Glucose is the primary source of fuel for the cells of the brain. Brain is dependent on a continuous supply of glucose diffusing from the blood into the interstitial tissue within the central nervous system and into neurons themselves. The physiological concentration of glucose in blood is maintained through highly regulated systemic mechanisms. Changes in glucose levels elicit a complex neuroendocrine response that prevents or rapidly corrects hyper or hypoglycaemia. Diabetes Mellitus is a chronic metabolic disorder resulting in hyperglycaemia (high plasma glucose level). Hypoglycaemia (low level of plasma glucose) is a common adverse effect of insulin treatment in individuals with diabetes. It is one of the most common and serious stress conditions challenging the body homeostasis. Severe hypoglycaemia with cognitive dysfunction is three times more common in intensively, rather than conventionally, treated insulin-dependent diabetes mellitus (IDDM) (Maran et al., 1995). When glucose levels fall below threshold glycaemic levels, neuroendocrine, autonomic nervous system (ANS) and metabolic glucose counter regulatory mechanisms are activated. These hypoglycaemic counter regulatory mechanisms can be blunted irreversibly by disease duration or by acute episodes of prior stress (Ertl & Davis, 2004). Although hypoglycaemia is associated with a number of physiological changes, the most profound effects are seen in the brain, where glucose is the major substrate for energy metabolism. Lack of glucose produces brain damage or even death if the deficit is prolonged.

The central nervous system (CNS) neurotransmitters play an important role in the regulation of glucose homeostasis. These neurotransmitters mediate rapid
intracellular communications not only within the central nervous system but also in
the peripheral tissues. They exert their function through receptors present in both
neuronal and non-neuronal cell surface that trigger second messenger signaling
pathways (Julius et al., 1989). Neurotransmitters have been reported to show
significant alterations during hyperglycaemia resulting in altered functions causing
neuronal degeneration (Bhardwaj et al., 1999). Chronic hyperglycaemia during
diabetes mellitus is a major initiator of diabetic micro-vascular complications like
retinopathy, neuropathy and nephropathy (Sheetz & King, 2002). Glucose processing
uses a variety of diverse metabolic pathways. Chronic hyperglycaemia can induce
multiple cellular changes leading to metabolic disorders.

Hypoglycaemia during insulin therapy causes damage to the brain specifically
because that is the organ which cannot withstand glucose deficiency. The functional
capability of the brain will deteriorate due to the frequent hypoglycaemic shock. If
glucose supply to the brain is not maintained, there is a decrease in cerebral electrical
activity, membrane breakdown with release of free fatty acids and altered amino acid
metabolism, including increased production of glutamate. Pathological studies in
humans and animals show that hypoglycaemia-induced neuronal death occurs
preferentially in the hippocampus, superficial layers of the cortex and striatum (Auer
et al., 1988; Auer & Siesjo, 1993; Auer, 2004). Because of the extensive neuronal
loss, one of the neurological sequelae associated with hypoglycaemia is cognitive
decline. According to clinical studies, significant learning and memory deficits
correlate with the frequency of hypoglycaemia not only in patients with type 1
diabetes, but also in the relatively younger group among the population with type 2
diabetes (Dey et al., 1997). Acute neuroglycopenia causes rapid deterioration of
cognitive function in humans with and without diabetes. Numerous clinical studies
suggest that intensive insulin treatment of type 1 diabetes is associated with an increased frequency of hypoglycaemic coma (Hannonen et al., 2003; Rovet, 1999) and cognitive impairment (Ryan et al., 1985; Langan et al., 1991; Wredling et al., 1990).

Glutamate, which is one of the excitatory amino acid neurotransmitters found only in the central nervous system, is believed to play a major role in the pathophysiology of hypoglycaemic brain injury. Numerous reports have documented that excessive glutamate, through NMDA/AMPA receptors, activate the excitotoxic process, which play an important role in the hypoglycaemic brain damage (Choi et al., 1998; Lipton & Nicotera, 1998; Duchen, 2000). Excess activation of NMDA receptors by glutamate increases cytoplasmic concentrations of sodium and calcium to levels that exceed the capacity of neuronal homeostatic mechanisms, thereby altering transmembrane ion gradients. Hypoglycaemia specifically increases the sensitivity of NMDA receptors to activation by glutamate, which results in a lower threshold for glutamate induced excitotoxicity (Jane et al., 1999).

