Urinary kallikrein excretion in Type 1 (insulin-dependent) diabetes mellitus

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Summary. Kidney haemodynamics appear to change after the early phases of diabetic nephropathy: increases in glomerular filtration rate and in renal plasma flow have been widely reported, while kidney size is increased. As the renal kallikrein-kinin system has been demonstrated to regulate kidney blood circulation, we have evaluated the excretion of urinary kallikrein in 87 Type 1 (insulin-dependent) diabetic patients with and without hyperfiltration. Urinary kallikrein excretion was measured in 24-h urine collections. The esterolytic activity was determined by fluorimetric assay. The excretion of urinary kallikreins was significantly higher in hyperfiltering patients (472 ± 125 esterase units/24 h) than in normofiltering (168 ± 77 esterase units/24 h) and control subjects (151 ± 39 esterase units/24 h), $p < 0.001$. Furthermore, we observed a positive correlation between urinary kallikrein excretion and glomerular filtration rate ($r = 0.785$). These data suggest that variations of kallikrein and kinin concentrations may play a role in the alteration of renal haemodynamics in Type 1 diabetes. It is possible that the kinin-kallikrein system, the renin-angiotensin system and the prostaglandins may interact to determine the haemodynamic alterations which are present in the diabetic disease.

Key words: Diabetic nephropathy, hyperfiltration, Type 1 (insulin-dependent) diabetes mellitus, urinary kallikrein excretion.

One-third of all patients with Type 1 (insulin-dependent) diabetes mellitus are at risk of developing a nephropathic complication [1, 2]. In the early phases of nephropathy these subjects show an increased glomerular filtration rate (GFR), elevated renal plasma flow (RPF) and increased kidney size [3–8]. Factors other than blood glucose levels such as atrial natriuretic factor [9, 10], vasodilatory prostaglandins [11] and kallikrein-kinin system could be associated with this circulatory alteration.

The renal kallikrein-kinin system has been shown to regulate kidney haemodynamics with a paracrine mechanism. Renal (glandular) kallikreins are a subfamily of serine-proteases which liberate potent kinin peptides from kininogen substrates through limited proteolysis. The renal kallikrein-kinin system has been shown to regulate kidney haemodynamics [12–14] by modulating such parameters as blood pressure, blood flow, renal vascular resistance and capillary permeability [15]. Decreased kallikrein excretion in Type 2 (non-insulin-dependent) diabetic patients with hypertension and nephropathy [16, 17] and elevated urinary kallikrein concentration in patients with poorly-controlled Type 1 diabetes has been reported [18]. Moreover, different reports have shown that untreated streptozotocin diabetic

rats show a reduced synthesis and excretion of active renal kallikrein [19–21].

Interactions between the renal kallikrein-kinin system, prostaglandins and the renin-angiotensin system have been described [22–26]. The relationship between urinary kallikrein excretion and hyperfiltration in incipient diabetic nephropathy has not yet been clearly established. The aim of our study is to evaluate the behaviour of urinary excretion of kallikrein in two groups of Type 1 diabetic patients with and without glomerular hyperfiltration.

Patients and methods

Patients

Eighty-nine Caucasian Type 1 diabetic patients without hypertension (blood pressure < 160/90 mm Hg), renal disease or any diabetic complications were recruited: of these 78 were normofiltering (group A) while 11 showed an increased GFR (group B); a group of 20 healthy subjects, matched for age and sex, with no family history of hypertension, served as controls. Each subject gave informed consent. The study was performed according to the Helsinki Declaration. The composition of usual patient diet was assessed by analysis of 3-day weighed food records. The test was performed in the morn-

Table 1. Age, sex, duration of Type 1 diabetes, blood pressure, BMI, creatinine, BUN, HbA$_{1c}$, overnight albumin excretion rate and creatinine clearance in control subjects, normofiltering (group A) and hyperfiltering (group B) diabetic subjects

