Changes of endothelin in streptozotocin-induced diabetic rats: effects of an angiotensin converting enzyme inhibitor, enalapril maleate

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Abstract

Endothelin-1 (ET-1) concentrations are increased in patients with diabetes mellitus, particularly those with diabetic retinopathy, or essential hypertension. We hypothesized that ET-1 might participate in the development and progression of diabetic microangiopathy. In this study, the effects of the angiotensin converting enzyme (ACE) inhibitor, enalapril maleate, on diabetic angiopathy were examined in streptozotocin (STZ)-induced diabetic (STZ-DM) rats by monitoring variations in renal function and ET-1 concentrations in blood and organ tissues.

Significant increases in kidney weight and in concentrations of urinary albumin, N-acetyl- β -D-glucosamidase (NAG) and serum ET-1 were observed in the STZ-DM rats as compared with the non-diabetic rats, and the concentration of ET-1 in the kidneys tended to be

increased. Microscopic and electron microscopic analyses showed increased mesangial cell proliferation, matrix expansion and enlarged mesangial area in the kidney of the diabetic rats. After administration of the ACE inhibitor, increased concentrations of urinary albumin and NAG in the STZ-DM rats were reduced to the control values with a slight improvement in the electron microscopic changes.

These data suggest that ET-1 may be involved in the development and progression of diabetic nephropathy and may explain, in part, why diabetes is liable to complicate hypertension. ACE inhibitor may help to restore diabetic nephropathy in the STZ-induced diabetic rats.

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Introduction

Diabetic vascular complications are important factors which influence the prognosis of diabetic patients. The development and progression of vascular endothelial cell damage is the basis of diabetic microangiopathy. In addition, hypertension may potentiate diabetic vascular complications.

Endothelin-1 (ET-1), a potent vasoconstricting factor that causes proliferation of vascular smooth muscle cells (Itoh *et al.* 1988, Yanagisawa *et al.* 1988, Inoue *et al.* 1989), is a peptide produced and secreted from vascular endothelial cells. It has been reported that ET-1 concentrations are increased in the sera of diabetic patients (Takahashi *et al.* 1990), particularly those with diabetic retinopathy (Kawamura *et al.* 1993), and that in diabetic rats endothelin A receptor antagonist and angiotensin converting enzyme (ACE) inhibitor could prevent the diabetic microangiopathy (Cooper *et al.* 1994, Dhein *et al.* 2000). This suggests that ET-1 may participate in the development and progression of diabetic microangiopathy. Serum

concentrations of ET-1, which has a potent vasoconstrictive action, are also increased in essential hypertension (Saito *et al.* 1990, Naruse *et al.* 1991).

Therefore, we have studied the involvement of ET-1 in the development and progression of diabetic vascular complications, especially diabetic nephropathy, the effects of an ACE inhibitor on renal function, and the ET-1 concentrations in sera and various organ tissues of streptozotocin-induced diabetic rats.

Materials and Methods

Preparation of diabetic rats

Diabetic rats were prepared by intraperitoneal administration of streptozotocin (STZ) at 60 mg/kg body weight to 10-week-old male Wistar rats (STZ-induced diabetic rats, STZ-DM). Those with a fasting blood glucose level greater than 200 mg/dl in the early morning were used for the experiments.

Treatment with ACE inhibitor

The STZ-DM rats were kept under diabetic conditions for 4 weeks, after which 5 mg/kg body weight of ACE inhibitor enalapril maleate (provided by Merck-Banyu Pharmaceutical Co., Ltd, Tokyo, Japan) dissolved in a 5% glucose solution was given orally, daily, for 4 weeks.

Methods of collecting blood samples and organs

Each blood sample was collected using cardiocentesis under anesthesia during fasting. The blood sample was then immediately centrifuged to obtain the serum which was stored at -20°C until assayed. At the same time, the removed organs (brain, heart, kidney and liver) were also immediately frozen and stored at -80°C .

