

Differential action of bradykinin on intrarenal regional perfusion in the rat: waning effect in the cortex and major impact in the medulla

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The renal kallikrein–kinin system is involved in the control of the intrarenal circulation and arterial pressure but bradykinin (Bk) effects on perfusion of individual kidney zones have not been examined in detail. Effects of Bk infused into renal artery, renal cortex or medulla on perfusion of whole kidney (RBF, renal artery probe) and of the cortex, outer- and inner medulla (CBF, OMBF, IMBF: laser-Doppler fluxes), were examined in anaesthetized rats. Renal artery infusion of Bk, 0.3–0.6 mg kg⁻¹ h⁻¹, induced no sustained increase in RBF or CBF. OMBF and IMBF increased initially 6 or 16%, respectively; only the IMBF increase (+10%) was sustained. Pre-treatment with L-NAME, 2.4 mg kg⁻¹ i.v., prevented the sustained but not initial transient elevation of medullary perfusion. Intracortical Bk infusion, 0.75–1.5 mg kg⁻¹ h⁻¹, did not alter RBF or CBF but caused a sustained 33% increase in IMBF. Intramedullary Bk, 0.3 mg kg⁻¹ h⁻¹, did not alter RBF or CBF but caused sustained increases in OMBF (+10%) and IMBF (+23%). These responses were not altered by pre-treatment with 1-aminobenzotriazole, 10 mg kg⁻¹ i.v., a cytochrome P-450 (CYP450) inhibitor, but were prevented or significantly attenuated by L-NAME or intramedullary clotrimazole, 3.9 mg kg⁻¹ h⁻¹, an inhibitor of CYP450 epoxygenase and of calcium-dependent K⁺ channels (K_{Ca}). Thus, cortical vasodilatation induced by Bk is only transient so that the agent is unlikely to control perfusion of the cortex. Bk selectively increases perfusion of the medulla, especially of its inner layer, via activation of the NO system and of K_{Ca} channels.

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Abbreviations ABT, 1-aminobenzotriazole; Bk, bradykinin; B₂, bradykinin type 2 receptor; CBF, cortical perfusion (laser-Doppler flux); CYP450, cytochrome P-450; EDHF, endothelium-derived hyperpolarizing factor; EETs, epoxyeicosatrienoic acids; 20-HETE, 20-hydroxyeicosatetraenoic acid; HOE 140, icatibant acetate – selective bradykinin B₂ receptor antagonist; IMBF, inner medullary perfusion (laser-Doppler flux); K_{Ca}, calcium-dependent K⁺ channel; MAP, mean arterial pressure; MS-PPOH, methylsulfonyl-propargyloxyphenylhexanamide – an inhibitor of CYP450 epoxygenases; NO, nitric oxide; OMBF, outer medullary perfusion (laser-Doppler flux); OMDVR, outer medullary descending vasa recta; RBF, renal blood flow; SNP, sodium nitroferricyanide(III).

Bradykinin (Bk), a major and functionally most important member of the kinin family, has two sources: it is both a paracrine agent generated by the tissue kallikrein–kinin system and a product of plasma kallikrein. Under most conditions it acts by stimulation of widely distributed constitutive B₂ receptors, which leads to activation of mediating systems, mostly of the NO cascade and the still elusive endothelium-derived hyperpolarizing factor (EDHF) (Clements, 2000; Imig, 2005; Moreau *et al.* 2005; Fleming & Busse, 2006). In the kidney, Bk modifies tubular transport and causes intrarenal vasodilatation (Vio *et al.*

1992; Navar *et al.* 1996; Imig, 2005). The latter action appears to be functionally important for modulation by Bk of autoregulation of renal blood flow and is postulated to be involved in the control of arterial pressure (Katori & Majima, 1996; Navar *et al.* 1996; Nafz *et al.* 1998; Tornel *et al.* 2000).

Considering the probable role of the medullary circulation in the long-term control of arterial pressure (Mattson, 2003; Bergström & Evans, 2004; Cowley, 2008), in the past two decades the main interest in renal vascular effects of Bk was focused on its influence on perfusion

of the medulla. While the vasodilator influence of Bk in this kidney zone seems clearly established, its possible contribution to the control of perfusion of the cortex or of the whole kidney remains uncertain. The relevant data, especially those from pharmacological studies using bolus injections or very short infusions (Braun *et al.* 1997; Dabisch *et al.* 2004) provide no information regarding the stability or transiency of the response. Remarkably, quite different responses to bolus injections as opposed to infusions of Bk were reported (Hoagland *et al.* 1999a).

In order to re-evaluate the intrarenal vascular action of Bk in different zones of the kidney, in the present study we compared the effects obtained with infusions of the agent into the renal artery and direct infusions into the renal cortex or renal medulla. Simultaneous measurements were conducted of the perfusion of the renal cortex as well as, separately, of the outer and inner medulla. Since quite often the responses to Bk tended to wane, we always continued the infusions until renal perfusion parameters stabilized at a new level. This helped distinguish between the transient and sustained phases of the response.

The long-established knowledge of the role of NO as a mediator of Bk-induced vasodilatation has been more recently supplemented by the sound evidence that non-NO mediation, by multiple factors described as EDHF, is also involved and can be of comparable importance (Roman, 2002; Imig, 2005; Moreau *et al.* 2005). In the present study, we re-examined the mediatory role of NO and EDHF, in order to characterize the contribution of either factor to the transient and sustained phases of post-Bk vasodilatation. In studies involving inhibition of NO synthesis, precautions were taken to prevent the associated depression of baseline renal haemodynamics. In EDHF studies we focused on its final common pathway of action i.e. the status of calcium-dependent K^+ channels and, in addition, examined the contribution of vasoactive products of CYP450-dependent enzymes.

