Postnatal Maturation of the Kallikrein-Kinin System in the Rat Kidney: From Enzyme Activity to Receptor Gene Expression

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ABSTRACT

During the course of aging, the balance between intrarenal hormones is disturbed. These age-related changes are well documented for the vasoconstrictor renin-angiotensin system, but comparable information on the renal kallikrein-kinin system is not yet available. The status of the kallikrein-kinin system was assessed by (1) kallikrein activity, measured by RIA; (2) maximum binding site density (Bmax) and affinity (Kd) of nonapeptide bradykinin (BK)-2 receptor, estimated by binding assays; (3) expression of BK2-receptor mRNA, detected by reverse transcription-polymerase chain reaction (RT-PCR) using specific BK2-oligonucleotide primers. These parameters were determined on renal glomeruli of 3-, 5-, 8-, 12- and 38-wk-old normotensive rats. Kallikrein activity increased from 3.2 to 7.7 ng BK/min per mg protein. The density of BK2 binding sites also rose from 12 to 40 femtomoles/mg protein with no difference in affinity. There was no change in specificity, which remained that expected of a BK2 receptor. The increase in the density of BK2 binding sites was associated with an augmented mRNA expression, whereas β-actin mRNA used as a control remained unchanged. The ratio of BK2 mRNA to β-actin mRNA indicated maximum steady expression after 8 wk of age. The data provide evidence that the renal kallikrein-kinin system develops postnatally.

Key Words: Glomerulus, BK2 receptor, ontogeny, mRNA, PGE2

Normal aging of the kidney is associated with progressive functional and structural deteriorations (1). Our understanding of the age-related changes in kidney functions may be improved by knowledge of the regulatory systems that control RBF, GFR, and solute excretion.

Kallikrein is a serine protease, and one of its major actions is to generate potent vasodilator and natriuretic substances, named kinins, from kininogen, which is synthesized in the liver and released into the circulation. Several lines of evidence favor the hypothesis that the renal kallikrein-kinin system could play a role in the regulation of renal functions, primarily water and electrolyte balance and blood pressure control (2,3). One of the primary interests in the renal kallikrein-kinin system arises from the suggestion made by many investigators that this system acts as a potential antihypertensive system, and on failure might be involved in the development of hypertension (3,4). A large number of investigators have reported a significant decrease in urinary kallikrein excretion in humans with essential hypertension (5), genetically hypertensive rats (6), and several animal models for secondary forms of experimental hypertension, such as the two kidney-one clip (2K-1C) renal hypertensive rats (7).

The physiological activity of the renal kallikrein-kinin system is carried out by the generated kinins. The nonapeptide bradykinin (BK) plays an important role in a number of biological processes, including pain, inflammation, vascular permeability, smooth muscle contraction, cell proliferation, and regulation of blood pressure (8-10). These effects of BK are mediated by cell-surface BK receptors. Several pharmacological studies have demonstrated the existence of at least two types of BK receptors, termed BK1 and BK2 (8.9). The BK1 receptor has a greater affinity for the endogenous BK metabolite des-Arg⁵-BK. The BK2 receptor, which is the more prevalent receptor type, has a greater affinity for the native BK. Cloning of the BK2 receptor from human and rat tissues (11-14) indicated that this receptor is a member of the superfamily of the seven transmembrane domain G-protein-coupled receptors. In the kidney, specific binding studies have reported the presence of BK2 receptor in cortical epithelial membrane (15), renomedullary interstitial cells (16), and in cortical tubules (17). We recently identified the presence of BK2 receptors in glomerular membranes (18), more precisely, on those membranes located on the mesangial cells (19).

