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Modulation by low sodium intake of glomerular response to cicletanine and atrial natriuretic factor

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1 The aim of the study was to investigate whether cicletanine (CIC), as a potential inhibitor of cyclic GMP phosphodiesterase, is able to restore glomerular response to atrial natriuretic factor (ANF) in rats under conditions of diet deprived of sodium. We examined the effects of CIC on glomerular filtration rate (GFR), natriuresis and nephrogenous cyclic GMP excretion in response to ANF and the effects of both agents on intracapillary volume and cyclic GMP accumulation in isolated glomeruli of rats on normal and low sodium diets.

2 CIC (0.25 mg min^{-1} kg^{-1} BW) of ANF (0.5 μg min^{-1} BW) alone, given in pharmacological doses, increased C_{in} significantly in normal sodium rats, whereas the effect of each agent was blunted in low sodium diet rats. Pretreatment with CIC restored the increase in C_{in} in response to ANF infusion in low sodium diet rats. In rats on either diet, there were no differences in the extent of diuresis and natriuresis induced by CIC or ANF alone. In contrast to FE_{Na}, combined effects of both agents on V and $U_{Na}V$ in rats on normal and low sodium diets were observed.

3 In normal sodium diet rats, CIC 10^{-4} M or ANF 10^{-6} M alone inhibited angiotensin II 10^{-6} M (AII)-induced decrease in intracapillary volume reflected by the glomerular [^{3}H]-inulin space (GIS). In contrast, CIC or ANF alone did not inhibit AII-induced decrease in GIS in low sodium diet rats. Both agents given together inhibited AII-induced decrease in GIS in low sodium diet rats.

4 CIC both alone and in combination with ANF increased nephrogenous cyclic GMP excretion and cyclic GMP accumulation in isolated glomeruli of rats on normal and low sodium diets. In rats on either diet, CIC abolished the difference in ANF-stimulated increase in nephrogenous cyclic GMP excretion and cyclic GMP accumulation in glomeruli.

5 These results suggest that CIC and ANF alone induce relaxation of glomeruli and a resultant increase in glomerular filtration rate in normal sodium diet rats; in contrast, these effects are blunted in the low sodium diet rats. CIC restores glomerular response to ANF in low sodium diet rats, apparently involving inhibition of the cyclic GMP phosphodiesterase.

Keywords: Cicletanine; ANF; kidney; glomerular effects; low sodium diet; cyclic GMP; cyclic GMP-phosphodiesterase

Introduction

Cicletanine (3-(4-chlorophenyl)-1,3-dihydro-7-hydroxy-6-methylfuro-[3,4-c] pyridine) is a novel agent that has been shown to possess vasorelaxant, natriuretic and diuretic properties in preclinical and clinical studies (Chabrier *et al*., 1988; Koltai *et al*., 1990). The mechanism(s) by which cicletanine (CIC) induces these biological effects has not been definitely established. The salidiuretic activity appears to be the result of an action of the sulphoconjugated metabolite of cicletanine, which inhibits the apical Na^+-dependent Cl^-/HCO_3^- anion exchanger in the cortical diluting segment (Garay *et al*., 1995). The mechanism of the vasodilating effect of cicletanine (CIC) seems to be complex and may involve stimulation of vascular prostaglandin synthesis and/or blockade of Ca^{2+}-channels either directly or indirectly via K^+-channel opening effects (Dorian *et al*., 1988; Calder *et al*., 1992a,b; Deitmer *et al*., 1992; Noack & Deitmer, 1993). The drug has also been shown to interact with vascular histamine receptors (Auguet *et al*., 1988), adrenoceptors (Malherbe *et al*., 1986) and muscarinic receptors (Rohmeiss *et al*., 1990) and with kinins (Plante *et al*., 1990). Moreover, it has been shown that inhibition of low K_m guanosine 3′:5′-cyclic monophosphate (cyclic GMP) phosphodiesterases by CIC may be partly responsible for the vasorelaxant effect of CIC as well as for the potentiation by CIC of the vasorelaxant actions of guanylate cyclase activators (Silver *et al*., 1990; 1991).

Atrial natriuretic factor (ANF), an activator of particulate guanylate cyclase, itself exerts vasorelaxation, natriuresis and diuresis (Brenner *et al*., 1990). It has also been shown, that both CIC and ANF-induced biological effects are dependent on dietary sodium intake (Cueno *et al*., 1986; Shenker, 1988; Jin *et al*., 1991). This indicates that CIC has an exaggerated antihypertensive effect in NaCl-supplemented spontaneously hypertensive rats.

Previous studies (Cueno *et al*., 1986; Shenker, 1988) as well as our own (Bizon *et al*., 1992; Kalinowski *et al*., 1995) have demonstrated that ANF-induced increase in glomerular filtration rate (GFR) is blunted during a low sodium diet. On the other hand, it was possible to restore the increased GFR in response to ANF in the low sodium diet rats by using zaprinast, a selective cyclic GMP-phosphodiesterase inhibitor (Kalinowski *et al*., 1995). Furthermore, we have shown that lack of a GFR-increasing response to ANF in the low sodium diet rats is primarily due to increased cyclic GMP hydrolysis in glomeruli.

