Stimulation of guanylate cyclase by atrial natriuretic factor in isolated human glomeruli

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A 23 amino acid synthetic peptide fragment of atrial natriuretic factor (ANF) stimulated guanylate cyclase activity in isolated human glomeruli in a concentration- and time-dependent manner. ANF activated particulate guanylate cyclase whereas it had no effect on soluble guanylate cyclase. These results demonstrate that the glomerulus is a target structure for ANF in humans. They also suggest that ANF-induced increase in glomerular filtration rate is due to a direct effect of this peptide on the glomerular cells mediated by activation of glomerular guanylate cyclase.

Atrial natriuretic factor *Isolated glomerulus* *Guanylate cyclase* *Cyclic GMP*

1. INTRODUCTION

It has become increasingly evident in the last 10 years that many extrarenal and renal humoral agents regulate the glomerular filtration rate through a change in the glomerular coefficient of ultrafiltration, K_f [1]. All the agents tested produced a decrease in K_f either directly as angiotensin II and arginine vasopressin or indirectly as parathyroid hormone and prostaglandins via stimulation of glomerular adenylate cyclase and cyclic AMP-induced local synthesis of renin and angiotensin II [2]. Reduction of K_f results in the decrease of glomerular filtration rate (GFR). In contrast with these hormones or mediators, atrial natriuretic factor (ANF) produces an increase of GFR in several animal species [3–5]. This peptide has also been demonstrated as vasodilatory in vitro [6] and it has been suggested that the relaxant effect of ANF may be mediated via increased tissue levels of cyclic GMP [7]. Studies in humans are scarce and provide no information on the effect of ANF on GFR [8]. Because of the limitations of in vivo studies particular to humans we investigated the effects of ANF on guanylate cyclase (GTP pyrophosphate (cyclizing), EC 4.6.1.2) in human glomeruli in vitro.

2. MATERIALS AND METHODS

Synthetic rat ANF (atriopeptin II), 23 amino acids, was donated by Ciba-Geigy (Basel, Switzerland). The following reagents and radiochemicals were also used: GTP, cyclic GMP, creatine kinase, phosphocreatine (disodium salt) from Boehringer (Mannheim, FRG); papaverine and sodium nitroprusside from Sigma (St. Louis, MO); cyclic [^{3}H]GMP (ammonium salt, 15 Ci/mmol) and [α-^{32}P]GTP (sodium salt, 600 Ci/mmol) from New England Nuclear (Dreieich, FRG).

Glomeruli were prepared as described [9]. Human renal tissue was obtained from cadaver kidneys judged to be unsuitable for transplantation (2 cases) or from the normal cortex of kidneys surgically removed for malignant tumors (2 cases). Isolated glomeruli were frozen in liquid nitrogen and stored until assayed (1–5 weeks). To separate the particulate and the soluble guanylate cyclase,

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glomeruli were homogenized using a Potter-Elvehjem apparatus. The particulate and soluble fractions were obtained by high-speed centrifugation (100000 × *g* for 60 min) of the homogenate. Glomerular proteins were determined according to [10].

Guanylate cyclase activity was assayed as described in [11] with minor modifications. The incubation medium (final volume, 100 μl) contained 20 mM Tris-HCl (pH 7.5), 5.5 mM $MnCl_2$, 5 mM $MgCl_2$, 0.1 mg/ml BSA, 0.25 mM papaverine, 10 mM creatine phosphate, 1.2 mg/ml creatine kinase, 0.5 mM GTP, 2.5 mM cyclic GMP and 0.1 μCi [α-^{32}P]GTP. The stimulator agents tested were diluted in 10 μl water. Isolated glomeruli or glomerular fractions (0.2–0.8 mg protein/ml) were added at time zero and the incubation was carried out for 10 min at 37°C. The reaction was stopped by addition of 20 μl of a solution containing 100 mM EDTA (sodium salt) and 30 nCi cyclic [^{3}H]GMP followed by immersion in boiling water for 3 min. Cyclic GMP was separated by successive filtrations on Dowex AG 50 WX4 (Biorad, Richmond, CA) and aluminium oxide (M. Woelm, Eschwege, FRG) columns. ^{3}H and ^{32}P radioactivities were determined by scintillation under appropriate conditions for discrimination between both isotopes. Guanylate cyclase activity was expressed as pmol of cyclic GMP formed/10 min per μg glomerular protein.

3. RESULTS

Guanylate cyclase activity in human glomeruli was 0.56 ± 0.06 and 1.25 ± 0.45 pmol·10 min^{-1}·μg^{-1} under basal conditions and in the presence of 0.1 mM sodium nitroprusside, respectively (means ± SE from 11 individual glomerular preparations). The latter agent was tested as a well-known activator of the enzyme devoid of tissue specificity [12]. Because of the absence of major individual differences between the 4 human kidneys utilized we pooled the results from the glomerular preparations irrespective of the original kidney.

