block kinin B_1 receptors, we looked for a possible residual agonist activity of the drug. Surprisingly, des-Arg^9-[Leu^8]-BK was a more potent vasoconstrictor on our kidney preparation than des-Arg^9-BK itself. A concentration-dependent response was observed which began at 0.1 μM and corresponded at 1 μM to an increase in renal vascular resistance similar to that observed with des-Arg^9-BK at a roughly ten times higher concentration (Figure 3).

Time-dependent sensitization to des-Arg^9-BK or BK

The vasoconstriction elicited by des-Arg^9-BK in the rat isolated kidney was also time-dependent ($P<0.001$) (Figure 4a). A weak vasoconstrictor response was already present 1 h after the onset of kidney perfusion and increased over time. Time-dependent sensitization was more pronounced at 3 μM

Table 1 Stability of the renal vasodilator responce elicited by BK over three experimental periods, at 30 min intervals

Period	*BK (30 nM) (% ACh- induced relaxation)*	*ACh (30 nM) (% relaxation vs $PGF_{2\alpha}$- induced preconstriction)*	*SNP (10 μM) (% relaxation vs $PGF_{2\alpha}$- induced preconstriction)*
First	40.8±4.4	62.6±7.8	/
Second	43.5±6.4	55.4±6.5	/
Third	42.2±5.3	59.1±7.8	96.8±20.3

Vasodilator responses are expressed as percentage reversal of the preconstriction induced by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or as a percentage of ACh-induced relaxation. The effects of SNP were only evaluated at the end of the experiment. Results are given by means±s.e.mean for five separate kidney preparations. Statistical analysis was performed by two-way ANOVA with repeated measurements. No significant difference was found in the responses over the three experimental periods. / not tested.

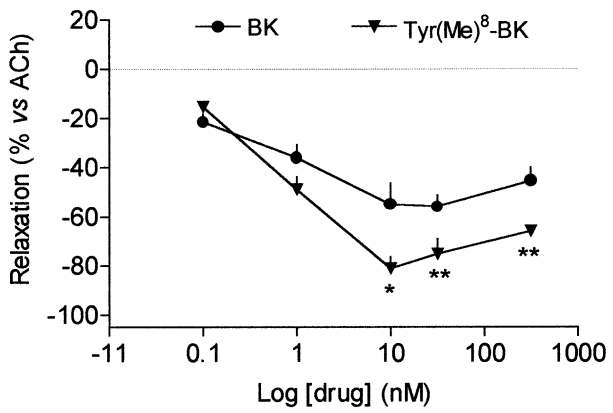

Figure 2 Concentration-dependent relaxation elicited by BK and Tyr(Me)^8-BK in the rat isolated perfused kidney. BK ($n=3-19$) and Tyr(Me)^8-BK ($n=4-6$) were tested as single concentrations separated by 30 min intervals. Responses are expressed as a percentage of the relaxation induced by 30 nM ACh in the same kidneys. Results are given as means±s.e.mean with $n=$the number of individual experiments for a given concentration. Statistical analysis was performed by unpaired Students *t*-test; $*P<0.05$; $**P<0.02$ *vs* BK.

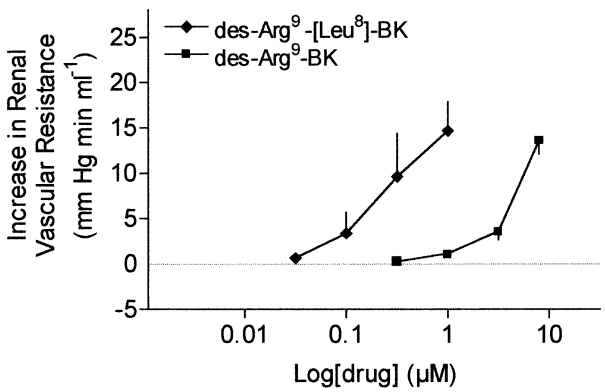

Figure 3 Concentration-dependent vasoconstriction elicited by des-Arg^9-BK ($n=3-11$) and des-Arg^9-[Leu^8]-BK ($n=4$) in the rat isolated perfused kidney. Responses were obtained at roughly 2 h after the onset of kidney perfusion. They are expressed as increases in renal vascular resistance. Results are given as means±s.e.mean with $n=$ the number of individual experiments for a given concentration.