Micropuncture studies (20) in the rat have shown that BK perfused into the renal artery decreased the glomerular ultrafiltration coefficient (Kf). Our lab then demonstrated that the activation of the glomerular BK2 receptor was associated with an increase in
intracellular calcium (21) as well as an inhibition in cAMP generation (22), leading to a contraction of the mesangial cells (23). The presence of BK2 binding sites in the glomerulus could be important in elucidating certain renal hemodynamic effects induced by BK. Although a role in the regulation of water, salt, and renal functions is generally well accepted, to our knowledge there is, as yet, very little information concerning the postnatal development of the renal kallikrein-kinin system in the rat. Moreover, we also reported that the density of glomerular BK2 binding sites showed opposite variations to acute changes of renal kallikrein activity induced by physiological conditions such as disturbances in sodium balance (24) and renovascular hypertension (25). In the report presented here, we examine the relationships among the three indexes of the kallikrein-kinin system under strictly physiological conditions. Changes in the renal kallikrein activity, BK2 receptor binding sites, and gene expression in the rat kidney are described during postnatal development.

**METHODS**

**Glomerular Preparation of Animals**

Male Sprague Dawley rats, 3, 5, 8, 12, and 38 wk of age, were housed in climate-controlled conditions with a 12-h light and dark cycle, and were fed with standard rat chow (UAR [A-04], IFA-Credo, Lyon, France; 104 mmol Na+/kg of food) and water ad libitum. The animals were killed by decapitation to allow exsanguination, the kidneys were quickly removed and decapsulated, and glomeruli were obtained as described previously (18). The cortex suspension is briefly passed twice through a 21-gauge needle to dissociate the tubule from the glomeruli. The glomeruli are then isolated by graded sieving through three consecutive steel sieves with decreasing pore sizes (180-, 125- and 75-μm), and the glomeruli are collected on the 75-μm sieve. In these conditions, we obtained approximately 15,000 glomeruli from one kidney, and under a light microscope, >95% of the glomeruli appeared to be decapsulated and free of adherent tubes and afferent and efferent arterioles. In preparations for mRNA studies, diethylpyrocarbonate (0.1%) was directly added in the buffer during glomerulus preparation to inactivate RNase. Each age group contained 12 rats organized as follows: 4 rats were used for kallikrein activity; 4 rats for glomerular morphometry, secretion of the prostaglandin E2 (PGE2), and binding studies; and 4 rats for mRNA extraction and RT-PCR studies.

**Glomerular Characterization**

An aliquot of the glomerular suspension was taken and the glomeruli were counted by using a counting cell (Sedgewick Rafter, from Graticules Limited, Tonbridge, England). Glomerular measurements were performed at ×100 magnification by using a light microscope (Leitz SM-Lux, Leitz, Paris, France) fitted with a micrometer, and all measurements were performed on 100-glomerulus samples. Because glomeruli are not perfectly circular, the mean glomerular diameter was determined as follows: (largest diameter + smallest diameter)/2. Glomerular theoretical surface area was determined by surface (S) = πr²). In preliminary experiments, we established an intra-assay variation coefficient of 3.3 ± 0.9% (N = 20) and an inter-assay variation coefficient of 4.4 ± 1.2% (N = 20). We also checked to make certain that the glomerular diameters of the same preparation remained unchanged for up to 3 h when periodically oxygenated with a 95% O₂-5% CO₂ mixture.

**Kallikrein Assays**

The activity of kallikrein was estimated by its kininogenase activity by using a kinin RIA as described previously (25). Since the major site for kallikrein synthesis and secretion are the granular connecting cells, which cannot be isolated, aliquots of tissue cortex were homogenized, heated citrate dog plasma was used as a kininogen source, BK (Bachem, Switzerland) was used as a standard, and [³²P]BK as a tracer. The BK generated was measured by RIA using BK antibodies, which only recognize whole BK but not BK fragments. Kininogenase activity was expressed in nanograms of BK generated per minute per mg protein (ng BK/min per mg prot).

