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B_2-KININ RECEPTOR LIKE BINDING IN RAT GLOMERULAR MEMBRANES

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SUMMARY:Incubation of a radiolabeled bradykinin analog, [^{125}I]-Tyr^8-BK with a crude membrane preparation obtained from isolated rat glomeruli revealed a time dependent binding. The binding was saturable, reversible and was a linear function of protein membrane concentration. The radiolabeled Tyr^8-BK bound to a single class of binding sites with an equilibrium dissociation constant (K_D) of 3.9±0.7nM and a density (B_{max}) of 31±5 fmol/mg protein. The BK-receptor complex was not affected by angiotensin II or by arginine vasopressin and atrial natriuretic factor. BK binding was reversed by bradykinin (Ki = 0.3 10^{-9} M), and by other kinin analogs in the following order of potency: Lys-BK, Met-Lys-BK, Thi^{5,8}-D Phe^7-BK. However, Des-Arg^9-BK had no effect on binding of the radiolabelled BK. These results are consistent with the presence of a B_2-kinin like receptor in rat glomeruli.

Nonapeptide bradykinin is involved in many biological processes including natriuresis, vasodilatation, inflammation and pain mediation (1). All these potent effects of bradykinin are presumably mediated via one or more specific receptors which have been classified in two types named B_1 and B_2 receptors (1). In addition to the classic and more prevalent kinin receptor (B_2-kinin receptor) which mediates vasorelaxant actions, some tissue may contain another kinin receptor (B_1-kinin receptor) under special conditions such as stress, anoxia, toxic exposure (1) which relays vasoconstrictive responses. These two receptors differ markedly by their affinity for kinins and kinin analogs. While bradykinin is the most potent agonist of B_2-kinin receptor, the presence of B_1-kinin receptor was investigated with DesArg^9-BK which is so far its most specific agonist. Initially the existence of these two receptor types was postulated following pharmacological studies of the biological effects of various kinin agonists and antagonists. More recently these pharmacological data were confirmed with binding studies using radiolabeled ligands. In this respect [^{3}H]-bradykinin binds to membranes from a variety of mammalian tissues including uterus, ileon, duodenum, heart and kidney homogenate(2). In the kidney , specific binding was further demonstrated successively in cortical epithelial membranes (3), in renomedullary interstitial cells (4) and in cortical collecting tubules (5). Bradykinin is considered as a potent vasoactive peptide with a large variety of renal biological effects. Micropuncture studies have shown a decrease in the glomerular capillary ultrafiltration coefficient in rat glomeruli following kinin perfusion (6). Since a large number of vasoactive compounds have been shown to regulate renal glomerular hemodynamics (7) we examined the binding of bradykinin in rat glomerular membranes.

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MATERIALS AND METHODS

Materials : Bradykinin related peptides were purchased from Sigma and Bachem. Radioactive iodine was from Amersham (specific radioactivity 2100 Ci/mmol).

Abbreviations: bradykinin (BK); lysyl-bradykinin (LBK); methionyl-lysyl-bradykinin (MLBK); tyrosine^{8}-bradykinin (Tyr^{8}-BK); Des-Arginine^{9}-bradykinine (Des-Arg^{9}-BK); thienyl^{5,8}-D-phenyl^{7}-bradykinin (Thi^{5,8}-D-Phe^{7}-BK); angiotensin II (A II), arginine-vasopressine (ADH); atrial-natriuretic factor (ANF).

Preparation of iodinated ligand : Tyr^{8}-BK, a synthetic analog of bradykinin with biological potency of the physiologic peptide was used as radiolabeled probe for BK receptor-like binding. Iodination of Tyr^{8}-BK was performed by the chloramin-T method and purification of the labeled product was achieved immediately by HPLC as described previously (8).Briefly, [^{125}I]-Tyr^{8}-BK was separated from unlabeled Tyr^{8}-BK by reverse phase chromatography using a C_{18} microbondapack column (Waters) eluted with 0.04 M triethylammonium formate pH 3.2, containing 21% acetonitrile at a flow rate of 1ml / min.The specific radioactivity was 160 ± 30 Ci/mmol , the ligand stored at - 70°C, showed the same specific binding for up to 20 days.

