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Although the association between diabetes mellitus and stress is well known, the correlation of diabetes mellitus with the principal neurotransmitters of stress namely 5-hydroxytryptamine (5-HT) and dopamine (DA) has not been studied systematically. The work in the present thesis is an attempt to explore the role of 5-HT and dopamine in diabetes mellitus and associated cardiovascular and renal complications.

Effect of 5-HT on glucose levels has been reported but is a subject of controversy. It is reported to produce both hypoglycemia and hyperglycemia in normoglycemic rats and mice. With the availability of specific 5-HT modulators and discovery of various 5-HT receptor subtypes, some of the researchers concluded that 5-HT produces hyperglycemia acting via 5-HT₁A, 5-HT₂A and 5-HT₂C receptors. However, there have been certain discrepancies in the methodology with respect to receptor subtypes. As far as dopamine is concerned, its direct effect on blood glucose levels has not been reported so far to our knowledge. In the first step we studied the effect of 5-HT and dopamine and their specific agonists and antagonists on blood glucose in normoglycemic rats.

5-HT or dopamine (1mg/kg and 2mg/kg) or their modulators were injected intraperitoneally in normoglycemic rats in different experimental sets to study their effect on blood glucose. Various 5-HT modulators used to study the interaction with 5-HT were buspirone (15mg/kg), α-methyl 5-HT (2mg/kg), 1-phenyl-biguanide (2mg/kg), pindolol (5mg/kg), sarpogrelate (1mg/kg), mianserin (10mg/kg), ondansetron (0.5mg/kg) and cisapride (2mg/kg). We also studied the interaction of fluoxetine (5mg/kg) and yohimbine (2mg/kg) with 5-HT-induced changes in serum glucose. Various dopamine modulators used to study the interaction with dopamine
were fenoldopam (1mg/kg), bromocriptine (2mg/kg), butaclamol (2mg/kg) and sulpiride (2mg/kg). We also studied the effect of levodopa (100mg/kg and 300mg/kg, orally) and its combination with carbidopa (10mg/kg, orally) on blood glucose in normoglycemic rats. Blood samples were drawn through tail vein before injection and then at 30, 60, 120 min time interval. The samples were subjected to glucose analysis.

5-HT produced dose dependent hyperglycemia in normoglycemic rats with decrease in insulin levels. 5-HT reuptake inhibitor fluoxetine potentiated 5-HT-induced hyperglycemia. 5-HT1A partial agonist buspirone potentiated 5-HT-induced hyperglycemia at a high dose of 15mg/kg, whereas, 5-HT1A receptor antagonist pindolol did not modify it suggesting no role of 5-HT1A receptor in the action of 5-HT on blood glucose. 5-HT2 agonist α-methyl 5-HT potentiated 5-HT-induced hyperglycemia, whereas, 5-HT2A antagonist sarpogrelate and 5-HT2A/2C antagonist mianserin inhibited it. Similarly, 5-HT3 agonist 1-phenyl biguanide potentiated 5-HT-induced hyperglycemia, whereas, 5-HT3 antagonist ondansetron inhibited it suggesting that 5-HT-induced hyperglycemia is mediated through 5-HT2A/2C and 5-HT3 receptors. 5-HT4 agonist cisapride did not affect 5-HT-induced hyperglycemia ruling out involvement of these receptors in the action of 5-HT on blood glucose.

Various studies have proposed that release of adrenaline from adrenal medulla may be one of the possible mechanisms involved in 5-HT-induced hyperglycemia. Adrenaline acts via α2 receptors located on β cells of pancreas to produce a decrease in insulin release and thereby causes an increase in blood glucose levels. To test this hypothesis, we studied the interaction of α2 adrenoceptor antagonist yohimbine with 5-HT. It was found that yohimbine significantly inhibited 5-HT-induced hyperglycemia. To investigate further, rats were adrenalectomized bilaterally and 5-
HT (1mg/kg) was injected 72 hrs after the surgery. 5-HT-induced hyperglycemia was inhibited significantly but partially in adrenalectomized rats.