Measurements

The animals were given regular laboratory chow and were randomly divided into the following four groups (8 rats in each group) and then treated as described in the parentheses: 1, control group (treated with saline instead of STZ, non-diabetic rats); 2, diabetic group (STZ-induced DM rats treated with saline instead of the ACE inhibitor); 3, ACE inhibitor-treated control group (non-diabetic rats treated with the ACE inhibitor); and 4, ACE inhibitor-treated diabetic group (STZ-induced DM rats treated with the ACE inhibitor).

The systolic and diastolic blood pressures (BP, mmHg) measured by noninvasive tail cuff plethysmography before death were 149 ± 3 (mean $\pm$ S.D., systolic) and 100 ± 7 (mean $\pm$ S.D., diastolic) in the control group, 134 ± 18 and 83 ± 20 respectively in the diabetic group, 113 ± 8 and 61 ± 16 respectively in the ACE inhibitor-treated control group, and 122 ± 4 and 73 ± 13 respectively in the ACE inhibitor-treated diabetic group.

Regarding the systolic BP, the difference between the ACE inhibitor-treated control group and the ACE inhibitor-treated diabetic group was statistically significant ($P=0.04$), while the others showed no significant difference.

Regarding the diastolic BP, a statistically significant difference was observed between the control group and the ACE inhibitor-treated diabetic group ($P=0.01$), while the others showed no significant difference.

The following data were obtained from each rat: body weight, weights of brain, heart, kidney and liver, fasting blood glucose (FBG) level measured by Glutest sensor (Sanwakagaku Laboratory, Nagoya, Japan), serum creatinine level (Perrone *et al.* 1992), amounts of urinary albumin (measured by enzyme immunoassay (Fielding *et al.* 1983)) and N-acetyl-f1-D-glucosamidase (NAG) (measured by the method reported previously by Noto *et al.* (1983)), and ET-1 concentrations in sera and tissues, brain, heart, kidney and liver. In addition, histological

examination was performed using electron microscopy for each specimen. Serum and urinary enalapril maleate (ACE inhibitor) levels were measured by the method reported previously (Ulm *et al.* 1982).

Assay for ET-1

Serum ET-1 concentrations were measured using an RIA (Ando *et al.* 1989). The ET-1 concentrations in the respective organ tissues were measured using the following method. After homogenization with 1.0 M acetic acid containing pepstatin (10 $\mu\text{g}/\text{ml}$) for 1 min, the homogenates were heated in boiling water for 10 min, and centrifuged at 55 000 g for 40 min at 4°C to obtain the supernatant. From the supernatant, the ET-1 was extracted with a Sep-pak C-8 column (Ando *et al.* 1989) and measured using an RIA kit (Sumikin Bioscience, Sagamihara, Japan). Intra- and interassay coefficients of variation were 10.0 and 12.4%. The recovery of ET-1 in this assay system was 97%.

Statistical analysis

Data are expressed as mean $\pm$ S.D. (standard deviation). Significant differences between the groups were determined by Kruskal-Wallis test or two-factor factorial ANOVA and/or repeated measures ANOVA, and differences were considered significant at $P<0.05$.

All experiments were performed in accordance with the guidelines on the handling of laboratory animals of our institution.

Results

Body weights

Body weights of the control group (non-diabetic rats) were increased at 4 weeks and at 8 weeks after the saline treatment, whereas those of the diabetic and the ACE inhibitor-treated diabetic group were significantly decreased after the STZ treatment in contrast to those of the non-diabetic groups (Table 1).

Organ weights (brain, heart, kidney and liver)

The weights of the kidneys in the diabetic group and the ACE inhibitor-treated diabetic group were significantly increased compared with those of the non-diabetic controls. However, there was no significant difference in the weights between the diabetic group and the ACE inhibitor-treated diabetic group (Table 1). The weights of brain, heart and liver were similar in the four groups (data not shown).

