Glycaemic control with insulin prevents the reduced renal dopamine D_1 receptor expression and function in streptozotocin-induced diabetes

Mónica Moreira-Rodrigues[1], Janete Quelhas-Santos[1], Paula Serrão[2], Cátia Fernandes-Cerqueira[1], Benedita Sampaio-Maia[1,3] and Manuel Pestana[1]

[1]Nephrology Research and Development Unit, Faculty of Medicine & Hospital S. João EPE, Porto, Portugal, [2]Institute of Pharmacology and Therapeutics, Faculty of Medicine and [3]Faculty of Dental Medicine, University of Porto, Porto, Portugal

Correspondence and offprint requests to: Manuel Pestana; E-mail: mvasconcelos@hsjoao.min-saude.pt

Abstract

Background. It was demonstrated in streptozotocin (STZ)-induced diabetic rats that the D_1 receptor agonist failed to promote sodium excretion as a result of reduced renal D_1 receptor expression and decreased receptor G protein coupling. The present study examined the influence of glycaemic control with insulin on the renal D_1 receptor dysfunction in STZ-induced type 1 diabetes.

Methods. Renal function, blood pressure, the natriuretic response to 5% volume expansion (VE) and the effects of the D_1 receptor agonist fenoldopam on natriuresis and on Na^+/K^+-ATPase activity in renal tubules were evaluated in uninephrectomized and sham-operated Wistar rats treated with STZ and compared with controls and STZ-treated rats made euglycaemic with insulin. D_1 receptor immunohistochemistry and protein abundance by western blot were also determined in all groups.

Results. Treatment of sham and uninephrectomized rats with STZ caused a 4-fold increase in glucose plasma levels compared to controls and euglycaemic diabetic rats. A blunted natriuretic response to VE was observed in both sham and uninephrectomized hyperglycaemic diabetic rats, and this was accompanied by failure of fenoldopam to increase natriuresis and to inhibit renal Na^+/K^+-ATPase activity. In contrast, in both sham and uninephrectomized euglycaemic diabetic rats, the natriuretic response to VE, the fenoldopam-induced natriuresis and the accompanied inhibition of Na^+/K^+-ATPase activity were similar to those of the corresponding controls. D_1 receptor immunodetection and protein abundance were reduced in hyperglycaemic diabetic rats, but not in euglycaemic diabetic animals.

Conclusions. We conclude that the renal expression and natriuretic response to D_1 receptor activation is compromised in both sham and uninephrectomized rats with STZ-induced diabetes. These abnormalities were prevented by lowering glucose blood levels with insulin, thus providing evidence for the involvement of hyperglycaemia in the disturbances that underlie the compromised dopamine-sensitive natriuresis and increase of blood pressure in type 1 diabetes.

Introduction

The epithelial cells of proximal tubules, but not the distal segments of the nephron, are endowed with a high aromatic L-amino acid decarboxylase activity, the indispensable enzymatic machinery for the conversion of filtered or circulating L-3,4-dihydroxyphenylalanine to dopamine [1,2]. Dopamine produced by proximal tubular cells behaves as an endogenous natriuretic hormone accounting for up to 60% of sodium excretion, namely during moderate sodium surfeit [3,4]. Renal dopamine primarily exerts its natriuretic effects via D_1-like dopamine receptors expressed at both the luminal and basolateral membranes of renal tubular cells [5,6]. At the level of the proximal tubules, the overall increase in sodium excretion produced by dopamine or D_1 receptor agonists results mainly from the inhibition of Na^+/K^+-ATPase and Na^+-H^+-exchanger 3 (NHE3) at the basolateral and apical membranes, respectively [7,8].

Type 1 diabetes mellitus is associated with sodium retention and increase of blood pressure that could be due to enhanced renal tubular reabsorption and/or impaired responses to natriuretic factors [9]. Accordingly, evidence was gathered that the ability of the kidney to excrete sodium and water after intravenous sodium chloride loading is decreased in patients with type 1 diabetes as well as in streptozotocin (STZ)-induced type 1 diabetic rats [9–11].

Several groups have reported a decrease in the endogenous production of dopamine in type 1 diabetes, thus suggesting that a reduced renal dopaminergic activity may contribute to decreased sodium excretion in type 1 diabetes [12–14]. More recently, Lokhandwala *et al.* clearly demonstrated that the D_1 receptor agonist SKF-38393 failed to promote sodium excretion as a result of reduced renal D_1 receptor expression and decreased receptor G protein coupling in STZ-induced diabetic rats [15]. Inasmuch as en-

dogenous renal dopamine plays an important role in maintaining sodium homeostasis, namely during moderate sodium surfeit, such an abnormality in renal D_1 receptor expression and function was suggested to account for sodium retention and increase blood pressure in type 1 diabetes.

The mechanisms underlying the decreased renal D_1 receptor expression and function in type 1 diabetes still remain to be fully elucidated. There seems to be considerable evidence linking reduced D_1 receptor expression and function with abnormal insulin and glucose levels in the central nervous system [16,17]. In fact, several investigators reported decreased D_1 receptors in brains of rats with STZ- or alloxan-induced diabetes [16,17]. On the other hand, when insulin treatment was initiated immediately after the onset of STZ-induced diabetes, the achievement of glycaemic control preserved natriuretic and diuretic responses to volume expansion (VE) [18]. However, at present it is not known whether the abnormalities of the renal D_1 receptor expression and function in type 1 diabetes can be restored by the correction of hyperglycaemia and hypoinsulinaemia.

To test this hypothesis, we examined in STZ-induced diabetic rats the influence of lowering glucose blood levels to normal with insulin on renal function, blood pressure and the effects of D_1 receptor agonist fenoldopam (10 μg/kg/min) on natriuresis (before, during and after 5% VE) as well as on proximal tubular Na^+/K^+-ATPase activity. Renal D_1 receptor immunohistochemistry and protein abundance by western blot were also determined. Because uninephrectomy was suggested to be associated with dopamine-sensitive enhanced natriuresis [19], the influence of glycaemic control with insulin on renal D_1 receptor expression and function in STZ-induced diabetes was evaluated in both uninephrectomized and sham-operated animals.

Materials and methods

In vivo studies

All *in vivo* investigations were performed in accordance with the European Directive number 86/609, transposed to Portuguese Law by Directive Law 129/92 and by Portaria 1005/92. Normotensive male Wistar-Han rats (Harlan, Barcelona, Spain), weighing 190–210 g, were selected after a 7-day period of stabilization and adaptation to blood pressure measurements. The animals were kept under controlled environmental conditions (12:12 h light/dark cycle and room temperature 22 ± 2°C); fluid intake and food consumption were monitored daily throughout the study. All animals were fed *ad libitum* throughout the study with ordinary rat chow (Panlab, Barcelona, Spain) containing 1.9 g/kg of sodium.

Blood pressure measurements. Blood pressure (both systolic and diastolic) and heart rate were measured throughout the study in conscious restrained animals, between 7.00 and 10.00 AM, using a photoelectric tail-cuff pulse detector (LE 5000, Letica, Barcelona, Spain). Four determinations were made each time and the means were used for further calculation.

Uninephrectomy. The surgical ablation of the right kidney was performed in anaesthetized rats (pentobarbital sodium, 60 mg/kg bw; i.p.), according to what was previously described [20]—uninephrectomized rats (U). Control rats were submitted to sham surgery under similar conditions; however, their kidneys remained intact—sham-operated rats (S). After total recovery from surgery (4–6 h), the rats were placed in animal facility, where they had free access to food and water.