	Control subjects	Group A	Group B
Age (years)	29 ± 7	30 ± 9	30 ± 10
Sex (male/female)	12/8	34/44	7/4
Duration diabetes (years)		12.5 ± 7.5	14 ± 2
Systolic blood pressure (mm Hg)	110 ± 10	115 ± 8	110 ± 10
Diastolic blood pressure (mm Hg)	70 ± 5	65 ± 10	70 ± 10
BMI (kg/m^2)	22 ± 1	21 ± 2	20 ± 2
Creatinine (μmol/l)	61 ± 7.6	61 ± 15	68 ± 7.6
BUN (mmol/l)	10.7 ± 2	11.8 ± 2.8	10 ± 2
HbA$_{1c}$ (%)	3.8 ± 0.5	7 ± 1.3	6.3 ± 1.4
AER (μg/min^{-1})	10 ± 10	10 ± 9	6.6 ± 4.4
Creatinine clearance (ml/min)	110 ± 8	110 ± 10	126 ± 46

BMI, Body mass index; BUN, blood urea nitrogen; AER, albumin excretion rate

ing following a standard light breakfast (150 g milk with 20 g of bread) for all groups.

Methods

To simultaneously evaluate GFR and RPF, a single i.v. bolus injection of ^{51}CrEDTA 1 μCu/kg and ^{125}I-Hippuran 0.2 μCu/kg (Sorin, Saluggia, Italy) was calibrated and made up to 10 ml with NaCl (0.9%), which was then administered 90 min after breakfast. Blood plasma was taken at 5, 10, 20, 30, 44, 50, 60, 80, 120, 180, 240 min after the injection. During the test the patients were kept euglycaemic and were in the supine position. Subjects were asked to avoid smoking during the study. GFR and RPF were measured as the clearance of the isotopes under the assumption that all the tracer is excreted, that the only route is renal and that the total amount of tracer excreted will be equal to the quantity injected.

The plasma clearance measurement techniques may be classified as follows: 1) For GFR: double exponential analysis utilized blood samples at 5, 10, 20 and 80, 120, 180, 240 min to obtain the two slopes λ and $\lambda 2$. The monoexponential analysis utilized only later plasma samples taken at 80, 120, 180, 240 min [27, 28]. The method from value distribution measurement utilized only the one taken at 180 min [29]. 2) For RPF: the double exponential analysis used all blood samples taken at 5, 10, 20 and 44, 50, 60 min. The monoexponential analysis utilized samples taken at 44, 50, 60 min. The method from value distribution measurement utilized the sample at 44 min.

We preferred to use the double exponential analysis because it is very reliable. In our laboratory the values of GFR and RPF in normal subjects range from 80 to 130 ml·min^{-1}·1.73 m^2 and from 500 to 650 ml·min^{-1}·1.73 m^2 respectively. Patients with GFR over 135 ml·min^{-1}·1.73 m^2 were considered hyperfiltering. Blood urea nitrogen was determined by Berthelot reaction, serum, urinary creatinine were measured by a modification of the Jaffé method with alkaline nitrate [30], glycated haemoglobin (HbA$_{1c}$) by the HPLC method, albuminuria by radioimmunoassay (Albumin DA; Sclavo, Siena, Italy). Systolic and diastolic blood pressure were evaluated

twice by Random-Zero mercury sphygmomanometer, by the same observer.

Urinary kallikrein excretion was measured in 24-h urine collections. Urine samples were stored under toluene at 4°C until assayed. The esterolytic activity was determined by modification of the Morita method [31] using L-propyl-L-phenylanil-L-arginil-7-amido-4-methylcoumarine-ester (Bachem; Feinchemikalien, AG Bubendorf, Switzerland) as substrate. Fluorimetric measurements were carried out with excitation at 380 nm and emission at 460 nm in a Perkin-Elmer fluorimeter. One esterase unit (EU) was defined as the amount of enzyme which hydrolyses 10 μmol of methylcoumarine/min at pH 8.0 and 37°C in a standard assay.

Statistical analysis

Data are expressed as the mean ± SEM. Differences between the three groups were assessed by univariate analysis of variance (ANOVA) with subsequent t-test applied where the differences were significant. Correlation was determined by linear regression analysis (performed with the computer programme Statview 512 Apple). Differences and correlations were considered significant at $p < 0.05$.

