Increased C-type natriuretic peptide mRNA expression in the kidney of diabetic rats

S-J Shin, J-D Wen, Y-J Lee, I-H Chen and J-H Tsai

Division of Endocrinology and Metabolism, Graduate Institute of Medicine, Kaohsiung Medical College, Kaohsiung 80317, Taiwan

(Requests for offprints should be addressed to S-J Shin, Division of Endocrinology and Metabolism, Kaohsiung Medical College, No 100, Shih-Chuan, 1st Rd, Kaohsiung 80317, Taiwan)

Abstract

To investigate the responsiveness of renal-synthesized C-type natriuretic peptide (CNP) to changes in water and electrolyte balance, we measured renal CNP mRNA levels, plasma CNP concentrations and urinary CNP excretion rates in streptozotocin-induced diabetic rats eating a normal (0.26% NaCl) or low (0.04% NaCl) salt diet. Using reverse transcription-PCR followed by Southern blot analysis, we found that renal cortical and medullary CNP mRNA levels were markedly enhanced in diabetic rats from the 14th day and remained enhanced with an accompanying elevation of urinary CNP excretion

rates for the entire 60-day study period. All increases of renal CNP mRNA and urinary CNP excretion rates in diabetic rats were attenuated in low salt diet-treated diabetic rats as well as insulin-treated diabetic rats. These results demonstrate that renal CNP synthesis is enhanced in diabetic rats and the increase of renal CNP mRNA is ameliorated by salt restriction and insulin treatment. These results imply that renal-synthesized CNP is responsive to the alteration of water and electrolyte homeostasis in diabetic rats.

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Introduction

Atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) are a family of structurally related but genetically distinct natriuretic peptides which mediate their cardiorenal function through a family of guanylyl cyclase receptors (Koller *et al.* 1991). Though initially identified in porcine brain (Sudoh *et al.* 1990), CNP immunoreactivity and mRNA have recently been found outside the brain (Komatsu *et al.* 1991, Stingo *et al.* 1992, Suga *et al.* 1992, Vollmar *et al.* 1993). Several investigations have also demonstrated that CNP protein (Komatsu *et al.* 1991, Ueda *et al.* 1991, Suzuki *et al.* 1993, Dean *et al.* 1994, Mattingly *et al.* 1994, Nir *et al.* 1994) and CNP mRNA (Suzuki *et al.* 1993, Dean *et al.* 1994, Terada *et al.* 1994) are detectable in the kidney, suggesting that the kidney is a site for CNP production (Mattingly *et al.* 1994). Although relatively little is known about the renal action of CNP, CNP may be involved in the regulation of water–electrolyte homeostasis as the ANP receptor-B (ANPR-B) is present in the kidney (Schultz *et al.* 1989, Canaan-Kuhl *et al.* 1992, Fraenkel *et al.* 1994, Terada *et al.* 1994) and CNP stimulates cGMP production by means of porcine kidney epithelial cells (Suga *et al.* 1990, Shigematsu *et al.* 1993, Terada *et al.* 1994). Furthermore, CNP stimulates natriuresis in rats (Sudoh *et al.* 1990, Tawaragi *et al.* 1991) and sheep (Charles *et al.* 1995) but apparently not in dogs (Clavell *et al.* 1993).

In animals and humans with diabetes, alterations in extracellular fluid and exchangeable sodium have been found (Ortola *et al.* 1987, Allen *et al.* 1990) and these alterations lead to changes in plasma ANP concentration (Ortola *et al.* 1987, Allen *et al.* 1990, Perico *et al.* 1992) and cardiac ANP mRNA expression (Matsubara *et al.* 1990). Despite the low expression of ANP gene message in the kidney, by use of reverse transcription-polymerase chain reaction (RT-PCR) we recently demonstrated that renal ANP mRNA levels and urinary ANP excretion rates are markedly increased in diabetic rats (Shin *et al.* 1997). Measurement of urine CNP concentrations (Mattingly *et al.* 1994) and renal CNP mRNA levels (Suzuki *et al.* 1993) has indicated that renal CNP synthesis could be higher than ANP synthesis. To date, no report has implied that renal-synthesized CNP is related to the intra-renal regulation of water–electrolyte homeostasis. In an attempt to find out whether intra-renal CNP synthesis is influenced in diseases with changes in water and electrolyte homeostasis, we measured renal CNP mRNA levels and urinary CNP excretion rates in streptozotocin-induced diabetic rats and in diabetic rats eating a salt-deficient diet.