Dopamine (DA), a major neurotransmitter in central nervous system is involved in the control of both motor and emotional behaviour (Vallone et al., 2000) and peripherally modulates insulin secretion in the pancreatic islets (Nogueira et al., 1994). DA is synthesised from tyrosine, stored in vesicles in axon terminals and released when the neuron is depolarised. DA interacts with specific membrane receptors to produce its effects. These effects are terminated by reuptake of dopamine into the presynaptic neuron by a dopamine transporter or by metabolic inactivation by monoamine oxidase B (MAO-B) or catechol-O-methyltransferase (COMT). DA plays an important role both centrally and peripherally. The recent identification of five dopamine receptor subtypes provides a basis for understanding dopamine's
central and peripheral actions. DA receptors are classified into two major groups: DA D₁ like and DA D₂ like. DA D₁ like receptors consists of DA D₁ and DA D₅ receptors. DA D₂ like receptors consists of DA D₂, DA D₃ and DA D₄ receptors. Stimulation of the DA D₁ receptor gives rise to increased production of cAMP. DA D₂ receptors inhibit cAMP production, but activate the inositol phosphate second messenger system (Seeman, 1980). An imbalance between dopaminergic neurotransmission and DA receptors is known to be associated with the symptomatology of numerous neuropsychiatric disorders, like schizophrenia, psychosis, mania and depression as well as neuropathological disorders, like Parkinson's disease and Huntington's disease (Carlsson, 1988, 1993; Bermanzohn & Siris, 1992; Brown & Gershon, 1993; Jakel & Maragos, 2000; Kostrzewa & Segura-Aguilar, 2003). Hyperglycaemia during diabetes is reported to damage dopaminergic functions. The progression of diabetes is associated with an impaired ability of the neurons in the CNS to release neurotransmitters resulting in behavioural changes (Broderick & Jacoby, 1989). The dopaminergic cells in particular are highly sensitive to excitotoxicity and oxidative stress when the energy metabolism is impaired (Callahan et al., 1998).

Despite our advances in the treatment of diabetes, hypoglycaemic episodes are often the limiting factor in achieving optimal blood sugar control. Recent therapeutic strategies aimed at closely controlling elevated glucose levels in diabetic individuals put them at risk for experiencing episodes of hypoglycaemia. Acute and recurrent hypoglycaemia cause transient or persistent alteration of cognitive functions, and can result in seizures or coma. The effects of acute or recurrent episodes of hypoglycaemia on the cells of the CNS are potentially harmful, and impose long-lasting damaging effects on the brain. The pathogenesis of hypoglycaemia induced nerve cell injury is largely unknown, but mechanisms that could result in damage to
cells of the CNS include excitotoxicity related to a dysregulation of the glutamate-glutamine cycle. To understand the effects of hypoglycaemia on the cells of the CNS, it is essential to characterize the response of CNS cells to reduced glycemic levels, to determine the extent of CNS cell injury induced by hypoglycaemia and to identify the mechanisms involved in hypoglycaemia induced cell or tissue damage in brain. The reports so far stated did not attempt to emphasis the functional correlation of dopaminergic and glutamergic receptors in hypoglycaemic and hyperglycaemic brain.

In the present study a detailed investigation on the alterations of dopamine and its receptors in the brain regions of streptozotocin induced diabetic and insulin induced hypoglycaemic rats were carried out. Glutamate receptor, NMDAR1 gene expression in the hypoglycaemic and hyperglycaemic brain was also studied. EEG recording in hypoglycaemic and hyperglycaemic will be carried out to measure brain activity. In vitro studies on glucose uptake and insulin secretion, with and without specific antagonists were carried out to confirm the specific receptor subtypes - DA D₁, DA D₂ and NMDA involved in the functional regulation during hyperglycaemic and hypoglycaemic brain damage. The molecular studies on the brain damage through dopaminergic and glutamergic receptors will elucidate the therapeutic role in the corrective measures of the damage to the brain during hypoglycaemia and hyperglycaemia. This has importance in the management of diabetes and anti-diabetic treatment for better intellectual functioning of the individual.
OBJECTIVES OF THE PRESENT STUDY

1. To quantify dopamine (DA) and homovanillic acid (HVA) content in the brain regions - hippocampus (Hippo), brainstem (BS), cerebral cortex (CC) and corpus striatum (CS) - of control and experimental rats using High Performance Liquid Chromatography (HPLC).

2. To study DA, DA-D_1, DA-D_2 receptors changes in the brain regions - Hippo, BS, CC and CS - of control and experimental rats.

3. To study DA- D_1 and DA-D_2 receptor gene expression in the brain regions - Hippo, BS, CC and CS of control and experimental rats using Real-Time PCR.

4. To study glutamate dehydrogenase (GDH) and malate dehydrogenase (MDH) activity in the brain regions - BS and CC - of control and experimental rats.

5. To study glutamate receptor, NMDAR1 gene expression in the brain regions - Hippo, BS, CC and CS of control and experimental rats.

6. To study the neurophysiological analysis of electrical activity in the frontal lobe of control and experimental rat brain using EEG recorder.

7. To study the role of DA, DA-D_1 and DA-D_2 receptors on glucose uptake by pancreatic islets *in vitro*. 
8. To study the role of glutamate and NMDA receptors on glucose uptake by pancreatic islets *in vitro*.

9. To study the role of DA, DA-D₁ and DA-D₂ receptors on glucose induced insulin secretion by pancreatic islets *in vitro*.

10. To study the role of glutamate and NMDA receptors on glucose induced insulin secretion by pancreatic islets *in vitro*. 