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In vivo and in vitro homologous desensitization of rat glomerular bradykinin B_2 receptors

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Abstract

We investigated the effects of bradykinin on glomerular bradykinin B_2 receptor functions and parameters in vivo, after intrarenal infusion of bradykinin, and in vitro, after incubation of isolated rat glomeruli with bradykinin. Bradykinin transiently increased renal plasma flow whereas a second challenge was ineffective. Scatchard analysis demonstrated the presence of two populations of bradykinin binding sites whose densities were similarly decreased by about 40% after intrarenal bradykinin infusion. This decrease was not altered by an acid wash suggesting internalization of the radiolabelled ligand. The effect of bradykinin was prevented by a bradykinin B_2 receptor antagonist. Pre-exposure of isolated rat glomeruli to bradykinin mimicked the in vivo results because there was a reduction in bradykinin-induced prostaglandin E_2 and prostagandin $F_{2\alpha}$ release. Rapid recovery was observed 15 min after washing out the bradykinin. Our results directly demonstrate a negative homologous down-regulation of B_2 glomerular bradykinin receptor density under both in vivo and in vitro conditions, an effect which involves a rapid sequestration of the receptor.

Keywords: Bradykinin; Glomerular hemodynamics; Glomerular prostaglandin; Kinin antagonist; Glomerular receptor; Receptor desensitization

1. Introduction

Several studies have indicated that intrarenal bradykinin infusion elicits effects on renal hemodynamics, namely an increase in renal blood flow and a decrease in renal vascular resistance with a minor or non-significant change in glomerular filtration rate (De Felice and Brousseau, 1988; Granger and Hall, 1985; Lortie et al., 1992; Stein et al., 1972; Willis et al., 1969; Yun et al., 1982). In most cases, the hemodynamic effects were observed during short infusion periods or after bolus injection of high doses of the peptide (Baylis et al., 1976; Mertz et al., 1984; Murthy et al., 1978; Willis et al., 1969). Bradykinin effects are mediated through activation of specific receptors which have been classified into bradykinin B_1 and B_2 receptors according to their respective affinity for des-[Arg^9]bradykinin and bradykinin (Regoli and Barabé, 1980). In nearly all fresh tissue preparations, including kidney preparations, spontaneously apparent kinin receptors belong to the B_2 class. Binding studies have been used essentially to localize the bradykinin-binding sites on isolated nephron segments or on renal cells in culture. The highest density of sites has been detected in the collecting tubule whereas only a low level of binding has been described in the glomerulus (Tomita and Pisano, 1984; Manning and Snyder, 1989; Vio et al., 1992). However, after re-examination using a ligand with a higher specific radioactivity, the presence of specific binding sites was demonstrated in rat crude glomerular membranes and in mesangial cells (Bascands et al., 1989; Emond et al., 1990, 1991). Moreover, the coexistence of functional B_1 and B_2 receptors in rat mesangial cells in culture has been

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reported recently (Bascands et al., 1993). The presence of intrarenal vascular bradykinin receptors has been indirectly demonstrated by the effects of intrarenal infusion of bradykinin either alone (Baylis et al., 1976; Thomas et al., 1982) or combined with a competitive kinin antagonist (Lortie et al., 1992; Siragy, 1993).

Interestingly, the density of the glomerular binding sites is decreased in rats fed on a low-sodium diet or submitted to water restriction, which therefore have an increased kallikrein concentration and activity in renal tissue and urine, whereas the density is unaffected in rats fed on a high-sodium diet, whose intrarenal kallikrein is unaltered (Emond et al., 1989). Conversely, the density of glomerular bradykinin-binding sites is increased in both clipped and unclipped kidneys during the early phase of two-kidney, one-clip Golblatt hypertension in rats whose intrarenal kallikrein activity is decreased (Emond et al., 1991). In addition, receptor affinity was not altered and remained as expected for a bradykinin B_2 receptor. Taken together, these observations suggest that there is an inverse relationship between the level of renal kallikrein and, presumably, of renal kinins and the availability of glomerular bradykinin binding sites. These results indirectly support the hypothesis of a desensitization of glomerular receptors in response to kinin stimulation but do not allow a definitive conclusion to be drawn concerning the response of the receptors to changes in bradykinin concentration.

It has been demonstrated that bradykinin stimulates the release of prostaglandins from glomerular and mesangial cells via bradykinin B_2 receptor activation (Uglesity et al., 1983; Emond et al., 1990, 1991). Whether or not the bradykinin-induced alterations in glomerular hemodynamics and in prostaglandin release, which are both dependent on the activation of bradykinin B_2 glomerular receptors, are altered by prior exposure to bradykinin, resulting in receptor desensitization, has not been examined.

This study was therefore designed, first, to determine the effects of prolonged intrarenal bradykinin infusion on the availability of glomerular receptors; and second, to examine the consequences of prior exposure of isolated glomeruli to bradykinin on the desensitization of glomerular bradykinin B_2 receptors and to investigate the possible mechanism of this rapid desensitization.