The purpose of the present studies was to evaluate whether CIC, as a potential inhibitor of cyclic GMP phosphodiesterase, is able to restore the glomerular response to ANF in low sodium diet rats. We examined the effects of CIC on GFR and natriuretic responses to ANF and the effects of both agents on intracapillary volume of isolated glomeruli under conditions of sodium deprivation. To evaluate the extent of the involvement of cyclic GMP phosphodiesterase inhibition of CIC in glomeruli, nephrogenous cyclic GMP excretion and cyclic GMP accumulation by isolated glomeruli were also measured.

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Methods

Experimental animals

Male Wistar rats weighing 200–250 g were maintained on a low (0.01% Na; Altromin C 1036) or a normal sodium diet (0.8% Na; Altromin C 1000) for five days before experimentation. Food was withheld 14 h before the experiments.

Clearance studies

Rats were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (40 mg kg^{-1} BW), a tracheostomy was performed and the animals were allowed to breathe spontaneously. Femoral vein and artery were catheterized with PE-50 tubing for infusion and blood sampling, respectively. Blood samples were drawn at the midpoint of each clearance period. For determination of cyclic GMP, blood samples were collected into tubes with EDTA at the midpoint of clearance periods. The mean arterial blood pressure (MAP) was monitored throughout the procedure via a pressure transducer attached to the arterial catheter. The bladder was also catheterized for urine collection. All animals were infused with saline vehicle: 140 mmol l^{-1} NaCl, 2% inulin, 0.2% sodium *p*-aminohippurate and 1000 u l^{-1} heparin at a constant infusion rate of 0.03 ml min^{-1}. Experiments were started after establishment of a steady diuresis i.e. about 1.5 h after the onset of the study; 10 min experimental clearance periods were obtained according to the following protocol.

Group 1: effect of CIC in normal and low sodium diet rats (n=7 on each diet) After two baseline collections, CIC (0.25 mg min^{-1} kg^{-1} BW) was infused and five clearance periods were obtained.

Group 2: effect of ANF in normal and low sodium diet rats (n=6 on each diet) After two baseline collections, vehicle (ethanol diluted in saline fusion) was infused for two clearance periods. ANF (5 μg kg^{-1} BW bolus then 0.5 μg min^{-1} kg^{-1} BW maintenance) was then added to the infusion and the next three clearance periods were obtained.

Group 3: effect of CIC plus ANF in normal and low sodium diet rats (n=7 on each diet) After two baseline collections, CIC (0.25 mg min^{-1} kg^{-1} BW) was infused for two clearance periods. Then ANF (5 μg kg^{-1} BW bolus then 0.5 μg min^{-1} kg^{-1} BW maintenance) was superimposed on the ongoing continuous infusion of CIC and the infusion was continued for three clearance periods.

In vitro *studies*

Isolation of glomeruli After decapitation of rats, the kidneys were immediately removed and placed in ice-cold phosphate buffered saline (PBS), pH 7.4 containing (mM): NaCl 137, KCl 2.7, Na_2HPO_4 8.1, KH_2PO_4 1.5, $CaCl_2$ 0.9, $MgCl_2$ 0.49 and glucose 5.6. Glomeruli were isolated by the gradual sieving technique, according to the method previously described (Angielski *et al*., 1990). Briefly, the kidneys were chopped to a paste-like consistency and strained through a nylon sieve (pore size 250 μm). The mash which passed through this sieve was suspended in ice-cold PBS. Then, the suspension passed through two consecutive nylon sieves (120 and 70 μm). The glomeruli retained on the top of the 70 μm sieve were washed off with ice-cold PBS. Glomeruli were resuspended in ice-cold PBS buffer. The tubular contamination was less than 5%, as assessed under the light microscope. Glomeruli were decapsulated and devoid of afferent and efferent arterioles.

Measurement of glomerular inulin space (GIS) GIS was measured according to the method of Savin & Terreros (1981) with modifications (Szczepanska-Konkel *et al*., 1991). Briefly, glomeruli were resuspended in PBS with 1% bovine serum albumin (BSA) at a concentration of about 10000 glomeruli ml^{-1}. After addition of 0.5 μC [^{3}H]-inulin (specific activity 8.55 MBq mg^{-1}) to the incubation volume of 100 μl per tube, the glomeruli were incubated at 37°C for 30 min, enough time to allow equilibration of the isotope. The samples were preincubated in the presence of different concentrations of CIC or the solvent (control vehicle) for 5 min, after which, AII 10^{-6} M was added and incubation was continued for a further 5 min. In the experiments with ANF 10^{-6} M, it was added at the same time as AII. The total incubation volume was 250 μl per tube.

The incubation was terminated by centrifugation ($5000 \times g$) of 200 μl aliquot of the glomeruli suspension through 100 μl ice-cold silicone oil in a Beckman Microfuge for 5 s. During this centrifugation, the glomeruli were spun through the oil and the medium remained behind. The tips of the tubes, containing glomeruli, were then cut off with a scalpel blade and placed in scintillation vials containing 500 μl of 0.3% Triton X-100 in which the glomeruli were solubilized; then 2 ml of scintillation cocktail was added to the scintillation vial. The supernate sample was also transferred to the scintillation vial in an identical manner. The ^{3}H radioactivity of the solubilized glomeruli and supernate was counted in a liquid scintillation counter.

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

$$\frac{^3\text{H radioactivity of pellet (c.p.m.)}}{^3\text{H radioactivity of supernate (c.p.m. pl}^{-1}\text{)}} \times \frac{1}{\text{No. of glomeruli in a pellet}}$$

The number of glomeruli in the suspension medium was counted with a light microscope at low magnification. Each value of GIS was determined in tri- or quadruplicate samples.

