Defective Dopamine Receptor Function in Proximal Tubules of Obese Zucker Rats

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Abstract—Some of the pathophysiological consequences of obesity include insulin resistance, increased renal sodium reabsorption, and the development of hypertension. Dopamine promotes renal sodium excretion via activation of \(D_1\)-like receptors present on the proximal tubules. Reduced dopamine-induced natriuresis and a defect in \(D_1\)-like receptor function have been reported in the proximal tubules of hypertensive animals. The present study investigated \(D_1\)-like dopamine receptors and associated G proteins as the initial signaling components in the proximal tubular basolateral membranes of obese Zucker and control lean Zucker rats. We found that the obese rats were hyperinsulinemic, hyperglycemic, and hypertensive compared with the lean rats. Dopamine produced concentration-dependent inhibition of Na,K-ATPase activity in the proximal tubules of lean rats, whereas the inhibitory effect of dopamine was reduced in obese rats. The \(D_1\)-like receptors measured by \([3H]SCH 23390 binding revealed an \(\approx 45\%\) decrease in \(B_{max}\) without a change in \(K_d\) in the basolateral membranes of obese rats compared with lean rats. Although we found an increase in \(G_{11\alpha}\) and no change in \(G_{s\alpha}\) in the basolateral membranes of obese rats, dopamine and SKF 38393 failed to stimulate G proteins as measured by \([35S]G\text{TP}_{i}\) binding in obese rats, suggesting a receptor–G protein coupling defect. We conclude that decrease in \(D_1\)-like dopamine receptor binding sites and diminished activation of G proteins, resulting perhaps from defective coupling, led to the reduced inhibition by dopamine of Na,K-ATPase activity in the proximal tubules of obese Zucker rats. Such a defect in renal dopamine receptor function may contribute to sodium retention and development of hypertension in obese rats. (Hypertension. 1999;34:1091-1096.)

Key Words: kidney ■ sodium pump ■ hypertension, obesity ■ insulin resistance ■ obesity

More than half of the US population is considered overweight, and nearly one quarter is clinically obese.\(^1\) Obesity is a known risk factor for chronic diseases such as heart disease, diabetes, and high blood pressure.\(^2\) Efforts have been made to elucidate some of the renal and cardiovascular changes associated with obesity.\(^3\)–\(^5\) It has been reported that alterations in sodium homeostasis lead to increased sodium retention in diabetes.\(^6\)–\(^7\) The increased sodium retention is suggested to play an important role in the development of hypertension in diabetic patients.\(^8\)

The renal dopaminergic system is known to play a significant role in promoting natriuresis and diuresis under various physiological conditions.\(^9\)–\(^10\) In spontaneously hypertensive rats, the natriuretic and diuretic responses to dopamine are reduced in comparison with Wistar-Kyoto rats.\(^11\)–\(^12\) Detailed studies have revealed that a defect exists in the \(D_1\)-like dopamine receptor–cellular signaling mechanisms present on the renal proximal tubules.\(^13\)–\(^14\) A similar defect in \(D_1\)-like dopamine receptor-mediated cellular signaling is reported in the proximal tubules of humans with primary hypertension.\(^15\) In an attempt to demonstrate a correlation between defective dopamine receptor and hypertension, it has been reported that mice lacking functional \(D_{1A}\) receptors (member of the \(D_1\)-like dopamine receptor) developed hypertension.\(^16\) The \(D_1\)-like dopamine receptors, via \(G_s\) and \(G_{q/11}\) proteins, are linked to the stimulation of adenyl cyclase and phospholipase C, which lead to the inhibition of the sodium-transporting proteins Na-H exchanger and Na,K-ATPase in the proximal tubules.\(^17\)–\(^19\) Because of a defect in \(D_1\)-like receptor–G protein coupling in the proximal tubules, dopamine and \(D_1\)-like receptor agonist were unable to stimulate adenyl cyclase and phospholipase C\(^13\)–\(^20\) and therefore failed to inhibit Na-H exchanger and Na,K-ATPase activities in hypertensive animals.\(^21\)–\(^22\)

