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Fenoldopam Treatment Improves Peripheral Insulin Sensitivity and Renal Function in STZ-Induced Type 2 Diabetic Rats

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ABSTRACT

Dopamine and diabetes mellitus are reported to have close link between them. We have studied the effect of six-week treatment with D₁ receptor agonist fenoldopam (1 mg/kg, i.p., daily) on glucose, lipid, and renal profile in streptozotocin (STZ)-induced (non-insulin dependent) type 2 diabetic rats. Streptozotocin (90 mg/kg, i.p.) was injected to two day old Sprague-Dawley pups. Streptozotocin produced hyperglycemia, hyperinsulinemia, hyperlipidemia, hypertension, increase in serum urea and creatinine by the time animals were 10 week old. Treatment with fenoldopam significantly decreased serum glucose, insulin, cholesterol, triglyceride, urea, creatinine, and blood pressure. During oral glucose tolerance test (OGTT), diabetic rats showed increase in AUCglucose and AUCinsulin. Fenoldopam significantly decreased AUCglucose in diabetic rats. Diabetic rats showed lower insulin sensitivity index (KITT) that was significantly increased by treatment with fenoldopam in diabetic rats. Diabetic rats showed decrease in urinary sodium. Fenoldopam treatment significantly increased urine output as well as urinary sodium indicating reduced sodium retention. Our data indicates fenoldopam treatment improves peripheral insulin sensitivity and renal function in STZ-induced type 2 diabetic rats.

Key Words: Fenoldopam; Non-insulin dependent diabetes mellitus; Insulin sensitivity; Renal function.

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INTRODUCTION

Involvement of dopamine in diabetes mellitus has been well documented. Glucose regulates dopamine release from substantia nigra neurons and dopaminergic activity is decreased in diabetic rats (1–4). Renal dopamine production is reduced in both, type 1 (insulin dependent) and type 2 (non-insulin dependent) diabetes mellitus leading to sodium retention which further aggravates hypertension associated with diabetes mellitus (5,6). Renal dopamine receptor function is defective in insulin resistant obese Zucker rats (7). The defect in receptor function is normalized with improvement in insulin sensitivity (8). This indicates the close link between insulin sensitivity and renal dopamine physiology. Dopamine receptor agonists SKF 38393 and bromocriptine, alone or in combination, are reported to ameliorate obesity and related metabolic dysfunctions like hyperglycemia, lipid profile, islet dysfunction in obese ob/ob or diabetic db/db mice (9–13). We have earlier reported beneficial effects of chronic treatment with D1-like receptor agonist fenoldopam on renal function in streptozotocin (STZ)-induced type 1 diabetic rats (14). Considering the close link between insulin sensitivity and peripheral dopamine receptor function, the present investigation was undertaken to study the effect of fenoldopam treatment on biochemical and renal parameters in STZ-induced type 2 (neonatal) diabetic rats.

MATERIALS AND METHODS

Induction of Diabetes

Streptozotocin at the dose of 90 mg/kg dissolved in normal saline was injected intraperitoneally to two day old Sprague-Dawley pups (15). Pups were weaned at three weeks of age and differentiated by sex. Female pups were selected for the study and housed in separate cages. Food and water was provided ad libitum till 10 weeks of age. Glucose level was estimated in 18 hour fasted rats and animals showing blood glucose >140 mg/dL were selected for study. Age matched Sprague-Dawley female rats were maintained as non-diabetic controls. Four groups were made as: non-diabetic control (SDcon), non-diabetic treated with fenoldopam (SDtr), diabetic control (Diacon), and diabetic treated with fenoldopam (Diatr). Treatment groups received fenoldopam (1 mg/kg, i.p.) dissolved in distilled water daily for six weeks.

Blood Sampling and Biochemical Analysis

At the end of six-week treatment, blood samples were collected from the tail vein into centrifuge tubes and allowed to clot for 30 min at room temperature. Blood samples were centrifuged at 3000 rpm for 20 min. Serum was separated and stored at −20°C until analysis was done. Serum samples were analyzed spectrophotometrically for glucose, cholesterol, triglyceride, creatinine, and urea (Bayer Diagnostics Kit, India). Serum insulin levels were estimated by radioimmunoassay method using the kit from Bhabha Atomic Research Center, Mumbai, India.
Oral Glucose Tolerance Test

Rats were fasted for 18 hours and glucose administered at the dose of 1.5 g/kg orally. Blood samples were collected at 0, 30, 60, and 120 min. Serum was separated immediately and analyzed for glucose and insulin. The results of oral glucose tolerance test (OGTT) are expressed as integrated areas under the curves (AUCs) over a period of 0–120 min.