STZ-induced type 1 diabetes. Seven days after sham surgery or uninephrectomy, the rats were divided in the following groups: (i) control vehicle groups: a single intraperitoneal injection of the vehicle of STZ (0.1 M sodium citrate, pH 4.5) was given to S and U rats—SV ($n = 20$) and UV ($n = 17$) groups; (ii) hyperglycaemic diabetic groups: type 1 diabetes was induced in S and U rats by a single intraperitoneal injection of STZ (65 mg/kg)—SSTZ ($n = 23$) and USTZ ($n = 19$) groups; and (iii) euglycaemic diabetic groups: two days after STZ injection, insulin (4 U/200 g/day) was administered daily in S and U rats until the end of the experiment—SSTZI ($n = 23$) and USTZI ($n = 19$) groups. Two days after STZ injection, hyperglycaemia was confirmed by checking the fasting blood glucose level using a blood glucose metre (Minifreestyle system, Abbot, CA, USA). All rats injected with STZ were considered hyperglycaemic diabetic rats because they presented blood glucose levels between 250 and 450 mg/dL. In addition, the glycaemic control with insulin was confirmed by blood glucose levels 2 days after the beginning of insulin treatment as well as in the end of the study. Because the present study aimed to examine the influence of glycaemic control with insulin on the renal D_1 receptor expression and function in STZ-induced diabetes, three in a total of 42 rats in the insulin-treated groups that presented blood glucose levels above 250 mg/dL at the end of the study were excluded.

Metabolic studies. In one series of experiments (SV, $n = 6$; SSTZ, $n = 8$; SSTZI, $n = 5$; UV, $n = 6$; USTZ, $n = 8$; USTZI, $n = 7$), 5 days after vehicle or STZ injection, the rats were placed in metabolic cages (Tecniplast, Buguggiate-VA, Italy) for the collection of 24-h urine for later quantification of sodium and creatinine. All animals received tap water and food *ad libitum*. Seven days after STZ or vehicle injection, the animals were anaesthetized with pentobarbital sodium (60 mg/kg; i.p.), and blood was collected from the heart in tubes containing lithium/heparin for later determination of biochemical parameters. The kidneys were rapidly removed and weighed. The kidneys were cut in half for paraffin fixation, and fragments from renal cortex were isolated and stored at −80°C for western blot studies.

VE. In another series of experiments (vehicle: SV, $n = 8$; SSTZ, $n = 8$; SSTZI, $n = 8$; UV, $n = 6$; USTZ, $n = 6$; USTZI, $n = 5$; fenoldopam: SV, $n = 6$; SSTZ, $n = 7$; SSTZI, $n = 8$; UV, $n = 5$; USTZ, $n = 5$; USTZI, $n = 6$), 7 days after STZ or vehicle treatment, the animals were anaesthetized with pentobarbital sodium (60 mg/kg followed by 20 mg/kg/h; i.p.). Thereafter, the rats were placed on a thermostatically controlled heating table to maintain a rectal temperature of 37°C. The rats were tracheostomized. The left jugular vein was catheterized by a PE50 tube (Becton Dickson, Lisboa, Portugal) for VE and infusion of fenoldopam (10 μg/kg/min; D_1-like receptor agonist; Sigma, St. Louis, MO, USA) or the vehicle (0.9% NaCl). After an abdominal incision, the urinary bladder was catheterized through a suprapubic incision for urine sampling. After the completion of surgical procedures, the infusion of fenoldopam or vehicle started at a rate of 5 mL/kg/h for 120 min; during this period, two consecutive 60-min urine samples were collected ($t = 0–120$ min, basal, B). After this stabilization period, the VE was started by infusion of isotonic saline (0.9% NaCl) at a rate of 100 mL/kg/h for 30 min (5% body weight); during this phase, one urine sample was collected ($t = 120–150$ min, VE). Thereafter, the infusion was again reduced to 5 mL/kg/h for 90 min; during this recovery period, urine sampling was performed every 30 min until the end of the experiment ($t = 150–240$ min, RVE). The urine was collected for later determination of sodium and creatinine. At the end of this experiment the kidneys were removed, the outer cortex was isolated and fragments were used for evaluation of proximal tubular Na^+/K^+-ATPase activity.

In vitro studies

Plasma and urine ionogram and biochemistry. In plasma and urine samples, the quantification of sodium was performed by ion-selective electrodes and creatinine by the Jaffé method. All determinations were performed by Cobas Mira Plus analyser (ABX Diagnostics, Geneva, Switzerland). Because high glucose concentrations are known to interfere with creatinine Jaffé assay [21,22], we calculated a linear equation using the creatinine values obtained when using a solution with a known concentration of creatinine and increasing glucose levels. This allowed us to correct the plasma creatinine levels (Pcreatc) using the following formula: Pcreatc = m * PgluN + PcreatD − (m * PgluD); m, slope of standard curve; PgluN, normal rat average plasma glucose; PcreatD, determined diabetic rat plasma creatinine; PgluD, determined diabetic rat plasma glucose. Creatinine clearance and fractional excretion of sodium were calculated as previously reported [20].

Na^+/K^+-ATPase activity. Na^+/K^+-ATPase activity in proximal tubules was measured in all groups of rats after VE and fenoldopam infusion by the method of Quigley and Gotterer [23] adapted in our laboratory with slight modifications as previously reported [24]. In brief, the renal proximal tubular cells were isolated and were pre-incubated for 10 min at 37°C fol-

Table 1. Body weight, renal function and metabolic balance in sham and uninephrectomized control rats (SV or UV), streptozotocin-treated rats (SSTZ or USTZ) and rats treated with streptozotocin plus insulin (SSTZI or USTZI)

	Sham			Uninephrectomy		
	SV	SSTZ	SSTZI	UV	USTZ	USTZI
Body weight, g	269.2 ± 6.8	213.0 ± 4.2[a]	259.4 ± 1.9[b]	259.3 ± 8.2	213.3 ± 3.7[a]	261.6 ± 6.8[b]
Renal mass, g	1.7 ± 0.1	2.0 ± 0.1[a]	1.7 ± 0.1[b]	1.1 ± 0.1	1.5 ± 0.1[a]	1.1 ± 0.1[b]
Renal mass/body weight, g/g	0.0063 ± 0.0001	0.0093 ± 0.0002[a]	0.0066 ± 0.0002[b]	0.0042 ± 0.0001	0.0070 ± 0.0004[a]	0.0042 ± 0.0001[b]
Plasma glucose, mg/dL	103.5 ± 5.2	385.6 ± 36.6[a]	121.6 ± 37.4[b]	99.7 ± 2.8	429.0 ± 20.0[a]	120.2 ± 18.9[b]
Urine glucose, mg/day	0.3 ± 0.2	4286.7 ± 732.5[a]	15.2 ± 9.4[b]	1.0 ± 0.5	5692.5 ± 727.7[a]	1.9 ± 1.7[b]
Na^+ intake, mmol/day	3.0 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.9 ± 0.1	2.9 ± 0.1
Plasma Na^+, mmol/l	131.8 ± 5.1	128.1 ± 3.6	130.6 ± 4.7	125.0 ± 5.0	123.3 ± 4.7	132.3 ± 2.5
Urine Na^+, mmol/day	2.3 ± 0.1	2.4 ± 0.1	2.7 ± 0.3	2.4 ± 0.1	2.4 ± 0.1	2.5 ± 0.1
Ccreat, mL/min	1.8 ± 0.2	2.0 ± 0.2	1.6 ± 0.3	1.5 ± 0.1	1.7 ± 0.2	1.3 ± 0.2
FE_{Na+}, %	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.1

