Renal dopamine D$_1$ receptor dysfunction is acquired and not inherited in obese Zucker rats

Anees Ahmad Banday, Tahir Hussain, and Mustafa F. Lokhandwala

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

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Banday, Anees Ahmad, Tahir Hussain, and Mustafa F. Lokhandwala. Renal dopamine D$_1$ receptor dysfunction is acquired and not inherited in obese Zucker rats. Am J Physiol Renal Physiol 287: F109–F116, 2004. First published April 6, 2004; 10.1152/ajprenal.00396.2003.—In essential hypertension, the defect in renal dopamine (DA) D$_1$ receptor function is intrinsic to proximal tubules as this phenomenon is also seen in primary proximal tubule cultures from spontaneously hypertensive rats (SHR) and essential hypertensive patients. Previously, a defect was reported in renal D$_1$ receptor function in obese Zucker rats. In the present study, we sought to determine whether this D$_1$ receptor dysfunction is intrinsic in these animals. In primary proximal tubular epithelial cells (PTECs) from lean and obese rats, DA inhibited Na-K-ATPase (NKA) activity in PTECs from both groups of rats. Basal NKA activity, D$_1$ receptor protein expression, and their coupling to G proteins were similar in cells from both groups. However, when PTECs from lean and obese rats were cultured in 20% serum from obese rats, DA failed to inhibit NKA activity, which was accompanied by a reduction in D$_1$ receptor expression and a defect in D$_1$ receptor-G protein coupling. No such defects in the inhibitory effect of DA on NKA activity, D$_1$ receptor numbers, or coupling were seen when PTECs from both lean and obese rats were grown in 20% serum from lean or rosiglitazone-treated obese (RTO) rats. RTO rat serum had normal blood glucose and reduced plasma levels of insulin compared with serum from obese rats. Furthermore, chronic insulin treatment of PTECs from lean and obese rats caused an attenuation in DA-induced NKA inhibition, a decrease in D$_1$ receptor expression, and D$_1$ receptor-G protein uncoupling. These results suggest that defective D$_1$ receptor function in obese Zucker rats is not inherited but contributed to by hyperinsulinemia and/or other circulating factors associated with obesity.

G proteins; insulin; Na-K-ATPase; obesity; rosiglitazone

The escalating global epidemic of overweight and obesity predicts a tremendous increase in type 2 diabetes and hypertension. Data from the Framingham Heart Study suggest that ~78% of essential hypertension in men and 65% in women can be directly attributable to obesity (17). However, the mechanism that links obesity with high blood pressure has not been fully elucidated.

Dopamine (DA) produced by the kidney is an intrarenal regulator of sodium transport and plays an important role in maintaining sodium homeostasis during an increase in sodium intake (20, 34). In conscious, as well as in anesthetized animals, up to 60% of sodium excretion during an acute volume load is mediated by DA (20, 26). This natriuretic action of DA results from the activation of D$_1$ receptors present on both basal and apical sides of proximal tubules. The D$_1$ receptors, through coupling with Gs and Gq proteins, activate adenyl cyclase and phospholipase C. The activation of the D$_1$ receptor and subsequent stimulation of effector enzymes (adenyl cyclase and phospholipase C) inhibit the activity of the Na/H exchanger (NHE3) and Na-K-ATPase (NKA) in proximal tubules (10, 12, 13, 48).

