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CO-OPERATION BETWEEN PARTICULATE AND SOLUBLE GUANYLYL CYCLASE SYSTEMS IN THE RAT RENAL GLOMERULI

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ANP and NO act *via* different receptors, although inducing the common intracellular messenger — cyclic GMP. However, interaction between both factors remains unclear. Our observations suggested that in the rat kidney glomeruli, activities of the ANP- and NO-dependent guanylyl cyclase systems may be mutually compensated. To check this, we have tested effects of ANP and sodium nitroprusside (SNP) on cGMP synthesis and relaxation of glomeruli contracted with angiotensin II. The glomeruli were isolated from Wistar rats receiving saline (Control), dexamethasone (DEX), deoxycorticosterone (DOCA) or N-ω-nitro-L-arginine methyl ester (NAME) for 1 or 2 days. In the DEX glomeruli exposed to 100 μM SNP, rate of cGMP synthesis was significantly higher then in the Control (26.3 *vs* 16.0 pmol/mg.prot./2min., $P<0.05$), while 1μM ANP was markedly less effective (2.8 *vs* 16.7 pmol/mg.prot./2min in Control, $P<0.01$). On the contrary, in NAME group 1μM ANP stimulated cGMP synthesis up to 35.6 pmol/mg.prot./2min whereas efficacy of SNP was slightly suppressed. High correlation coefficient ($r = 0.979$, $p<0.01$) indicates interrelationship between NO- and ANP-dependent cGMP synthesis. Ability of the glomeruli to relax in response to ANP or SNP was in accord to their ability to cGMP generation. This was confirmed by high correlation ($r = 0.845$, $p<0.001$) between degree of relaxation and rate of cGMP synthesis. Our results support strongly the hypothesis that both, ANP and NO dependent systems co-operate in regulation of the function of kidney glomeruli.

Key words: *kidney, glomeruli, guanylyl cyclase.*

INTRODUCTION

Atrial natriuretic peptide (ANP) and nitric oxide (NO) are crucial relaxatory factors in the cardiovascular system. In the kidney, besides modulating the blood flow, they play an important role in the control of glomerular filtration rate and tubular transport processes (1, 2). Despite entirely different ways of generation, structure, and mechanism of interaction

with the target cells, natriuretic peptides and NO act via the common intracellular messenger, cyclic GMP. Interaction between both factors is still subject of investigations. NO-induced inhibition of ANP secretion (3) and ANP-dependent modulation of NO synthesis (4) were observed, however, interrelationship between the target systems remains unclear. We have shown an increased activity of the soluble guanylyl cyclase with simultaneous inhibition of the ANP-dependent system (5) in the kidney glomeruli from rats injected for 2 days with dexamethasone. On the other hand, administration of L-NAME, an inhibitor of NO synthesis, doubled activity of the particulate guanylyl cyclase in the rat glomeruli, whereas NO-dependent system seemed to be suppressed (6). These observations suggest that in the kidney glomeruli activities of the particulate and soluble guanylyl cyclase systems may be mutually compensated. Therefore, in this study we have tested ability to cGMP synthesis as well as relaxing potencies of ANP and sodium nitroprusside (SNP) in the glomeruli isolated from rats injected with dexamethasone, L-NAME or deoxycorticosterone.

MATERIAL AND METHODS

Animals and material sampling.

Male Wistar rats, weighing 180—250 g with free access to the standard diet (Altromin C1000, Germany) and drinking tap water were housed in metabolic cages three days prior beginning of the study. The animals were divided into four experimental groups; (I) NAME — receiving N-ω-nitro-L-arginine methyl ester dissolved in saline, s.c., 40 mg/kg, every 12 hours for one day; (II) DEX — receiving dexamethasone, 4 mg/kg i.m.. every 24 hours for two days; (III) DOCA — receiving deoxycorticosterone, 5 mg/kg i.m. every 24 hours for two days, and (IV) Control — receiving equal to each group volume of saline solution for two days. Urine collection to the vessels containing 20 µl 10% thymol in isopropanol was initiated together with the first injection. The last injection was made 1 hour prior to the blood collection and excision of the kidneys. The animals were anaesthetised with ether and two samples of blood were drawn from the abdominal aorta: one to the heparinized syringe and the other one to the syringe containing EDTA. For assay of NO metabolites (NO_2/NO_3), plasma was deproteinized with 30% $ZnSO_4$, 50 µl per ml of heparinized plasma, centrifuged and stored in aliquots at -20°C. For cGMP analysis, plasma proteins were precipitated with 200 µl 30% $HClO_4$ per millilitre of EDTA-plasma. The supernatant was neutralised with 5N KOH and subsequently with 5N K_2CO_3, centrifuged, frozen and stored in aliquots at -20°C. Urine samples were divided into aliquots, frozen and stored in -20°C until analysis.

