Reduced renal dopamine D1 receptor function in streptozotocin-induced diabetic rats

Aditi Marwaha, Anees Ahmad Banday, and Mustafa F. Lokhandwala

Heart and Kidney Institute, College of Pharmacy, University of Houston, Houston, Texas 77204

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Marwaha, Aditi, Anees Ahmad Banday, and Mustafa F. Lokhandwala. Reduced renal dopamine D1 receptor function in streptozotocin-induced diabetic rats. Am J Physiol Renal Physiol 286: F451–F457, 2004. First published November 11, 2003; 10.1152/ajprenal.00227.2003.—Dopamine, via activation of renal D1 receptors, inhibits the activities of Na-K-ATPase and Na/H exchanger and subsequently increases sodium excretion. Decreased renal dopamine production and sodium excretion are associated with type I diabetes. However, it is not known whether the response to D1 receptor activation is altered in type I diabetes. The present study was designed to examine the effect of streptozotocin-induced type I diabetes on renal D1 receptor expression and function. Streptozotocin treatment of Sprague-Dawley rats caused a fourfold increase in plasma levels of glucose along with a significant decrease in insulin levels compared with control rats. Intravenous administration of SKF-38393, a D1 receptor agonist, caused a threefold increase in sodium excretion in control rats. However, SKF-38393 failed to produce natriuresis in diabetic rats. SKF-38393 caused a concentration-dependent inhibition of Na-K-ATPase activity in renal proximal tubules of control rats. However, the ability of SKF-38393 to inhibit Na-K-ATPase activity was markedly diminished in diabetic rats. D1 receptor numbers and protein abundance as determined by [3H]SCH-23390 ligand binding and Western blot analysis were markedly reduced in diabetic rats compared with control rats. Moreover, SKF-38393 failed to stimulate GTPyS binding in proximal tubular membranes from diabetic rats compared with control rats. We conclude that the natriuretic response to D1 receptor activation is reduced in type I diabetes as a result of a decrease in D1 receptor expression and defective receptor G protein coupling. These abnormalities may contribute to the sodium retention associated with type I diabetes.

G proteins; hyperglycemia; Na-K-ATPase; natriuresis; SKF-38393

DOPAMINE, BY ACTIVATING D1 receptors on the renal proximal tubules, inhibits the sodium transporters Na-K-ATPase and Na/H exchanger and promotes sodium excretion (2, 10). A direct correlation exists between urinary dopamine levels and sodium excretion. Moreover, dopamine produced during intravenous sodium chloride loading accounts for almost 60% of sodium excretion during that period, suggesting that endogenously produced dopamine plays an important role in sodium homeostasis (7, 14).

Type I diabetes is associated with sodium retention, which could be due to decreased renal sodium excretion (23). Also, type I diabetes is associated with hypoinsulinemia and hyperglycemia. Glucose-fed rats have a decreased urinary excretion of sodium and water (28), indicating that the hyperglycemia associated with diabetes might be responsible for altered sodium and water excretion. It is also reported that the ability of the kidney to excrete sodium and water after intravenous sodium chloride loading is decreased in type I diabetic patients as well as in streptozotocin (STZ)-induced type I diabetic rats (26, 28, 35, 36). Furthermore, glucose infusion in patients prevents renal dopamine mobilization (35). This observation is in parallel with the observation of decreased renal dopamine production in type I diabetic patients (21, 36), thus suggesting that decreased renal dopamine may contribute to decreased sodium excretion in type I diabetes. Another possible contributing factor to the decreased ability to excrete sodium could be a decreased response to the activation of renal dopamine D1 receptors. However, at present it is not known whether the natriuretic response to the activation of renal dopamine D1 receptors is altered in type I diabetes.

