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URODILATIN AND ß-ANF: BINDING PROPERTIES AND ACTIVATION OF PARTICULATE GUANYLATE CYCLASE

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Summary: Urodilatin (ANF-(95-126)) and ß-ANF, the antiparallel dimer of ANF-(99-126), are naturally occurring members of the ANF family. We studied their receptor binding properties in human platelets and Triton-solubilized membranes from bovine adrenal cortex and their ability to activate particulate guanylate cyclase in bovine adrenal cortex. In human platelets containing R_2-receptors not coupled to particulate guanylate cyclase urodilatin binds with similar affinity as ANF-(99-126) (K_D: 55 pM), whereas ß-ANF has an affinity lower than the truncated ANF-(103-123) (K_D: 295 pM and 154 pM). Scatchard analysis indicates one binding site for urodilatin as well as for ß-ANF. In adrenal cortex containing predominantly R_1-receptors coupled to particulate guanylate cyclase, urodilatin binds with a higher affinity (K_D: 30 pM) than ANF-(99-126) (K_D: 52 pM) and stimulates to a similar extent to ANF-(99-126) (about twofold at 1µM), whereas ß-ANF has a smaller affinity (K_D: 120 pM) and stimulates particulate guanylate cyclase to a lower extent than ANF-(99-126). The data from platelets and adrenal cortex show that ß-ANF has low binding affinities but stimulates particulate guanylate cyclase, whereas urodilatin appears to be a physiological R_1-agonist.

Urodilatin (ANF-(95-126) is a recently discovered NH_2-terminal extended member of the family of atrial natriuretic peptides (1). It is secreted in the kidney by a different posttranslational processing of the prohormone ANF-(1-126). Whereas ANF-(99-126) is the circulating form in plasma, urodilatin appears to be the major form of ANF-like material in urine (2) and is not biologically inactivated by the enzyme that inactivates ANF-(99-126) (3). In animal experiments urodilatin like ANF-(99-126) showed natriuretic, diuretic as well as vasodilatory effects (2). Binding properties and effects on particulate guanylate cyclase (PGC) are not known for urodilatin.

ß-ANF, the antiparallel dimer of ANF-(99-126), is one of the atrial natriuretic factors which has been isolated from the human heart (4). It is also found in the circulation, especially in patients with heart failure (5). When administered intravenously to healthy human subjects, ß-ANF had smaller effects on blood pressure but a more pronounced effect on diuresis and natriuresis compared to ANF-(99-126) (6).

Hirata and collegues (7) have shown that ß-ANF binds to vascular smooth muscle cells with equal affinity as ANF-(99-126), while the formation of intracellular cyclic GMP was significantly lower compared to ANF-(99-126). However, it is not known to which ANF receptor ß-ANF binds. There are at least two subtypes of ANF membrane surface receptors, one coupled to particulate guanylate cyclase (R_1), the other devoid of guanylate cyclase activity (R_2). ANF acts, at least in part, by binding to the R_1-receptor coupled with guanylate cyclase (8,9) through activation of this enzyme. Therefore we have compared the binding affinities of urodilatin and ß-ANF with those of other ANF analogs in Triton-solubilized membranes from bovine adrenal cortex (BAC) and in human platelets. We also measured stimulation of particulate guanylate cyclase in Triton-solubilized membranes from adrenal cortex. We found that ß-ANF stimulates particulate guanylate cyclase, but has low binding affinities in both tissues studied. Urodilatin showed characteristics of a typical R_1-agonist in binding and activation of particulate guanylate cyclase.

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MATERIALS AND METHODS

Materials: Biosynthetic rat ANF-(99-126) and human urodilatin (ANF-(95-126) were obtained from Bissendorf Biochemicals, Wedemark, FRG. Rat atriopeptin I (ANF-(103-123)) and rat atriopeptin III (ANF-(103-126)) were purchased by Peninsula, Belmont, CA, USA. ß-ANF was from Peptide Institute, London, GB. Triton X-100, hydroxyethylpiperazineethanesulphonic acid (HEPES), ethyleneglycoltetraacetic acid (EGTA), polyethylenimine (PEI), polyethyleneglycol (PEG), prostacyclin (PGI_2), bovine serum albumin and phenylmethylsulfonylfluoride (PMSF) were from Sigma Chemicals, Deisenhofen, FRG. Percoll™ was from Pharmacia, Freiburg, FRG and aprotinine was from Bayer Leverkusen, FRG. [^{125}I]-iodo-rANF-(99-126) were obtained from Amersham, Braunschweig, FRG. All other chemicals were of analytical grade.