Fasting blood glucose (FBG) and serum creatinine levels

The FBG level was measured in blood samples drawn from the tail vein of each rat in the early morning after

Table 1 Body and kidney weights and fasting blood glucose (FBG) levels in control (non-diabetic), diabetic and ACE inhibitor-treated control and diabetic rats. The values are means $\pm$ S.D. ($n=8$ in each group)

Group	Body weight (g)			FBG (mmol/l)			Kidney weight (g)
	Initial	4 weeks	8 weeks	Initial	4 weeks	8 weeks	
Control (non-diabetic)	319 $\pm$ 5	346 $\pm$ 6 ^c	392 $\pm$ 8 ^c	5.3 $\pm$ 0.2	5.5 $\pm$ 0.3	5.6 $\pm$ 0.5	2.96 $\pm$ 0.07
Diabetic	320 $\pm$ 4	309 $\pm$ 9 ^a	297 $\pm$ 14 ^a	5.4 $\pm$ 0.3	15.3 $\pm$ 0.6 ^{ac}	15.6 $\pm$ 0.9 ^{ac}	3.42 $\pm$ 0.32 ^a
ACE inhibitor-treated control	317 $\pm$ 6	348 $\pm$ 9 ^c	394 $\pm$ 9 ^c	5.4 $\pm$ 0.3	5.5 $\pm$ 0.4	5.5 $\pm$ 0.6	2.99 $\pm$ 0.13
ACE inhibitor-treated diabetic	320 $\pm$ 5	309 $\pm$ 10 ^{ab}	295 $\pm$ 17 ^{ab}	5.5 $\pm$ 0.4	15.0 $\pm$ 0.7 ^{abc}	15.9 $\pm$ 1.0 ^{abc}	3.25 $\pm$ 0.24 ^{ab}

^a $P=0.04$, compared with those in control non-diabetic rats; ^b $P=0.04$, compared with those in ACE inhibitor-treated control rats; ^c $P=0.04$, compared with those in the initial period.

Table 2 Serum and renal tissue ET-1 levels in control, diabetic, ACE inhibitor-treated control and diabetic rats. The values are means $\pm$ S.D. ($n=8$ in each group)

			4 weeks after the treatment	
	Control (non-diabetic)	Diabetic	ACE inhibitor-treated control	ACE inhibitor-treated diabetic
Serum ET-1 level (pg/ml)	5.02 $\pm$ 3.01	8.69 $\pm$ 3.15*	4.92 $\pm$ 3.09	5.14 $\pm$ 3.23**
Renal tissue ET-1 level				
pg in whole kidney	66.9 $\pm$ 39.2	109.8 $\pm$ 53.1*	72.4 $\pm$ 45.2	87.8 $\pm$ 53.0
pg/mg protein	0.86 $\pm$ 0.28	1.19 $\pm$ 0.20	0.91 $\pm$ 0.32	1.01 $\pm$ 0.31
pg/mg DNA	8.2 $\pm$ 2.2	11.4 $\pm$ 2.7	8.4 $\pm$ 2.7	8.8 $\pm$ 2.4

* $P=0.04$, statistically significant, compared with that of control rats; ** $P=0.04$, statistically significant, compared with that of diabetic rats.

The protein and DNA concentrations were determined by Bio-Rad protein assay kit (Bradford 1976) and the method reported by Labarca and Paigen (1980).

overnight fasting. FBG levels in the diabetic group and the ACE inhibitor-treated diabetic group were significantly increased, but there was no significant difference between the two diabetic groups (Table 1). Serum creatinine levels in each group varied from 0.8 to 1.4 mg/dl respectively and they showed no statistically significant changes (data not shown).

NAG concentrations

The amount of urinary NAG was significantly ($P=0.04$) increased in the diabetic group compared with the control group and this increase was restored to normal by treatment with the ACE inhibitor. Urinary NAG levels were: control (non-diabetic) group 10.1 ± 4.3 U/l, diabetic group 25.7 ± 19.5 U/l, ACE-inhibitor-treated control group 9.6 ± 5.8 U/l and ACE-inhibitor-treated diabetic group 10.5 ± 8.7 U/l.