Incubation of isolated glomeruli with ANF resulted in an increase in glomerular guanylate cyclase activity. The time course of activation (fig.1) shows that the maximal effect of ANF on cyclic GMP production occurred at 10 min and

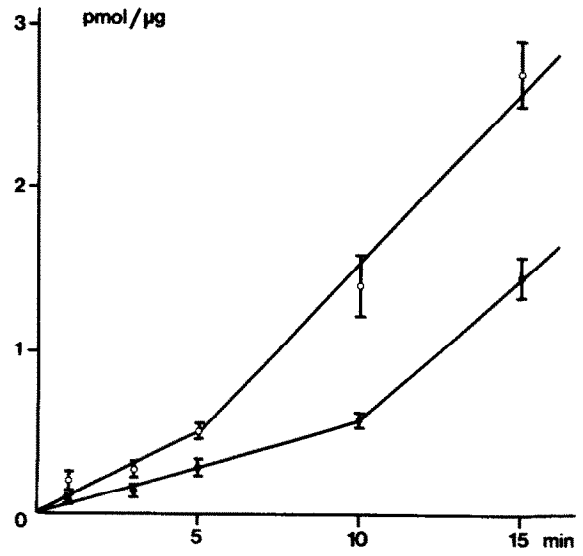

Fig.1. Formation of cyclic GMP as a function of time in human glomeruli under basal conditions (●—●) and in the presence of 0.1 μM ANF (○—○). Each point represents the mean and each vertical bar twice the SE of 4 individual experiments (3 values for each experiment).

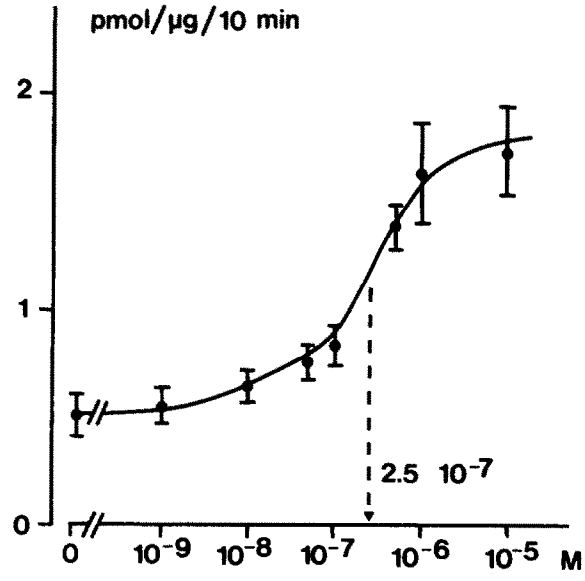

Fig.2. Effect of ANF concentration on guanylate cyclase activity present in whole human glomeruli. Each point represents the mean and each vertical bar twice the SE of 4 experiments (3 values for each experiment). The dotted line indicates the concentration of ANF corresponding to 50% of maximal stimulation.