**In Vitro Glomerular Production of PGE₂**

PGE₂ was measured by enzyme immunoassay as currently performed in the laboratory (25). The production of PGE₂ was assessed by the amount released by freshly isolated glomeruli in the incubation medium. Approximately 1500 to 2000 glomeruli were used for each incubation in a final volume of 300 μL. The amount of prostaglandins secreted was estimated after a 5-min incubation at 37°C in the presence of 10 nM BK. The incubation medium was centrifuged at 4,000 x g and the supernatant was stored frozen until assayed for determination of prostaglandins. The amount of PGE₂ secreted in the medium was assayed directly by specific enzyme immunoassays (Cayman Chemical Corp, Ann Arbor, MI). Briefly, this assay is done on the basis of the competition between free prostaglandin and the acetylcholinesterase-linked prostaglandin used as a tracer for the respective rabbit-prostaglandin antiserum. The prostaglandin antiserum was coated onto 96-well plates (NUNC Certified, Poly-Lab, Strasbourg, France) via a mouse-monoclonal rabbit antibody. The enzymatic tracer reacts with the Ellman Reagent added to the well, and the amount of colored substance released is inversely proportional to the amount of prostaglandin present in the well. The data for PGE₂ release are expressed in pg of PGE₂ secreted per min incubation per milligram of glomerular protein (pg/min per mg prot).

**Binding Studies**

Preparation of crude renal glomerulus membranes and [³²P]-[Tyr²]BK were obtained as described previously and used in binding studies as currently performed in the laboratory (19,21). Saturation studies were conducted at 37°C for 30 mins in the presence of an amount of [³²P]-[Tyr²]BK increasing from 0.2 to 10 nM. Dissociation constant (Kd) and maximum binding site density (Bmax) values were calculated from computer-assisted analysis of the data by using the kinetic-EBDA-LI-GAND program (Elsevier-Biosoft, Cambridge, United Kingdom), and were expressed as femtomoles of iodinated BK bound per milligram of protein (fmol/mg protein). For competition studies, fixed amounts of membrane extract were incubated for 30 min at 37°C in triplicate, with an increasing amount of unlabeled BK or antagonist (from 10⁻¹² to 10⁻⁴ M) in the presence of 2 nM of [³²P]-[Tyr²]BK.
Protein Determination

In each experiment, samples of renal cortex or glomerulus suspension were used for determination of the protein content. After solubilization for 15 min at 100°C with 1 M NaOH, proteins were measured by using the method of Lowry et al. (26) with serum albumin as a standard.

Statistical Analysis

Results are expressed as mean ± SE of at least three to five separate experiments. An unpaired t test or analysis of variance was performed when appropriate, and in all comparisons, differences were considered significant at P < 0.05.

RNA Isolation and RT-PCR Procedure

Total RNA was extracted from frozen powdered renal cortex (approximately 100 to 200 mg) or from frozen glomeruli according to the method described by Chomczynski and Sacchi (27). To study the expression of BK2 receptor mRNA at the different ages, polyA+ RNA was isolated using the Promega reagent kit (Promega, Charbonnières, France).

First-strand cDNA was synthesized by using 1 μg of total RNA or PolyA+ RNA, oligonucleotides (polydT16) for priming, RNase inhibitor (32 U), 1,4-dithiothreitol (DTT) (25 mM), dNTPs (5 mM), 5 X RT buffer, and 200 U RT (T-MLV from GBCO-BRL, France Life Technologies, Cergy Pontoise, France) were used. After RNA denaturation (10 min at 70°C), the reaction was carried out at 42°C for 50 min with a Perkin Elmer TC-480 (Toulouse, France) heated to 95°C for 5 min and chilled on ice.

Polymerase Chain Reaction

Five μl of each cDNA preparation were amplified. PCR amplification was performed by adding 140 pmol of each primer, 2 U Taq polymerase (Appligene, Illkirch, France), 0.5 mM dNTPs in a final volume of 100 μl PCR buffer containing either 3% formamide for BK2-receptor mRNA amplification, or 10% dimethyl sulfoxide for β-actin mRNA amplification. The samples were denatured for 3 min at 95°C, then PCR was performed for 30 cycles (1 min at 94°C, 1 min 30 s at 58°C, 1 min at 72°C). PCR was carried out using a Perkin Elmer DNA Thermal Cycler (Sigma, Saint Quentin Fallavier, France). In order to evaluate the PCR products comparatively and confirm the integrity of the RNA, we amplified a housekeeping gene, that of β-actin. The PCR products for BK2 receptor or β-actin were amplified at the same time from the same cDNA and then electrophoresed. After analysis, the ratio of BK2 to β-actin band density was determined.