Values are means ± SE; SV, $n = 6$; SSTZ, $n = 8$; SSTZI, $n = 5$; UV, $n = 6$; USTZ, $n = 8$; USTZI, $n = 7$. Ccreat, creatinine clearance; FE, fractional excretion.
[a]Significantly different from values in control rats (SV or UV; $P < 0.05$, Newman–Keuls test).
[b]Significantly different from values in streptozotocin-treated rats (SSTZ or USTZ; $P < 0.05$, Newman–Keuls test).

lowed by rapid freezing at −80°C and subsequent thawing to allow cell permeabilization. The protein content (1.0 mg/mL) in cell suspension was determined by the Bradford method [25]. The cellular suspension was used to evaluate ouabain-resistant (ouabain, 1 mM; Sigma, St. Louis, MO, USA) and total Na^+/K^+-ATPase activity. The reaction was initiated by addition of 4 mM ATP. The released inorganic phosphate in the supernatant was measured by spectrophotometry, as a result of Na^+/K^+-ATPase activity. Ouabain-sensitive Na^+/K^+-ATPase activity was determined as the difference between total and ouabain-resistant Na^+/K^+-ATPase.

Immunohistochemistry staining. Kidney sections, 4 μm thick (Microm STS HM 340E, Walldorf, Germany) of paraffin-embedded formalin-fixed specimens, were deparaffinized in xylene and rehydrated through graded ethanol solutions. The sections were incubated with 15 μg/mL anti-D_1 receptor primary polyclonal antibody (Chemicon International, CA, USA) for two overnights at 4°C. Immunostainning was carried out with an anti-rabbit secondary biotinylated antibody (diluted 1/200; Vector Laboratories, Peterborough, England) followed by diaminobenzidine (DAB 97% TLC, Sigma, St. Louis, MO, USA) and haematoxylin counterstaining. The results were observed in a light microscope (Leica Application Suite Software, Leica Microsystems, Wetzlar, Germany).

Semiquantitative immunoblotting. Renal cortex homogenates (lyses buffer supplemented with phosphatases and proteases inhibitors; Pierce, Rockford, USA) were mixed in sample buffer (Bio-Rad Laboratories, CA, USA) and denaturated at 95°C for 5 min. Samples containing 80 μg of cell protein were separated by SDS–PAGE with 10% polyacrylamide gel and then electroblotted onto nitrocellulose membranes (Bio-Rad Laboratories, CA, USA). Blots were blocked for 1 h with PBS-casein solution (Bio-Rad Laboratories, CA, USA) at room temperature with constant shaking and were then incubated with the primary antibodies, rabbit polyclonal anti-D_1 receptor (1/100; Chemicon International, CA, USA) and mouse polyclonal anti-β-actin (1/10 000; Santa Cruz Biotechnology, Santa Cruz, USA), overnight at 4°C. The immunoblots were subsequently washed and incubated with fluorescently labelled donkey anti-rabbit secondary antibody (1:20 000; IRDye^{Tm} 700 nm, Rockland, PA, USA) or the fluorescently labelled goat anti-mouse secondary antibody (1:20 000; IRDye^{Tm} 700 nm, Rockland, PA, USA) for 60 min at room temperature and protected from light. The membrane was washed and imaged by scanning at both 800 nm and 700 nm, with an Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA). The intensity values of D_1 receptor protein were expressed as arbitrary units (AU) after normalization by β-actin protein. AU was set as the average value of the control group.

Statistics

Results are means ± Standard Error (SE) of values for the indicated number of determinations. Statistical analysis used a one-way ANOVA to compare differences among groups. The Newman–Keuls test was used

for multiple comparisons when significant differences were detected. A $P < 0.05$ was assumed to denote a significant difference.

Results

Seven days after the intraperitoneal injection of STZ, there was an ~4-fold increase in blood glucose levels in both sham and uninephrectomized rats which was accompanied by marked increases in glycosuria compared with control animals (Table 1). Hyperglycaemia and glycosuria were completely ameliorated in both sham and uninephrectomized STZ-injected rats that received insulin therapy (Table 1). In addition, by day 7, both sham and uninephrectomized STZ-treated rats did not increase their body weight, whereas STZ-injected rats that were treated with insulin gained weight rapidly, reaching values not significantly different from the corresponding control animals at the end of the experimental period (Table 1). The kidney weight at the end of the experiment was significantly greater in hyperglycaemic diabetic groups than in corresponding controls or euglycaemic diabetic groups (Table 1). Also, the kidney weight per body weight ratio was significantly greater in hyperglycaemic diabetic groups than in corresponding controls and euglycaemic diabetic groups (Table 1).

There were no differences in daily intake, plasma levels or urinary excretion of sodium among sham or uninephrectomized groups of rats (Table 1). In addition, creatinine clearance and fractional excretion of sodium did not differ among sham or uninephrectomized groups of animals (Table 1). Blood pressure did not differ significantly among sham groups (Figure 1A). In contrast, in uninephrectomized groups, both systolic and diastolic blood pressure was significantly higher in hyperglycaemic diabetic rats than in control and euglycaemic diabetic animals (Figure 1B).
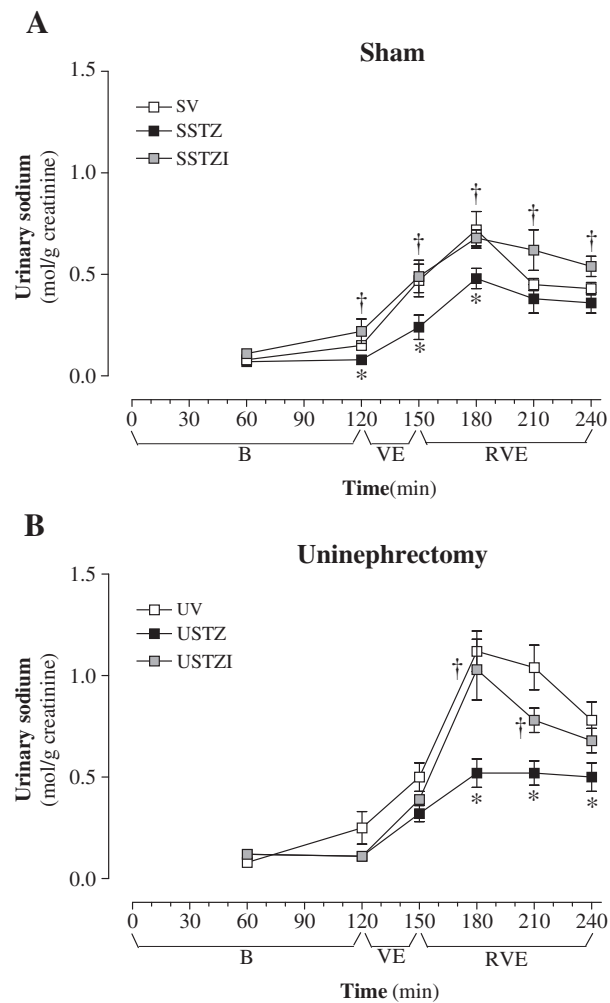
The urinary sodium excretion before ($t = 0$–120 min, B), during ($t = 120$–150 min, VE) and after ($t = 150$–240 min, RVE) VE with isotonic saline, in sham and uninephrectomized groups of rats, is depicted in Figure 2. As can be