It is believed that obesity is a primary cause of essential hypertension and type 2 diabetes mellitus or non–insulin-dependent diabetes mellitus.\(^23\) It is also known that obesity increases tubular sodium reabsorption, which is suggested to be closely linked to the enhanced activation of antinatriuretic renin-angiotensin and sympathetic systems.\(^4\)–\(^23\) On the other hand, renal production of dopamine is decreased in patients with type 2 diabetes mellitus.\(^24\) In addition, exogenously infused dopamine produced markedly lower natriuretic response in insulin-treated patients with type 2 diabetes mellitus.\(^25\) On the basis of defective dopamine receptor signaling and reduced natriuretic response to dopamine in hyperten-
sion, we hypothesized that a lower natriuretic response to dopamine administration in type 2 diabetes mellitus may be a consequence of defective/reduced dopamine receptor function, leading to an impairment in the D2-like receptor-mediated cellular signaling mechanisms in the proximal tubules. Therefore, in the present study we used obese Zucker rats to measure D2-like receptor and its associated G proteins, which are involved in the inhibition of Na,K-ATPase in the proximal tubules. Obese Zucker rats share many similarities with obese humans who have insulin resistance and type 2 diabetes mellitus and develop hypertension.26

Methods
Male obese and lean Zucker rats (aged 11 to 12 weeks) were purchased from Harlan Sprague-Dawley, Inc (Indianapolis, Ind). The rats were maintained in plastic cages and housed in the animal care facility, where they were fed normal rodent chow and tap water ad libitum.

General Parameters
For blood pressure measurement, the rats were anesthetized with sodium pentobarbital (50 mg/kg body wt IP). After a midline incision, the aorta was catheterized below the kidney, and blood pressure was measured with a Statham pressure transducer and recorded on a Grass polygraph for 30 minutes. A blood sample was collected from the aorta in EDTA-coated tubes for measurement of glucose and insulin levels. Blood glucose was measured with a glucose analyzer (1500 Sidekick, YSI). Plasma insulin levels were measured with a glucose analyzer (1500 Sidekick, YSI). Plasma insulin was measured by radioimmunoassay with the use of a kit (Linco Research Inc).

Isolation and Enrichment of Proximal Tubules
After blood pressure measurement and blood samples were withdrawn, rats were used for the preparation of renal proximal tubules.19 Protein was measured with the use of a kit (Pierce).

Na,K-ATPase Assay
The proximal tubular suspension (1 mg protein per milliliter) in Krebs’ buffer was incubated without or with dopamine (10 nmol/L to 1 μmol/L) at 37°C for 20 minutes. After incubation, the tubules were permeabilized by rapid freezing in dry ice/acetone and thawing. Na,K-ATPase activity was measured,19 and activity was expressed as nanomoles inorganic phosphate per milligram protein per minute.

Membrane Preparation
Rats were anesthetized with pentobarbital (50 mg/kg IP). After a midline abdominal incision, the kidneys were excised and placed in ice-cold Tris-buffered saline (pH 7.4), and the outer cortex was removed above the corticomedullary junction. Basolateral membranes from the cortices were prepared by the colloidal suspension of silica (Percoll)/sucrose density gradient method.27

Receptor-Ligand Binding
Binding of [3H]SCH 23390 to the basolateral membranes was performed according to the previously described method.28 To generate saturation isotherm, the ligand concentration was varied from 1 to 60 nmol/L. Cold SCH 23390 (10 μmol/L) was used for determining the nonspecific binding.

Western Blot Analysis of Sodium Pump and G Proteins
The basolateral membrane proteins (25 μg protein in Laemmli buffer) were separated by SDS–polyacrylamide gel electrophoresis and then electrophoretically transferred onto immobilon P membrane (blot). The blot was incubated with the antibodies for Gα/11a and Gα and monoclonal antibody for the α-subunit of Na pump, followed by incubation with anti-rabbit or anti-mouse IgG–horse-radish peroxidase conjugate. The signal was detected with the use of chemiluminescent substrate.

The antibodies for G proteins were polyclonal, purified, and antipeptides. The amino acid sequence of the peptides, specific for Gα/11a subunit of Na pump was a gift from Dr Carlos Pedemonte of our department. The antibodies for respective G protein α-subunits (performed by Calbiochem-Novabiochem).

[35S]GTPyS Binding Assay
GTP binding assay was performed as earlier described.29 Briefly, the reaction mixture contained the following (mmol/L): HEPES 25 (pH 8.0), MgCl2 15, EDTA 1, dithiothreitol 1, NaCl 100, plus ~100 000 cpm [35S]GTPyS, 5 μg membrane protein, and the agonists. Bound [35S]GTPyS was separated from free by rapid filtration on Whatman GF/C filters. Nonspecific [35S]GTPyS binding was determined in the presence of 100 μmol/L cold GTPyS. The [35S]GTPyS was expressed as nanomoles of [35S]GTPyS bound per milligram protein.

