Rosiglitazone restores renal D_{1A} receptor-G\(_s\) protein coupling by reducing receptor hyperphosphorylation in obese rats

Meghna Trivedi and Mustafa F. Lokhandwala

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

Submitted 27 September 2004; accepted in final form 22 March 2005

Rosiglitazone restores renal D_{1A} receptor-G\(_s\) protein coupling by reducing receptor hyperphosphorylation in obese rats. Am J Physiol Renal Physiol 289: F298–F304, 2005. First published March 29, 2005; doi:10.1152/ajprenal.00362.2004.—Dopamine D_{1A} receptor function is impaired in obesity-induced insulin resistance, contributing to sodium retention. We showed previously that uncoupling of D_{1A} receptors from G proteins is responsible for diminished natriuretic response to dopamine in obese Zucker rats (OZRs). We hypothesized that overexpression of G protein-coupled receptor kinases (GRKs) leads to increased phosphorylation of D_{1A} receptors, which in turn causes uncoupling of the receptors from G proteins in proximal tubules of OZRs. We also examined effects of an insulin sensitizer, rosiglitazone, in proximal tubules of OZRs. We found that basal and agonist-induced coupling of D_{1A} receptors to G\(_s\) proteins was impaired in proximal tubules of OZRs compared with lean Zucker rats (LZRs). Moreover, basal serine phosphorylation of D_{1A} receptors was elevated two- to threefold in proximal tubules of OZRs compared with LZRs. Fenoldopam increased D_{1A} receptor phosphorylation in proximal tubules of LZRs but not OZRs. Compared with that in LZRs, GRK4 expression in OZRs was elevated 200–300% in proximal tubule cell lysates and GRK2 expression was ~30% higher in plasma membranes isolated from proximal tubules of OZRs. Rosiglitazone treatment restored basal and agonist-induced coupling of D_{1A} receptors to G\(_s\) proteins and reduced basal serine phosphorylation of D_{1A} receptors, GRK4 expression, and translocation of GRK2 to the plasma membrane in proximal tubules of OZRs. Furthermore, in the first part of the study, we examined the potential importance in preventing genesis of hypertension (3). Moreover, in renal proximal tubules, these receptors couple to mainly G\(_s\) and G\(_q\) proteins among all the G proteins (16, 21). Because D_{1A} receptor subfamily includes D_{1A} and D_{1B} receptors, only the D_{1A} receptors are implicated in the natriuretic response of dopamine and its analogs in obesity remains unclear.

Radioligand binding and [\(^{35}\)S]guanosine 5′-O-(3-thiotriphosphate) (GTP\(_\gamma\)S) binding experiments done in our laboratory indicate that the impairment in D_{1A}-like receptor function in obese Zucker rats may be due to decreased D_{1A}-like receptor binding sites and, perhaps more likely, to uncoupling of D_{1A}-like receptors from G proteins in the plasma membrane of renal proximal tubules (15, 39). Although the D_{1A}-like receptor subfamily includes D_{1A} and D_{1B} receptors, only the D_{1A} receptors could be a reason for 1) a nonsignificant difference in basal coupling and 2) only a minor difference in agonist-induced coupling of D_{1A} receptors to G proteins in obese vs. lean Zucker rats in our previous study (37). Therefore, in the first part of the study, we examined the potential defects in basal and agonist (fenoldopam)-induced coupling of D_{1A} receptors specifically to G\(_s\) proteins in the proximal tubules of obese Zucker rats by performing coimmunoprecipitation experiments. Coupling of D_{1A} receptors with G\(_q\) proteins could not be measured because these two proteins do not coimmunoprecipitate (41). Furthermore, we determined whether improvement in insulin sensitivity with rosiglitazone corrects this coupling defect of D_{1A} receptors.

