

Impaired response to angiotensin II in Type 1 (insulin-dependent) diabetes mellitus. Role of prostaglandins and sodium-lithium countertransport activity

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Summary. The pathogenesis of diabetic nephropathy remains elusive. A role for renal prostaglandins in antagonizing the hormonal effects of renin-angiotensin II has been postulated as a putative factor leading to hyperfiltration in patients with Type 1 (insulin-dependent) diabetes mellitus. Our aim was to elucidate the effects of angiotensin II on kidney haemodynamics and on blood pressure in eight normal subjects, in nine normotensive, in nine hypertensive with normal sodium-lithium countertransport activity in erythrocytes, in seven hypertensive without and in eight hypertensive Type 1 diabetic patients with microalbuminuria and with high sodium-lithium countertransport activity in erythrocytes. Angiotensin II infusion ($4 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 60 min) decreased the glomerular filtration rate to a greater extent in normal subjects (-20%), than in normotensive patients (-5% $p < 0.01$), in hypertensive patients with normal sodium-lithium countertransport activity in erythrocytes (-8% $p < 0.01$) in hypertensive patients with high sodium-lithium countertransport (-6% $p < 0.01$) and in hypertensive microalbuminuric patients (-5% $p < 0.01$) with Type 1 diabetes. The urinary excretion rate of vasodilatory prostaglandins was two-three fold higher in all patients than in normal subjects. Acute indomethacin treatment restored a normal response to angiotensin II infusion in normotensive patients, but did not change the renal haemodynamic response in normal subjects. With regard to hypertensive patients with and without microalbuminuria indomethacin treatment restored a normal response to angiotensin II

in some but not all patients. An inverse relation was found between angiotensin II-induced decrease in the glomerular filtration rate and the sodium-lithium countertransport activity in erythrocytes during indomethacin treatment. Hypertensive and microalbuminuric patients with a sodium-lithium countertransport activity higher than $0.41 \text{ mmol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$ (the upper limit in normal subjects) also had a greater intimal plus medial thickness of the carotid artery using an ultrasonic imaging technique. Chronic indomethacin administration (30 days) significantly decreased the baseline overnight fasting glomerular filtration rate in normotensive and in hypertensive patients with normal but not in hypertensive and microalbuminuric patients with high sodium-lithium countertransport activity.

In conclusion these results demonstrate that: (1) excessive synthesis of vasodilatory prostaglandins antagonizes the regulation of renal haemodynamics by angiotensin II, at least partially accounting for hyperfiltration in Type 1 diabetes, (2) elevated sodium-lithium countertransport activity in erythrocytes identifies a subgroup of patients with Type 1 diabetes and hypertension, with and without microalbuminuria, in whom the normalization of urinary excretion rate of prostaglandins does not restore a normal response to angiotensin II.

Key words: Prostaglandins, angiotensin, sodium-lithium countertransport, hypertension, diabetic nephropathy.

One third of the patients with Type 1 (insulin-dependent) diabetes mellitus are at risk of developing renal disease [1, 2]. This subset of patients usually has an increased rate of glomerular filtration (GFR), microalbuminuria and elevation of arterial blood pressure prior to the development of overt nephropathy [3–5]. Moreover, it has recently been shown that high sodium-lithium countertransport activity in erythrocytes, a genetic marker of essential hypertension [6–7], characterizes patients with Type 1

diabetes and overt nephropathy [8], microalbuminuria [9] and glomerular hyperfiltration [10] although not all reports confirm this finding [11]. It has also been suggested that diabetic nephropathy is most common in patients with a family history of hypertension [9]. Patients with diabetic nephropathy often have elevated blood pressure, which has been presumed to be a consequence of renal damage. However, blood pressure in patients with Type 1 diabetes and microalbuminuria is often raised be-

Table 1. Mean \pm SEM clinical and biochemical parameters^a of control subjects and Type 1 (insulin-dependent) diabetic patients (CD, H₁D, H₂D, H₃D).^a Data refer to values obtained on the 2nd day of admission during 200 mEq/day sodium and 100 mEq/day potassium intake

	Normotensive control subjects (C)	Normotensive diabetic patients (CD)	Hypertensive diabetic patients with normal Na ⁺ /Li ⁺ CT (H ₁ D)	Hypertensive diabetic patients with high Na ⁺ /Li ⁺ CT (H ₂ D)	Hypertensive, microalbuminuric diabetic patients with high Na ⁺ /Li ⁺ CT (H ₃ D)	
Age (years)	29 \pm 3	28 \pm 4	30 \pm 3	30 \pm 2	36 \pm 2 ^{a,c}	
Gender (male:female)	4:4	4:5	4:5	4:3	4:4	
Duration of antihypertensive treatment (years)	–	–	2.3 \pm 0.4	2.1 \pm 0.2	4.4 \pm 0.9 ^e	
Duration of diabetes (years)	–	7 \pm 2	8 \pm 2	8 \pm 2	15 \pm 2 ^e	
Smokers (%)	26	31	26	21	22	
Blood pressure (mm Hg)	Systolic	120 \pm 2	119 \pm 3	155 \pm 3 ^b	158 \pm 3 ^b	162 \pm 4 ^b
	Diastolic	80 \pm 2	78 \pm 2	97 \pm 2 ^b	95 \pm 3 ^b	97 \pm 4 ^b
Body mass index (kg/m ²)	20 \pm 1	21 \pm 1	22 \pm 1	22 \pm 2	23 \pm 2	
Plasma sodium concentration (mmol/l)	140 \pm 2	139 \pm 3	140 \pm 2	139 \pm 2	140 \pm 3	
Plasma potassium concentration (mmol/l)	4.0 \pm 0.1	3.9 \pm 0.1	4.1 \pm 0.1	4.0 \pm 0.2	4.0 \pm 0.3	
Sodium excretion (mmol/24 h)	110 \pm 12	115 \pm 17	120 \pm 20	102 \pm 9	97 \pm 8	
Potassium excretion (mmol/24 h)	107 \pm 17	99 \pm 22	110 \pm 24	104 \pm 19	99 \pm 10	
Albumin excretion rate (μ g/min)	6 \pm 2	8 \pm 3	7 \pm 3	10 \pm 3	46 \pm 6 ^{b,c}	
Plasma glucose (mmol/l)	4.7 \pm 0.2	6.4 \pm 0.2 ^b	6.4 \pm 0.3 ^b	7.2 \pm 0.3 ^b	7.1 \pm 0.3	
Plasma renin activity (ng angiotensin I/ml/h)	1.9 \pm 0.2	1.1 \pm 0.2 ^a	1.3 \pm 0.3	1.2 \pm 0.2	1.4 \pm 0.3	
Serum creatinine (mmol/l)	116 \pm 17	107 \pm 17	120 \pm 12	119 \pm 17	126 \pm 16	