Preparation of adrenal cortex membranes: Fresh bovine adrenal glands were obtained from the local slaughterhouse. The cortex was homogenized with a Potter-Elvehjem homogenizer and suspended in a 20 mM HEPES-buffer, pH 7.4 containing 5 mM EDTA. The particulate fraction was obtained by twofold high-speed centrifugation (100 000 x g for 15 min at 4°C). The pellet was resuspended in a 20 mM HEPES buffer containing 0.5 mM EDTA. Membranes were solubilized with Triton X-100 (final concentration 1% v/v) for 10 min at 4°C. Solubilized membranes were recovered by centrifugation (100 000 x g for 30 min at 4°C) in the supernatant fraction. Aliquots were frozen in liquid nitrogen and stored at -70°C until assayed. Protein was measured by the method of Bradford with bovine serum albumin as a standard (10).

Preparation of platelets: Human platelets were prepared as described elsewhere (11). In brief, blood was anticoagulated with citrate, carefully layered on a discontinuous Percoll™-gradient (specific gravity 1.07 g x ml^{-1}) and centrifuged for 10 min at 2000 x g and 24°C. The interphase was removed and platelets were washed twice in binding assay buffer (134 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 5.5 mM glucose, 0.36 mM NaH_2PO_4, 5 mM HEPES, 12 mM $NaHCO_3$, 3 g x l^{-1} bovine serum albumin, 70 mg x l^{-1} PGI_2, pH 7.4) by centrifugation at 2000 x g for 10 min at 24°C onto a Percoll-cushion (specific gravity 1.07 g x ml^{-1}). The final interphase was removed and the platelet concentration was adjusted to a final concentration of 500 000 platelets x ml^{-1}.

Determination of guanylate cyclase activity: Guanylate cyclase activity was assessed as previously described (12). The reaction mixture (100 ml) contained 1 mM 1-methyl-3-isobutyl-xanthine, 100 mM EDTA, 5.5 mM creatine phosphate, 50 mg creatine phosphokinase, 3 mM $MnCl_2$ and 1 mM guanosine 5'-triphosphate (GTP) in a 50 mM triethanolamine/HCl buffer, pH 7.5. The tubes were preincubated for 10 min at 37°C and the reaction was started by the addition of 20 ml enzyme solution (protein concentration 0.65 mg x ml^{-1}). The reaction was stopped after 10 min by the addition of 1 ml of boiling EDTA (30 mM). Formed cyclic GMP was measured by radioimmunoassay as described in detail elsewhere (12). Results shown are means from at least four experiments done in duplicates. Standard errors of the mean of the different experiments were less than 10% and were omitted for clarity.

Determination of binding properties: Triton X-100-solubilized membranes from BAC (protein concentration 0.6 mg x ml^{-1}) or platelets (500 000 x ml^{-1}) were mixed with 8 pM [^{125}I]-iodo-rANF-(99-126) (specific activity 2000 Ci x mmol^{-1}) and with increasing concentrations (0 to 256 nM) of the following unlabelled ANF analogs: ANF-(99-126), ANF-(103-126), ANF-(103-123), ß-ANF or urodilatin (ANF-(95-126)). The mixture was incubated for 150 min (platelets) or for 90 min (BAC) at 24°C under constant agitation. In the case of Triton-solubilized membranes the reaction was terminated by coprecipitation of protein with rabbit gammaglobulin (final concentration 0.2 mg x ml^{-1}) and PEG (buffered in 0.1 M phosphate, pH 7.4, final concentration 0.18 g x ml^{-1}). Protein-unbound hormone was separated from protein-bound hormone by rapid filtration through PEI (0.33%)-treated glass-fibre filters (GF/C, Whatman). Protein-bound radioactivity was counted in a gammacounter (Zinsser HG-16) with 80% efficiency. Dissociation constants were calculated with the program LIGAND (14). Results shown are from at least three experiments done in triplicates.