The data so obtained were related to % change from the basal GIS. The basal GIS, expressed as 100%, was 684 ± 18 and 663 ± 21 pl per glomerulus for glomeruli isolated from normal and low sodium diet rats, respectively.

ANF-stimulated cyclic GMP accumulation by isolated glomeruli Cyclic GMP accumulation was measured according to the method previously described (Angielski *et al*., 1990). The glomeruli were resuspended (12000 ml^{-1}) in ice-cold Hank's salt solution containing (mM): NaCl 110, KCl 5.0, Na_2HPO_4 2.5, $CaCl_2$ 1.0, $MgSO_4$ 1.0, glucose 5.0, $NaHCO_3$ 25.0, sodium succinate 5.0, equilibrated with 95% O_2 and 5% CO_2. This suspension of glomeruli was preincubated in the presence of cicletanine (100 μM) or vehicle (ethanol diluted with buffer in ratio 1/2000) for 1 min at 37°C in a shaking water bath. Following preincubation, ANF (0.5 μM) was added and incubation was continued for 2 min. Incubation was stopped by addition of ice-cold absolute ethanol (1 : 1 v/v). The samples were sonicated for 2 min and centrifuged at $2500 \times g$ for 15 min. The supernatants were collected and the precipitates were washed with ethanol : water (2 : 1), vortexed and again centrifuged. Supernatants were combined and quickly evaporated to dryness at 50°C under the stream of nitrogen. Dried samples were resuspended in assay buffer and cyclic GMP content was determined with a commercial kit. Pellets were solubilized and protein concentration was measured. Values are given as picomol cyclic GMP mg^{-1} protein.

Other analytical methods

Urine volume was determined gravimetrically and sodium concentration by flame photometry. Inulin clearance (C_{in}) and *p*-aminohippurate clearance (C_{PAH}) were regarded as markers of GFR and effective renal plasma flow, respectively. Inulin was measured according to Heyrowsky (1956), *p*-aminohippurate was measured according to Bratton & Marshall (1939). Excretion of nephrogenous cyclic GMP was expressed as picomol cyclic GMP excreted in urine relative to C_{in}. Cyclic

GMP concentration was determined by a radioimmunological method with commercial kits.

The protein content of glomeruli was determined by the method of Lowry *et al*. (1951).

Statistical analysis

Results are presented as the mean ± s.e.mean. Statistical significance was assessed by using the Mann-Whitney non-paired test for results in the different animals or the Wilcoxon paired test for results in the same animals; $P<0.05$ was considered to be statistically significant.

Materials

Cicletanine was a gift from Institute Henri Beaufour (Le Plessis-Robinson, France). Before the experiments, cicletanine was dissolved in absolute ethanol as a stock solution at concentration of 0.2 M. Atrial natriuretic factor (human, 28 amino acids) was purchased from Peninsula Laboratories Inc. (Belmont, Ca.), angiotensin II was from Sigma Chemical Co. (St. Louis, U.S.A.), silicone oil AR 20 was from Wacker-Chemie GmbH (Monachium, Germany), cyclic GMP-RIA kits and [^{3}H]-inulin were from Amersham (Buckinghamshire, U.K.). Low sodium diet (Altromin C 1036) and normal sodium diet (Altromin C 1000) were obtained from Altromin GmbH (Lage, Germany).

Results

Clearance studies

Group 1 In the normal sodium diet rats, infusion of CIC alone at a dose of 0.25 mg min^{-1} kg^{-1} BW caused a significant increase in C_{in}, urinary flow (V), sodium excretion ($U_{Na}V$) and fractional excretion of sodium FE_{Na} within the first 40 min of infusion reaching the highest rates between the 10th and 30th min (Table 1). In the low sodium diet rats, C_{in} did not change significantly throughout the infusion of CIC alone, whereas V, $U_{Na}V$, FE_{Na} increased significantly. In rats on both diets, CIC induced a significant increase in nephrogenous cyclic GMP excretion that did not alter markedly during the experimental time course. There were no differences in the CIC-induced nephrogenous cyclic GMP excretion in the rats on neither diet.

Group 2 In the normal sodium diet rats, infusion of ANF alone at a dose of 0.5 μg min^{-1}kg^{-1} BW elicited transient increase in C_{in}, V, $U_{Na}V$, FE_{Na} within 20 min with the peak in the first clearance period. In the low sodium diet rates, C_{in} did not change markedly throughout the infusion of ANF alone, whereas V, $U_{Na}V$, FE_{Na} increased significantly. In rats on both diets, ANF infusion caused a significant increase in nephrogenous cyclic GMP excretion only within the first 10 min; the increase of nephrogenous cyclic GMP excretion in response to ANF infusion in the low sodium diet rats was markedly lower as compared to the normal sodium diet rats.

Group 3 In rats on both diets, infusion of CIC in combination with ANF (in a period of the peak response of GFR to CIC or ANF alone in (groups 1 and 2 in normal sodium diet rats) caused a significant increase in C_{in} and in nephrogenous cyclic GMP excretion as compared to the respective period during infusion of CIC alone. In the normal sodium diet rats, increases in both C_{in} and nephrogenous cyclic GMP excretion induced by coadministration of CIC and ANF were comparable to the effects of ANF alone in Group 2. In the low sodium diet rats, increase in C_{in} induced by coadministration of CIC and ANF was accompanied by an increase in nephrogenous cyclic GMP excretion to the level achieved during the ANF infusion alone in the normal sodium diet rats.