Amplification in the absence of cDNA did not yield any bands other than those of the primers for BK2-receptor mRNA and β-actin mRNA at the bottom of the gel (not shown). The BK2-receptor primers were designed in the 3' untranscribed region of the cDNA from the sequence of the rat gene (11). Primer 1 was defined by bases 2955–2975 5'-ACCAGAGATAGGATGCCCAGCTC-3' (sense) and Primer 2 was defined by bases 3451–3473 5'-ACAGTGTTGACCTGAGCCAGAC-3' (antisense). The cDNA amplification product was predicted to be 518 base pairs in length. The sequences of sense and antisense primers for β-actin (28) were defined by bases (2846–2865, exon 5) 5'-CCTAGGACCATGAAGATCAA-3' (sense) and by bases (3171–3196 exon 6) 5'-TTCTGGCGCAAAGTTAGGTGTGTCGA-3' (antisense). The expected size of the PCR product for β-actin was 227 base pairs.

Optimization of PCR Conditions

To achieve optimal conditions for PCR, we first synthesized cDNA from 1 μg of total RNA obtained from normal Sprague-Dawley rat renal cortex and amplified it, increasing the cycle number from 15 to 50 for BK receptor and from 10 to 40 for β-actin. We next studied the PCR product for BK receptor and β-actin as a function of increasing amount of RT solution (cDNA). PCR amplification was performed for 35 and 25 cycles for BK2 receptor and β-actin respectively.

Relative Quantification of PCR Products

After amplification, 20 μl of each PCR product was electrophoresed through a 2% agarose gel and stained with etidium bromide. The gel was photographed with Polaroid Type 665 film (Positive/Negative) (Poly-Lab, Strasbourg, France) over ultraviolet (UV) light at the same exposure and developing time. The bands on the negative film were scanned by using an image-analysis system (Elecophor™, CRIIS, Toulouse, France), which calculated the densitometric values of each band. Then, for relative quantification, arbitrary values were assigned as described in the figure legends.

Southern Blot Analysis

In order to verify that the amplified fragment corresponded to authentic BK2-receptor transcript, and to obtain a fuller definition of the variations observed on the agarose gel electrophoresis of the PCR products stained with etidium bromide, we hybridized Southern blots of electrophoresed PCR products with the rat BK2-receptor mRNA probe obtained by amplification of 200 ng of genomic rat DNA with our specific BK2-receptor primers and labeled with ([α-32P]-dCTP) by random priming (Amersham megaprime kit, Les Ulis, France). After amplification, 40 μl of each PCR product was electrophoresed through a 1.8 to 2% agarose gel, then the gel was denatured, neutralized, and transferred overnight onto a standard nylon membrane (Hybond™-N, Amersham) with 20 × SSC (sodium chloride/sodium citrate) as the transfer buffer (29). The DNA was fixed to the nylon under UV radiation (254 nm) in an UV-crosslinker (Stratagene, La Jolla, CA). After hybridization in stringent conditions with the 32P probe (42°C overnight), the nylon was washed once with SSC 2×/0.1% sodium dodecyl sulfate (SDS) at room temperature and twice at 65°C for 30 min. Autoradiography was performed for 1 h at ~80°C by using Amersham hyperfilm-MP film with intensifying screens, developed and analyzed as described above.

To confirm that the PCR products were really BK2-receptor cDNA, they were sequenced using the CircumVent™ thermal cycle dideoxy DNA sequencing kit from Biolabs (Ozyme, Montigny Le Bretonneux, France).