RESULTS

Binding affinities in human platelets: The specific binding of urodilatin and ß-ANF in comparison with ANF-(99-126) and ANF-(103-123) is shown in figure 1. The order of potency in competing for [^{125}I]-iodo-rANF-(99-126) was typical for the known properties of ANF-R_2 receptors (ANF-(99-126): 55 pM; ANF-(103-123): 150 pM). Scatchard transformation revealed one receptor site for urodilatin (see inset figure 1) and ß-ANF (not shown). When analysed with nonlinear curve fitting, an apparent affinity constant of 55 pM was obtained for urodilatin. The antiparallel dimer of ANF-(99-126), ß-ANF, had a binding affinity even lower than ANF-(103-123) (295 pM and 154 pM, respectively).

Binding affinities in Triton-X-100-solubilized membranes from bovine adrenal glands: Specific binding of urodilatin and ß-ANF to Triton-solubilized membranes from BAC are shown in figure

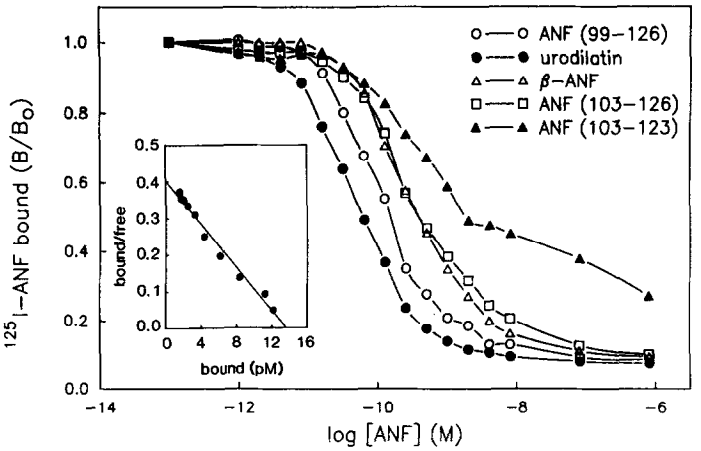

Figure 1: Competition curves for ANF-(99-126) (open circles), urodilatin (closed circles), ß-ANF (open triangles) and ANF-(103-123) (closed triangles). Percoll™-isolated human platelets were incubated with 8 pM [125I]-iodo-rANF-(99-126) and increasing concentrations of the respective analog for 150 min at 24°C. The reaction was stopped by rapid filtration through PEI-treated glass fiber filters. Inset: Scatchard plot for urodilatin.

2. The membrane preparation exhibited the following apparent binding affinities (K_D): ANF-(99-126): 52 pM; ANF-(103-126): 127 pM; ANF-(103-123): 201 pM. A straight line was obtained after Scatchard transformation for urodilatin (see inset figure 2) and ß-ANF (not shown) suggesting one binding site for urodilatin as well as for ß-ANF. Nonlinear curve fitting revealed a apparent affinity constant of 30 pM for urodilatin. ß-ANF showed a apparent affinity constant of 120 pM which is comparable to ANF-(103-126).

Stimulation of particulate guanylate cyclase activity by urodilatin: The dose-response curve of particulate guanylate cyclase to various ANF analogs is shown in figure 3. Urodilatin (ANF-(95-126)) activated particulate guanylate cyclase about twofold at a concentration of 1µM

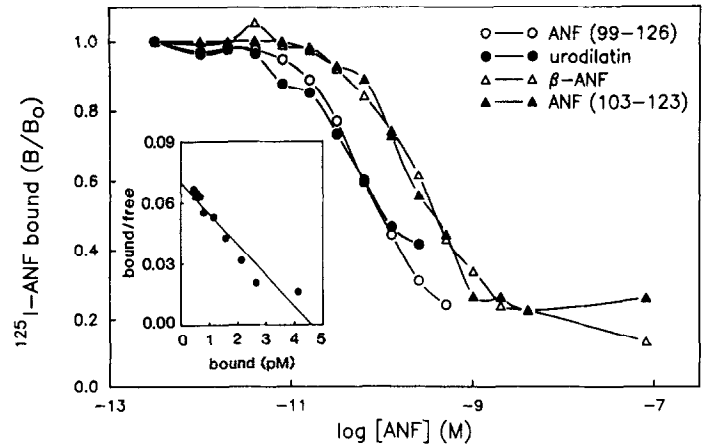

Figure 2: Competition curves for ANF-(99-126) (open circles), urodilatin (closed circles), ß-ANF (open triangles), ANF-(103-126) (open squares) and ANF-(103-123) (closed triangles). Triton X-100- solubilized membranes from bovine adrenal gland were incubated with 8 pM [125I]-iodo-rANF-(99-126) and increasing concentrations of the respective analog for 90 min at 24°C. The reaction was stopped by coprecipitation with polyethyleneglycol / gammaglobulin and subsequent rapid filtration through PEI-treated glass fiber filters. Inset: Scatchard plot for urodilatin.

