Discussion

In the present investigation 5-hydroxytryptamine (5-HT) was found to produce dose dependent increase in serum glucose levels in overnight fasted rats. We also found that 5-HT reuptake inhibitor fluoxetine that increases synaptic 5-HT availability potentiated 5-HT induced hyperglycemia. This is in accordance with previous reports (Yamada et al, 1999). 5-HT per se or its agonists are reported to induce hyperglycemia in rats in the recent past (Yamada et al, 1999; Chaouloff and Jeanrenaud, 1987; Chaouloff et al, 1990a,b; Sugimoto et al, 1992; Baudrie and Chaouloff, 1992; Sugimoto et al, 1996b). Yamada et al. (1997) have reported that hyperglycemia induced by various 5-HT receptor agonists is mediated through central, peripheral, or the both, types of 5-HT receptors.

Previous studies have reported the involvement of 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors in 5-HT induced hyperglycemia (Baudrie and Chaouloff, 1992; Sugimoto et al, 1996b). 5-HT_{1A} partial agonist buspirone potentiated 5-HT induced hyperglycemia at a high dose of 15 mg/kg, whereas, 5-HT_{1A} receptor antagonist pindolol did not modify 5-HT induced hyperglycemia. 5-HT_{2} receptor agonist α methyl 5-hydroxytryptamine potentiated, whereas, 5-HT_{2A} antagonist sarpogrelate and 5-HT_{2A/2C} antagonist mianserin inhibited 5-HT induced hyperglycemia. Thus, our study supports the involvement of 5-HT_{2A} receptors in 5-HT induced hyperglycemia. Involvement of 5-HT_{1A} receptors can not be confirmed at this stage.

Earlier studies have ruled out the involvement of 5-HT_{3} receptors in 5-HT induced hyperglycemia. (Sugimoto et al, 1996a; Yamada et al, 1997; Yamada et al, 1998). In the present study, 5-HT_{3} receptor agonist 1-phenyl-biguanide per se did not produce significant alteration in glucose levels, however, it potentiated hyperglycemic action of 5-HT. Ondansetron, a specific 5-HT_{3} receptor antagonist inhibited 5-HT-
induced hyperglycemia. These results clearly indicate that 5-HT3 receptors may be involved in glycemic control. Carvalho et al. (2002) using m-chlorophenyl-biguanide and ondansetron have reported that central stimulation of 5-HT3 receptors produces hyperglycemia. Potentiation of 5-HT-induced hyperglycemia by peripherally administered 5-HT3 agonist 1-phenyl biguanide and inhibition by peripherally administered 5-HT3 ondansetron suggests that even peripheral 5-HT3 receptors may be involved in 5-HT induced hyperglycemia. 5-HT induced hyperglycemia was associated with decrease in serum insulin levels. 5-HT affects pancreatic islet function through central and peripheral mechanisms. Five week infusion of 5-HT into the ventromedial hypothalamus has been reported to impair pancreatic islet function (Luo et al, 1999). In the pancreas, 5-HT is mainly present in beta cells of the islets of Langerhans (Ekholm et al, 1971; Cetin 1992). It inhibits basal as well as glucose-mediated insulin release from the beta cells of pancreas in isolated pancreatic islets (Pontiroli et al, 1978). It can be hypothesized that 5-HT induced hyperglycemia is due to decrease in insulin release from beta cells by 5-HT.

Previous studies have proposed release of adrenaline due to stimulation of adrenal medulla by 5-HT as the reason for 5-HT induced hyperglycemia (Yamada et al, 1998). Adrenaline acts via α2 receptors located on β cells of pancreas to decrease insulin release resulting in increase in blood glucose (Lacey et al, 1993). Pretreatment with α2 receptor antagonists like yohimbine block adrenaline or α2 receptor agonist induced decrease in insulin release (Lacey et al, 1993; Niddam et al, 1990). In the present study, pretreatment with yohimbine or adrenaloctomy significantly inhibited 5-HT induced hyperglycemia. However, the inhibition was not complete indicating that involvement of adrenergic system is not the only mechanism for 5-HT-induced hyperglycemia.
Fig. J: Proposed mechanism for 5-HT-induced hyperglycemia in normoglycemic rats.
Thus in nutshell, our data suggest that 5-HT induces hyperglycemia in rats possibly due to either inhibition of insulin release from β cells of pancreas and/or release of adrenaline from adrenal medulla that in turn acts via α2 adrenoceptors pancreatic β cells to inhibit insulin release.