Methods

The experimental procedures were approved by the extramural IV Local Ethical Committee (Warsaw); the standards and regulations used are equivalent to those in the UK (Drummond, 2009). Experiments were designed to examine effects of infusion of bradykinin (Bk, Sigma Chemical Co., St Louis, MO, USA) into the left renal artery or renal medullary or cortical interstitium on ipsilateral total and regional perfusion of the kidney. Effects of bradykinin were also examined in rats pre-treated with agents that were likely to modify the response.

Male Wistar rats weighing 280–330 g, maintained on dry pellet diet and given free access to water, were anaesthetised with intraperitoneal thiopentobarbital

(Thiopental, Biochemie GmbH, Vienna, Austria) ($100 \text{ mg (kg body weight)}^{-1}$). The experimental techniques, procedures and measurements, including that of the renal artery blood flow (RBF) and laser-Doppler fluxes in the superficial renal cortex (CBF) and in the outer and inner medulla (OMBF and IMBF), were described in detail previously (Bądryńska *et al.* 2003; Bądryńska & Sadowski, 2008). The laser-Doppler flux is a product of the number of blood cells moving and of their mean velocity; for brevity, and in agreement with the accepted usage, the abbreviations used (CBF, OMBF and IMBF) denote fluxes and not flows. The CBF probe (0.5 mm in diameter) was placed on the kidney surface, and OMBF and IMBF probes (0.4 mm in diameter) were placed at depths of 3 and 5 mm from the surface, respectively. In some series, CBF measurements had to be discarded because of problems with probe calibration; in these cases, RBF was taken as a measure of whole cortical perfusion. Earlier studies have shown effectiveness of vasodilator agents infused into the inner medulla of the rat kidney (Lu *et al.* 1992), in contrast to doubtful value of the procedure in the rabbit (Kalyan *et al.* 2002). The cannulas for renal artery, intracortical and intramedullary infusions were all 32G.

Experimental infusion protocols

Renal artery infusions of bradykinin (Bk)

(1) *In untreated rats* ($n = 19$). Bk dissolved in isotonic saline was infused at a rate of $0.3\text{--}0.6 \text{ mg kg}^{-1} \text{ h}^{-1}$ until renal perfusion parameters stabilized at a new level; this occurred usually within 20–30 min of the infusion. After termination of the infusion, 20–30 min recovery observations were made. The Bk dose used had no significant effect on mean arterial pressure (MAP). In preliminary experiments, a 5-fold greater Bk dose ($1.5 \text{ mg kg}^{-1} \text{ h}^{-1}$) was also tested: it still did not affect MAP but the renal haemodynamic responses to Bk were less pronounced than with the smaller, ultimately accepted dose.

(2) *In rats pre-treated with intravenous L-NAME* ($n = 6$). Before Bk administration, the rats received L-NAME (N^ω -nitro-L-arginine methyl ester, Sigma, Switzerland), 2.4 mg kg^{-1} infused i.v. during 10 min.

(3) *In rats pre-treated with intravenous L-NAME and receiving SNP* ($n = 11$). To attenuate the decrease in baseline renal perfusion which follows L-NAME administration, sodium nitroprusside (sodium nitroferricyanide(III), SNP, Sigma-Aldrich) was co-infused with Bk into the renal artery, at about $0.6 \text{ mg kg}^{-1} \text{ h}^{-1}$. This manoeuvre was successfully used to nullify such disturbing effects of L-NAME in studies of the

responses to Bk in the rat hindlimb vascular bed (Dabisch *et al.* 2004).

Renal interstitial infusions of Bk

(1) In untreated rats ($n = 15$)

a) Bk was infused into the renal cortical tissue ($n = 8$) via two cannulas placed in the renal cortex, at a total rate of $0.75\text{--}1.5\text{ mg kg}^{-1}\text{ h}^{-1}$ (2 ml h^{-1}). The stabilization of perfusion parameters occurred after 30–50 min.

b) Bk was infused into the medulla ($n = 15$) at $0.3\text{ mg kg}^{-1}\text{ h}^{-1}$ (1 ml h^{-1}) using a single cannula with the tip placed at or just beneath the outer–inner medullary border. In a parallel series, an inhibitor of bradykinin B_2 receptors, HOE 140 (icatibant acetate, Sigma), was infused into the medulla at $0.12\text{ mg kg}^{-1}\text{ h}^{-1}$.

(2) In rats treated with intravenous or intramedullary L-NAME, ABT or clotrimazole. In separate groups, intramedullary infusions of Bk as described above were conducted in rats pre-treated with intravenous L-NAME (2.4 mg kg^{-1} ; $n = 11$), rats receiving L-NAME infusion into the medulla, simultaneously with Bk, at a rate of $0.12\text{ mg kg}^{-1}\text{ h}^{-1}$ ($n = 12$), and rats pre-treated with intravenous 1-aminobenzotriazole (ABT, Fluka, Buchs, The Netherlands) (10 mg kg^{-1} infused during 10 min; $n = 6$). In another group ($n = 13$), we examined the responses to Bk of rats receiving clotrimazole (Sigma-Aldrich). To increase solubility, the substance was transformed into clotrimazole hydrochloride, and $100\text{ }\mu\text{l}$ of 40% 2-hydroxypropyl- β -cyclodextrin (Sigma) was added per 6 ml of saline solvent. Clotrimazole HCl was infused into the medulla at $3.9\text{ mg kg}^{-1}\text{ h}^{-1}$ (1.5 ml h^{-1}) for about 30 min, which *per se* did not affect renal perfusion parameters. Subsequently, Bk infusion was started at the usual rate (1 ml h^{-1}) while clotrimazole infusion rate and its solvent infusion rate was reduced to one-third of the initial value, to keep the total volume of the infusion constant at 1.5 ml min^{-1} . During the post-Bk recovery period, the initial clotrimazole infusion rate was restored.