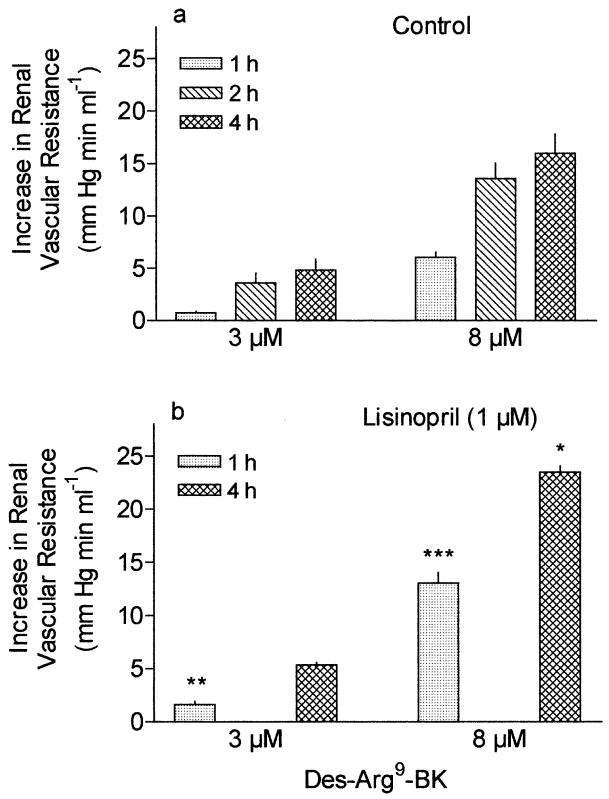

Figure 4 Time-dependent sensitization to des-Arg^9-BK in the rat isolated perfused kidney. Des-Arg^9-BK (3 and 8 μM) was tested 1, 2 and 4 h after the onset of kidney perfusion in the control study (a) ($n=7-11$). A comparable study was performed in the presence of lisinopril (b) ($n=3$). Responses are expressed as increases in renal vascular resistance. Results are given as means$\pm$s.e.mean with $n=$ the number of individual experiments for a given concentration. A two-way ANOVA analysis of data from (a) showed a significant time factor ($P<0.001$) and concentration factor ($P<0.001$) with significant interaction ($P<0.05$). *$P<0.05$, **$P<0.01$ and ***$P<0.001$ *vs* the corresponding response in the control group.

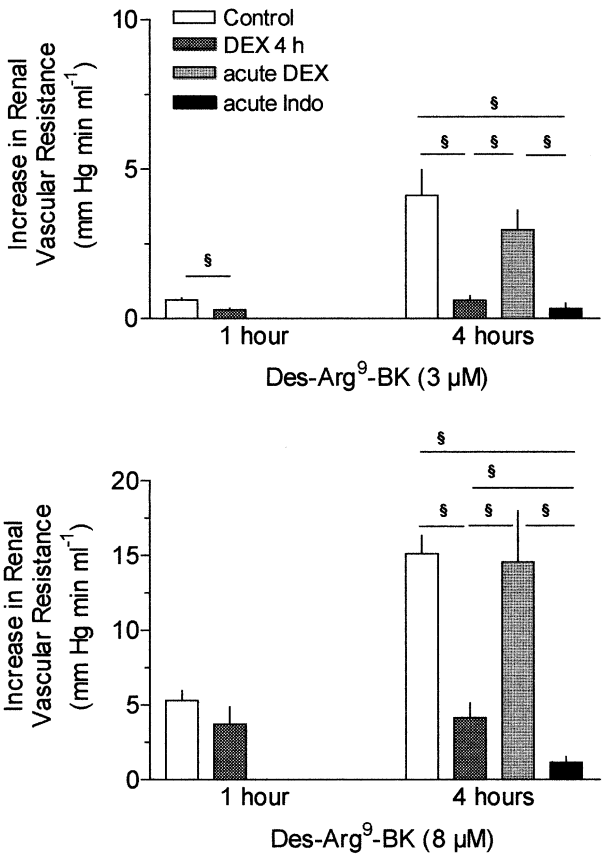

Figure 5 Effects of DEX and indomethacin on the time-dependent sensitization to des-Arg^9-BK in the rat isolated perfused kidney. Control vasoconstrictor responses to des-Arg^9-BK ($n=7-11$) were compared with responses elicited in kidneys taken from rats pretreated by and perfused with DEX (30 mg kg^{-1} i.p. 3 h before the experiment and 10 μM in the perfusate throughout the experiment, $n=5$), or in kidneys acutely exposed to DEX (10 μM, 10 min, $n=3$) or to indomethacin (Indo 30 μM, 10 min, $n=3$). Responses are expressed as increases in renal vascular resistance. Results are given as means$\pm$s.e.mean with $n=$ the number of individual experiments. Data were analysed by Student's *t*-test or one-way ANOVA followed by a modified *t*-test according to Bonferroni; $^{\S}P<0.001$ vs control at any concentration and time of perfusion.

des-Arg^9-BK than at 8 μM, with respectively 6 and 3 fold increases in vasoconstrictor responses over a 3 h perfusion. In contrast, the NA-elicited vasoconstriction did not vary with the duration of perfusion.