A

Sham

B
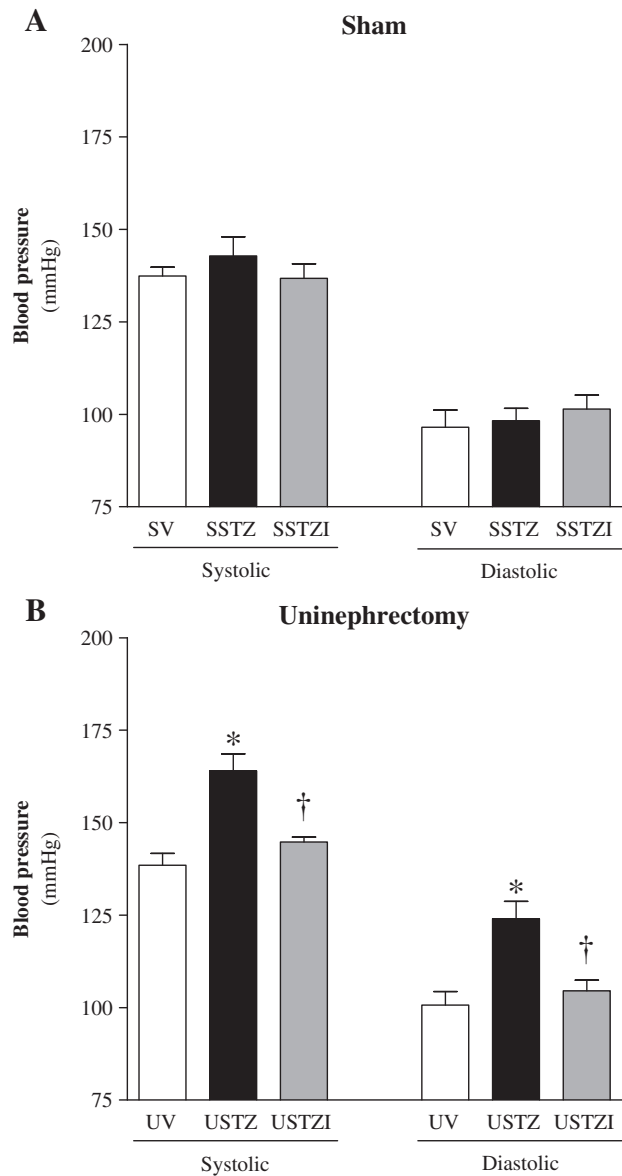
Uninephrectomy

Fig. 1. Systolic and diastolic blood pressure in (**A**) sham and (**B**) uninephrectomized control rats (SV and UV, empty square), streptozotocin-treated rats (SSTZ and USTZ, filled square) and rats treated with streptozotocin plus insulin (SSTZI and USTZI, shaded square). Bars represent means of 5–8 animals per group (SV, $n = 6$; SSTZ, $n = 8$; SSTZI, $n = 5$; UV, $n = 6$; USTZ, $n = 8$; USTZI, $n = 7$), and error bars represent SE. *Significantly different from values in control rats (SV or UV; $P < 0.05$, Newman–Keuls test). †Significantly different from values in streptozotocin-treated rats (SSTZ or USTZ; $P < 0.05$, Newman–Keuls test).

A

Sham

B

Uninephrectomy

Fig. 2. Urinary sodium excretion (mol/g creatinine) before [$t = 0$–120 min, basal (B)], during ($t = 120$–150 min, VE) and after [$t = 150$–240, recovery (RVE)] 5% volume expansion (VE) with isotonic saline in (**A**) sham and (**B**) uninephrectomized control rats (SV and UV, empty square), streptozotocin-treated rats (SSTZ and USTZ, filled square) and rats treated with streptozotocin plus insulin (SSTZI and USTZI, shaded square). Symbols represent means of 5–8 animals per group (SV, $n = 8$; SSTZ, $n = 8$; SSTZI, $n = 8$; UV, $n = 6$; USTZ, $n = 6$; USTZI, $n = 5$), and error bars represent SE. *Significantly different from values observed in control rats (SV or UV; $P < 0.05$, Newman–Keuls test). †Significantly different from values in streptozotocin-treated rats (SSTZ or USTZ; $P < 0.05$, Newman–Keuls test).

observed, the natriuretic responses to VE were significantly decreased in hyperglycaemic diabetic groups compared to corresponding controls or euglycaemic diabetic animals. In addition, the natriuretic responses to VE in euglycaemic diabetic groups were similar to those observed in the corresponding control groups (Figure 2).

The effect of the D_1-like receptor agonist fenoldopam on the accumulated urinary sodium excretion before ($t = 0$–120 min, B), during ($t = 120$–150 min, VE) and after ($t = 150$–210 min, RVE) VE, in sham and uninephrectomized groups of rats, is depicted in Figure 3. In both

sham and uninephrectomized rats, intravenous administration of fenoldopam (10 µg/kg/min) caused significant increases in urinary sodium excretion during the recovery phase in the control groups, whereas in hyperglycaemic diabetic groups fenoldopam failed to increase the urinary sodium excretion in sham and only slightly increased natriuresis in uninephrectomized rats (Figure 3A and B). In contrast, fenoldopam caused significant increases in urinary sodium excretion in euglycaemic diabetic groups that were similar to those observed in the corresponding control groups (Figure 3). It is interesting to observe that the effect of fenoldopam on the accumulated urinary sodium excretion in both control and euglycaemic diabetic groups was more pronounced in uninephrectomized rats than in sham animals (Figure 3).

A

B
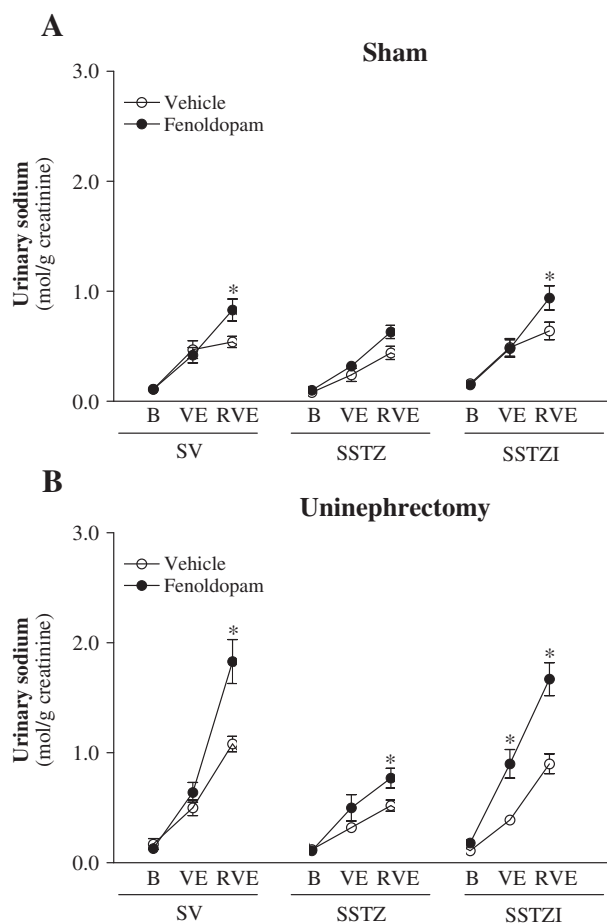
Fig. 3. Accumulated urinary sodium excretion (mol/g creatinine) in fenoldopam (10 µg/min/kg, filled circle) and vehicle (empty circle) treated rats before ($t = 0–120$ min, B), during ($t = 120–150$ min, VE) and after ($t = 150–210$ min, RVE) 5% volume expansion with isotonic saline. Studies were carried out in (**A**) sham and (**B**) uninephrectomized control rats (SV and UV), streptozotocin-treated rats (SSTZ and USTZ) and rats treated with streptozotocin plus insulin (SSTZI and USTZI). Bars represent means of 5–8 animals per group (vehicle: SV, $n = 8$; SSTZ, $n = 8$; SSTZI, $n = 8$; UV, $n = 6$; USTZ, $n = 6$; USTZI, $n = 5$; fenoldopam: SV, $n = 6$; SSTZ, $n = 7$; SSTZI, $n = 8$; UV, $n = 5$; USTZ, $n = 5$; USTZI, $n = 6$), and error bars represent SE. *Significantly different from values observed in vehicle-treated rats ($P < 0.05$, Newman–Keuls test).

