Renal excretion of kallikrein and eicosanoids in patients with Type 1 (insulin-dependent) diabetes mellitus. Relationship to glomerular and tubular function

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Summary. Glomerular filtration rate, renal plasma flow, renal tubular sodium reabsorption (derived from lithium clearance) and renal excretion rates of kallikrein, prostaglandin E₂ and systemic and renally-derived metabolites of prostacyclin and thromboxane A₂ were measured in patients with Type 1 (insulin-dependent) diabetes mellitus and in normal subjects. Diabetic patients with glomerular hyperfiltration had greater active kallikrein and prostaglandin E₂ excretion than patients with normal glomerular filtration rate or than normal control subjects. Both active kallikrein and prostaglandin E₂ excretion correlated directly with glomerular filtration rate. Active kallikrein excretion correlated directly with the reabsorption of sodium in the distal tubule. The excretion rates of 6-keto prostaglandin F_{1α}, 2,3 dinor 6-keto prostaglandin F_{1α}, thromboxane B₂, 2,3 dinor thromboxane B₂ and 11-dehydro thromboxane B₂ excretion were not dif-

ferent between the groups. This study confirms in man our previous finding of increased renal kallikrein production in the hyperfiltering streptozotocin-diabetic rat model. Given that kinins generated by kallikrein are extremely potent vasodilators and stimulate the renal production of eicosanoids that also regulate glomerular function, our findings suggest that increased kallikrein activity and prostaglandin E₂ production may contribute to renal vasodilatation and hyperfiltration in human diabetes. The localization of kallikrein in the distal connecting tubule makes it plausible that altered sodium transport in the distal tubule may be a signal to increase generation of kallikrein.

Key words: Type 1 (insulin-dependent) diabetes mellitus, glomerular filtration rate, kallikrein, prostaglandin E₂, prostacyclin, thromboxanes, renal tubule.

An increase in glomerular filtration rate (GFR) above normal occurs in the early course of Type 1 (insulin-dependent) diabetes mellitus [1]. Both retrospective and prospective studies in man show that hyperfiltration is predictive of progression to proteinuria [2, 3]. In diabetic rats with hyperfiltration, the accompanying raised intraglomerular pressure accelerates the development of nephropathy [4, 5]. Glomerular haemodynamic measurements in these rats show that reduced afferent arteriolar resistance is the principal cause of glomerular capillary hyperperfusion and raised intracapillary pressure [6, 7].

The factors which mediate glomerular vasodilatation in diabetes remain uncertain. Increased circulating levels of glucagon and growth hormone may together contribute, but are probably not independently responsible [8, 9]. Amongst vasoactive substances produced within the kidney, animal model studies support a role for vasodilatory eicosanoids [10]. We recently reported that renal levels and excretion of kallikrein are increased in streptozotocin-diabetic rats with hyperfiltration, and treatment with a kallikrein inhibitor reduced GFR and renal plasma

flow (RPF) to normal [11]. In an earlier clinical study, we found that active kallikrein excretion is increased in poorly controlled Type 1 diabetes, and falls with improved diabetic control [12]. These changes were not studied in relation to renal haemodynamics.

Several findings suggest that kallikrein and its kinin product participate in regulating glomerular function. Kallikrein is synthesized and secreted from distal connecting tubule cells, but this portion of the nephron makes a second loop, after the macula densa, and encircles the afferent arteriole [13, 14]. Kallikrein resides in basolateral membranes of connecting tubule cells, and adjacent principal cells produce kininogen substrate [15]. Therefore, enzyme and substrate are located such that kinins could be generated in the interstitium surrounding the preglomerular circulation. In vitro, kinins applied anti-lumenally reduce the resistance of perfused afferent arterioles [16]. Finally, kinins stimulate production of vasodilatory eicosanoids (prostaglandin (PG) E₂ and prostacyclin) by afferent arterioles and glomerular mesangial cells [17, 18].

In the present study of Type 1 diabetic patients, we measured GFR, RPF and the simultaneous renal excretion rates of kallikrein, prokallikrein, 6-keto-PGF_{1 α} (the stable metabolite of prostacyclin), PGE₂ and thromboxane (TX) B₂ (the stable metabolite of TXA₂). The excretion of systemically derived metabolites of PGI₂ and TXA₂ were also measured. Renal tubular sodium handling was assessed by measurement of lithium clearance.