MATERIALS

Male Sprague-Dawley rats were from the IFA-Credo farm (Lyon, France). The commercial origin of the materials used was as follows: radioactive iodine as sodium salt was from Amersham (67.5 TBq/mmol). BK-related peptides were purchased from Sigma (Saint Quentin Fallavier, France). HOE-140 (D-Arg-(Hyp7, Thr8, D-Tic7, Oic8)-BK) was a gift from P.B. Schöllkens (Hoechst, Frankfurt, Germany). The enzyme immunoassay (EIA) used for PGE2 measurement was from Cayman Chemical (Ann Arbor, MI). dNTPs was from Bioprobe® Systems (Montreuil-Sous-Bois, France) and [α-32P]-dCTP from Gibco-BRL. Oligonucleotides for primers were synthesized by Institut Pasteur (Paris, France).
TABLE 1. Glomerular parameters

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>Body Wt (g) (N = 12)</th>
<th>Kidney Wt (g) (N = 12)</th>
<th>Glomerular Protein Content (ng/Glomerulus) (N = 20)</th>
<th>Mean Glomerular Diameter (μm) (N = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>40 ± 4</td>
<td>0.23 ± 0.03</td>
<td>48 ± 2</td>
<td>94 ± 2</td>
</tr>
<tr>
<td>5</td>
<td>110 ± 7</td>
<td>0.43 ± 0.05</td>
<td>54 ± 2.6</td>
<td>132 ± 2</td>
</tr>
<tr>
<td>8</td>
<td>210 ± 5</td>
<td>1.15 ± 0.1</td>
<td>83 ± 4.3</td>
<td>172 ± 4</td>
</tr>
<tr>
<td>12</td>
<td>350 ± 10</td>
<td>1.39 ± 0.25</td>
<td>97 ± 5.2</td>
<td>176 ± 4</td>
</tr>
<tr>
<td>38</td>
<td>600 ± 25</td>
<td>1.46 ± 0.5</td>
<td>123 ± 7.5</td>
<td>228 ± 4</td>
</tr>
</tbody>
</table>

*Body and kidney weight, values are mean ± SE of 12 rats. The glomerular protein content for each age is the mean value ± SE of 20 determinations. The value for the mean glomerular diameter for each age represents the mean value ± SE of 100 glomeruli.

(3000Ci/mmol) and (α35S) dATP (>1000 Ci/mmol) were from ICN (Costa Mesa, CA).

RESULTS

Effect of Age on Glomerular Parameters

As shown in Table 1, the glomerular protein content and the glomerular diameter increased continuously with age.

Age-Related Variations in Renal Cortex Kallikrein Activity

As shown in Figure 1, the renal kallikrein activity increased with age. The increase occurred during the first weeks of postnatal life, from 3.7 ± 0.5 ng BK/min per mg prot at Week 3 to 6.6 ± 1.2 ng BK/min per mg prot at Week 8, and then remained stable until Week 38. The slight increase at Week 12 is not significantly different from the values obtained at Weeks 8 or 38.

Effect of Age on the Number of BK Binding Sites

Because the glomerular protein content and glomerular diameter increased with age, we also expressed the number of BK binding sites in fmol/mg glomerular protein and in fmol/μm². Whatever the type of expression used for the results, the same pattern of evolution was observed. Over the age range studied, the number of BK binding sites increased during the first 8 wk of postnatal life (Figure 2). It then decreased to a lower steady level, up to 38 wk. No variations in affinity were observed, and the mean Kd value for each age (3, 5, 8, 12, 38 wk of age) remained in the nanomolar range and was respectively 2.1 ± 0.5; 2.4 ± 0.2; 2.7 ± 0.3; 2.5 ± 0.25; and 2.85 ± 0.3 nM (N = 4) for each age. Specificity of BK2 binding sites of glomeruli from the different age groups was assessed on the basis of competition experiments and the Kᵢ of different BK antagonists are given in Table 2. The specificity of the BK binding sites remained that of BK2 binding sites, since the specific BK1 antagonist des-Arg9-Leu8-BK was without effect. Furthermore, no significant varia-
TABLE 2. *K* values