Hypertension per se, or in association with obesity, is a major health problem in many Western countries and an increasing one within other countries (3, 18, 19). Hypertension is a complex syndrome that has multifactorial origins. For example, essential hypertension has a heritability as high as 30–50%, but its genetic cause(s) is yet to be determined. Felder et al. (14) reported that in human essential hypertension a single nucleotide polymorphism of GRK4 results in an increase in the activity of the kinase, which leads to D$_1$-receptor phosphorylation and its uncoupling from G proteins in renal proximal tubules. The constitutive activity of GRK4 was retained when these cells were cultured. Unlike essential hypertension, which has a genetic origin, the obese Zucker rat is a genetic model of obesity (7, 11) that also exhibits a moderate hypertension. Previously, we have reported that DA failed to inhibit NKA and NHE3 activity in proximal tubules from obese Zucker rats compared with their lean littermates (22, 23, 25). A decrease in receptor number and defective coupling of the receptor and the G protein/effector enzyme complex could be the probable cause for this impaired renal D$_1$ receptor function (23, 47). We found that treatment of obese Zucker rats with rosiglitazone lowered plasma insulin and glucose levels and also restored D$_1$ receptor function (47). Therefore, it appears that elevated levels of circulating factors, which include but are not limited to insulin, glucose, triglycerides (TGs), and free fatty acids, may have contributed to defective DA receptor function (44, 47). In a recent study, we have shown that chronic exposure of proximal tubular epithelial cells (PTECs) from Sprague-Dawley (SD) rats to insulin blunted the ability of SKF-38393, a D$_1$-receptor agonist, to inhibit NKA activity. A decrease in D$_1$ receptor number and defective receptor-G protein coupling led to the failure of SKF-38393 to inhibit NKA activity in insulin-exposed PTECs (2).

It has been reported that renal proximal tubular cells from essential hypertensive patients retain the D$_1$ receptor defect for seven to eight culture passages, indicating that D$_1$ receptor dysfunction in these patients is of genetic origin (14, 38). In view of these findings, we undertook the present study to determine whether defective renal proximal tubular D$_1$ receptor function in obese Zucker rats has a genetic origin or is contributed by circulating factors.

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We examined D₁ receptor function in lean and obese Zucker rat primary proximal tubule cells 1) grown under similar culture conditions, 2) grown in serum from lean, obese, and rosiglitazone-treated obese (RTO) Zucker rats, and 3) grown under similar culture conditions followed by chronic insulin exposure.

METHODS

Materials. Cell culture media were purchased from GIBCO BRL. R(+)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride (SKF-38393 hydrochloride), a D₁ receptor agonist and active enantiomer of (±)-SKF-38393, was purchased from Sigma (RBI). ⁸⁶RbCl, R(+)-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol hydrochloride ([¹⁸]H)SCH-23390 hydrochloride), a D₁ receptor antagonist, and ³⁵S-labeled guanosine 5'-(γ-thio)triphasphate ([³⁵]S)-GTPyS were purchased from New England Nuclear Life Sciences. Antibodies were purchased from Alpha Diagnostic and Calbiochem-Novabiochem. All other chemicals of the highest purity available were purchased from Sigma.

Rosiglitazone treatment. Nine-week-old male obese and lean Zucker rats (Harlan, Indianapolis, IN) were housed in plastic cages with free access to normal rodent chow and tap water. Obese rats were divided into two groups: one was treated with rosiglitazone (n = 10), and other treated with vehicle (1% aqueous carboxymethyl cellulose, n = 10) served as the control. The rats were dosed (3 mg·kg⁻¹·day⁻¹) daily via oral gavage for 2 wk. Lean Zucker rats received no treatment. At the end of the treatment, blood was collected from the aorta in EDTA-coated tubes for plasma insulin measurement and in glass beakers for serum analysis. Blood glucose and triglycerides (TG) were measured with a glucose analyzer (Roche Diagnostic) and TG analyzer (Polymer Technology Systems), and insulin was measured by a RIA commercial kit (Linco Research).

Cell culture. The proximal tubule fragments were purified as previously described (2, 37). The isolated proximal tubules were resuspended in complete media containing DMEM-F-12 (1:1) supplemented with 0.573 ng/ml insulin, 5 μg/ml transferrin, 40 ng/ml hydrocortisone, 5 ng/ml selenium, 4 μg/ml 3,3,5-triiodo- L -thyronine, 10 ng/ml EGF, 1.2 mg/ml sodium bicarbonate, 0.29 mg/l 1-glutamine, 25 IU/ml penicillin, and 25 μg/ml streptomycin. Lean and obese Zucker rat renal proximal tubule cells grown under similar or different culture conditions were used to investigate D₁ receptor function. The experimental groups comprised lean and obese Zucker rat PTECs cultured in complete media and 10% FCS (group 1) and lean and obese Zucker rat PTECs grown in complete media and supplemented with 20% rat serum from lean, obese, or RTO Zucker rats (group 2).