Subjects and methods

Protocol

Thirty-one Type 1 diabetic patients and nine normal control subjects were studied. The protocol was approved by the ethical committee (Leeds) and the Institutional Review Board for Human Research (South Carolina). Informed consent was obtained from all subjects. Subjects were asked to avoid coffee, tea and cigarettes on the study day. Most diabetic patients had their usual insulin dose by subcutaneous injection. In the fasting state or after a light, low protein breakfast, each subject had venous cannulae inserted and then rested at least 1 h prior to GFR and RPF measurement. Subjects drank 300 ml water every 30 min, beginning immediately after cannula insertion and continuing throughout the study.

GFR and RPF were measured in 10 of the diabetic subjects (South Carolina) by the direct clearance of inulin and para-aminohippurate (PAH), respectively, given by constant infusion [19]. The average of three consecutive 30 min clearance measurements was calculated for each patient. In 21 diabetic (8 with hyperfiltration) and 9 control subjects (Leeds), single injection clearances of ⁵¹Cr-EDTA [20] and ¹²⁵I-iodohippuran [21] were used to measure GFR and RPF. Because renal plasma clearance of ⁵¹Cr-EDTA underestimates GFR relative to inulin, EDTA clearances were corrected according to the method of Brochner-Mortensen [22]. The single injection iodohippuran clearances were corrected according to the method of Blafox and Merrill [21]. The upper limit of normal GFR was calculated as the mean + 2SD of the GFR of the normal control subjects (132 ml·min⁻¹·1.73 m⁻²). The diabetic patients were divided into normal or hyperfiltering subgroups.

Arterial blood pressure was measured using a Dinamap monitor (Critikon, Tampa, Fla. USA) at 30 min intervals throughout the study and mean diastolic and systolic pressures during the clearance measurements were calculated for each subject. Plasma glucose was measured at the beginning, midpoint and end of the clearance determinations. From urine collected during the clearance measurements the renal excretion rates of kallikrein (active and prokallikrein) and eicosanoids were measured. Samples for eicosanoid measurement were obtained only from the 30 subjects who underwent lithium clearance determination. Albumin excretion rate was assessed from the mean of at least two timed overnight urine collections obtained prior to the clearance studies.

From the total group of 40 subjects, 30 subjects (9 controls, 13 diabetic patients with normal GFR, and 8 hyperfiltering patients) underwent a lithium clearance determination simultaneously with GFR and RPF measurement. Lithium carbonate (650 mg) was given orally at 22.00 hours the evening before the study. Lithium concentration was determined in serum obtained at the beginning and end of the GFR and RPF measurements and in an aliquot of urine. Proximal and distal tubular reabsorption rates were calculated as previously described [23].

Assays

Inulin and PAH in plasma and urine were measured as previously described [24, 25]. Lithium was measured by atomic absorption spectrometry. Urinary kallikrein was measured by RIA using a polyclonal

Table 1. Age, duration of Type 1 (insulin-dependent) diabetes, HbA_{1c} and overnight albumin excretion rate in control and diabetic subjects

	Age (years)	Duration of diabetes (years)	HbA _{1c} (%)	Albumin excretion rate (μg·min ⁻¹)
Normal control subjects	31 ± 2	—	6.2 ± 0.1	5 ± 1
Normal GFR diabetic patients	31 ± 2	11 ± 1	8.2 ± 0.4	12 ± 4
Hyperfiltering diabetic patients	26 ± 2	10 ± 2	9.0 ± 1.0	7 ± 2

GFR, Glomerular filtration rate

antibody raised against human urinary active kallikrein [26]. A parallel sample was treated with trypsin to convert prokallikrein to active kallikrein. Assay of total urinary kallikrein in this sample allowed determination of prokallikrein concentration by subtracting the concentration of active kallikrein in the untreated sample [26]. The stable metabolites of TXA₂ (TXB₂, 2,3 dinor TXB₂, and 11-dehydro-TXB₂) and PGI₂ (6-keto-PGF_{1 α} , and 2,3 dinor 6-keto-PGF_{1 α}), as well as PGE₂, were measured in urine by gas chromatography, mass spectrometry [27]. Urine was stored at -40°C prior to measurement of eicosanoid and kallikrein concentrations. Urinary albumin was measured by RIA and HbA_{1c} by isoelectric focussing. The glucose concentration in plasma and urine was measured with a Glucose Analyzer 2 (Beckman, Fullerton, Calif., USA).