In nutshell, results of our studies on characterization of 5-HT receptors on glycemic control suggest that (a) 5-HT induces hyperglycemia that is associated with decrease in insulin levels in normoglycemic rats involving 5-HT2A/C and 5-HT3 receptors. (b) Stimulation of adrenergic system may be one of the mechanisms for the action of 5-HT-induced hyperglycemia.

Like 5-HT, dopamine also produced hyperglycemia at the dose of 1 mg/kg and 2mg/kg in normoglycemic rats with decrease in insulin levels. Pretreatment with DA1 agonist fenoldopam and SKF 38393, DA2 agonist bromocriptine, DA1 antagonist butaclamol and DA2 antagonist sulpiride neither altered per se glucose levels nor produced any effect on DA-induced hyperglycemia. It is thus possible that DA-induced hyperglycemia may not involve directly the peripheral dopamine receptors, but produces hyperglycemia after being converted into nor-adrenaline or adrenaline. To check the possible involvement of central DA receptors we studied the effect of levodopa on blood glucose. It was found in our studies that levodopa produces dose dependent increase in blood glucose. Carbidopa potentiated 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.

Since, we found that 5-HT and DA receptor stimulation produces hyperglycemia, 5-HT and DA antagonists may be presumed to prevent hyperglycemia induced by streptozotocin (STZ). Brain monoamine levels are affected in diabetes mellitus. STZ induced diabetes is reported to alter 5-HT levels in various areas of brain and affects the responses of various peripheral tissues to 5-HT. Based on the
results of characterization of 5-HT receptors, we selected 5-HT2A antagonist sarpogrelate, 5-HT3 antagonist ondansetron and D1 like receptor agonist fenoldopam to study the effect of chronic treatment with these drugs on various parameters in streptozotocin (STZ)-induced type 1 and type 2 diabetic rats.

To induce insulin dependent diabetes (type 1), STZ (45mg/kg) dissolved in saline was injected in Wistar/Sprague Dawley rats through tail vein. Animals showing blood glucose level more than 140mg/dl, 48 hr after the STZ injection, were selected for the experiment. Animals were divided into four groups with six animals in each group: non-diabetic control (NDCon), non-diabetic treated (NDTr), diabetic control (DCon) and diabetic treated (DTr). Treatment groups received sarpogrelate, ondansetron or fenoldopam (1mg/kg, i.p.) dissolved in distilled water daily for six weeks. Non insulin dependent diabetes (type 2) was induced by injecting STZ intraperitoneally at the dose of 90mg/kg dissolved in normal saline to two day old Wistar/Sprague Dawley pups. Pups were weaned at three weeks of age and differentiated by sex. At ten weeks of age, glucose levels were estimated in 18 hour fasted rats and animals showing blood glucose more than 140 mg/dl were selected for study. Age matched Wistar/Sprague Dawley rats were maintained as non-diabetic controls. Four groups were made as: non-diabetic control (NDCon), non-diabetic treated (NDTr), diabetic control (DCon) and diabetic treated (DTr). Treatment groups received sarpogrelate, ondansetron or fenoldopam (1mg/kg, i.p.) dissolved in distilled water daily for six weeks.

At the end of six-week treatment, blood samples were collected from the tail vein into centrifuge tubes and serum was separated. Serum samples were analyzed spectrophotometrically for glucose, cholesterol, triglyceride, creatinine, and urea. Serum insulin levels were estimated by radioimmunoassay method. To study the
peripheral glucose disposal, oral glucose tolerance test (OGTT) was performed. Glucose was administered orally at the dose of 1.5g/kg to 18 hr fasted rats. Blood samples were taken at 0, 30, 60 and 120 min time intervals and curve was plotted using either glucose concentration or insulin concentration versus time and areas under the curve for glucose (AUC_{glucose}) and insulin (AUC_{insulin}) were calculated respectively. To evaluate peripheral insulin sensitivity, insulin at the dose of 0.2IU/100g body weight was administered through tail vein to 6 hr fasted rats. Blood samples were taken at 0, 10, 20 and 30 min time intervals and blood glucose was measured. Insulin sensitivity index (K_{ITT}) was calculated using a formula \( K_{ITT} = \frac{0.693/t_{1/2 \text{ (glucose)}}}{100} \). Blood pressure was recorded by tail-cuff method using Harvard blood pressure monitor.