We hypothesized that renal dopamine D1 receptor function is reduced in type I diabetes. To test this hypothesis, we measured the effect of the dopamine D1 receptor agonist SKF-38393 on urinary sodium and water excretion in STZ-induced diabetic rats. We also measured the inhibition of Na-K-ATPase activity by SKF-38393 and Na-K-ATPase protein expression in renal proximal tubules of diabetic and control rats. In addition, we determined the D1 receptor expression in the renal proximal tubules of diabetic and control rats. Finally, we measured D1 receptor and G protein coupling and Gso and Gq/11α protein expression in the proximal tubular membranes of diabetic and control rats.

MATERIALS AND METHODS

Materials. [3H]SCH-23390 and [35S]GTPyS were purchased from DuPont New England Nuclear Life Sciences (Boston, MA). The rabbit polyclonal D1A receptor antibodies and horseradish peroxidase-conjugated goat anti-rabbit antibodies were purchased from Alpha Diagnostic Intl (San Antonio, TX). Mouse monoclonal α1, Na-K-ATPase antibodies, and anti-mouse secondary antibodies were purchased from Upstate Biotechnology (Lake Placid, NY) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. The rabbit polyclonal Gso and Gq/11α antibodies were purchased from Calbiochem Novabiochem (San Diego, CA). All the other chemicals were purchased from Sigma (St. Louis, MO).

Animals and induction of diabetes. Male Sprague-Dawley rats (250–300 g) were obtained from Harlan (Indianapolis, IN). The rats were maintained in the University of Houston animal care facility. They were kept at 22°C on a 12:12-h dark-light cycle with free access to standard rat chow (Purina Mills, St. Louis, MO) and tap water. Rats were divided into two groups: 1) STZ-treated group in which type I diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg) and 2) control group in which the rats were given a single intraperitoneal injection of the vehicle (5 mM sodium citrate, pH 4.5). Experiments were performed 7 days after the injection of STZ or vehicle and after fasting the rats overnight.

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Surgical procedures for renal function studies. Rats were anesthetized with Inactin (100 mg/kg ip). Tracheotomy was performed to facilitate breathing. To measure the blood pressure and heart rate and to collect blood samples, the left carotid artery was catheterized with PE-50 tubing. This tubing was connected to a Statham P23AC pressure transducer. Similarly, the left jugular vein was catheterized for infusing saline or drug. For collecting urine samples, a midline incision was performed and the left ureter was catheterized with PE-10 tubing connected to tygon tubing. At the completion of the surgery, normal saline (1% body wt/ml) was infused continuously throughout the experimental period to maintain a stable urinary output. Blood pressure and heart rate were continuously recorded on a Grass polygraph (model 7D, Grass Instrument, Quincy, MA).

Experimental protocol for renal function studies. The effect of SKF-38393 on sodium and water excretion was determined both in STZ-treated (diabetic) and vehicle-treated (control) rats (n = 7 per group). The protocol consisted of 45-min stabilization period after the surgery followed by five consecutive 30-min collection periods: C1, C2, D, R1, and R2. During C1 and C2, saline alone was infused; during D, SKF-38393 (1 μg/kg "" min"" in saline) was infused; and during R1 and R2 (recovery), only saline was infused. Urinary samples were collected throughout the 30-min periods, and blood samples were collected at the end of each period. Plasma was separated from blood samples at 1,500 g for 15 min at 4°C. Urine and plasma samples were stored at −20°C until analyzed for creatinine and sodium.

Urine and plasma analysis. Sodium concentration in the urine and plasma was measured using a flame photometer 480 (Ciba Corning Diagnostics, Norwood, MA). Plasma and urinary creatinine levels were measured by creatinine analyzer (model 2, Beckman, CA). Blood glucose was measured by glucose analyzer (Accuchek Advantage, Roche). Plasma insulin was measured by radioimmunoassay using a rat insulin kit (RI-13k, Linco Research, St. Charles, MI). Hematocrit (%) was measured using a standard microcappillary reader.

Evaluation of renal function. Urinary volume was measured gravimetrically, and urine flow (UF; μl/min) was calculated. Urinary sodium excretion (UNaV; μmol/min) was calculated as UF × UNa/V. The glomerular filtration rate (GFR; ml/min) was calculated based on the clearance of creatinine. The fractional excretion of sodium (FENa; %) was calculated based on clearance of sodium and creatinine.