2. Materials and methods

2.1. Surgical preparation

Experiments were performed on male Wistar-Kyoto rats (WKY, obtained from Iffa-Credo, l'Arbresle, France), aged 9.7 ± 1.1 weeks and weighing 242 ± 24 g. All animals were fed on a standard rat food (UAR A04: sodium content, 104 ± 3 μmol/g). They were fasted the night before an experiment but were allowed free access to water. All experiments reported were conducted in conformity with guiding principles for the care and use of animals.

After induction of anesthesia with an intraperitoneal injection of sodium pentobarbital (50 mg/kg), the animals were placed on a servo-controlled heating table to maintain body temperature at 37°C. The trachea, the left carotid artery and the left jugular vein were cannulated. Direct arterial blood pressure was monitored (Statham P10EZ pressure transducer connected to a TA4000 recorder, Gould Electronics, Ballainvilliers, France). One jugular cannula was used for infusing a Ringer-lactate solution containing 3% gelatin (Plasmion, Roger Bellon Laboratories) to replace fluid losses during surgical preparation (100 μl/min equivalent to 1.25 ml/100 g body weight). This cannula was then used to infuse a mixture made up of Plasmion solution for one half and isotonic dextrose solution for the other half, at 20 μl/min throughout the remainder of the experiment. A second jugular cannula was used to infuse 0.9% NaCl (10 μl/min), sodium pentobarbital (75 μg/min per kg), inulin (0.22 mg/min; Inutest, Laevosan-Gesellschaft) and *p*-aminohippurate (0.13 mg/min; Nephrotest, Biol. Arbeitsgen.) to establish and maintain inulin and *p*-aminohippurate plasma levels at 20 mg/100 ml and 2 mg/100 ml, respectively. A ventral midline incision was made and both ureters were catheterized (Biotrol 1) for urine collection. The right suprarenal artery was carefully cannulated as previously described (Smits et al., 1983), under the lens of a stereomicroscope (SZ3, Olympus, Tokyo, Japan), with a very thin catheter made of stretched PE-10 tubing and infused with 0.9% NaCl at the rate of 10 μl/min. The rats were allowed to equilibrate for 1 h after the completion of surgery.

2.2. Animal groups and clearance experiments

Right and left kidney hemodynamics were evaluated separately for three 20-min clearance periods. The first period was the control period (10 μl/min, vehicle infusion only), the second period was the test period and the third period was either a recovery period or a second stimulation period with the test substance. The animals were divided into groups as follows. Group 1 (C, $n = 15$) received saline as vehicle into the right renal artery for three periods. Group 2 (BK, $n = 21$) received 20 ng/min bradykinin alone during the second period. Group 3 (BK + BK, $n = 7$) received bradykinin alone during the second and the third periods. Group 4 (BK + Antag, $n = 14$) received the same dose of bradykinin with 200 ng/min of a bradykinin B_2 receptor antagonist during the second period. Urine

from the left and right kidneys was collected separately while blood samples were taken at the midpoint of each collection period. At the end of an experiment, the rats were killed by quick aortic bleeding. Both kidneys were immediately excised, decapsulated, weighed and stored (−80°C) until binding assays were performed.

The doses of bradykinin and bradykinin B_2 receptor antagonist used in these experiments were determined in pilot experiments with the help of a slotted sensor placed around the right renal artery of WKY rats and connected to an electromagnetic flowmeter (1401, Skalar Medical, Delft, Netherlands). The dose of bradykinin was determined as the lowest dose whose infusion into the suprarenal artery always increased right renal blood flow by at least 0.5 ml/min without any extrarenal action. The bradykinin B_2 receptor antagonist (D-Arg^0-[Hyp^3,D-Phe^7]BK) was synthetized by D. Regoli and its selectivity and affinity have been systematically assayed and characterized in our glomerular system (Regoli and Barabé, 1980; Rhaleb et al., 1989; Regoli et al., 1990). The dose of antagonist was determined as the lowest dose whose concurrent infusion into the suprarenal artery always suppressed this effect of bradykinin.

Glomerular filtration rate was equated with the clearance of inulin, and effective renal plasma flow with the clearance of *p*-aminohippurate. Renal blood flow was calculated as the ratio of renal plasma flow to 1-hematocrit. The filtration fraction was calculated as the ratio of glomerular filtration rate to renal plasma flow. Renal vascular resistances were calculated as the ratio of mean arterial pressure to mean renal blood flow.