Effects of lisinopril on renal vasoconstriction elicited by des-Arg^9-BK

Lisinopril (1 μM) enhanced the renal vasoconstriction elicited by des-Arg^9-BK (Figure 4b). Potentiation was particularly pronounced at 8 μM peptide since a doubling of the early response (1 h perfusion) and a 0.5 fold increase in the late response (4 h perfusion) were observed. Lisinopril, however, had no effect *per se* on renal vascular resistance and also did not affect NA-elicited vasoconstriction.

Effects of R 715 on renal vasoconstriction elicited by des-Arg^9-BK

R 715 (8 μM in the presence of lisinopril) inhibited by more than 50% the vasoconstrictor response elicited by des-Arg^9-BK after a 4 h kidney perfusion. For 8 μM des-Arg^9-BK, the residual response corresponded to an increase in renal vascular resistance of 8.8 ± 0.8 mmHg min ml^{-1} (*vs* 23.5 ± 0.6 mmHg min ml^{-1} in the presence of lisinopril alone, $n=5$ and 3 respectively, $P<0.001$). However R 715 did not affect NA-elicited vasoconstriction. At 8 μM, R 715 retained some partial agonist activity, as shown by the increase in renal vascular resistance of 6.0 ± 1.1 mmHg min ml^{-1} ($n=5$).

Effects of DEX and indomethacin on time-dependent sensitization to des-Arg^9-BK

To investigate the contribution of injury processes in the time-dependent sensitization to des-Arg^9-BK, experiments were performed in the presence of DEX. In kidneys obtained from DEX-pretreated rats and exposed to DEX throughout the experiment, sensitization to des-Arg^9-BK no longer occurred (Figure 5). Indeed, similar vasoconstrictor responses were obtained with 8 μM des-Arg^9-BK after 1 h and 4 h perfusion (3.7 ± 1.1 and 4.2 ± 1.0 mmHg min ml^{-1} respectively, $n=5$). Prolonged exposure to DEX (10 μM) alone similarly prevented time-dependent sensitization (not shown). DEX however left the early vasoconstrictor response to des-Arg^9-BK (8 μM) unchanged (Figure 5). Acute exposure to DEX (10 μM, 10 min) did not affect the response to des-Arg^9-BK in contrast to acute exposure to indomethacin (30 μM, 10 min) that abolished it (Figure 5). All these treatments were without effect on NA-elicited vasoconstriction.

Effects of actinomycin D on time-dependent sensitization to des-Arg^9-BK

The effects of actinomycin D (2 μM) were evaluated on the vasoconstriction elicited by des-Arg^9-BK after a 2 h kidney perfusion since time-dependent sensitization was already obvious at that time in the perfused kidney. Actinomycin D prevented the sensitization to the B_1 receptor agonist. Indeed, responses to 3 and 8 μM des-Arg^9-BK (1.6 ± 0.2 and 3.0 ± 0.6 mmHg min ml^{-1}, $n=5$) remained markedly lower than control responses (3.6 ± 0.6 and 13.6 ± 1.5 mmHg min ml^{-1}, $n=11$, $P<0.05$ and $P<0.001$ respectively). Actinomycin D left NA-elicited vasoconstriction unchanged.

Effects of DEX on the vasodilator response to BK

The effects of DEX (pretreatment and 10 μM in the perfusate) were also evaluated on the renal vasodilation elicited by BK. Here also the evaluation was performed 2 h after the onset of kidney perfusion, since endothelium-dependent vasodilation often deteriorated over a longer perfusion time. DEX did not affect the relaxation elicited by BK (53.5 ± 3.9 *vs* 55.8 ± 4.3% ACh-induced relaxation in corresponding control group, $n=6$ and 19 respectively). The vasodilator responses to ACh and SNP also were left unchanged by DEX.