Table 1 Comparison of CIC (0.25 mg min^{-1} kg^{-1} BW) and ANF (5 μg kg^{-1} bolus then 0.5 μg min^{-1} kg^{-1} BW)-induced changes in renal function in normal and low sodium diet rats

	Clearance period (min)	V *(µl min^{-1})*	$U_{Na}V$ *(µmol min^{-1})*	*FE_{Na} (%)*	C_{in} *(ml min^{-1})*	C_{PAH} *(ml min^{-1})*	*ncyclic GMP (pmol min^{-1})*	*MAP (mmHg)*
				Normal sodium diet				
Group 1								
Baseline	−10–0	25±6	1.6±0.3	0.8±0.2	1.61±0.09	6.82±0.70	6.4±1.8	122±3
CIC	10–20	42±6*	5.4±0.4**	2.2±0.1**	2.10±0.11*	7.92±0.49	28.2±5.3*	120±3
CIC	20–30	55±7**	5.0±1.0**	2.0±0.2**	2.13±0.08*	8.30±0.82	36.9±8.9*	117±5
Group 2								
Baseline	−10–0	19±3	1.8±0.3	0.7±0.2	1.87±0.15	7.86±0.48	7.8±1.3	118±3
Vehicle	10–20	22±3	2.0±0.4	0.8±0.1	1.73±0.10	8.07±0.53	5.2±1.8	120±5
+ANF	20–30	58±7**	14.9±5.4**	4.3±0.3**	2.49±0.12**	8.33±0.55	444±36**	104±3**
Group 3								
Baseline	−10–0	28±5	1.7±5	0.8±0.1	1.76±0.11	7.92±0.32	6.4±3.0	120±3
CIC	10–20	61±8**	6.1±1.1**	2.2±0.2**	2.18±0.12*	7.68±0.50	24.5±7.2*	118±3
CIC+ANF	20–30	112±13**	22.0±6.1**	5.7±0.5**	2.65±0.19**	8.26±0.84	528±52**	102±4**
				Low sodium diet				
Group 1								
Baseline	−10–0	19±5	0.9±0.3	0.4±0.1	1.75±0.11	7.22±0.50	4.8±2.9	120±3
CIC	10–20	39±5*	5.0±0.7**	1.9±0.2**	1.92±0.14	8.62±0.65	26.8±4.5*	120±2
CIC	20–30	57±7**	3.9±1.1**	1.6±0.2**	1.88±0.12	8.26±0.42	30.5±7.8*	117±5
Group 2								
Baseline	−10–0	22±5	1.2±0.3	0.5±0.1	1.67±0.08	7.12±0.46	7.1±1.6	122±3
Vehicle	10–20	21±4	1.1±0.4	0.5±0.2	1.75±0.12	7.80±0.35	5.2±3.2	119±3
+ANF	20–30	52±8**	15.4±2.2**	6.3±0.6**	1.73±0.05	7.78±0.28	243±43**	103±5**
Group 3								
Baseline	−10–0	20±4	0.9±0.3	0.4±0.2	1.82±0.12	7.28±0.52	9.8±1.8	120±3
CIC	10–20	41±7*	6.5±0.6**	2.5±0.4**	1.85±0.09	7.71±0.60	31.9±5.9*	116±4
CIC+ANF	20–30	98±14**	18.1±2.0**	5.2±0.5**	2.56±0.15**	7.95±0.38	487±41^{++}	98±3**

V = urinary flow; $U_{Na}V$ = sodium excretion; FE_{Na} = fractional excretion of sodium; C_{in} = inulin clearance; C_{PAH} = *p*-aminohippurate clearance; ncyclic GMP = nephrogenous cyclic GMP excretion. Values are means ± s.e.mean. *$P<0.05$, **$P<0.01$ vs baseline; $^{++}P<0.01$ vs ANF alone as shown in the low sodium diet rats.

In rats on both diets, the superimposed infusion of ANF on an ongoing infusion of CIC elicited a further significant increase in V, $U_{Na}V$ and FE_{Na}. In contrast to FE_{Na}, combined effects of both agents on V and $U_{Na}V$ were observed.

In all groups of rats (1, 2 and 3) on neither diet, C_{PAH} did not change during infusion of CIC or/and ANF. MAP decreased markedly during infusion of ANF; CIC in dose tested, given alone or in combination with ANF, did not effect MAP significantly.

Effect of CIC and ANF on AII-induced decrease in GIS

Consistent with our previous observations (Szczepanska-Konkel *et al*., 1991), in rats on both diets, AII 10^{-6} M rapidly decreased GIS, which was maximal at 5 min and lasted up to 30 min.

