Distribution of atrial natriuretic factor receptors in dog kidney fractions

André De Léan, Patrick Vinay* and Marc Cantin

Clinical Research Institute of Montreal, 110 Pine avenue, West, Montreal, Quebec H2W 1R7 and **Department of Physiology, Faculty of Medicine, University of Montreal, Canada*

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Specific receptors for atrial natriuretic factor were studied in purified glomeruli, proximal tubules, thick ascending limbs of Henle's loops and collecting ducts from dog kidney. Glomeruli contain the highest concentration of receptor sites ($pK = 9.9$, $B_{max} = 200$ fmol/mg protein), followed by collecting ducts ($pK = 9.4$, $B_{max} = 150$ fmol/mg). Low levels of receptor sites were also detectable in thick ascending limbs of Henle's loops ($pK = 9.4$, $B_{max} = 36$ fmol/mg) while proximal tubules were completely devoid of specific binding sites. These results indicate that the glomeruli appear to be the primary site of interaction of artrial natriuretic factor in kidney cortex but that it might also act in the medulla on lower nephron tubular function.

Atrial natriuretic factor *Vasoactive peptide* *Peptide receptor (Dog kidney)* *Glomerular function* *Tubular function*

1. INTRODUCTION

Atrial natriuretic factor (ANF) contained in specific granules of atrial cardiomyocytes [1,2] seems to be released in response to various hemodynamic conditions associated with atrial distension or stretch [3–6] by inducing diuresis, natriuresis and vasorelaxation [1–7]. Various sites of action of ANF have been documented, including arterial muscular tone [8,9], urinary sodium and water excretion [10,11], adrenal mineralocorticoid secretion [12,13] and pituitary vasopressin secretion [14]. The mode of action of ANF on kidney function is still debated [7]. The hormone appears to enhance directly the glomerular filtration rate and both sodium and water filtration fractions [15,16]. An inhibitory effect on tubular reabsorption has also been proposed [16,17]. Specific high-affinity receptor sites have been documented in aorta [18], adrenal cortex [19], posterior lobe of the pituitary gland [14] and in kidney cortex [18]. We have documented the distribution of ANF receptors in glomeruli and tubules purified from dog kidney. The results perfectly correlate with a parallel study on ANF-induced cGMP production in the same preparations [20] as well as with the autoradiographic localization of the in vivo uptake of ^{125}I-ANF in rat kidney [21].

2. MATERIALS AND METHODS

2.1. *Dog kidney fractionation*

Glomeruli, proximal tubules, thick ascending limbs of Henle's loops and collecting ducts were purified from dog kidney as described [22]. Two kidneys were excised and rinsed in ice-cold Krebs-Henseleit. The cortex, red medulla and white medulla were dissected, minced and digested for 45 min with collagenase in Krebs-Henseleit buffer at 37°C. Glomeruli were purified from the cortical digest by passing through a 90 μm sieve which retained the glomeruli. The remaining fraction completely devoid of glomeruli was fractionated on a Percoll gradient to purify proximal tubules [22]. Red and white medulla collagenase digests were

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similarly fractionated on a Percoll gradient to prepare thick ascending limbs of Henle's loops and collecting ducts, respectively. All tissue fractions were kept on ice until assayed.

2.2. *ANF receptor assay*

(Arg^{101}-Tyr^{126}) ANF was iodinated with chloramine-T [19] to a specific activity not less than 1000 Ci/mmol. Kidney fractions were homogenized in ice-cold buffer containing 20 mM $NaHCO_3$ and 1 mM EDTA in a Teflon-glass homogenizer, centrifuged at 40000 × *g* for 10 min, then washed once with buffer and resuspended in ice-cold incubation buffer containing 50 mM Tris-HCl (pH 7.4) at 25°C, 100 mM NaCl, 5 mM $MnCl_2$ and 0.1 mM EDTA. ANF receptor binding was performed by incubating at 25°C for 90 min 30000 cpm ^{125}I-ANF (15 fmol) with 25, 50 or 100 μg membrane protein for glomeruli, collecting ducts and Henle's loops, respectively, in a total volume of 1 ml incubation buffer supplemented with 0.2% bovine serum albumin and containing the indicated concentration of unlabeled ANF. The reaction was stopped by dilution with 4 ml ice-cold incubation buffer and filtration through GF/C glass fiber filters (Whatman) pretreated with 1% polyethyleneimine. The filters were then immediately rinsed with 20 ml ice-cold incubation buffer and ^{125}I-ANF trapped on the filters was counted in an LKB Quatro gamma counter with an efficiency of 80%. Protein was measured using the Bradford assay kit (BioRad).

2.3. *Data analysis*

Competition curves were analyzed by nonlinear least-squares curve fitting using a model for the law of mass action and including provision for multiple ligands and classes of binding sites together with nonspecific binding [23]. A model involving 2 classes of binding sites was retained only when the resulting goodness of fit was statistically significantly better than that for a single class of sites.