Materials and Methods

Animal experiments

Male Wistar rats weighing 284–334 g were individually housed in metabolic cages. Diabetes was induced by a

Table 1 Characteristics of normal control rats (NC), diabetic rats (DM), insulin-treated diabetic rats (ITDM) and low salt diet-treated diabetic rats (LSD-DM) on the 60th day after the administration of citric buffer or streptozotocin. Values are means ± S.E.M.

	NC (n=8)	DM (n=8)	ITDM (n=7)	LSD-DM (n=7)
Body weight (g)	467 ± 7	247 ± 17 ^a	463 ± 8 ^c	232 ± 28 ^{ae}
Blood glucose (mmol/l)	6.9 ± 0.5	23.4 ± 1.7 ^a	6.2 ± 1.4 ^c	21.2 ± 1.8 ^{ae}
Urine amount (ml/day)	15 ± 1	172 ± 28 ^a	23 ± 9 ^c	116 ± 17 ^{aef}
Urine sodium (mmol/l)	0.93 ± 0.11	1.58 ± 0.17 ^a	1.03 ± 0.22 ^d	0.32 ± 0.12 ^{ace}
PRA (ng/ml/h)	9.5 ± 1.2	6.2 ± 0.8 ^b	10.6 ± 2.2 ^d	21.6 ± 1.8 ^{ace}
Plasma CNP (pmol/l)	1.9 ± 0.2	1.8 ± 0.2	1.5 ± 0.2	1.7 ± 0.2
Urine CNP (pmol/day)	1.0 ± 0.1	4.8 ± 0.6 ^a	1.5 ± 0.3 ^d	1.9 ± 0.3 ^{bd}

^a*P*<0.001, ^b*P*<0.05 vs NC; ^c*P*<0.001, ^d*P*<0.05 vs DM; ^e*P*<0.001, ^f*P*<0.05 vs ITDM.

single peritoneal injection of 55 mg/kg streptozotocin (STZ; Sigma, St Louis, MO, USA). Twenty-four hours later, induction of diabetes was confirmed by the measurement of tail blood glucose levels (Accutrend Glucose, Boehringer Mannheim, Mannheim, Germany). Rats with blood glucose levels >19.4 mmol/l were regarded as diabetic and were randomly assigned to one of three groups: diabetic rats (DM), insulin-treated diabetic rats (ITDM) and low salt diet-treated diabetic rats (LSD-DM). Rats in the ITDM group received insulin (human ultralente insulin; Novo-Nordisk, Copenhagen, Denmark) designed to achieve blood glucose levels between 4.5 and 8.3 mmol/l. Rats in the LSD-DM group were fed a diet containing 0.04% NaCl (No. 960232, ICN Pharmaceuticals Inc., Irvine, CA, USA). Twenty-nine weight- and age-matched rats were studied as normal controls (NC) and were given 0.26% NaCl food (catalog no. 905453, ICN Biochemicals, Irvine, CA, USA). These diabetic and normal rats were used in two experiments. In Experiment 1, rats (NC, *n*=8; DM, *n*=8; ITDM, *n*=7; LSD-DM, *n*=7) were killed by decapitation to collect blood for the measurement of plasma CNP, plasma renin activity (PRA) and plasma sodium on the 60th day after injection of STZ or citric buffer, and the kidney cortex, the outer and inner medulla were immediately removed for CNP mRNA analysis. CNP immunoreactivity in the urine of the four groups of rats was also measured on the 4th, 7th, 14th,

28th and 42nd day. In Experiment 2, rats from the DM group were killed on the 2nd (*n*=5), 7th (*n*=6), 14th (*n*=6), and 28th (*n*=5) day and rats from the normal group were also killed on the 2nd (*n*=5), 7th (*n*=6), 14th (*n*=5) and 28th (*n*=5) day, for renal CNP mRNA determination.

