Introduction
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Diabetes mellitus in rats as well as in human beings is associated with regionally specific changes in brain monoamines (Lackovic et al, 1990) and the turnover rate of monoamines is reportedly decreased in diabetic rats (Chen and Yang, 1991). Mental illness can be a manifestation of a diabetic brain state or a sort of ‘cerebral diabetes’ (Holden, 1995). Mania and positive schizophrenia have been reported to be associated with hyperglycemia, hyperdopaminergia, hyperserotonergia, whereas, depression and negative schizophrenia have been associated with hypoglycemia, hypodopaminergia and hyposerotonergia. This indicates link between diabetes mellitus and neurotransmitters like 5-HT and dopamine. The current project was initiated to explore this link further with respect to diabetes mellitus and insulin resistance.

The association between 5-HT and its role in glucose control has been a subject of controversy for last couple of decades. 5-HT is shown to have differential effects on blood glucose levels, that is, hyperglycemia and hypoglycemia. Earlier studies reported that 5-HT does produce hypoglycemia. For example, in mice, it has been demonstrated that 5-HT precursor 5-hydroxytryptophan (5-HTP) produces hypoglycemia, and the effects of 5-HT are due to formation of 5-HT (Furman and Wilson, 1980; Wilson and Furman, 1982; Endo, 1985). It was shown that 5-HTP causes accumulation of 5-HT in liver and 5-HT then causes increase in serum insulin levels resulting into hypoglycemia (Endo, 1985). 5-HT when injected in rats or dogs, is reported to produce hypoglycemia (Glagliardino et al, 1971; Lechin et al, 1975; Yamada et al, 1989). These studies and the studies involving incubation of isolated pancreatic islets with 5-HT revealed that 5-HT stimulates increase in insulin release from pancreatic islets (Lechin et al, 1975). This action of 5-HT was later shown to be
mediated through 5-HT$_1$ and 5-HT$_2$ receptors using specific antagonists like methysergide and ketanserin (Sugimoto et al, 1990). The \textit{in vivo} hypoglycemic action of 5-HT was seen in the dose range of 20-80 mg/kg when given intraperitoneally. However, during these early years some researchers reported 5-HT to produce hyperglycemia (Jacoby and Bryce, 1978; Botros and Saba, 1968). Sirek et al (1966) reported 5-HT as a hyperglycemic substance released by growth hormone. According to Jacoby and Bryce (1978), 5-HT at the doses of 5-25 mg/kg, i.p., produced increase in blood glucose in dose dependent manner in overnight fasted rats. It also increased insulin in dose independent manner. The changes in blood glucose and insulin by 5-HT were speculated to be secondary to epinephrine and/or norepinephrine release (Jacoby and Bryce, 1978).

During 90s, the workers who had earlier reported 5-HT-induced hypoglycemia, reported 5-HT-induced hyperglycemia at much lower doses (1-10 mg/kg, i.p.) in rats (Yamada et al, 1990). Both, centrally and peripherally acting 5-HT receptor agonists are reported to cause hyperglycemia. Centrally, 5-HT$_{1A}$ receptor agonist 8-hydroxy-2-di-n-(propylamine) tetralin (8-OH-DPAT) and 5-HT$_{1A}$ receptor partial agonists including buspirone and ipsapirone are reported to induce hyperglycemia in rats (Chaouloff and Jeanrenaud, 1987; Chaouloff et al, 1990a,b; Sugimoto et al, 1992). Similarly, 5-HT$_2$ receptor agonists like 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and 1-(3-chloro-phenyl) piperazine (mCPPP) are reported elicit hyperglycemia (Baudrie and Chaouloff, 1992; Sugimoto et al, 1996b). Like central 5-HT receptors, peripheral 5-HT receptors have shown to be involved in glycemic control. Peripheral administration of 5-HT or a peripheral 5-HT receptor agonist can elevate plasma glucose levels in rats (Baudrie and Chaouloff, 1992; Yamada et al, 1995; Sugimoto et al, 1996a). Peripherally acting 5-HT$_{1A/1D}$ receptor
agonist N, N-di-propyl-5-carboxamidotryptamine (DP-5-CT) also elicits hyperglycemia in rats (Laude et al, 1990). Moreover, inhibition of 5-HT reuptake by using fluoxetine and fluvoxamine produces hyperglycemia in rats (Yamada et al, 1990). These studies also showed that central or peripheral 5-HT receptor mediated hyperglycemia is associated with adrenaline release from the adrenal medulla as adrenomedullation has been reported to inhibit hyperglycemia.