2.3. Assays of bradykinin binding to glomeruli from infused kidneys

Both kidneys from the rats used in the clearance experiments were used in binding assays. Preparation of crude glomerular membranes, of iodinated ligand, binding assays and binding-data analysis have been previously validated and described in detail (Emond et al., 1989, 1991). Briefly, in each group of rats, three or four pools of glomerular membranes were prepared from four right or left kidneys. Cortical glomeruli were isolated by graded sieving, collected on the last 75 μm sieve, resuspended, centrifuged and homogenized in a manual tissue grinder (Kontes). The crude membrane preparation was centrifuged at 40 000 × *g* for 20 min and the pellet was used the same day. [Tyr^0]BK was iodinated according to the chloramine-T method and purified by high-pressure liquid chromatography to reach a specific radioactivity of 280 ± 40 Ci/mmol (Girolami et al., 1987). The ligand was aliquoted and stored at −70°C and used for up to 20 days. For saturation studies, an aliquot of the glomerular membrane extract (50–100 μg of protein) was incubated in triplicate, 30 min at 4°C, in the binding buffer in the presence of increasing amounts of ^{125}I-[Tyr^0]BK (0.2–20 nM). Unlabeled [Tyr^0]BK was used at 10 μM. The final assay volume was 0.4 ml. The composition of the binding buffer was as follows: 5 mM phosphate, 10 mM NaCl, 0.32 M sucrose, 2.5 mM phenanthroline, 10 μM leupeptin, 0.05% bacitracin, 1 mM benzamidine, 2 μM captopril, 1 μM phosphoramidon, 0.1% lysosyme. The effectiveness of the sequential addition of the various protease inhibitors has been previously reported (Emond et al., 1990). Washing buffer (5 mM potassium phosphate buffer, pH 7.2, 0.32 M sucrose without any inhibitor) was added at the end of the incubation time and the total volume was filtered on a GF/C Millipore filter (1.2 μm) that had been soaked prior to the assay in 0.1% polyethylenimine for 24 h to reduce non-specific binding. The filters were washed 4 more times with 4 ml of the washing buffer and the filter-bound radioactivity was determined in a gamma counter (Cristal Multiradioimmunoassay Packard).

Specific binding was calculated as the difference between total and non-specific binding obtained in the absence and in the presence of unlabeled [Tyr^0]BK (10 μM), respectively. Results were analyzed by Scatchard analysis using the Kinetic EBDA LIGAND computerized program (Munson and Rodbard, 1980). Results are means ± S.E. of, at least, three independent assays carried out with the different membrane pools from each group of rats. Results are expressed as fmol of iodinated bradykinin bound per mg of protein (fmol/mg protein).

2.4. Effect of bradykinin, [Tyr^0]BK and ^{125}I-[Tyr^0]BK on free cytosolic Ca^{2+} of cultured mesangial cells

The intracellular calcium concentration ($[Ca^{2+}]_i$) of cultured mesangial cells was determined by the use of the calcium-sensitive fluorescent probe, fura 2-acetomethyl ester, as currently performed in the Laboratory and recently reported (Bascands et al., 1993).

2.5. In vitro desensitization study

The bradykinin pretreatment consisted of a 20-min incubation of isolated glomeruli in the presence or absence of 10^{-6} M bradykinin at either 37 or 4°C. The incubation mixture was then centrifuged for 30 s at 4°C. The pellets were washed 3 times consecutively with the washing buffer and submitted to an acid wash to remove the bradykinin bound during pretreatment. It has been demonstrated previously that such an acid wash removes the cell surface-bound radioactivity (Haigler et al., 1980). In control experiments with crude glomerular and mesangial cell membrane preparations, in order to avoid internalization of the radioligand, we

did not detect any binding with ^{125}I-[Tyr^0]BK after the acid wash procedure, indicating that this protocol removed all the membrane-bound radioactivity. The acid wash was performed on all the glomeruli obtained from in vivo or in vitro studies, the pellets being incubated for 6 min at 4°C in the binding buffer adjusted to pH 2.5 with a 90% acetic acid solution. The pellets were further washed with the washing buffer, and acid was eliminated by centrifugation (20000 × g, 15 min at 4°C). The binding studies were then conducted on these different samples as described in the binding section. The acid-resistant binding was considered as an index of internalized radioligand.

2.6. Prostaglandin secretion by isolated glomeruli

Glomeruli were isolated from untreated rats as described previously. Each incubation contained approximately 2000 glomeruli whose secretion of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ was measured during a 5-min incubation period at 37°C as indicated in previous reports (Emond et al., 1991). Seven different types of incubation were carried out as follows: (i) normal untreated glomeruli; (ii) glomeruli incubated with 10^{-7} M bradykinin alone; (iii) glomeruli incubated with 10^{-7} M bradykinin associated with 10^{-6} M D-Arg^0-[Hyp^3,D-Phe^7]BK, a bradykinin B_2 receptor antagonist; in groups (iv) to (vii) glomeruli initially incubated for 20 min with 10^{-6} M bradykinin and then incubated for an additional 5-min period: (iv) in the presence of 10^{-7} M bradykinin alone, (v) in the presence of 10^{-7} M bradykinin in combination with 17 μM arachidonic acid, (vi) in the presence of 10^{-7} M bradykinin after glomeruli had been allowed to recover for 15 min in the absence of bradykinin, and (vii) in the presence of 10^{-7} M bradykinin after glomeruli had been allowed to recover for 30 min in the absence of bradykinin. Incubation was stopped by centrifugation (30 s at 2000 × g). The supernatant was removed and stored frozen (−70°C) until prostaglandin measurements were performed. The pellets were kept for protein determination.