Urinary albumin levels

The concentration of urinary albumin was also increased significantly ($P=0.04$) in the diabetic group compared with the control group. The treatment of the STZ-DM rats with the ACE inhibitor for 4 weeks partly restored albumin excretion. Urinary albumin levels were: control

group $(2.2 \pm 1.2) \times 10^{-2}$ mmol/l, diabetic group $(9.6 \pm 6.0) \times 10^{-2}$ mmol/l, ACE inhibitor-treated control group $(2.0 \pm 1.7) \times 10^{-2}$ mmol/l and ACE inhibitor-treated diabetic group $(5.4 \pm 4.7) \times 10^{-2}$ mmol/l.

Serum ET-1 concentrations

There was a significant increase in the serum ET-1 concentrations in the diabetic group as compared with controls, and treatment with the ACE inhibitor significantly reduced the ET-1 serum levels (Table 2).

ET-1 concentrations in organ tissues

The ET-1 concentrations in the whole kidneys of the diabetic group increased significantly as compared with the non-diabetic rats (Table 2). The ET-1 contents in the kidneys were compared as a function of the amounts of protein and DNA in the kidney tissues. There were no differences in the amounts of protein and DNA between the rats of each group. The ET-1 concentrations revised by protein or DNA concentrations tended to increase in the diabetic group as compared with that in the control group, but those in the ACE inhibitor-treated diabetic group did not differ from those in the control and diabetic groups (Table 2).

Table 3 Cardiac, hepatic and cerebral ET-1 levels in control, diabetic, ACE inhibitor-treated control and diabetic rats. The values are means ± s.d. (n=8 in each group)

	4 weeks after the treatment			
	Control (non-diabetic)	Diabetic	ACE inhibitor- treated control	ACE inhibitor- treated diabetic
Cardiac ET-1 (pg/g wet weight)	27.6 ± 9.3	38.8 ± 14.9	30.8 ± 13.9	41.0 ± 16.7
Hepatic ET-1 (pg/g wet weight)	6.9 ± 4.5	6.2 ± 3.8	5.9 ± 3.7	4.6 ± 1.6
Cerebral ET-1 (pg/g wet weight)	25.1 ± 4.3	27.6 ± 5.1	23.2 ± 5.9	22.1 ± 3.5

Figure 1 Histological examination of the renal tissue from the STZ-induced diabetic rats by electron microscopic images (× 8300). Mesangial cell proliferation and matrix expansion were observed in the diabetic rat (middle panel), differing from the non-diabetic rat (control) (left panel). The right panel shows the situation in the ACE inhibitor-treated diabetic rats, which is slightly improved compared with that of the diabetic rats.

There were no significant differences in the ET-1 concentrations in the heart, liver and brain among the four groups (Table 3).

Microscopic and electron microscopic findings in the kidney tissue specimens

Increased mesangial cell proliferation, matrix expansion and enlarged mesangial area were observed in the diabetic rats but not in the non-diabetic rats (Fig. 1). In the electron microscopic findings, treatment with the ACE inhibitor resulted in slight improvements in the histological abnormalities (Fig. 1) (Table 4).

Enalapril maleate (ACE inhibitor) levels in serum and urine

Serum and urinary enalapril maleate levels in control (non-diabetic) and diabetic rats were undetectable. Serum enalapril maleate level in ACE inhibitor-treated control

rats was 12.1 ± 2.5 µg/l (mean ± s.d.), and that in ACE inhibitor-treated diabetic rats was 13.2 ± 2.1 µg/l. Urinary enalapril maleate level in ACE inhibitor-treated control rats was 10.1 ± 8.9 µg/l, and that in ACE inhibitor-treated diabetic rats was 10.5 ± 7.6 µg/l.

There were no statistically significant differences in enalapril levels which were enough to inhibit ACE activity (Cohen & Kurz 1982, Cohen *et al.* 1983) in serum and in urine between ACE inhibitor-treated normal and diabetic rats.