STZ-induced diabetes is reported to affect serotonergic system. STZ diabetes produces a decrease in precursor levels of 5-HT in the brain coupled with a subsequent decrease in synthesis and turn over of 5-HT (Manjarrez-Gutierrez et al, 2000). Interestingly, depletion of brain 5-HT prior to STZ injection, by pretreatment with 5,7-DHT or parachlorophenyl alanine (PCPA), inhibits diabetogenic effects of STZ (Yang and Lin, 1995). Plasma concentrations of 5-HT are reported to be high in diabetic than in normal subjects (Martin et al, 1995). Contractile responses to 5-HT are attenuated in rat aorta (Hattori et al, 1995; James et al, 1994; Sikorski et al, 1993), gastric fundus smooth muscles (Zhu and Sakai, 1996) and blood vessels from diabetic rats (James and Hodgson, 1995). Attenuation of contractile responses to 5-HT in aorta is reported to be due to reduced activation of protein kinase C (James and Hodgson, 1997). Contractile response to 5-HT is increased in detrusor smooth muscle of STZ-diabetic rat bladder (Kodama and Takimoto, 2000). This effect is related to smooth muscle hypertrophy and/or hyperplasia and it is indicated that this effect is mediated by activation of 5-HT2A receptors.

If 5-HT induces hyperglycemia and 5-HT levels are high in diabetes mellitus, it is possible that chronic treatment with 5-HT antagonists will produce change in blood glucose in diabetic animals. As mentioned before 5-HT2A and 5-HT3 receptors are involved in glycemic control in rats. Hence, we studied the effect of chronic treatment with 5-HT2A antagonist sarpogrelate and 5-HT3 receptor antagonist ondansetron in STZ induced type 1 and type 2 diabetic rats.
Insulin dependent diabetes mellitus (type 1) was induced by injecting STZ in adult rats. STZ destroys pancreatic beta cells and hypoinsulinemic diabetes is induced (Johansson and Tjalve, 1978). On the other hand, to make the animals non-insulin dependent diabetic (type 2), STZ is injected to younger pups rather than adult animals. STZ injection to 2 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 to 15 weeks of age, the rats are reported to have an impaired glucose disposal rate and significant beta cell secretory dysfunction (Bonnevie-Nielsen et al, 1981). 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 to 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 (Blondel et al, 1989; Weir et al, 1981). However, in contrast to adult rats treated with STZ, the beta cells of the treated neonates partially regenerate (Wang et al, 1996). 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 (Blondel et al, 1989; Weir et al, 1981; Wang et al, 1996; Boner-Weir et al, 1981; Iwase et al, 1994). 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 fed 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 (Schaffer and Wilson, 1993).
Discussion