Preparation of renal proximal tubular suspension. A separate group of STZ-treated and control rats (n = 5 per group) was used for the preparation of proximal tubular suspension. An in situ enzyme digestion procedure as previously described (6) was used to isolate renal proximal tubules. The proximal tubular suspension was used for the Na-K-ATPase assay and membrane preparation for subsequent experiments. Protein was determined by bichoronic acid method (Pierce Chemical, Rockford, IL) using bovine serum albumin as a standard.

Effect of SKF-38393 on Na-K-ATPase activity. Na-K-ATPase activity was determined by the method of Quigley and Gotterer (27) with slight modification as reported earlier (6). To determine the SKF-38393-induced Na-K-ATPase inhibition, proximal tubular suspensions (1 mg protein/ml) from both groups were incubated with or without SKF-38393 (10−8 to 10−6 mol/l) at 37°C for 15 min. The tubules were lysed by rapid freezing and thawing with dry ice and acetone. Tubular suspension (0.1 mg protein/ml) was used to assay ouabain (4 mM)-sensitive Na-K-ATPase activity, using end-point phosphate hydrolysis of ATP (4 mM). The inorganic phosphate released was determined colorimetrically.

Preparation of proximal tubular membranes. Proximal tubular suspensions were homogenized in homogenization buffer (10 mM Tris-HCl, 250 mM sucrose, 2 mM phenylmethylsulfonyl fluoride, protease inhibitors, and 5 mM MgCl2) at 4°C. After homogenization, tubules were centrifuged at 20,000 g for 25 min at 4°C. The upper fluffy layer of the pellet was resuspended in the homogenization buffer and used for Western blotting and radioligand binding studies.
The SKF-38393-mediated response is significantly decreased in STZ-treated rats, compared with control rats (Fig. 1). The maximal inhibition of ~14% was produced by $10^{-6}$ mol/l SKF-38393 in the proximal tubules of STZ-treated rats compared with ~33% inhibition in control rats. Basal Na-K-ATPase activity (nmol Pi·mg protein·1·min$^{-1}$) in proximal tubules of STZ-treated rats was significantly higher (122.7 ± 12.6) than in control rats (93.8 ± 20.0). These results show that despite the high basal Na-K-ATPase activity, inhibition of Na-K-ATPase activity by SKF-38393 is less in the STZ-treated rats. To investigate a possible cause for the observed increase in the basal activity of significantly elevated and urinary creatinine levels were significantly decreased in STZ-treated rats (Table 1), resulting in a decreased GFR (Table 1), thereby indicating a decreased renal function.

**Effect of SKF-38393 on renal and cardiovascular parameters in STZ-treated and control rats.** Intravenous administration of SKF-38393 (1 μg·kg$^{-1}$·min$^{-1}$) failed to increase $U_{Na}V$ and $F_{Ena}$ in the STZ-treated rats (Fig. 1, B and C). In control rats, SKF-38393 caused significant increases in $U_{Na}V$ and $F_{Ena}$ and these variables remained elevated during the recovery phase. There was a significant increase in the UF after the intravenous administration of SKF-38393 in STZ-treated rats as well as in control rats (Fig. 1A). UF increased by 48.5% in the STZ-treated rats and by 90.2% in control rats, and it recovered progressively to the basal values by R2 in both groups. The SKF-38393-mediated response is specifically due to activation of dopamine D$_1$ receptors as this response is blocked by the D$_1$ receptor antagonist SCH-23390 in various tissues (4, 34). No changes in the mean arterial pressure, heart rate, and GFRs were produced by SKF-38393 in either of the groups (data not shown). The basal (C1 and C2) UF (before administration of SKF-38393) was significantly higher in the STZ-treated group, whereas the $U_{Na}V$ was significantly lower compared with the control group (Fig. 1, A and B).