Results

Our data showed no significant differences in age, sex and duration of diabetes in groups A and B (Table 1). The value of HbA$_{1c}$ in normofiltering and hyperfiltering patients were similar (7 ± 1.3% vs 6.3 ± 1.4%; p = NS). Albumin excretion rate was 10 ± 9 μg/min (range 0.2–24 μg/min) in group A and 6.6 ± 4.4 μg/min (range 1.0–15 μg/min) in group B, p = NS (Table 1).

The excretion rate of urinary kallikreins was 151 ± 39 EU·24 h·incubation min in control subjects, 168 ± 77 EU·24 h·incubation min in normofiltering patients and 472 ± 125 EU·24 h·incubation min in hyperfiltering patients. The excretion of urinary kallikreins was significantly higher in hyperfiltering patients than in normofiltering and control subjects; $p < 0.001$ (Fig. 1).
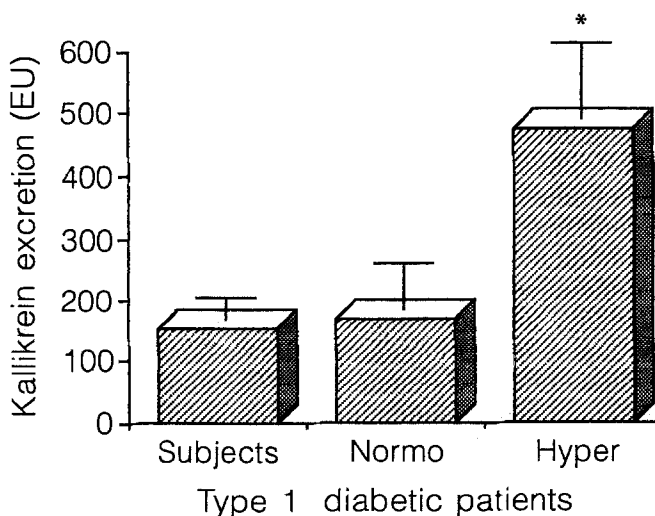

Fig. 1. Urinary kallikrein excretion in control subjects (n = 20), normofiltering Type 1 diabetic patients (n = 78) and hyperfiltering Type 1 diabetic patients (n = 11). EU, esterase units. * $p < 0.001$
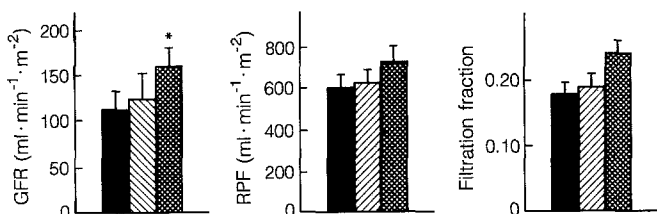
Fig. 2. Glomerular filtration rate (GFR), renal plasma flow (RPF) and filtration fraction in normal subjects (■), in normofiltering Type 1 diabetic patients (▨) and in hyperfiltering Type 1 diabetic patients (▩) * p < 0.001
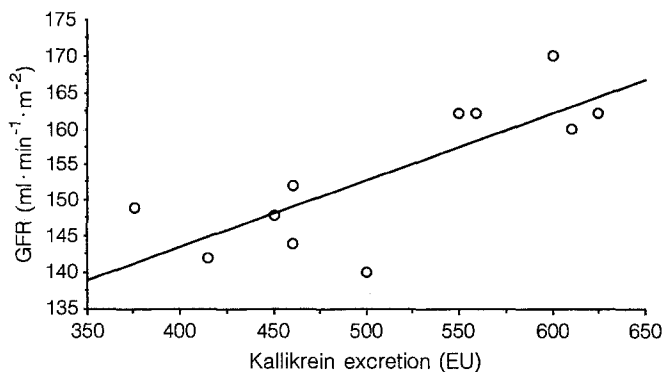

Fig. 3. Correlation between urinary kallikrein excretion (esterase units, EU/24 h) and glomerular filtration rate (GFR) (ml·min^{-1}· m^{-2}) in Type 1 diabetic patients with hyperfiltration ($n = 11$) (group B); ($r = 0.785, p < 0.04$)