3. RESULTS

Specific high-affinity receptor sites for ANF were measured by competitive binding assay using rat ^{125}I-ANF. Purified glomeruli displayed the highest level of ANF specific binding. Typical competition curves displayed an IC_{50} for ANF of 250–300 pmol/l (fig.1). The curves were also flatter with a slope factor of 0.6–0.7. When these curves were analyzed according to models for 1 or 2 classes of sites, the model for both a high- and a low-affinity class of binding sites was statistically significantly better ($p < 0.01$). The class with high affinity displayed a p*K* of 9.9 ± 0.3 and a density of 205 ± 102 fmol/mg protein while the lower affinity component was characterized by a p*K* of 8.0 ± 0.3 and a larger capacity of 4800 ± 3200 fmol/mg protein (table 1). Following removal of all glomeruli by sieving, the collagenase digest of kidney cortex provided a source for proximal tubules. That preparation was completely devoid of receptor sites for ^{125}I-ANF and absolutely no specific binding could be documented above nonspecific binding level (850 cpm/tube). These results indicate that all high-affinity binding sites in dog kidney cortex are located in glomeruli. Fig.1 also shows that specific binding sites for ANF were also present in the medulla, especially in collecting ducts obtained from white medulla (148 ± 43 fmol/mg protein), and to a smaller extent in thick ascending limbs of Henle's loops prepared from red medulla (36 ± 8 fmol/mg protein). Only high-affinity binding sites could be documented in these 2 preparations with p*K* of 9.4 (table 1).

Table 1

Distribution of ANF binding sites in dog kidney fractions

Fraction	p*K*[a]	*R*[a] (fmol/mg protein)
Glomeruli (n = 4)[b]	9.9 ± 0.3[c] 8.0 ± 0.3	205 ± 102 4800 ± 3200
Proximal tubules (n = 3)	–	0[d]
Thick ascending Henle's loops (n = 3)	9.4 ± 0.1	36 ± 8
Collecting ducts (n = 7)	9.4 ± 0.2	148 ± 43

[a] p*K*, $\log_{10}$ of equilibrium constant (reciprocal of dissociation constant); *R*, total binding capacity
[b] Number of experiments
[c] Mean ± SE
[d] No specific binding above nonspecific level

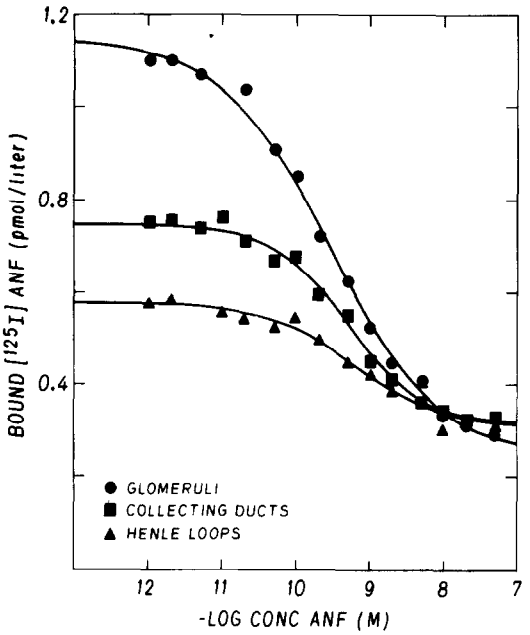

Fig.1. Competitive binding curves for ANF in dog kidney fractions. ^{125}I-ANF (15 pM) was incubated at 25°C for 90 min with varying concentrations of unlabeled ANF and 26, 44 and 97 μg protein/ml of membranes from glomeruli, collecting ducts or Henle's loops, respectively. Membrane-bound ^{125}I-ANF was separated by filtration and washing on GF/C glass fiber filters.

4. DISCUSSION

These results confirm and extend a previous report by Napier et al. [18] of specific receptor sites for ANF in kidney cortex as well as in a porcine kidney tumor cell line of medullary origin. The localization of ANF binding sites also perfectly correlates with that for ANF-induced cGMP production reported by Tremblay et al. [20] in a parallel study. Interestingly, ANF-sensitive particulate guanylate cyclase has been documented in all target organs also containing ANF receptor binding sites, strongly suggesting a close association of ANF receptor with guanylate cyclase in the plasma membrane of target cells. The localization within kidney cortex of ANF binding sites only in the glomeruli was also observed by Bianchi et al. [21]. These results strongly suggest that the glomeruli and not the proximal tubules constitute the primary site of interaction of ANF in kidney cortex. Micropuncture studies of the effect of ANF on kidney function have also documented a primary influence of glomerular filtration rate with a secondary increase in water and sodium excretion rate [15]. The same studies could not however preclude changes in medullary blood flow distribution or in ion transport by lower nephron segments [15]. The observation of specific receptor sites in ascending limbs of Henle's loops and especially in collecting ducts suggests that ANF might also modulate ion and water transport. The presence of a low level of ANF uptake in vasa recta documented by Bianchi et al. [21] could not be confirmed in these studies because vascular structures were not recovered in the collagenase digests.

The localization of ANF binding sites in the kidney strikingly correlates with that for vasopressin [24]. Vasopressin receptors not coupled with adenylate cyclase activation have been identified in glomeruli and appear to correspond to the same V1 subclass also found in vascular smooth muscle [24]. V2 subtype receptors are localized to Henle's loops and collecting ducts and are positively coupled to adenylate cyclase [24]. It is not yet known whether glomerular and collecting duct receptors for ANF might correspond to distinct subtypes. Further characterization of ANF receptor components in these various kidney fractions will help to ascertain their similarity as well as their mechanism of action.

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