RNA isolation and reverse transcription

Total RNA was extracted from the renal cortex, outer and inner medulla using a modified guanidinium isothiocyanate method (Chomczynski & Sacchi 1987). Two micrograms total RNA from each sample were reverse transcribed by incubating with 20 µl reverse transcription mixture containing 20 pmole oligo (dT)₁₈ primer, 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM MgCl₂, 20 units RNase inhibitor, 0.5 mM dNTPs, and 500 U MMLV reverse transcriptase (Clontech Laboratories Inc., Palo Alto, CA, USA) at 37 °C for 2 h. The reverse transcriptase was inactivated by heating for 5 min at 94 °C.

Polymerase chain reaction amplification and Southern hybridization

Primers for the PCR were designed to flank at least one putative intron site for rat CNP and β-actin. For CNP, the sense primer (5'-TGCTCGCGCTACTCTCACT-3') corresponded to base pair (bp) 35–53; the antisense

Table 2 Urinary CNP excretion rate (pmol/day) of normal control rats (NC), diabetic rats (DM), insulin-treated diabetic rats (ITDM) and low salt diet-treated diabetic rats (LSD-DM) on days 0, 4, 7, 14, 28 and 42. Values are means ± S.E.M.

	NC (n=8)	DM (n=8)	ITDM (n=7)	LSD-DM (n=7)
Day				
0	1.2 ± 0.1	1.0 ± 0.2	0.9 ± 0.1	1.2 ± 0.2
4	1.2 ± 0.1	1.4 ± 0.2	1.5 ± 0.3	1.4 ± 0.2
7	0.6 ± 0.1	2.1 ± 0.4 ^b	1.1 ± 0.2	1.6 ± 0.7
14	1.0 ± 0.1	4.6 ± 0.5 ^a	3.0 ± 0.3 ^{af}	1.9 ± 0.3 ^{ceg}
28	0.9 ± 0.3	3.2 ± 0.4 ^a	1.7 ± 0.4 ^f	1.7 ± 0.3 ^f
42	0.8 ± 0.3	4.3 ± 0.4 ^a	1.6 ± 0.3 ^d	1.9 ± 0.5 ^{ce}

^a*P*<0.001, ^b*P*<0.01, ^c*P*<0.05 vs NC; ^d*P*<0.001, ^e*P*<0.01, ^f*P*<0.05 vs DM; ^g*P*<0.05 vs ITDM.

Figure 1 CNP gene expression in rat brain and kidney. Two micrograms total RNA from rat brain, rat renal cortex, outer medulla and inner medulla were used for RT and amplified with primers described in Materials and Methods. (A) Ethidium bromide-stained PCR products of 28 cycle-amplified RT product of brain (lane 1), renal cortex (lane 2), outer medulla (lane 3) and inner medulla (lane 4), negative control, 28 cycle-amplification with CNP primers in the absence of cDNA template (lane 5), 28 cycle-amplification of 2.5 µg genomic DNA prepared from rat brain tissue (lane 6). M: molecular weight markers (Marker VI; Boehringer Mannheim). (B) Southern blot hybridization of PCR product with a ^{32}P -labeled 247 bp rat CNP cDNA probe of 1/20 PCR product of rat brain (lane 1), PCR product of renal cortex (lane 2), outer medulla (lane 3), inner medulla (lane 4), negative control (lane 5), 1/40 PCR product of genomic DNA (lane 6).