The concentrations of prostaglandins E_2 and $F_{2\alpha}$ were measured by specific enzymo-immunoassays (EIA) using Cayman chemical kits, as currently used in the Laboratory according to the manufacturer's instructions and previously described in detail (Emond et al., 1990, 1991). Results are means ± S.E. of four incubations and are expressed as pg of prostaglandins secreted per min of incubation per mg of protein (pg/min per mg protein).

2.7. Protein determination

Proteins were measured by the Lowry method after solubilization with 1 M sodium hydroxide for 15 min at 100°C (Lowry et al., 1951).

2.8. Drugs

Bradykinin was purchased from Sigma Chemicals (France), the bradykinin B_2 receptor antagonist (D-Arg^0-[Hyp^3,D-Phe^7]BK was synthesized in the laboratory of D. Regoli and ^{125}I-sodium salt was purchased from Amersham UK.

2.9. Statistical analysis

The values are reported as means ± S.E. For clearance data, differences in a given group were analyzed using the Wilcoxon signed-rank test. For other results, multiple means were compared using a single factor analysis of variance (ANOVA). Results with P values less than 0.05 were considered statistically significant.

3. Results

3.1. Effect on renal hemodynamics of bradykinin intrarenal infusion

As shown in Fig. 1, no change in renal plasma flow, glomerular filtration rate, filtration fraction or renal vascular resistances was detected in the left non-infused kidney. In addition, none of the tested substances induced any change in blood pressure or hematocrit (not shown). In the right kidney infused for 20 min with bradykinin, no change in glomerular filtration rate was detected whereas + 20 ± 4, − 10 ± 2 and − 15 ± 4% significant changes ($P < 0.001$) were observed in renal plasma flow, filtration fraction and renal vascular resistances, respectively. These effects were no longer observed when bradykinin was infused in the presence of the bradykinin B_2 receptor antagonist D-Arg^0-[Hyp^3,D-Phe^7]BK. No significant alteration in right kidney renal plasma flow, filtration fraction or renal vascular resistances was observed after a second 20-min intrarenal bradykinin infusion.

3.2. Binding studies on glomerular membranes from bradykinin-infused rats

Under our experimental conditions, we achieved maximum steady binding after 30 min of incubation. The total binding represented approximately 1% of the added radioactivity and the specific and non-specific binding were 65 ± 11% and 33 ± 9% respectively of the total binding. A rapid 50% dissociation of ^{125}I-[Tyr^0]BK occurred in less than 3 min. The remaining free iodinated ligand was not altered after a 30-min incubation, as estimated by radioimmunoassay after HPLC analysis. Moreover, ^{125}I-[Tyr^0]BK was able to increase the free cytosolic Ca^{2+} content of cultured adherent mesangial cells. As shown in Fig. 2, [Tyr^0]BK, ^{125}I-[Tyr^0]BK and bradykinin induced very similar pat-

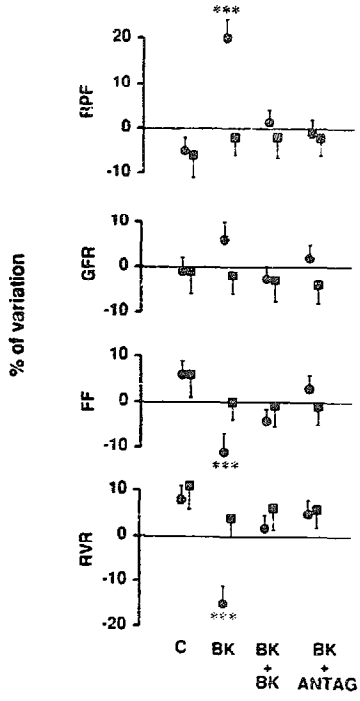

Fig. 1. Changes in renal vascular resistance (RVR), filtration fraction (FF), glomerular filtration rate (GFR) and renal plasma flow (RPF) in rats infused with 0.9% NaCl vehicle (C), after a single 20-min infusion of 20 ng/min bradykinin (BK), after two successive 20-min infusions of 20 ng/min bradykinin (BK+BK) or after infusion of a mixture of 20 ng/min bradykinin and 200 ng/min of a bradykinin B_2 receptor antagonist (BK+Antag). Results are expressed as the percent change of the indicated parameters measured during the second period except for the group receiving two successive BK infusions, where changes were calculated during the third period. The values of the first period are taken as 100%, each animal being used as its own control. Results are means ± S.E. for right (circles) and left kidneys (squares). Statistical significance of differences between before and during infusion of test substances: *** $P < 0.001$.

terms of increases in ($[Ca^{2+}]_i$). A typical saturation binding curve of bradykinin with glomerular membranes obtained from control animals is shown in Fig. 3