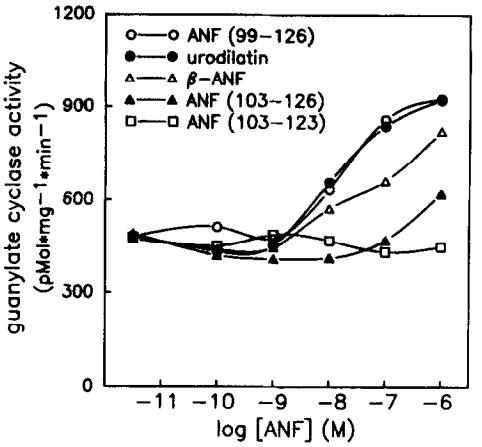

Figure 3: Activation of particulate guanylate cyclase by urodilatin (closed circles) and various other ANF-analogs (ANF-(99-126) (open circles); ß-ANF (open triangles); ANF-(103-126) (closed triangles); ANF-(103-123) (open squares). Triton X-100-solubilized membranes from bovine adrenal glands were incubated as described in "Materials and Methods" with 3 mM Mn^{2+} and 1 mM GTP.

similar to ANF-(99-126). ß-ANF activated particulate guanylate cyclase by about 75% at 1µM. ANF-(103-123) failed to activate the enzyme up to 1µM, whereas ANF-(103-126) activated it by 30% at 1µM.

DISCUSSION

The results of the present study show that urodilatin (ANF-(95-129) binds with a higher affinity to the guanylate cyclase-coupled ANF-R_1 receptor of BAC and activates the enzyme to the same extent compared to ANF-(99-126), the most abundant form of ANF circulating in plasma. These data are compatible with animal experiments where equimolar infusions of urodilatin in dogs had higher effects on the kidneys compared to ANF-(99-126) (15). Until now it is not known whether urodilatin circulates in plasma. It has been hypothesized that urodilatin is secreted luminally into collecting ducts (2) and that this tissue is the main target of urodilatin. De Léan et al (16) have shown that collecting ducts contain ANF receptors with high affinity and Tremblay and coworkers (17) have demonstrated ANF-sensitive guanylate cyclase activity in this tissue. We have chosen the two non-kidney tissues bovine adrenal cortex and human platelets for our experiments since it had been demonstrated that human platelets contain almost only R_2-receptors (18, 19), whereas on the other hand bovine adrenal cortex has been shown to contain predominantly R_1-receptors (20). We propose that our binding data and the activation of PGC measured in BAC are transferable to kidney tissue.

Hirata et al have published binding data of ß-ANF on intact vascular smooth muscle cells (7). They found almost identical binding affinities for ANF-(99-126) and for ß-ANF. This is in contrast to our data where ß-ANF had much lower affinities in both tissues studied. These conflicting results may be explained by the finding that most of the ß-ANF is converted to ANF-(99-126) in the presence of intact cells (7).

An additional interesting result is the discrepancy between the binding affinity of ß-ANF to membranes from bovine adrenal cortex and its ability to activate particulate guanylate cyclase activity. While the binding affinity was comparable to ANF-(103-126), ß-ANF activates particulate guanylate cyclase to a greater extent than this analog. One possible explanation is that ß-ANF rather than ANF-(103-126) promotes or stabilizes a dimeric or oligomeric complex after binding to the R_1-receptor. This hypothetical dimerization may lead to an higher coupling efficacy, a mechanism already shown for the epidermal growth factor receptor (21).

In conclusion, the results of the present study show that ß-ANF binds with lower affinity to both tissues studied and activates particulate guanylate cyclase less than ANF-(99-126), while urodilatin binds with equal or higher affinity to the different ANF receptors and activates particulate guanylate to the same extent compared to ANF-(99-126).

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