Some of the studies ruled out the involvement of 5-HT$_3$ receptors in 5-HT induced hyperglycemia. Sugimoto et al (1996a) reported that peripheral 5-HT$_3$ receptor agonist 2-methyl-5-HT does not produce any effect on blood glucose, insulin or glucagons levels. Similarly, hyperglycemia produced by 5-HT receptor agonists 5-methoxytryptamine and 5-carboxamidotryptamine is not inhibited by specific 5-HT$_3$ receptor antagonist tropisetron (Yamada et al, 1997; Yamada et al, 1998). However, a recent study by Carvalho et al (2002) has shown the stimulation of central 5-HT$_3$ receptors by m-chlorophenylbiguanide (mCPBG) causes increase in blood glucose that is blocked by pretreatment with 5-HT$_3$ receptor antagonist ondansetron.

Considering the controversial reports on the effect of 5-HT on blood glucose, we decided to investigate the effect of 5-HT receptor antagonists sarpogrelate and ondansetron on blood glucose and to characterize the role of 5-HT receptors in 5-HT action.

As mentioned earlier, monoamine levels are affected in diabetes mellitus. Streptozotocin-induced diabetes is reported to decrease 5-HT precursor levels coupled with subsequent decrease in synthesis of 5-HT in brain (Manjarrez-Gutierrez et al, 2000; Chu et al, 1986; Crandall et al, 1981). Inversely, depletion of brain 5-HT with parachloro phenyl alanine (PCPA) causes inhibition of STZ-induced hyperglycemia. This effect is reported to be independent of pancreatic islets (Yang and Lin, 1995).
However, effect of STZ diabetes on brain 5-HT levels is also not devoid of controversies. According to some studies brain 5-HT levels are increased during STZ diabetes in rats (Lackovic and Salkovic, 1990; Lackovic et al, 1990). This makes the understanding of the effect of diabetic state on brain 5-HT more complicated.

Diabetic state also affects the responses of various isolated tissues to 5-HT. 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). This 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). However, in detrusor smooth muscle of STZ-diabetic rat bladder contractile response to 5-HT is increased significantly (Kodama and Takimoto, 2000). This effect is hypothesized to be related to smooth muscle hypertrophy and/or hyperplasia and it is indicated that this effect is mediated by activation of 5-HT$_{2A}$ receptors.

5-HT is one of the strongest inducers of platelet aggregation. 5-HT is not synthesized in platelets but is taken up from the circulation and stored in secretory granules. The main function of platelets is to plug holes in injured endothelial cells. Conversely, the functional integrity of endothelium is critical for the action of platelets (Furchgott and Vanhoutee, 1989). The endothelial surface is exposed to platelets, because the shear forces of the circulating blood favor centrifugal stratification of platelets (Gibbons and Dzau, 1994). Release of endothelium-derived relaxing factor antagonizes the vasoconstrictor action of 5-HT (Furchgott and Vanhoutee, 1989). The net effect of platelet aggregation is critically determined by the functional status of endothelium (Hawiger, 1992; Ware and Heistad, 1993). When platelets make contact with injured endothelium, they release substances that promote
platelet adhesion and release of 5-HT (Furchgott and Vanhoutee, 1989). 5-HT binding to platelet 5-HT$_{2A}$ receptors elicits an aggregation response that is markedly enhanced in the presence of collagen. Diabetes is frequently associated with coronary artery disease. Experimental studies indicate that blood platelets are involved in the development of atherosclerosis (Wolf, 1978; Lewis and Kottke, 1977; Sevitt, 1986). Platelets are activated and aggregate at the sites of coronary artery stenosis and endothelial injury (Bush et al, 1984; Ashton et al, 1986; Eidt et al, 1989). Activated platelets release 5-HT in substantial quantities causing vasoconstriction Ashton et al, 1986; Golino et al, 1989). 5-HT further causes platelet aggregation mediated through 5-HT$_2$ receptors. Increased platelet aggregation is a feature of diabetes and accordingly 5-HT levels are higher in diabetics (Barrads et al, 1988). 5-HT$_{2A}$ receptor antagonist sarpogrelate is reported to inhibit 5-HT induced platelet thrombus formation (Takano, 1995). Sarpogrelate is an effective antiplatelet aggregation agent in diabetic set up also. In patients with type 1 diabetes, antiplatelet therapy of sarpogrelate is a useful antithrombin therapy as it suppresses the production of intrinsic coagulants by activated platelets and decreases endothelial cell damage via adhesion molecules (Shouzu et al, 2000). Sarpogrelate is equally effective in type 2 diabetes as it is reported to be inhibitory to enhanced platelet aggregability under these conditions (Pietraszek et al, 1993).