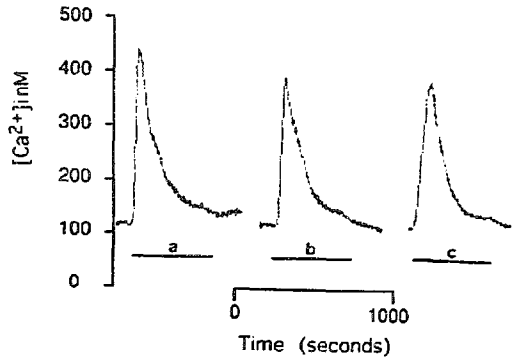

Fig. 2. Effects of 10^{-7} M bradykinin (a), [Tyr^0]BK (b) and ^{125}I-[Tyr^0]BK (c) on the intracellular Ca^{2+} content ($[Ca^{2+}]_i$) of adherent mesangial cells. Each trace shows a typical response obtained at 3 three times.

and the results of Scatchard analysis of binding assays on glomerular membranes obtained from control, bradykinin-infused and bradykinin B_2 receptor antagonist-infused rats are summarized in Table 1. Data obtained for glomerular membranes from control rats revealed the presence of two classes of bradykinin-binding sites for ^{125}I-[Tyr^0]BK. A small population (B_{max} = 14.1 ± 1.9 fmol/mg protein) of very-high-affinity binding sites (K_d = 0.4 ± 0.1 nM) was detected together with a larger one (B_{max} = 117.3 ± 14.6 fmol/mg protein) of high-affinity sites (K_d = 6.8 ± 2.1 nM). It can be seen that no change occurred, in any group, in the binding parameters of glomerular membranes obtained from the left non-infused kidney. In contrast, a significant and similar decrease in the density of the two populations of binding sites was observed in the right kidneys of rats infused for 20 min with bradykinin. Compared to control conditions, bradykinin induced similar decreases (53 ± 9% and 42 ± 7%, $P < 0.01$) in the density of the very high- and the high-affinity binding sites. Interestingly, at the end of infusion in the

Table 1
Affinity (K_d) and density (B_{max}) of very high-affinity bradykinin-binding sites (Site 1) and of high-affinity sites (Site 2) for glomerular membranes prepared from control rats, or from rats infused with bradykinin (BK) alone or simultaneously with bradykinin and a bradykinin B_2 receptor antagonist (D-Arg^0-[Hyp^3,D-Phe^7]BK (BK + B_2 antagonist) as described in Materials and methods

	Site 1		Site 2	
	K_d (nM)	B_{max} (fmol/mg protein)	K_d (nM)	B_{max} (fmol/mg protein)
Control				
Left kidney	0.4 ± 0.1	13.9 ± 3.5	6.6 ± 1.7	104.0 ± 10.0
Right kidney	0.4 ± 0.1	14.1 ± 1.9	6.8 ± 2.1	117.3 ± 14.6
BK				
Left kidney	0.4 ± 0.1	11.7 ± 1.4	5.8 ± 1.1	113.2 ± 16.5
Right kidney	0.5 ± 0.1	6.5 ± 1.2 [a,b]	4.2 ± 1.5	66.5 ± 6.1 [a,b]
BK + B_2 antagonist				
Left kidney	0.4 ± 0.2	11.2 ± 2.1	6.2 ± 1.2	96.0 ± 9.[illegible]
Right kidney	0.4 ± 0.1	11.9 ± 2.7	4.8 ± 1.1	97.3 ± 12.8

Results are means ± S.E. of three distinct experiments run in triplicate. Statistical difference: [a] $P < 0.01$ when compared to values of the right infused kidney of the control group; [b] $P < 0.01$ when compared to values of the left non-infused kidney in the same group.

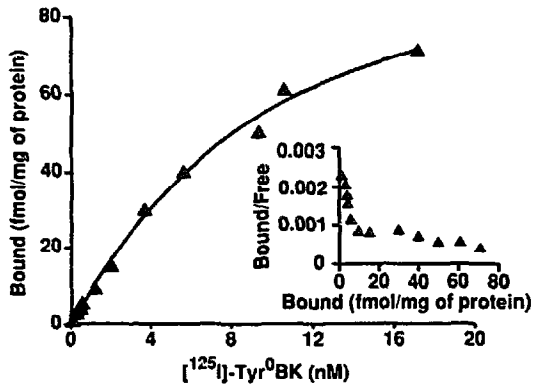

Fig. 3. Saturation binding curves for increasing amounts of ^{125}I-[Tyr^0]BK bound to freshly prepared rat glomerular membranes as described in Materials and methods. Inset shows Scatchard plot analysis. Results are means ± S.E. of six independent experiments.