In a separate group of rats (n = 5), the effect of time alone on UF, $U_{Na}V$, and $F_{Ena}$ was studied. Urine samples were collected for five intervals, C1, C2, C3, C4, and C5 during which saline (1% body wt ml/h) was infused. There was no significant difference in UF, $U_{Na}V$, and $F_{Ena}$ in any of the intervals (Table 2). These results indicate that time alone did not alter the renal function in these rats and the diuretic and natriuretic response produced by SKF-38393 was drug specific.

**Effect of SKF-38393 on Na-K-ATPase activity in renal proximal tubules of STZ-treated and control rats.** SKF-38393 caused a concentration-dependent ($10^{-8}$ to $10^{-6}$ mol/l) inhibition of Na-K-ATPase activity in proximal tubules from control animals. However, the ability of SKF-38393 to inhibit Na-K-ATPase activity was significantly diminished in the STZ-treated animals (Fig. 2A). The maximal inhibition of ~14% was produced by $10^{-6}$ mol/l SKF-38393 in the proximal tubules of STZ-treated rats compared with ~33% inhibition in the control rats. Basal Na-K-ATPase activity (nmol Pi·mg protein·1·min$^{-1}$) in proximal tubules of STZ-treated rats was significantly higher (122.7 ± 12.6) than in control rats (93.8 ± 20.0). These results show that despite the high basal Na-K-ATPase activity, inhibition of Na-K-ATPase activity by SKF-38393 is less in the STZ-treated rats. To investigate a possible cause for the observed increase in the basal activity of

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**Table 1. Effects of streptozotocin treatment on basal parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Streptozocin Treated</th>
</tr>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>304.3±10</td>
<td>310.0±6.5</td>
</tr>
<tr>
<td>Day 7</td>
<td>317.1±7.5*</td>
<td>277.1±8.4*†</td>
</tr>
<tr>
<td>Fasting blood glucose, mg/ml</td>
<td>112.9±3.9</td>
<td>398.0±11.5†</td>
</tr>
<tr>
<td>Fasting plasma insulin, mg/ml</td>
<td>0.55±0.09</td>
<td>0.16±0.02†</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>106.3±5.8</td>
<td>105.8±6.8</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>280.0±10</td>
<td>290.0±11.6</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>1.074±0.04</td>
<td>1.206±0.05</td>
</tr>
<tr>
<td>Kidney weight/body weight, %</td>
<td>0.34±0.009</td>
<td>0.43±0.01‡</td>
</tr>
<tr>
<td>Urine creatinine, mg/dl</td>
<td>132.9±22.8</td>
<td>67.4±9.7†</td>
</tr>
<tr>
<td>Serum creatinine, mg/dl</td>
<td>0.37±0.03</td>
<td>0.57±0.06†</td>
</tr>
<tr>
<td>Glomerular filtration rate, ml/min</td>
<td>0.66±0.08</td>
<td>0.37±0.05‡</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>45.67±1.2</td>
<td>46.17±0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 7 (body weight, fasting blood glucose, fasting plasma insulin, glomerular filtration rate, urine creatinine, serum creatinine) or 6 (blood pressure, heart rate, kidney weight, hematocrit). *P < 0.05 from day 1 (in the same group), using Student’s paired t-test. †P < 0.05 from control rats using unpaired t-test. ‡P < 0.0001 from control rats using Student’s unpaired t-test.
Na-K-ATPase in STZ-treated rats, we performed Western blot analysis of the α1-subunit of Na-K-ATPase. There was a ∼35.5% increase in the expression of α1-subunit of the Na-K-ATPase in the proximal tubular membranes of STZ-treated rats compared with control rats (Fig. 3B). A single band with molecular size ∼95 kDa was detected by the primary antibodies.