^a $p < 0.05$, ^b $p < 0.01$, CD, H₁D, H₂D and H₃D patients vs C subjects; ^c $p < 0.05$ H₃D vs H₂D, H₁D and CD Na⁺/Li⁺ CT = sodium-lithium countertransport

fore any evidence of an impairment in renal function [3, 4, 9].

These findings suggest that the mechanisms behind hypertension in Type 1 diabetes are still poorly understood.

It is well established that a relative or absolute overactivity of the renin-angiotensin system can cause or sustain hypertension and renal abnormalities. With regard to the hormonal regulation of kidney haemodynamics, the interaction of prostaglandins (PG) and renin-angiotensin II (Ang II) is known to modulate renal haemodynamics and sodium handling. In normal man PGI₂ can stimulate the release of renin in the juxtaglomerular apparatus, and in turn Ang II in the renal interstitium, which is responsible for adjusting the vascular tone of the efferent glomerular artery and tubular sodium reabsorption [12, 13]. Ang II in turn stimulates the release of PGE₂ from renal interstitial cells [13], which inhibits sodium reabsorption in the renal medulla. An increased renal synthesis of PGE₂ and PGI₂ is frequently found in patients with Type 1 diabetes [14–17], together with low blood levels of renin and angiotensin II [14].

On the other hand an abnormal interaction of PGs and the renin-angiotensin system appears to characterize all patients with Type 1 diabetes [15–16], whereas only a subset of patients develops hypertension and nephropathy [1, 2, 9]. To gain further insights into the role of PGs and renin-angiotensin II in determining abnormalities in kidney function as well as hypertension in Type 1 diabetes, we studied the response to angiotensin II in diabetic patients with and without hypertension and microalbumin-

uria before and after indomethacin treatment. The rationale of the selection criterion of diabetic patients was that hypertension, microalbuminuria and abnormalities in kidney haemodynamics have been shown to be associated in patients, who are at risk for developing diabetic nephropathy [3–5]. However, since it is not yet clearly established whether microalbuminuria alone or microalbuminuria together with hypertension and hyperfiltration can predict overt nephropathy, we studied Type 1 diabetic patients in whom these pathological conditions had occurred either together or separately.

Patients and methods

Twenty-four patients with Type 1 diabetes and hypertension, attending the Department of Internal Medicine, University of Padova, were recruited for the study in the North East region of Italy. A matched group of eight normotensive healthy subjects and nine normotensive Type 1 diabetic patients served as control subjects (Table 1).

Informed consent was obtained from all study participants and the study protocol was approved by the Ethical Committee of the University of Padova.

All subjects were Caucasian with normal serum creatinine concentrations. The presence of diabetes or impaired glucose tolerance was excluded by an abbreviated 75 g glucose tolerance test (baseline and 2 h blood glucose concentration) in non-diabetic subjects using the American Diabetes Association criteria. The same criteria were used to diagnose Type 1 diabetes in diabetic patients [18].

Blood pressure (diastolic phase V) was measured in the sitting position after 10 min rest to the nearest 2 mm Hg using a Hawksley random zero sphygmomanometer (12 \times 35 cm cuff) and hypertension was defined as a blood pressure of \geq 145 mm Hg systolic and/or \geq 90 mm Hg diastolic in the absence of anti-hypertensive treatment.

Causes of secondary hypertension were excluded by a complete medical examination which also included a 12-lead ECG. All subjects had normal liver and endocrine function, except for diabetes, and were following an isocaloric diet containing 50% carbohydrate, 25% fat and 25% protein. Patients were admitted to the metabolic ward of the Internal Medicine Department of the University of Padova for one week prior to the study and all anti-hypertensive medication was discontinued for at least 20 days prior to admission. No patient was taking amiloride or oral contraceptives. Patients were given a 200 mEq Na, 100 mEq K intake with a fluid intake of 1,500 ml/day in the week preceding the renal haemodynamic evaluation both during placebo and indomethacin treatment. Timed overnight urine collections for urinary albumin measurement were performed at least four times over a period of 6 months preceding the study. The mean of all values for each patient was used for calculation.

Procedures

The day before the angiotensin II infusion the diabetic patients omitted the injection of intermediate-acting insulin and received short-acting insulin only at breakfast and lunch. At about 18.00 hours a constant intravenous infusion of insulin at a rate of 15 mU · kg⁻¹ · h⁻¹ using a Harvard pump (Harvard Apparatus, Mills, Mass., USA) was started in order to achieve and maintain euglycaemia. Solid food, alcohol, coffee, tea and smoking but not tap water were prohibited after 22.00 hours. On the morning of the angiotensin II infusion test the overnight constant insulin infusion was stopped in the diabetic patients and both normal subjects and diabetic patients were connected to an artificial pancreas (Biostator, Miles Ames, Cavenago, Italy) in run mode 9:1 to perform a completely automatized blood glucose clamp at 4.7 mmol/l. Thus, all experiments were performed under comparable conditions of fluid infusion (0.5 ml/min 154 mmol/l NaCl infusion) and Biostator driven euglycaemia. Diabetic patients received 14 ± 6 IU of regular insulin and normal subjects 2 ± 1 IU over the 330 min of the study. Experiments were performed under conditions of steady-state water diuresis and tracer blood concentrations as previously described [19–22]. Priming doses of 50 µCi of ⁵¹Cr-EDTA (Amersham, Amersham, UK) and 30 µCi of ¹²⁵I-hippurate (Amersham) followed by a constant infusion of 0.7 µCi/min and 0.2 µCi/min of the two tracers respectively, were given via an antecubital vein of the opposite arm for determination of GFR and renal plasma flow (RPF) [19–22]. Measurements were started after 120 min of equilibration and with a urine osmolarity consistently below 75 mosmol/kg. Patients remained supine throughout the experiment standing only to void urine. Three 30-min urine collections were made at the start of the experiment. At the midpoint of each urine collection period, blood samples were drawn, pulse rate taken, and blood pressure (diastolic phase V) was measured to the nearest 2 mm Hg using a standard mercury sphygmomanometer by a single observer (P.F.). Urine volume was measured and aliquots taken in plain tubes. A 3.5 ml aliquot was also taken in a tube containing 20 µl gelatine (10 g/dl) for protein measurements. Specimens for hormone measurements were collected in chilled tubes containing EDTA (12 mg) and aprotinin (500 kallikrein-inhibitor U/ml blood) (Antagosan, Behring, Rome, Italy). All urine and blood samples were frozen immediately and stored at -20 °C until the assay.