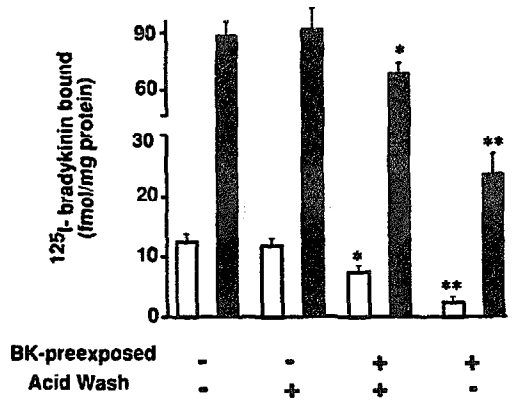

Fig. 4. Effects of pre-incubation of isolated glomeruli with 10^{-6} M of bradykinin for 20 min at 37°C on the density of very high-affinity BK-binding sites (white bars) and of high-affinity BK-binding sites (dark bars). The density of binding sites was calculated from Scatchard plot analysis of ^{125}I-[Tyr^0]BK binding to fresh isolated glomeruli prepared from normal rats incubated in the absence or presence of 10^{-6} M bradykinin. After the period of incubation, glomeruli were washed with a washing buffer or submitted to an acid wash. Statistical difference: $^*P < 0.05$ and $^{**}P < 0.01$ when the density of the BK-binding sites is compared with that in the control group (without bradykinin-pre-exposure and acid wash).

presence of the bradykinin B_2 receptor antagonist D-Arg^0-[Hyp^3,D-Phe^7]BK, glomerular membranes did not exhibit any significant alteration in the density of bradykinin binding sites (Table 1).

3.3. Binding studies on isolated glomeruli after in vitro exposure to bradykinin

The effect of bradykinin pretreatment on the density of binding sites is summarized in Fig. 4. In order to check whether the acid wash induced alterations in binding parameters, we first examined the effect of this procedure on untreated glomeruli. As shown in Fig. 4, the acid wash of untreated glomeruli had no effect on the binding parameters of the two populations of binding sites. Pre-exposure of isolated glomeruli to 10^{-6} M bradykinin for 20 min at 37°C resulted in significant ($P < 0.05$) and similar decreases in the density of both populations of binding sites (very high affinity site: $B_{max} = 7.3 \pm 1.1$ vs. 11.8 ± 1.2 fmol/mg protein; high affinity site: $B_{max} = 68.9 \pm 8.2$ vs. 92.3 ± 13.2 fmol/mg protein). In the absence of an acid wash, the decrease was even more pronounced, probably indicating residual surface binding of bradykinin (Fig. 4).

3.4. Internalization studies

As demonstrated in Fig. 5, when the binding studies were performed at 37°C, internalization showed a transient increase, reaching a 37% maximum value after 20 min of incubation, followed by a slow decrease to 20% after 60 and up to 180 min. In contrast, when experi-

Table 2
Prostaglandin E_2 and prostaglandin $F_{2\alpha}$ secretion by isolated glomeruli

	Prostaglandin E_2 (pg/min per mg protein)	Prostaglandin $F_{2\alpha}$ (pg/min per mg protein)
(i) Control	94 ± 12	72 ± 8
(ii) BK 10^{-7} M	168 ± 18 [a]	149 ± 16 [a]
(iii) BK 10^{-7} M + antagonist 10^{-6} M	102 ± 14 [b]	88 ± 12 [c]
(iv) Pre BK 10^{-6} M + BK 10^{-7} M	128 ± 13 [b]	96 ± 11 [c]
(v) Pre BK 10^{-6} M + AA + BK 10^{-7} M	118 ± 17 [b]	91 ± 9 [c]
(vi) Pre BK 10^{-6} M + 15 min + BK 10^{-7} M	148 ± 20 [d]	139 ± 16 [d]
(vii) Pre BK 10^{-6} M + 30 min + BK 10^{-7} M	154 ± 14 [d]	162 ± 19 [d]

Prostaglandins E_2 and $F_{2\alpha}$ secretion was measured when isolated glomeruli were incubated for 5 min at 37°C in the absence (i) or the presence of 10^{-7} M bradykinin alone (ii) or in combination with a bradykinin B_2 receptor antagonist (D-Arg^0-[Hyp^3,D-Phe^7]BK, 10^{-6} M), (iii). In the other four groups, glomeruli were previously incubated for 20 min with bradykinin (10^{-6} M). This preincubation was followed by a second 5-min incubation in the presence of 10^{-7} M bradykinin alone (iv) or in combination with 17 μM arachidonic acid (AA), (v). In the last two groups, after the preincubation, glomeruli were washed and allowed to recover either for 15 min (vi) or 30 min (vii) with a bradykinin-free incubation medium before the second 5-min incubation in the presence of bradykinin 10^{-7} M. Results are means ± S.E. in pg/min per mg protein ($n = 4$ in each group). Statistical differences: [a] $P < 0.01$: BK 10^{-7} M vs. control. [b] $P < 0.05$, [c] $P < 0.01$: vs. BK 10^{-7} M. [d] $P < 0.01$: pre BK 10^{-6} M + recovery + BK 10^{-7} M vs. pre BK 10^{-6} M + BK 10^{-7} M.

