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CHARACTERIZATION OF RENAL GLOMERULAR ENDOTHELIN RECEPTORS IN THE RAT

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Specific receptors for Endothelin (Et) have been identified in the glomerulus of rat kidney. Human ^{125}I-ET binds to a single population of high affinity receptors in glomerular membranes from normal rats with a mean equilibrium dissociation constant of 200 pM. The binding was time and temperature-dependent, saturable and reversible. The Et-receptor complex was not affected by either vasopressin, atrial natriuretic factor or angiotension II.

Endothelin (Et) is a 21 amino acid peptide, which has been recently isolated from porcine aortic endothelial cells (1). Porcine (1) and human (2) Et are identical and share some homology to rat Et (3). The cloning and sequence analysis of the complementary DNA of its precursor, prepioendothelin (1) has aroused interest in its potential physiologic or pathophysiologic significance. The present study was designed to determine the presence of specific receptors for Et in rat glomeruli.

MATERIALS AND METHODS

Endothelin was purchased from the Peptide Institute Inc. (Osaka, Japan), labelled by the lactoperoxidase method and purified by HPLC.

Protein concentration was measured by the Coomassie Blue method as modified by Bradford (4) using bovine serum albumin as standard.

Male Sprague-Dawley rats weighing 250-300 g were anesthetized with sodium pentobarbital (30 mg/kg body weight, i.p.), their kidneys were removed and the cortex was separated and placed in ice-cold 0.9% NaCl. Rat glomeruli were isolated by graded sieving (5). The glomerular isolation thechnique have been described in detail elsewhere (6).

The glomerular suspension was homogenized for 1 min with a Polytron, centrifuged at 30000 g for 30 min, and resuspended in 1 ml 0.05 M Tris-HCl (pH 7.2). An aliquot was taken for protein determination. 50 μg of glomerular membrane proteins were incubated in duplicate for 120 min at 20°C, in the presence of increasing concentration of unlabelled Et (10^{-13} to 10^{-7} M) and 40 pM ^{125}I-Et in a final volume of 1 ml. The assay buffer contained 50 mM

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Tris-HCl (pH 7.2), 1 μM aprotinin, 0.1% bacitracin, 0.5% bovine serum albumin (BSA), 5 mM $MgCl_2$, and 0.4% PMSF. The reaction was stopped by dilution with 3.5 ml of Tris-HCl (pH 7.2) and rapid filtration through Tris-containing 0.2g%/BSA-treated Whatman GF/C filters, which were then rinsed three times with 3 ml of Tris-HCl (pH 7.2) and counted in a LKB-Gamma counter (Turku, Finland) with 65% efficiency. Non specific binding was defined as radioactivity bound in the presence of saturating concentrations (10^{-6}M) of unlabeled Et and represented 3-6% of total radioactivity. To determine association rates, glomeruli were incubated with 25-125 pM ^{125}I-h Et for varying periods of time up to 240 min. Dissociation rates were studied at 0^{o}C and 20^{o}C in competitive binding inhibition studies, glomerular membranes were incubated with human Et and either ANF (99-126), angiotensin II or AVP.

RESULTS AND DISCUSSION

The binding data were analysed by processing the raw data with the computer-based program EBDA (7) and then the density and affinity of binding sites were determined using the computer-based LIGAND program (8). Competition experiments were performed to avoid the effect of changes in specific activity of the labelled ligand, since previous studies had already shown that ^{125}I-Et and unlabelled Et to be similar in saturation and competition experiments.

The binding of Et to glomerular membranes from normal rats varied linearly as a function of protein concentration up to 75 μg. Binding was time- and temperature-dependant (fig. 1). Specific binding reached equilibrium at 90-120 min of incubation at 20^{o}C. The non-specific binding represented less than 5% of the total cpm. Unrelated peptides such as angiotensin II, AVP or ANF (99-126) (fig. 2) failed to compete with ^{125}I-Et binding. Competitive binding inhibition data from experiments with unlabelled hEt afforded a best

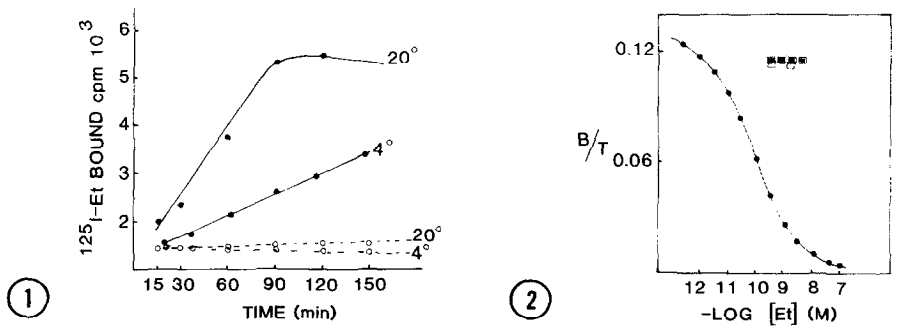

Figure 1: Binding of ^{125}I-Et to 50 μg of glomerular membrane proteins as a function of time at 4^{o}C and 20^{o}C. The dotted lines represent the non specific binding.

Figure 2: Representative competition curve of Et in glomerular membranes. Kd = 182 pM. Bmax = 420 fmol/mg prot.
□—□ , ○—○ , ⊡—⊡ represent displacement by angio II, vasopressin and ANF (99-126) respectively.

fit to a single affinity binding model, and showed a mean value for the *equilibrium dissociation constant* (Kd) of 200 ± 32 pM. The density of binding sites (Bmax) was 450 ± 122 fmol/mg protein. No degradation of the peptide was noticed in presence of protease inhibitors after separation of the incubation media on reverse phase HPLC (not shown).

The association rate constant at 20°C was estimated by examining the binding at two different concentrations of ^{125}I-Et at various times. The dissociation experiments were performed by adding 10^{-6} M of unlabelled Et to membranes after equilibrium had been reached (120 min) and the reversibility of the complex was followed up to 180 min.

The kinetic constants (K+1, K-1) were obtained from the integrated rate equation or from the second order (association) and pseudo-first order (dissociation) equations in order to determinate the dissociation constant (Kd). The non equilibrium dissociation constants were 282 pM derived from integrated rate equation from association curves and 82 pM from the association and dissociation curves.

The present study demonstrates then the presence of specific Et receptors in rat glomeruli. Rakugi (7) reported that Et inhibits renin release from isolated rat glomeruli: the administration of ET resulted in severe renal vasoconstriction, fall in the glomerular capillary ultrafiltration coefficient and glomerular filtration rate (8). The presence of specific Et receptors in the glomeruli is in agreement with this physiological phenomena reported.

It is possible that Et by regulating its glomerular receptors may play an important role in glomerular function under certain physiological or pahtophysiological conditions, such as renal failure.

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