Insulin or fatty acid treatment. At ~85–90% confluence (passage 1), lean and obese Zucker rat PTECs (group 1) cultured in complete media and 10% FCS were serum starved and incubated with insulin (50–100 nM in DMEM-F-12) or without insulin (control, DMEM–F-12 only) for 24 h. In a separate set of experiments, cells were incubated with palmitic acid, steric acid, and oleic acid (50/100 M) for 24 h. Cells starved in DMEM–F-12 served as the control. Cells from group 2 were studied without any exposure to insulin or fatty acids. The culture conditions are conducive to the growth of renal proximal tubules that retain characteristics of renal proximal tubule cells (2).

Na-K-ATPase assay. At ~80–85% confluence, lean and obese Zucker rat cells cultured in 10% FCS (group 1) or lean and obese Zucker rat cells cultured in 20% rat serum (group 2) were serum starved for 24 h in DMEM-F-12 media. Cells were incubated without or with DA for 10 min at 37°C. NKA activity was measured by ⁸⁶Rb⁺ uptake, as described in detail earlier (2). NKA activity was determined as the difference between ⁸⁶Rb⁺ uptake in the absence (total uptake) or presence (ouabain-insensitive) of ouabain (1 mM). Ouabain inhibited ~70% of total activity. Insulin- and fatty acid-treated cells were washed with PBS and stabilized in DMEM-Ham’s F-12 for 2–3 h to eliminate the acute effect of insulin on NKA activity (2). Cells were incubated with or without DA for 10 min at 37°C, and NKA activity was determined as mentioned above.

[¹⁸]H]SCH-23390 binding. The cells were scraped and suspended in sucrose buffer (in mM: 10 Tris·HCl and 50 sucrose, pH 7.6). The cells were homogenized and processed for membrane preparation as reported earlier (2). Fifty micrograms of membrane protein were incubated with 4 nM [¹⁸]H]SCH-23390, a D₁-receptor antagonist, in 25 μl (final volume) of binding buffer for 120 min at 25°C. Nonspecific binding was determined in the presence of 1 μM cold SCH-23390.

Western blotting of D₁ receptor proteins. Membrane proteins were resolved by SDS-PAGE and transblotted as described earlier (2). The specific antibodies (Abs) were used to detect D₁ receptor proteins. Horseradish peroxidase-conjugated secondary Abs were used to probe the primary Abs, and bands were visualized with a chemiluminescence reagent kit and recorded on X-ray film for densitometry.

[³⁵]S]GTPyS binding. A GTP binding assay was performed as described in our previous publications (2, 24). The reaction mixture of 90 μl (final volume) contained (in mM) 25 HEPES, 15 MgCl₂, 1 dithiothreitol, and 100 NaCl (pH 8.0) as well as 5 μg protein and ~100,000 counts/min of [³⁵]S]GTPyS with or without D₁-receptor agonist SKF-38393. Nonspecific binding of [³⁵]S]GTPyS, determined in the presence of 100 μM cold GTPyS, was always ~<2% of total binding.

Statistical analysis. The difference between means was evaluated using an unpaired t-test or analysis of variance with the Newman-Keuls multiple test, as appropriate. P < 0.05 was considered statistically significant.

RESULTS

As shown in Table 1, the body weights of 11- to 12-wk-old obese and RTO rats were significantly higher than those of lean rats. Plasma insulin levels in obese rats were about eight times higher than in lean rats, whereas plasma glucose was ~50% higher in obese rats than in lean rats. These data confirm that obese Zucker rats are hyperinsulinemic with moderate hyperglycemia. Also, the blood TG levels in obese rats were 10 times higher than in lean rats, suggesting defective lipid metabolism, a hallmark of obesity and insulin resistance. Rosiglitazone completely normalized the plasma glucose levels, as there was no significant difference in plasma glucose levels in lean or RTO rats. Furthermore, treatment of obese rats with rosiglitazone reduced plasma insulin and TG levels by 73 and 72%, respectively (Table 1).