Angiotensin II infusion

After the assessment of basal GFR and RPF, all subjects received an infusion of angiotensin II amide (Hypertensina, 4 ng · min⁻¹ · kg⁻¹ diluted in 60 ml (154 mmol/l NaCl, Ciba Geigy, Basle, Switzerland) for 60 min using a Harvard infusion pump (Harvard Apparatus). The constant infusion of ⁵¹Cr-EDTA and ¹²⁵I-hippurate continued throughout the angiotensin II infusion period to assess the changes in GFR and RPF. Blood pressure was monitored every 10 min with the cuff positioned over the brachial artery of the arm containing the sampling catheter (Study 1). All subjects received a 0.9% (weight/volume) 154 mmol/l NaCl infusion containing 0.5% human

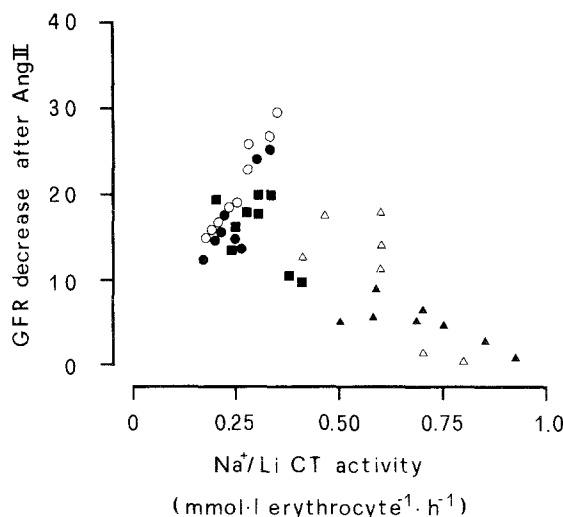


Fig. 1. Sodium-lithium countertransport (Na⁺/Li⁺ CT) in erythrocytes and angiotensin II (Ang II)-induced decrease in glomerular filtration rate (GFR) during indomethacin treatment in eight normotensive control subjects (●) and in 33 hypertensive patients with Type 1 (insulin-dependent) diabetes, nine normotensive (○) and seven hypertensive with high (Δ), nine hypertensive with normal (■) and eight hypertensive with high Na⁺/Li⁺ CT activity and microalbuminuria (▲) Type 1 diabetic patients. R_s = 0.76, p < 0.01, Spearman Correlation Coefficient

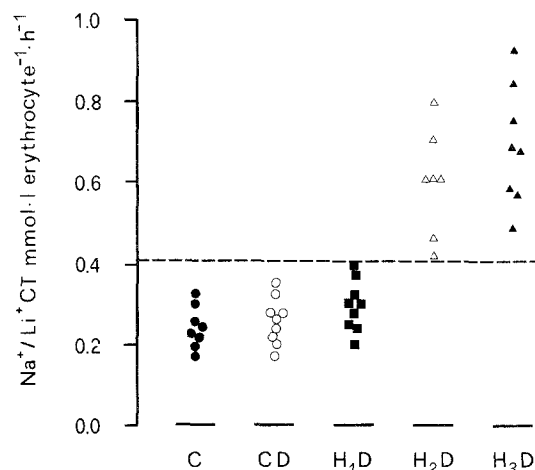


Fig. 2. Sodium-lithium countertransport (Na⁺/Li⁺ CT) activity in erythrocytes in eight control subjects (C), in nine normotensive (CD) and seven hypertensive with high (H₂D), nine hypertensive with normal (H₁D) and eight hypertensive with high sodium-lithium countertransport activity and microalbuminuria (H₃D) Type 1 (insulin-dependent) diabetic patients. The broken line indicates the upper limit of normal population

albumin at an infusion rate of 50 ml over 150 min (placebo). One week after Study 1, all subjects were given a second angiotensin II infusion with procedures identical to those of Study 1 except that indomethacin (Liometacen, Master, Pharma, Parma, Italy) was given orally to all subjects (50 mg twice per day) for the preceding 7 days. On the day of the angiotensin II infusion indomethacin was administered intravenously throughout the experimental period (15 µg · min⁻¹ · kg⁻¹) diluted in 154 mmol/l NaCl containing 0.5% human serum albumin at an infusion rate of 50 ml per 150 min (Study 2).

Baseline overnight fasting glomerular filtration rate was measured in all diabetic and non-diabetic subjects after 30 days of indomethacin treatment (50 mg twice daily) following the procedures described previously.

