Vol. 147, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1987 Pages 135-139

IDENTIFICATION OF AN ATRIAL NATRIURETIC PEPTIDE SPECIFIC RECEPTOR IN HUMAN KIDNEY

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Received July 10, 1987

SUMMARY: A specific receptor for human atrial natriuretic peptide (h-ANP) was identified in the human kidney using the radioligand binding assay. Samples were prepared from non-malignant renal tissues obtained at nephrectomy of patients with renal carcinoma. Binding studies using [^{125}I]hANP were performed at 0°C for 20 minutes and terminated by a rapid filtration technique. Scatchard plot analysis revealed [^{125}I]hANP bound to a single class of binding site (Kd=0.4±0.2 nM) with a density of 16±4 fmol/mg protein in the renal cortex (n=7). The binding was rapid and maximal binding was obtained within 20 minutes after the start of incubation. Radioligand displacement was observed in a dose dependent fashion when cold hANP was entered into the reaction mixture.. However, unrelated agents, such as angiotensin II or l-epinephrine, did not affect the binding. This is the first time characterization of the hANP receptor in the human kidney has been conducted using a Scatchard plot analysis.

De Bold et al. first reported the presence of a biologically active factor in the rat atrium. This factor was shown to possess strong diuretic action on rats when administered intravenouly (1). Rat and human atrial natriuretic peptides (ANP) have been isolated and their amino acid sequences determined (2,3). In experimental animals, ANP has been shown to cause direct vasorelaxation of vessels, and strong diuresis and natriuresis in kidneys (4). Specific receptors for ANP have been characterized in various animal tissues, including kidney, vessel and adrenal gland. Among these, kidney has been shown to possess the highest density of ANP receptors (5,6). Increased levels of intracellular cyclic GMP has been pointed out as an expression of ANP's effect (7).

In clinical trials involving human subjects (8), injection of ANP intravenously causes strong natriuresis and diuresis, as well as a lowering of the blood pressure. This suggests the presence of specific receptors for ANP in human kidney also. However, characterization of this putative ANP receptor by the radioligand binding assay has not been conducted pre-

0006-291X/87 $1.50

viously with human kidney. In this study we have demonstrated the presence of an ANP specific receptor in human kidney using such an assay.

MATERIALS AND METHODS

Materials Synthetic alpha-human ANP(1-28) (hANP), Met(O)^{12}alpha-human ANP(1-28), rat ANP IV and parathyroid hormone were purchased from Peptide Research Institute Inc., Osaka, Japan. [^{125}I]hANP was kindly given by Dinabott Ltd., Tokyo, Japan. The specific activity of [^{125}I]hANP was 1241-1634 Ci/g. L-epinephrine, l-norepinephrine were purchased from Sigma Chemical Co., St.Luis, Missouri, U.S.A.. Angiotensin II was purchased from Ciba Geigy(Japan) Ltd., Tokyo, Japan.

Membrane preparation Human kidney was obtained at nephrectomy of patients with renal carcinoma. Non-malignant tissue was immediately separated, rapidly frozen, and stored at -80°C until use. All the tissues were used within 2 months of acquisition. After removal of the renal capsule, the renal cortex was minced with scissors, homogenized in a Brinkmann Polytron, two 10 second pulses at a setting of 5.5, and suspended in a buffer containing 50 mM TrisHCl, 10 mM $MgCl_2$, 0.3% bovine serum albumin and 0.1% bacitracin pH7.5 at 0°C. The homogenate was centrifuged at 400 x g for 10 minutes at 4°C. The pellet was discarded, and the supernatant was passed through double layers of gauze. This was followed by centrifugation at 28,000 x g for 10 minutes at 4°C. The resulting pellet was resuspended with Potter-Elvejheim Teflon glass tissue homogenizer to a final concentration of 0.2-3.0 mg of protein/ml in the buffer described above. The protein concentration was determined by the method of Lowry et al. (9) using bovine serum albumin as a standard.

[^{125}I]hANP binding assay The binding studies were carried out at 0°C in a buffer containing 50 mM TrisHCl, 10 mM $MgCl_2$, 0.3% BSA and 0.1% bacitracin, pH7.5. 50 microliters of this buffer containing various concentration of [^{125}I]hANP (0.01-2 nM), with or without the competing agent, were added to 100 microliters of the membrane suspension. The binding was allowed to proceed for 20 minutes. Isolation of the ligand/membrane complex was performed by immediate filtration through Millipore filters with polyethilenimine-treated Whatman GF/C filter (10) with 4 ml of ice-cold incubation buffer. The filters were washed twice with an additional 4 ml of the cold buffer. The filters were dried and radioactivity was counted in a PGD Auto Gamma, Packard Instrument Company Inc., ILL, U.S.A.. All assays were performed in duplicate. Non-specific binding were determined in the presence of 10^{-5} M of cold hANP.