STZ produced loss of body weight, hyperphagia, polydypsia, hyperglycemia, hypoinsulinemia, increased serum cholesterol and triglyceride levels and hypertension in type 1 diabetic rats. Treatment with sarpogrelate or ondansetron did not change STZ-induced loss of body weight, increase in food intake and water intake of diabetic rats. Treatment with sarpogrelate or ondansetron significantly decreased fasting serum glucose and increased serum insulin levels. However, 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). Diabetic rats showed significant increase in AUC_{glucose} and decrease in AUC_{insulin} during OGTT. Treatment with sarpogrelate or ondansetron significantly decreased AUC_{glucose} (33% and 24% respectively) however, AUC_{insulin} was not significantly altered. Insulin sensitivity index (K_{ITT}) was not different in diabetic rats as compared to non-diabetic rats. STZ produced hypercholesterolemia and hypertriglyceridemia in diabetic rats. Treatment with sarpogrelate or ondansetron
significantly decreased the total cholesterol (21% and 19% respectively). In addition, ondansetron treatment also significantly decreased serum triglycerides (28% with ondansetron and 16% with sarpogrelate). The decrease in lipid levels in diabetic rats may possibly be due to increase in insulin levels seen with the treatment of sarpogrelate and ondansetron. Sarpogrelate and ondansetron significantly lowered blood pressure in diabetic rats. 5-HT levels are higher in diabetics and 5-HT is reported to inhibit basal as well as glucose mediated insulin release by affecting early steps in the beta cell stimulus-secretion coupling. Sarpogrelate and ondansetron being 5-HT antagonists possibly act as insulin releasers by inhibiting insulin action. The beneficial effects observed on glucose and lipid levels and blood pressure are possibly due to their action as insulin releasers.

Diabetes coexists with heart failure and development of cardiomyopathy. Platelets are involved in the development of coronary artery disease with which diabetes is frequently associated. 5-HT is one of the strongest inducers of platelet aggregation. Beneficial effects of sarpogrelate have been correlated with reduction in platelet aggregation in both, type 1 and type 2 diabetes mellitus. Demand for glucose uptake is increased and there is defective myocardial glucose transport in diabetic heart. In view of the importance of 5-HT receptors in cardiomyopathy and higher platelet aggregability associated with diabetes mellitus, we studied the effect of sarpogrelate (5mg/kg, orally for 6 weeks) on left ventricular developed pressure (LVDP), heart rate, the rate of ventricular pressure development (+dP/dt) and the rate of ventricular pressure decline (-dP/dt) in STZ-diabetic rats. STZ diabetes produced cardiac dysfunctions as reflected by significant decrease in heart rate, LVDP, +dP/dt and −dP/dt. Sarpogrelate treatment significantly prevented STZ-induced alteration in LVDP, heart rate, +dP/dt and −dP/dt.
As mentioned earlier, impaired glucose uptake by diabetic heart is associated with diabetic cardiomyopathy. We studied effect of sarpogrelate on glucose transporters GLUT 1 and GLUT 4 in plasma membrane and cytosol of the cardiomyocytes obtained from diabetic rats by Western blotting. There was a significant decrease in protein levels of GLUT 1 and GLUT 4 in the cardiac membrane from STZ-diabetic rats. Insulin partially prevented STZ-induced decrease in GLUT 4. However, sarpogrelate produced increase in both GLUT 4 and GLUT 1 levels in cardiac membrane. In the cardiac cytosol, STZ-diabetes did not produce any change in GLUT 1 but there was an increase in GLUT 4 level. Treatment with sarpogrelate produced increase in protein levels of both GLUT 4 and GLUT 1 in STZ-diabetic rats. These results indicate that 5-HT2A receptors are involved in glucose transport mechanisms and increase in glucose transporters in cardiomyocytes by sarpogrelate may be independent of insulin. It is also possible that 5-HT2A receptor inhibition causes an increase in the biogenesis of glucose transporters and hence enhancing the glucose transport in cardiomyocytes.