Preparation of crude glomerular membranes : Sprague Dawley rats (Body weight 150 ± 20g) were used. The kidneys were removed under anesthesia and immediately placed in cold saline. The cortex was dissected with scissors and the glomeruli were isolated by graded sieving (9). The cortical tissue was extensively washed with phosphate-buffered saline : 0.01M, pH 7.4, 0.14M NaCl and passed successively through 180, 125 and 75 µm sieves The glomeruli were collected on the last sieve, resuspended in Tris-HCl 0.05M, pH 7.4 containing EDTA 5mM and centrifuged at 500 x g for 5 min. The pellet was resuspended in the same buffer (1 ml per 10 kidneys) and homogenized in a manual tissue grind (KONTES). This crude membrane preparation was centrifuged at 40000 x g for 20 min, the pellet was stored frozen at -70°C until use up to 2 months later.Proteins were measured by the Lowry method (10) after digestion of the crude membrane glomerular suspension with 2N sodium hydroxide (4/1, vol/vol, 15 min at 100°C).

Binding assay of [^{125}I]-Tyr^{8}-BK : Aliquots (100 to 500 µg of protein) of the membrane extract were incubated in triplicate for 25 min at 37°C in the assay buffer (potassium-phosphate 5 mM, sucrose 0.32M, phenanthroline 2.5mM, leupeptine 10 µM, bacitracin 0.05 %, benzamidin 1mM, captopril 2 µM, BSA 0.2 %) in the presence of increasing concentrations of bradykinin agonist or antagonist and 2.1nM of [^{125}I]-Tyr^{8}-BK. The final assay volume was 0.4 ml. At the end of the incubation time, 4 ml of washing buffer (5 mM potassium phosphate buffer pH 7.2, sucrose 0.32 M) was added. This total volume was filtered on a RAWP Millipore filter (1.2 µm). The filter was washed four additionnal times with 4 ml of washing buffer. The filter-bound radioactivity was determined in a Cristal Multi RIA Packard gamma counter. The specific binding was determined by subtracting cpm bound in the presence of excess unlabeled kinin (10^{-4} M) from cpm bound in the membrane preparation without added unlabeled peptide. Non-specific binding was taken to be the difference between the cpm retained on the filter alone and the cpm determined in the presence of excess unlabeled peptide. Results were calculated using the Kinetic EBDA Ligand computerized program (11) and expressed as mean ± SEM of triplicate measurements of three independent experiments.

RESULTS

Incubation of iodinated BK with a crude membrane preparation of isolated rat glomeruli was time-dependent and reached a plateau after 25 minutes incubation at 37°C and was stable up to 60 minutes as shown in figure 1. Rapid dissociation of [^{125}I]-Tyr^{8}-BK from the crude membrane preparation was observed following the addition of 10^{-4}M unlabeled bradykinin (Figure 1). The total binding represented approximatively 1% of the added radioactivity, the specific and non-specific binding were 58±9 and 33±10 % respectively of the total binding when 2.1nM of ligand was used.