Isolation of the kidney glomeruli

For each experiment one Control and one NAME, DEX or DOCA rat were used. The kidneys were excised immediately after the blood collection. Than the kidneys were placed in the ice-cold phosphate-buffered saline (PBS), pH 7.4, containing (in mM): 137 NaCl, 2.7 KCl, 8.1 Na_2HPO_4, 1.5 KH_2PO_4, 0.9 $CaCl_2$, 0.49 $MgCl_2$, 5.6 glucose. Glomeruli were isolated by the gradual sieving technique, as described previously (6, 7). Briefly, the kidneys were chopped to a paste-like consistency and strained through the sieves (pore size 250 µm, 70 µm and 120 µm). Suspension of

glomeruli in the ice-cold PBS was centrifuged in 15 ml aliquots for 2 minutes at 400 × g, at +4°C. The supernatant was removed and the glomeruli were resuspended in the fresh PBS and centrifuged again. The procedure was repeated 3 times. After the last centrifugation, the glomeruli were suspended in 5.5 ml PBS and assessed under the light microscope. The final preparation consisted of decapsulated glomeruli without arterioles and the tubular contamination was below 6%. Entire procedure was carried out in the ice bath and lasted approximately 2 hours.

Stimulation of the glomeruli to the cGMP synthesis.

To check ability of the glomeruli to the cGMP synthesis, suspensions of about 6000 glomeruli in 450 µl PBS containing 1mM 3-isobutyl—1-methylxantine (IBMX), 75 U superoxide dismutase (SOD) and 5 mM cysteine were preincubated for 10 minutes in the shaking bath, at 37°C (5). Incubation was started by adding 50 µl PBS (Blank), or PBS solutions of SNP (final concentration 0.01—100 µM) or ANP (final concentration 0.01—1.0 µM). Incubation with ANP was performed in the absence of cysteine. After 2 minutes, incubation was stopped by adding 100 µl of the 30% perchloric acid to the samples. The precipitate dissolved in 1 ml 0.2% SDS/0.2 N NaOH was used for determination of protein content. Remaining supernatant was neutralised with 5N KOH and 5N K_2CO_3, centrifuged, frozen and stored at −20°C for cGMP analysis.

In some experiments, prior to incubation with ANP, glomeruli from the Control group were preincubated 10 minutes with 1 µM dexamethasone or with 1 mM L-NAME dissolved in the PBS, followed by incubation with varying concentrations of SNP or ANP. The rates of guanylyl cyclase activation were similar to these in not preincubated glomeruli, thus excluding direct effect of glucocorticoid or L-NAME on cGMP synthesis.

Isolation of cytosolic and membrane-bound fractions from the glomeruli

The procedure was carried out as described previously (8, 9). About 40,000 freshly isolated glomeruli were sonicated for 2 minutes in 2 ml of the ice-cold homogenizing buffer containing: 50 mM Tris-HCl pH 8.2, 250 mM sucrose, 1 mM EDTA-Na_2, 1 mM 1,4-dithiothreitol, 0.1 mM PMSF. After 60 minutes ultracentrifugation at 100,000 × g, +4°C, the supernatant was immediately frozen and stored in liquid nitrogen to determine activity of the soluble isoform of guanylyl cyclase (sGC). The remaining pellet was sonicated for 30 seconds in washing buffer A: 500 mM KCl, 50 mM Tris-HCl pH 8.2, 1 mM EDTA-Na_2, 1 mM 1,4-dithiothreitol, 0.1 mM PMSF. After 30 minutes ultracentrifugation at 100,000 × g, the supernatant was discarded, and remaining pellet was sonicated for 30 seconds in washing buffer B: 50 mM Tris-HCl pH 8.2, 1 mM EDTA-Na_2, 1 mM 1,4-dithiothreitol, 0.1 mM PMSF. The suspension was again ultracentrifuged at 100,000 × g for 30 minutes, supernatant was discarded and the pellet was suspended in 1400 µl of homogenising buffer and stored in the liquid nitrogen for measurement of activity of the particulate isoform of guanylyl cyclase (pGC).

Activities of soluble and particulate guanylyl cyclases

Samples containing 15—20 µg protein/tube were preincubated 5 minutes in the shaking bath at 37°C in a final volume 500 µl of 20 mM Tris-HCl buffer pH 7.6, containing 1 mM IBMX, 5 units creatine phosphokinase and 15 mM creatine phosphate, with varying concentrations of SNP or ANP. Incubation was started by addition of 1 mM GTP and 4 mM $MgCl_2$. After 10 minutes, 100 µl 30% ice-cold $HClO_4$ was added to each tube, excess of acid was neutralised with 5N KOH and 5N K_2CO_3, samples were centrifuged and the supernatant was stored at −20°C until cGMP analysis.

cGMP radioimmunoassay

The cGMP was determined in duplicate by the modified radioimmunoassay method (10), with use of the rabbit anti-cGMP antibodies obtained in our laboratory. (8,5'-^{3}H) cGMP was used as a radioligand, and radioactivity was counted in the liquid scintillation counter (Beckman LS 5801).