Diabetes co-exists with incidences of heart failure and development of specific cardiomyopathy (Page and Watkins, 1977). 5-HT regulates cardiovascular function through central (McCall and Clement, 1994) as well as peripheral mechanisms (Lee and Wu, 1999; Sharma et al, 1999). 5-HT$_{2A}$ antagonist sarpogrelate is reported to inhibit vascular smooth muscle cell migration and proliferation induced by 5-HT (Sharma et al, 1999). The beneficial effects of 5-HT antagonists however, have not
been studied except for anti-thrombotic effects of sarpogrelate in diabetic situation. In diabetic cardiomyopathy myocardial glucose transport is reported to be defective (Katz et al, 1995). A reduction in the capacity of myocardial glucose transport can be detrimental to the diabetic patient during periods of increased myocardial work or ischemia that is common with diabetes when the demand for glucose uptake is increased (Hall et al, 1995). Glucose is carried across the plasma membrane by a family of glucose transporters including GLUT 1 and GLUT 4 (James et al, 1989). Myocardial GLUT 4 and GLUT 1 are also shown to be decreased in diabetes (Camps et al, 1992; Garvey et al, 1993; Stanley et al, 1994; Kainulainen et al, 1994). It is reported that GLUT 1 is recruited to plasma membrane by various types of glucose transport stimuli, including insulin (Fischer et al, 1995), 5-HT (Fischer et al, 1995), and catecholamines (Fischer et al, 1996). In view of the importance of peripheral 5-HT receptor antagonists in cardiomyopathy and higher platelet aggregability associated with diabetes mellitus, we studied the effect of chronic treatment with 5-HT$_{2A}$ antagonist sarpogrelate and 5-HT$_{3}$ antagonist ondansetron in STZ-diabetic rats with a view to project peripheral 5-HT receptors as a potential target for anti-diabetic drugs.

Like 5-HT, various evidences indicate an association of dopamine with diabetes at central as well as peripheral level. Various researchers have reported increase in brain dopamine levels during diabetes whereas, others have stated decrease in dopamine levels. According to Lackovic et al (1990), there is an increase in brain dopamine levels in diabetics. The possible reason for this may be a decrease in turnover rate of monoamines in diabetic rats (Chen and Yang, 1991). These findings are supported by an observation that dopamine metabolites dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) are reduced in straita of
hyperglycemia rats suggesting increases in dopamine levels in this brain region (Lirr. et al, 1994). This was further strengthened by Ramakrishnan et al (1996) as they found an increase in brain dopamine due to decrease turnover rate of monoamines. Earlier Trulson and Himmel (1983) found decrease in dopamine synthesis in diabetic rats, but still there was increase in dopamine levels in some areas of brain. This is explained on the basis of possible decrease in the monoamine oxidase enzyme activity (Mayanil et al, 1982) again hinting at lower turnover rate of monoamines in diabetes. Kamei and Saitoh (1997) reported increase in dopamine levels in mesolimbic system in brain resulting in hyperlocomotory activity in diabetic mice. These findings support the hypothesis of Holden (1995) that neuro-disorders are manifestation of diabetic brain state where dopamine levels vary in direct proportion with brain glucose levels. However, some results contradicting this hypothesis are also reported. According to Saller (1984), dopaminergic activity is decreased in non insulin dependent diabetes mellitus. Here it is interesting to note that NIDDM that is associated with hyperglycemia and hyperinsulinemia, also presents a situation of hypodopaminergia. Insulin induced hypoglycemia is reported to increase stratal as well as adrenal dopamine content (Toner and Stamford, 1997; Gripois et al, 1987). Dopamine $D_2$ receptor antagonist haloperidol is reported to produce potentiation of 2-deoxy D-glucose induced hyperglycemia suggesting decreased dopaminergic activity in hyperglycemic state (Baudrie and Chaouloff, 1991). Decrease in dopamine levels and dopaminergic neuronal activity in striatum and limbic system is also reported in STZ-diabetic as well as genetically diabetic bio breed (BB) rats (Murzi et al, 1996; Kono and Takada, 1994).

Chronic diabetic state is accompanied by alteration in receptor density as well as receptor sensitivity of various receptor systems and these alterations are thought to
play some definite role in complications associated with diabetes mellitus. Few of the researchers have discussed the status of mainly central dopaminergic receptor system in diabetic state. However, again as with 5-HT, reports appear to contradict with each other. Kirouac and Ganguly (1993) reported an upregulation of brain D₁ receptors in spontaneously hypertensive rats with established hypertension. Lim et al (1994) reported that affinity of striatal D₁ receptors is significantly increased without changes in number of receptors, while up regulation of D₂ receptor occurs without changes in affinity in STZ diabetic rats. However, Salkovic and Lackovic (1992) reported decrease in D₁ receptor number in alloxan-induced diabetic rats. So from the available reports if one has to believe in view of the majority of researchers, it can be hypothesized that brain dopamine levels are higher in diabetic rats that results in down regulation of dopaminergic receptor system either by decrease in number or by affinity of receptors at the central level. However, status of peripheral dopaminergic receptor system in diabetes is not known yet. So, we decided to study the effect of peripherally administered dopamine on blood glucose and to characterize the dopaminergic receptors in the effect of dopamine on blood glucose.