After termination of experiments, the rats were killed with a lethal intravenous dose of sodium thiopental. The position of the intrarenal probes and of the infusion needles was checked at the kidney's cross-section.

Statistics

The differences between means were evaluated by repeated measures analysis of variance and by Student's paired *t* test. For inter-group comparison, the classical one-way ANOVA was used, followed by modified Student's *t* statistics; Bonferroni's correction was used in case of multiple comparisons. The standard error of the mean (S.E.M.) was used as a measure of data dispersion, and $P < 0.05$ was accepted as the significance level.

Results

Renal artery infusions of bradykinin

(1) In untreated rats. Bk infusions did not alter MAP. An example of renal haemodynamic responses to Bk is given in the left panel of Fig. 1. It is seen that the responses of RBF and OMBF showed two phases: an initial increase followed by a decrease to baseline; for IMBF the second phase was slightly below the initial phase level. The results are summarized in Fig. 2 (top panel) which shows the averaged values, separately for the initial 10–12 min and the final level. There was an initial 6% increase in RBF, followed by a decrease below baseline whereas CBF did not change at all. OMBF and IMBF increased initially 6% and 16%, respectively. This was followed by a decrease to baseline in the former and by a decrease to a level 10% above baseline in the latter, which was still significantly different from control. In summary, effects of Bk on cortical perfusion (RBF, CBF) and on OMBF, if any, were modest and transient. A sustained increase in perfusion was seen for IMBF only.

(2) In rats pre-treated with intravenous L-NAME. Under L-NAME treatment the initial post-bradykinin increases in RBF, OMBF and IMBF, as seen without pre-treatment, appeared enhanced (Fig. 2, middle panel). However, in the second phase all the perfusion parameters decreased to baseline despite continued infusion. Thus, there was no sustained increase in perfusion of the inner medulla. These results should be interpreted with caution because intravenous L-NAME substantially decreased baseline perfusion parameters and increased arterial blood pressure. Therefore another group of L-NAME-treated rats received, in addition, a renal artery infusion of sodium nitroprusside (SNP), which largely improved baseline perfusion and also reduced the increase in blood pressure (Table 1).

(3) In rats receiving intravenous L-NAME and SNP. In this group there was no initial post-bradykinin increase in RBF (Fig. 2, bottom panel). This suggests that the 50% reduction of the baseline value after L-NAME given alone (Table 1) was a necessary condition for this increase. On the other hand, the increases in OMBF and IMBF were significant but limited to the initial phase, similar to those seen with the treatment with L-NAME alone (Fig. 2, middle panel). Overall, L-NAME given with or without SNP did not interfere with the initial transient post-bradykinin increase in medullary perfusion (OMBF and IMBF) but prevented the sustained increase.

Since we thought that intravascular route of administration may not be appropriate for Bk, a paracrine agent synthesised largely in the tissue, experiments were performed using infusions into the renal cortex or medulla.

Renal interstitial infusions of bradykinin

(1) *In untreated rats.* Cortical interstitial infusions of Bk did not alter RBF or CBF. By contrast, it caused an increase in perfusion of the medulla, which was substantial (+24%) but transient for OMBF, and even more substantial (+28%) but sustained for IMBF (Fig. 3, right upper panel).

A sample response to intramedullary Bk is shown in the right hand panel of Fig. 1. Compared to effects of renal artery infusions, the initial increase in IMBF was followed by a more substantial decrease in the second phase. Mean responses to Bk for all groups are given in Fig. 3. In untreated rats, intramedullary Bk did not alter RBF or CBF but there was a significant increase in medullary perfusion, both OMBF and IMBF. Intramedullary infusion of bradykinin B₂ receptor inhibitor (HOE140) induced significant but minor (less than 1%) decreases in OMBF and IMBF without changing RBF (data not shown). In summary, renal interstitial infusions of Bk did not increase the whole kidney or cortical blood flow, in contrast to a modest transient increase observed with renal artery infusion (Fig. 2). In the medulla, the increases were sustained not only for IMBF but also for OMBF, even though both parameters were lower in the second than in the first phase of Bk infusion.

(2) *In rats treated with intravenous or intramedullary L-NAME, intravenous ABT or intramedullary clotrimazole.* The treatment with intravenous L-NAME tended to attenuate the initial transient increases in OMBF and IMBF and virtually abolished the sustained (second phase) increase in IMBF. However, with intravenous L-NAME the baseline perfusion of the kidney was greatly reduced (Table 1), which might *per se* influence the response to Bk. Intramedullary infusion of L-NAME was associated with only minor, if any, reduction of baseline renal perfusion (Table 1). The response to Bk was similar to that seen with intravenous L-NAME, with one exception: the elevation of OMBF remained significant in the second phase of Bk infusion. Taken together, L-NAME treatment abolished or attenuated the Bk-dependent sustained increases in OMBF and IMBF; however, a significant transient elevation of the perfusion was maintained.

In rats treated with intravenous ABT, the Bk-induced increases in renal medullary perfusion were quite comparable with those observed in untreated rats. The increase in RBF was significant but minor (+3%).