Insulin Sensitivity Index

Insulin sensitivity index ($K_{ITT}$) was calculated as per the method of Alford et al. (16). Rats were fasted for six hours and human insulin (Actrapid, Novo Nordisk, India) was injected through tail vein at the dose of 0.2 IU/100 g body weight. Blood samples were collected at 10, 20, 30, and 60 min time interval. Blood glucose was estimated and plotted against time on semi log paper to calculate $t_{1/2}$. Insulin sensitivity index was calculated as:

$$K_{ITT} = \left(\frac{0.693}{t_{1/2}}\right) \times 100$$

Measurement of Blood Pressure

Blood pressure was recorded by tail-cuff method using Harvard blood pressure monitor (Kent, UK). Trained rats were restrained and tail cuff introduced. Tail cuff pressure was first raised to 200 mmHg and then slowly released. During the decline in pressure, the point at which there was an increase in magnitude of deflection of the pulse analyzer was recorded as the systolic blood pressure.

Measurement of Renal Parameters

Rats were placed in metabolic cages and urine was collected for 12 hours. Urinary sodium was measured using flame photometer.

Drugs

Streptozotocin was purchased from Sigma Chemical Co. (St. Louis, MO) and fenoldopam was a kind gift from Dr. M. F. Lokhandwala, University of Houston, USA.

Statistical Analysis

The results were analyzed statistically using one way ANOVA followed by Tukey multiple test to determine the level of significance. Value of $P < 0.05$ was considered significant.
RESULTS

General Features of Diabetic Rats

Body weight and food intake of diabetic rats were not significantly different from non-diabetic rats. However, water intake in diabetic animals was higher than non-diabetic control rats. Fenoldopam treatment produced further increase in water intake in diabetic rats (Table 1).

Serum Glucose, Insulin, and Lipid Levels of Diabetic Rats

Neonatal STZ diabetic rats had moderate, but stable hyperglycemia and hyperinsulinemia. Fenoldopam treatment significantly decreased glucose and insulin levels (Fig. 1). AUC\(_{\text{glucose}}\) and AUC\(_{\text{insulin}}\) were significantly greater in diabetic rats as compared to non-diabetic control rats during OGTT. Treatment with fenoldopam significantly lowered AUC\(_{\text{glucose}}\) in diabetic rats, but AUC\(_{\text{insulin}}\) remained unchanged (Fig. 2). Insulin sensitivity index was significantly lower in diabetic rats that was significantly increased with fenoldopam treatment (Fig. 3). Diabetic rats showed significantly higher serum total cholesterol and triglyceride. Fenoldopam treatment significantly decreased lipid levels (Fig. 4).

Renal Parameters

Urine volume in diabetic rats remained unchanged; however, fenoldopam treatment significantly increased urine volume in both non-diabetic and diabetic rats. Urinary sodium content was significantly lower in diabetic rats as compared to non-diabetic rats. Fenoldopam treatment did not show significant effect on urinary sodium in treated rats (Fig. 5). Diabetic rats showed significantly higher serum urea and creatinine levels that were significantly decreased by fenoldopam treatment in diabetic rats (Fig. 6).

Table 1. Effect of six week treatment with fenoldopam on general variables of diabetic rats.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-diabetic control</th>
<th>Non-diabetic treated</th>
<th>Diabetic control</th>
<th>Diabetic treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>218.30 ± 9.36</td>
<td>224.20 ± 7.68</td>
<td>238.00 ± 5.14</td>
<td>231.00 ± 7.14</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>25.13 ± 3.47</td>
<td>25.00 ± 2.04</td>
<td>26.25 ± 3.14</td>
<td>25.00 ± 2.04</td>
</tr>
<tr>
<td>Water intake (mL/day)</td>
<td>27.50 ± 1.44</td>
<td>37.50 ± 1.44*</td>
<td>39.00 ± 0.57*</td>
<td>47.50 ± 1.44**</td>
</tr>
</tbody>
</table>

Note: Data is shown as mean ± S.E.M. Number of animals in each group = 6.
*Significantly different than non-diabetic control.
**Significantly different than diabetic control (one way ANOVA followed by Tukey test, P < 0.05).
Diabetic rats showed higher blood pressures and bradycardia. Fenoldopam treatment significantly lowered blood pressure, but no effect was observed on heart rate (Fig. 7).