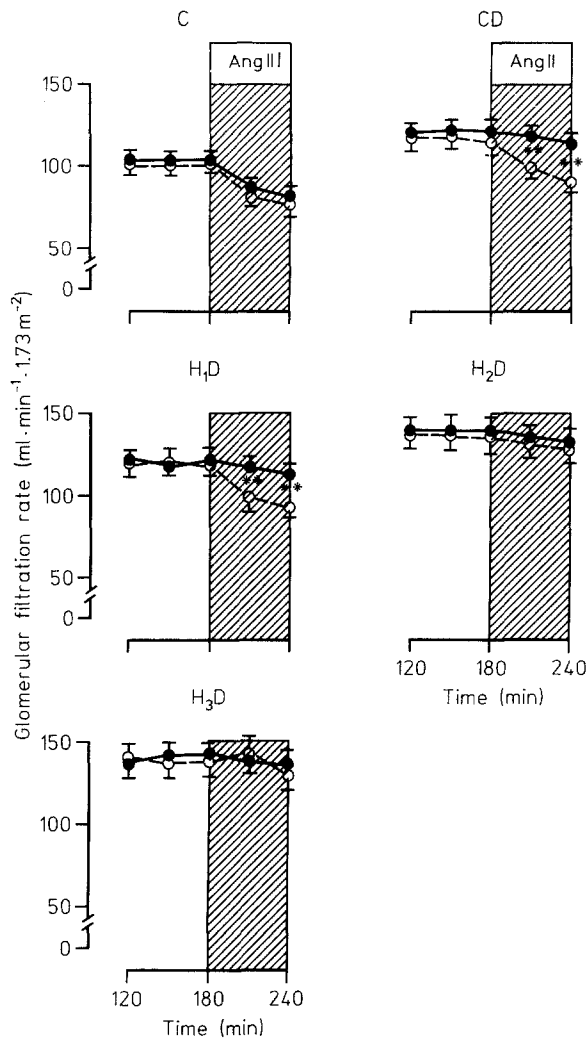


Fig. 3. Mean \pm SEM glomerular filtration rate at baseline and during angiotensin II infusion (Ang II) in eight control subjects (C), in nine normotensive (CD), in nine hypertensive with normal (H_1D), in seven hypertensive with high (H_2D) and in eight hypertensive with high sodium-lithium activity and microalbuminuria (H_3D) Type 1 (insulin-dependent) diabetic patients. Continuous line indicates the placebo Study 1 and broken line the indomethacin Study 2. ** $p < 0.01$ placebo vs indomethacin

Measurements

GFR and RPF were estimated by counting ^{51}Cr and ^{125}I in plasma and urine [19–22]. Albumin concentrations in urine [23] and free insulin [24] in plasma were measured by RIA technique. Blood glucose was measured by a glucose oxidase method (Beckman Instruments, Fullerton, Calif., USA) [25]. Plasma renin activity was measured by RIA technique [26]. Haemoglobin A_{1c} levels were determined using the BioRad (Richmond, Calif., USA) microcolumn chromatographic technique [27]. Plasma proteins and plasma albumin were measured using a Technicon RA-1000 multichannel autoanalyser. Haematocrit was determined by coulter counter. Sodium was measured in plasma and urine by flame photometry with lithium as an internal standard as indicated elsewhere [19, 22]. Urinary PGE_2 , 6-keto $PGF_{1\alpha}$, and $T \times B_2$ were measured by RIA technique. 1000 cpm 3H- PGE_2 (160 Ci/mmol, Amersham) 3H-6-keto $PGF_{1\alpha}$ (151 Ci/mmol, Amersham) 3H- $T \times B_2$ (200 Ci/mmol, Amersham), were added to 5 ml urine samples acidified to pH 4.0 with hydrochloric acid, and extracted on an octadecyl silicic microcolumn (Sep-Pak, Waters Milano, Italy) following the method of Powell [28]. The extracts were re-suspended in methanol and eluted isocratically with

acetonitrile: water:benzene: acetic acid 30: 69.7: 0.2: 01 on a C18 reverse phase micro-Bondapak column (Waters) at a flow rate of 2 ml/min [29–31]. This procedure is able to isolate urinary $T \times B_2$ from 2.3 dinor $T \times B_2$ [32]. The retention time of PGE_2 was 660, of 6-keto $PGF_{1\alpha}$, 300, of $T \times B_2$, 450. Recovery was $55 \pm 19\%$ for PGE_2 , $82 \pm 17\%$ for 6-keto $PGF_{1\alpha}$, $41 \pm 10\%$ for $T \times B_2$. For RIA of PGE_2 , anti- PGE_2 serum was purchased from the Pasteur Institute, Paris, France; for RIA of 6-keto $PGF_{1\alpha}$ and $T \times B_2$ a kit was purchased from Amersham. After overnight incubation, separation of antibody-bound and free fractions was obtained with charcoal-dextran. A fasting blood sample was taken in a 30 ml heparin-treated syringe for measurements of Na^+/Li^+ countertransport as described by Caneva et al. [6] and modified by Mangili et al. [8]. In order to have a reference value for Na^+/Li^+ countertransport activity in erythrocytes in normal subjects, 35 normotensive normal subjects with no family history of hypertension were identified in the Internal Medicine Department, University of Padova and in the Metabolic Medicine Department of UMDS, Guy's Hospital, London. Mean plus 2 SD Na^+/Li^+ countertransport activity in these subjects was $0.41 \text{ mmol} \cdot L \text{ erythrocytes}^{-1} \cdot h$. B mode ultrasound imaging technique was used to determine the intimal plus medial thickness in both common carotid arteries. The average value of the two measurements was then used for statistical comparison. This echographic technique has been recently validated comparing ultrasound imaging and intimal plus medial thickness measurements on pathological examination [33].

Calculations and statistical analysis

Clearances were calculated using the standard formula and protein excretion rate by multiplying concentrations by the urine flow rate. Renal vascular resistance (RVR) was calculated as $[MBP \times (1 - H_cT) / RPF] \times 100$, where MBP is mean blood pressure (calculated as diastolic blood pressure + 1/3 pulse pressure), H_cT is haematocrit and RPF is renal plasma flow. Results at baseline and at each hour time-point represent the mean of the measurements made within each one-hour period. Data were analysed by two-way analysis of variance for repeated measurements. If two-factor analysis of variance revealed a significant difference between groups and between times, Newman-Keuls test was used to determine the specific levels between which the difference occurred. Spearman-analysis was used to assess the correlation coefficient between the decrease in GFR and Na^+/Li^+ countertransport during indomethacin infusion along with angiotensin II administration. Chi-square statistical analysis was used when appropriate. Data are given as mean \pm SEM unless otherwise stated.