Discussion

Diabetic nephropathy is an important factor determining the prognosis of diabetic patients. In diabetic nephropathy, increased intraglomerular pressure due to renal glomerular vascular lesions induces microalbuminuria. When this lesion advances further apparent and persistent proteinuria

Table 4 The maximum and minimum widenings of mesangial matrix in the electron microscopic image ($\times 8300$) of control, diabetic and ACE inhibitor-treated rats. The values are means $\pm$ s.d. Numbers in parentheses are the number of the electron microscopic images of the rat kidney

Group	Maximum widening (μm)	Minimum widening (μm)
Control (non-diabetic) (5)	1.75 $\pm$ 0.22	0.69 $\pm$ 0.04
Diabetic (5)	3.31 $\pm$ 0.09*	2.01 $\pm$ 0.20*
ACE inhibitor-treated diabetic (5)	2.20 $\pm$ 0.47**	0.90 $\pm$ 0.05**

* $P=0.01$, statistically significant, compared with that in control non-diabetic rats; ** $P=0.03$, statistically significant, compared with that in diabetic rats.

due to glomerular sclerosis occurs leading to deterioration of renal function and chronic renal failure. ACE inhibitors have specific actions (Heeg *et al.* 1987, Remuzzi *et al.* 1991, Gansevoort *et al.* 1993) in reducing the progression of diabetic nephropathy.

The findings in our animal model, namely the increases in kidney weight, urinary albumin and NAG concentrations, mesangial cell proliferation, matrix expansion and enlarged mesangial area, are associated with diabetic nephropathy.

In the STZ-DM rats, treatment with enalapril maleate effectively reduced the levels of urinary NAG and albumin, and there were slight improvements in histological abnormalities. These data suggest that ACE inhibitors reduce the progress of diabetic nephropathic dysfunction (Hoher *et al.* 1998) and its associated morphology.

Brenner, Hostetter and colleagues have proposed the glomerular hyperfiltration theory (Hostetter *et al.* 1981, Brenner *et al.* 1982). This suggests that the rise in blood osmotic pressure with hyperglycemia and the increase in circulating blood volume promotes the hypersecretion of atrial natriuretic peptide (ANP) which causes the dilatation of glomerular afferent arterioles. Hyperglycemia also causes the production of angiotensin II, which stimulates arteriolar constriction but acts more strongly on the glomerular efferent arterioles. These mechanisms induce an imbalance between the constriction and dilatation of the afferent and efferent arterioles, resulting in increased intraglomerular pressure, and the subsequent development and progression of diabetic nephropathy (Hostetter *et al.* 1981, Brenner *et al.* 1982).

ACE inhibitors cause dilatation of the efferent arterioles more selectively than the afferent arterioles (Anderson *et al.* 1986, Fujiwara *et al.* 1991) and inhibit degradation of bradykinin, which also dilates the efferent arterioles (Kon *et al.* 1993). This may decrease intraglomerular pressure and improve glomerular hyperfiltration. It has also been demonstrated using cultured mesangial cells that angiotensin II acts directly on mesangial cell proliferation and matrix expansion which constitute glomerular sclerosis (Ichikawa & Harris 1991, Ray *et al.* 1991).

In the ACE inhibitor-treated diabetic group, the urinary NAG concentration was decreased to the normal

level. NAG is an enzyme located in renal glomeruli and proximal tubular cells and is released into the urine when renal parenchymal cells are damaged.

The reduction in urinary NAG levels in STZ-DM rats by the ACE inhibitor may be due to decreased intra-glomerular pressure (Anderson *et al.* 1986, Fujiwara *et al.* 1991) and the slight increase in the permeability of glomerular basement membranes (Wiegmann *et al.* 1992). As the ACE inhibitor did not affect blood glucose in the diabetic rats, the reduction of urinary albumin and NAG secretion was not attributable to improvements in glycemic control.