Intramedullary clotrimazole greatly attenuated or abolished the Bk-induced increase in medullary perfusion; however, a modest 6% elevation of IMBF (compared to +23% in untreated rats) persisted and was significant in the second phase of infusion ($P = 0.043$).

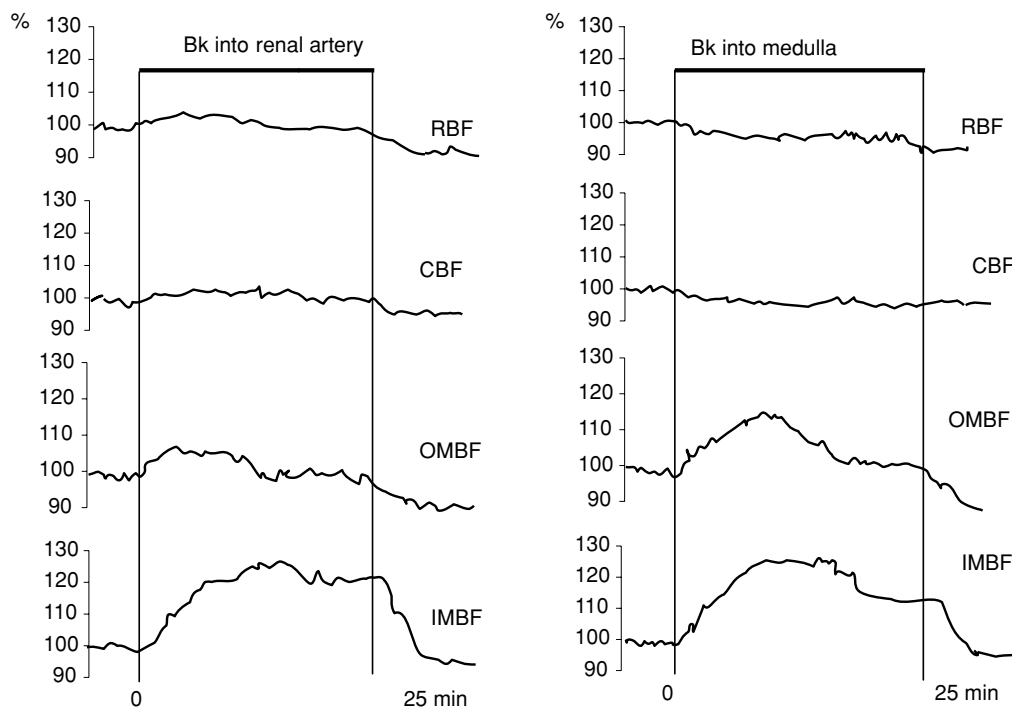


Figure 1. Two sample experiments showing intrarenal perfusion responses to bradykinin

Bradykinin was infused into the renal artery ($0.6 \text{ mg kg}^{-1} \text{ h}^{-1}$) or into the renal medullary tissue ($0.3 \text{ mg kg}^{-1} \text{ min}^{-1}$). RBF – total renal blood flow measured by renal artery (Transonic) probe; CBF, OMBF, IMBF – laser-Doppler fluxes measured on kidney surface, and in the outer and inner medulla, respectively.

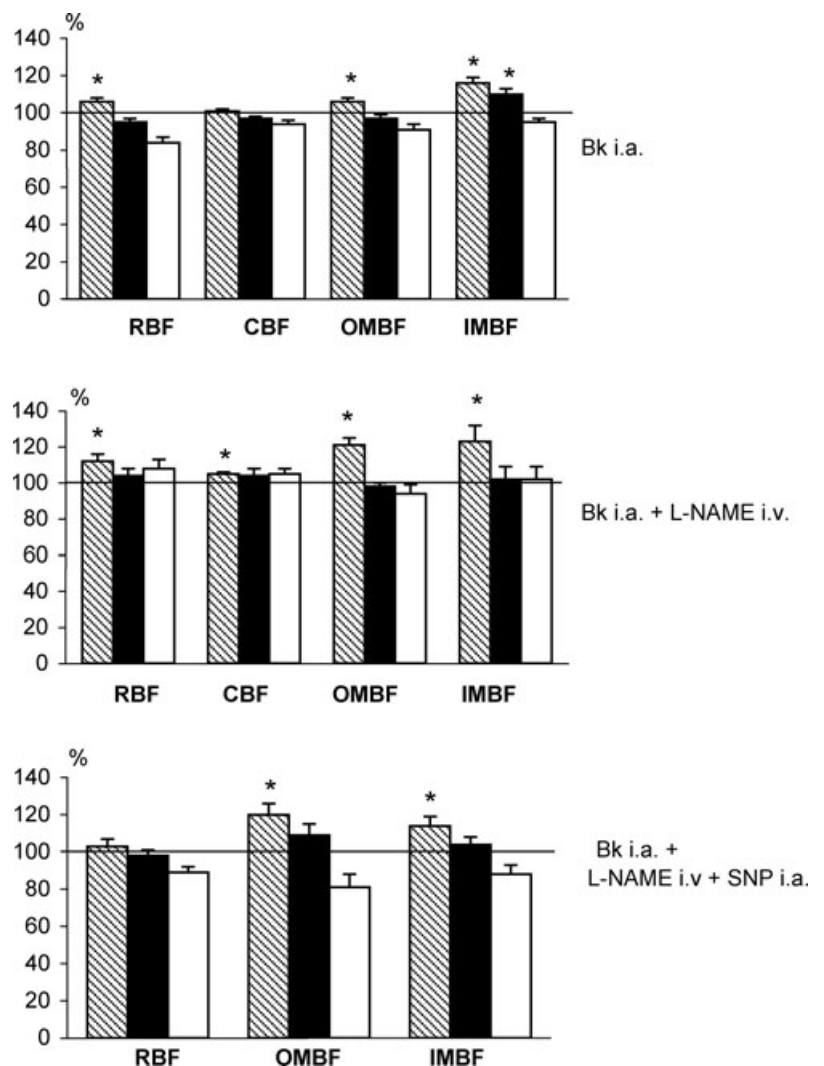
Table 1. Basal values of MAP and renal perfusion parameters (measured before infusion of bradykinin, pooled data from various groups)