Results

Both normotensive and hypertensive-microalbuminuric Type 1 diabetic patients had higher baseline GFR and RPF values (normotensive Type 1 diabetic patients: 122 ± 7 , $p < 0.05$, and 545 ± 49 , $p < 0.01$, $\text{ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$; hypertensive-microalbuminuric Type 1 diabetic patients: 131 ± 8 , $p < 0.05$, and 576 ± 51 , $p < 0.01$, $\text{ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$) than normal subjects (106 ± 6 and $509 \pm 37 \text{ ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$).

Ang II-induced decrease in GFR and RPF was lower in normotensive diabetic patients (-6 ± 2 and $-64 \pm 13 \text{ ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$, -5% and -12% , $p < 0.01$ and $p < 0.01$, respectively) and in hypertensive-microalbuminuric diabetic patients (-7 ± 2 and -71 ± 13 , $p < 0.01 \text{ ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$; -6% and -13% , $p < 0.01$ and $p < 0.01$ vs normal subjects, respectively) than in normal subjects (-21 ± 3 and $-174 \pm 26 \text{ ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$; -20% and -34% , respectively). The urinary excretion

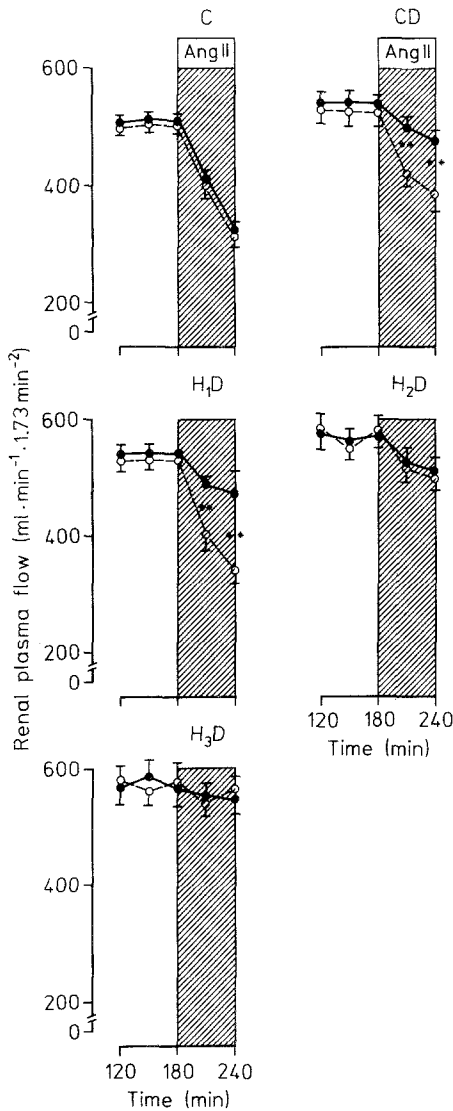


Fig. 4. Mean \pm SEM renal plasma flow in eight control (C) subjects, in nine normotensive (CD), in nine hypertensive with normal (H₁D) and in seven hypertensive with high (H₂D) and in eight with high sodium-lithium countertransport activity and microalbuminuria (H₃D) Type 1 diabetic patients. Continuous line indicates the placebo Study 1 and broken line indomethacin Study 2. ** $p < 0.01$ placebo vs indomethacin

rate of PGE₂ (normotensive diabetic patients: 1913 \pm 117 pg/min, $p < 0.01$; hypertensive microalbuminuric diabetic patients: 1872 \pm 173 pg/min, $p < 0.01$) of 6-ketoPGF_{1 α} (normotensive diabetic patients: 982 \pm 106 pg/min, $p < 0.01$; hypertensive microalbuminuric diabetic patients: 934 \pm 103 pg/min) but not of T \times B₂ (normotensive diabetic patients: 383 \pm 60, $p < 0.01$; hypertensive-microalbuminuric diabetic patients: 379 \pm 88 pg/min) was significantly higher than in normal subjects (PGE₂: 736 \pm 112; 6ketoPGF_{1 α} : 413 \pm 72; and T \times B₂: 256 \pm 36). Baseline values of GFR and RPF were not significantly influenced by indomethacin treatment in normal subjects or diabetic patients. On the contrary, indomethacin enhanced the Ang II-induced decrease in GFR in the normotensive, hypertensive and microalbuminuric Type 1 diabetic patients (-18 ± 3 ml \cdot min⁻¹ \cdot 1.73 m⁻²; -15% $p < 0.01$ placebo vs indomethacin) but not in normal subjects. Similarly indomethacin enhanced the Ang II-induced decrease in RPF in the normotensive and the hypertensive and microalbuminuric Type 1 diabetic patients (-135 ± 29 ml \cdot min⁻¹ \cdot 1.73 m⁻²; -25% $p < 0.01$ placebo vs indomethacin) but not in the normal subjects.

However, the action of Ang II on kidney haemodynamics was not similar in all Type 1 diabetic patients during cyclooxygenase inhibition. The administration of indomethacin was capable of restoring normal patterns of Ang II-induced changes in GFR and RPF in some, but not in all, patients with Type 1 diabetes.