Discussion

Our results show that BK and related peptides are able to induce opposite tonic effects in the *in vitro* perfused rat renal vasculature. These peptides produced either a renal B_2 receptor-mediated vasodilation or a B_1 receptor-mediated vasoconstriction. Moreover, the B_1 and B_2 receptor-mediated tonic responses were regulated in an opposite way. Desensitization to the B_2 receptor-mediated vasodilation became obvious soon after exposure to the agonist, while sensitization to the B_1 receptor-mediated vasoconstriction appeared over the time of *in vitro* perfusion of the kidney.

Like in many other vascular beds (Regoli & Barabé, 1980), the main response to BK in the isolated perfused kidney was a vasodilation. A maximum response of about 60% relaxation as compared to ACh is somewhat lower than the 85% relaxation previously reported by Fulton *et al.* (1992) in a similar rat kidney preparation. The calculated pD_2 value of 8.9 is compatible with apparent affinity for the kinin B_2 receptor described in other vascular bioassays (Regoli & Barabé, 1980; Félétou *et al.*, 1994; Regoli *et al.*, 1998). Tyr(Me)^8-BK, a selective B_2 receptor agonist, also elicited renal relaxation at nanomolar concentration. The involvement of B_2 receptors was confirmed by the inhibition of BK-elicited renal vasodilation using the selective B_2 receptor antagonist, icatibant (Hock *et al.*, 1991). However, a rather low degree of relaxation was observed when BK was perfused at increasing concentrations in a full concentration-response curve. We therefore hypothesized that BK-induced relaxation was attenuated in our kidney preparation possibly for several reasons: (i) desensitization of kinin B_2 receptor; (ii) occurrence of a vasoconstrictor response; (iii) local catabolism of the peptide or (iv) any combination of these. The first two possibilities are analysed in the present study. Preliminary results indicate a role of local catabolism of BK by ACE (Bagaté *et al.*, 1997).

The occurrence of desensitization to the vasodilator response of BK was obvious from the blunted relaxation we observed when BK was sequentially perfused as compared to perfusions at 30 min intervals. Rapid desensitization of B_2 receptors has often been described. Indeed, in rat mesangial cells, functional desensitization resulted in down-regulation of the calcium response to BK (Bascands *et al.*, 1993). In Chinese hamster ovary cells stably expressing the human B_2 receptor, exposure to [^{3}H]-BK at 37°C resulted within 5 min in 80% receptor-mediated ligand internalization (Austin *et al.*, 1997). Such an internalization and sequestration process may also have occurred for the renal vascular B_2 receptors in the study of Guimaraes *et al.* (1986) within the 20 min exposure to the kinins. In our preparation, the process seemed to be fully reversible within 30 min. The stability of the B_2 receptor-mediated vasodilation over three experimental periods extending over about 2 h and the inability of DEX to alter this response comply with the lack of time-related induction of the B_2 receptor in rat renal vasculature.

In the present study, a vasoconstrictor component in the response to BK was revealed after inhibition of B_2 receptors by icatibant suggesting the involvement of B_1 receptor-mediated vasoconstriction. This effect most likely contributed to the reduction of the relaxation induced by BK when compared with Tyr(Me)^8-BK which lacks affinity for the B_1 receptor. In support to this interpretation, des-Arg^9-BK, a selective B_1 receptor agonist, caused constriction in our renal vascular preparation and this response was antagonized by R 715, a selective B_1 receptor antagonist (Gobeil *et al.*, 1996). Due to a limited supply of the drug, we were not able to look for a complete inhibition by R 715 at a higher concentration. We used the antagonist at an equimolar concentration to the agonist, although complete inhibition would require a 100 times higher ratio (Nsa Allogho *et al.*, 1997). Des-Arg^9-[Leu^8]-BK exhibited agonist activity with an even higher apparent affinity than des-Arg^9-BK itself. Such an agonist activity has also been observed in rat colonic epithelium (Teather & Cuthbert, 1997) and is now recognized as a particular behaviour of des-Arg^9-[Leu^8]-BK in rat and mouse species (Marceau *et al.*, 1998). Noteworthy, des-Arg^9-BK was only active at micromolar concentration in our study as was also observed previously in the rat kidney by Guimaraes *et al.* (1986). This activity was far below the nanomolar affinity derived from binding studies on cloned mouse B_1 receptor (Marceau *et al.*, 1998). The inhibition by lisinopril of des-Arg^9-BK catabolism by ACE increased the maximum response to the kinin but did not affect its apparent affinity. The presence of B_1 receptors has often been reported in other blood vessels in which vasoconstriction appeared as the predominant response, although relaxation sometimes also occurred (Marceau, 1995). In contrast to the results reported herein, a B_1 receptor-mediated relaxation has been documented in canine renal arteries, suggesting further species differences (Rhaleb *et al.*, 1989).