primer (5'-TTGGGGTGCTCGTGCAGAA-3') corresponded to bp 263–281 of rat CNP cDNA. For β -actin, the sense primer (5'-CGTAAAGACCTCTATGCCAA-3') corresponded to bp 2748–2767; the antisense primer (5'-AGCCATGCCAAATGTCTCAT-3') corresponded to bp 3203–3222 of rat β -actin gene. The size of the expected PCR products was 247 bp for CNP and 349 bp for β -actin. For amplification, PCR was performed at a final concentration of $1 \times$ PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl_2), 0.2 mM dNTP, 0.4 µM sense and antisense oligos, and 2.0 units Taq DNA polymerase (Boehringer Mannheim, Indianapolis, IN, USA) to a total volume of 50 µl. The amplification cycles were 45 s at 94 °C, 45 s at 60 °C and 90 s at 72 °C in a Perkin-Elmer Cetus 9600 thermocycler (Perkin-Elmer Cetus, Norwalk, CT, USA). The cycles for the amplification of CNP mRNA and β -actin mRNA were repeated 28 and 22 times respectively in renal tissues, and the amplified products were electrophoresed on 1.8% agarose gels and transferred to nylon membranes (Schleicher & Schuell GmbH, Dassel, Germany). The

blots were hybridized with ^{32}P -labeled, randomly-primed, 247 bp rat CNP cDNA prepared by PCR cloning of rat brain extracts for 16 h at 42 °C, according to the standard technique. After each hybridization, the blots were washed twice in a solution containing 0.1% sodium dodecyl sulfate (SDS) and $2 \times$ SSC (0.3 M NaCl, 30 mM sodium citrate) for 15 min at room temperature, and then twice in 0.1% SDS and $0.2 \times$ SSC at 65 °C. Blots were exposed to Kodak XAR (Eastman Kodak Company, Rochester, NY, USA) film at -70 °C. A radioisotope-labeled probe for β -actin used as an internal control was also made by the primer extension method. After autoradiography, the X-ray film was scanned by a laser densitometer (Molecular Dynamics, Sunnyvale, CA, USA), and the data analyzed by M D ImageQuant software release version 3.22. The value obtained for each CNP PCR product was normalized to its β -actin level. To control the accuracy of RT-PCR followed by Southern hybridization, serially diluted RNA of renal tissues was subjected to RT-PCR amplification. Moreover, intra-assay variation of semi-quantitative RT-PCR followed by Southern blot analysis from renal tissues was tested.

Radioimmunoassay

CNP-like immunoreactivities (ir-CNP) from samples of plasma and urine were determined by a radioimmunoassay (RIA, Peninsula Laboratories, Inc., Belmont, CA, USA) after extraction as previously described (Lee *et al.* 1996). Briefly, 2 ml plasma or 5 ml urine were passed through Sep-Pack C^{18} cartridges (Waters Associates, Milford, MA, USA) and eluted with 5 ml 60% acetonitrile (ACN) containing 0.1% trifluoroacetic acid (TFA). The eluate was lyophilized and reconstituted for RIA. Duplicate samples were tested for RIA. Serial dilutions (1/1, 1/2, 1/4, 1/8, 1/16) of plasma and urine samples were subjected to RIA for ir-CNP. The correlation coefficients between ir-CNP levels and the times of dilution were $r=0.992$ and $r=0.975$. The intra- and interassay coefficients of variation were 5.7% and 10.2% for plasma ir-CNP, and 6.3% and 11.2% for urine ir-CNP. PRA was also determined by RIA (DuPont Company, Billeerica, MA, USA). Concentrations of urinary sodium were determined in an automatic analyser (Nova 5, Nova Biochemical, Newton, MA, USA).

Statistical analysis

Data were expressed as means $\pm$ S.E.M. To test the differences among the four groups, analysis of variance was performed. If a difference was found, unpaired Student's *t*-test was used for comparison between two groups. Linear regression analysis was used to test the correlation between the RNA concentrations and the corresponding densities when a serial dilution of RNA was subjected to RT-PCR

Figure 2 Validation of RT-PCR analysis as a method of determining the relative levels of CNP mRNA in different samples. (A) Results of Southern blot analysis of RT-PCR products of rat renal cortex (○), outer medulla (OM) (●) and inner medulla (IM) (■) with ^{32}P -labeled CNP cDNA for CNP of different amplification cycles. According to the densitometric analysis (lower panel), the cycle number selected for PCR amplification for CNP was 28 and is within the exponential phase of PCR amplification. (B) Results of Southern blot analysis of RT-PCR products for CNP of serially diluted RNA extracted from rat renal cortex (○), outer medulla (●) and inner medulla (■). Densitometric analysis (lower panel) showed good correlation ($r=0.943$ for renal cortex; $r=0.958$ for outer medulla; $r=0.944$ for inner medulla) between the RNA amount and the RT-PCR product when the RNA amount was under $2.4\text{ }\mu\text{g}$.

followed with Southern hybridization. A P value <0.05 was considered statistically significant.