The Na^+/K^+-ATPase activity in proximal tubular cells of sham and uninephrectomized groups of rats after fenoldopam infusion is depicted in Figure 4. As can be observed, the Na^+/K^+-ATPase activity in renal proximal tubules from hyperglycaemic diabetic groups was significantly higher than that observed in corresponding controls and euglycaemic diabetic groups (Figure 4). In addition, the activity of proximal tubular Na^+/K^+-ATPase did not differ between control groups and corresponding euglycaemic diabetic groups (Figure 4).

The distribution of D_1 receptor immunohistochemistry staining was markedly decreased in the renal cortex from hyperglycaemic diabetic groups in comparison with both corresponding controls and euglycaemic diabetic groups (Figure 5). No immunostaining was detected in negative control slices (data not shown).

In both sham and uninephrectomized groups, the D_1 receptor protein expression levels in the renal cortex from

A

B
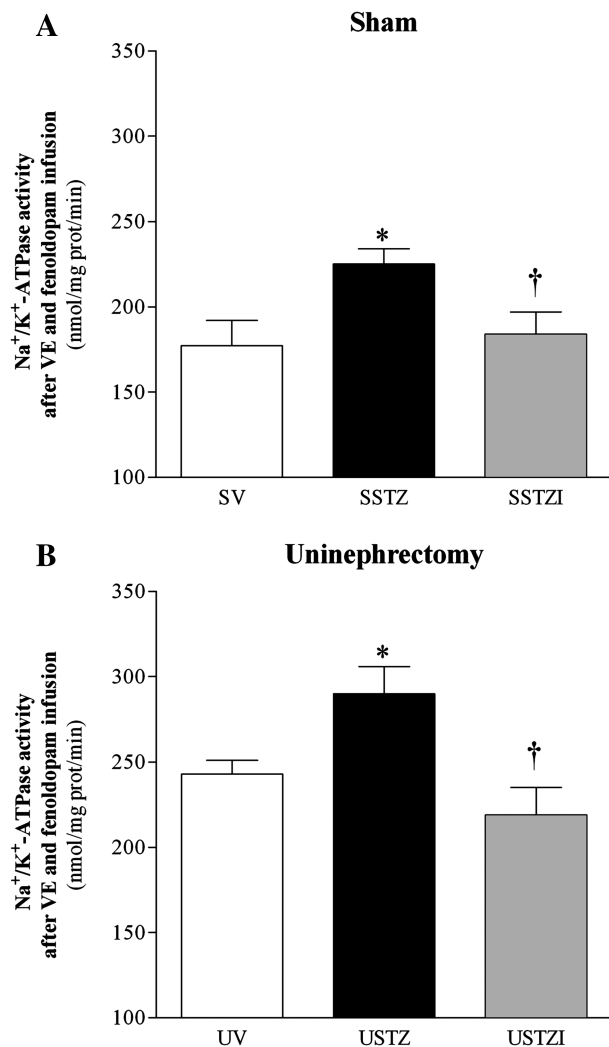
Fig. 4. Na^+/K^+-ATPase activity in renal proximal tubules from fenoldopam (10 µg/min/kg) treated (**A**) sham and (**B**) uninephrectomized rats after 5% volume expansion (VE). Studies were carried out in control rats (SV and UV, empty square), in streptozotocin-treated rats (SSTZ and USTZ, filled square) and in rats treated with streptozotocin plus insulin (SSTZI and USTZI, shaded square). Bars represent means of 5–8 animals per group (SV, $n = 6$; SSTZ, $n = 7$; SSTZI, $n = 8$; UV, $n = 5$; USTZ, $n = 5$; USTZI, $n = 6$), and error bars represent SE. *Significantly different from values observed in control rats (SV or UV) ($P < 0.05$, Newman–Keuls test). †Significantly different from values observed in streptozotocin-treated rats (SSTZ or USTZ; $P < 0.05$, Newman–Keuls test).

hyperglycaemic diabetic rats were markedly decreased by $49 \pm 20\%$ and $46 \pm 10\%$, respectively, in comparison with the corresponding control groups (Figure 6). In contrast, in both sham and uninephrectomized groups, the D_1 receptor protein expression levels in the renal cortex from euglycaemic diabetic rats did not differ from those observed in the corresponding controls (Figure 6).

Discussion

Consistent with the findings of a previous study by Lokhandwala *et al.* [15], our present results demonstrate that in STZ-induced diabetes, a model of type 1 diabetes, there is a reduced renal D_1 receptor expression which is

A-Sham

| SV | SSTZ | SSTZI |

B-Uninephrectomy

| UV | USTZ | USTZI |
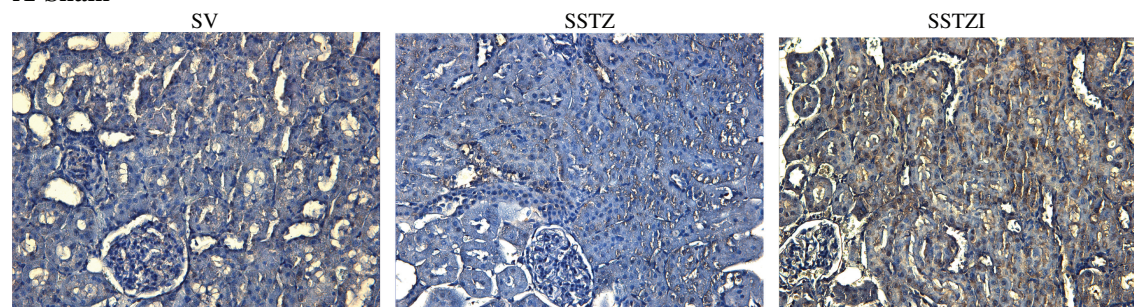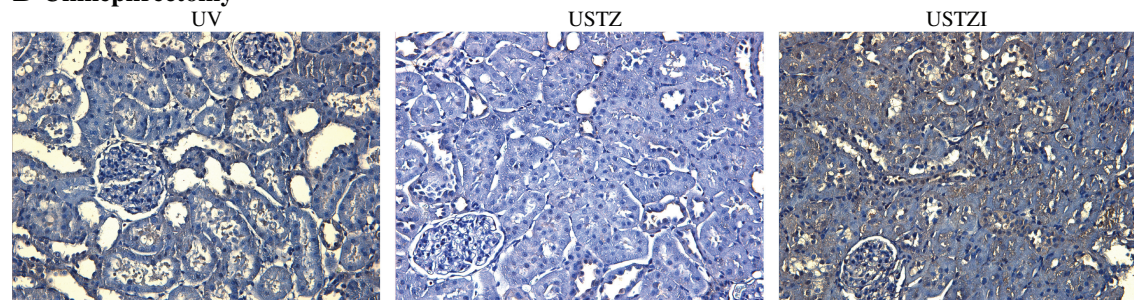
Fig. 5. Representative light microscopy images of immunohistochemical detection of dopamine D_1 receptor expression in rat kidney paraffin slices (magnification ×200) from (**A**) sham and (**B**) uninephrectomized control rats (SV and UV), streptozotocin-treated rats (SSTZ and USTZ) and rats treated with streptozotocin plus insulin (SSTZI and USTZI).