Uncoupling of several G protein-coupled receptors (GPCRs) from G proteins, due to hyperphosphorylation of GPCRs resulting from overexpression of GPCR kinases (GRKs) that phosphorylate the receptors, has been reported in various pathological conditions, such as heart failure and hypertension (2, 4, 8, 12, 22, 31, 35, 38, 40). It is possible that similar overexpression of GRKs resulting in hyperphosphorylation of D_{1A} receptors contributes to impaired G protein coupling and defective signaling. Moreover, hyperphosphorylation of D_{1A} receptors also would explain reduced binding sites of these...
receptors on the plasma membrane, because phosphorylated receptors are targeted for endocytosis (9). Therefore, in the second part of this study, we examined the underlying mechanisms of impaired D1A receptor-Gs protein coupling in proximal tubules of obese Zucker rats by determining serine phosphorylation of D1A receptors as well as expression of GRK isoforms. Even though the above-mentioned mechanism has been established in animal models of hypertension and aging (4, 8, 22, 31, 35, 38), conditions other than obesity, therapeutic remedy for this dysfunction in dopamine pathway has not been addressed. Hence, we also determined the effects of rosiglitazone treatment on hyper-serine phosphorylation of D1A receptors as well as on expression of GRK isoforms in the proximal tubules of the obese Zucker rats to establish whether improvement in insulin sensitivity will correct the defects in these pathways.

METHODS

Material

The following chemicals and materials were purchased from the source indicated: rabbit anti-rat D1A receptor polyclonal antibodies (Chemicon, Temecula, CA); horseradish peroxidase-conjugated anti-rabbit antibodies and chemiluminescence substrate (Alpha Diagnostics, San Antonio, TX); anti-mouse phosphoserine antibodies and rabbit anti-G6P monoclonal antibodies (Calbiochem, San Diego, CA); mouse anti-human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) polyclonal antibodies, mouse anti-human GRK2 monoclonal antibodies, mouse anti-human GRK4 monoclonal antibodies, horseradish peroxidase-conjugated anti-mouse antibodies, and protein A/G-agarose (Santa Cruz Biotechnology, Santa Cruz, CA); Immobilon P membrane (Millipore, Bedford, MA); X-ray films (Kodak, Rochester, NY); and rat insulin radioimmunoassay kit (Linco Research, St. Charles, MO). All other chemicals were purchased from Sigma (St. Louis, MO) and were of the highest grade available.

Animals and Drug Treatment

Male obese and lean rats (Charles River Laboratories, Wilmington, MA) were maintained in an animal care facility with a 12:12-h light-dark cycle and provided standard rat chow (Purina Mills, St. Louis, MO) and tap water ad libitum. Twenty-four obese and lean rats (9–10 wk old) were randomly assigned to either rosiglitazone maleate (9–10 mg/kg) or distilled water at 37°C for 15 min and then used for coimmunoprecipitation experiments with a previously described method (19). Samples I) without the addition of D1A receptor antibodies and 2) without proximal tubular lysate but with D1A receptor antibodies served as negative controls in the experiments. No D1A receptor protein band was detected in these lanes.

Preparation of Plasma Membranes

Plasma membranes were prepared from the frozen-thawed proximal tubular lysate as described previously (37).

Immunoprecipitation-Immunoblotting to Detect Gsα Subunit Coupled to D1A Receptors

Immunoprecipitation of D1A receptors. Proximal tubular cell lysates were first treated with either vehicle (distilled water) or fenoldopam (1 μmol/l) at 37°C for 15 min and then used for coimmunoprecipitation experiments with a previously described method (19). Samples I) without the addition of D1A receptor antibodies and 2) without proximal tubular lysate but with D1A receptor antibodies served as negative controls in the experiments. No D1A receptor protein band was detected in these lanes.

Immunoblotting of Gsα subunit. The immunoprecipitated samples were resolved using 10% SDS-PAGE, and the proteins were electrotransferred on Immobilon P membrane. Immunoblotting of Gsα subunit was performed as described previously (25). The same Immobilon P membranes were stripped of the antibody complex with stripping buffer and were used for immunoblotting of D1A receptors as described previously (37). Band density of Gsα subunits was normalized to band density of D1A receptors.

Immunoprecipitation-Immunoblotting to Detect Serine-Phosphorylated D1A Receptors

Immunoprecipitation of D1A receptors. Proximal tubules were treated with either vehicle (distilled water) or fenoldopam (1 μmol/l) at 37°C for 25–30 min. After freeze-thaw, D1A receptors were immunoprecipitated from these proximal tubular cell lysates as described previously (4). Samples I) without the addition of D1A receptor antibodies and 2) without proximal tubular lysate but with D1A receptor antibodies served as negative controls in the experiments. No D1A receptor protein band was detected in these lanes.