**DISCUSSION**

Streptozotocin injection to two day old pups is reported to induce beta cell injury that is followed by limited regeneration, primarily as a result of ductal budding, rather than mitosis of preexisting beta cells, creating a short term normalization of glycemia. At 6–15 weeks of age, the rats are reported to have an impaired glucose disposal rate and significant beta cell secretory dysfunction (15). There have been numerous variations of this model of...
non-insulin dependent diabetes mellitus. Nonetheless, all variations are based on the premise that neonatal rats treated with STZ (80–100 mg/kg) at birth or within the first five days following birth experience severe pancreatic beta cell destruction, accompanied by a decrease in pancreatic insulin stores and a rise in plasma glucose levels (17,18). However, in contrast to adult rats treated with STZ, the beta cells of the treated neonates partially regenerate (19). Following the initial spike in plasma glucose, the STZ treated neonate rat becomes normoglycemic by three weeks of age. In the next few weeks, the beta cell number increases, the extent dependent up the age at which animal is treated with STZ (17–21). It is reported that although 10 week old n2 (i.e., neonate treated with STZ on day 2 of the birth) STZ Wistar rats exhibit normal nonfasting glucose and insulin levels, they are markedly glucose intolerant. By six months of age, a glucose challenge provokes a condition of severe hyperglycemia and hyperinsulinemia in these animals (22). D₁-like receptor agonist fenoldopam is reported to act through D₁-like dopamine receptors at

Figure 2. Effect of fenoldopam treatment (1 mg/kg, i.p. daily for six weeks) on AUC<sub>glucose</sub> and AUC<sub>insulin</sub> in STZ-induced type 2 diabetic rats. Key: SDCon, non-diabetic control; SDtr, non-diabetic treated with fenoldopam; Diacon, diabetic control; Diatr, diabetic treated with fenoldopam. Each bar represents mean ± S.E.M. Number of animals in each group = 6. *, significantly different from non-diabetic control; **, significantly different from diabetic control. P < 0.05.
**Figure 3.** Effect of fenoldopam treatment (1 mg/kg, i.p. daily for six weeks) on $K_{ITT}$ in STZ-induced type 2 diabetic rats. Key: SDCon, non-diabetic control; SDtr, non-diabetic treated with fenoldopam; Diacon, diabetic control; Diatr, diabetic treated with fenoldopam. Each bar represents mean ± S.E.M. Number of animals in each group = 6. *, significantly different from non-diabetic control; **, significantly different from diabetic control. $P < 0.05$.

**Figure 4.** Effect of fenoldopam treatment (1 mg/kg, i.p. daily for six weeks) on serum cholesterol and triglyceride in STZ-induced type 2 diabetic rats. Key: SDCon, non-diabetic control; SDtr, non-diabetic treated with fenoldopam; Diacon, diabetic control; Diatr, diabetic treated with fenoldopam. Each bar represents mean ± S.E.M. Number of animals in each group = 6. *, significantly different from non-diabetic control; **, significantly different from diabetic control. $P < 0.05$. 
the dose of 0.3–20 mg/kg when given intraperitoneally or subcutaneously (23,24) and we have reported fenoldopam (1 mg/kg, i.p.) to produce beneficial effects in STZ-induced type 1 diabetes in rats (14). In the present study, diabetic animals showed increase in water intake as compared to non-diabetic control animals, however, body weight and food intake remained unchanged. Treatment with fenoldopam produced increase in water intake in both, non-diabetic and diabetic rats. D1-like receptor agonists are known to produced diuresis and natriuresis by inhibition of Na, K-ATPase and Na, H-exchanger proteins that are responsible for retention of water and sodium ions in the body (25,26). The increase in water intake seen in the present study could possibly be an attempt to make up for the loss of water due to fenoldopam treatment. Streptozotocin produced hyperglycemia, hyperinsulinemia, and dyslipidemia that are typical features of non-insulin dependent (type 2) diabetes mellitus in rats. Treatment with fenoldopam significantly decreased serum glucose and insulin levels in diabetic rats. Dopaminergic agonists are reported to reduce