RESULTS

The binding of [^{125}I]hANP to human renal cortical membranes was rapid and saturable. Fig. 1 shows the time course of [^{125}I]hANP binding to human renal cortical membranes, a representative of several experiments. Maximum binding was obtained within 20 minutes after the start of incubation, then gradually the amount of binding was decreased. The apparent rate constant of the pseudo-first order reaction, Kap, was calculated to be 0.17 min^{-1} (R=0.98) (11).

Scatchard plot analysis (Fig. 2) revealed the existence of a single class of binding site with high affinity (Kd=0.4±0.2 nM, Bmax=16±4 fmol/mg protein, n=7) in the human renal cortical membrane. Hill's coefficiency

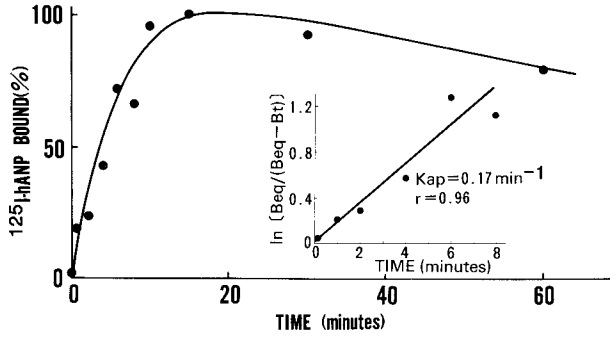
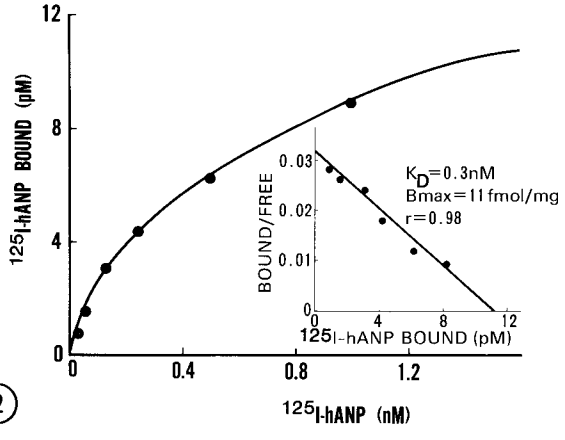


Fig. 1. Kinetic analysis of [^{125}I]hANP binding to the human renal cortical membranes as a function of time is shown. The specific binding of the radioligand was rapid and saturable. Maximal binding was obtained within 20 minutes after the start of incubation. Insert; Pseudofirst order kinetic plot of [^{125}I]hANP binding. Data were used to determine Bt (amount of [^{125}I]hANP bound at time "t") and Beq (amount of [^{125}I]hANP bound at equibrium). This line (r=0.96) has a slope, Kap, equal to the observed rate constant of the pseudofirst order reaction.

was 0.98, which suggests either a lack of cooperation between receptors, or the presence only of receptors with similar affinities.

Competition study of various agents with [^{125}I]hANP for the binding to human renal cortical membranes were performed (Fig. 3). Binding of [^{125}I]hANP was competed by its related peptides, i.e., unlabeled alpha-hANP(1-28), Met(O)^{12}alpha-hANP(1-28) and rat ANP IV, in a dose dependent



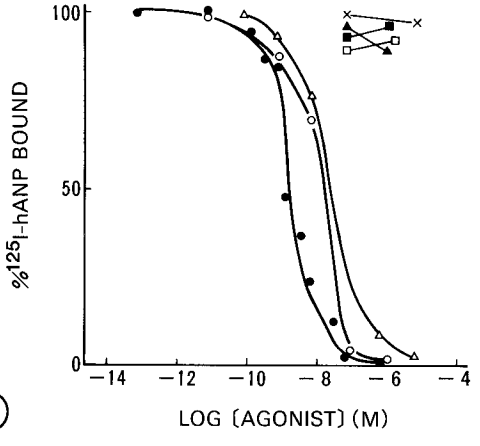


Fig. 2. A representative saturation curve of [^{125}I]hANP binding to the membranes from the human renal cortex is shown. Insert; Scatchard plot analysis showed a straight line, indicating a single class of binding site. Hill's coefficiency is 0.98. Kd=0.3 nM, Bmax=11 fmol/mg protein.

Fig. 3. Competition study of various agents with [^{125}I]hANP for the binding to the human renal cortical membranes. Kd of each agent is as follows; alpha-hANP(1-28) (●-●), 0.78 nM, Met(O)^{12}alpha-hANP(1-28) (o-o), 12 nM, rat ANP IV (△-△), 19 nM. Angiotensin II (▲-▲), l-norepinephrine (■-■), l-epinephrine (□-□), and parathyroid hormone (x-x), all showed Kd of 10^{-6} M.

fashion(10^{-4}-10^{-13} M). Kd of each peptide against the binding of [^{125}I]-hANP to human renal cortical membranes were 0.78 nM, 12 nM and 19 nM, each respectively. Unrelated agents, such as angiotensin II, l-epinephrine l-norepinephrine, parathyroid hormone, all showed Kd of $>10^{-6}$ M. Each Kd was determined from a function of the EC_{50} of each agent, that is, $Kd = EC_{50}/(1+[ANP]/Kanp)$, [ANP] represents the concentration of [^{125}I]hANP used. Kanp is the Kd of hANP itself (11).