	No treatment (31–39)	L-NAME i.v. (14–19)	L-NAME i.m. (12)	L-NAME i.v. + SNP i.a. (10)
MAP (mmHg)	114 ± 2	123 ± 2	123 ± 3	117 ± 2
RBF (ml min ⁻¹)	8.4 ± 0.4	4.1 ± 0.2	8.1 ± 0.5	7.6 ± 0.3
CBF (PU)	566 ± 15	399 ± 13	–	–
OMBF (PU)	174 ± 7	90 ± 3	170 ± 18	140 ± 7
IMBF (PU)	141 ± 5	105 ± 6	132 ± 12	120 ± 7

Means ± s.e.m. The ranges of *n* values for individual groups are given in brackets. Abbreviations: i.m., intramedullary; i.a., into renal artery; SNP, sodium nitroprusside; PU, perfusion units. Other denotations as in Figs 1 and 2.

Overall, L-NAME and especially clotrimazole treatment greatly attenuated or abolished the response of medullary perfusion to Bk. The ultimate statistical evaluation of inter-group differences was performed on three groups of rats: untreated, treated with intramedullary L-NAME

and treated with clotrimazole. The ABT group was excluded because this treatment was clearly without effect. The group of rats treated with intravenous L-NAME was excluded because this treatment induced profound changes in baseline perfusion, which *per se* may have



been a source of independent inter-group variability. The classical one-way ANOVA was used for evaluation of both absolute and per cent post-bradykinin changes, separately for OMBF and IMBF and separately for the initial and second-phase (sustained) responses. ANOVA showed that the treatments (L-NAME, clotrimazole) significantly altered the responses to Bk, with one exception. The OMBF response to Bk in the second phase of Bk infusion was significantly altered by the treatments when absolute values were considered ($F = 3.33$, $P = 0.047$) but not for the data expressed as a percentage of control values ($F = 1.99$, $P < 0.15$). Figure 4 compares the Bk-induced increases ($\Delta\%$) in OMBF and IMBF of three groups of rats: untreated, treated with intramedullary L-NAME and those

treated with intramedullary clotrimazole. Also shown are the results of Student's t test for independent variables, used for two inter-group comparisons (untreated *vs* L-NAME and untreated *vs* clotrimazole, the required significance level after Bonferroni's correction: $P < 0.025$). It is seen that intramedullary L-NAME did not significantly affect the response of OMBF but did reduce the response of IMBF. On the other hand, clotrimazole almost abolished the responses to Bk of both OMBF and IMBF.

Discussion

In order to characterise more clearly the influence of Bk on renal regional perfusion, we compared effects seen with