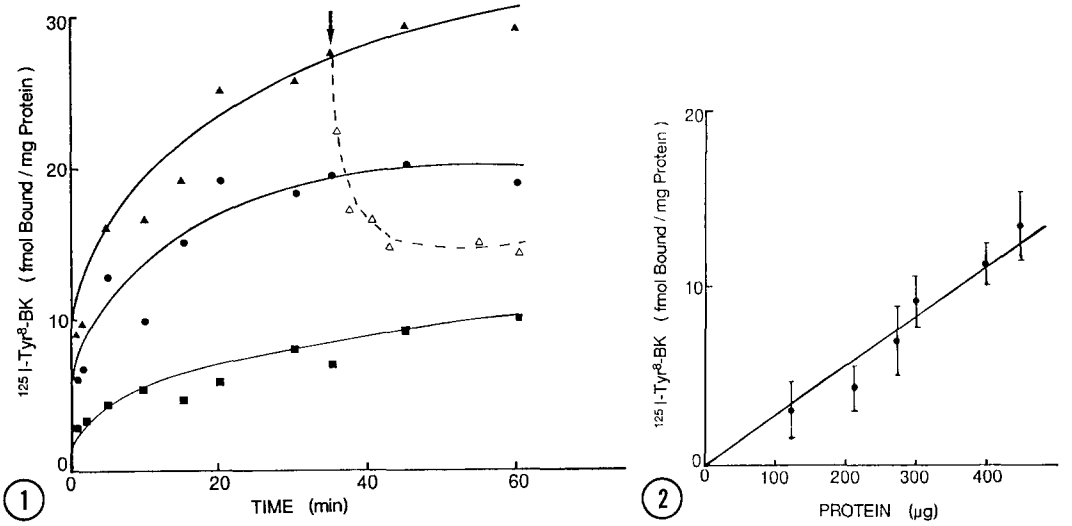


Figure 1 : Time course of [^{125}I]-Tyr^{8}-bradykinin (1.4nM) binding to membranes from rat glomeruli : (▲) Total binding, (■) non specific binding, (●) specific binding. Reversibility of [^{125}I]-Tyr^{8}-bradykinin binding was determinated by adding (arrow) unlabeled peptide (10^{-4}M) after incubation for 35 minutes at 37°C . Data are presented as mean of triplicate determinations from a representative experiment.Similar results were obtained in three independent experiments.

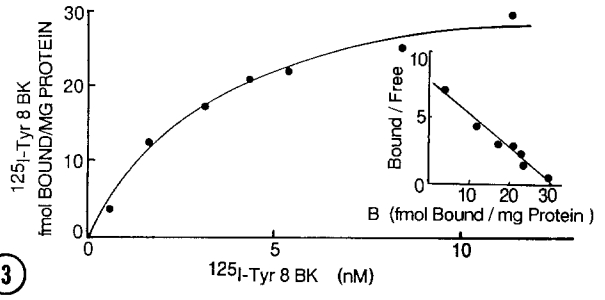
Figure 2: Effect of increasing membrane protein concentration on specific binding of [^{125}I]-Tyr^{8}-bradykinin. Incubations were performed for 30 min at 37°C. Data are presented as mean ± S.E.M. of triplicate determinations in three independent experiments.

Figure 2 shows that specific binding of bradykinin was a linear function of protein concentration from 100 to 450 µg.

No degradation of the iodinated bradykinin during the incubation time was observed . To check this point, the peptide was separated from the incubation medium, repurified by HPLC and either reincubated with membrane preparation or tested according to the bradykinin radioimmunoassay already described (12). No difference was observed between fresh and repurified iodinated bradykinin.

The specific binding of [^{125}I]-Tyr^{8}-BK was saturable as shown in figure 3 and Scatchard analysis gave a straight line indicating a single population of binding sites with an equilibrium dissociation constant (K_D) of 3.9±0.7 nM and a maximum number of binding sites (B_{max}) of 31±5 fmol/mg protein. The computed Hill coefficient was 0.96±0.1 indicating the absence of positive or negative cooperativity.

Competition for radioligand binding was assessed with various kinin analogs and other unrelated peptides (Fig 4) .The order of potency in displacing [^{125}I]-Tyr^{8}-BK binding was as follow BK>LBK>MLBK> Thi^{5,8}-D Phe^{7}-BK. However Des-Arg^{9} -BK up to 10^{-4}M did not significantly affect [^{125}I]-Tyr^{8}-BK binding. Relative potencies of kinins and other peptides are summarized in table 1. Peptides not belonging to the bradykinin superfamily such as angiotensin II ,arginin vasopressin and atrial natriuretic factor did not compete for [^{125}I]-Tyr^{8}-BK binding at concentrations up to 10^{-5} M.



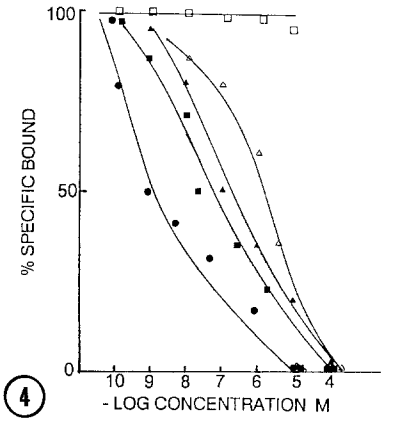


Figure 3 : Saturation with an increasing amount of [^{125}I]-Tyr^{8}-bradykinin and Scatchard analysis (inset) of [^{125}I]-Tyr^{8}-bradykinin binding to membranes from rat glomeruli. Data are presented as mean of triplicate determinations from a representative experiment.Similar results were obtained in three independent experiments. The following values were obtained: K_D=3.9±0.7nM and B_{max}= 31±5 fmol/mg protein .