Immunoblotting of serine-phosphorylated D1A Receptors. The immunoprecipitated samples (20 μl) were resolved using 10% SDS-PAGE, and the proteins were electrotransferred onto an Immobilon P membrane. Immunoblotting of serine-phosphorylated D1A receptors with the use of anti-phosphoserine antibodies was performed as previously described (4). Moreover, these immunoprecipitated samples were used for immunoblotting of D1A receptors as described previously (37). Band density of serine-phosphorylated D1A receptors was normalized to band density of D1A receptors.

Immunoblotting of GRK2 and GRK4

Proximal tubular cell lysates or plasma membranes were used to prepare loading samples containing SDS-Laemmli and bromphenol blue for immunoblotting. These loading samples (protein: 30–40 μg of proximal tubular cell lysates and 10 μg of plasma membranes) were then resolved using 10% SDS-PAGE and electrophoretically blotted onto an Immobilon P membrane. The membrane blots were incubated with primary monoclonal mouse anti-GRK2 (1:125) and anti-GRK4 (1:250) antibodies followed by horseradish peroxidase-conjugated secondary antibodies (1:4,000). The bands were detected with chemiluminescence substrate on X-ray films and were densitometrically quantified using Scion Image software provided by NIH. The same Immobilon P membranes were also used for immunoblotting of GAPDH for normalization as described previously (10).

Data Analysis

Data are represented as means ± SE of the number (n) of experiments. The results were analyzed using either Student’s unpaired t-test or one-way ANOVA followed by the Newman-Keuls multiple comparison test to assess the significance of differences between groups (lean and obese rats treated with either vehicle or rosiglitazone). The probability of type I error was fixed at 0.05.
zone) as well as within groups. Statistical analysis was done using GraphPad Prism version 3.02 (GraphPad Software, San Diego, CA). Statistical significance was considered at $P < 0.05$.

RESULTS

Body Weight, Fasting Blood Glucose, and Plasma Insulin

Obese rats weighed significantly more than lean rats in both vehicle and rosiglitazone treatment groups (Table 1). Treatment with rosiglitazone (3 mg·kg$^{-1}$·day$^{-1}$ for 15 days) caused significant weight gain in obese rats but not in lean rats (Table 1). Rosiglitazone treatment normalized fasting blood glucose levels and significantly reduced plasma insulin levels in obese treated rats (Table 1). Fasting blood glucose and plasma insulin levels were not significantly different between lean control and lean treated rats. These results suggest that obese rats are insulin resistant and that rosiglitazone treatment improves insulin sensitivity in these animals.

Rosiglitazone Treatment Restores Basal and Agonist-Induced $G_{i}$ Protein Coupling of $D_{1A}$ Receptors in Proximal Tubules of Obese Rats

In proximal tubular cell lysates of vehicle-treated obese rats, fenoldopam did not increase immunoprecipitation of $G_{i}$ subunit with $D_{1A}$ receptors (Fig. 1A). However, there was an approximately threefold increase in coimmunoprecipitation of $G_{i}$ subunit with $D_{1A}$ receptors after fenoldopam treatment of proximal tubules in the three other groups of rats (Fig. 1A). Moreover, the basal amount of $G_{i}$ subunit immunoprecipitated with $D_{1A}$ receptors was significantly reduced in proximal tubules of obese rats compared with lean rats (Fig. 1A), indicating a reduction in $D_{1A}$ receptors in the high-affinity state in proximal tubules of obese rats. When obese rats were treated with rosiglitazone, this decrease in basal $D_{1A}$-$G_{i}$ interaction was restored to the same level seen in lean rats (Fig. 1A). Interestingly, rosiglitazone treatment significantly increased the amount of $G_{i}$ subunit coimmunoprecipitated with $D_{1A}$ receptors in both lean and obese Zucker rats (Fig. 1A). Dopamine $D_{1A}$ receptor protein density was not significantly different among all four groups of rats (Fig. 1B). Therefore, variation in immunoprecipitated $D_{1A}$ receptors was caused by limitation of experimental technique and was compensated by normalizing density of $G_{i}$ subunit with that of $D_{1A}$ receptors.