DISCUSSION

The present study demonstrate, for the first time, the presence of a single class of binding site for alpha-hANP in the human renal cortical membranes using the radioligand binding assay. In the recent animal studies, the glomeruli were shown to possess the highest affinity for ANP (5,6,13,14). Thus it had been hypothesized that the main site of action and the highest binding of ANP in the human kidney existed in the renal cortex rather than in the medulla. High concentration of [^{125}I]hANP binding sites in human renal cortex was also pointed out previously by radioautographic study (12). In our study, however, whether glomeruli are actually responsible for this obtained value of affinity for hANP is unknown. Further localization of hANP receptors in the human kidney is pending.

The data complied here are comparable to those of Napier et al. (14), who demonstrated the presence of ANP specific receptors in the rat renal cortical membranes. However, the value of Kd reported in their study (0.05 nM) is different from the ones given here (0.4±0.2 nM). This is probably due to differences in the experimental protocol, including differences in the methods of membrane preparation and in the subject species utilized, i.e., rat versus human. Together with other studies (7,14), the receptor identified here might play a key role in diuretic and natriuretic action of hANP in human kidney.

In experimental animals, ANP is known to block the responses of vascular strips to angiotensin II or norepinephrine (15). Similarly, interference to ANP binding sites by those agents appeared possible. However, our data showed no evidence of such interferences in the human renal cortex. Thus, hANP binds its receptor independently from those active substances in the human renal cortex.

Involvement of ANP receptors in the development of high blood pressure in genetically hypertensive animals has been suggested (16). In human, increased diuretic and natriuretic responses to hANP administration have been observed in hypertensive subjects, compared with those in normotensive subjects (17). Thus, different Kd or Bmax values appeared possible between

those two groups. However, those differences could not be established due to the small sample size in this study. Further studies are required to evaluate the involvement of ANP receptors in various pathophysiological phenomena involving the human kidney.

REFERENCES

1. De Bold A.J., Borenstein H.B., Veres A.T., and Sonnenberg H. (1981) Life Sci. 28, 89-94.
2. Kangawa K. and Matsuo H. (1984) Biochem. Biophys. Res. Commun. 118, 131-139.
3. Kangawa K., Fukuda A., and Matsuo H. (1985) Nature 313, 397-400.
4. Currie M.G., Geller D.M., Cole B.R., Boylan J.G., Yusheng W., Holmberg S.W., and Needleman P. (1983) Science 221, 71-73.
5. Bianchi C., Gutkowska J., Thibault G., Garcia R., Genet J., and Cantin M. (1985) Histochem. 82, 441-452.
6. Murphy K.M.M., McLaughlin L.L., Michener M.L., and Needleman P. (1985) Eur. J. Pharmacology 111, 291-292.
7. Tremblay J., Gerzer R., Vinay P., Pang S.C., Beliveau R., and Hamet P. (1985) FEBS Lett. 181, 17-22.
8. Richards A.M., Nicholls M.G., and Ikram H. (1985) Lancet 1, 545-549.
9. Lowry O.H., Rosebrough N.J., Farr A.L., and Randall R.J. (1951) J. Biol. Chem. 193,265-275.
10. Bruns R.F., Lawson-Wendling K., and Pugsley T.A. (1983) Anal. Biochem. 132, 74-81.
11. Williams L.T., Mullikin D., and Lefkowitz R.J. (1976) J. Biol. Chem. 251, 6915-6923.
12. Manthyh C.R., Kruger L., Brecha N.C., and Manthyh P.W. (1986) Hypertension 8, 712-721.
13. Carrier F., Thibault G., Schiffrin E.L., Garcia R., Gutkowska J., Cantin M., and Genet J. (1985) Biochem. Biophys. Res. Comm. 132, 666 -673.
14. Napier M.A., Vandlen R.L., Albers-Schornberg G., Nutt R.F., Brady S., Lyle T., Winquist R., Faison E.P., Heinel L.A., and Blaine E.H. (1984) Proc. Natl. Acad. Sci. 81, 5946-5950.
15. Kleinert H.D., Maack T., Atlas S.A., Januszewicz A., Sealy J.E., and Laragh J.H. (1985) Hypertension 6(Suppl. I), I-143-I-147.
16. Hirata Y., Ganguli M., Tobian L., and Iwai J. (1984) Hypertension 6(Suppl. I), 148-155.
17. Richards A.M., Nichools M.G., and Espiner E.A. (1985) Hypertension 7, 812-817.