CIC alone, similar to ANF alone, did not affect the basal [^{3}H]-inulin space (GIS) in isolated glomeruli from normal and low sodium diet rats. In the normal sodium diet rats, CIC inhibited the AII-induced decrease in GIS in a concentration-dependent manner, reaching statistical significance at concentrations of 10^{-6} M ($88 \pm 4\%$ of basal GIS) and 10^{-4} M ($102 \pm 4\%$ of basal GIS) (Figure 1). This effect of CIC was maximal in the fifth minute and was maintained also in the tenth minute of the experiment. By contrast, in the low sodium diet rats, CIC did not inhibit the AII-produced decrease in GIS even at a concentration of 10^{-4} M. ANF 10^{-6} M prevented the decrease in GIS produced by AII in the normal sodium diet rats. ANF 10^{-6} M had no effect on AII-produced decrease in GIS in the low sodium diet rats. However, when glomeruli isolated from low sodium diet rats were preincubated with CIC 10^{-4} M, AII-induced decrease in GIS was prevented by ANF 10^{-6} M.

The combined effect of CIC 10^{-4} M and ANF 10^{-6} M on AII-induced decrease in GIS was also tested in the normal sodium diet rats; the combined effect of both agents was comparable to the effect of CIC 10^{-4} M or ANF 10^{-6} M alone.

Glomerular CIC and ANF-stimulated cyclic GMP accumulation

In the absence of the phosphodiesterase inhibitor, the mean basal cyclic GMP accumulation by glomeruli isolated from the normal sodium diet rats was higher than from the low sodium

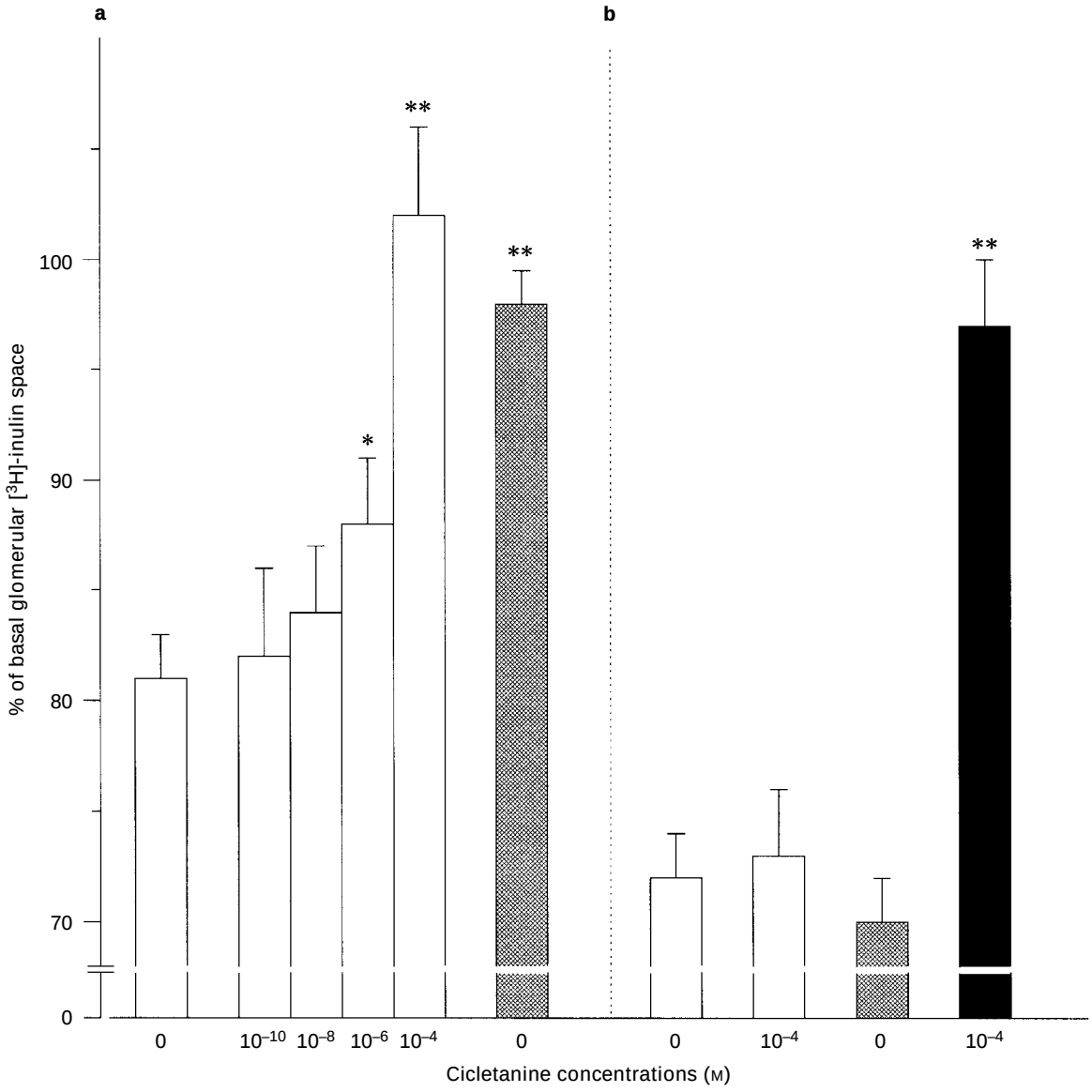

Figure 1 Effect of cicletanine (CIC) and atrial natriuretic factor (ANF) on angiotensin (AII)-induced decrease in glomerular [^{3}H]-inulin space (GIS) in (a) normal and (b) low sodium diet rats. Each column represents the mean $\pm$ s.e.mean of % of basal glomerular [^{3}H]-inulin space. Different concentrations of CIC were added to the glomeruli incubation mixture (10^{-10}–10^{-4} M). Open columns - control (AII 10^{-6} M, alone); cross-hatched columns - ANF 10^{-6} M; solid columns - CIC plus ANF 10^{-6} M. $n = 4$–6. *$P < 0.05$; **$P < 0.01$ vs controls.