<table>
<thead>
<tr>
<th>Drugs</th>
<th>3</th>
<th>5</th>
<th>8</th>
<th>12</th>
<th>38</th>
</tr>
</thead>
<tbody>
<tr>
<td>BK (nM)</td>
<td>0.35±0.12</td>
<td>0.28±0.15</td>
<td>0.38±0.12</td>
<td>0.37±0.14</td>
<td>0.42±0.15</td>
</tr>
<tr>
<td>des-Arg9Leu8 BK (M)</td>
<td>&gt;10^-4</td>
<td>&gt;10^-4</td>
<td>&gt;10^-4</td>
<td>&gt;10^-4</td>
<td>&gt;10^-4</td>
</tr>
<tr>
<td>(D-ArgHyp3-D-Phe7)BK (nM)</td>
<td>1.16±0.08</td>
<td>1.27±0.06</td>
<td>1.25±0.1</td>
<td>1.34±0.09</td>
<td>1.37±0.08</td>
</tr>
<tr>
<td>HOE-140 (nM)</td>
<td>0.16±0.02</td>
<td>0.13±0.04</td>
<td>0.12±0.03</td>
<td>0.15±0.06</td>
<td>0.22±0.05</td>
</tr>
</tbody>
</table>

*Comparison of the potency of BK antagonists for competition in ^25l-(Tyr^9)BK binding to glomerular membranes. The mean *K* values were calculated from the following equation: *K* = EC_50/(1 + (L)/K_0), where K_0 is the dissociation constant for ^25l-(Tyr^9)BK. The EC_50 values were calculated from competition studies, and (L) is the concentration of ^25l-(Tyr^9)BK used in the assay (2 nM). Values are means ± SE of triplicate determinations of three independent experiments.

Figure 3. Effect of 10 nM BK on PGE_2 production by 1500 to 2000 freshly isolated glomeruli. Values are expressed as means ± SE. Triplicate determinations of four separate glomeruli suspensions obtained from four rats were performed. *P < 0.01, compared with the 3-week value.

Effect of Age on Glomerular PGE_2 Release Stimulated by BK

The functional effect of the BK2 receptor on glomerul was assessed by BK-induced PGE_2 release. The results are shown in Figure 3. A progressive increase in PGE_2 secretion in response to 10 nM of BK was observed from Week 3 to Week 12. This rise in BK-induced PGE_2 secretion was parallel to those observed for the density of glomerular BK2 receptors. At the 38th week of postnatal life, a small decline was observed and values similar to those obtained for glomeruli from the 5th week were obtained. Interestingly, the diminished response at the 38th week corresponded to a decrease in the number of BK2 binding sites and thus probably indicates the stabilized functional status of the BK2 receptor of the adult rat.

Relative Quantitation of PCR Products

To evaluate the sensitivity and linear response of our scanned densitometric analysis system, we first analyzed PCR products from bacteriophage lambda DNA provided as a control template in Perkin Elmer PCR kit Number 808–008, using serial twofold dilution of the PCR product.

Each diluted aliquot (20 μl) was then electrophoresed through a 2% agarose gel, stained with ethidium bromide, and then quantitatively analyzed as described in "Methods." Figure 4A shows a good relationship between the dilution and the relative value of the densitometric data of each band.

To achieve optimal conditions for PCR, we first synthesized cDNA from 1 μg of total RNA obtained from normal Sprague-Dawley rat renal cortex, and amplified it with an increasing cycle number from 15 to 50 cycles for BK receptor and from 10 to 40 PCR cycles for β-actin (Figure 4C and 4D). The PCR products for BK2 receptor and β-actin increased in a linear manner from 25 to 40 cycles for BK2 and from 15 to 35 cycles for β-actin. We then chose 35 cycles for BK2-mRNA receptors and 25 cycles for β-actin. As shown in Figure 4B, the PCR products for BK2 and β-actin increased as a function of the increasing amount of the RT product used for PCR amplification. Taken together, these results show that under the conditions defined above, PCR can be used in a semiquantitative manner to assess mRNA variation in rat renal cortex.