Statistical analysis

GFR, RPF and lithium clearance are expressed per 1.73 m² body surface area. All data are expressed as the mean ± SEM. Differences between the three groups of subjects were assessed by one-way analysis of variance (ANOVA), with subsequent *t*-tests applied where ANOVA was significant. Data were logarithmically transformed if non-linear. Correlations were calculated by linear regression analysis.

Results

The age, duration of diabetes, HbA_{1c} and albumin excretion rate are shown in Table 1 for diabetic patients with normal or elevated GFR and for control subjects. The age of the three groups was similar, and there was no difference in duration of diabetes, HbA_{1c} or albumin excretion between the two groups of diabetic patients. All subjects were normotensive and there were no differences in blood pressure between the groups. Average blood pressure in the control, normal GFR diabetic and hyperfiltering diabetic patients was 132/81, 126/79 and 123/78 mm Hg respectively. Blood pressure did not show a change during the renal function measurements. During the clearance studies, mean plasma glucose in control, normal GFR diabetic and hyperfiltering diabetic subjects was 4.1 ± 0.2, 6.2 ± 0.9 and 7.4 ± 1.3 mmol·l⁻¹ respectively (*p*, NS). The mean GFR in hyperfiltering patients was 151 ± 6 ml·min⁻¹ (*p* < 0.001) compared to 110 ± 2 in normal filtering patients and 109 ± 4 in control subjects (Fig. 1). Although RPF tended to be higher in hyperfiltering patients it was not significantly increased, nor was the

Fig. 1. Glomerular filtration rate, renal plasma flow and filtration fraction in the normal subjects (□), in normal glomerular filtration rate diabetic patients (▨) and in hyperfiltering diabetic patients (■). * $p < 0.001$ vs each of the other two groups

Fig. 2. Active and prokallikrein excretion rates in the normal subjects (□), in normal glomerular filtration rate diabetic patients (▨) and in hyperfiltering diabetic patients (■). * $p < 0.05$ vs each of the other two groups

filtration fraction significantly increased in the hyperfiltering patients.

Active kallikrein and prokallikrein excretion rates are shown in Figure 2. Active kallikrein excretion was increased by more than 50% in hyperfiltering diabetic patients compared to normal filtering patients or normal subjects ($p < 0.05$). Active kallikrein excretion was nearly identical in the latter two groups. In the three groups of subjects collectively, active kallikrein excretion correlated directly with GFR ($r = 0.37$, $p < 0.02$). There was no difference in prokallikrein excretion between the three groups (Fig. 2).

Excretion of TXB_2 and 6-keto- $\text{PGF}_{1\alpha}$, the metabolites of TXA_2 and PGI_2 produced intrarenally, showed no significant differences between the groups, expressed either as an absolute excretion rate or as quantity per milligram of urinary creatinine (Table 2). In addition, the excretion of dinor metabolites of systemically derived TXA_2 and PGI_2 , as well as 11-dehydro TXB_2 , were unchanged in diabetic patients. However, PGE_2 excretion was increased in hyperfiltering diabetic patients compared with either normal filtering diabetic or control subjects. PGE_2 excretion ($\text{pg} \cdot \text{min}^{-1}$) correlated with GFR ($r = 0.36$, $p = 0.05$) but did not correlate with the excretion rate of active kallikrein.

Lithium clearance together with calculated fractional and absolute sodium reabsorption rates are given in Table 3. Sodium and water excretion rates were not different between any of the groups. However, the absolute reabsorption of sodium in the proximal tubule was significantly greater in the hyperfiltering patients compared with normal GFR diabetic patients or normal control subjects ($p < 0.001$ vs both). Taking normal and diabetic subjects together, active kallikrein excretion correlated directly with both fractional ($r = 0.46$, $p < 0.02$, Fig. 3) and absolute ($r = 0.38$, $p < 0.05$) distal tubular sodium reabsorption and inversely with sodium excretion ($r = -0.36$, $p = 0.05$). Eicosanoid excretion rate did not correlate with distal sodium reabsorption.

Discussion

These data show that renal excretion of active kallikrein is increased in Type 1 diabetic patients with glomerular hyperfiltration, compared to diabetic patients with normal GFR or normal subjects. These findings are consistent with our previous observations in the streptozotocin-diabetic rat, in which inhibition of kallikrein with aprotinin reduced GFR to normal [11]. The latter suggests that increased kallikrein excretion in diabetic humans or rats with hyperfiltration is not secondary to raised GFR.