diet rats (2.9 ± 0.2 vs 1.4 ± 0.1 pmol mg^{-1} protein, respectively, $P < 0.05$) (Figure 2). In rats on both diets, in the presence of CIC and ANF, the increase of cyclic GMP accumulation was transient with the peak in the second minute of glomeruli incubation (data not shown). Hence, this incubation time was chosen for the following experiments. Addition of CIC 10^{-4} M to the incubation mixture produced an increase in basal cyclic GMP accumulation by glomeruli from the normal and low sodium diet rats (up to 5.0 ± 0.4 and up to 4.8 ± 0.2 pmol mg^{-1} protein, respectively); there were no differences in cyclic GMP accumulation by glomeruli in rats on neither diet. Similarly, addition of ANF 10^{-6} M to the incubation mixture induced an increase in cyclic GMP accumulation in rats on both diets. However, ANF-stimulated cyclic GMP accumulation was markedly lower in glomeruli isolated from the low than the normal sodium diet rats (7.1 ± 0.5 vs 12.5 ± 1.1 pmol mg^{-1} protein, respectively, $P < 0.05$). In the low sodium diet rats, preincubation of glomeruli with CIC 10^{-4} M markedly potentiated ANF-stimulated cyclic GMP accumulation by glomeruli; in the presence of CIC, there were no differences in ANF-stimulated cyclic GMP accumulation by glomeruli from low and normal sodium diet rats (22.4 ± 1.0 and 24.1 ± 1.2 pmol mg^{-1} protein, respectively).

Discussion

Clearance studies

These studies showed that the GFR response to both CIC and ANF, given in pharmacological doses, depends on dietary sodium loading. In rats maintained on a normal sodium intake an increase in GFR was induced by CIC or ANF alone, whereas in low sodium diet rats this effect was markedly blunted. On the other hand, we did not observe any significant differences in the diuresis and natriuresis induced by these agents in rats on either diet. The natriuretic and diuretic responses to the pharmacological doses of CIC and ANF with no increase in GFR in low sodium diet rats suggests glomerular resistance to the effects of these agents. In contrast to the infusion of CIC or ANF alone, both agents given together increased GFR in low sodium diet rats. CIC also potentiated an increase in GFR induced by ANF in normal sodium diet rats. In addition, the combined effects of both agents on V and $U_{Na}V$ with no change in the responses in FE_{Na} in normal and low sodium diet rats were observed. This suggests that an increase in natriuresis during coadministration of CIC and ANF is implicated by the increase in sodium glomerular filtration load.