Data Analysis
The values are presented as mean±SEM and were subjected to ANOVA for concentration response curves, followed by the Fisher protected least significant difference post hoc test and t test for single concentration responses. P<0.05 was considered statistically significant.

Chemicals
[35S]GTPyS (1200 Ci/mmol) and [3H]SCH 23390 (81.4 Ci/mmol) were purchased from DuPont NEN. Dopamine was purchased from Sigma Chemical Co, and SKF 38393 and SCH 23390 were purchased from Research Biochemicals International. Anti-Gα/11a and anti-Gα were purchased from Calbiochem-Novabiochem, and anti-α of Na pump was a gift from Dr Carlos Pedemonte of our department. Other chemicals were from either Sigma or Fisher Scientific Co.

Results
General Parameters
The general parameters are given in Figure 1. The obese Zucker rats (598±20 g) were heavier (by ∼200 g) than the lean rats (393±14 g). The systolic and diastolic blood pressure readings of the obese rats (170±4/148±4 mm Hg) were significantly higher (P<0.05) than those of the lean rats (129±3/114±3 mm Hg). Blood glucose level in the obese rats was 40% higher (P<0.05) and levels of plasma insulin were ∼8 times greater in the obese rats than in the lean rats. In addition, plasma renin activity (nanograms angiotensin I per milliliter per hour) in obese rats (14.14±1.8) was not significantly different than that in lean rats (18.27±1.16).

Effect of Dopamine on Na,K-ATPase Activity in Proximal Tubules
Dopamine (10 nmol/L to 1 μmol/L) inhibited Na,K-ATPase activity in the proximal tubules of the lean rats. The maximal inhibition of ∼30% was produced by 1 μmol/L dopamine (Figure 2, top). The inhibition of Na,K-ATPase activity by dopamine was significantly attenuated in the proximal tubules of obese rats (Figure 2, top). Na,K-ATPase control activity (nanomoles per milligram protein per minute) in lean rats (253±20) was not different from that in obese rats (275±30). This observation is further supported by Western
blot analysis of the Na pump α-subunit (≈95 kDa) in the basolateral membranes (Figure 2, bottom). The density of the α-subunit of Na pump in the lean rats was comparable to that in the obese rats.

Figure 1. Body weight, blood pressure, blood glucose, and plasma levels of insulin in lean and obese Zucker rats. Values represent mean±SE from 6 to 10 rats. *Significantly different from lean rats (P<0.05, Student’s t test).

[3H]SCH 23390 Binding
[3H]SCH 23390 bound in a saturable manner to the basolateral membranes from both the lean and the obese rats. As seen in Figure 3, Scatchard analysis of the data revealed a significant decrease in receptor number (≈45%) in the obese rat membranes (Bmax: 1537±422 fmol per milligram protein in lean rats and 866±96 fmol per milligram protein in obese rats). The dissociation constant (Kd) values in lean (37±11 nmol/L) and obese (35±5 nmol/L) rats were not significantly different.

Effect of Dopamine and SKF 38393 on [35S]GTPγS Binding
As shown in Figure 4, dopamine (0.1 to 100 μmol/L) elicited stimulation of [35S]GTPγS binding in the membranes from both the lean and the obese rats. However, it failed to stimulate binding in obese rats. Similarly, SKF 38393 stimulated [35S]GTPγS binding in the membranes of lean rats but failed to stimulate binding in obese rats. The basal binding of [35S]GTPγS in the membranes of lean (6.06±0.3 pmol/mg protein) and obese (6.44±0.34 pmol/mg protein) rats was not different.

Antibody for Gq/11α recognized a single band (42 kDa), whereas antibody for Gsα recognized 2 bands (45 and 48 kDa) in the basolateral membranes subjected to Western blotting. The densitometric analysis of the bands revealed a significant increase (48%) in Gq/11α in the membranes of obese Zucker rats compared with lean rats (Figure 5, top), whereas the densities of Gsα were not different in the basolateral membranes of obese rats compared with lean rats (Figure 5, bottom).
Discussion

In this study we investigated D₁-like dopamine receptors and coupled G proteins in the proximal tubules of obese and lean Zucker rats. We found a reduced number of D₁-like receptor binding sites and reduced stimulation of G proteins by dopamine and SKF 38393 in obese rats compared with lean rats. It is suggested that the reduction in D₁-like receptor binding sites and subsequent failure to stimulate G protein led to the reduced inhibition by dopamine of Na,K-ATPase activity in the proximal tubules of obese rats. Whether such a reduction in D₁-like receptors, as well as its function in the basolateral membranes of obese Zucker rats, is a generalized phenomenon or specific to the kidney remains to be determined.