accompanied by lack of natriuretic response to the D_1-like receptor agonist fenoldopam. The present study extended this observation to uninephrectomized rats with STZ-induced diabetes. The finding in uninephrectomized rats with STZ-induced diabetes that the renal D_1 receptor dysfunction was accompanied by increases in both systolic and diastolic blood pressure agrees well with the notion that the renal dopamine system plays an important role in keeping uninephrectomized rats within sodium balance [19]. Achievement of glycaemic control with insulin restored the renal D_1 receptor expression as well as the natriuretic response to fenoldopam in both sham and uninephrectomized rats with STZ-induced diabetes. Moreover, insulin therapy prevented the increase of blood pressure in uninephrectomized rats with STZ-induced diabetes. Taken together, our data suggest that hyperglycaemia impairs renal D_1 receptor expression and function, which contributes to sodium retention and high blood pressure in type 1 diabetes.

In the present study, the impaired renal D_1 receptor expression and function could have been due to hyperglycaemia, hypoinsulinaemia or both. Thus, one cannot exclude that the reversal of renal D_1 receptor dysfunction in STZ-induced diabetes may have been mediated, at least in part, by direct or indirect insulin-induced actions in proximal tubular cells. However, several arguments are against this possibility: (i) chronic exposure to insulin of opossum kidney cells, a proximal tubular cell line, caused renal D_1 receptor dysfunction [26]; (ii) in addition, in Sprague-Dawley rats, elevated levels of plasma insulin for 3 weeks caused hypertension which was accompanied by renal dopamine D_1 receptor dysfunction [27]; (iii) furthermore, in obese Zucker rats, a rat model of type 2 diabetes, renal D_1 receptor dysfunction was observed and this was restored by lowering

blood glucose to normal values by rosiglitazone treatment [28–30]. Interestingly, hyperglycaemia in obese Zucker rats was accompanied by hyperinsulinaemia which was also restored to normal by rosiglitazone treatment thus reinforcing the view that hyperglycaemia may in fact impair renal D_1 receptor function in both type 1 and type 2 diabetes, independently of insulin blood levels.

In type 1 diabetes, there is considerable evidence linking altered expression and function of dopamine receptors with hyperglycaemia and hypoinsulinaemia in other organs, namely in the central nervous system [16,17]. In fact, hyperglycaemia was reported to suppress the firing of central dopaminergic neurons [31], and animal studies provided evidence that chronic hyperglycaemia decreases striatal dopaminergic transmission [32]. Moreover, many of the attenuated central dopaminergic functions in type 1 diabetes were normalized by insulin treatment [33]. The results of the present study extend these observations to the kidney and further demonstrate that both the reduced expression of renal D_1 receptors and the failure of fenoldopam to promote natriuresis in STZ-induced diabetes are prevented by decreasing blood glucose levels to normal with insulin. Evidence to date indicates that protein kinase C is activated by elevation of glucose levels [34,35]. Inasmuch as the increase in protein kinase C activity was associated with GRK-2 upregulation which leads to D_1 receptor serine hyperphosphorylation and receptor desensitization [36], one can hypothesize that the increase in the activity of protein kinase C may serve as a link between hyperglycaemia and the impaired renal D_1 receptor expression and function in STZ-induced diabetes.

Several earlier reports showed increased expression and basal activity of Na^+/K^+-ATPase in the STZ-induced dia-

A

Sham

B

Uninephrectomy
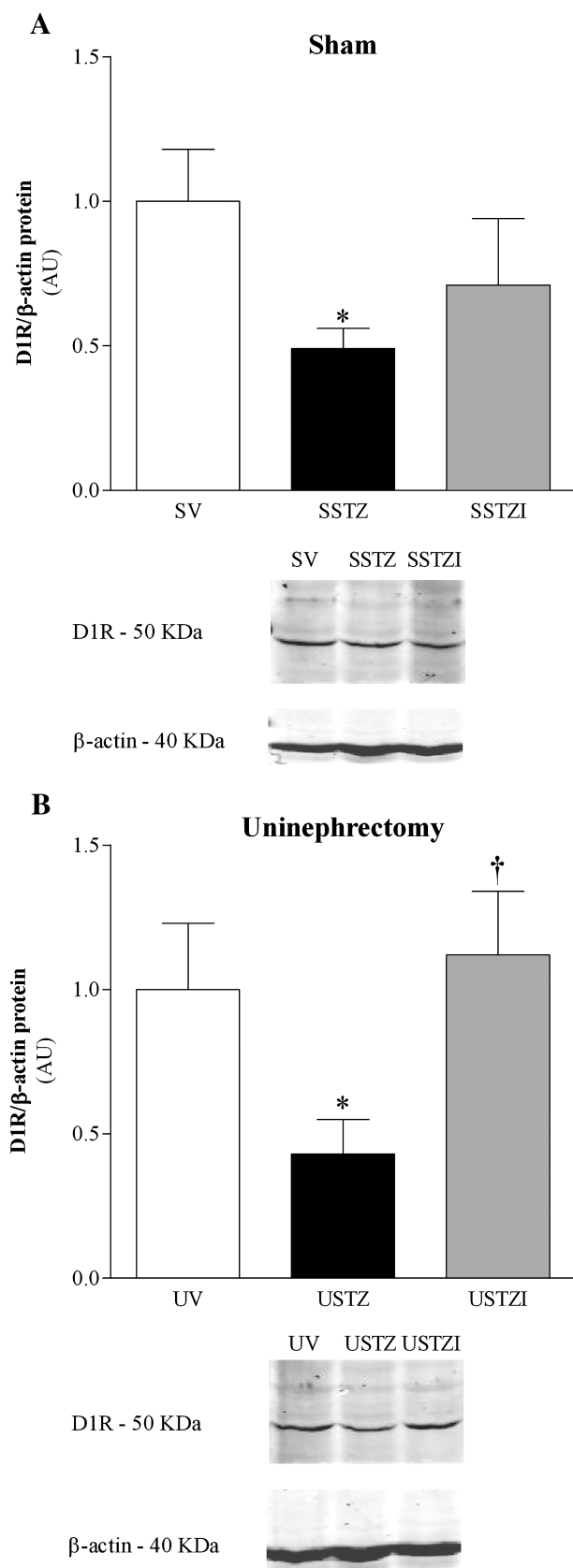
betic kidney [37,38], suggesting a state of sodium retention in these animals. Given that the natriuretic effects of dopamine are more prominent after VE or high salt diet [39], we felt it was worthwhile to examine the natriuretic response to fenoldopam in STZ-induced diabetes, before, during and after VE with isotonic saline. The rats with STZ-induced diabetes presented a blunted natriuretic response to VE and this was accompanied by failure of fenoldopam to increase the urinary sodium excretion in STZ-induced diabetes. Because the natriuretic response to D$_1$-like receptor agonists is produced by inhibition of Na$^+$/K$^+$-ATPase, the blunted natriuretic response to fenoldopam in STZ-induced diabetes is most likely due to a decrease in the fenoldopam-mediated inhibition of Na$^+$/K$^+$-ATPase compared with corresponding controls and euglycaemic diabetic animals. In agreement with this view are the results showing that the activity of Na$^+$/K$^+$-ATPase in proximal tubules was increased in hyperglycaemic diabetic rats after VE and fenoldopam infusion, in comparison with corresponding controls and euglycaemic diabetics.