STZ-induced type 2 diabetic animals showed hyperglycemia, hyperinsulinemia, increased levels of serum cholesterol and triglyceride and high blood pressure that are characteristics of type 2 diabetes mellitus. Treatment with sarpogrelate and ondansetron did not produce significant effect on these values. Diabetic rats showed significantly higher AUC_{glucose} and AUC_{insulin}. Sarpogrelate significantly decreased AUC_{insulin}. Diabetic rats also showed significant decrease in insulin sensitivity as depicted by decrease in K_{ITT}. It was not altered with the treatment with sarpogrelate and ondansetron. As seen from the results of type 1 diabetic rats, sarpogrelate and ondansetron are suggested to act by releasing insulin. Sarpogrelate and ondansetron do not improve insulin sensitivity as seen from K_{ITT}. 

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These drugs failed to produce beneficial effects in non insulin dependent diabetes, a condition of hyperinsulinemia and where insulin sensitizers not releasers are effective.

In conclusion, 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.

Like 5-HT, dopamine is also associated with glycemic control and diabetes mellitus. Decreased central and peripheral dopaminergic activity is reported in various animal models of diabetes mellitus. According to some studies, dopaminergic D$_1$ and D$_2$ like receptors are up regulated with or without changes in their number and affinity in diabetes and hypertension, whereas, decreased D$_1$ like receptor number is also reported in alloxan induced diabetic rats. Peripheral dopaminergic system is involved in the regulation of glucose and lipid metabolism. D$_1$ and D$_2$ like dopamine receptor agonists cause reduction in basal lipolysis as well as inhibition of activities of various key enzymes regulating glucose and lipid metabolism like glucose-6-phosphatase, lipoprotein lipase, phosphoenol pyruvate carboxykinase (PEPCK). Renal dopaminergic system is involved in regulation of renal sodium secretion. Renal sodium absorption is increased in diabetes mellitus and this is a contributing factor to hypertension associated with diabetes. D$_1$ agonists are reported to reduce blood pressure in hypertensive rats owing to natriuresis. Considering the available literature, we studied the effect of D$_1$ receptor agonist fenoldopam on metabolic and renal parameters in STZ-induced type 1 and type 2 diabetic rats.
In our studies, fenoldopam treatment did not produce change in STZ-induced hyperphagia, polydypsia and loss of body weight in adult rats. Treatment with fenoldopam significantly decreased glucose levels in diabetic rats, however, insulin levels were not affected with the treatment. Diabetic rats showed increase in AUC_{glucose} and decrease in AUC_{insulin} during OGTT. Fenoldopam did not significantly alter these values. Insulin sensitivity index was not affected in diabetic rats. STZ produced hyperlipidemia in rats that was unchanged with fenoldopam treatment. These results suggest that fenoldopam does not affect the hypoinsulinemic status of type 1 diabetic rats and thus fails to improve metabolic functions in these animals.

Peripheral dopaminergic system is reported to be linked with insulin sensitivity. Dopaminergic agonists like D_1 receptor agonist SKF 38393 and D_2 receptor agonist bromocriptine produce beneficial effects on glucose and lipid profile in insulin resistant ob/ob and db/db mice and conversely, insulin resistant animals have loss of dopamine function. Similarly, insulin resistant obese Zucker fa/fa rats show loss of renal dopamine function. In our studies with type 2 diabetic rats, treatment with fenoldopam significantly decreased serum glucose, insulin, AUC_{glucose}, serum cholesterol, triglyceride and blood pressure. Diabetic rats showed decreased K_{ITT} indicating reduced insulin sensitivity that was significantly increased by fenoldopam. The increase in insulin sensitivity is possibly responsible for improved glucose and lipid profile as well as decrease in blood pressure in fenoldopam treated diabetic rats.

As mentioned earlier, kidney is one of the most important organs for dopamine action. Dopamine is synthesized locally in kidney. It acts via D_1 receptors to produce renal vasodilatation, increase in glomerular filtration rate, diuresis and natriuresis. Alteration in sodium homeostasis leads to sodium retention in diabetes.
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mellitus. Increased sodium retention plays an important role in development of hypertension in diabetics. Renal dopamine production is reduced in type 1 and type 2 diabetes mellitus that is associated with an increase in total body sodium and impaired ability to excrete sodium.