The upregulation of kinin B_1 receptor has recently emerged as an important finding in the kallikrein-kinin system (Marceau, 1995; Marceau *et al.*, 1998). Upregulation of B_1 receptor was described in various smooth muscle preparations in response to tissue injury, whether injury was provoked by tissue isolation, long term (hours) incubation/perfusion on experimental inflammation evoked by various agents (lipopolysaccharide, cytokines, angioplasty, ischaemia). In these studies, B_1 receptor upregulation was usually prevented by DEX and inhibitors of RNA or protein synthesis. We now for the first time report a spontaneous sensitization to the vascular response elicited by B_1 receptor activation in the rat isolated

kidney. As in other preparations, DEX prevented the sensitization to the B_1 receptor agonist, des-Arg^9-BK. Since the isolated kidney was not perfused in aseptic conditions, cytokines might have been generated locally from endothelial or vascular smooth muscle cells (Libby *et al*., 1986a,b). Glucocorticoids block the synthesis of cytokines and also directly repress transcription factors activated in inflammatory states (Barnes & Adcock, 1993). Alternatively, glucocorticoids inhibit the synthesis of prostaglandins *via* the synthesis of lipocortin-1, an inhibitor of phospholipase A_2 activity, or the repression of phospholipase A_2 and cyclo-oxygenase gene transcription. DEX might have acted at the genomic level since acute exposure to DEX was ineffective on the B_1 receptor-mediated response that had previously been allowed to sensitize. In contrast, the acute inhibition by indomethacin of des-Arg^9-BK-mediated vasoconstriction corroborates the contribution of eicosanoids in this response, probably caused by thromboxane A_2 as also shown in the rat portal vein (Campos & Calixto, 1994). Finally, the sensitization to des-Arg^9-BK was prevented by the inhibition of RNA synthesis, with actinomycin D thus further supporting *de novo* synthesis of B_1 receptors. The spontaneous time-dependent sensitization of B_1 receptor-mediated response reported herein is in line with the recently described *in vivo* sensitization induced by bacterial lipopolysaccharide in rat glomerular efferent arterioles which appeared to be directly linked to overexpression of B_1 receptor mRNA (Marin-Castaño *et al*., 1998).

Renal vasoconstriction mediated by kinin B_1 receptor was observed in our study as soon as 1 h after beginning kidney perfusion and this response persisted after DEX pretreatment. Whether this means that a constitutive B_1 receptor was present in the rat renal vasculature is however not clear. Indeed, part of the time-dependent vascular response to B_1 receptor activation has been suggested to be resistant to treatment with glucocorticosteroids (Deblois *et al*., 1988). Moreover, B_1 receptor mRNA and functional B_1 receptors have not been detected in efferent glomerular arterioles from control rats (Marin-Castaño *et al*., 1998). The pathophysiological relevance of B_1 receptor induction in renal blood vessels remains to be established. This induction might occur in renal diseases associated with the release of inflammatory cytokines due to glomerulonephritis, sepsis or ischaemia. The B_1 receptor-mediated vasoconstriction might then mask the B_2 receptor-mediated vasodilation in as much as the former is induced and the later down-regulated. Such a reversal of BK response has indeed been described in bovine mesenteric arteries after the upregulation of B_1 receptor by interferon-γ (De Kimpe *et al*., 1994). Besides, inflammed vascular tissues have been shown to be enriched in endothelial carboxypeptidase M activity which might contribute to enhanced local synthesis of the B_1 agonist, des-Arg^9-BK (Schremmer-Danninger *et al*., 1998). Whether this really occurs in renal diseases remains however to be elucidated. In this respect, the use of a non peptide selective B_1 receptor antagonist as recently synthesized would be helpful (Altamura *et al*., 1999).

In conclusion, the present study provides clear evidence for the presence of both kinin B_1 and B_2 receptors in the rat renal vasculature. Although the B_2 receptor is constitutively expressed and rapidly desensitized after exposure to the agonist, sensitization to B_1 receptor-mediated vasoconstriction occurred in kidneys injured by *in vitro* perfusion and was linked to *de novo* synthesis of B_1 receptors. It may therefore be expected that B_2 receptor-mediated renal vasodilation could be replaced by a vasoconstrictor response in inflammatory states with elevated local kinin levels.

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