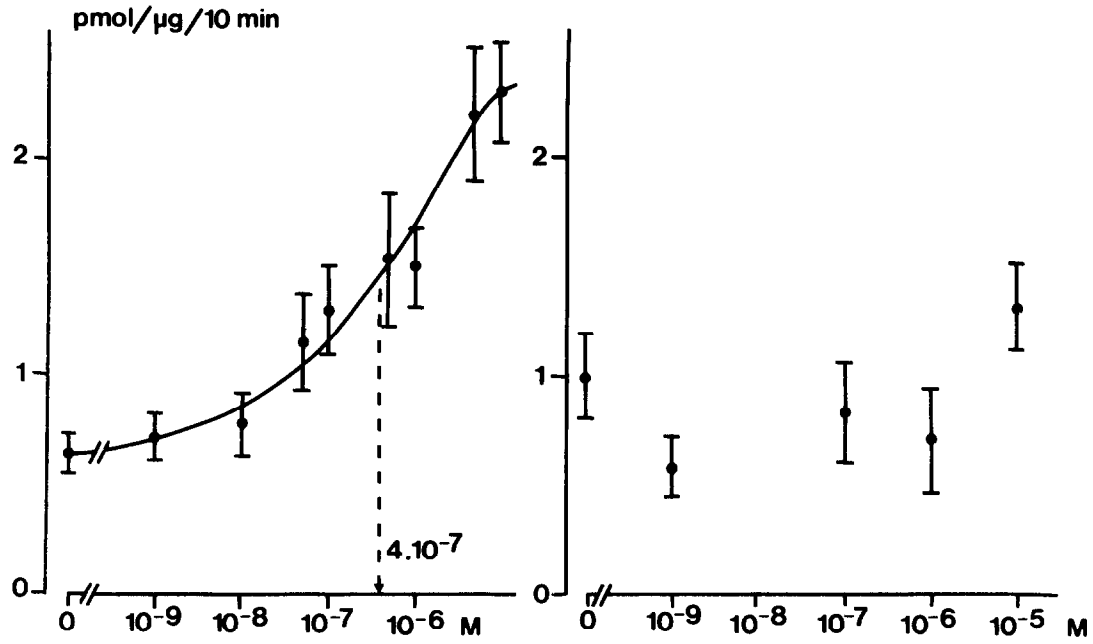

Fig.3. Effect of ANF concentration on guanylate cyclase activity present in the particulate fraction (left) and the soluble fraction (right) of homogenized human glomeruli. Each point represents the mean and each vertical bar twice the SE of 3 experiments (3 values for each experiment). The dotted line indicates the concentration of ANF corresponding to 50% of maximal stimulation in the particulate fraction.

persisted at 15 min. A maximum stimulation of 3–4-times the basal value was observed at concentrations of 1–10 μM. The lowest concentration of ANF at which a significant response was first obtained (threshold dose) was 0.05 μM and the concentration corresponding to 50% of the maximum stimulation (EC_{50}) was 0.25 μM (fig.2). Guanylate cyclase activity was linearly correlated with the glomerular concentration of protein between 0.2 and 0.8 mg/ml both under basal conditions and in the presence of 0.1 μM ANF (r = 0.93 for 11 couples of values). The slope of the regression line obtained with ANF (1.23 pmol · 10 $min^{-1} \cdot \mu g^{-1}$) was close to the value of guanylate cyclase activity observed at an identical concentration.

ANF stimulated guanylate cyclase activity in the glomerular particulate fraction but had no effect on the soluble enzyme (fig.3). The dose-response of the particulate guanylate cyclase to ANF was similar to that observed with whole glomeruli. Maximum stimulation (3.8-times the basal value) occurred at 10 μM. The threshold and EC_{50} were 0.05 and 0.4 μM, respectively.

4. DISCUSSION

These findings indicate for the first time that ANF stimulates particulate guanylate cyclase in a concentration-dependent fashion in human isolated glomeruli. Guanylate cyclase activity has been demonstrated in rabbit and rat glomeruli [13,14]. Glomeruli contain a much higher guanylate cyclase activity than tubules if both are isolated simultaneously from the same animal. Torres et al. [15] have also shown that glomeruli have a higher content of immunoreactive cyclic GMP than tubules. The role of cyclic GMP in the regulation of GFR has been studied essentially by determining variation of the intracellular level of this cyclic nucleotide. Carbamylcholine, a cholinergic analogue, and to a lesser degree, histamine and serotonin produced an increase in glomerular cyclic GMP [15,16]. However, little is known on the regulation of glomerular guanylate cyclase activity. ANF produced an increase in the tissue levels of cyclic GMP in rat kidneys and aortas [17] and activated guanylate cyclase activity in

rabbit aortas [7]. We show in this study that it also stimulates guanylate cyclase activity in human isolated glomeruli over a similar range of concentrations. The threshold dose (0.05 μM) corresponds approximately to the expected initial plasma concentration obtained after administration of an intravenous bolus of 100 μg ANF in adult humans, if we consider that ANF is distributed in the plasma volume. Under such conditions, Richards et al. [8] observed a clear increase in urine volume and urinary sodium excretion. As in the other preparations, ANF had a specific effect on particulate compared to soluble guanylate cyclase. It is well documented that cyclic GMP formation is stimulated by hormones only in intact cells supporting the view that guanylate cyclase activation is an event relatively distal to hormone-receptor interaction with probably increases in cytosolic calcium or oxygen reactive species as intermediary steps. In contrast, sodium nitroprusside and sodium azide have the capacity to stimulate cyclic GMP production both in broken and intact cells through the soluble form of guanylate cyclase [12]. ANF is thus particular since it stimulates guanylate cyclase activity in a cell-free system similarly to what occurs for the hormones linked to adenylate cyclase. This may be due to the fact that ANF like the peptide hormones in general, binds to membrane receptors [18] which must be in close vicinity with the enzyme they regulate.

This study demonstrates that the human glomerulus is a target structure for ANF and that cyclic GMP is the secondary messenger. It is likely that the increase of this nucleotide within the glomeruli in response to ANF is involved in the relaxation of the glomerular mesangial cells which show characteristics of modified smooth-muscle cells and contract upon addition of angiotensin II and arginine-vasopressin [19]. Relaxation of the mesangial cells may result in the increase of the filtration surface and thus of K_f. ANF may also inhibit the effects of the vasoconstrictor hormones on mesangial cell contractility in accordance with the findings reported using isolated perfused kidneys [20]. Thus, the stimulation of GFR by ANF appears to be the consequence of a direct effect of this peptide on the glomerular cells mediated by activation of the glomerular guanylate cyclase.

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