Results

The characteristics of control, diabetic, insulin-treated diabetic and low salt diet-treated diabetic rats on the 60th day after the administration of STZ or citric buffer are shown in Table 1. The mean blood glucose, daily urine amount and sodium excretion values were significantly increased, whereas mean body weight was significantly decreased in diabetic rats compared with the control group. The mean PRA value was significantly lower, whereas the mean urinary CNP excretion rate was significantly higher in diabetic rats compared with control rats. All these changes in diabetic rats were ameliorated in the insulin-treated diabetic rats. Significantly lower urinary sodium excretion rates and daily urine amounts, and no significantly different values for mean blood glucose and body weight were observed in low salt diet-treated diabetic rats compared with diabetic rats. The mean urinary CNP excretion rate was significantly decreased while the mean PRA value was markedly increased in the low salt diet-treated diabetic rat group compared with the diabetic group. There was no difference in plasma CNP values among the four groups. As shown in Table 2, mean daily urinary CNP excretion rates

were significantly increased from the 7th day after STZ administration and remained elevated for the entire study period in the diabetic group compared with the control group. The elevation of the daily urinary CNP returned to near normal values after strict glycemic control in the insulin-treated diabetic group. In the low salt diet-treated diabetic rats, daily urinary CNP excretion rates were significantly decreased from the 14th day compared with the diabetic group.

RT-PCR coupled with Southern blot analysis revealed a 247-bp product from rat renal cortex, outer and inner medulla as well as brain tissue RNA extracts (Fig. 1). As the CNP primer sets used in the current study span a 394-bp intron, the 247-bp product amplified by these primers could not be of genomic origin. To determine the relative changes in tissue CNP mRNA expression, the yield of CNP PCR products was normalized to the amount of β -actin cDNA amplified from the tissue samples. This method has been used in several studies (Lee *et al.* 1996, Shin *et al.* 1997). Experiments were designed to determine the accuracy of the semi-quantitative RT-PCR. First, the amplification cycles of the PCR procedure were evaluated. Figures 2A and 3A show the results of different PCR cycles in the renal cortex and the outer and inner medulla. Secondly, a serial dilution of RNA subjected to RT-PCR coupled with Southern hybridization showed good correlation of the RNA dosage (Figs 2B and 3B). The correlation coefficients between

Figure 3 Validation of RT-PCR analysis as a method of determining the relative levels of β -actin mRNA in different samples. (A) Results of Southern blot analysis of RT-PCR products of rat renal cortex (○), outer medulla (OM) (●) and inner medulla (IM) (■) with ^{32}P -labeled β -actin cDNA for β -actin of different amplification cycles. According to the densitometric analysis (lower panel), the cycle number selected for PCR amplification for β -actin was 22 and is within the exponential phase of PCR amplification. (B) Results of Southern blot analysis of RT-PCR products for β -actin of serially diluted RNA extracted from rat renal cortex (○), outer medulla (●) and inner medulla (■). Densitometric analysis (lower panel) showed good correlation ($r=0.907$ for renal cortex; $r=0.948$ for outer medulla; $r=0.958$ for inner medulla) between the RNA amount and the RT-PCR product when the RNA amount was under $2.4\ \mu\text{g}$.

RNA concentrations and corresponding densities were $r=0.943$ and $r=0.907$ from the renal cortex, $r=0.958$ and $r=0.948$ from the outer medulla, and $r=0.944$ and $r=0.958$ from the inner medulla for CNP and β -actin respectively. Thirdly, the intra-assay test of RT-PCR followed by Southern blot analysis from one rat renal tissue was carried out. Consistent amounts ($2\ \mu\text{g}$) of RNA extracts were reverse transcribed followed by PCR amplification and Southern hybridization. The coefficients of variation of CNP and β -actin mRNA were, respectively, 3.3% and 3.5% for the renal cortex ($n=8$), 8.1% and 5.6% for the outer medulla ($n=8$), and 2.9% and 4.1% for the inner medulla ($n=8$). These results indicate that the semi-quantitative RT-PCR used in the present study was able to measure a small change in the relative amounts of a specific mRNA with reasonable accuracy.