Sequencing of the PCR product confirmed complete identity, between base 2955 and base 3473, with the rat cDNA sequence previously published (11).

mRNA Expression of the BK2 Receptor During Aging

As shown in Figure 5, BK2-receptor mRNA expression exhibited the same patterns of evolution as those obtained for the variations in the density of BK2 binding sites. We can notice a specific increase in BK2-receptor mRNA expression in 8 and 12-wk-old rats. When compared to the 3-wk-old rats, the expression found in 8-wk-old rats was 2.5-fold higher (Figure 5C and 5D). The specificity of the increase was confirmed by the expression of a housekeeping gene (β-actin), which does not show any variations (Figure 5A), and as a consequence, the ratio of BK to β-actin band intensity was significantly increased at the 8th and 12th weeks of age (Figure 5B).

DISCUSSION

It has been known, for a long time, that during the initial 9 wk of postnatal life in the rat, the functional maturation of the nephrons progress from the jux-

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Figure 4. Optimization of PCR conditions. (A) Comparison of densitometric signal against dilution of a PCR product (bacteriophage lambda DNA). The densitometric value (arbitrary units) is plotted against dilution. (B) The figure represents the relative band intensity of PCR product for BK-receptor and β-ACTIN as a function of increasing amount of RT solution (0.5 to 8 µl). PCR amplification for the BK receptor and for β-actin was performed, respectively, for 35 and 25 cycles. An arbitrary value of 100 was assigned to the sample containing 8 µl of RT product. Each point is the mean ± SE of four independent experiments. (C) and (D) PCR amplification for, respectively, BK-receptor and β-actin mRNA. The data represents the relative band intensity as an increasing function of cycle number. For the BK receptor, an arbitrary value of 100 was assigned to the sample amplified over 50 cycles. For β-actin, an arbitrary value of 100 was assigned to the sample amplified over 40 cycles. Each point is the mean ± SE of four independent experiments.

tamedullary region towards the outer cortex (30). The ontogeny of some renal vasoactive systems has been reported in the rat, such as that of α1 and α2-adrenoceptors (31), renal natriuretic peptide (32), dopamine-1 receptor (33), oxytocin receptors (34), and, of course, the renin-angiotensin system (35–37). The report presented here extends the knowledge of the postnatal maturation of these renal vasoactive systems to the renal kallikrein-kinin system investigated at the cortical level. The maturation of the renal kallikrein-kinin system was essentially characterized by an increase in the mRNA expression of the glomerular B2 receptor associated with an increase in the BK-binding capacity and a rise in BK-induced glomerular PGE2 release. In addition, we found an increase in cortical kallikrein activity, which is consistent with an enhancement of in vivo kinin generation and thereby of B2-receptor activation. The fact that kallikrein activity has been measured in whole-cortex homogenate and not in isolated glomeruli can be intriguing. The primary explanation is that the major sites for kallikrein synthesis and secretion are the granular connecting cells and, to our knowledge, it is not possible to obtain such isolated cells: the cortical homogenate appears to be the best compromise available for assessment of the renal activity of the system. The relationships between kallikrein activity primarily located in the distal tubule and glomerular B2-receptor activation have been previously extensively discussed (2,3,24). It is well admitted that the anatomical data and in vitro studies support the possibility that kallikrein present in the distal tubule may act upstream to its synthesis and secretion site through the release of kinin in the renal circulation and/or in the interstitial space.