Characterization of D₁ receptor function in PTECs from lean and obese Zucker rats. At ~80–85% confluence, lean and obese Zucker rat PTECs cultured in 10% FCS were serum starved for 24 h and studied for D₁ receptor expression and function.

Table 1. General and biochemical parameters of lean, obese, and rosiglitazone-treated obese Zucker rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean (g)</th>
<th>Obese (g)</th>
<th>RTO (g)</th>
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<tr>
<td>Body weight (g)</td>
<td>265.0 ± 7.0</td>
<td>475.0 ± 14.0*</td>
<td>488.0 ± 18.0*</td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>5.5 ± 0.3</td>
<td>8.3 ± 1.8*</td>
<td>5.8 ± 0.28*</td>
</tr>
<tr>
<td>Insulin (nmol/l)</td>
<td>0.62 ± 0.1</td>
<td>4.92 ± 0.4*</td>
<td>1.3 ± 0.20†</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>≤50.0</td>
<td>520.0 ± 6.0*</td>
<td>145.0 ± 30.0*</td>
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</table>

Values are means ± SE. RTO, rosiglitazone-treated obese mice. Triglyceride (TG) analyzer (Polymer Technology Systems) can analyze a sample with TG levels ranging from ~50 to ~500 mg/dL. Serum from obese rats was diluted for TG estimation. *Significantly different compared with lean values; †significantly different compared with obese values (1-way ANOVA-Newman-Keuls; n ≥ 8 animals/group, P < 0.05).
As shown in Fig. 1A, the specific binding of the D₁-receptor antagonist [³H]SCH-23390 was similar in lean and obese PTEC membranes, suggesting an equal number of D₁-like receptors on the cell membrane. D₁A receptor protein was measured by Western blotting using D₁A receptor-specific antibody. This antibody labeled a D₁A-specific band with a molecular mass of ~50 kDa (Fig. 1C). Similar to ligand binding, densitometric analysis of bands revealed that basal expression of the D₁A receptor is similar in PTECs from lean and obese Zucker rats (Fig. 1B).

The functional responsiveness of the D₁ receptor was performed by measuring D₁ receptor ligand-induced stimulation of G proteins and DA-induced inhibition of NKA activity. Incubation of membranes with 10⁻⁶M SKF-38393, a D₁-receptor agonist, elicited an equal stimulation of [³⁵S]GTPγS binding, suggesting intact receptor-G protein coupling (Fig. 1D). Furthermore, DA (10 nM-1 μM) produced a concentration-dependent inhibition of NKA activity in both groups of PTECs (Fig. 2). These results along with light microscopy (data not shown) suggest that D₁ receptors are functional in both lean and obese PTECs and also confirm that culture conditions are conducive to normal cell growth (2).

Effect of DA on NKA activity in PTECs cultured in rat serum. Because we did not observe any morphological (light microscopy; data not shown) or D₁ receptor functional difference in lean or obese Zucker rat PTECs cultured in 10% FCS, further experiments were performed to study the effect of serum from obese rats on D₁ receptor function in PTECs from lean and obese Zucker rats. PTECs (from the same animal) cultured in 20% serum from lean or RTO rats served as the control. The three cultures from the same animal grown in different sera were studied in parallel. As shown in Table 2, DA (1 μM) significantly inhibited the activity of NKA in obese rat PTECs cultured in serum from lean and RTO rats. However, DA-induced inhibition of NKA activity was completely

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**Fig. 1.** A: [³H]SCH-23390-specific binding in lean and obese proximal tubule epithelial cell (PTEC) membranes. B: bar graph (means ± SE) of densitometric values from 5 experiments (animals). C: dopamine D₁A receptor Western blot analysis for renal proximal tubular homogenate. D: effect of SKF-38393 on [³⁵S]-labeled guanosine 5’-(γ-thio)-triphosphate ([³⁵S]GTPγS) binding from lean and obese rat PTEC membranes. *Significantly different from control (1-way ANOVA-Newman-Keuls, P < 0.05).