NO_2/NO_2 assay

Total NO_2^- and NO_3^- (NO_2/NO_3) content, as a marker of NO production, was measured as described previously (11, 12). The nitrates in the samples were reduced to nitrite with nitrate reductase (13) and the nitrite was determined according to the Griess reaction (14). Briefly, 50 µl of mixture containing: 0.1 M potassium-phosphate buffer pH 7.5, 5 µM FAD, 0.3 µM NADPH and 80 mU nitrate reductase, was added to 75 µl of urine or deproteinized heparinized plasma and incubated for 60 minutes at the room temperature. The reaction was terminated by heating the tubes in the boiling water bath for 3 minutes. Subsequently, 625 µl of Griess reagent were added to each sample, and absorbance at 548 nm was measured after 20 minutes incubation at the room temperature.

Measurement of Glomerular Inulin Space (GIS)

GIS was measured according to the previously described method (15, 16). Immediately after isolation, average amount of 2000 glomeruli/tube was suspended in 200 µl of PBS buffer containing 1% bovine serum albumin and 0.5 µCi (^{3}H)-inulin and preincubated in the shaking water bath at 37°C for 30 minutes, for equilibration of the isotope. Incubation was started by adding 50 µl angiotensin II (final concentration 1 µM) dissolved in the PBS or 50 µl mixture of angiotensin II with varying concentrations of SNP or ANP. For measurements of basal intracapillary volume of glomeruli, equal volume of PBS was used. After 5 minutes of incubation, 200 µl of suspension of glomeruli were transferred to the microtube containing 100 µl of the ice-cold silicone oil AR20 and centrifuged at +4°C for 5 s, at 5,000 × g (Beckman Microfuge). During centrifugation the glomeruli were spun through the oil and the medium remained on the surface. Tips of the vials containing pellets of glomeruli were cut off and suspended in 500 µl of Triton X-100 0.3% for 2 hours. After the pellets were solubilised, 2 ml of scintillation cocktail (650 ml xylene, 350 ml Triton X-100, 7 g PPO and 0.5 g POPOP) were added, and the samples were left overnight at the room temperature. 50 µl aliquots of supernatant were treated in an identical manner. Radioactivity of the samples was measured in the liquid scintillation counter (Beckman LS 5801). Each sample was measured in triplicate.

The ^{3}H-inulin space of an isolated glomerulus (GIS) was calculated as follows:

$$\frac{^3\text{H radioactivity of the pellet (cpm)}}{^3\text{H radioactivity of the supernatant (cpm/pl)}} \times \frac{1}{\text{No. of glomeruli in a pellet}}$$

Results are expressed in picoliters (pl) per glomerulus.

Degree of relaxation in per-cent (%) was calculated as follows:

$$\frac{\text{GIS(ANP/SNP)} - \text{GIS (AngII)}}{\text{GIS (Basal)} - \text{GIS (AngII)}} \times 100$$

where: GIS(Basal) is GIS of freshly prepared glomeruli,
GIS(Ang.II) is GIS of glomeruli precontracted with 1 µM angiotensin II,
GIS(ANP/SNP) is GIS after adding ANP or SNP.

Other Analyses

Urine and plasma analyses for sodium, potassium and creatinine content were performed by means of routine clinical laboratory methods and equipment (Abbott, Spectrum EPX). The pH value of the urine and urinary hydrogen excretion was measured immediately after collection. Titratable acidity was evaluated from the amount of 0.1mM NaOH used for titrating sample containing 0.1 ml urine, 0.9 ml water and 400 μl 0.1N HCl, up to pH 7.42. Ammonia ions were determined using formalin titrimetric method (17).

Protein content was determined by the method of Lowry (18) and by the BCA method (19), using bovine serum albumin as a standard.

Reagents

N-ω-nitro-L-arginine methyl ester (NAME), 3-isobutyl—1-methylxanthine (IBMX), phenylmethylsulfonyl fluoride (PMSF), superoxide dismutase (SOD), guanosine triphosphate (GTP), 3',5'-cyclic guanosine monophosphate (cGMP), flavine adenine dinucleotide disodium salt (FAD) and angiotensin II (Ang II) were purchased from Sigma Chemical Co. (St. Louis, USA). Dexamethasone (Dexaven) and deoxycorticosterone (Desoxycortonum) were manufactured by Polfa (Jelenia Gora, Poland). Nitrate reductase from Aspergillus species was from Boehringer (Mannheim, Germany), (^{3}H)-inulin, specific radioactivity 1.73 Ci/mmol was purchased from Amersham Life Science (Little Chalfont, England). 3',5' cyclic (8,5'-^{3}H) guanosine monophosphate ammonium salt, specific radioactivity 35.5 Ci/mmol was from DuPont NEN Products (Boston, MA, USA), α-atrial natriuretic polypeptide, human, 28 amino acids (ANP) was from Peninsula Laboratories Inc. (Belmont, Ca., USA). Creatine phosphate and phosphocreatine kinase were from Calbiochem AG (La Jolla, CA, USA). 1,4-dithiothreitol (DTT) was from Carl Roth (Karlsruhe, Germany), silicone oil AR 20 was from Salben-Wacker-Chemie GmbH (Muenchen, Germany).