Obesity is a metabolic disorder with multiple pathophysiological consequences, including insulin resistance, increased renal sodium reabsorption, and development of hypertension. Several investigators have attempted to understand the mechanisms responsible for the development of hypertension in obese individuals as well as in animal models.3–5 Obese Zucker rats provide a useful model in which the possible candidate hormones and/or their cellular signaling pathways have been investigated to identify a relationship between the development of hypertension and obesity.4,30 In the present study, obese Zucker rats were found to exhibit high plasma insulin levels along with higher blood glucose levels compared with their control lean Zucker rats, suggesting insulin resistance. Moreover, this insulin resistance in obese rats was associated with high blood pressure. Higher levels of plasma insulin and blood glucose found in this study are in agreement with earlier studies.4,30

Endogenously produced kidney dopamine and exogenously administered dopamine and D₁-like agonists promote sodium excretion, at least in part, via the activation of D₁-like dopamine receptors and subsequent inhibition of Na-H exchanger and Na,K-ATPase in the proximal tubules. Unlike in hypertensive animals and patients in which renal dopamine production is not affected, patients with type 2 diabetes mellitus have a reduced renal dopamine production; the mechanism for this abnormality is not yet known. However, some studies suggest that increased levels of plasma insulin may play a role in reducing the production of renal dopamine, perhaps by inhibiting either the conversion of L-dopa to dopamine or the tubular uptake of L-dopa. In addition to lower renal dopamine production, a suppressed natriuretic response to exogenously infused dopamine has been reported in patients with type 2 diabetes mellitus, which would suggest that the dopamine receptors and/or coupled cellular signaling system may also be defective in type 2 diabetes mellitus. In the present study we observed that dopamine-mediated inhibition of Na,K-ATPase activity was attenuated in the proximal tubules of obese Zucker rats. This suggested that a defect or downregulation may exist in D₁-like dopamine receptor-mediated cellular signaling that causes a reduction in dopamine-mediated inhibition of Na,K-ATPase in the proximal tubules of obese rats. We measured D₁-like receptor

Figure 4. Effect of dopamine and SKF 38393 on [³⁵S]GTPγS binding in the basolateral membranes from lean and obese Zucker rats. Values are mean±SE from 4 rats determined in triplicate. The basal values of [³⁵S]GTPγS in lean and obese rats were 6.06±0.3 and 6.44±0.34 pmol [³⁵S]GTPγS bound per milligram protein, respectively. *Significantly different within the group from control (P<0.05, ANOVA); #significantly different between groups (P<0.05, Student’s t test).

Figure 5. Western blot of basolateral membranes from lean and obese Zucker rats for Gq11α and Gaα. Left lanes on blots represent lean rats, and right lanes represent obese rats. Bars represent mean±SE of densities of bands from 3 to 4 rats in each group. *Significantly different from lean rats (P<0.05, Student’s t test).
binding sites and G proteins as the initial components of the signaling pathway in the basolateral membranes from obese and lean Zucker rats to identify the site(s) of defects that contribute to the reduction in dopamine-induced inhibition of Na,K-ATPase. It is likely that this phenomenon may contribute and/or lead to a reduced natriuretic response to dopamine in obese Zucker rats, as reported in subjects with type 2 diabetes mellitus.\textsuperscript{25} It should be noted that the reduced inhibition of Na,K-ATPase by dopamine may not be due to alterations per se in the number or the activity of Na pumps in obese rats. This was evident from the finding that there was neither no significant difference in either the control activity of Na,K-ATPase or the quantity of α-subunit, the sodium-transporting part of the enzyme, as measured by Western blotting between obese and lean rats.