**Fig. 2.** Effect of dopamine (DA) on Na-K-ATPase (NKA) activity in PTECs from lean and obese Zucker rats. Values are means ± SE of 5 experiments (animals) performed in triplicate. *Significantly different from control (1-way ANOVA-Newman-Keuls, P < 0.05).
absent in PTECs cultured in serum from obese Zucker rats. When PTECs from lean rats were cultured in 20% serum from lean or RTO animals, DA (1 μM) caused a 28% inhibition in NKA activity (lean rat serum, control vs. DA: 14.0 ± 0.8 vs. 10.0 ± 0.3; RTO rat serum, control vs. DA: 13.5 ± 0.8 vs. 10.5 ± 0.5 nmol 86Rb-mg protein⁻¹ h⁻¹, P < 0.05 control vs. DA). However, DA (1 μM)-induced inhibition of NKA was attenuated when these PTECs were grown in serum from obese rats (control vs. DA: 13.03 ± 0.5 vs. 11.8 ± 0.4 nmol 86Rb-mg protein⁻¹ h⁻¹).

Effect of rat serum on D₁ receptor expression and G protein coupling in PTEC membranes. Obese rat cells cultured in obese rat serum showed a 43% decrease in [³H]SCH-23390 binding compared with cells grown in serum from lean or RTO rats. There was no difference in [³H]SCH-23390 membrane binding between cells cultured in lean or RTO rat serum (Table 2). Similar to ligand binding, densitometric analysis of Western blotting revealed a 50% decrease in membrane receptor protein abundance in cells cultured in obese rat serum compared with lean or RTO rat serum (Table 2). A similar decrease in D₁ receptor number was observed when cells were harvested from lean rats and cultured in obese rat serum ([³H]SCH-23390 binding, lean rat serum: 89.0 ± 7.0, obese rat serum: 46.0 ± 3.0 and RTO rat serum: 87.0 ± 5.0 fmol/mg protein; Western blot densitometry, lean rat serum: 99.4 ± 2.0, obese rat serum: 65 ± 3 and RTO rat serum: 95.6 ± 3.9 arbitrary units, P < 0.05 obese rat serum vs. lean or RTO rat serum).

As shown in Table 2, SKF-38393 elicited equal stimulation of [³⁵S]GTPγS binding in obese rat PTEC membranes cultured in lean or RTO rat serum. Similar stimulation of [³⁵S]GTPγS binding was observed when cells harvested from lean rats were cultured in lean (control vs. SKF-38393: 102.7 ± 5.0 vs. 129.4 ± 7.0, P < 0.05) or RTO rat serum (control vs. SKF-38393: 98.9 ± 5.0 vs. 121.60 ± 6.0 fmol/mg protein, P < 0.05). However, SKF-38393 was unable to stimulate [³⁵S]GTPγS binding in PTEC membranes from obese rats (Table 2) as well as those from lean rats (control vs. SKF-38393: 95.8 ± 3.0 vs. 101.0 ± 6.0 fmol/mg protein) cultured in obese rat serum.

In separate experiments, serum from obese rats was also able to attenuate D₁ receptor function in PTECs harvested from RTO rats and SD rats (results not shown).

Effect of insulin on the effect of DA on NKA activity in PTECs from lean and obese Zucker rats. The basal characterization of lean and obese PTECs revealed no difference in their morphological and D₁ receptor functional parameters. Furthermore, serum from obese Zucker rats diminished D₁ receptor function to a similar extent in PTECs from both lean and obese rats. Because obese Zucker rats are associated with hyperinsulinemia and mild hyperglycemia, we sought to determine the effect of chronic insulin (100 nM) exposure in PTECs from lean and obese rats on D₁ receptor function. As shown in Table 3, DA (1 μM) caused significant inhibition of NKA activity in control cells, but not in insulin-pretreated cells from lean and obese rats. It should be noted that the NKA activity in this protocol was measured after 3 h of stabilization of cells in insulin-free medium, and we observed no difference in basal NKA activity between control and insulin-treated cells. Also, basal NKA activity in control or insulin-treated cells is similar to basal activity observed (see Fig. 2).