In the diabetic group, the ET-1 concentration tended to increase in the kidney. This change may be due to ET-1 in the renal cells because ET-1 exists not only in the vascular endothelial cells but also in the mesangial cells or tubular cells (Kosaka *et al.* 1989, Simonson & Dunn 1990, Kohan 1991). Fukui *et al.* (1994) reported that increases in ET-1 mRNA (2, 3 kilobases (kb)) in isolated glomeruli from diabetic rat kidney were partly reversed by enalapril treatment. However, MacCumber *et al.* (1989) detected two forms of ET mRNA, 3.7 and 2.5 kb, in rat tissues and assumed that the 2.5-kb transcript represents ET-1 and the 3.7-kb transcript reflects ET-3, so that kidney, eye and brain predominantly express ET-3 while lung expresses both ET-1 and ET-3. Moreover, employing *in situ* hybridization, they confirmed that renal ET mRNA was prominently localized to the medulla (MacCumber *et al.* 1989). We have tried to conduct Northern analysis of ET-1 mRNA in our experimental rat kidney. However, it was unsuccessful, probably because we used an unsuitable probe for renal ET mRNA or did not extract the microorganism from the tissue specimens. Serum ET-1 concentration which was augmented in diabetic rats was significantly decreased by the ACE inhibitor treatment. This mechanism is not clear, but it might be caused by a decrease in angiotensin II, since angiotensin II has been reported to promote the production and secretion of ET *in vivo* (Moreau *et al.* 1997) and *in vitro* (Emori *et al.* 1989).

The ET-1 concentration in the kidney was not significantly changed by the administration of the ACE inhibitor nor was it correlated with serum ET-1 concentration. The renin-angiotensin system within the vascular wall (Unger

& Gohlke 1990) may be affected by ACE inhibition. ET-1 induces the constriction of glomerular interlobular and arcuate arteries, and afferent and efferent arterioles (Simonson *et al.* 1989) and also mesangial cell proliferation, causing a reduction in renal blood volume and glomerular filtration and a decrease in the urinary Na excretion (Goetz *et al.* 1988, Miller *et al.* 1989). Olbrich *et al.* (1996) observed that ACE inhibitor treatment could prevent an impairment of NO-dependent regulation in small resistance vessels. These data, including the changes of ET-1 concentration due to ACE inhibition, may at least partly cause the reduction of urinary albumin and NAG secretion.

In conclusion, the increased urinary albumin and NAG secretion in STZ-induced diabetic nephropathy was reduced by ACE inhibition. ACE inhibitors may, in part, exert their effects in diabetic microangiopathy by reducing ET-1 levels.