Figure 4 : Displacement of [^{125}I]-Tyr^{8}-bradykinin binding to membranes from rat glomeruli by BK analogs. Residual binding of [^{125}I]-Tyr^{8}-bradykinin Data are presented as mean of triplicate determinations in three independent experiments. (●) bradykinin; (■) lysyl-bradykinin; (▲) methionyl-lysyl-bradykinin; (△) Thi$^{5-8}$-D-Phe^{7}-bradykinin; (□) Des-Arg^{9}-Bradykinin. Data are presented as mean ± S.E.M. of triplicate determinations from a representative experiment .Similar results were obtained in three independent experiments.

DISCUSSION

These results extend the work of previous investigators regarding the presence of bradykinin receptors in the kidney. So far, the binding of bradykinin has never been characterized in the glomerulus. The first binding studies of bradykinin in mammalian kidney (2) were conducted using ^{3}H-Bradykinin and crude membrane extracts prepared from a whole kidney homogenate and no binding characteristics were given. Using a large number of BK analogs , the possible presence of

TABLE 1: Comparison of potency of kinins and unrelated peptides for [^{125}I]-Tyr^{8}-bradykinin binding. The Ki (mean Ki) were calculated from the equation $Ki = EC_{50} / (1+[L] / K_D)$ where K_D is the dissociation constant for [^{125}I]-Tyr^{8}-bradykinin (3.9 nM) , the EC_{50} values was obtained from competition studies and [L] the concentration of [^{125}I]-Tyr^{8}-bradykinin used in the assay (2.1 nM) . All experiments were performed in triplicate (values are mean ± S.E.M.).

PEPTIDE	Ki (M)
BK	$0.3\pm0.1\ 10^{-9}$
LBK	$4.5\pm1\ 10^{-8}$
MLBK	$1.9\pm0.4\ 10^{-7}$
Thi^{5,8}-D Phe^{7}-BK	$1.3\pm0.3\ 10^{-6}$
Des-Arg^{9}-BK	$> 10^{-4}$
AVP	$> 10^{-5}$
ANF	$> 10^{-5}$
A II	$> 10^{-5}$

a B_2-kinin receptor-like binding activity was also suggested in renomedullary interstitial cells (4). In rabbit, using microdissected nephron segments, BK binding was observed in the cortical collecting tubule and in the outer medullary collecting tubule, however a small but significant binding was also observed in all the other nephron segments including the glomerulus(5). These authors reported a K_D = 12.9 nM and a B_{max} = 73 fmol/mg protein for the highest binding sites of the collecting tubule. The nature of the modulated cellular event remained unknown; In our study, binding was only investigated in isolated glomeruli . We found a higher K_D= 3.9nM but a lower B_{max} = 31 fmol/mg protein. However it is difficult to compare these two studies mainly because they were conducted on different species and different radiolabelled bradykinins were used. In our study we used [^{125}I]-Tyr^8-BK because of its high specific radioactivity, although this analog does not belong to the natural kinin family, it behaves as a partial agonist of bradykinin exhibiting a weaker biological activity (1) and is now widely used for radioligand studies.

Since the glomerulus had angiotensin, vasopressin and atrial natriuretic peptide (ANF) receptors, bradykinin could have bound to one of these peptide receptors, the fact that neither angiotensin II , vasopressin nor ANF affected bradykinin binding ruled out that possibility. The other possibility that non-receptor binding sites , e.g. kininases , could interfere with direct binding to the receptor was carefully examined . In this respect, bradykinin binding was not modified by the addition of bacitracin and captopril indicating that bradykinin was not bound to a kininase II , also known as angiotensin II-converting enzyme , recently identified in rat and human glomerulus (13). High affinity bradykinin binding with post proline cleaving enzyme (PPCE , E.C: 3.4.21.26), a serine protease belonging to the prolyl endopeptidase family, has recently been reported (14) . However it is unlikely that in our study bradykinin binding occurred with PPCE as this enzyme is mainly found in the cytosol (15). Futhermore the purified enzyme bound preferentially with [^{125}I]-Tyr^1-BK and not with [^{125}I]-Tyr^8-BK (14) which was the radioligand used in our study .