Effect of insulin on D₁ receptor expression and G protein coupling in PTEC membranes from lean and obese Zucker rats. The specific binding of [³H]SCH-23390, a D₁ receptor ligand, was reduced by 42% in membranes from 100 nM

Table 3. Effect of insulin on [³H]SCH-23390 specific binding, D₁A receptor protein abundance, [³⁵S]GTPγS binding, and Na-K-ATPase activity in PTECs

<table>
<thead>
<tr>
<th>PTECs from Lean Rats</th>
<th>PTECs from Obese Rats</th>
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<tbody>
<tr>
<td></td>
<td>DMEM</td>
</tr>
<tr>
<td>Na-K-ATPase, nmol 86Rb-mg protein⁻¹·min⁻¹</td>
<td>Control</td>
</tr>
<tr>
<td>[³H]SCH-23390, fmol/mg protein</td>
<td>102.0 ± 5.0</td>
</tr>
<tr>
<td>Western blot densitometry, arbitrary units</td>
<td>100.7 ± 2.3</td>
</tr>
<tr>
<td>[³⁵S]GTPγS bound, fmol/mg protein</td>
<td>118.0 ± 7.3</td>
</tr>
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</table>

Values are means ± SE of 5 experiments (animals) performed in triplicate. PTECs were incubated with 100 nM insulin-DMEM for 24 h. Cells incubated with DMEM alone served as the control. *Significantly different from DMEM; †significantly different from respective control (1-way ANOVA-Newman-Keuls, P < 0.05).
D1 receptor function in obese Zucker rats

Our results show that impaired D1 receptor function in proximal tubules of obese Zucker rats is caused by circulating factors. We found that PTECs from both lean and obese Zucker rats expressed functional D1 receptors. Further experiments revealed that culturing of PTECs from lean or obese animals in obese rat serum blunted the DA-induced inhibition of NKA, whereas cells cultured in lean or rosiglitazone-treated rat serum exhibited no defect in D1 receptor function. Furthermore, consistent with our recent report in PTECs from SD rats, the present findings demonstrate that chronic insulin exposure of PTECs from lean or obese rats attenuated DA-induced inhibition of NKA.

Obese Zucker rats (fa/fa) are homozygous for a mutation in the leptin receptor gene and develop pronounced hyperinsulinemia and severe obesity with relatively mild hyperglycemia (7, 11, 35, 45). In addition to secondary endocrine abnormalities, these rats also exhibit mild hypertension (1, 8). The mechanisms responsible for altered renal function and hypertension in obese Zucker rats are not clear. However, as has been reported with other forms of hypertension, the increased blood

Fig. 3. Effect of DA on NKA activity in control and chronically insulin-treated PTECs from Sprague-Dawley rats. Values are means ± SE of 3 experiments (animals) performed in triplicate. *Significantly different from control (1-way ANOVA-Newman-Keuls, P < 0.05).
pressure in obese Zucker rats is accompanied by impaired pressure-natriuresis (1, 16). In these animals, impaired pressure-natriuresis is mainly due to increased renal sodium reabsorption because the glomerular filtration rate and renal blood flow are increased by \(-50\%\) (1). The increased sodium reabsorption may be attributable, at least in part, to reduced DA-induced inhibition of sodium transporters and, subsequently, decreased sodium excretion (22, 23, 25).