In order to elucidate the reasons accounting for the differences in the renal haemodynamic response to Ang II infusion in Type 1 diabetic patients during indomethacin administration, we investigated the relationship between the absolute decrease in GFR following Ang II challenge and the activity of sodium lithium countertransport activity in erythrocytes. A highly significant inverse correlation was found between these two parameters (Fig. 1), suggesting that those patients with elevated sodium-lithium countertransport activity in erythrocytes were to some extent resistant to the action of Ang II, even during cyclooxygenase inhibition. To gain further insights into the nature of such a relationship we divided the hypertensive

Table 2. Mean \pm SEM values of filtration fraction, renal vascular resistance and diastolic blood pressure during placebo and indomethacin treatment at baseline (Base) and during angiotensin II infusion (Ang II) in control subjects and in CD, H₁D, H₂D and H₃D patients

		Normotensive control subjects (C)		Normotensive diabetic patients (CD)		Hypertensive diabetic patients with normal Na ⁺ /Li ⁺ CT (H ₁ D)		Hypertensive diabetic patients with high Na ⁺ /Li ⁺ CT (H ₂ D)		Hypertensive and microalbuminuric diabetic patients with high Na ⁺ /Li ⁺ CT (H ₃ D)	
		Base	Ang II	Base	Ang II	Base	Ang II	Base	Ang II	Base	Ang II
Filtration fraction (%)	Placebo	20.4 \pm 1.3	24.6 \pm 1.4 ^b	21.4 \pm 1.4	23.9 \pm 1.5 ^b	21.5 \pm 1.8	23.8 \pm 1.9 ^b	24.1 \pm 1.8	25.12 \pm 1.6 ^b	24.2 \pm 1.6	25.1 \pm 1.4 ^b
	Indomethacin	20.3 \pm 1.4	24.0 \pm 1.6 ^b	20.4 \pm 1.5	23.7 \pm 1.4 ^b	21.6 \pm 1.7	26.9 \pm 1.7 ^{b,d}	23.9 \pm 1.6	26.2 \pm 1.4 ^b	23.8 \pm 1.7	26.5 \pm 1.4 ^b
Renal vascular resistance $\frac{\text{mm Hg}}{\text{ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}}$	Placebo	119 \pm 12	206 \pm 14 ^b	111 \pm 21	135 \pm 19 ^a	127 \pm 16	135 \pm 20 ^a	120 \pm 12	131 \pm 15 ^b	121 \pm 10	131 \pm 14 ^b
	Indomethacin	120 \pm 15	210 \pm 17 ^b	112 \pm 17	175 \pm 15 ^c	128 \pm 11	206 \pm 23 ^c	119 \pm 13	130 \pm 12 ^b	119 \pm 9	130 \pm 12 ^b
Diastolic blood pressure (mmHg)	Placebo	82 \pm 2	93 \pm 1 ^b	83 \pm 2	90 \pm 1 ^a	99 \pm 3	106 \pm 3 ^a	100 \pm 2	104 \pm 2 ^b	100 \pm 2	106 \pm 3 ^b
	Indomethacin	81 \pm 1	94 \pm 2 ^b	84 \pm 2	94 \pm 1 ^a	98 \pm 2	109 \pm 2 ^a	100 \pm 2	107 \pm 2 ^b	100 \pm 2	106 \pm 3 ^b

^a $p < 0.05$, ^b $p < 0.01$ Ang II vs Base; ^c $p < 0.05$, ^d $p < 0.01$ indomethacin vs placebo
Na⁺/Li⁺ CT = sodium-lithium countertransport

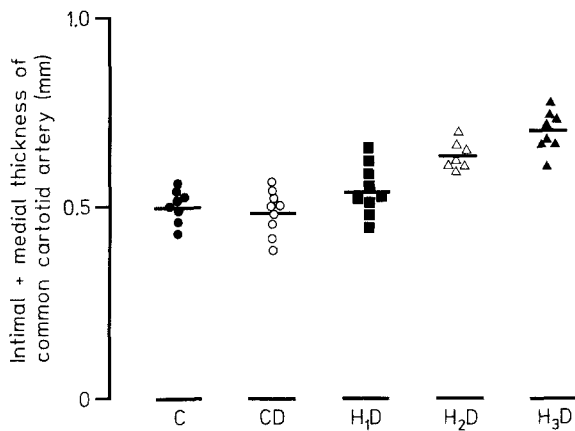


Fig. 5. B mode ultrasound imaging of intimal plus medial thickness of common carotid arteries in the five groups of control subjects (C) and in normotensive (CD) and hypertensive with normal (H_1D) with high sodium-lithium countertransport activity (H_2D) with high sodium-lithium countertransport activity and microalbuminuria (H_3D) Type 1 diabetic patients

patients with Type 1 diabetes in two subgroups: i. e. with an activity of sodium-lithium countertransport in erythrocytes lower (H_1D) and higher (H_2D) than $0.41 \cdot L$ erythrocytes $^{-1} \cdot h^{-1}$; that is the mean value plus 2 SD of the sodium-lithium countertransport in normotensive normal subjects.

Figure 2 shows the sodium-lithium countertransport activity in the erythrocytes of the normotensive control subjects (C), of normotensive (CD) and hypertensive Type 1 diabetic patients with normal (H_1D) and high (H_2D) sodium-lithium countertransport activity and hypertensive diabetic patients with microalbuminuria and high sodium-lithium countertransport activity (H_3D). The five groups of normal and diabetic subjects were comparable for gender, smoking habits, and body mass index (Table 1). Age and diabetes duration were similar in the CD, H_1D and H_2D patients. H_3D were older and had a significantly longer duration of diabetes. The three groups of hypertensive patients (H_1D and H_2D and H_3D) had similar blood pressure levels, but duration of antihypertensive treatment was longer in H_3D . Plasma sodium and potassium concentrations were similar in normal control subjects (C) and CD, H_1D , H_2D and H_3D diabetic patients, as was sodium and potassium excretion rate (Table 1). Plasma renin activity was significantly lower only in CD on ad-

mission to the hospital ward (Table 1). Serum creatinine was similar in all four groups of subjects and patients (Table 1). Albumin excretion rate was similar in C subjects ($6 \pm 2 \mu g/min$) and CD (8 ± 3), in H_1D (7 ± 3) and H_2D (10 ± 3) patients, whereas significantly higher values were found in H_3D patients ($46 \pm 6 p < 0.05$ vs C, CD, H_1D , H_2D) (Table 1).

The response to Ang II infusion during placebo and indomethacin treatment was then evaluated separately in these five groups of normal subjects and patients with Type 1 diabetes identified by different activities of sodium-lithium countertransport. This different approach in the analysis of the results of the present study revealed that baseline GFR were more elevated in H_2D and H_3D than in H_1D hypertensive patients (136 ± 7 and $138 \pm 6 ml \cdot min^{-1} \cdot 1.73 m^{-2}$ vs 117 ± 5 , $p < 0.01$ and $p < 0.01$, respectively) (Fig. 3). Similar differences were found with regard to RPF.