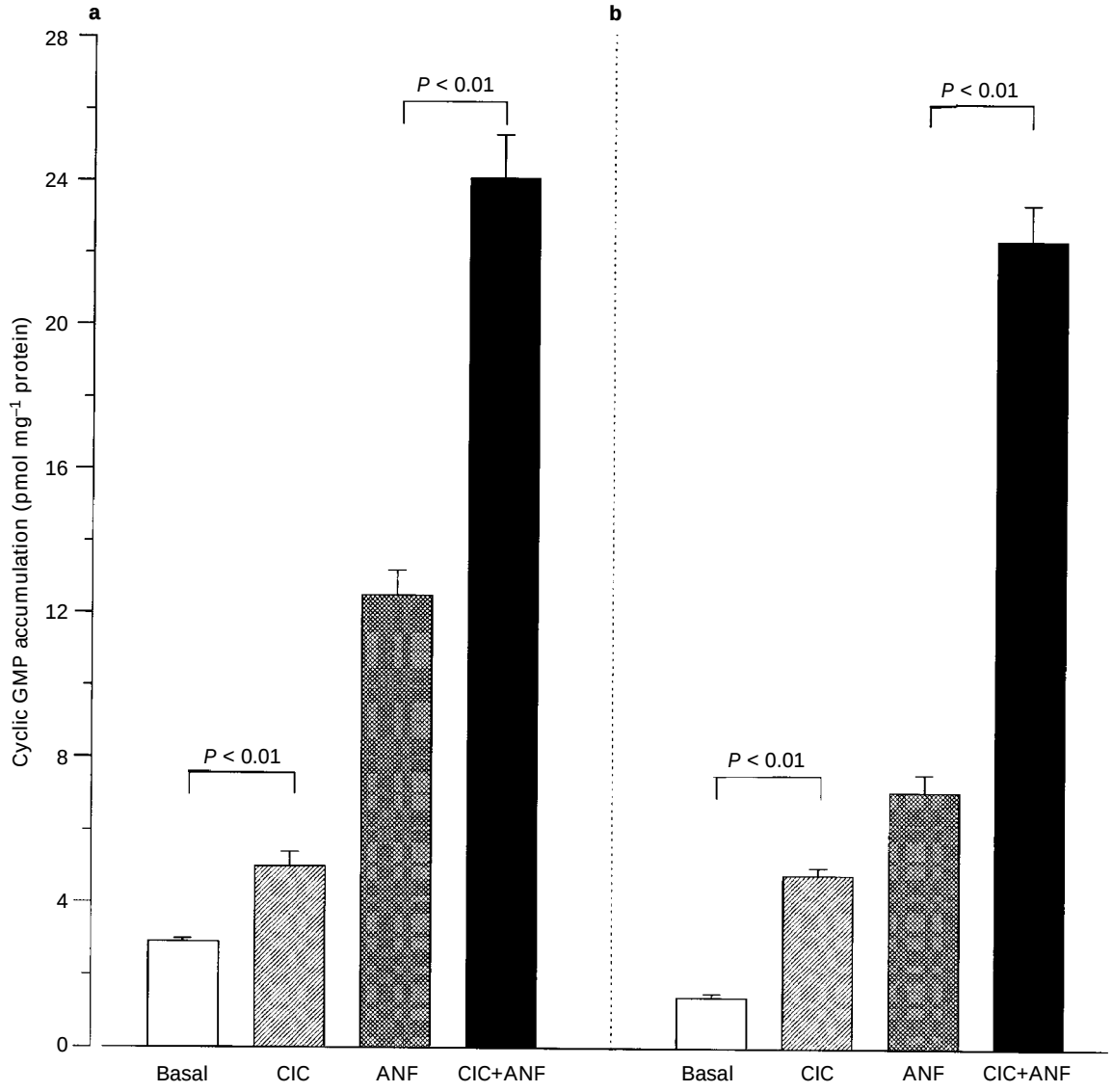

Figure 2 Effect of cicletanine (CIC) and atrial natriuretic factor (ANF) on cyclic GMP accumulation by glomeruli isolated from (a) normal and (b) low sodium diet rats. Glomeruli were preincubated with CIC 10^{-4} M or vehicle for 1 min at 37°C; then, ANF 10^{-6} M was added and incubation was continued for 2 min. Each column represents the mean ± s.e.mean of the values of cyclic GMP accumulation by isolated glomeruli in the 2nd minute of glomeruli incubation. Open columns - basal (vehicle); hatched columns - CIC; cross-hatched columns - ANF; solid columns - CIC plus ANF. $n = 4-6$.

Effect of CIC and ANF on AII-induced decrease in GIS

The *in vivo* experiments indicated a potential interaction of CIC with an activator of particulate guanylate cyclase in the regulation of glomerular filtration rate. To evaluate this possibility in rats on normal and low sodium diets, studies with [^{3}H]-inulin to measure the extracellular (largely intracapillary) volume of glomeruli (GIS) isolated from rats were performed. An increase and decrease in GIS during glomeruli incubation with tested substances reflects a relaxation and contraction of the glomeruli, respectively (Arriba *et al*., 1988; Fujiwara *et al*., 1989). This observation is consistent with results of the morphological studies of Hornych *et al*. (1972), who demonstrated by scanning electron microscopy that vasoactive agents, such as AII, caused a mechanical distortion of the glomerular capillaries restricting their size. The effect of vasoactive substances on glomerular intracapillary volume may be associated with changes in filtration surface area and resultant modification of ultrafiltration coefficient (Dworkin *et al*., 1983; Arriba *et al*., 1988). In our experiments, CIC and ANF alone completely prevented AII-induced contraction of glomeruli isolated from the normal sodium diet rats. In contrast, neither CIC nor ANF alone were able to induce relaxation of AII-contracted glomeruli isolated from the low sodium diet rats. Even a 10 fold higher concentration of CIC (10^{-3} M) or 100 fold higher concentration of ANF (10^{-4} M) had no effect on GIS of AII-contracted glomeruli isolated from the low sodium diet rats (data not shown). However, both CIC and ANF given together produced relaxation of AII-contracted glomeruli isolated from the low sodium diet rats. Hence, the experiments on isolated glomeruli contracted by AII correspond with the effects of CIC and/or ANF on GFR in an *in vivo* model of rats on both diets. We did not expect the combined effects of both agents on relaxation of AII-contracted isolated glomeruli from the low sodium diet rats. In the presence of CIC 10^{-4} M or ANF 10^{-6} M alone, the values of GIS did not differ from the basal GIS in rats on normal sodium diet. The isolated decapsulated glomeruli in the buffer free of vasoactive agents have globular structure and maximal volume, which is reflected by basal GIS. It is noticeable that, without a vasoconstrictor like AII, we were unable to demonstrate a relaxing effect of CIC on isolated glomeruli. In a similar way, the vasorelaxant properties of ANF on isolated glomeruli (Bianchi *et al*., 1986; Arriba *et al*., 1988) have also been shown to occur only in the presence of vasoconstrictors. It seems that, *in vivo*, glomerular size is probably regulated in a tonic fashion by different vasoactive substances and CIC could interact to antagonize the constrictor effects of these substances.

It is widely accepted that contraction of relaxation of mesangial cells is associated with changes in glomerular filtration surface area and a resultant decrease or increase in the ultrafiltration coefficient (Savin & Terreros, 1981; Arriba *et al*., 1988). Mesangial cells contain much more actinomyosin than other glomerular cell types (Andrews, 1981) and are known to possess specific AII and ANF receptors (Brenner *et al*., 1990; Sechi *et al*., 1992). It appears that CIC may interact in the intracellular processes evoked by AII and ANF in mesangial cells. However, the glomerular relaxant effect of CIC by an indirect effect on endothelial cells can also not be excluded. It has been shown, that the vasorelaxant effect of CIC is partially endothelium-dependent and related to nitric oxide (Bukoski *et al*., 1993).

