Glycaemic control with insulin prevents the reduced renal dopamine D1 receptor expression and function in streptozotocin-induced diabetes

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Abstract

Background. It was demonstrated in streptozotocin (STZ)-induced diabetic rats that the D1 receptor agonist failed to promote sodium excretion as a result of reduced renal D1 receptor expression and decreased receptor G protein coupling. The present study examined the influence of glycaemic control with insulin on the renal D1 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 D1 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. D1 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. D1 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 D1 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.

Keywords: blood pressure; dopamine; glycaemic control; natriuresis; streptozotocin and type 1 diabetes mellitus

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 D1-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 D1 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 D1 receptor agonist SKF-38393 failed to promote sodium excretion as a result of reduced renal D1 receptor expression and decreased receptor G protein coupling in STZ-induced diabetic rats [15]. Inasmuch as en-
dogeneous renal dopamine plays an important role in maintaining sodium homeostasis, namely during moderate sodium surfeit, such an abnormality in renal D₁ 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 D₁ receptor expression and function in type 1 diabetes still remain to be fully elucidated. There seems to be considerable evidence linking reduced D₁ receptor expression and function with abnormal insulin and glucose levels in the central nervous system [16,17]. In fact, several investigators have reported decreased D₁ 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 glycemic control preserved natriuretic and diuretic responses to volume expansion (VE) [18]. However, at present it is not known whether the abnormalities of the renal D₁ receptor expression and function in type 1 diabetes can be restored by the correction of hyperglycaemia and hypoinsulinemia.

To test this hypothesis, we examined in STZ-induced diabetic rats the influence of lowerance glucose blood levels to normal with insulin on renal function, blood pressure and the effects of D₁ 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 D₁ 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 glycemic control with insulin on renal D₁ 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 anesthetized rats (pentobarbital sodium, 60 mg/kg; 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. When urine samples were collected (t = 60 min), 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 anesthetized 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; STZ, n = 8; SSTZI, n = 8; UV, n = 6; USTZ, n = 8; USTZI, n = 5; fenoldopam: SV, n = 6; STZ, n = 7; SSTZI, n = 8; UV, n = 5; USTZ, n = 5; USTZI, n = 6), 7 days after STZ or vehicle treatment, the animals were anesthetized 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 PE-50 tube (Becton Dickson, Lisboa, Portugal) for VE and infusion of fenoldopam (10 μg/kg/min; D₁-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 creatinine and sodium.

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 plasma and urine creatinine values (Pcrea and PcreaD) using the following formula: Pcrea = m * PguaN + PcreaD – (m * PguaD); m, slope of standard curve; PguaN, normal rat average plasma glucose; PcreaD, determined diabetic rat plasma creatinine; PguaD, determined diabetic rat plasma glucose. Creatinine clearance and fractional excretion of sodium were calculated as previously reported [20].

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)–STZ (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—SSTZ (n = 23) and USTZI (n = 19) groups. Two days after STZ injection, hyperglycaemia was confirmed by measuring blood glucose level using a blood glucose metre (Minifreestyle system, Abbott, 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 glycemic 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 glycemic control on the renal D₁ 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.
bain-sensitive Na+/K+-ATPase activity was determined as the difference in Na+ excretion.

The results are means ± Standard Error (SE) of values for the indicated number of determinations. Statistical analysis used a one-way ANOVA to evaluate ouabain-resistant (ouabain, 1 mM; Sigma, St. Louis, MO, USA) and ouabain-sensitive Na+/K+-ATPase activity. Ouanty determination by the Bradford method [25].

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)

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>SSTZ</th>
<th>SSTZI</th>
</tr>
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<tbody>
<tr>
<td><strong>Body weight, g</strong></td>
<td>269.2 ± 6.8</td>
<td>213.0 ± 4.2</td>
<td>259.4 ± 1.9</td>
</tr>
<tr>
<td><strong>Renal mass, g</strong></td>
<td>1.7 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td><strong>Renal mass/body weight, g/g</strong></td>
<td>0.0063 ± 0.0001</td>
<td>0.0093 ± 0.0002</td>
<td>0.0066 ± 0.0002</td>
</tr>
<tr>
<td><strong>Plasma glucose, mg/dL</strong></td>
<td>103.5 ± 5.2</td>
<td>385.6 ± 36.6</td>
<td>121.6 ± 37.4</td>
</tr>
<tr>
<td><strong>Urine glucose, mg/day</strong></td>
<td>0.3 ± 0.2</td>
<td>4286.7 ± 732.5</td>
<td>15.2 ± 9.4</td>
</tr>
<tr>
<td><strong>Na+ intake, mmol/day</strong></td>
<td>3.0 ± 0.1</td>
<td>2.9 ± 0.1</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td><strong>Plasma Na+, mmol/l</strong></td>
<td>131.8 ± 5.1</td>
<td>128.1 ± 3.6</td>
<td>136.0 ± 4.7</td>
</tr>
<tr>
<td><strong>Urine Na+, mmol/day</strong></td>
<td>2.3 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td><strong>Creat, μmol/min</strong></td>
<td>1.8 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td><strong>FE Na+, %</strong></td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
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Values are means ± SE; SV, n = 6; SSTZ, n = 8; SSTZI, n = 5; UV, n = 6; USTZ, n = 8; USTZI, n = 7. Creat, creatinine clearance; FE, fractional excretion.

*Significantly different from values in control rats (SV or UV; P < 0.05, Newman–Keuls test).

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
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 D₁-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).
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 hyperglycaemic diabetic rats were markedly decreased by 49 ± 20% and 46 ± 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
accompanied by lack of natriuretic response to the D₁-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 D₁ 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 D₁ 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 D₁ receptor expression and function, which contributes to sodium retention and high blood pressure in type 1 diabetes.

In the present study, the impaired renal D₁ receptor expression and function could have been due to hyperglycaemia, hypoinsulinaemia or both. Thus, one cannot exclude that the reversal of renal D₁ 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 D₁ 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 D₁ receptor dysfunction [27]; (iii) furthermore, in obese Zucker rats, a rat model of type 2 diabetes, renal D₁ 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 D₁ 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 D₁ 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 D₁ 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 D₁ 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-
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 D1-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 D1 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 D1-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 D1 receptor protein levels (D1R, ~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).](https://academic.oup.com/ndt/article/25/9/2945/1940094)
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 D1 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 D1 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 D1-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 secondary messenger cyclic guanosine monophosphate (cGMP) may recruit silent D1 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 D1 receptors and biologically active ANP receptors.

**Conclusion**

In summary, our results demonstrate that the renal expression and natriuretic response to D1 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.

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**Conflict of interest statement.** None declared.

**References**


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

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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 kidney microvascular endothelial cells (RMEC) and larger cortical vessel endothelial cells (EC) by flow cytometry based on differential expression of human leucocyte antigen (HLA)-

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