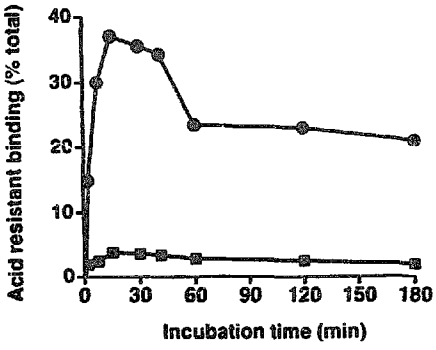

Fig. 5. Effects of changes in incubation time with 10^{-6} M bradykinin and temperature on the density of acid-resistant bradykinin B_2-binding sites from glomerular membranes. Experiments were performed at 37°C (circles) and at 4°C (squares).

ments were conducted at 4°C, a change in acid-resistant binding was observed, indicating the absence of significant internalization.

3.5. Desensitization of bradykinin-mediated prostaglandin release by isolated glomeruli

In glomeruli obtained from normal rats, bradykinin induced a dose-dependent increase in both prostaglandin E_2 and prostaglandin $F_{2\alpha}$, with an $ED_{50} = 0.5 \times 10^{-7}$ M (not shown). Table 2 shows bradykinin-mediated prostaglandin release by isolated glomeruli. Compared to control glomeruli, 10^{-7} M bradykinin elicited an approximately 100% increase in the amount of the prostaglandins secreted (prostaglandin E_2: 168 ± 18 vs. 94 ± 12 pg/min per mg protein; prostaglandin $F_{2\alpha}$: 149 ± 16 vs. 72 ± 8 pg/min per mg protein, $P < 0.01$). The stimulating effect of bradykinin disappeared completely when the incubation was performed in the presence of the bradykinin B_2 receptor antagonist D-Arg^0-[Hyp^3,D-Phe^7]BK (Table 2). The bradykinin-induced increase in prostaglandin secretion was significantly reduced (Δ prostaglandin $E_2 = -67 \pm 6\%$; Δ prostaglandin $F_{2\alpha} = -75 \pm 8\%$) when isolated glomeruli were previously incubated for 20 min in the presence of 10^{-6} M bradykinin (prostaglandin $E_2 = 128 \pm 13$ vs. 168 ± 18 pg/min per mg protein; prostaglandin $F_{2\alpha} = 96 \pm 11$ vs. 149 ± 16 pg/min per mg protein; $P < 0.05$, $P < 0.01$). This reduction of bradykinin-stimulated prostaglandin secretion was independent of the addition of arachidonic acid. Furthermore, when glomeruli had been incubated beforehand for 20 min in the presence of 10^{-6} M bradykinin, and then washed and allowed to recover for 15 or 30 min without bradykinin, a complete recovery of the prostaglandin secretory capacity was observed (Table 2).

4. Discussion

This study documents the time dependence of the renal effects of bradykinin in anesthetized rats with regard to the availability of glomerular receptors. Our observations provide new information on two points: first, the effects of strictly intrarenal bradykinin infusion on glomerular hemodynamics are only transient; and second, the brevity and spontaneous reversibility of bradykinin action on the glomerulus are probably related to a negative autoregulation of the bradykinin B_2 receptors.

Regarding the effects of bradykinin infusion, this study confirms the direct renal action of bradykinin (Granger and Hall, 1985; Lortie et al., 1992; Madeddu et al., 1992; Stein et al., 1972; Willis et al., 1969). It appears that this objective was achieved for the following three reasons: (i) the low doses of bradykinin used caused no extrarenal alteration, namely changes in arterial pressure or hematocrit, that could affect renal response; (ii) contralateral kidney hemodynamics were not affected; (iii) no effect was observed in the kidney infused with both bradykinin and a specific bradykinin B_2 receptor antagonist. Therefore, the reported alterations can be ascribed to bradykinin and to the binding to B_2-type receptors. Low doses of bradykinin induced an increase in renal blood flow, a decrease in renal vascular resistances with no significant change in glomerular filtration rate and, as a consequence, a decrease in filtration fraction. The statistically insignificant rise in glomerular filtration rate in the face of an increased renal plasma flow indicates that the glomerular autoregulatory mechanisms were maintained. This may imply changes in the balance between afferent and efferent arteriolar resistances, namely a predominant efferent arteriolar vasodilation, and/or in the glomerular ultrafiltration coefficient (K_f). In this regard, a micropuncture study of the determinants of single-nephron glomerular filtration rate in Munich-Wistar rats has indicated that bradykinin elicits a decrease in K_f (Baylis et al., 1976). This explanation is consistent with the recent identification of bradykinin B_2 receptors in intact adherent mesangial cells (Bascands et al., 1989, 1993; Emond et al., 1990), and with the demonstration that bradykinin is able to induce a rapid and long-lasting contraction of cultured rat mesangial cells with a significant decrease in the planar surface area of the cells, and thus, probably, is able to reduce the glomerular K_f (Bascands et al., 1994).