Peripheral dopaminergic system is reported to be involved in regulation of lipid and glucose metabolism. D₁ and D₂-like receptor agonists cause reduction in basal lipolysis as well as reduction in lipoprotein lipase (LPL) activity (Cincotta et al, 1995). In the liver, these dopamine receptor agonists are reported to decrease glucose-6-phosphatase (G6P-ase) activity and reduction in phosphoenolpyruvate carboxykinase (PEPCK) activity (Scislowski et al, 1999). Dopamine receptor agonists also decrease body weight by increasing oxygen consumption and reducing de novo lipogenesis (Scislowski et al, 1999). Dopamine D₁ receptor agonist SKF 38393 and dopamine D₂ receptor agonist bromocriptine alone or in combination are reported to
ameliorate obesity and related metabolic dysfunction like hyperglycemia, lipid profile, islet dysfunction in obese ob/ob or diabetic db/db mice (Liang et al, 1998a,b; Cincotta et al, 1997; Cincotta et al, 1999; Scislowski et al, 1999). Jetton et al (2001) reported that dopaminergic agonist attenuates hyperglycemia and hyperlipidemia in ob/ob mice by improving aberrations in the beta cell’s glucose sensing apparatus. This enhances insulin storage and/or retention and stabilizes hyperplasia and thus reduces basal insulin levels.

Kidney can be considered as one of the most important peripheral target organ for the action dopamine. The synthesis of dopamine has been shown in kidney (Hussain and Lokhandwala, 1998). Within kidney, by ligand binding, autoradiographic studies, microanatomical localization, the presence of D_{1A}, D_{1B}-D_{1} like and D_{2}, D_{3} – D_{2} like receptors have been shown (Felder et al, 1989a; Lokhandwala and Amenta, 1991; Yamaguchi et al, 1993; Amenta et al, 1999). Dopamine causes renal vasodilation, increases cortical and medullary blood flow and enhances glomerular filtration rate. Moreover, dopamine causes diuresis and natriuresis by inhibiting renal sodium reabsorption (Glodberg, 1972; Lokhandwala and Hegde, 1989a; Lokhandwala and Amenta, 1991). The D_{1}-like dopamine receptors cause stimulation of adenylyl cyclase and phospholipase C via G_{s} and G_{q/11} proteins that leads to the inhibition of the sodium transporting proteins Na^{+}, H^{+}-exchanger and Na^{+}, K^{+}-ATPase in the proximal tubules of the kidneys (Felder et al, 1990; Vyas et al, 1992a). It has been reported that alteration in sodium homeostasis leads to increased sodium retention in diabetes (O’Hare et al, 1986, DeChatel et al, 1977). The increased sodium retention is suggested to play an important role in development of hypertension in diabetic patients (Feldt-Rasmussen et al, 1987). Diabetic nephropathy is one of the major complications associated with diabetes mellitus. Renal production
of dopamine is reduced in both type 1 (insulin dependent) and type 2 (non-insulin dependent) diabetes mellitus (Carranza et al, 2001; Tsuchida et al, 2001) and this reduced dopamine production is associated with an increase in total body sodium and impaired ability to excrete sodium load (Segars et al, 1995; Segars et al, 1996). The D₁-like receptor binding sites are reported to be reduced in obese Zucker rats which show distinct insulin resistance that is also a feature of type 2 diabetes mellitus. There is diminished activation of G-proteins by dopamine that accounts for reduced inhibition of Na⁺, H⁺-exchanger and Na⁺, K⁺-ATPase proteins (Hussain et al, 1999).

According to the available literature, it is clear that dopaminergic system plays a major role in regulation of glucose and lipid metabolism. The treatment with dopaminergic agonists causes restoration of this metabolism which is imbalanced in diabetic state to a great degree. The treatment with these agonists also normalizes the insulin function possibly through improving insulin sensitivity, but the direct physiological evidence for this is still not available. On the other hand, sodium retention is observed in diabetic rats that is due to loss of renal dopamine function that is in turn because of insulin resistance. Considering these facts, we decided to study the effect of dopaminergic agonist fenoldopam on glucose and insulin profile in STZ-diabetic rats and its effect on diabetic renal function.