The mean of GFR in hyperfiltering patients was 161 ± 19 ml·min^{-1}·m^{-2} ($p < 0.001$) compared to 125 ± 26 ml·min^{-1}·m^{-2} in normofiltering Type 1 diabetic patients and 112 ± 20 ml·min^{-1}·m^{-2} in control subjects. Although the RPF tended to be higher in hyperfiltering patients, neither the RPF nor the filtration fraction (FF) significantly increased (Fig. 2). Furthermore, we observed a positive correlation, in hyperfiltering patients, between urinary kallikrein excretion and GFR ($r = 0.785, p < 0.04$) (Fig. 3). There was no correlation between excretion of urinary kallikrein and each of the following parameters: albumin excretion rate ($r < 0.022$), and creatinine clearance ($r < 0.025$), HbA$_{1c}$ ($r < 0.026$), duration of diabetes ($r < 0.09$) in hyperfiltering Type 1 diabetic patients. Furthermore no correlation was observed in normofiltering Type 1 diabetic patients between kallikrein excretion and all parameters mentioned above.

Discussion

This study shows that diabetic subjects with glomerular hyperfiltration have increased excretion of urinary kallikrein, compared to diabetic patients with normal GFR or control subjects. Our data are in accordance with the study of Harvey et al. [32] that showed an increase in renal active kallikrein excretion in Type 1 diabetic patients with hyperfiltration.

Previously Harvey et al. [21] observed a rise in the concentration of both urinary and renal kallikreins in strepto-

zotocin-induced diabetic rats with moderately hyperglycaemic values and increased GFR. Conversely, Mayfield et al. [18] showed that kallikrein excretion is increased in patients with poorly-controlled Type 1 diabetes; while strict glycaemic control decreases urinary kallikrein excretion. This observation could be explained by the direct effect of hyperglycaemia on glomerular hyperfiltration. Nevertheless all our hyperfiltering and normofiltering Type 1 diabetic patients were in good metabolic control with similar HbA$_{1c}$ values.

The observation that injection of aprotinin [21, 33, 34], a protease inhibitor, reduces GFR and RPF in rats, suggests a further correlation between kallikrein activity and haemodynamic alterations. Although aprotinin does not specifically inhibit the kallikreins, other proteases known to be inhibited by aprotinin have no effect on renal activity suggesting that the effect of aprotinin is due to a direct kallikrein inhibition.

Levy et al. [35] found a direct relationship between RPF and urinary kallikrein excretion in humans; many studies in animals also suggest a direct relationship between the renal kallikrein-kinin system and renal blood flow [12–14]. In our study we found a slight increase of RPF and FF in hyperfiltering Type 1 diabetic patients. Furthermore, the urine volume did not change in all three groups studied excluding a washout effect secondary to GFR changes.

Moreover, urinary kallikrein excretion is related to protein intake: an increase in renal kallikrein synthesis and excretion with a rise in GFR and RPF has been noticed in non-diabetic and in streptozotocin-diabetic rats with high protein intake [36]. On the other hand several studies have found that GFR and RPF are reduced in rats kept on a protein-restricted diet in contrast to rats fed with high-protein diet [37–40]; such parameters are also reduced in humans receiving a low protein-diet [41]. Mauer et al. [42] reported that increasing protein in the diet from 20% to 50% raised both GFR and RPF in normal rats. In our study the diabetic hyperfiltering patients had a protein intake lower than 19% similar to normofiltering patients; 30% of the total amount of protein present in our usual diet derived from vegetables. According to Jones et al. [43, 44] a meal of vegetable proteins has no effect on kidney function and no abnormalities in renal haemodynamics have been reported in vegetarians [45].

It was shown that in rats [46] and in normal man [47] on chronic high and low sodium diets urinary kallikrein excretion was positively correlated to urinary volumes but not to sodium excretion [46]. Although the intake of sodium was not controlled in our patients, analysis of the food record showed no significant differences in dietary amount of sodium in the three groups (data not shown). There was no significant change in urinary volume and sodium excretion between normofiltering and hyperfiltering Type 1 diabetic patients. In conclusion we observed an increased kallikrein excretion in Type 1 diabetic hyperfiltering patients, positively correlated to GFR. The increase in kallikrein excretion may represent a marker of the early phase of diabetic nephropathy.

Further investigations will be required to study the synthesis and secretion of kallikrein in the different phases of

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diabetic nephropathy and to evaluate the role of the kallikrein-kinin system in the pathogenesis of this complication.

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