STZ injected to adult rats produced loss of body weight, hyperphagia and polydypsia. The loss of body weight could be due to dehydration and catabolism of fats and proteins (Hofteizer and Carpenter, 1973). Sarpogrelate and ondansetron treatments did not produce any change in body weight, hyperphagia and polydypsia in diabetic rats. These parameters were unaltered in type 2 diabetic rats. Type 1 diabetic rats showed significant hyperglycemia and hypoinsulinemia, whereas, in type 2 diabetic rats significant hyperglycemia and hyperinsulinemia was observed. Treatment with sarpogrelate and ondansetron significantly decreased fasting but not fed serum glucose with significant increase in serum insulin levels in type 1 diabetic rats. However, the effect of sarpogrelate on glucose and insulin levels was greater (56% decrease in glucose and 72% increase in insulin) as compared to ondansetron (25% decrease in glucose and 26% increase in insulin). Sarpogrelate and ondansetron did not alter glucose and insulin levels in type 2 diabetic rats. As mentioned earlier, 5-HT is reported to inhibit basal as well as glucose-mediated insulin release from the beta cells of pancreas in isolated pancreatic islets (Pontiroli et al., 1978). 5-HT inhibits glucose-induced insulin release by affecting early steps in the beta cell stimulus-secretion coupling (Lindstorm and Sehlin, 1982, 1983). Sarpogrelate and ondansetron, being 5-HT antagonists, possibly act as insulin releasers by inhibiting 5-HT action. During oral glucose tolerance test (OGTT), type 1 diabetic rats showed significant increase in AUC$_{\text{glucose}}$ and decrease in AUC$_{\text{insulin}}$ indicating decreased glucose disposal, whereas, insulin sensitivity index ($K_{\text{ITT}}$) was unaltered indicating no change in peripheral insulin sensitivity in these animals. Type 2 diabetic rats showed significant increase in AUC$_{\text{glucose}}$ and AUC$_{\text{insulin}}$, and a decrease in $K_{\text{ITT}}$. This indicates the abnormal glucose disposal despite of hyperinsulinemia due to decreased peripheral insulin sensitivity in type 2 diabetes. In type 1 diabetic rats, treatment with
sarpogrelate or ondansetron significantly decreased $\text{AUC}_{\text{glucose}}$ (33% and 24% respectively) however, $\text{AUC}_{\text{insulin}}$ was not significantly altered. Insulin sensitivity index ($K_{\text{ITT}}$) was not different in type 1 diabetic rats as compared to non-diabetic rats. This can be explained on the basis of increase in insulin levels by these two drugs. $\text{AUC}_{\text{glucose}}$, $\text{AUC}_{\text{insulin}}$, and $K_{\text{ITT}}$ remained unchanged with the treatment in type 2 diabetic rats. As mentioned before, sarpogrelate and ondansetron probably act as insulin releasers. Type 2 diabetic rats being hyperinsulinemic, the drug that can improve insulin sensitivity rather than release is desirable in such condition. Sarpogrelate and ondansetron failed to produce any change in insulin sensitivity index. These drugs also failed to affect $\text{AUC}_{\text{glucose}}$ and $\text{AUC}_{\text{insulin}}$ during OGTT.

STZ produced significant increase in serum cholesterol and triglyceride in type 1 and 2 diabetic rats. Sarpogrelate significantly decreased serum cholesterol; however, serum triglyceride remained unchanged. Ondansetron significantly decreased serum cholesterol and triglyceride. Treatment with these drugs did not alter lipid levels in type 2 diabetic rats. Role of 5-HT in lipid metabolism is still unclear. In the normal physiological state, triglycerides undergo lipolysis in adipose tissue forming free fatty acids that enter the circulation. These free fatty acids are ultimately reesterified to triglycerides. In diabetes mellitus, the rate of lipolysis exceeds the rate of esterification and thus high levels of free fatty acids are observed in blood. All these effects are due to decrease in insulin level in type 1 diabetes mellitus (Nakai et al., 1985; Satoh et al., 1987). The reduction in serum cholesterol and triglyceride by sarpogrelate and ondansetron observed in the present study may be due to increase in insulin level by these drugs. Additionally, 5-HT is also reported to have lipolytic action on adipocytes increasing plasma levels of free fatty acids (Martinez-Conde et al, 1984). It is also possible that the reduction in serum lipid levels with these agents
Discussion

may be due to their antagonistic action at 5-HT receptors. However, ineffectiveness of sarpogrelate and ondansetron on lipid levels in type 2 diabetic rats suggests the positive effect seen in type 1 diabetic rats is due to increase in insulin levels. 5-HT plays a role in the regulation of blood pressure. Activation of 5-HT$_2$ receptors results in pressor response associated with large increases in sympathetic activity (McCall and Clement, 1994). The decrease in blood pressure with sarpogrelate and ondansetron treatment in type 1 diabetic rats may possibly be due to antagonism at 5-HT receptors as well as improved glucose and lipid profile as these drugs did not significantly decrease blood pressure in type 2 diabetic rats.