Figure 4 shows the autoradiographs of RT-PCR amplification of CNP and β -actin mRNA in the renal cortex and the outer and inner medulla from diabetic and normal rats on the 60th day. The ratios of the densitometry measures of the PCR products for CNP and β -actin mRNA from rat renal cortex and outer and inner medulla in the diabetic group were significantly increased compared with the control group. No significant change in the CNP mRNA expression in the renal tissues was observed among insulin-treated diabetic, low salt diet-treated diabetic and NC groups.

Figure 5 represents the autoradiographs of RT-PCR amplification of CNP and β -actin mRNA in the renal cortex of diabetic and control rats on the 2nd, 7th, 14th and 28th day, and of insulin-treated diabetic rats on the 28th day. The ratio of the PCR products for CNP and for β -actin mRNA in the diabetic group was significantly increased on the 14th and 28th day compared with the control group and on the 28th day compared with the insulin-treated diabetic group.

Discussion

In the present study, we have demonstrated that renal CNP synthesis is enhanced in diabetic rats and that renal-derived CNP may be related to the regulation of sodium excretion by the kidney, based on the following findings: (1) PCR amplification identified the presence of CNP mRNA in the rat kidney; (2) renal CNP mRNA expression was significantly increased in diabetic rats; (3) urinary ir-CNP excretion was also increased in diabetic rats compared with normal rats; (4) the enhancement of renal CNP synthesis in diabetic rats returned to the near normal range in insulin-treated and salt-restricted diabetic rats.

CNP has been identified in the cultured renal cells of rat and human kidneys (Komatsu *et al.* 1991, Ueda *et al.*

Figure 4 Representative autoradiographs (upper panels) of the amplification of CNP and β -actin mRNA by RT-PCR followed by Southern blot analysis in the renal cortex (A), outer medulla (B) and inner medulla (C) from normal control (NC, $n=8$), diabetic (DM, $n=8$), insulin-treated diabetic (ITDM, $n=7$) and low salt diet-treated diabetic (LSD-DM, $n=7$) rats on the 60th day after administration of citric buffer or streptozotocin. Lower panels: relative ratios of the densitometry readings for RT-PCR amplification of CNP and β -actin mRNA in renal cortex, outer medulla and inner medulla from the upper panels. ^a $P<0.01$ compared with NC group; ^b $P<0.001$ compared with DM group.

1991, Suzuki *et al.* 1993, Dean *et al.* 1994, Mattingly *et al.* 1994, Nir *et al.* 1994). Several investigations have also demonstrated CNP mRNA in the rat kidney (Suzuki *et al.* 1993, Dean *et al.* 1994, Terada *et al.* 1994) and cultured mouse proximal cells (Suzuki *et al.* 1993). CNP mRNA has even been found in microdissected nephron segments of the proximal convoluted tubule, the cortical collecting duct and the medullary thick limbs of rats (Dean *et al.* 1994). The current study also demonstrates that CNP mRNA can be detected in the rat renal cortex, outer and inner medulla using RT-PCR coupled with Southern blot analysis. Furthermore, an abundance of renal ANPR-B mRNA was detected in animal kidneys and cultured renal cells (Schultz *et al.* 1989, Canaan-Kuhl *et al.* 1992, Fraenkel *et al.* 1994, Terada *et al.* 1994). Thus, these studies suggest that CNP has an important autocrine or paracrine function in the mammalian kidney.