Rosiglitazone Treatment of Obese Zucker Rats Corrects Hyper-Serine Phosphorylation of $D_{1A}$ Receptors in Proximal Tubules

In proximal tubules of vehicle-treated obese rats, basal serine phosphorylation of $D_{1A}$ receptors was two- to threefold higher compared with that of vehicle-treated lean rats (Fig. 2A). However, when obese rats were treated with rosiglitazone, basal serine phosphorylation of $D_{1A}$ receptors was reduced to the levels seen in lean rats (Fig. 2A). There was no significant difference in basal serine phosphorylation of $D_{1A}$ receptors in proximal tubules of vehicle- and rosiglitazone-treated lean rats (Fig. 2A). In addition, fenoldopam failed to further increase serine phosphorylation of $D_{1A}$ receptors in proximal tubules of vehicle-treated obese rats (Fig. 2B). However, in rosiglitazone-treated obese rats, there was a two- to threefold increase in serine phosphorylation of $D_{1A}$ receptors when proximal tubules were treated with fenoldopam (Fig. 2B). There was no significant difference in the percent increase in $D_{1A}$ receptor phosphorylation by fenoldopam in vehicle-treated lean, rosiglitazone-treated lean, and rosiglitazone-treated obese rats. These hyper-serine-phosphorylated $D_{1A}$ receptors in proximal tubular cell lysates mainly represented $D_{1A}$ receptors on the plasma membranes, because no detectable band for serine-phosphorylated $D_{1A}$ receptors was observed in cytoplasmic compartments of proximal tubules from these rats (data not shown).

Overexpression of GRK4 in Proximal Tubules of Obese Zucker Rats is Corrected by Rosiglitazone Treatment

There was a more than twofold increase in GRK4 expression in proximal tubular cell lysates of vehicle-treated obese rats compared with vehicle-treated lean rats (Fig. 3). When obese rats were treated with rosiglitazone, GRK4 expression in proximal tubular cell lysates was significantly reduced, although it was not completely normalized to the levels seen in lean rats (Fig. 3). In addition, GRK4 expression in proximal tubular lysates was not significantly different between vehicle- and rosiglitazone-treated lean rats (Fig. 3).

Translocation of GRK2 to Plasma Membrane in Proximal Tubules of Obese Zucker Rats is Corrected by Rosiglitazone Treatment

There was no significant difference in GRK2 expression in proximal tubular cell lysates among all four groups of animals (Fig. 4A). However, because GRK2 is located mainly in the cytosolic fraction, we wanted to determine whether GRK2 is translocated to the plasma membranes, which is a site for GRK2 action. We detected an $\sim$30% increase in GRK2 immunoreactivity in plasma membranes isolated from proximal tubules of vehicle-treated obese rats compared with vehicle-treated lean rats (Fig. 4B). Moreover, rosiglitazone treatment significantly reduced GRK2 protein density on the plasma membrane in proximal tubules of obese Zucker rats (Fig. 4B). GRK2 immunoreactivity on the plasma membrane was not affected by rosiglitazone treatment in lean Zucker rats (Fig. 4B).

| Table 1. Effects of rosiglitazone treatment on baseline parameters in lean and obese Zucker rats |
|--------------------------|----------------|----------------|----------------|----------------|
|                          | Baseline Parameters | Lean Control | Obese Control | Lean Treated | Obese Treated |
| Body weight before treatment, g | 329.7±11.3 | 463.5±9.7* | 323.2±5.3 | 457.3±9.3† |
| Body weight after treatment, g | 344.7±8.8 | 535.0±6.7* | 350.3±4.1 | 559.3±10.1†‡ |
| Fasting blood glucose, mmol/l | 5.7±0.2 | 8.9±0.3* | 5.4±0.3 | 5.3±0.4‡ |
| Plasma insulin, mmol/l | 0.19±0.02 | 1.26±0.08* | 0.21±0.02 | 0.43±0.03†‡ |