Chronic diabetes mellitus is associated with depressed heart functions and diabetic cardiomyopathy. It is characterized by decreased left ventricular developed pressure (LVDP), decreased rate of ventricular pressure development (+dP/dt), decreased rate of ventricular pressure decline (-dP/dt) and decreased heart rate. In the present study, type 1 diabetes produced decrease in LVDP, +dP/dt, -dP/dt and heart rate. Treatment with sarpogrelate produced significant increase in LVDP, +dP/dt, -dP/dt and heart rate. In addition, the diabetic rats showed increase in heart weight to body weight and left ventricle weight to body weight ratios indicating hypertrophy of the heart. Sarpogrelate significantly decreased heart weight to body weight ratio indicating reduction in hypertrophy. Circulating levels of 5-HT are increased in diabetics as well as patients with coronary artery disease as compared to the normal subjects. This increase in 5-HT levels may be due to release of 5-HT from platelets as well as mast cells (van Den Berg et al, 1989). As mentioned earlier, 5-HT receptor sensitivity is altered in various tissues in STZ diabetic rats. We have reported the decreased positive inotropic effect of 5-HT owing to the down regulation of 5-HT$_{2A}$ receptors in STZ diabetic hearts (Umrani et al, In press). Sarpogrelate treatment
restores the 5-HT$_{2A}$ receptor sensitivity and reverses the inotropic effects of 5-HT, thus improves the heart function. However, chronic hyperglycemia may alter the hemodynamic responses in experimental animals due to more than one factor. The attenuation in the ability to generate contractile force has been suggested to be due to the depression in the ATPase activity of contractile proteins (Pierce and Dhalla, 1981; Pierce and Dhalla, 1985a; Dillmann, 1980; Malhotra et al, 1995) and alteration in the sarcolemmal membrane (Pierce and Dhalla, 1983; Pierce et al, 1983). The defects in cardiac relaxation have been attributed to the depression in the sarcoplasmic reticular calcium uptake (Penpparkgul et al, 1981; Ganguly et al, 1987) and the sarcolemmal calcium exchange activities (Makino et al, 1987). It has also been reported that cardiac mitochondria isolated from diabetic animals have reduced capacity to accumulate calcium (Pierce and Dhalla, 1985b). All this evidence lead to the conclusion that condition of calcium overload may present in mitochondrial cells in chronic diabetes (Dhalla et al, 1985, Pierce and Dhalla, 1984).

5-HT causes very strong smooth muscle cell contraction that is considered to be associated with cardiovascular diseases like hypertension (Frishman et al, 1995). 5-HT causes an increase in [Ca$^{2+}$]$_i$ of smooth muscle cells via 5-HT$_2$ receptor through the release of calcium from intracellular stores as well as the influx of extracellular calcium (Doyle et al, 1986; Kanaide et al, 1987; Hirafuji et al, 1998). 5-HT induced influx may occur through both, voltage dependent and independent channels (Hirafuji et al, 1999). Chronic treatment with 5-HT$_{2A}$ antagonist sarpogrelate may have led to the suppression of this resultant calcium overload leading to improvement in cardiac function.

Normal glucose homeostasis is the result of a balance between the appearance of glucose in the blood and its cellular uptake and use (Olefsky, 1993). These
processes are controlled by the action of hormones (i.e. insulin and glucagon) and by a family of membrane glucose transporters (Thorens et al, 1990; Kayano et al, 1990). A reduced ability of myocardium to take up and metabolize glucose has been demonstrated in both type 1 and 2 diabetes and the function of these glucose transporters is modified in diabetes (Sivitz et al, 1989; Muekler, 1990). In non-stimulated condition these transporters are located in the cytosolic pools and in response to the insulin release these transporters get translocated in the plasma membrane (Fischer et al, 1997). In type-1 diabetes due to hypoinsulinemia, the process of translocation may be affected and hence the number of transporters in membrane may decrease further increasing the blood glucose levels. Moreover, as mentioned earlier, 5-HT levels are high in diabetes and 5-HT is one of the stimuli for the translocation of glucose transporters. In the present study both GLUT 1 and GLUT 4 levels were found to be decreased in cardiomyocytes from STZ-diabetic rats. This is consistent with the earlier findings (Garvey et al, 1993; Stanley et al, 1994). Insulin was found to prevent only GLUT 4 in STZ-diabetic rats. However, sarpogrelate, a specific 5-HT$_{2A}$ receptor antagonist was found to increase both GLUT 1 and GLUT 4 levels in diabetic rats. These results indicate that not only 5-HT$_{2A}$ receptors are involved in glucose transport mechanisms but also that increase in glucose transporters in cardiomyocytes by sarpogrelate may be independent of insulin. Recently, Hajduch et al (1999) have reported that rat and human skeletal muscles both express 5-HT$_{2A}$ receptors and that specific 5-HT$_{2A}$ receptor agonists can stimulate glucose uptake in skeletal muscles by a mechanism which does not depend upon components that participate in the insulin signaling pathway. Our results support similar findings in cardiomyocytes.
Although, it has been accepted that GLUT 4 translocation is the major mechanism for increasing glucose uptake in various tissues, there are several issues that require attention. It has been reported that GLUT 1 is also highly expressed in cardiomyocytes (Fischer et al, 1997). In our studies also we found that the expression of GLUT 1 is decreased in STZ-diabetic rats and it was not altered by insulin. A more interesting observation in our study was that while decrease in GLUT 4 in membrane was associated with increase in cytosol, treatment with sarpogrelate caused a further increase in GLUT 4 cytosol. An area that is beginning to attract attention is the biogenesis of GLUT 4 vesicle and the mechanisms determining this process (Rea and James, 1997; Cheatham, 2000). It is possible that 5-HT$_2A$ receptor inhibition causes an increase in the biogenesis of glucose transporters and hence enhancing the glucose transport in cardiomyocytes.