All chemicals were of the highest purity grade commercially available.

Statistics

Results are expressed as means ± SE from not less than four experiments. Statistical analysis was made using Mann-Whitney test and $p<0.05$ was considered statistically significant.

RESULTS

Renal function in vivo

Administration of L-NAME to the rats resulted in 20% decrease in urinary creatinine excretion with concomitant drop in sodium excretion (25%), whereas no significant change in diuresis was observed (*Table 1*). Inhibitory effect of L-NAME on the NO synthesis was confirmed by the two-fold reduction in plasma as well as in urinary NO_2/NO_3 excretion (*Table 2*). Nevertheless, plasma cGMP concentration and urinary cGMP excretion did not differ from those in the Control group. Adverse effect was observed in the rats treated with dexamethasone (DEX-1) for 24 hours. Increase in the creatinine excretion (by 14%) was paralleled by a marked increase in sodium

excretion (by 49%). Also, diuresis exceeded over two-fold this in the Control (*Table 1*). This was accompanied by significant increase in NO_2/NO_3 (by 91%) and cGMP (by 42%) excretion (*Table 2*). Almost all tested parameters returned to the normal levels after 48 hours of the experiment (DEX-2), except of the still marked increase in the NO_2/NO_3 and cGMP excretion (*Table 2*). However, significant decrease (by 55%) in the plasma NO_2/NO_3 concentration was paralleled by only 26% drop in plasma cGMP level.

Table 1. Effect of L-NAME and dexamethasone (DEX) on renal function *in vivo*. Urine was collected an analysed as described in Methods. DEX-1 and DEX-2 represent data from the first and the second day of experiment, respectively. Values are mean ± SE from nine experiments. Significance levels *versus* Control are: * $P < 0.05$, ** $P < 0.01$.

Experimental group	Diuresis ($ml \times 24\ h^{-1} \times 100\ g\ b.w.^{-1}$)	Creatinine excretion ($mg \times 24\ h^{-1} \times 100\ g\ b.w.^{-1}$)	Na^+ excretion ($mmol \times 24\ h^{-1} \times 100\ g\ b.w.^{-1}$)
Control	6.0 ± 0.8	2.76 ± 0.13	0.99 ± 0.07
NAME	7.2 ± 0.6	2.22 ± 0.13 **	0.74 ± 0.09 *
DEX-1	14.1 ± 1.4 **	3.15 ± 0.14 *	1.48 ± 0.17 **
DEX-2	8.8 ± 0.8 *	2.86 ± 0.08	0.85 ± 0.08

Table 2. Effect of L-NAME and dexamethasone (DEX) on plasma level and urinary excretion of cyclic GMP and NO_2/NO_3. Collection of the blood and urine followed by cGMP and NO_2/NO_3 analysis were performed as described in Methods. DEX-1 and DEX-2 represent data from the first and the second day of experiment, respectively. Values are mean ± SE from nine experiments. Significance levels *versus* Control are: * $P < 0.05$, ** $P < 0.005$.

Experimental group	Urine		Plasma	
	cGMP ($nmol \times 24\,h^{-1} \times 100\,g\ b.w.^{-1}$)	NO_2/NO_3 ($\mu mol \times 24\,h^{-1} \times 100\,g\ b.w.^{-}$)	cGMP ($nmol \times l^{-1}$)	NO_2/NO_3 ($\mu mol \times l^{-1}$)
Control	26.5 ± 2.6	1.49 ± 0.11	34.5 ± 3.8	26.3 ± 2.0
NAME	24.5 ± 3.1	0.60 ± 0.21 **	37.4 ± 3.5	14.0 ± 2.9 **
DEX-1	37.7 ± 4.1 *	2.85 ± 0.29 **	—	—
DEX-2	38.8 ± 3.2 **	1.85 ± 0.17 *	25.5 ± 2.4 *	11.9 ± 1.4 **

Synthesis of cGMP in the glomeruli.

Ability of the glomeruli to synthesise the cyclic GMP is shown in *Table 3*. In the presence of SNP, rate of cGMP synthesis in glomeruli from NAME group was very similar to that in the Control. However, in the presence of

ANP, cyclic GMP synthesis in NAME glomeruli was significantly higher, exceeding two-fold the Control level at 1 μM ANP (35.6±6.0 *vs* 16.7±4.2 pmoles/mg.protein/2 min.; $p<0.01$). These differences were reflected by activities of particulate and soluble guanylyl cyclases isolated from the glomeruli. Stimulation of particulate guanylyl cyclase by ANP was more effective in NAME than in the Control group (0.64±0.31 *vs* 0.23±0.04 pmoles/mg.protein/10 min.; $p<0.05$). Activity of the soluble form, in turn, was suppressed in NAME group, at 100 μM SNP achieving only 69% of the Control activity (1.6±0.3 *vs* 2.3±1.0 pmoles/mg.protein/10 min.).