Relatively little is known about the renal function of CNP. In anesthetized rats (Sudoh *et al.* 1990, Tawaragi *et al.* 1991) and conscious sheep (Charles *et al.* 1995), CNP was shown to be mildly diuretic and natriuretic, although other reports have demonstrated that intrarenal arterial administration of CNP has no effect on urine volume or urine sodium excretion (Clavell *et al.* 1993). Our current results show that urinary ir-CNP excretion and CNP mRNA expression in renal cortex and medulla were markedly enhanced after the induction of diabetes. The enhancement of renal CNP mRNA and urinary ir-CNP levels in diabetic rats were ameliorated after insulin treatment achieved near normal glycemic control. Mattingly *et al.* (1994) have recently reported that human urine contains both the high molecular form, CNP-53, and the lower molecular form, CNP-22. This finding is in

accordance with Suzuki *et al.* (1993) who have demonstrated that CNP-53 is produced by cultured renal cells. Since only CNP-22 has been identified in plasma (Togashi *et al.* 1992) and CNP is the most rapidly degraded of the natriuretic peptides by neutral endopeptidase (Kenny *et al.* 1993), urinary CNP is mostly derived from renal production rather than from filtered peptide. The present study shows that the increase of urinary ir-CNP excretion rate parallels the enhancement of renal CNP mRNA levels in diabetic rats. Additionally, urinary CNP excretion in diabetic rats was markedly increased compared with normal rats, but no difference in circulating plasma CNP concentration was found between the two groups. Based on these observations, we suggest that the elevation of urinary CNP excretion is most likely attributable to increased renal CNP synthesis in diabetic rats.

Using RT-PCR followed by Southern blot analysis, we compared the relative amount of CNP mRNA expression in the renal tissues, a method used in our previous reports (Lee *et al.* 1996, Shin *et al.* 1997). This method has proved to be reliable for comparing the changes of a gene product in relatively low abundance. Our previous reports demonstrated that renal ANP mRNA is significantly enhanced and the elevation of urinary ANP excretion correlated well with urinary sodium excretion in diabetic rats (Shin *et al.* 1997) and deoxycorticosterone acetate (DOCA)-treated rats (Lee *et al.* 1996). These findings support the proposal that renal-synthesized natriuretic peptide may be related to the change in water-electrolyte balance (Greenwald *et al.* 1991). In animals and humans with diabetes, alterations in extracellular and exchangeable sodium have been found (Ortola *et al.* 1987, Allen *et al.* 1990) and have been thought to lead to changes in plasma ANP

Figure 5 (A) Representative autoradiographs of the amplification of CNP and β -actin mRNA by RT-PCR followed by Southern blot analysis in the renal cortex from normal control rats (NC) on the 2nd ($n=5$), 7th ($n=5$), 14th ($n=6$) and 28th ($n=5$) day, diabetic rats (DM) on the 2nd ($n=5$), 7th ($n=6$), 14th ($n=6$) and 28th ($n=5$) day, and insulin-treated diabetic rats on the 28th ($n=5$) day after the administration of citric buffer or streptozotocin. (B) Relative ratios of the densitometry readings for RT-PCR amplification of CNP and β -actin mRNA in renal cortex from (A). ^a $P<0.001$ compared with NC; ^b $P<0.001$ compared with DM group.

concentration (Ortola *et al.* 1987, Allen *et al.* 1990, Perico *et al.* 1992) and cardiac ANP mRNA expression (Matsubara *et al.* 1990). Furthermore, in the present study, we show that CNP mRNA expression in renal cortex and medulla is markedly enhanced in diabetic rats, and that this enhancement could be attenuated by salt-restricted dietary administration. Recently, Fraenkel *et al.* (1994) reported that ANPR-B receptor mRNA levels were increased approximately twofold in the renal cortex of sodium-depleted sheep. Additionally, Mattingly *et al.* (1994) have reported that increased urinary CNP excretion was found in patients with congestive heart failure. According to these studies, it is conceivable that local renal CNP may regulate sodium excretion, independently of circulating CNP, in electrolyte-volume disorders.

In conclusion, our results show that the rat kidney is a site of CNP synthesis and that renal CNP synthesis is increased in diabetic rats. These findings support the concept that renal-synthesized CNP may be related to the renal regulation of water-electrolyte homeostasis in diabetic rats.

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