In summary, it is suggested that 5-HT$_2A$ receptors may be looked upon as a novel target to develop anti-diabetic drugs to prevent the cardiovascular complications associated with diabetes mellitus considering (a) the close association between 5-HT$_2A$ receptors and glucose transporters, (b) the beneficial effects of 5-HT$_2A$ receptor antagonists like sarpogrelate in cardiovascular abnormalities and (c) the link between cardiac dysfunction and glucose metabolism.
**Fig.K:** Proposed mechanism for glucose lowering effect of Sarpogrelate in diabetic rats.
Dopamine produced hyperglycemia at the doses of 1 mg/kg and 2 mg/kg. Dopamine D₁ receptor agonist fenoldopam and SKF 38393 and D₂ agonist bromocriptine did not produce change in dopamine induced hyperglycemia. These agonists themselves did not produce significant change in serum glucose. Moreover, D₁ receptor antagonist butaclamol and D₂ antagonist sulpiride did not inhibit dopamine induced hyperglycemia. These results suggest that dopamine induced change in serum glucose is not mediated via peripheral dopamine receptors. Levodopa produced dose dependent increase in blood glucose. Orally administered levodopa is taken by the adrenergic neurons and decarboxylated by DOPA-decarboxylase to dopamine. Peripherally it is also converted to nor-adrenaline (Standaert and Stern, 1993). In our study, carbidopa was found to potentiate levodopa-induced hyperglycemia indicating involvement of central dopaminergic system. Thus, DA-induced hyperglycemia observed in the present study may be either due to activation of central dopaminergic system or peripheral adrenergic system. However, further detailed studies would be needed to support this hypothesis.

Dopamine is closely associated with diabetes mellitus. Although the role of dopamine in diabetes is controversial, central dopaminergic activity appears to be increased in diabetic animals (Lackovic et al, 1990a; Chen and Yang, 1991; Lim et al, 1994; Ramkrishnan et al, 1996; Kamei and Saitoh, 1997; Holden, 1995). Decreased central as well as peripheral dopaminergic activity is also reported in various animal models of diabetes mellitus (Sailer 1984; Toner and Stamford, 1997; Gripois et al, 1987; Murzi et al, 1996; Kono and Takada, 1994).
Discussion

DOPAMINE / L-DOPA

Nor-adrenergic Neurons
(Peripheral)

$\alpha_2$ adrenoceptors

Pancreas

Decrease in Insulin Release

HYPERGLYCEMIA

Central Nervous System

Fig.L: Proposed mechanism for DA-induced hyperglycemia in normoglycemic rats.
In the present study, fenoldopam did not change hyperphagia, polydypsia and loss of body weight in type 1 diabetic rats, whereas in type 2 diabetic rats water intake was significantly increased with the treatment. Fasting serum glucose levels were significantly higher in type 1 as well as type 2 diabetic rats as compared to non diabetic controls. Diabetes mellitus is characterized by decrease in glucose uptake by skeletal muscle and adipose tissue as well as increase in hepatic glucose production. This is due to lack of insulin action owing to hypoinsulinemia in type 1 diabetics and insulin resistance in type 2 diabetics. Although insulin levels were not changed, fasting glucose levels were significantly decreased in type 1 diabetic rats with fenoldopam treatment. The decrease in glucose levels may be due to increase in glucose uptake by skeletal muscle or adipose tissue. Dopaminergic agonists are reported to reduce the activities of hepatic gluconeogenic enzymes like glucose 6 phosphatase (G-6-P) and phosphoenol pyruvate carboxykinase (PEPCK) (Scisłowski et al, 1999). So the decrease in fasting glucose by fenoldopam may also be due to decrease in hepatic glucose production in type 1 diabetic rats. Fenoldopam treatment significantly decreased serum glucose in type 2 diabetic rats along with decrease in serum insulin and increase in insulin sensitivity index ($K_{ITT}$). The decrease in serum glucose in type 2 diabetic rats may be attributed to improved insulin sensitivity and antigluconeogenic activity of fenoldopam. Fenoldopam treatment did not affect $AUC_{glucose}$ and $AUC_{insulin}$ in type 1 diabetic rats indicating unchanged abnormal glucose disposal in these animals. $K_{ITT}$ did not change in type 1 diabetic rats as compared to non diabetic controls and fenoldopam had no effect on the index in type 1 diabetics. However, significant increase in $K_{ITT}$ was obtained with fenoldopam in type 2 diabetic rats leading to improved glucose disposal as seen from decreased $AUC_{glucose}$ in these rats as compared to diabetic control group. $AUC_{insulin}$ in type 2
diabetic rats was significantly higher than non diabetic controls and fenoldopam did not affect it.