Table 3. Activities of guanylyl cyclase systems in the glomeruli from the Control, L-NAME, dexamethasone and deoxycorticosterone receiving rats. Renal glomeruli were isolated, incubated with SNP or ANP and cGMP was determined as described in Methods. Results are means ± SE from four (DOCA group), eight (DEX) or ten (Control and NAME groups) experiments. Significance levels *versus* Control are: * $P < 0.05$, ** $P < 0.01$.

Experimental group	cGMP synthesis (pmoles/mg.protein/2 minutes)			
	+ANP 0.1 μM	+ANP 1 μM	+SNP 1 μM	+SNP 100 μM
Control	4.3±1.9	16.7±4.2	1.9±2.4	16.0±4.0
NAME	11.6±2.3*	35.6±6.0**	1.2±0.5*	14.1±3.0
DEX	—	2.8±1.6**	10.8±3.3*	26.3±5.2*
DOCA	—	9.1±4.2	1.5±0.9	21.0±4.6

Adversely, in rats receiving dexamethasone (DEX group), activity of NO-dependent guanylate cyclase system was markedly elevated. An increase in cyclic GMP synthesis was particularly apparent at 1 μM SNP (10.8±3.3 *vs* 1.9±2.4 pmoles/mg.protein/2 min. in the Control glomeruli; $p<0.05$). At 100 μM SNP, cyclic GMP synthesis exceeded the Control value by 64% for the intact glomeruli, and by 30% for the soluble guanylyl cyclase (3.2±1.0 *vs* 2.3±1.0 pmoles/mg.protein/10 min.). In contrast, 1 μM ANP had only slight effect on generation of cGMP, which was 6 times lower in DEX than in the Control group. Activities of particulate guanylyl cyclases isolated from DEX and Control glomeruli were, however, similar. No significant changes in ability to generate cGMP were observed in glomeruli from mineralocorticoid-treated (DOCA) rats.

Relationship between rates of the cGMP synthesis in glomeruli at the highest effective activator concentrations, i.e., 1 μM ANP and 100 μM SNP is shown at *Fig. 1*. High correlation coefficient ($r = -0.979$, $p<0.01$) suggests strongly that activities of ANP- and NO-dependent systems in the kidney

glomeruli regulate each other so that decrease in efficiency of one of the systems is compensated by an increase in activity of the other one.

In order to check whether observed alterations in guanylyl cyclase activities affect function of the glomeruli, we have tested ability of glomeruli to relax.

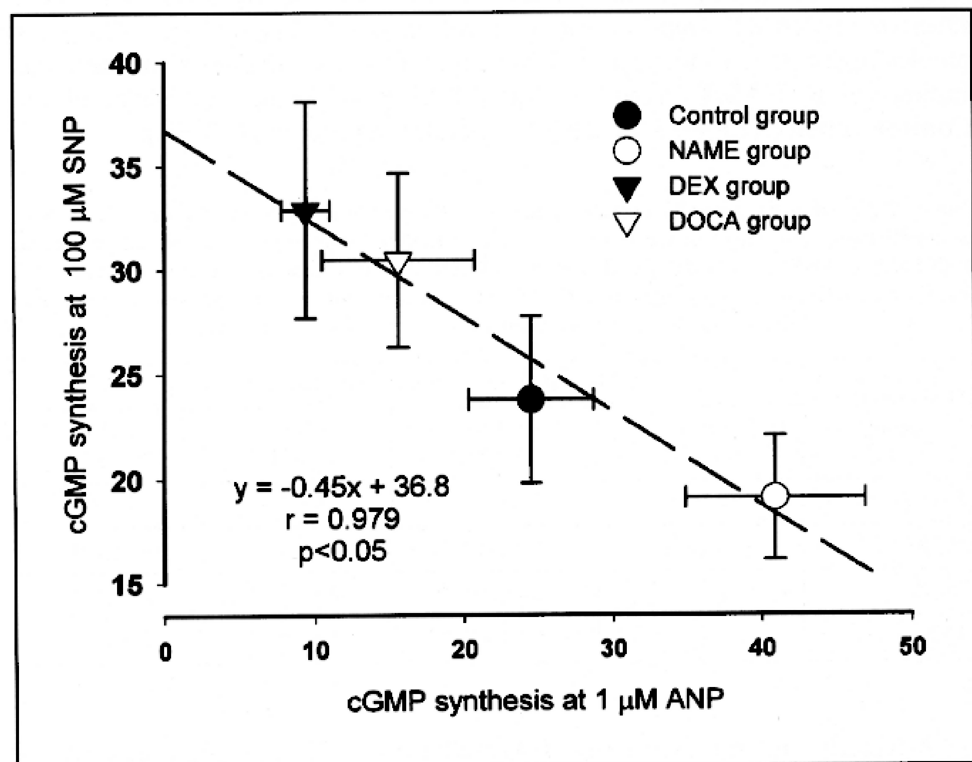

Fig. 1. Correlation between SNP- and ANP-stimulated cyclic GMP synthesis in the isolated kidney glomeruli.
Rates of cGMP increase at 100 µM SNP and 1 µM ANP are expressed in pmol/mg protein/2 minutes and are mean ± SE from ten (Control and NAME groups), eight (DEX group) or four (DOCA group) experiments.