The binding of $[^35\text{S}]\text{SCH 23390}$ revealed that the number of D\textsubscript{1}-like dopamine receptor binding sites was reduced by \approx \text{45\%} in the basolateral membranes of obese rats without a change in affinity ($K_a$) to the ligand. We earlier reported a similar reduction in D\textsubscript{1}-like receptor binding sites in the proximal tubules of old Fischer 344 rats compared with adult rats.\textsuperscript{28} However, Fischer 344 rats were not hypertensive.\textsuperscript{28} It is not known whether the reduction in D\textsubscript{1}-like receptor binding sites is due to a decrease in the synthesis of receptors or internalization and degradation of receptors. The decrease in D\textsubscript{1}-like receptor binding sites may be partly responsible in the attenuation of dopamine-mediated inhibition of Na,K-ATPase in the proximal tubules of obese Zucker rats.

Quantification of $G_{\alpha/11}$ and $G_{\gamma}$ proteins and agonist-induced activation of G proteins were performed to assess the next component of the D\textsubscript{1}-like receptor signaling pathway. Western blot analysis of the basolateral membranes revealed an increase in the α-subunit of $G_{\alpha/11}$ protein and no change in the α-subunit of $G_{\gamma}$ protein in obese rats compared with lean rats. However, while dopamine and SKF 38393 caused a concentration-dependent activation of G proteins (as measured by $[^35\text{S}]\text{GTP\gammaS}$ binding) in lean rats, this response was absent in obese rats. We reported a similar defect in G protein activation by dopamine in the basolateral membranes of old Fischer 344 rats in which D\textsubscript{1}-like receptors were reduced and the levels of G proteins were increased.\textsuperscript{28} A 45\% reduction in D\textsubscript{1}-like dopamine receptors may not account for the total failure of dopamine and SKF 38393 to stimulate G proteins, as measured by $[^35\text{S}]\text{GTP\gammaS}$ binding, in obese rats. It is possible that G proteins in obese rats may be defective and/or may not be available for interacting with D\textsubscript{1}-like receptors. In experiments conducted in spontaneously hypertensive rats, it was discovered that D\textsubscript{1}-like dopamine receptor numbers were not altered,\textsuperscript{13} but the activation of G proteins\textsuperscript{29} and the subsequent stimulation of second messengers were significantly reduced compared with Wistar-Kyoto rats.\textsuperscript{13,20} The D\textsubscript{1}-like receptors were unable to bind to the agonist with high affinity because of a defect in a receptor–G protein coupling.\textsuperscript{13} A recent study in the proximal tubules of essential hypertensive subjects suggested that agonist-independent phosphorylation of D\textsubscript{1} dopamine receptors may be the molecular cause of the defect in the receptor–G protein coupling.\textsuperscript{15} It is yet to be determined whether a similar mechanism of receptor–G protein uncoupling exists in the proximal tubules of obese rats. However, the failure of dopamine and SKF 38393 to activate G proteins suggests that there may be little or no stimulation to these agonists of the effector enzymes (phospholipase C and adenylyl cyclase) in the proximal tubules of obese Zucker rats.

The mechanism(s) responsible for reduced D\textsubscript{1}-like dopamine receptor function in the proximal tubules of obese Zucker rats is not known. However, it is possible that the reduction in D\textsubscript{1}-like dopamine receptor binding sites and a defect in coupling with the G proteins may be 2 different independent phenomena. It is known that the defect in D\textsubscript{1}-like receptor–G protein coupling in the proximal tubules of spontaneously hypertensive rats exists even before the development of hypertension,\textsuperscript{32} when such defect is proposed to have contributed to the development of hypertension. The obese Zucker rats used in this study are hypertensive. Furthermore, it is also important to determine in future studies whether the defective dopamine receptor function is related to hypertension, obesity, or insulin-resistant phenotype in these animals. Younger obese Zucker rats aged 3 to 4 and 7 to 8 weeks may be used to delineate the mechanisms of such a receptor defect when these animals have not yet developed hypertension, obesity, and insulin resistance.

In summary, we found that obese Zucker rats were hypertensive and displayed higher levels of plasma insulin and blood glucose. The D\textsubscript{1}-like dopamine receptor binding sites were reduced and the activation of G proteins by dopamine and D\textsubscript{1}-like agonists was diminished, which may account for the reduced inhibition of Na,K-ATPase by dopamine in the proximal tubules of obese rats. This is the first demonstration of such a defect in the ability of dopamine to inhibit Na,K-ATPase, which may contribute to increased sodium reabsorption and development of hypertension in obese Zucker rats.

**References**