Serum cholesterol and triglyceride levels were significantly higher in type 1 and type 2 diabetic rats indicating hyperlipidemia in these animals. Fenoldopam treatment significantly decreased cholesterol and triglyceride levels in type 2 diabetic rats only, whereas, it did not change lipid levels in type 1 diabetic rats. Dopaminergic agonists bromocriptine and SKF 38393 are reported to reduce basal lipolysis and adipose tissue lipoprotein lipase (LPL) activity resulting in reduced serum free fatty acid (FFA) concentrations (Scislowski et al, 1999; Cincotta and Meier, 1995). 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. Insulin is one of the strongest contributors to the homeostasis of lipid metabolism. Insulin treatment is reported to reverse hypercholesterolemia in STZ induced diabetic rats (Nakai et al, 1985). Insulin also regulates synthesis and secretion of triglycerides in liver (Iwai et al, 1989; Murthy and Shipp, 1981). Our results suggest that fenoldopam is more effective in type 2 diabetes mellitus for better glucose and lipid profile by improving insulin sensitivity. STZ produced increase in blood pressure in type 1 and type 2 diabetic rats. Fenoldopam treatment significantly lowered blood pressure in diabetic rats. Heart rate was significantly decreased in type 1 but not in type 2 diabetic rats. Fenoldopam did not have effect on heart rate. Fenoldopam is reported to produce decrease in blood pressure, renal vasodilatation, natriuresis and diuresis with minimum changes in renal hemodynamics (Mann et al, 1981; Ackerman et al, 1982). Increased sodium retention is suggested to play an important role in the development of hypertension in diabetics (Feldt-Rasmussen et al, 1987). Hence, the decrease in
blood pressure in the present study with fenoldopam treatment in diabetic rats may possibly due to a combined effect of vasodilatation, natriuresis and diuresis.

Endogenously produced kidney dopamine as well as exogenously administered dopamine and D₁-like agonists promote sodium excretion via activation of D₁-like dopamine receptors and subsequent inhibition of Na⁺, K⁺-ATPase and Na⁺, H⁺ exchanger in proximal tubules (Hegde et al, 1989; Felder et al, 1990; Chen and Lokhandwala, 1993). In the present study, type 1 and type 2 diabetes produced increase in urine volume and decrease in urinary sodium excretion. Fenoldopam treatment produced diuresis and natriuresis leading to significant reduction in sodium retention. Type 1 and type 2 diabetic rats showed significant increase in serum creatinine and urea levels indicating deteriorated renal function. Treatment with fenoldopam produced significant decrease in serum creatinine and urea. Considering the beneficial effects of fenoldopam treatment on urinary sodium excretion and serum urea, creatinine levels, it is clear that fenoldopam improves renal function in both, type 1 and type 2 diabetes mellitus.