An enhanced dopamine-sensitive natriuresis was previously reported in uninephrectomized rats indicating that the renal dopaminergic system may play an important role in keeping uninephrectomized rats within sodium balance [19]. In the present study, the renal D$_1$ receptor dysfunction in STZ-induced diabetes was accompanied by increases in both systolic and diastolic blood pressure in uninephrectomized rats, but not in sham animals. Thus, it is likely that the impaired renal dopaminergic activity in STZ-induced diabetes may be more deleterious for sodium balance and blood pressure control in conditions of reduced renal mass. The finding in uninephrectomized rats that blood pressure values in euglycaemic diabetic rats with restored renal D$_1$-like receptor function were similar to those observed in corresponding controls is in agreement with this view.

Our present results in uninephrectomized rats with STZ-induced diabetes agree well with the previous findings of others, showing that early treatment with insulin, started at the time diabetes is induced by STZ, prevents the development of hypertension in Wistar rats submitted to 25% reduction in renal mass [40]. In that study, the prevention of hypertension by early treatment with insulin was accompanied by prevention of albuminuria, VE and appearance of a plasma Na$^+$/K$^+$-ATPase inhibitor. However, when the same insulin treatment was started after 4 weeks of sustained diabetes, this post-treatment failed to ameliorate the hypertension and most of the changes associated with it [40]. On the other hand, evidence was gathered that insulin treatment ini-

Fig. 6. Representative immunoblots of dopamine D$_1$ receptor protein levels (D$_1$R, ~50 kDa) in renal cortex from (**A**) sham and (**B**) uninephrectomized control rats (SV and UV, empty square), streptozotocin-treated rats (SSTZ and USTZ, filled square) and rats treated with streptozotocin plus insulin (SSTZI and USTZI, shaded square). Results are expressed as arbitrary units (AU) after normalization for β-actin protein expression (~40 kDa). AU was set as the average value of the control groups (SV or UV). Bars represent means of 4–5 animals per group (SV, $n = 5$; SSTZ, $n = 5$; SSTZI, $n = 4$; UV, $n = 5$; USTZ, $n = 5$; USTZI, $n = 5$), and error bars represent SE. *Significantly different from values observed in control rats (SV or UV; $P < 0.05$, Newman–Keuls test). †Significantly different from values observed in streptozotocin-treated rats (SSTZ or USTZ; $P < 0.05$, Newman–Keuls test).

tiated immediately after the onset of diabetes restores natriuretic and diuretic responses to VE, whereas delayed insulin treatment does not fully restore the natriuretic response to VE [18]. Thus, even though early insulin treatment may prevent the renal D_1 receptor dysfunction in both sham and uninephrectomized rats with STZ-induced diabetes, it remains to be established whether post-treatment is able to ameliorate the decrease in renal D_1 receptor expression and function and most of the changes associated with it.

Renal dopamine can act in conjunction with other natriuretic hormones and can oppose the effects of antinatriuretic hormones [39]. There is an abundance of evidence suggesting that the natriuretic response to atrial natriuretic peptide (ANP) requires an intact renal dopaminergic system [41]. The inhibitory effect of ANP on basolateral Na^+/K^+-ATPase occurs via a D_1-dependent mechanism, with a synergistic inhibition of Na^+/K^+-ATPase by the two hormones [41]. On the other hand, it has been shown that ANP and its intracellular second messenger cyclic guanosine monophosphate (cGMP) may recruit silent D_1 dopamine receptors from the interior of the cells towards the plasma membrane, thus sensitizing the tubular cells to the effect of dopamine [41]. Interestingly, a blunted natriuretic response to ANP infusion was previously reported in STZ-induced diabetic rats and this was associated with a decrease in the number of biologically active ANP receptors in the kidneys of these rats providing an explanation for the reduced renal response to the hormone [42]. Importantly, the decreased number of biologically active ANP receptors in the kidneys of rats with STZ-induced diabetes was associated with a decreased production of cGMP by renal tubular cells and those changes were prevented by reduction of glucose plasma levels to normal, with insulin [42]. Although our study was not designed to evaluate the relationship between ANP and dopamine resistance in STZ-induced diabetes, the combined data strongly suggest that failure to observe natriuresis in rats with STZ-induced diabetes could be due, at least in part, to a combined dysfunction of renal D_1 receptors and biologically active ANP receptors.

Conclusion

In summary, our results demonstrate that the renal expression and natriuretic response to D_1 receptor activation is compromised in both sham and uninephrectomized rats with STZ-induced diabetes and further show that this is accompanied in uninephrectomized rats by increase in blood pressure. These abnormalities were prevented in both sham and uninephrectomized rats with STZ-induced diabetes by lowering blood glucose levels to normal, with early insulin therapy, thus providing evidence for the involvement of hyperglycaemia in the disturbances that underlie the compromised dopamine-sensitive natriuresis and increase of blood pressure in type 1 diabetes.

References

1. Hayashi M, Yamaji Y, Kitajima W, Saruta T. Aromatic L-amino acid decarboxylase activity along the rat nephron. *Am J Physiol* 1990; 258: F28–F33
2. Soares-da-Silva P. Source and handling of renal dopamine: its physiological importance. *News Physiol Sci* 1994; 9: 128–134
3. Hughes JM, Beck TR, Rose CE Jr, Casey RM. The effect of selective dopamine-1 receptor stimulation on renal and adrenal function in man. *J Clin Endocrinol Metab* 1988; 66: 518–525
4. Hughes JM, Ragsdale NV, Felder RA, Chevalier RL, King B, Carey RM. Diuresis and natriuresis during continuous dopamine-1 receptor stimulation. *Hypertension* 1988; 11: I69–I74
5. Hussain T, Lokhandwala MF. Renal dopamine receptor function in hypertension. *Hypertension* 1998; 32: 187–197
6. Jose PA, Raymond JR, Bates MD, Aperia A, Felder RA, Carey RM, Eisner GM, Jose PA. The renal dopamine receptors. *J Am Soc Nephrol* 1992; 2: 1265–1278
7. Felder CC, Albrecht FE, Campbell T, Eisner GM, Jose PA. cAMP-independent, G protein-linked inhibition of Na+/H+ exchange in renal brush border by D1 dopamine agonists. *Am J Physiol* 1993; 264: F1032–F1037
8. Jose PA, Eisner GM, Felder RA. Paracrine regulation of renal function by dopamine. In: DW Seldin, G Giebisch (eds). *The Kidney Physiology and Pathophysiology.* Philadelphia: Lippincot Williams and Wilkins, 2000; 915–930
9. O'Hare JP, Roland JM, Walters G, Corall RJ. Impaired sodium excretion in response to volume expansion induced by water immersion in insulin-dependent diabetes mellitus. *Clin Sci (Lond)* 1986; 71: 403–409
10. Patel KP, Zhang PL. Reduced renal responses to volume expansion in streptozotocin-induced diabetic rats. *Am J Physiol* 1989; 257: R672–R679
11. Zhang PL, Patel MB, Patel KP. Renal responses to acute volume expansion and atrial natriuretic factor in streptozotocin-induced diabetic rats. *Diabetes Res Clin Pract* 1991; 14: 37–46
12. Madacsy L, Sulyok E, Klujber L, Vamosi I, Barkai L, Baranyai Z. Decreased urinary excretion of dopamine and sodium in diabetic children with incipient nephropathy. *Padiatr Padol* 1991; 26: 253–256
13. Segers O, Gerlo E, Dupont AG, Somers G. Decreased urinary dopamine excretion and disturbed dopamine/sodium relationship in type 1 diabetes mellitus. *Diabet Med* 1995; 12: 229–234
14. Stenvinkel P, Saggar-Malik AK, Wahrenberg H, Diczfalusg U, Bolinder J, Alvestrand A. Impaired intrarenal dopamine production following intravenous sodium chloride infusion in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1991; 34: 114–118
15. Marwaha A, Banday AA, Lokhandwala MF. Reduced renal dopamine D1 receptor function in streptozotocin-induced diabetic rats. *Am J Physiol Renal Physiol* 2004; 286: F451–F457
16. Saitoh A, Morita K, Sodeyama M, Kamei J. Effects of the experimental diabetes on dopamine D1 receptor-mediated locomotor-enhancing activity in mice. *Pharmacol Biochem Behav* 1998; 60: 161–166
17. Salkovic M, Lackovic Z. Brain D1 dopamine receptor in alloxan-induced diabetes. *Diabetes* 1992; 41: 1119–1121
18. Tang D, Yu T, Khraibi AA. Effects of insulin on renal interstitial hydrostatic pressure and natriuretic response to volume expansion in diabetic rats. *Am J Physiol Regul Integr Comp Physiol* 2004; 286: R751–R755
19. Vieira-Coelho MA, Serrao P, Guimaraes JT, Pestena M, Soares-da-silva P. Concerted action of dopamine on renal and intestinal Na (+)-K(+)-ATPase in the rat remnant kidney. *Am J Physiol Renal Physiol* 2000; 279: F1033–F1044