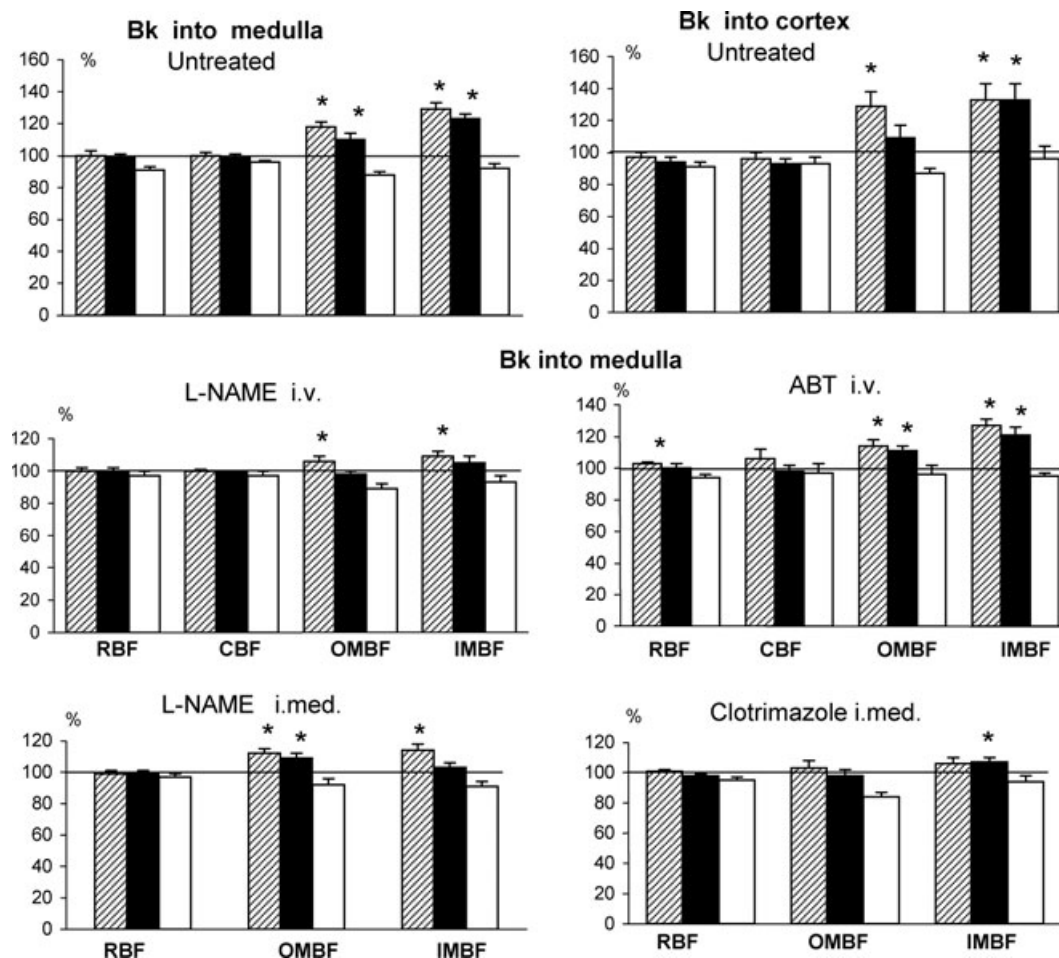


Figure 3. Intrarenal perfusion responses to bradykinin infused into the renal medullary or cortical interstitium

Bradykinin (Bk) was infused into the renal cortex, $0.75\text{--}1.50\text{ mg kg}^{-1}\text{ h}^{-1}$ (right hand upper panel) or into the medulla, $0.3\text{--}0.6\text{ mg kg}^{-1}\text{ h}^{-1}$ (all the other panels). Means ($\%$) \pm s.e.m. The striped, black and open columns refer to the initial phase of Bk infusion, the final level established during the infusion, and the recovery level after Bk has been withdrawn, respectively. The rats were either untreated ($n = 15$ for intramedullary and $n = 8$ for intracortical Bk) or pre-treated with L-NAME ($2.4\text{ mg kg}^{-1}\text{ h}^{-1}$ i.v., $n = 11$, or $0.12\text{ mg kg}^{-1}\text{ h}^{-1}$ into renal medulla, $n = 12$), ABT (10 mg kg^{-1} i.v., $n = 12$) or clotrimazole ($3.6\text{ mg kg}^{-1}\text{ h}^{-1}$ into renal medulla, $n = 13$). In two groups, CBF measurements had to be discarded because of technical problems. *Significantly different from pre-bradykinin baseline as tested by paired Student's t test.

different routes of administration: into the renal artery, and into the renal cortical or renal medullary interstitium. With all these routes, systemic vasodilatation and the effect on arterial blood pressure are nullified or minimized. Renal artery infusions result in uniform distribution of the agent throughout the kidney. However, intravascular delivery may be optimal in the case of circulating vasoactive substances, possibly also of Bk generated by plasma kallikrein, whereas paracrine agents synthesised in the tissue reach the vascular smooth muscle from the side of the interstitium. Thus, infusions directly into the tissue (interstitium) may be thought to better mimic Bk diffusion from the major sites of synthesis, e.g. in the cells of the renal connecting or distal tubule (Vio *et al.* 1992; Clements, 2000). An appropriate alternative to renal artery infusions is administration of agents into the renal cortical interstitium. We showed recently that PGE₂ infused into the renal cortex was distributed and acted throughout the kidney, in the same way as seen with renal artery infusions (Badzyńska & Sadowski, 2008).

Does Bk participate in the control of cortical or whole kidney perfusion?

Due to the increasing interest in the potential contribution of kinins to the control of arterial pressure (Katori & Majima, 1996; Navar *et al.* 1996; Nafz *et al.* 1998; Tornel *et al.* 2000) and considering the postulated role of the renal medulla in this control (Mattson, 2003; Bergström & Evans, 2004; Cowley, 2008), during the past two decades attention was focused on Bk effects on perfusion of the renal medulla (Navar *et al.* 1996; Clements, 2000; Tornel *et al.* 2000). However, there is ample evidence indicating that kinins may affect vessel tone throughout the kidney.

Numerous reports from whole kidney studies indicated that exogenous Bk increased total renal blood flow (Webster & Gilmore, 1964; Flamenbaum *et al.* 1979; Yun *et al.* 1982; Granger & Hall, 1985; Matsumura *et al.* 1999; Patel & Smith, 2000) and similar results were reported from experiments with the isolated perfused rat kidney (Gardes *et al.* 1990; Fulton *et al.* 1992; Bagate *et al.* 1997; Pompermayer *et al.* 2002; Wang *et al.* 2003). Vasodilator effects of Bk were also demonstrated in isolated preparations of vessels (especially of the glomerular arterioles) that were as a rule harvested from the renal cortex and not from its juxtamedullary layer (Edwards, 1985; Yu *et al.* 1998; Ren *et al.* 2002; Wang *et al.* 2005). The conclusions derived from studies of whole kidney perfusion or studies of isolated outer- or midcortical vessels were usually extrapolated to the vessels determining perfusion of the medulla. The studies showing specifically juxtamedullary arteriolar vasodilatation (Imig *et al.* 2001) or dilatation of isolated outer medullary descending vasa recta (OMDVR) (Pallone *et al.* 1998) were relatively few in number.

The vast functional evidence on the action of kinins on perfusion of the whole kidney agrees well with the multiple data indicating the presence of bradykinin B₂ receptors throughout the cortex (Vio *et al.* 1992); taken together these data indicate that the renal cortical vasculature has the potential to respond to Bk. On the other hand, it can be doubted whether Bk, an agent synthesised mostly in the connecting tubule and collecting duct cells, can access the vasculature in the outer or middle cortex. It could also be speculated that the cortical vasculature is affected by Bk generated by plasma kallikrein. Given the uncertainty regarding the genuine role of Bk in the control of intrarenal regional circulation, we thought that examination of the effects obtained with the delivery of the agent to different tissue compartments and to different regions of the kidney might help clarify some fundamental questions.