Effect of ANP and SNP on the relaxation of the glomeruli

Freshly isolated kidney glomeruli seem to be completely relaxed. Therefore, effect of relaxing factors can be checked only when glomeruli are precontracted with agents such as angiotensin II, adenosine or endothelin. For this purpose we have used 1 µM angiotensin II (Ang II), concentration that maintains glomeruli contracted for at least 10 minutes (16). Initial intracapillary volume of the glomeruli (Glomerular Inulin Space, GIS), was similar in all tested groups and balanced between 600 and 850 pl/glomerulus. Addition of Ang II

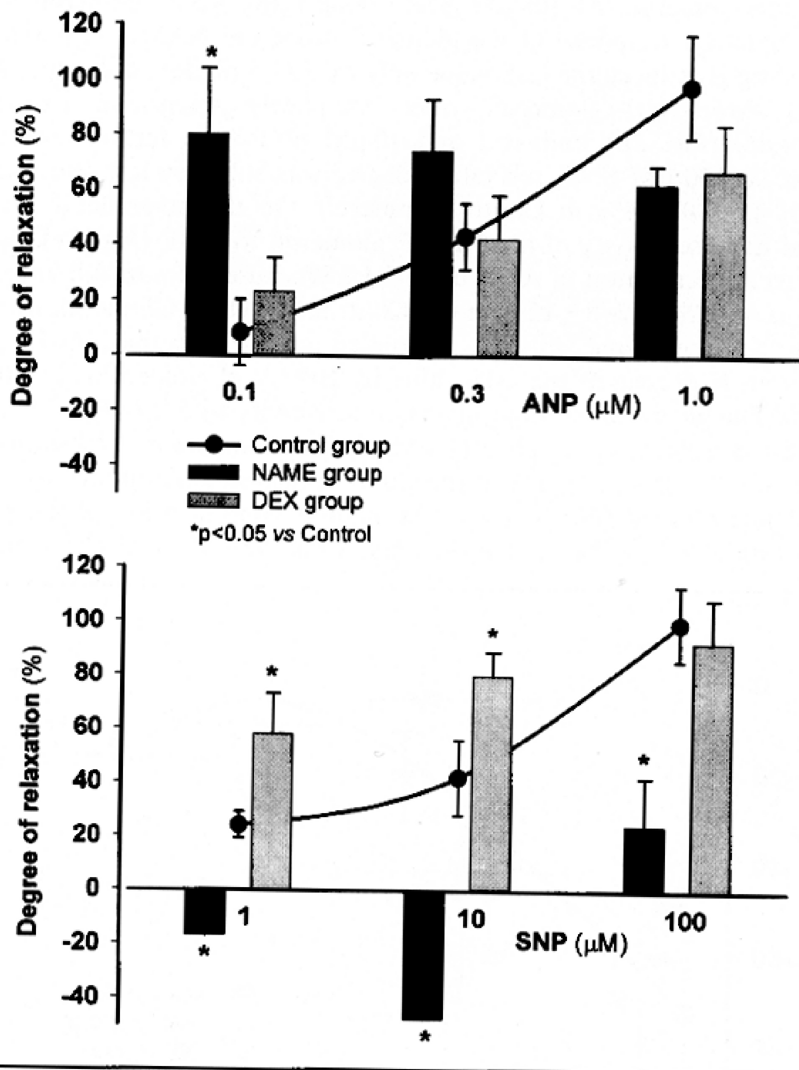

Fig. 2. Effect of SNP and ANP on relaxation of the kidney glomeruli from the Control, NAME and DEX rats.