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References

- Anderson S, Rennke HG & Brenner BM 1986 Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. *Journal of Clinical Investigation* **77** 1993–2000.
- Ando K, Hirata Y, Shichiri M, Emori T & Marumo F 1989 Presence of immunoreactive endothelin in human plasma. *FEBS Letters* **245** 164–166.
- Bradford M 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72** 248–254.
- Brenner BM, Meyer TW & Hostetter TH 1982 Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *New England Journal of Medicine* **307** 652–659.
- Cohen ML & Kurz KD 1982 Angiotensin converting enzyme inhibition in tissues from spontaneously hypertensive rats after treatment with Captopril or MK-421. *Journal of Pharmacology and Experimental Therapeutics* **220** 63–69.
- Cohen ML, Kurz KD & Schenck KW 1983 Tissue angiotensin converting enzyme inhibition as an index of the disposition of enalapril (MK-421) and metabolite MK-422. *Journal of Pharmacology and Experimental Therapeutics* **226** 192–196.
- Cooper ME, Rumble J, Komers R, Du HC, Jandeleit K & Chou ST 1994 Diabetes-associated mesenteric vascular hypertrophy is attenuated by angiotensin-converting enzyme inhibition. *Diabetes* **43** 1221–1228.
- Dhein S, Hochreuther S, Aus Dem Spring C, Bollig K, Hufnagel C & Raschack M 2000 Long-term effects of the endothelin (A) receptor antagonist LU 135252 and the angiotensin-converting enzyme inhibitor trandolapril on diabetic angiopathy and nephropathy in a chronic type I diabetes mellitus rat model. *Journal of Pharmacology and Experimental Therapeutics* **293** 351–359.
- Emori T, Hirata Y, Ohra K, Shichiri M & Marumo F 1989 Secretory mechanism of immunoreactive endothelin in cultured bovine endothelial cells. *Biochemical and Biophysical Research Communications* **160** 93–100.
- Fielding BA, Price DA & Houlton CA 1983 Enzyme immunoassay for urinary albumin. *Clinical Chemistry* **29** 355–357.
- Fujiwara Y, Kitamura E, Ochi S, Shin S, Fukunaga M, Yokoyama K, Fukuhara Y, Ueda N, Kamada T & Orita Y 1991 Isotopic measurement of glomerular intracapillary volume as a quantitative index for mesangial cell contractility. *Contributions to Nephrology* **95** 12–21.
- Fukui M, Nakamura T, Ebigara I, Makita Y, Osada S, Tomino Y & Koide H 1994 Effects of enalapril on endothelin-1 and growth factor gene expression in diabetic rat glomeruli. *Journal of Laboratory and Clinical Medicine* **123** 763–768.
- Gansevoort RT, DeZeeuw D & DeJong PE 1993 Dissociation between the course of the hemodynamic and antiproteinuric effects of angiotensin I converting enzyme inhibition. *Kidney International* **44** 579–584.
- Goetz KL, Wang BC, Madwed JB, Zhu JL & Leadley Jr RJ 1988 Cardiovascular, renal, and endocrine responses to intravenous endothelin in conscious dogs. *American Journal of Physiology* **255** R1064–R1068.
- Heeg JE, DeJong PE, Van Der Hem GK & DeZeeuw D 1987 Reduction of proteinuria by angiotensin converting enzyme inhibition. *Kidney International* **32** 78–83.
- Hoche B, Lun A, Priem F, Neumayer HH & Raschack M 1998 Renal endothelin system in diabetes: comparison of angiotensin-converting enzyme inhibition and endothelin-A antagonism. *Journal of Cardiovascular Pharmacology* **31** (Suppl 1) S492–S495.
- Hostetter TH, Troy JL & Brenner BM 1981 Glomerular hemodynamics in experimental diabetes mellitus. *Kidney International* **19** 410–415.
- Ichikawa I & Harris RC 1991 Angiotensin actions in the kidney: renewed insight into the old hormone. *Kidney International* **40** 583–596.
- Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K & Masaki T 1989 The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *PNAS* **86** 2863–2867.
- Itoh Y, Yanagisawa M, Ohkubo S, Kimura C, Kosaka T, Inoue A, Ishida N, Mitsui Y, Onda H, Fujino M & Masaki T 1988 Cloning and sequence analysis of cDNA encoding the precursor of a human endothelium-derived vasoconstrictor peptide, endothelin: identity of human and porcine endothelin. *FEBS Letters* **231** 440–444.
- Kawamura M, Ohgawara H, Naruse M, Iwasaki N, Naruse K, Hori S & Omori Y 1993 Plasma endothelin levels in diabetic patients with retinopathy. *Journal of the Japanese Diabetes Society* **36** 125–129.
- Kohan DE 1991 Endothelin synthesis by rabbit renal tubule cells. *American Journal of Physiology* **261** F221–F226.
- Kon V, Fogo A & Ichikawa I 1993 Bradykinin causes selective efferent arteriolar dilation during angiotensin I converting enzyme inhibition. *Kidney International* **44** 545–550.
- Kosaka T, Suzuki N, Matsumoto H, Itoh Y, Yasuhara T, Onda H & Fujino M 1989 Synthesis of the vasoconstrictor peptide endothelin in kidney cells. *FEBS Letters* **249** 42–46.
- Labarca C & Paigen K 1980 A simple, rapid, and sensitive DNA assay procedure. *Analytical Biochemistry* **102** 344–352.
- MacCumber MW, Ross CA, Glaser BM & Snyder SH 1989 Endothelin: visualization of mRNAs by *in situ* hybridization provides evidence for local action. *PNAS* **86** 7285–7289.
- Miller WL, Redfield MM & Burnett Jr JC 1989 Integrated cardiac, renal, and endocrine actions of endothelin. *Journal of Clinical Investigation* **83** 317–320.
- Moreau P, d'Uscio LV, Shaw S, Takase H, Barton M & Luscher TF 1997 Angiotensin II increases tissue endothelin and induces vascular