The factors responsible for increased kallikrein in diabetes-induced hyperfiltration are not certain. From the present data, it is unlikely that washout accounts for the increased excretion because urine flow rates were similar in the three groups, and active kallikrein was increased whereas prokallikrein showed no difference. Renal kallikrein excretion increases in response to dietary sodium restriction and there is evidence to suggest that sodium-retaining steroids stimulate renal kallikrein production and excretion [28]. In a previous study of Type 1 diabetic patients on constant sodium intake, increased excretion of active kallikrein was related to poor glycaemic control [12]. In the present study HbA_{1c} was not significantly different between the three groups. However, few patients with poor control were studied; also, since in this study sodium intake was not strictly controlled, inter-subject variation in sodium intake could have increased the variation in active kallikrein excretion and obscured the relationship with glycaemic control. Mayfield et al. [12] have clearly shown the effect of intensive insulin treatment on active kallikrein excretion but further studies are needed to investigate the relationship of changes in active kallikrein to changes in GFR. Although sodium intake was not controlled in the current study, urinary excretion did not differ significantly between the groups, suggesting that sodium intake was neither different nor responsible for the increased active kallikrein excretion in the hyperfiltering patients. Acute water diuresis does not alter kallikrein excretion [28].

To further examine the relationship between kallikrein excretion and tubular handling of sodium in diabetes, we measured proximal and distal tubular reabsorption rates by lithium clearance [23]. The validity of the lithium clearance technique depends on two assumptions: firstly, that

Table 2. Urinary excretion of renal and systemically derived eicosanoids, expressed relative to creatinine excretion and as absolute excretion rate

	pg · mg urinary creatinine ⁻¹					
	TXB ₂	Dinor TXB ₂	11-Dehydro TXB ₂	6-Keto PGF _{1α}	Dinor 6-Keto PGF _{1α}	PGE ₂
Normal control subjects	45 ± 6	252 ± 46	993 ± 267	225 ± 36	802 ± 115	118 ± 47 ^b
Normal GFR diabetic patients	37 ± 4	232 ± 38	885 ± 122	236 ± 46	892 ± 143	168 ± 40 ^a
Hyperfiltering diabetic patients	58 ± 11	269 ± 55	931 ± 103	200 ± 36	796 ± 132	435 ± 139
	pg · min ⁻¹					
	TXB ₂	Dinor TXB ₂	11-Dehydro TXB ₂	6-Keto PGF _{1α}	Dinor 6-Keto PGF _{1α}	PGE ₂
Normal control subjects	53 ± 9	289 ± 50	1139 ± 293	256 ± 39	915 ± 128	133 ± 54 ^b
Normal GFR diabetic patients	42 ± 3	289 ± 62	1025 ± 117	277 ± 49	1053 ± 154	196 ± 44
Hyperfiltering diabetic patients	61 ± 12	280 ± 61	957 ± 94	212 ± 43	841 ± 158	427 ± 125

^a $p < 0.05$, ^b $p < 0.02$ vs hyperfiltering diabetic patients.

TX, Thromboxane; PG, prostaglandin; GFR, glomerular filtration rate

Table 3. Glomerular and tubular function in normal control subjects, in normal GFR Type 1 diabetic patients and hyperfiltering diabetic patients

	GFR (ml · min ⁻¹)	C _{Li} (ml · min ⁻¹)	Proximal reabsorption of sodium		Distal reabsorption of sodium		Urine flow (ml · min ⁻¹)	Urinary Na excretion (μmol · min ⁻¹)	Urine glucose (mmol · l ⁻¹)
			Fractional (%)	Absolute (mmol · min ⁻¹)	Fractional (%)	Absolute (mmol · min ⁻¹)			
Normal control subjects	109 ± 4	23 ± 2	79 ± 2	11.9 ± 0.4	94 ± 1	3.0 ± 0.3	6.7 ± 0.3	222 ± 32	0.3 ± 0.01 ^b
Normal GFR diabetic patients	109 ± 3	24 ± 1	78 ± 1	11.8 ± 0.4	95 ± 1	3.2 ± 0.2	6.4 ± 0.7	185 ± 28	2.0 ± 1.0
Hyperfiltering diabetic patients	144 ± 5 ^a	26 ± 1	82 ± 1	16.3 ± 0.5 ^a	96 ± 1	3.5 ± 0.2	6.1 ± 0.4	141 ± 25	3.9 ± 2.4