Figure 5. Effect of fenoldopam treatment (1 mg/kg, i.p. daily for six weeks) on urine volume and urinary sodium in STZ-induced type 2 diabetic rats. Key: SDCon, non-diabetic control; SDtr, non-diabetic treated with fenoldopam; Diacon, diabetic control; Diatr, diabetic treated with fenoldopam. Each bar represents mean ± S.E.M. Number of animals in each group = 6. *, significantly different from non-diabetic control; **, significantly different from diabetic control. P < 0.05.
the activities of hepatic gluconeogenic enzymes, such as glucose 6 phosphatase and phosphoenol pyruvate carboxykinase (PEPCK) (12). This may be one of the possible mechanisms for decrease in serum glucose level found in the present study. Oral glucose tolerance test and insulin index revealed decreased insulin sensitivity in diabetic rats. Fenoldopam treatment significantly decreased AUC glucose indicating improved glucose disposal. Treatment with fenoldopam also increased the insulin sensitivity index significantly in diabetic rats. Serum cholesterol and triglyceride levels were significantly higher in diabetic rats as compared to non-diabetic control. Fenoldopam treatment significantly reduced both, serum cholesterol, and triglyceride levels in diabetic rats. It is reported that dopaminergic agonists bromocriptine and SKF 38393 reduce de novo lipogenesis in ob/ob mice (12). Additionally, dopaminergic agonists also reduce basal lipolysis and adipose tissue lipoprotein lipase (LPL) activity resulting in reduced serum free fatty acid (FFA) concentrations (12,27). The reduction in serum lipid levels with fenoldopam treatment seen in diabetic rats could be attributed to possible decrease in de novo lipogenesis and basal lipolysis in addition to improved insulin sensitivity seen with fenoldopam treatment.

**Figure 6.** Effect of fenoldopam treatment (1 mg/kg, i.p. daily for six weeks) on serum urea and creatinine in STZ-induced type 2 diabetic rats. Key: SDCon, non-diabetic control; SDtr, non-diabetic treated with fenoldopam; Diacon, diabetic control; Diatr, diabetic treated with fenoldopam. Each bar represents mean ± S.E.M. Number of animals in each group = 6. *, significantly different from non-diabetic control; **, significantly different from diabetic control. \( P < 0.05. \)
Endogenously produced kidney dopamine as well as 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 (25,26). Fenoldopam is known to produce hypotension, renal vasodilatation, natriuresis, and diuresis with minimum changes in renal hemodynamics (28,29). In the present study, urine volume did not show significant change, however, urinary sodium was significantly decreased in diabetic rats indicating sodium retention that is an established feature of diabetes mellitus. Increased sodium retention is suggested to play an important role in development of hypertension in diabetic patients (30). Although fenoldopam did not change urinary sodium significantly, it significantly increased urine volume (2 fold) effectively reducing sodium retention. The diabetic rats showed higher blood pressure as compared to non-diabetic controls. Treatment with fenoldopam significantly decreased blood pressure in diabetic rats. Decreased sodium retention, decrease in hyperglycemia due to improved insulin sensitivity, and direct effect of fenoldopam on vasculature may be responsible for this decrease in blood pressure with fenoldopam treatment.

Figure 7. Effect of fenoldopam treatment (1 mg/kg, i.p. daily for six weeks) on blood pressure and heart rate in STZ-induced type 2 diabetic rats. Key: SDCon, non-diabetic control; SDtr, non-diabetic treated with fenoldopam; Diacon, diabetic control; Diatr, diabetic treated with fenoldopam. Each bar represents mean ± S.E.M. Number of animals in each group = 6. *, significantly different from non-diabetic control; **, significantly different from diabetic control. P < 0.05.
Fenoldopam Treatment and Type 2 Diabetic Rats

Diabetic rats showed increase in serum urea and creatinine that is an indication of deteriorated renal function. Fenoldopam treatment significantly decreased serum urea and creatinine. Recently it is shown that insulin sensitivity and renal dopamine function are closely interrelated (7) and improvement in insulin sensitivity restores impaired renal dopamine function in obese Zucker rats (8). Decrease in serum urea and creatinine in STZ type 2 diabetic rats in the present study may be due to improvement in insulin sensitivity that was observed with fenoldopam treatment.

In conclusion, our data suggest that STZ-induced type 2 diabetes in rats produces an alteration in dopamine receptor sensitivity that may be responsible for renal dysfunctions. Treatment with fenoldopam causes improvement in peripheral insulin sensitivity and renal functions in STZ-induced type 2 diabetic rats.

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