Furthermore, indomethacin treatment restored a normal response to Ang II in CD and H_1D but not in H_2D and H_3D diabetic patients with Type 1 diabetes (Figs. 3 and 4). Diabetic patients also had an impaired blood pressure and renal vascular resistance response to Ang II (Table 2). The inhibition of cyclooxygenase by indomethacin ameliorated the response in filtration fraction and in renal vascular resistance to Ang II challenge in the CD and H_1D but not in the H_2D and H_3D patients (Table 2). Interestingly, no significant differences in the renal and systemic haemodynamic response to Ang II were shown by normal subjects between the two studies with placebo and indomethacin treatments. The urinary excretion rate of PGE_2 , 6keto $PGF_1\alpha$ and $T \times B_2$ were markedly and similarly inhibited both in normal subjects and CD, H_1D , H_2D and H_3D patients with Type 1 diabetes by indomethacin treatment (Table 3).

Baseline overnight fasting GFR after 30 days of indomethacin treatment was $104 \pm 4 ml \cdot min^{-1} \cdot 1.73 m^{-2}$ in C subjects (NS vs placebo); 111 ± 5 ($p < 0.05$) in CD patients; 113 ± 6 ($p < 0.01$) in H_1D patients and 120 ± 7 (NS vs placebo) in H_2D patients and 128 ± 6 (NS vs placebo) in H_3D patients. Thus, chronic indomethacin treatment resulted in a significant decrease in GFR only in CD and H_1D patients but not in normal subjects (C) or hypertensive and microalbuminuric diabetic patients with high sodium-lithium countertransport activity (H_2D and H_3D).

Table 3. Mean \pm SEM prostaglandin urinary excretion rate during placebo and indomethacin (baseline plus angiotensin II infusion periods) in control subjects and in CD, H_1D , H_2D and H_3D patients

		Normotensive control subjects (C)	Normotensive diabetic patients (CD)	Hypertensive diabetic patients with normal Na^+/Li^+ CT (H_1D)	Hypertensive diabetic patients with high Na^+/Li^+ CT (H_2D)	Hypertensive and microalbuminuric diabetic patients with high Na^+/Li^+ CT (H_3D)
PGE_2 (pg/min)	Placebo	736 ± 112	1913 ± 117^d	2017 ± 213^d	1901 ± 217	1818 ± 244^d
	Indomethacin	211 ± 33^b	519 ± 103^b	411 ± 103^b	519 ± 37^b	$622 \pm 59^{a,c}$
6-keto $PGF_1\alpha$ (pg/min)	Placebo	413 ± 72	982 ± 106^c	1221 ± 144^c	914 ± 229	821 ± 184^c
	Indomethacin	106 ± 22^b	318 ± 104^a	294 ± 98^b	2741 ± 41^b	261 ± 69^b
$T \times B_2$ (pg/min)	Placebo	256 ± 36	383 ± 60	419 ± 103^c	501 ± 136	477 ± 118
	Indomethacin	102 ± 17^b	66 ± 21^b	113 ± 32^b	131 ± 39^b	124 ± 31^a

^a $p < 0.05$, ^b $p < 0.01$ Indomethacin vs Placebo; ^c $p < 0.05$, ^d $p < 0.01$ CD, H_1D , H_2D and H_3D patients vs C subjects. PGE_2 = prostaglandin E_2 . Na^+/Li^+ CT = sodium-lithium countertransport, 6-keto $PGF_1\alpha$ = prostaglandin 6-keto $PGF_1\alpha$ and $T \times B_2$ = thromboxane B_2

Ultrasound B mode imaging of common carotid arteries showed that the intimal plus medial thickness of the arteries was significantly lower (0.50 ± 0.01 mm) in C subjects, (0.49 ± 0.01 mm) in CD patients, and (0.53 ± 0.01 mm) in H₁D patients than (0.64 ± 0.03 mm $p < 0.01$) in H₂D patients and (0.69 ± 0.04 mm $p < 0.01$) in H₃D patients (Fig. 5).

Discussion

The findings of the present study demonstrate that in patients with Type 1 diabetes: (1) the renal and systemic responsiveness to Ang II is impaired, (2) the urinary excretion rate of vasodilatory PGs is high, (3) the administration of the cyclooxygenase inhibitor, indomethacin, restores a normal renal and systemic response to Ang II and overnight fasting baseline glomerular filtration rate in diabetic patients with normal sodium-lithium countertransport activity in erythrocytes and (4) an elevated sodium-lithium countertransport activity identifies a subset of diabetic patients, with hypertension and microalbuminuria and with carotid artery hypertrophy, in whom renal haemodynamics remain abnormal despite the inhibition of excessive prostaglandin synthesis.

In accordance with previous reports, Type 1 diabetic patients show a higher renal excretion rate of PGs [15–17]. Patients with Type 1 diabetes have increased urinary excretion rates mainly of vasodilatory PGs, independent of the degree of metabolic control [15, 16] and, as suggested by the present study, also of the levels of blood pressure. It is not clear whether such abnormalities in PGs metabolism play a role in the pathogenesis of glomerular haemodynamic disturbances in Type 1 diabetes. In both human and animal diabetes a role for renal PGs in the hyperfiltration phenomenon has been advocated by some workers but denied by others [34–37].

The current finding that indomethacin administration restored a normal renal and systemic response to Ang II in diabetic patients who had elevated urinary excretion rate of PGs but not in normal subjects in whom the renal synthesis of eicosanoids was low, supports the view that a resistance to Ang II in Type 1 diabetes is secondary to an excessive renal synthesis of PGs, which antagonizes the modulation of renal haemodynamics by the renin-Ang II system.