Earlier it has been reported that the inhibitory effects of dopamine on Na⁺, K⁺-ATPase and Na⁺, H⁺-exchanger activities are significantly reduced in proximal tubules of hyperinsulinemic obese Zucker rats as compared to lean rats (Hussain et al, 1999; Hussain et al, 2001). It is also observed that the reduced inhibition of these transporters is associated with a significant reduction in D₁ receptor number in proximal tubular membranes of obese rats (Hussain et al, 1999; Hussain et al, 2001). In the present study, we found that lowering of plasma insulin levels in obese Zucker rats restores the ability of dopamine to inhibit the sodium transporters in proximal tubules and normalizes the membrane D₁ receptor number to the level of lean rats. There have been reports suggesting a negative correlation between renal
dopaminergic function and plasma insulin levels (Segers et al, 1996a; Segers et al, 1996b; Shigetomi et al, 1995). Inability of dopamine to inhibit the $\text{Na}^+$, $\text{K}^+$-ATPase activity in proximal tubules of type 2 diabetic Wistar fatty rats is also reported (Tsuchida et al, 2001). Additionally, exogenous infusion of dopamine produces attenuated natriuresis and diuresis in type 2 diabetics as compared to control subjects (Segers et al, 1996a). This study has suggested that there might be a defect in dopamine receptor and effector coupling resulting in the reduced ability of dopamine to inhibit sodium transporters causing diminished natriuresis and diuresis. This notion is supported by studies performed in obese Zucker rats (Hussain et al, 1999; Hussain et al, 2001). The present study confirms these findings.

Insulin influences not only the carbohydrate and lipid metabolism, but it is also a potent growth hormone that affects cellular transcription. Insulin has been shown to affect the functional response to a number of hormones by altering the receptor number or ligand affinity (Hu et al, 1996; Nickenig et al, 1998; Valiquette et al, 1995). It is possible that in type 2 diabetic patients, hyperinsulinemia might down-regulate the $\text{D}_1$ receptor and its function, which subsequently leads to a diminished natriuretic response to exogenously infused dopamine (Segers et al, 1996a). In the present study, treatment with rosiglitazone lowered plasma insulin by 50% of the control obese rats and the lowering plasma insulin in obese Zucker rats produced significant restoration of the ability of dopamine to inhibit the activity of both, $\text{Na}^+$, $\text{K}^+$-ATPase and $\text{Na}^+$, $\text{H}^+$-exchanger in proximal tubules. Since the basal activity of sodium transporters, especially of $\text{Na}^+$, $\text{K}^+$-ATPase was not affected by rosiglitazone treatment, the ability of dopamine to cause inhibition of $\text{Na}^+$, $\text{K}^+$-ATPase suggests the restored sensitivity of $\text{D}_1$ receptors and their responsiveness to dopamine.
Additionally, we also found that D₁ receptor numbers on proximal tubular membrane of the treated obese rats were normalized to the level seen in lean rats.

It is suggested that the Gq-PLC-PKC pathway is involved in D₁ receptor mediated inhibition of Na⁺, K⁺-ATPase (Satoh et al, 1993), whereas, D₁ receptor coupled Gs-cAMP-PKA pathway leads to the inhibition of Na⁺, H⁺-exchanger (Felder et al, 1990) in proximal tubules. A complete restoration of D₁ receptor number and a partial ability of dopamine to inhibit Na⁺, K⁺-ATPase suggests that Gq-PLC-PKC pathway is possibly not fully restored with rosiglitazone induced insulin sensitization. While the complete restoration of dopamine-mediated inhibition of Na⁺, H⁺-exchanger suggests that Gs-cAMP-PKA pathway is fully restored. It may be speculated that further lowering of the plasma insulin levels in obese rats would be required to restore the remaining deficiency in D₁ receptor function, as it relates to the Gq-PLC-PKC pathway. However, the mechanism of insulin that regulates D₁ receptors and associated signaling pathways that influence sodium transporters is yet to be determined.

Numerous studies have shown that obese Zucker rats develop high blood pressure (Felder et al, 1990; Alonso-Galicia et al, 1989; Kurtz et al, 1989). It has also been reported that the natriuretic response to volume expansion in obese rats is attenuated as compared to lean rats (Zeigler and Patel, 1991) suggesting impaired mechanisms responsible for natriuresis, including dopaminergic system. In the present study, we found that rosiglitazone treatment in obese Zucker rats caused an increase in urinary sodium excretion and a reduction in blood pressure compared with control obese rats. Significant restoration of renal dopamine receptor function may be a possible reason for the natriuresis and decrease in blood pressure observed in our study.
**Fig. M:** A possible link between Insulin action and renal dopamine receptor function in proximal convoluted tubule of kidney.
In summary, hyperinsulinemia causes down-regulation of D₁ receptor function in proximal convoluted tubules and lowering of plasma insulin levels leads to the restoration of renal D₁ receptor function and increase in sodium excretion. Thus, insulin seems to play a significant role in the regulation of renal functions through D₁ receptors.