We found that increases in whole kidney or cortical perfusion induced by Bk were modest and subsided despite continued infusion. Notably, the increases occurred exclusively with renal artery infusion of Bk while intracortical (or intramedullary) infusion was without effect. This finding agrees with the observation that the isolated

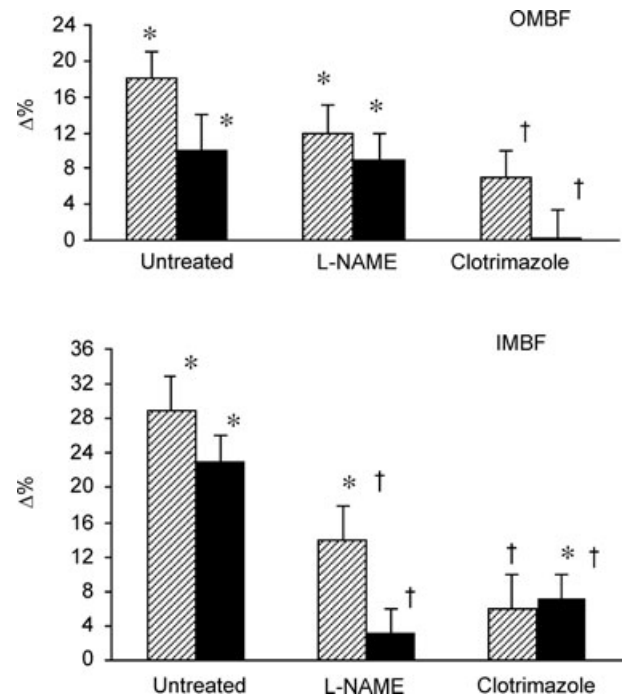


Figure 4. A summary of the effects of intramedullary bradykinin on outer- and inner medullary perfusion

Means ($\Delta\%$) \pm S.E.M. for rats that were either untreated or treated with intramedullary L-NAME or intramedullary clotrimazole (doses and *n* values as described for Fig. 3). The striped and black columns refer to the initial phase of Bk infusion and the final level established during the infusion, respectively. *Significantly different from pre-bradykinin baseline as tested by paired *t* test. †Significantly different from the corresponding change in untreated rats, as tested by unpaired Student's *t* test.

glomerular afferent arterioles dilated in response to Bk applied from the luminal but not from the perivascular side (Yu *et al.* 1998). The transiency of Bk-induced vasodilatation is probably not uncommon but was rarely reported (Wang *et al.* 2003); many experimental protocols, as those using bolus injections, precluded its detection. On the other hand, we saw that renal artery infusion of Bk induced a distinct sustained increase in inner medullary perfusion.

The failure of Bk infused into the renal artery or into the interstitium of the cortex to induce a sustained increase in cortical perfusion suggests that the endogenous kinin, even if it accessed cortical microvessels, be it from the luminal or interstitial side, would not affect local circulation in a sustained manner. An elevation of plasma Bk which follows renal artery infusion might mimic increased release of the agent by plasma kallikrein, e.g. in inflammatory conditions; however, since the vasodilatation was minor and only transient, such an effect is also unlikely to be of functional significance. It could be suspected that at concentrations higher than those induced in this study, Bk could still lead to sustained cortical vasodilatation. However, we saw that increasing the dose 5-fold tended to reduce rather than increase vasodilator responses to renal artery infusions of Bk (data not shown). Others have reported that after increasing Bk dose, renal vasoconstriction instead of vasodilatation was seen (Yu *et al.* 1998; Hoagland *et al.* 1999b).

The background of the waning of the vasodilator response to Bk (especially in the cortex), as seen after renal artery infusions, is unclear. When activated by an agonist, rapid desensitization of bradykinin B₂ receptor has been described (Moreau *et al.* 2005). Alternatively, poor response of paracrine systems known to mediate the vasodilatation, such as NO or EDHF, should be considered. The rate of synthesis and the amount of bioavailable NO, a major mediator of Bk action, is much smaller in the cortex than in the medulla and its rapid exhaustion can be imagined and could account for the disappearance of Bk-induced vasodilatation. Our results of renal artery Bk infusions under NO blockade do not support this interpretation. Unlike NO, epoxyeicosatrienoic acids (EETs) are abundant in the rat cortex (Roman, 2002; Moreau *et al.* 2005) and have established vasodilator potency. In the rabbit cortex these epoxygenase products effectively oppose vasoconstrictor influence of vasopressin V₁ receptor stimulation (Rajapakse *et al.* 2004). Thus, paucity of EETs is an unlikely mechanism of the waning of the response. In a recent study, we found a transient cortical vasodilatation in response to renal artery infusion of prostaglandin E₂, followed by sustained decrease in cortical perfusion (Bądzynska & Sadowski, 2008). Apparently, transient cortical vascular responses to vasodilators are not uncommon, hence the rationale of using prolonged infusions rather than bolus injections.

In general, smaller responses to vasodilator agents in the cortical compared to the medullary circulation are not surprising; possible reasons for this phenomenon have been discussed in detail (Evans *et al.* 2004).