20. Sampaio-Maia B, Serrao P, Guimaraes JT, Vieira-Coelho MA, Pestena M. Renal dopaminergic system activity in the rat remnant kidney. *Nephron Exp Nephrol* 2005; 99: e46–e55
21. Da Rin G, Amici G, Virga G, Bardin C, Calzavara P, Bocci C. Correction of glucose concentration interference on Jaffe kinetic creatinine assay in peritoneal dialysis. *Am J Nephrol* 1995; 15: 480–487
22. Mak TW, Cheung CK, Cheung CM, Leung CB, Lam CW, Lai KN. Interference of creatinine measurement in CAPD fluid is dependent on glucose and creatinine concentrations. *Nephrol Dial Transplant* 1997; 12: 184–186
23. Quigley JP, Gotterer GS. Distribution of (Na+-K+)-stimulated ATPase activity in rat intestinal mucosa. *Biochim Biophys Acta* 1969; 173: 456–468
24. Sampaio-Maia B, Moreira-Rodrigues M, Serrao P, Pestena M. Blunted renal dopaminergic system activity in puromycin aminonucleoside-induced nephrotic syndrome. *Nephrol Dial Transplant* 2006; 21: 314–323
25. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248–254
26. Banday AA, Asghar M, Hussain T, Lokhandwala MF. Dopamine-mediated inhibition of renal Na, K-ATPase is reduced by insulin. *Hypertension* 2003; 41: 1353–1358
27. Ahmad Banday A, Lokhandwala MF. Defective renal dopamine D1 receptor function contributes to hyperinsulinemia-mediated hypertension. *Clin Exp Hypertens* 2006; 28: 695–705
28. Hussain T, Becker M, Beheray S, Lokhandwala MF. Dopamine fails to inhibit Na, H-exchanger in proximal tubules of obese Zucker rats. *Clin Exp Hypertens* 2001; 23: 591–601
29. Hussain T, Beheray SA, Lokhandwala MF. Defective dopamine receptor function in proximal tubules of obese Zucker rats. *Hypertension* 1999; 34: 1091–1096
30. Umrani DN, Banday AA, Hussain T, Lokhandwala MF. Rosiglitazone treatment restores renal dopamine receptor function in obese Zucker rats. *Hypertension* 2002; 40: 880–885
31. Saller CF, Chiodo LA. Glucose suppresses basal firing and haloperidol-induced increases in the firing rate of central dopaminergic neurons. *Science* 1980; 210: 1269–1271
32. Sandyk R. The relationship between diabetes mellitus and Parkinson's disease. *Int J Neurosci* 1993; 69: 125–130
33. Rutledge LP, Ngong JM, Kuperberg JM, Samaan SS, Soliman KF, Kolta MG. Dopaminergic system modulation of nociceptive response in long-term diabetic rats. *Pharmacol Biochem Behav* 2002; 74: 1–9
34. Craven PA, DeRubertis FR. Protein kinase C is activated in glomeruli from streptozotocin diabetic rats. Possible mediation by glucose. *J Clin Invest* 1989; 83: 1667–1675
35. Lee TS, Saltsman KA, Ohashi H, King GL. Activation of protein kinase C by elevation of glucose concentration: proposal for a mechanism in the development of diabetic vascular complications. *Proc Natl Acad Sci U S A* 1989; 86: 5141–5145
36. Banday AA, Fazili FR, Lokhandwala MF. Oxidative stress causes renal dopamine D1 receptor dysfunction and hypertension via mechanisms that involve nuclear factor-kappaB and protein kinase C. *J Am Soc Nephrol* 2007; 18: 1446–1457
37. Khadouri C, Barlet-Bas C, Doucet A. Mechanism of increased tubular Na-K-ATPase during streptozotocin-induced diabetes. *Pflugers Arch* 1987; 409: 296–301
38. Ku DD, Meezan E. Increased renal tubular sodium pump and Na+, K+-adenosine triphosphatase in streptozotocin-diabetic rats. *J Pharmacol Exp Ther* 1984; 229: 664–670
39. Aperia AC. Intrarenal dopamine: a key signal in the interactive regulation of sodium metabolism. *Annu Rev Physiol* 2000; 62: 621–647
40. Chen S, Yuan CM, Haddy FJ, Pamnani B. Effect of administration of insulin on streptozotocin-induced diabetic hypertension in rat. *Hypertension* 1994; 23: 1046–1050
41. Brismar H, Holtback U, Aperia A. Mechanisms by which intrarenal dopamine and ANP interact to regulate sodium metabolism. *Clin Exp Hypertens* 2000; 22: 303–307
42. Sechi LA, Valentin JP, Griffin CA *et al*. Receptors for atrial natriuretic peptide are decreased in the kidney of rats with streptozotocin-induced diabetes mellitus. *J Clin Invest* 1995; 95: 2451–2457

Proliferative potential of human kidney endothelial cells: bone marrow-derived cells may not be required for high proliferation

Kimberly A. Muczynski, Nicolae Leca and Susan K. Anderson

Department of Medicine, Division of Nephrology University of Washington, Seattle, WA 98195, USA

Correspondence and offprint requests to: Kimberly A. Muczynski; E-mail: kzynski@u.washington.edu

Abstract
Background. Proliferative potential of a single cell, defined as the number of progeny it gives rise to, has been used to define a hierarchy of endothelial progenitor cells in blood. Cells with high proliferative potential are presumed to have greater capacity for endothelium repair. Based on results with commercially available endothelial cells, it has been proposed that a proliferative hierarchy of endothelial cells also exists within blood vessels. It is unknown whether such vessel-derived highly proliferative endothelial cells originate from the bone marrow or whether the supply of precursors is limited to pre-existing cells that reside within vessels.
Methods. In this study, we isolated normal human renal microvascular endothelial cells (RMEC) and larger cortical vessel endothelial cells (EC) by flow cytometry based on differential expression of human leucocyte antigen (HLA)-