Values are means ± SE; $n = 5–7$. Statistical comparisons were made with 1-way ANOVA followed by Newman-Keuls multiple comparison test. *$P < 0.05$; obese control vs. lean control rats. †$P < 0.05$; obese treated vs. lean treated rats. ‡$P < 0.05$; obese control vs. obese treated rats.
DISCUSSION

Our results show that uncoupling of D\textsubscript{1A} receptors from G\textsubscript{s} proteins coexists with hyper-serine phosphorylation of D\textsubscript{1A} receptors, overexpression of GRK4, and translocation of GRK2 to the plasma membrane. Furthermore, rosiglitazone, by improving insulin sensitivity, corrects overexpression of GRK4 and translocation of GRK2, reduces basal serine phosphorylation of D\textsubscript{1A} receptors, and thus restores G\textsubscript{s} protein coupling of D\textsubscript{1A} receptors in proximal tubules of obese Zucker rats.

In our attempt to identify mechanisms leading to impaired D\textsubscript{1A} receptor signaling in obese Zucker rats, we have previously demonstrated that these defect(s) exist(s) at both the receptor and receptor-G protein level (15, 39). It is possible that hyperphosphorylation of D\textsubscript{1A} receptors causes both of these defects. It is well documented that hyperphosphorylated GPCRs are uncoupled from G proteins (9). Furthermore, these receptors are then targeted for endocytosis and subsequent degradation (9). This, again, would explain why there is a 50% reduction in the receptor binding density on the plasma membrane (15, 39).

To study the coupling between D\textsubscript{1A} receptors and G\textsubscript{s} proteins, we performed coimmunoprecipitation experiments in proximal tubules of lean and obese Zucker rats treated with either vehicle or rosiglitazone. A: proximal tubular cell lysates from lean control (LC, vehicle), obese control (OC, vehicle), lean treated (LT, rosiglitazone), and obese treated (OT, rosiglitazone) rats were treated with either distilled water (vehicle; V) or fenoldopam (F; 1 µmol/l) at 37°C for 15 min and used for immunoprecipitation of D\textsubscript{1A} receptors. Immunoprecipitated samples were then used for immunoblotting of G\textsubscript{s} subunit and D\textsubscript{1A} receptors. Top: representative immunoblot of G\textsubscript{s} subunit and D\textsubscript{1A} receptors. Bottom: densitometric analysis of G\textsubscript{s} subunit band density, normalized to D\textsubscript{1A} receptor protein density (means ± SE; n = 4). *P < 0.05, fenoldopam vs. vehicle treatment; #P < 0.05, obese vs. lean; $P < 0.05,$ rosiglitazone vs. vehicle treatment (unpaired Student’s t-test).