- hypertrophy; reversal by ET(A)-receptor antagonist. *Circulation* **96** 1593–1597.
- Naruse M, Kawana M, Hifumi S, Naruse K, Yoshihara I, Oka T, Kato Y, Monzen C, Kurimoto F, Ohsumi K, Hosoda S & Demura H 1991 Plasma immunoreactive endothelin, but not thrombomodulin, is increased in patients with essential hypertension and ischemic heart disease. *Journal of Cardiovascular Pharmacology* **17** (Suppl 7) S471–S474.
- Noto A, Ogawa Y, Mori S, Yoshioka M, Kitakaze T, Hori T, Nakamura M & Miyake T 1983 Simple, rapid spectrophotometry of urinary N-acetyl-beta-D-glucosaminidase, with use of a new chromogenic substrate. *Clinical Chemistry* **10** 1713–1716.
- Olbrich A, Rosen P, Hilgers RD & Dhein S 1996 Fosinopril improves regulation of vascular tone in mesenteric bed of diabetic rats. *Journal of Cardiovascular Pharmacology* **27** 187–194.
- Perrone RD, Madias NE & Levey AS 1992 Serum creatinine as an index of renal function: new insights into old concepts. *Clinical Chemistry* **38** 1933–1953.
- Ray PE, Aguilera G, Kopp JB, Horikoshi S & Klotmen PE 1991 Angiotensin II receptor-mediated proliferation of cultured human fetal mesangial cells. *Kidney International* **40** 764–771.
- Remuzzi A, Peticucci E, Ruggenti P, Mosconi L, Limonta M & Remuzzi G 1991 Angiotensin converting enzyme inhibition improves glomerular size-selectivity in IgA nephropathy. *Kidney International* **39** 1267–1273.
- Saito Y, Nakao K, Mukoyama M & Imura H 1990 Increased plasma endothelin levels in patients with essential hypertension. *New England Journal of Medicine* **322** 205.
- Simonson MS & Dunn MJ 1990 Endothelin-1 stimulates contraction of rat glomerular mesangial cells and potentiates β -adrenergic-mediated cyclic adenosine monophosphate accumulation. *Journal of Clinical Investigation* **85** 790–797.
- Simonson MS, Wann S, Mene P, Dubyak GR, Kester M, Nakazato Y, Sedor JR & Dunn MJ 1989 Endothelin stimulates phospholipase C, NA^+/H^+ exchange, c-fos expression, and mitogenesis in rat mesangial cells. *Journal of Clinical Investigation* **83** 708–712.
- Takahashi K, Ghatei MA, Lam HC, O'Halloran DJ & Bloom SR 1990 Elevated plasma endothelin in patients with diabetes mellitus. *Diabetologia* **33** 306–310.
- Ulm EH, Hichens M, Gomez HJ, Till AE, Hand E, Vassil TC, Biollaz J, Brunner HR & Schelling JL 1982 Enalapril maleate and a lysine analogue (MK-521): disposition in man. *British Journal of Clinical Pharmacology* **14** 357–362.
- Unger T & Gohlke P 1990 Tissue renin-angiotensin systems in the heart and vasculature: possible involvement in the cardiovascular actions of converting enzyme inhibitors. *American Journal of Cardiology* **65** 3I–10I.
- Wiegmann TB, Herron KG, Chonko AM, MacDougall ML & Moore WV 1992 Effect of angiotensin-converting enzyme inhibition on renal function and albuminuria in normotensive type 1 diabetic patients. *Diabetes* **41** 62–67.
- Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K & Masaki T 1988 A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* **332** 411–415.

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