**Dopamine D1 receptor density in proximal tubular membrane of STZ-treated and control rats.** Saturable specific binding of [3H]SCH-23390 was observed in control as well as STZ-treated rats (Fig. 3A). B<sub>max</sub> values were significantly lower in the proximal tubules of the STZ-treated rats (43.79 ± 9.4 fmol/mg protein) compared with the control rats (115.57 ± 23.8 fmol/mg protein; Fig. 3B). The K<sub>d</sub> values of [3H]SCH-23390 binding did not differ in the STZ-treated (14.12 ± 0.6 nM) and control rats (15.03 ± 1.6 nM) (Fig. 3C). Nonspecific binding accounted for ∼25% of the total binding. When we specifically measured the D<sub>1A</sub> receptor protein expression, there was a ∼43% reduction in the D<sub>1A</sub> receptor protein abundance in proximal tubular membranes of STZ-treated rats compared with control rats (Fig. 3D). A single band with molecular size ∼55 kDa was detected by the primary antibodies. These results demonstrate that there is a decreased D<sub>1</sub> receptor density in proximal tubular membrane of STZ-treated rats.

**Effect of SKF-38393 on [35S]GTPγS binding in renal proximal tubular membrane of STZ-treated and control rats.** D<sub>1</sub> receptor activation by SKF-38393 elicited a concentration-dependent (10<sup>–8</sup> to 10<sup>–6</sup> mol/l) stimulation of [35S]GTPγS binding in proximal tubular membranes from control rats. However, SKF-39393 failed to stimulate the [35S]GTPγS binding in proximal tubular membranes from STZ-treated rats (Fig. 4). The maximal stimulation of ∼3% was produced by 10<sup>–6</sup> mol/l SKF-38393 in the proximal tubular membranes of STZ-treated rats compared with ∼23% stimulation in the control rats. Basal [35S]GTPγS binding (pmol/mg protein) in proximal tubular membrane of STZ-treated rats (0.759 ± 0.167) was not significantly different from control rats (0.625 ± 0.132).

The abundance of G<sub>so</sub> and G<sub>q/11</sub>α proteins, known to be coupled with D<sub>1</sub> receptors, was also measured in the membranes from control and STZ-treated animals. There was no significant change in the band density of either G<sub>so</sub> or G<sub>q/11</sub>α in STZ-treated rats compared with the control rats (Fig. 5, A and B).

**DISCUSSION**

The present study demonstrates that in STZ-induced diabetic rats, a model of type I diabetes, there is reduced renal dopamine D<sub>1</sub> receptor function. The lack of natriuretic response to the D<sub>1</sub> receptor agonist SKF-38393 is most likely due to a decrease in the ability of SKF-38393 to inhibit Na-K-ATPase, which in turn is due to a decrease in dopamine D<sub>1</sub> receptor expression and a defect in the coupling of the D<sub>1</sub> receptor to the G protein. This abnormality in D<sub>1</sub> receptor expression along with defective receptor G protein coupling and function may contribute to sodium retention seen in type I diabetes.

Our study suggests that hyperglycemia may be one of the causes of renal dopamine D<sub>1</sub> receptor dysfunction in type I diabetes. We previously reported that in obese Zucker rats, a model of type II diabetes, the inhibitory effects of dopamine on Na-K-ATPase and Na/H exchanger were significantly reduced (10, 11). The reduced inhibition in obese Zucker rats could have been due to hyperglycemia, hyperinsulinemia, or both. Lowering the blood glucose to normal values and decreasing

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**Table 2. Time course for renal parameters in control rats**