B: proximal tubules were treated with either distilled water (V) or fenoldopam (F; 1 µmol/l) at 37°C for 25–30 min. Proximal tubular cell lysates were used for immunoprecipitation of D\textsubscript{1A} receptors and immunoblotting of serine-phosphorylated D\textsubscript{1A} receptors and total D\textsubscript{1A} receptors. Top: representative immunoblots of serine-phosphorylated D\textsubscript{1A} receptors and total D\textsubscript{1A} receptors. Bottom: densitometric analysis of band density of serine-phosphorylated D\textsubscript{1A} receptor protein, normalized to immunoprecipitated D\textsubscript{1A} receptor protein density (means ± SE; n = 6). Serine (P), serine phosphorylated. *P < 0.05, obese vs. lean (1-way ANOVA followed by Newman-Keuls multiple comparison test).
which the ability of \( G_\alpha \) to coimmunoprecipitate with \( D_{1A} \) receptors was measured in renal proximal tubules isolated from four groups of rats. The basal coupling of \( D_{1A} \) receptors to \( G_\alpha \) proteins was reduced in the proximal tubules of obese compared with lean Zucker rats. This observation indicates a loss of \( D_{1A} \) receptors in the high-affinity state that are precoupled to \( G_\alpha \) proteins. In addition, fenoldopam-induced coupling of the \( D_{1A} \) receptors to \( G_\alpha \) proteins was decreased in obese Zucker rats compared with lean rats. Rosiglitazone treatment, which improved insulin sensitivity, restored basal as well as fenoldopam-induced coupling of \( D_{1A} \) receptors to \( G_\alpha \) proteins in proximal tubules of obese Zucker rats. Interestingly, rosiglitazone also increased basal \( G_\alpha \) coupling of \( D_{1A} \) receptors in proximal tubules of the insulin-sensitive lean rats, indicating that this effect of rosiglitazone may be independent of its insulin-sensitizing activity. Moreover, the increase in basal \( G_\alpha \) coupling of \( D_{1A} \) receptors by rosiglitazone treatment also was not caused by changes in the level of expression of \( G_\alpha \) or \( G_\beta \\eta \) proteins, because rosiglitazone treatment did not alter the expression of these proteins in the proximal tubules of both lean and obese Zucker rats (unpublished observation). Restoration of fenoldopam-induced \( G_\alpha \) protein coupling of \( D_{1A} \) receptors by rosiglitazone treatment was, however, selectively present in obese Zucker rats and not in lean rats. Therefore, the restoration of fenoldopam-induced coupling of \( D_{1A} \) receptors to \( G_\alpha \) proteins is the result of improved insulin sensitivity in obese Zucker rats. It is noteworthy that although dopamine \( D_{1A} \) receptor also couple to \( G_\alpha \) proteins, we could not evaluate this interaction using our experimental technique because these receptors do not coimmunoprecipitate with \( G_\alpha \) proteins (41).

To explore the possible mechanisms of the defective \( D_{1A} \) receptor-\( G \) protein coupling, we measured the basal serine phosphorylation of \( D_{1A} \) receptors in obese and lean Zucker rats. It has been reported that increased phosphorylation at serine residues in \( D_{1A} \) receptors is responsible for the attenuation of the natriuretic effects of dopamine in both spontaneously hypertensive rats (SHR) and old Fischer 344 rats (4, 31). The basal serine phosphorylation of \( D_{1A} \) receptors was found to be higher in the proximal tubules of the obese Zucker rats compared with that in the lean rats. Unlike the lean rats, in proximal tubules of obese rats, fenoldopam failed to increase serine phosphorylation of \( D_{1A} \) receptors because these receptors were already hyperphosphorylated. This hyper-serine phosphorylation of \( D_{1A} \) receptors could explain the uncoupling of \( D_{1A} \) receptors from the \( G_\alpha \) proteins and subsequent failure of agonists to stimulate second messengers in proximal tubules of obese rats. It is relevant to note that \( D_1 \) (analogous to \( D_{1A} \)) receptors in the proximal tubular culture from essential hypertensive patients are hyper-serine phosphorylated (8, 31). Furthermore, the proximal tubular culture from these patients do not exhibit a fenoldopam-mediated increase in cAMP cum-

Fig. 3. G protein-coupled receptor kinase 4 (GRK4) protein expression in proximal tubules of lean and obese Zucker rats treated with either vehicle or rosiglitazone. Proximal tubular cell lysates from LC (vehicle), OC (vehicle), LT (rosiglitazone), and OT (rosiglitazone) rats were used for immunoblotting of GRK4 and GAPDH. Top: representative immunoblots of GRK4 and GAPDH. Bottom: densitometric analysis of band density of GRK4 proteins, normalized to GAPDH density (means \( \pm \) SE; \( n = 5 \)). *\( p < 0.05 \), lean vs. obese; \( \# p < 0.05 \), control vs. treatment (1-way ANOVA followed by Newman-Keuls multiple comparison test).

Fig. 4. GRK2 protein expression in proximal tubules of lean and obese Zucker rats treated with either vehicle or rosiglitazone. Proximal tubular cell lysates (A) or plasma membranes (B) from LC (vehicle), OC (vehicle), LT (rosiglitazone), and OT (rosiglitazone) rats were used for immunoblotting of GRK2 and GAPDH. Top: representative immunoblots of GRK2 and GAPDH. Bottom: densitometric analysis of band density of GRK2 proteins, normalized to GAPDH density (means \( \pm \) SE; \( n = 5 \)). *\( p < 0.05 \), lean vs. obese; \( \# p < 0.05 \), control vs. treatment (1-way ANOVA followed by Newman-Keuls multiple comparison test).
pared with proximal tubules from normotensive humans (31). Similarly, dopamine fails to increase cAMP in proximal tubules of obese Zucker rats (14). Therefore, it appears that an increase in the basal serine phosphorylation of D1A receptors in the proximal tubules of the insulin-resistant obese Zucker rats leads to their uncoupling from the G proteins and loss of activation of downstream signaling components in these tubules.