A large number of bradykinin agonists and antagonists have been developed in the last decade (16). Replacement of the proline residue in position 7 in the bradykinin aminoacid sequence (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) is a key modification which converts BK agonists into antagonists. In this respect we have tested both agonists, such as: bradykinin, lysyl-bradykinin, methionyl-lysyl-bradykinin and antagonists such as Thi$^{5-8}$-D-Phe^7-bradykinin. A significant reduction in binding was observed indicating that kinin agonists as well as kinin antagonists bound to this receptor site.

Using guinea pig ileon and rabbit aorta, Regoli and Barabe (1) described different kinds of kinin related biological responses which included either vasorelaxant actions (B_2-kinin receptor) or vasoconstrictive responses (B_1-kinin receptor). Recently a renal vasoconstrictive effect of kinin was observed suggesting the possible presence of a B_1-kinin receptor (17).Usually the presence of a B_1-kinin receptor is investigated using the B_1-kinin specific agonist Des-Arg^9-bradykinin. When Des-Arg^9-bradykinin was added to incubation medium no displacement of binding was observed, suggesting a very weak affinity of the glomerular membrane preparation for this ligand. These results are therefore consistent with the characteristics of the classic B_2-kinin receptor.

In conclusion the presence of a high affinity bradykinin binding site in rat glomerular membrane has been demonstrated. This site specificity and affinity appear consistent with those expected of a B_2-kinin receptor. However to demonstrate that these binding data are linked to a receptor site, the nature of the modulated cellular event will have to be investigated. Furthermore, since the glomerulus is a heterogenous structure which includes a large number of different cells, the location of this bradykinin glomerular target is still to be determinated.

REFERENCES

1 Regoli, D. and Barabe, J. (1980) Pharmacol. Rev. 32, 1-46.
2 Innis, R.B., Manning D.C., Stewart J.M., Snyder S.H. (1981) Proc.Natl.Acad. Sci. U.S.A. 78,2630-2634.
3 Cox, H.M., Munday, K.A., Poat, J.A. (1984) Clin. and Exp.-Theory and Practice A6, 1983.
4 Fredrick, M.J., Abel, F.C., Rightsel, W.A., Muirhead E.E., Odya C.E. (1985) Life Sci. 37,331-338.
5 Tomita, K., Pisano J.J. (1984) Am. J. Physiol. 246, F732-F737.
6 Baylis, C., Deen, W.M., Myers, B.D., Brenner, B.M. (1976) Am. J. Physiol. 230, 1148-1158.
7 Dworkin, L.D., Ichikawa, I., Brenner, B.M. (1983) Am. J. Physiol. 244, F95-F104.
8 Girolami, J.P., Alhenc-Gelas, F., Dos Reiss, M.L., Bascands, J.L., Suc J.M., Corvol, P., Menard, J. (1986) Adv. Exp. Biol. Med. A, 137-145.
9 Foidart, J.B., Dechenne, C.A., Mahieu, P., Creutz, C.E., De Mey J. (1979) Invest. Cell Pathol. 645-667.
10 Lowry, O.M., Rosebrough, N.J., Faar, A.L., Rrandall, R.. (1951) J. Biol. Chem. 193, 265-275.
11 Munson, P.J., Robard, D. (1980) Anal, Biochem. 107, 220-239.
12 Alhenc-Gelas, F., Marchetti, J., Allegrini, J., Corvol, P., Menard, J. (1981). Biochem. Biophys. Acta 677, 477-488.
13 Chansel, D., Morin, J.P., Borghi, H., Ardaillou, N., Ardaillou, R. (1987). FEBS Letters 22,247-252.
14 Odya , C.E. ,Dally ,R.D. , Georgiadis , K.E. (1987) Biochem. Pharmacol. 36, 39-49 .
15 Ward P., Bausback, H.H., Odya , C.E. (1987) Biochem. Pharmacol. ,36, 3187-3193.
16 Vavrek, R.J., Stewart, J.M. (1985) Competitive antagonists of Bradykinin. Peptides 6, 161-164.
17 Guilmaraes, J.A., Vieira, M.A.R., Camargo, M.J.F., Maack, T. (1986) Eur. J. Pharmacol. 130, 177-185.