To assess renal function, urine samples were collected over a period of 12 hours by placing animals in metabolic cages. Urinary sodium content was measured by using flame photometry. Animals were sacrificed and kidneys were quickly excised from all groups and washed with cold saline to remove as much of the blood as possible. Kidneys were dried on filter paper and dry weight was recorded immediately. Kidneys were used for estimation of DNA and RNA. STZ produced an increase in urinary volume, urinary sodium, serum urea and creatinine indicating deteriorated kidney function in type 1 diabetic rats. We also found an increase in kidney weight to body weight ratio, decrease in total DNA and increase in total RNA content of the kidney indicating renal hypertrophy. Fenoldopam treatment produced significant increase in urine volume and urinary sodium thus decreasing sodium retention. Fenoldopam treatment produced decrease in blood pressure of diabetic rats that may be a direct result of decreased sodium retention. Fenoldopam treatment significantly decreased serum urea and creatinine in diabetic rats. In addition, significant decrease in kidney weight/body weight ratio and increase in total DNA was recorded in diabetic rats treated with fenoldopam. These results suggest improvement in deteriorated renal function with fenoldopam treatment. Similar to type 1 diabetic rats, type 2 diabetic rats also showed increase in serum urea and creatinine levels and decrease in urinary sodium content. Fenoldopam treatment produced significant decrease in serum urea and creatinine in type 2 diabetic rats also. Fenoldopam treatment significantly increased urinary volume and urinary sodium
content in diabetic rats indicating decreased sodium retention. We hypothesize that *peripheral dopamine function and insulin resistance are inversely proportional* and insulin resistant state leads to loss of peripheral dopamine function.

To test this hypothesis, we studied the effect of insulin sensitizer rosiglitazone (10 mg/kg, orally for 4 weeks) on renal dopamine function of obese Zucker fa/fa rats. Animals were divided into three groups, namely obese control (ObCon), obese treated with rosiglitazone (ObTr) and age matched lean control rats (Lean). At the end of the treatment, urine samples were collected and analyzed for urinary sodium and potassium using flame photometry. Blood pressure was measured by catheterizing aorta. Blood samples were collected from aorta for measurement of blood glucose and plasma insulin. Kidneys were excised and digested in hyaluronidase and collagenase enzyme mixture. Renal proximal tubular cells were isolated using ficoll gradient method. The proximal tubular suspension was used to measure Na\(^+\), K\(^+\)-ATPase activity spectrophotometrically and Na\(^+\), H\(^+\)-exchanger activity fluorometrically using BCECF dye. The tubules were further used for membrane preparation and radioligand binding was performed using \(^{[3]H}\) SCH 23390 for D\(_1\) receptors by generating saturation isotherm. Binding studies were used to obtain D\(_1\) receptor density and affinity. Obese Zucker rats showed significantly higher serum glucose, insulin and blood pressure values as compared to age matched lean rats. Urinary volume was significantly higher in obese rats as compared to lean rats and radioligand studies revealed decreased D\(_1\) receptor density and affinity in proximal convoluted tubules of obese rats. Treatment with rosiglitazone significantly decreased glucose, insulin and blood pressure in obese rats. This was associated with increased urinary sodium. In the isolated proximal tubules obtained from untreated lean rats, dopamine caused concentration-dependent inhibition of Na\(^+\), K\(^+\)-ATPase and Na\(^+\), H\(^+\)-exchanger.
activities but this inhibitory effect was absent in untreated obese rats along with decrease in D₁ receptor number and affinity. Rosiglitazone treatment significantly restored the inhibitory effect of dopamine on Na⁺, K⁺-ATPase and Na⁺, H⁺-exchanger in obese rats. This was accompanied by a complete restoration of D₁ receptor numbers in proximal tubular membranes of treated obese rats though the receptor affinity did not alter significantly. Our data suggest a direct role of insulin in D₁ receptor regulation. It is concluded that hyperinsulinemia causes down-regulation of D₁ receptor function and improvement in insulin sensitivity leads to the restoration of renal D₁ receptor function and increase in sodium excretion.

Thus, our study suggests 5-HT/ DA receptors as a future target for newer antidiabetic drugs with particular reference to cardiovascular complications and renal dysfunction.