<table>
<thead>
<tr>
<th>Renal Parameter</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>UF, μl/min</td>
<td>2.83±0.31</td>
<td>3.05±0.39</td>
<td>2.69±0.31</td>
<td>2.97±0.49</td>
<td>2.76±0.66</td>
</tr>
<tr>
<td>U&lt;sub&gt;Na&lt;/sub&gt;V, μmol/min</td>
<td>0.16±0.03</td>
<td>0.16±0.04</td>
<td>0.18±0.05</td>
<td>0.17±0.04</td>
<td>0.17±0.03</td>
</tr>
<tr>
<td>FE&lt;sub&gt;Na&lt;/sub&gt;, %</td>
<td>0.21±0.07</td>
<td>0.18±0.07</td>
<td>0.23±0.1</td>
<td>0.21±0.09</td>
<td>0.19±0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 5. UF, urine flow; U<sub>Na</sub>V, urinary sodium excretion; FE<sub>Na</sub>, fractional excretion of sodium; C1–C5, collection periods.
the plasma insulin levels in the obese rats by rosiglitazone treatment restored renal dopamine D₁ receptor expression and function (37). In another follow-up study, the role of insulin was determined. Chronic exposure of proximal tubular cell culture to insulin caused both a reduction in D₁ receptor expression and decreased receptor G protein coupling, indicating that hyperinsulinemia per se was responsible for D₁ receptor dysfunction under these experimental conditions (3). Because the present study involved STZ-induced diabetic rats (type I diabetes) in which there was actually hypoinsulinemia and only blood glucose levels were elevated, this study demonstrates that in the setting of type I diabetes, hyperglycemia can also be responsible for causing renal dopamine receptor dysfunction.

Because inhibition of Na-K-ATPase resulting from activation of D₁ receptor on proximal tubules is responsible for a natriuretic response to D₁ receptor agonists, the absence of a natriuretic response to D₁ receptor activation in STZ-treated rats is most likely due to a decrease in the SKF-38393-mediated inhibition of Na-K-ATPase compared with the control animals. When we examined the expression and basal activity of the Na-K-ATPase, we found increased expression of the Na-K-ATPase that might have contributed to the observed

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**Fig. 3.** A: representative saturation curve for the specific [³H]SCH-23390 binding in proximal tubular membranes of STZ-treated (○) and control (○) rats. The total number of D₁ receptors on the proximal tubular membrane as determined by binding of [³H]SCH-23390, a D₁ receptor antagonist, to the proximal tubular membrane. Unlabeled SCH-23390 (10 μmol/l) was used for determining nonspecific binding. Specific binding was calculated as the difference between total binding and nonspecific binding. B: maximum number of binding sites (Bₘₐₓ) values. C: dissociation constant (Kₛ) in STZ-treated and control rats. Bars and lines represent means and SE, respectively, n = 3; *P < 0.05 compared with control rats using Student’s unpaired t-test. D: Western blot analysis of D₁A receptor in the proximal tubular membrane of STZ-treated and control rats. Left: representative of Western blots, a single band at ~55 kDa was observed. Right: densitometric value bars and lines represent means and SE, respectively, n = 3; *P < 0.05 compared with proximal tubular membranes of control rats using Student’s paired t-test.

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**Fig. 4.** Effect of SKF-38393 on [³⁵S]GTP₆S binding in renal proximal tubular membranes from control (○) and STZ-treated (●) rats. Experiments were performed in triplicate; proximal tubular membranes from both groups were incubated with [³⁵S]GTP₆S, unlabeled GTP₆S (for nonspecific) and SKF-38393 (10⁻⁸-10⁻⁶ mol/l) at 30°C for 60 min. Symbols and lines represent means and SE, respectively, n = 6; *P < 0.001 from control basal within the same group using ANOVA and Newman-Keuls test. #P < 0.05 from control rats using Student’s unpaired t-test. Values are expressed as percent stimulation compared with the basal binding. Basal GTP₆S binding in STZ-treated group is 0.759 ± 0.167 pmol/mg protein and 0.625 ± 0.132 pmol/mg protein for the control group.
increase in the basal activity of the enzyme in STZ-induced diabetic rats. Our results are in agreement with several earlier reports showing increased expression and basal activity of Na-K-ATPase in the STZ-induced diabetic kidney (16–18, 38). The decreased basal sodium excretion in STZ-induced diabetic rats is in parallel with the increased basal Na-K-ATPase activity, suggesting a state of sodium retention in these animals. In the normal state, sodium retention leads to an increase in renal dopamine tonus, and the natriuretic effects of dopamine are more prominent under this condition (2). However, in pathophysiological conditions like hypertension and diabetes, which are associated with increased sodium retention, overactivity of antinatriuretic hormones and underactivity of natriuretic hormones have been described (2).