Uncoupling of several GPCRs from G proteins has been reported in various pathological conditions, such as heart failure and hypertension. This uncoupling is due to hyperphosphorylation of GPCRs resulting from overexpression of GRKs that phosphorylate the receptors (5). For example, in cardiomyocytes of patients and animals with heart failure, GRK2 expression is increased, which results in hyperphosphorylation of β-adrenergic receptors, rendering the receptors incapable of coupling to G proteins and activating downstream pathways (2, 12, 40). Similarly, in proximal tubules of both SHR (35, 38) and old Fisher 344 rats (22), uncoupling of D1A receptors from the G proteins is associated with hyperphosphorylation of serine residues in the D1A receptors (4, 31). This hyper-serine phosphorylation of D1A receptors is caused by the overexpression of GRK4 in proximal tubules of SHR rats (8). In this study, we found that not only was the basal level of expression of GRK4 increased in proximal tubules of obese Zucker rats, but there also was a translocation of GRK2 to the proximal tubular plasma membranes in these animals. Although GRK overexpression and hyper-serine phosphorylation of D1A receptor coexist in the proximal tubules of the obese Zucker rats, we have not performed experiments to link these two phenomena. However, ample evidence in the literature supports the notion that increased GRK expression causes agonist-independent hyper-serine phosphorylation of D1A receptors in proximal tubules of the kidney (4, 8, 31).

One consistent finding of our study was that the insulin-sensitizing drug rosiglitazone restored D1A receptor-G protein coupling, decreased D1A receptor hyper-serine phosphorylation, and also decreased the overexpression of GRK isoforms selectively in the proximal tubules of obese Zucker rats and not the lean rats. The obese Zucker rat is an established model of insulin resistance syndrome, which is exhibited by symptoms like hyperglycemia, hyperinsulinemia, and hypertriglyceremia. It is possible that these metabolic defects interfere with renal dopamine D1-like receptor function in obese Zucker rats. For instance, triglyceride-derived fatty acids are responsible for hyperactivation of PKC in obesity, which interferes with insulin signaling pathways (6, 17, 24, 32). Similar hyperactivation of PKC in the proximal tubules of the obese Zucker rats may activate cellular components such as GRKs, which may interfere with dopamine function. Treatment of the obese rats with rosiglitazone, by normalizing the metabolic abnormalities in these animals (18, 28, 29, 36), may simultaneously restore D1A receptor coupling and function in proximal tubules of obese Zucker rats.

In summary, we have established that D1A receptors are uncoupled from the G proteins in the proximal tubules of the obese Zucker rats in both basal as well as agonist-stimulated states. This uncoupling of D1A receptors from G protein may be due to hyperphosphorylation of these receptors, which in turn is caused by overexpression of GRK4 and translocation of GRK2 to the plasma membrane in the proximal tubules of the obese Zucker rats. Furthermore, all of these defects are secondary to insulin resistance syndrome, because they are corrected by rosiglitazone treatment of obese Zucker rats. Therefore, the renal dopamine receptor dysfunction in the proximal tubules of the obese Zucker rats is a consequence of insulin resistance-related uncoupling of D1A receptors from G proteins.

ACKNOWLEDGMENTS

This study was conducted as part of a PhD dissertation submitted in partial fulfillment of the requirements for the designated degree at the University of Houston (M. Trivedi).

GRANTS

This project was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-58743.

REFERENCES

17. Inoguchi T, Sonta T, Tsobouchi H, Etoh T, Kakimoto M, Sonoda N, Sato N, Sekiguchi N, Kobayashi K, Sumimoto H, Utsumi H, and Nawata H. Protein kinase C-dependent increase in reactive oxygen...