Various authors have also reported a close relationship between sodium retention and hypertension in human essential hypertension and SHR (14, 50) and suggested that the failure in D1 receptor-mediated inhibition of sodium transporters in renal proximal tubules leads to a diminished natriuretic response to endogenously produced or exogenously administered DA (9, 15, 24). These defects in D1 receptor function were reproduced in cultured proximal tubules from essential hypertensive patients and SHR for several passages (14, 38, 50). To test whether this phenomenon is observed in obese Zucker rats, the present studies were conducted in PTECs from lean and obese animals. Our results show that DA caused a concentration-dependent inhibition of NKA in PTECs from lean and obese Zucker rats. Basal NKA activity was also similar in both groups of PTECs. Furthermore, there was no change in D1 receptor number, receptor protein abundance, or ligand-induced stimulation of G proteins in PTECs from lean compared with obese rats. These results show that renal proximal tubule cells from obese Zucker rats with impaired D1 receptor function do not retain the specific D1 receptor defects once are they are cultured. Also, the PTECs from obese rats show similar D1 receptor expression and function as observed in PTECs from their lean Zucker rat littermates. The possible confounding effect of dedifferentiation is unlikely because Sanada et al. (38) have shown the preservation of the response to forskolin and parathyroid hormone-related peptide in human proximal tubule cells studied after several passages. Also, there are other reports suggesting that the genetic defect is retained under in vitro culture conditions (50). Felder et al. (14) reported the hyperphosphorylation of the D1 receptor due to a ligand-independent increase in GRK activity in proximal tubular cell cultures obtained from patients with essential hypertension. In our study, the D1 receptor-G protein uncoupling observed in proximal tubules from obese Zucker rats was not present when we prepared primary cultures of these tubules. This suggests that the D1 receptor defect observed in proximal tubules of obese Zucker rats is probably induced by either paracrine factors or circulating factors associated with obesity. Our results support the latter notion because we found that culturing of PTECs from obese or lean rats in serum from obese rats blunted the DA-induced NKA inhibition, reduced D1 receptor expression, and caused D1 receptor-G protein uncoupling. When PTECs from both groups were cultured in serum from lean or rosiglitazone-treated rats, there was no difference in D1 receptor number and in the ability of DA to inhibit NKA activity. We have earlier reported that DA failed to inhibit NKA and NHE3 activity in proximal tubules of obese Zucker rats compared with their lean Zucker rat littermates (22, 23, 47). The inability of DA to inhibit NKA activity may have resulted from decreased D1 receptor number and D1 receptor-G protein uncoupling (22, 23, 47). Treatment of obese Zucker rats with rosiglitazone normalized blood glucose and caused a significant decrease in plasma insulin, TG, and free fatty acid levels (29, 33, 47). Rosiglitazone, while reducing the levels of these elevated factors, also effectively restored renal responsiveness to DA and normalized membrane D1 receptors to the levels seen in lean rats (47).

Our results in obese Zucker rats demonstrate that the D1 receptor defect in these animals is not intrinsic to renal proximal tubules, as is the case with human essential hypertension. A plausible explanation for these differences could be that obese Zucker rats mimic human syndrome X, often exhibiting hyperglycemia, hyperphagia, hyperlipidemia, hyperinsulinemia, insulin resistance, and hypertension (30). Multiple mechanisms have been proposed to explain the relationship between obesity and hypertension, including increased sympathetic activity, increased activity of the renin-angiotensin-aldosterone system, increased cardiac output, increased mechanical pressure from intestinal fat around organs, hyperinsulinemia, insulin resistance, changes in vascular reactivity, activated function of voltage-dependent Ca\(^{2+}\) channels in vascular smooth muscle, impaired endothelial function, and/or altered pressure-natriuresis (8, 16, 32, 49, 51). It is possible that some of these factors are altering the D1 receptor function in obese rats and thus contributing to sodium retention and hypertension. It should be noted that the blood pressure difference between hypertensive and normotensive controls has been attributed to two to six genetic loci (21, 31, 52). In human essential hypertension, a single nucleotide polymorphism of G protein-coupled receptor kinase GRK4\(\gamma\) increases GRK activity and causes the serine phosphorylation and uncoupling of the D1 receptor from its G protein/effect enzyme in renal proximal tubules. Moreover, expressing GRK4\(\gamma\) gene in transgenic mice produces hypertension and reduces the diuretic and natriuretic effects of a D1-like agonist (14).