A decrease in cortical or whole kidney perfusion after blockade of B₂ receptors would provide an indication of the agent's tonic vasodilator influence in the cortex. However, systemic administration of a kinin antagonist decreased renal papillary blood flow without affecting outer cortical flow or glomerular filtration rate (Roman *et al.* 1988). Decreases in the total or cortical blood flow after inhibition of B₂ receptors were seen only when basal activity of the tissue kinin–kallikrein system had been increased, e.g. by sodium restriction or pre-treatment with inhibitors of kininase II (i.e. angiotensin converting enzyme) (Navar *et al.* 1996; Siragy *et al.* 1997). It will be noticed that after such inhibition an increase in tissue Bk was only modestly greater in the medulla than in the cortex (Matsuda *et al.* 1999). Thus, it is likely that Bk participates in the control of the renal cortical circulation under such unusual conditions.

Bk effects on medullary perfusion. Mediatory role of NO, CYP450 and K_{Ca} channels

Increases in medullary perfusion in response to Bk were demonstrated with all the three administration routes. They were always sustained in the case of IMBF whereas OMBF remained elevated with intramedullary but not with renal artery or intracortical infusion. In agreement with our observations, intramedullary Bk was reported to increase perfusion of the exposed renal papilla in Munich–Wistar rats (Mattson & Cowley, 1993; Tornel *et al.* 2000).

It is not clear why Bk had a greater or/and more persistent effect on perfusion of the inner compared to outer medulla, as observed with all the three infusion routes. Given the uncertainty regarding the relative importance of juxtamedullary glomerular arterioles and OMDVR as determinants of perfusion of either sub-zone (Evans *et al.* 2004), and in the absence of the knowledge of the post-infusion distribution and concentration of Bk in the kidney, speculations based on perfusion measurements alone are unwarranted.

L-NAME pre-treatment did not abolish the initial phase of the post-Bk increase in medullary perfusion, which suggests that this transient and perhaps non-specific response was not or was only in part mediated by NO. On the other hand, the sustained phase of the increase was almost always abolished by L-NAME. There was only one exception: in rats receiving L-NAME infusion into the medulla (but not those given L-NAME i.v.), the OMBF elevation persisted throughout Bk infusion (Figs 3 and 4). Possibly, with infusion into the outer–inner medullary

border area, L-NAME did not access the juxtamedullary glomerular arterioles and these vessels were able to release NO in response to Bk. On the whole, our results show that Bk induces a stable increase in the perfusion of the inner medulla and that this action is largely mediated by NO. This suggests that NO mediates the prolonged tonic influence of the kinin while it does not participate in the initial phase of vasodilatation.

There is vast evidence that Bk action is mediated not only by NO but also by EDHF; the latter term is used to describe multiple factors, such as vasodilator EETs, the products of CYP450-dependent epoxygenase (Fulton *et al.* 1992; Imig *et al.* 2001; Ren *et al.* 2002; Imig, 2005; Fleming & Busse, 2006). EETs induce vasodilatation via hyperpolarization and relaxation of vascular smooth muscle cells secondary to activation of Ca^{2+} -dependent K^+ channels (K_{Ca}) (Fleming & Busse, 2006). To explore the role of EETs in the Bk-induced vasodilatation we re-examined effects of Bk after treatment with ABT, a CYP450 inhibitor that was shown to inhibit both 20-hydroxyecosatetraenoic acid (20-HETE) and EET synthesis in normal rats (Maier *et al.* 2000). We chose to first apply non-specific inhibition of CYP450 rather than selective inhibition of EETs synthesis because many of the established inhibitors of epoxygenase (especially anti-mycotic imidazole derivatives) are in fact direct inhibitors of K_{Ca} channels. We found that ABT treatment did not alter the vasodilatation induced by Bk. The dosage used was previously found to significantly decrease RBF in Wistar rats maintained both on standard and on a high-sodium diet (E Kompanowska-Jeziarska & M Kuczeriszka unpublished results from our laboratory), suggesting inhibition of vasodilator EETs. However, considering that acute ABT administration may not have sufficiently depleted tissue stores of CYP450-dependent epoxygenase and without direct demonstration of the disappearance of EETs from the tissue, the data are not entirely conclusive.

In a separate experimental series, we found that clotrimazole, an inhibitor of CYP450 epoxygenase but also a potent direct inhibitor of K_{Ca} channels (Wu *et al.* 1999), almost totally abolished (OMBF) or greatly reduced (IMBF) the medullary vascular response to Bk, both its initial and sustained phase (Figs 3 and 4). This indicates that Bk induces vasodilatation by smooth muscle relaxation secondary to activation of K_{Ca} channels; to what extent this is mediated by EETs remains uncertain (see above). Imig and co-workers (2001) reported that dilatation of the afferent arteriole of the isolated perfused juxtamedullary nephron induced by Bk was largely inhibited both by miconazole, another imidazole derivative that probably has direct action on K_{Ca} channel activity, and also by MS-PPOH, a structurally dissimilar selective epoxygenase inhibitor. Overall, our data indicate that in the kidney of anaesthetized rats the NO system and an increase in the activity of K_{Ca} channels

contribute to Bk-induced vasorelaxation. There is no doubt, however, that in different animal species, vascular beds and experimental settings, Bk may exert its vaso-relaxant action by a variety of complex mechanisms and along multiple biochemical pathways (Wang *et al.* 2003).

In summary, exogenous bradykinin applied by intravascular route or to the interstitium of the cortex or medulla failed to increase cortical perfusion in a sustained manner; this suggests that the agent has little, if any, role in the physiological control of whole cortex or whole kidney perfusion. By contrast, Bk invariably and substantially increased perfusion of the medulla, especially of its inner subzone. This effect was mediated by the NO system and by activation of K_{Ca} channels.

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Author contributions

Both authors contributed comparably to all aspects of the experimental work, evaluation of the results and manuscript preparation.

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