To our knowledge, this is the first study to report a reduced natriuretic response to dopamine D1 receptor activation in STZ-induced diabetic rats. Several groups have reported a decrease in the endogenous production of dopamine in the type I diabetic kidney (5, 19, 36). Our study demonstrates that in addition to a reduction in endogenously produced dopamine, there also exists a reduction in the responsiveness to exogenously administered D1 receptor agonist in these animals. Moreover, intrarenal dopamine can act in conjunction with other natriuretic hormones and can oppose the effects of antinatriuretic hormones (1, 2). Natriuretic responses to atrial natriuretic peptide are reduced in STZ-induced diabetic rats (25). It is reported that the natriuretic response to atrial natriuretic factor requires an intact renal dopamine system (8), suggesting that failure to observe natriuresis during atrial natriuretic factor administration in STZ-induced diabetic rats could be due, in part, to a defect in renal D1 receptor function. Dopamine opposes the effects of antinatriuretic hormones including ANG II (39). Interestingly, the renin-angiotensin system is activated in type I diabetes (22); also, renal cortical AT1 receptor protein and circulating ANG II levels are increased (40). Therefore, it is likely that increased AT1 receptor function in diabetes is partly due to a decreased opposing influence of dopamine.

A decrease in D1 receptor expression on the proximal tubular membrane is a likely cause of reduced inhibition of Na-K-ATPase and the reduced natriuretic response to SKF-38393, as we observed that the Bmax for the D1 receptor was significantly reduced in STZ-induced diabetic rats. There was also a defective D1 receptor G protein coupling, in the proximal tubular membranes of STZ-induced diabetic rats. Earlier we reported that in a model of type II diabetes, there is a 50% reduction in D1 receptor number and decreased coupling of G proteins with D1 receptor (11).

Several groups reported alterations in G proteins in different tissues in type I diabetes, and a decrease in Gsα has been reported in gastrointestinal smooth muscles, adipocytes, and retina (24); decreased levels of Gq/11α subunit in gastric smooth muscles cells from spontaneous diabetic WBN/Kob (WBN/Kob) rats have also been reported (20). In our study, the possibility of a reduced renal Gsα and Gq/11α protein pool contributing to the observed decrease in receptor G protein coupling was eliminated because the Western blot analysis of these proteins showed no change in the band density in diabetic rats compared with the control. Our study demonstrates that a decrease in D1 receptor expression and defective receptor G protein coupling accounts for failure of SKF-38393 to inhibit Na-K-ATPase, thus resulting in reduced natriuretic response.

Decreased expression and function of dopamine receptors in type I diabetes are not unique to the kidney. There seems to be considerable evidence linking reduced expression and function of dopamine receptor with abnormal insulin and glucose levels even in the central nervous system. Several investigators reported decreased D1 receptors in brains of STZ- or alloxan-induced diabetic rats (30, 31). Moreover, many of the central dopaminergic functions such as dopamine-mediated nociceptive response are attenuated in type I diabetes and insulin treatment normalizes this response (29). In addition, hyperglycemia has been reported to suppress the firing of central dopaminergic neurons (32) and animal studies indicate that chronic hyperglycemia decreases striatal dopaminergic transmission (33). Therefore, hyperglycemia and hypoinsulinemia have been reported to alter central dopamine expression and function. The results of our study extend these findings to the kidney and demonstrate that SKF-38393 fails to promote sodium excretion, as a result of reduced D1 receptor expression and decreased receptor G protein coupling in this animal model of diabetes. Inasmuch as endogenous kidney dopamine plays an important role in maintaining sodium homeostasis during increases in sodium intake, such an abnormality in renal D1 receptor function could account for sodium retention seen in type I diabetes. Further studies are needed to fully elucidate the role of hyperglycemia per se in renal dopamine D1 receptor function and to determine whether correcting this abnormality would lead to restoration of the renal D1 receptor G protein coupling and function.
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