Only a few studies have reported age-related changes in the kallikrein-kinin system: one in humans (38) and one in female rats (39). Both studies reported a decrease in urinary kallikrein excretion. One study in fetal and newborn lambs (40) reported an increase in urinary kallikrein excretion during the last trimester of gestation and an even further rise after birth. These increases correlate well with the rise in plasma aldosterone concentration, but a good correlation was also observed between the increase in urinary kallikrein excretion and the rise in RBF, which occurs during fetal and postnatal life. Two more recent studies focused on kallikrein mRNA expression during nephron maturation. With Northern blotting, Clemments et al. (41) detected kallikrein mRNA in the kidney of 1-day-old rats, reaching a maximum expression at 60 days old. Using an in situ hybridization technique, El-Dahr and Chao (42) detected mRNA kallikrein expression in the upper limb of S-shaped bodies of 1-day-old rats. Moreover, the transition from newborn to adult life was associated with a fourfold increase in
renal mRNA accumulation. Both of these studies showed that the renal kallikrein gene is developmentally regulated. Since we observed a progressive increase in the renal kallikrein activity during the first 8 wk of postnatal life, reaching a steady level up to the 38th week, our data are consistent with these observations and with the hypothesis that kallikrein plays a role in the maturation of renal functions. It is well known that BK is a natriuretic and diuretic peptide (2-5) that can reduce (20) the glomerular ultrafiltration coefficient (Kf). Direct evidence for a tonic involvement of the B2-receptor activation in the control of glomerular hemodynamics came from the effect of kinin-receptor antagonists, which demonstrate that BK increases RBF with minor changes in GFR (43). In addition, we recently showed (23) that BK induces in vitro contraction via B2 receptor activation, a possible explanation consistent with reduction of Kf previously reported (20). On the other hand, the early postnatal period is accompanied by an increase in both Kf (44) and single-nephron GFR (30). Although we do not demonstrate any direct link between B2-receptor maturation and maturation of glomerular functions in the report presented here, the increase in the glomerular B2-receptor expression may be relevant with maturational development of glomerular ultrafiltration in the rat.

To our knowledge, no previous work has yet documented the maturation of the renal kallikrein-kinin system regarding the BK-receptor mRNA expression, the BK binding on its receptor, and the functionality of the BK receptor altogether. It is therefore difficult to compare our results with those of others. However, it is well-known that the kallikrein-kinin system is often investigated in parallel with the renin-angiotensin system. Many authors have studied the maturation of the renin-angiotensin system and especially the role of angiotensin II (All) in nephrogenesis and postnatal maturation of the kidney. Pohlova and Jelinek (35) reported that in rats between 20 and 40 days of age, the plasma angiotensinogen concentration increases with age. Wallace et al. (36) also reported an increase in renal renin content throughout the first 6 wk of postnatal life. They also describe an increase in plasma renin activity and concentration during birth and at 3 wk of age, followed by a decrease to adult values. The maximum plasma concentration of All was not attained until 5 wk of age. Grima et al. (45) found an increase in angiotensin-converting enzyme activities during 3 to 8 wk of age in rat lungs and aorta, but they noted a very large decrease in the renal cortex.

Furthermore, a recent study (46) that used quantification of the 125I-[Sar1]All binding (autoradiography) reported a significant rise in All binding between Day 3 and the 8th week of postnatal life of Sprague-Dawley rats, with a spike at 2 wk. Taken together, these studies indicate a very early postnatal maturation in the glomerular All receptors, occurring almost in the first 2 wk of postnatal life, followed by a slight de-
crease at the 8th week, and remaining stable thereafter.

Because it is well-known that the protein content and the surface area of glomeruli increase with age, we normalized our results by expressing the number of BK2-binding sites either in fmol/mg glomerular protein or in fmol/μm². Both types of expression gave the same pattern of evolution. Moreover, such variations are consistent with BK2-receptor mRNA expression and with functional studies showing the age-related increase in PGE₂ release by glomeruli in response to BK stimulation. Taken together, our data indicate that the postnatal maturation of glomerular BK2 receptors occurs during the first 8 wk of life, which is similar to the postnatal maturation previously reported for other renal receptors such as α-adrenoceptors (31), oxytocin receptors (34), and All (46).

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REFERENCES