Our studies also demonstrate a striking time-dependent change in the renal action of bradykinin. While bradykinin intrarenal infusion was maintained at the same rate, glomerular hemodynamics had returned to basal values 40 min after the start of the infusion. Our results clearly demonstrate that the effects of exogenous bradykinin on the kidney are transient and that

they vanish spontaneously after 20 min of infusion. In fact, the renal effects of bradykinin have been almost exclusively observed after either a bolus injection (Nasjletti et al., 1975; Seino et al., 1988) or after a short intrarenal infusion of higher doses (μg/range) than ours (Webster and Gilmore, 1964; Baylis et al., 1976; Thomas et al., 1982). Only one study reported the transient effect of bradykinin during a 2-h intrarenal infusion of similar doses in anesthetized dogs (Flamenbaum et al., 1979). The bradykinin-induced increase in renal blood flow was transient at 15 min after the onset of infusion, whereas the secretion of renin was high at 60 min and remained high until 120 min. Accordingly, the authors suggested that this rise could explain the lack of sustained renal hyperemia. Another possibility is that exposure to bradykinin might alter the receptors' availability, a hypothesis that we tested further by examining the characteristics of the glomerular bradykinin binding sites.

The present results confirm recent studies by our group that have indicated that bradykinin has the ability to interact with a heterogeneous population of glomerular binding sites (Emond et al., 1990, 1991; Bascands et al., 1993). However, this study extends our knowledge by showing that both populations of glomerular binding sites undergo rapid desensitization: the density of the two populations of sites was reduced to approximately the same extent by the 20-min intrarenal infusion of bradykinin whereas their affinity remained unchanged. This desensitization clearly depends on the agonist binding to bradykinin receptors because it was not observed after a 20-min intrarenal infusion of bradykinin plus a bradykinin B_2 receptor antagonist. The desensitization could also be demonstrated with freshly isolated rat glomeruli whose incubation with 10^{-6} M bradykinin for 20 min at 37°C elicited a similar significant decrease in the density of the two populations of sites. Furthermore, the process of receptor desensitization can be associated with a modification of glomerular function as demonstrated by the decrease in prostaglandin production. That the same reduction in prostaglandin secretion was observed when the preincubation with bradykinin was performed either in the presence or in the absence of arachidonic acid demonstrates that this effect results from the decreased density of bradykinin B_2 receptors and not from a lack of substrate. Another point worth discussing is the relationship between the magnitude of the decrease in binding site density and that of prostaglandin secretion observed after bradykinin preexposure. Although the physiological significance of the heterogeneity of the bradykinin-binding site population remains unclear, the discrepancy between the magnitude of the desensitization assessed by the binding site density (−42 to −53%) and by the level of prostaglandin secretion (−67 to −75%) suggests a disparity between the linkage to the receptors and the functional response. This is in agreement with the hypothesis that the binding of bradykinin to one site can lead to a large signal amplification involving the activation of several G-proteins (Burch and Kyle, 1992). Another report indicated such an association between a partial desensitization of bradykinin-binding site density and a non-proportional and greater decrease in inositol phosphate production (Wolsing and Rosenbaum, 1991). Since bradykinin B_2 receptors belong to the seven transmembrane G protein-coupled receptor superfamily (McEachern et al., 1991), several mechanisms of desensitization are possible and have been described. A three-state model for the binding of agonists to the bradykinin B_2 receptor has recently been proposed (Leeb-Lundberg et al., 1994). This model includes two G-protein-coupled states that differ in their dissociation rates and a G-protein-uncoupled state. According to this three-state model, in bovine myometrial membrane, the decrease in cell surface receptors is associated with both internalization and uncoupling (Munoz and Leeb-Lundberg, 1992; Leeb-Lundberg et al., 1994). A homologous autoregulation of bradykinin receptors, similar to our observation, has previously been detected with human fibroblasts: a 5-min incubation in the presence of 10^{-6} M bradykinin induced a significant decrease (up to 90%) in the density of these receptors and in the release of prostaglandin I_2 with no change in the dissociation constant (Roscher et al., 1984). As in our studies, after a 15-min incubation in the presence of bradykinin (10^{-6} M), a further incubation of human fibroblasts in the absence of bradykinin for 30 min at 37°C (after washing out bradykinin from the incubation medium) almost completely restored both the number of receptors and the bradykinin-induced prostaglandin I_2 release. Finally, in rat fibroblasts, another possibility of desensitization was reported. The decrease in cell surface receptor number was associated with a change in the affinity of these receptors. Indeed, prolonged exposure of rat fibroblasts to bradykinin caused a concentration-dependent decline in a high-affinity population of binding sites and the appearance of an equal number of lower-affinity binding sites, suggesting that the desensitization had occurred through an alteration in ligand affinity (Roberts and Gullick, 1990). With regard to this matter, our studies demonstrate that the desensitization of the glomerular bradykinin B_2 receptor occurred without conversion to a low-affinity population of binding sites.

In summary, the direct demonstration of a negative homologous autoregulation of the bradykinin receptors probably accounts for: (i) the lack of sustained hemodynamic effects when the intrarenal infusion of bradykinin is maintained; (ii) the previously reported decrease in the density of glomerular binding sites

when the renal kallikrein activity is increased (Emond et al., 1989); and (iii) the increased density when kallikrein activity is decreased (Emond et al., 1991).

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