CIC and ANF-stimulated nephrogenous cyclic GMP excretion and glomerular cyclic GMP accumulation

Silver *et al*. (1990) demonstrated that in aortic smooth muscle contracted with phenylephrine, CIC produced concentration-related vasorelaxation which occurred at the concentration range corresponding to the inhibition of low K_m cyclic GMP phosphodiesterases. In our clearance experiments, infusion of CIC alone significantly increased nephrogenous cyclic GMP excretion to the same extent in rats on both diets. ANF-stimulated nephrogenous cyclic GMP excretion was markedly lower in the low than in the normal sodium diet rats. Infusion of CIC increased the ANF-stimulated nephrogenous cyclic GMP excretion in the low sodium diet rats to the level observed in the normal sodium diet rats which coincided with restoration of the ANF-induced increase in GFR. In isolated glomeruli, CIC increased the cyclic GMP accumulation in rats on both diets; the difference demonstrated in basal cyclic GMP accumulation by glomeruli isolated from normal and low sodium diet rats was completely abolished in the presence of CIC. Cicletanine potentiated ANF-stimulated cyclic GMP accumulation by isolated glomeruli from rats on both diets. In addition, CIC completely abolished the difference in ANF-stimulated cyclic GMP accumulation by glomeruli isolated from rats in the low and normal sodium diets. Similar effects of CIC on nephrogenous cyclic GMP excretion and glomerular cyclic GMP accumulation in the presence or absence of ANF were previously demonstrated by us in rats on both diets in experiments with zaprinast, a selective cyclic GMP-phosphodiesterase inhibitor (Kalinowski *et al*., 1995). Furthermore, we have shown directly that the cyclic GMP-phosphodiesterase activity in the soluble fraction of the renal cortex was markedly higher in rats on low than normal sodium diets. Hence, it appears that the blunted glomerular response to CIC or ANF is due rather to an increased breakdown than to decreased formation of cyclic GMP in low sodium diet rats.

It is likely that CIC may inhibit low K_m cyclic GMP phosphodiesterase functionally in glomerular cells by virtue of its ability to potentiate the action of endogenous activators of guanylate cyclase relaxing glomeruli, such as ANF. However, our data indicating that CIC potentiates the cyclic GMP response to ANF through inhibition of cyclic GMP phosphodiesterase are not so straightforward. CIC alone induced cyclic GMP excretion to the same extent in rats on both diets, whereas the increase in GFR induced by CIC was only observed in the normal sodium diet rats. Similarly, CIC was unable to relax AII-contracted glomeruli from low sodium diet rats despite comparable accumulation of cyclic GMP in glomeruli of rats on both diets. All these data may be interpreted as suggesting that CIC not only inhibits cyclic GMP phosphodiesterase activity in glomeruli, but may cause relaxation by additional mechanism(s) in rats on a low sodium intake, possibly by interaction with prostaglandin and/or inhibition of other Ca^{2+} intracellular-dependent regulatory systems (i.e. opening of K^+-channels). The final glomerular response to CIC may be masked by the renin-angiotensin system (Greven *et al*., 1995), which is activated in a low sodium diet. CIC stimulates renal prostaglandin and prostacyclin synthesis (Hornych *et al*., 1989; Deby *et al*., 1989; Damase *et al*., 1991; Uehara *et al*., 1991; Emond *et al*., 1992; Tsunoda *et al*., 1993). On the other hand, the actions of prostaglandins (PG) I_2 and E_2 on the glomerular microcirculation appear to depend on an intermediate action of AII (Schor *et al*., 1981): blocking of AII receptors by saralasin, transformed renal actions of PGI_2 and PGE_2 from vasoconstrictors to vasodilators. An effect of CIC on the opening of K^+ channel was previously postulated by us, since in the rat glomeruli CIC-induced relaxation was antagonized by glibenclamide, an inhibitor of the ATP-sensitive K^+ channel (Szczepanska-Konkel *et al*., 1991). Cyclic GMP activates the K^+ channel (Fujino *et al*., 1991) and thus, it seems likely that the decreased basal level of cyclic GMP in glomeruli in a low sodium diet facilitates the inhibition of the glibenclimide-sensitive K^+ current which contributes to depolarization of the cell membrane in glomeruli. At present, it is difficult to define the crucial mechanism(s) by which CIC may antagonize glomerular constriction in a low sodium diet, the more so that the low sodium diet probably affects renal function by altering levels of various mediators and messengers, including prostaglandins, kinins and cyclic nucleotides (Siragy *et al*., 1994).

It is worth emphasizing that, from a clinical point of view, CIC restores the glomerular response to ANF under conditions of dietary sodium deprivation. It may be useful to give diuretics that can inhibit cyclic GMP phosphodiesterase in

combination with a low sodium diet to treat hypertension, or in the setting of diuretic resistance and sodium and water retension.

In conclusion, the present study demonstrates that, similar to ANF, CIC induces relaxation of glomeruli and a resultant increase in glomerular filtration rate. However, the glomerular response to CIC and ANF may be blunted by a low sodium diet intake. Moreover, it appears that CIC potentiates the action of a particulate guanylate cyclase activator by the involvement of cyclic GMP phosphodiesterase inhibition in low sodium diet rats. Further studies are needed to determine the extent of cyclic GMP phosphodiesterase inhibition of CIC, with respect to other proposed mechanisms of action of this agent.

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