^a $p < 0.001$, ^b $p < 0.02$ vs each of the other two groups. GFR, Glomerular filtration rate; C_{Li}, lithium clearance; Na, sodium

lithium, sodium and water are reabsorbed in similar proportions in proximal convoluted and straight tubules. Micropuncture measurements support this assumption [29–31]. Secondly, that lithium is not reabsorbed in the distal nephron (loop of Henle, distal tubule and collecting duct). This has been tested in man using diuretics which act on the distal tubule [32], and at moderate or high sodium intake, lithium is not reabsorbed in this section of the nephron. The subjects in our study were on unrestricted sodium intake and urinary sodium excretion suggested a moderate sodium intake. Finally, glucose excretion by the diabetic subjects in our study was minimal and therefore unlikely to have influenced lithium clearance.

Lithium clearance measurements indicated that the increased filtration of water and sodium in hyperfiltering patients is compensated largely by increased reabsorption in the proximal tubule. There was no correlation between kallikrein excretion and proximal tubular sodium reabsorption rate. However, kallikrein excretion rate correlated directly with reabsorption of sodium in the distal tubule and inversely with renal sodium excretion. Although a correlation between distal sodium reabsorption and kallikrein excretion rate does not prove a causal relationship, other observations are in support of such a relationship. Mineralocorticoid-stimulated distal sodium reabsorption is associated with increased kallikrein [28]. In addition, thiazide diuretics, which inhibit sodium reabsorption in the connecting tubule [33], the site of kallikrein produc-

tion [13, 14], reduce kallikrein excretion [34]. It is also interesting that distal nephron sodium reabsorption is stimulated by insulin [35, 36] and prolonged hyperinsulinaemia may increase renal kallikrein synthesis [37]. Therefore, it is possible that the higher systemic levels of insulin in patients treated with insulin contribute to increased renal kallikrein production.

Fig. 3. The relationship between active kallikrein excretion rate and fractional sodium reabsorption in the distal tubule in normal subjects (○), in normal glomerular filtration rate diabetic patients (●) and in hyperfiltering diabetic patients (△). For all subjects: $r = 0.46$, $p < 0.02$

Finally, we found increased renal excretion of PGE_2 in hyperfiltering patients, whereas patients with normal GFR had excretion similar to control subjects. Excretion of renal and systemic (dinor and 11-dehydro) metabolites of PGI_2 and TXA_2 were not altered in either group of diabetic patients. Four previous studies have measured urinary eicosanoids in Type 1 diabetic patients with hyperfiltration. Two studies found increased 6-keto- $\text{PGF}_{1\alpha}$ excretion and no change in TXB_2 excretion [38, 39]. One of these also found no change in urinary PGE_2 [39]. A third study found no change in PGE_2 or 6-keto- $\text{PGF}_{1\alpha}$ excretion [40]. In contrast to our study, in which eicosanoids were measured in urine from timed collections during clearance measurements with water loading, the previous studies measured overnight or 24-h excretion. However, Hommel et al. [41] using a study design similar to ours found increased urinary PGE_2 patients with increased GFR. GFR and PGE_2 excretion were both reduced by indomethacin. In all of these previous studies urinary eicosanoids were measured by direct RIA. We employed gas chromatography-mass spectrometry, which separately quantitates the excretion of metabolites of systemically produced PGI_2 and TXA_2 from the renal metabolites of these eicosanoids [27]. This eliminates the possible cross-reactivity of the systemic metabolites, which are excreted in much higher concentrations in urine than TXB_2 and 6-keto- $\text{PGF}_{1\alpha}$.

The possibility that increased renal kallikrein and PGE_2 production could be related is suggested by studies of isolated glomerular arterioles and cultured mesangial cells. In these tissues, kinins stimulate PGE_2 production [17, 18]. Moreover, PGE_2 production is increased in glomeruli isolated from streptozotocin-diabetic rats with hyperfiltration [42], a model which also shows increased kallikrein excretion [11].

In summary, our study shows that Type 1 diabetic patients with glomerular hyperfiltration demonstrate the same renal kallikrein abnormality previously found in a rat model of diabetic hyperfiltration. The increase in renal kallikrein excretion may be related to the changes in distal tubular sodium transport and renal eicosanoid abnormalities seen in diabetes.

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