Degree of relaxation, computed as relative change in Glomerular Inulin Space (%), as described in Methods. Glomeruli were precontracted with 1 μM angiotensin II (Ang II) followed by addition of increasing concentrations of ANP (upper panel) or SNP (lower panel). Results are presented as means ± SE from four (Control group) or five (NAME and DEX groups) experiments. Significance levels *versus* Control are: $^{*}P < 0.05$.

to suspension of Control glomeruli decreased the GIS value by 73 ± 8 pl (10.3 ± 0.9%) (*Fig. 2*). Following supplementation of the incubation medium with increasing concentrations of ANP (*Fig. 2A*) or SNP (*Fig. 2B*) progressively

relaxed the glomeruli. At 100 μM SNP or at 1 μM ANP, glomeruli relaxed back completely. Response of the glomeruli from the NAME rats was quite distinct. Ang II reduced the GIS value only by 51 ± 5 pl/glomerulus ($p < 0.05$ *vs* Control). Moreover, glomeruli were completely insensitive to sodium nitroprusside (*Fig. 2B*), and at 1 and 10 μM SNP even further contraction occurred. At 100 μM SNP, relaxation degree was still very low and was only 24 ± 18% *vs* 100 ± 14% in Control glomeruli. On the other hand, we have observed hypersensitivity of the NAME glomeruli to ANP (*Fig. 2A*). Already the lowest concentration of ANP used (0.1 μM) caused almost full relaxation, as high as 80 ± 24% *vs* 8 ± 12% in the Control ($P < 0.05$). Glomeruli from rats receiving dexamethasone, in turn, responded adversely to the NAME group. Angiotensin II decreased the GIS value by 104 ± 9 pl/glomerulus ($p < 0.05$ *vs* Control). The glomeruli showed increased sensitivity to SNP (*Fig. 2B*) and at the lowest concentration applied (1 μM), half of the maximal relaxation was obtained (58 ± 15% *vs* 24 ± 5% in the Control, $p < 0.05$). Simultaneously, effect of ANP was blunted (*Fig. 2A*) and the highest concentration of the peptide (1 μM) caused 67 ± 17% relaxation only, while 98 ± 19% in the Control.

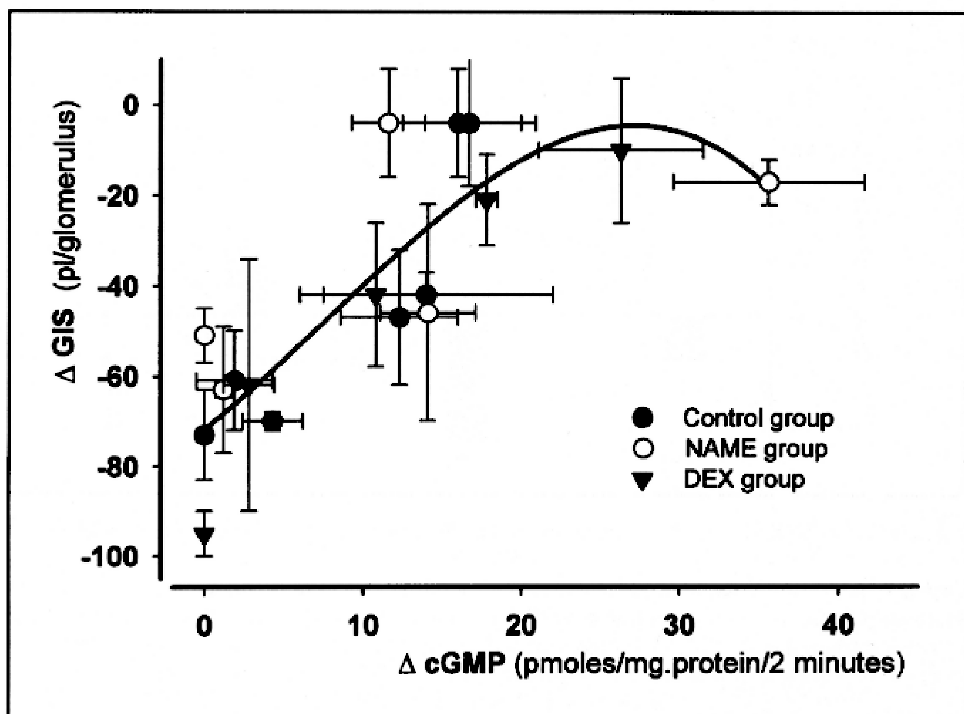

Fig. 3. Correlation between relaxation of the glomeruli and rate of the cGMP synthesis. Values of rates of ANP- and SNP-stimulated cyclic GMP synthesis (Δ cGMP) were taken from *Table 3* and from our previously published data (5, 6). ΔGIS represents difference between GIS values before and after adding AngII and SNP or ANP, as described in Methods.

Results of the present as well as previous (5, 6) experiments indicate that there is consistence between ability of the glomeruli to relax and to the cGMP synthesis. This observation was confirmed by a high correlation ($r = 0.845$, $p < 0.001$) between ANP- and SNP-induced rates of the cGMP synthesis and corresponding increases in the glomerular intracapillary volumes (*Fig. 3*). Calculated activity of the GC systems, necessary to achieve 50% relaxation of the glomeruli, is approximately 11 pmol cGMP/mg protein/2 min., whereas full relaxation occurs at 27 pmoles of cGMP/mg protein/2 minutes. It seems likely that in the kidney glomeruli, degree of relaxation is dependent rather on final concentration of cGMP, than on its source of cGMP.