The mechanism underlying the development of hypertension in type 2 diabetes has begun to be elucidated through the use of several animal models including obese Zucker rats. A growing body of evidence has been accumulated demonstrating that insulin resistance is an important risk factor in the genesis of hypertension. It is obviously difficult to single out unequivocally which of the factors is responsible for hypertension as well as the D1 receptor impairment observed in ex vivo studies or in vivo obese models. However, there are reports suggesting a negative correlation between renal D1 receptor function and plasma insulin levels (39, 40). Recently, we were able to show that chronic insulin exposure of PTECs from SD rats causes a reduction in D1 receptor number and receptor-G protein uncoupling with the subsequent failure of SKF-38393, a D1-like agonist, to inhibit NKA activity (2). In the present studies, we also found that chronic insulin exposure of PTECs from lean and obese Zucker rats blunts the DA-induced NKA inhibition. This inability of DA to inhibit NKA may be attributable to reduced receptor number and receptor-G protein uncoupling. These studies suggest that hyperinsulinemia, commonly associated with obesity, could be an important contributing factor for D1 receptor dysfunction and subsequent hypertension. Furthermore, in support of our findings in obese Zucker rats, it has been shown that in type 2 diabetic patients the infusion of low doses of dopamine elicited a suppressed natriuretic response compared with normal volunteers and that this reduced natriuretic response was further exaggerated when patients were pretreated with insulin (39, 40). These studies suggest that impairment of the renal dopaminergic system and
subsequent blunted response to dopamine in type 2 diabetic patients may be due to hyperinsulinemia.

Insulin may play a role in the development of hypertension associated with syndrome X, or risk factor clustering, a common age-related syndrome that is expressed as hyperinsulinemia and lipid abnormalities (28, 41). Insulin is one the hormones that positively regulate NKA and epithelial Na+ channel (ENaC) activity. Although the role of NKA in the development of hypertension is still under debate, as of now ENaC is the only sodium transport protein for which genetic evidence exists for involvement in the genesis of both hypertension (Liddle’s syndrome) and hyponatremia (pseudohyponatremia type 1) (6, 27, 41–43, 46). The regulation of ENaC involves a variety of hormonal signals including insulin, but the molecular mechanisms behind this regulation are mostly unknown. Recently, Blazer-Yost and others (5, 36) reported that insulin-induced trafficking of ENaC in renal cells is mediated by phosphorylidyinositol 3-kinase. Despite the elevated levels of circulating insulin and increased abundance of β-ENaC in obese Zucker rats, the role of ENaC in the genesis of hypertension in these animals is yet to be determined (4).

Both obese rat serum and insulin (>1 nM) led to the complete loss of DA-induced NKA inhibition. We speculate that in addition to reduction in D1 receptor number, insulin or obese rat serum may also alter the cell signaling downstream of the D1 receptor. This may be explained, in part, by the absence of G protein stimulation in PTECs cultured in obese rat serum or treated with insulin. Thus the reduction in receptor number and D1 receptor-G protein uncoupling may act synergistically to blunt D1 receptor function in obese Zucker rats.

In conclusion, our results demonstrate that D1 receptor dysfunction in obese Zucker rats is not intrinsic but is induced by elevated plasma levels of circulating factors. The observation that PTECs grown in serum from RTO rats, with reduced levels of insulin and other circulating factors, do not exhibit D1 receptor abnormalities supports the concept that D1 receptor dysfunction in obese Zucker rats is caused by elevated levels of circulating factors. Furthermore, chronic exposure of PTECs to insulin caused decreased D1 receptor expression and receptor-G protein uncoupling with subsequent D1 receptor dysfunction. Thus these results provide substantial evidence that hyperinsulinemia may be a contributing factor for impaired renal D1 receptor function in obese Zucker rats.

GRANTS

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