These results in human subjects confirm a preliminary report of Inman et al. [38] and Sarubbi et al. [39] in diabetic rats, showing that the vascular bed in Type 1 diabetes does not properly respond to Ang II action. Furthermore, it has also been reported that the administration of prostacyclin (PGI₂) in human pregnancy attenuates both pressor and adrenocortical changes following Ang II challenge [40]. Indomethacin has been shown to eventually restore a normal responsiveness to Ang II in diabetic rats [38].

From a general point of view these findings lend support to the belief that vasodilatory PGs play an important intrarenal role in modulating the contractile effects of Ang II in humans [13]. With regard to the patients with Type 1 diabetes an attenuated hormonal action of Ang II,

secondary to an excessive intrarenal synthesis of vasodilatory PGs, could help to determine a chronic arteriolar vasodilation leading to glomerular hyperfiltration.

On the other hand the present study shows that a subset of diabetic patients with hypertension and microalbuminuria without clinical nephropathy, had an impaired response to Ang II, even when the excessive synthesis of PGs had been significantly reduced by cyclooxygenase inhibitors.

In order to elucidate the mechanisms accounting for the different haemodynamic behaviour of this latter group of patients with Type 1 diabetes, we investigated the relationship between the renal response to Ang II and the activity of sodium-lithium countertransport in erythrocytes during indomethacin infusion. The rationale for such a study was suggested by previous reports that hyperfiltration is particularly evident only in a subset of patients with Type 1 diabetes [41] and is associated with elevated exchange in sodium-lithium countertransport erythrocytes [10]. A highly significant inverse correlation was found between the decrease in GFR and RPF and the rate of activity of sodium-lithium countertransport in the erythrocytes, even if the urinary excretion rate of PGs had been markedly inhibited by indomethacin in all diabetic patients.

Interestingly, the clinical features of these diabetic patients are quite similar to those shown by non-diabetic patients with “non-modulating” essential hypertension, who also have high average values of sodium-lithium countertransport activity in the erythrocytes and high frequency of positive family history of hypertension [42, 43].

More recently the clinical characteristics of “non-modulating” hypertension, that is the abnormal response to Ang II and the capacity to handle oral sodium intake, have been found to be genetically inherited in subjects with essential hypertension [43].

However, the finding of an association between elevated sodium-lithium countertransport activity in erythrocytes and the impaired response to Ang II raises the question of whether these two phenomena are simply associated or whether such abnormalities in all membrane cation transport systems play a mechanistic role in determining altered haemodynamics at kidney level in a subset of diabetic patients. With regard to this latter hypothesis it has been shown that sodium-lithium countertransport activity is a mode of operation of sodium-hydrogen antiport [44, 45] and a good agreement has been found between the exchange rates of sodium-lithium and sodium-hydrogen in the erythrocytes of human subjects [46]. Theoretically, altered function of the sodium-hydrogen exchanger might explain several abnormalities at vascular and tissue level in the subset of diabetic patients characterized by elevated sodium-lithium countertransport activity. Changes in activity of the sodium-hydrogen exchanger would link abnormalities in cell growth with abnormalities in sodium homeostasis because the exchanger has been shown to be critically involved in both growth factor-stimulated cell division [7, 47] and agonist-stimulated sodium influx [48, 49]. Increased sodium-hydrogen exchange might result in a more sustained or more rapid mitogenetic response to growth factors [50]. Conversely, the binding of vasocon-

strictor agents could enhance sodium-hydrogen antiport activity contributing to the abnormal vasoreactivity demonstrated by the diabetic patients with elevated sodium-lithium countertransport activity in the present study [7].

The results of the present study demonstrate that diabetic patients with elevated sodium-lithium countertransport in erythrocytes had an intimal plus medial thickness of the common carotid artery greater than that of normal subjects and of diabetic patients with normal sodium-lithium countertransport activity. Yap et al. [51] recently showed that amongst patients with essential hypertension, the subjects with an elevated sodium-lithium countertransport, despite levels of blood pressure similar to those with normal sodium-lithium countertransport, had electrocardiographic left ventricular hypertrophy. We have recently reported similar findings in Type 1 diabetic patients using ultrasound techniques [52].

It can be suggested that high sodium-lithium countertransport activity in erythrocytes is associated with cardiac and vascular abnormalities which can precede the increase in blood pressure and further deteriorate the deleterious effects of hypertension on cardiac function in a cohort of subjects characterized by genetic factors predisposing to cardiovascular cell replication and hyperplasia. With regard to this view it has been reported that cardiac hypertrophy does occur in spontaneously hypertensive rats, who are genetically characterized by high sodium-hydrogen antiport activity, before the appearance of increased blood pressure [53, 54]. Also in humans, left ventricular muscle mass has been shown to be higher in the normotensive offspring of hypertensive parents than in control subjects with similar blood pressure, suggesting that factors other than a simple increase in blood pressure could contribute to determine cell growth and replication in these subjects [55].

In summary, the results of this study demonstrate that an excessive synthesis of vasodilatory PGs antagonizes the regulation by the renin-Ang II hormonal system of the systemic and renal haemodynamics in patients with Type 1 diabetes.

On the other hand a subgroup of hypertensive patients with Type 1 diabetes before the development of overt clinical nephropathy, who are identified by elevated sodium-lithium countertransport in erythrocytes, is characterized by abnormalities at functional, renal and morphological vascular level. It is interesting to point out that a similar behaviour with regard to the response to Ang II and to the other clinical parameters was also shown by the hypertensive subjects with high sodium-lithium countertransport and microalbuminuria. Since microalbuminuria has been shown to predict the development of overt nephropathy in Type 1 diabetes [4-5] it can be postulated that the clinical and physiological features described by the present study in Type 1 diabetic patients are to some extent associated with those diabetic patients who have a susceptibility to diabetic nephropathy. In these subjects the normalization of the urinary excretion rate of PGs does not restore a normal response to Ang II infusion and baseline glomerular filtration rate. It is suggested that abnormalities in cell growth and function at arteriolar level, cause a lower compliance to vasoconstrictor stimuli in a

subset of patients with Type 1 diabetes, i.e. a vascular stiffness, which may determine tissue and particularly glomerular hyperfiltration and possibly a susceptibility to diabetic complications.

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