DISCUSSION

In the present study we have demonstrated that there is compensatory interaction between both cGMP generating systems in the kidney glomeruli (*Tab. 3, Fig. 1*). Adaptive changes in ability to synthesise cGMP could be observed not only in the intact glomeruli but also in isolated subcellular fractions (5, 6). Moreover, the biochemical properties seem to be synchronized with haemodynamic functions of glomeruli reflected by their capability relax *in vitro* (*Figs. 2* and *3*). Also *in vivo,* lack of significant changes in plasma and urinary cGMP, despite of apparent blockade of endogenous NO synthesis (*Tab. 2*) suggests that complementary cyclic GMP producing system was activated.

The question is, what kind of physiological mechanism(s) could co-ordinate activities of both guanylyl cyclase – dependent systems. Role of the common product, cyclic GMP, is not clear. To-date, there is no explicit evidence that cGMP feeds its total synthesis back. Reports on modulation of guanylyl cyclase ligands by cyclic GMP are also inconsistent. It was shown in the cell culture that formation and release of nitric oxide do not depend on the cyclic GMP (20, 21). Also, SNP-stimulated elevation of cGMP synthesis did not influence ANP-dependent cyclic GMP production in isolated coronary arteries (22). However, 8-bromo-cGMP down-regulated ANP clearance receptors in cultured endothelial cells (23), effect observed also by induction of NO in smooth muscle cells (24). There are also conflicting data showing that ANP both inhibits (4, 25) and stimulates (26, 27) induction of nitric oxide synthase. Our observations made in cultured podocytes indicate that 3-hour incubation with dexamethasone decreases, while 18-hour incubation increases activity of soluble guanylyl cyclase (28). This suggests that increased activity of the sGC may be due to preceding decrease in cGMP synthesis.

Other mechanisms of the interrelationship between guanylyl cyclase systems include accessibility of agonists to their receptors. Such possibility may

be supported by observed in our present and previous studies (6) discrepancy between activities of guanylyl cyclases in the intact glomeruli and in the subcellular fractions. Maximal activities of guanylyl cyclases in the DEX glomeruli differed significantly from the Control values, while differences in the subcellular fractions were not as much pronounced. Glucocorticoids inhibit the free radical production in glomeruli and other tissues (29, 30), thus prolonging the half-life of NO and enhancing its activity (31). In the intact glomeruli from dexamethasone-receiving rats, exogenous nitric oxide could have better access to the intracellular target enzyme, whereas isolated guanylyl cyclases were completely exposed to their activators. Therefore the enzymes could achieve their maximal activities, which were similar for DEX and Control groups (*Tab. 3*), (5). On the other hand, glucocorticoids may limit amount of ANP reaching its target enzyme. Dexamethasone upregulates the clearance receptors for ANP (32), which may account for higher uptake/inactivation of the peptide, and thus, for declined activation of the pGC. This could explain observed by us drop in response to ANP in the intact DEX glomeruli whereas activity of isolated particulate guanylyl cyclase remained unaltered (data not shown).

Inhibition by glucocorticoid of endogenous NO synthesis leading to sensitization of soluble guanylyl cyclase could be another reason for the increased response to SNP in the DEX glomeruli. A drastic drop in plasma NO_2/NO_3 level (*Tab. 2*) in rats receiving dexamethasone could support such hypothesis. However, glucocorticoids are known to prevent rather the iNOS induction by inflammatory agents (33, 34), than to suppress the constitutive enzymes. On the other hand, dexamethasone increases mRNA levels for endothelial and inducible NOS in rats (35) and elevated NO production could directly affect the particulate guanylyl cyclase. As it was reported (36, 37), binding of the natriuretic peptides induces di- or oligomerization of the pGC monomers what, in turn, activates the catalytic unit. Extracellular ligand-binding domain of particulate guanylyl cyclase is rich in cysteine residues (38). As nitric oxide reacts readily with sulfhydryl groups (39), one may not exclude that it may hinder activation of the enzyme by S-nitrosylation of the pGC monomers. And conversely, reduced supply of NO in NAME group could attenuate physiological inactivation of particulate guanylyl cyclase, thus increasing efficacy of the membrane-bound cGMP generating system (*Tab. 3, Fig. 2A*).

One should also consider an indirect regulation of guanylyl cyclases by vasoconstrictors opposing their action. Ang II, by activating protein kinase C and increasing the intracellular calcium level, may inhibit the particulate guanylyl cyclase system (40). Therefore, observed by us contracting effect of angiotensin II, which was increased in DEX and suppressed in NAME glomeruli, may reflect respective decrease and increase in capability of ANP dependent guanylyl cyclase system (*Tab. 3* and *Fig. 2*)

Presented in this paper results support the hypothesis that atrial natriuretic peptide and nitric oxide co-operate in regulation of glomerular function. Interaction between both factors is based on mutual compensatory modulation of particulate and soluble guanylyl cyclase systems. However, further investigations are necessary to find out the mechanism(s) driving this reciprocity.

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