**Introduction**

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. Diabetes Mellitus is a chronic metabolic disorder resulting in hyperglycaemia - high plasma glucose level. Hypoglycaemia - low level of plasma glucose, is a relatively common episode primarily affecting diabetic patients receiving treatment with insulin or other hypoglycaemic drugs and patients suffering from insulinoma. Strict glycemic control with low target ranges invariably carries a risk of inadvertent hypoglycemic episodes. Several studies have nevertheless reported a potentially higher incidence of hypoglycemia in patients under strict glycemic control (Van den Berghe *et al.*, 2005; Krinsley & Grover, 2007; Thomas *et al.*, 2007). Hypoglycaemia impose alterations upon both the central (CNS) and peripheral (PNS) nervous systems. It is one of the most common and serious stress conditions challenging the body homeostasis. Hypoglycaemia can lead to brain damage and long-term cognitive impairment (Wieloch, 1985; Gazit *et al.*, 2003). Severe hypoglycaemia with cognitive dysfunction is three times more common in intensively, rather than conventionally, treated insulin-dependent diabetes mellitus (IDDM) (Maran *et al.*, 1994). The 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. Children and adults exposed to hypoglycaemia can
develop long-term impairment of cognitive function (Blattner, 1968; Hawdon, 1999;
Karp, 1989; Ryan et al., 1985; Vannucci & Vannucci, 2001) and are at risk of
epilepsy (Kaufman, 1998). Hypoglycaemia can affect all aspects of life, including
employment, driving, recreational activities involving exercise and travel and
measures should be taken in all of these situations to avoid this potentially dangerous
side-effect of insulin therapy (Frier, 2008).

Ageing is the biological process characterized by the progressive and
irreversible loss of physiological function accompanied by increasing mortality with
advancing age. It is a complex physiological phenomenon associated with a multitude
of biological changes at the molecular level, which is eventually manifested at the
tissue and organism level. Normal human ageing is associated with a progressive
impairment of glucose tolerance. Total glucose stimulated insulin secretion has been
described as being not changed, suppressed or increased as the animal ages.
Neurotransmitters have been reported to show significant alterations during diabetes
resulting in altered functions causing neuronal degeneration. Age related changes in
the capacity of β-cell for proliferation affect the insulin production and contribute to a
decrease in glucose tolerance with advance in age.

The CNS neurotransmitters play an important role in the regulation of glucose
homeostasis. These neurotransmitters mediate rapid intracellular communications not
only within the CNS 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
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complications like retinopathy, neuropathy and nephropathy (Sheetz & King, 2002). Dopaminergic dysfunction in hippocampus (Robinson et al., 2009) and glutamatergic dysfunction in cerebral cortex (Joseph et al., 2008) and cerebellum (Joseph et al., 2007) during hypoglycaemia and hyperglycaemia is suggested to contribute to cognitive and memory deficits.

The brain and other tissues require glucose in order to function properly. Studies suggest that acute or chronic hypoglycaemia leads to neurological dysfunction and injury. 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. Hypoglycaemia-induced brain injury is a significant obstacle to optimal blood glucose control in diabetic patients. Prolonged insulin-induced hypoglycaemia causes widespread loss of neurons and permanent brain damage with irreversible coma. As in brain injury associated with ischaemia and neurodegenerative conditions, altered neurotransmitter action appears to play a role in hypoglycaemic brain injury (Auer & Seisjo, 1993; Auer, 2004). 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 & 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.,
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, 1999).

Attention has been focussed on glutamate as a potential mediator of hypoglycaemic brain injury (Aral et al., 1998; Cavaliere et al., 2001; Marinelli et al., 2001). Severe hypoglycaemia triggers a cascade of events in vulnerable neurons that may culminate in cell death even after glucose normalization (Sang et al., 2003, 2004, 2005, 2007). Glutamate receptor activation and excitotoxicity has long been recognized as an upstream event in this cascade (Wieloch, 1985). In brain, glutamate accumulation is reported to cause neuronal degeneration (Atlante et al., 1997; Berman & Murray, 1996; Budd & Nicholas, 1996).

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. Recent reports suggest that if intensive insulin therapy is to be used, great effort must be
taken to avoid hypoglycemia (Bilotta et al., 2008). 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 role of glutamergic receptor subtypes in hypoglycaemic and hyperglycaemic adult and old brain.

In the present study a detailed investigation on the alterations of glutamate and its receptors in the brain regions of streptozotocin induced diabetic and insulin induced hypoglycaemic adult and old rats were carried out. Glutamate receptor subtypes- NMDAR1, NMDA2B, mGluR5 and GLAST glutamate transporter gene expression in the hypoglycaemic and hyperglycaemic adult and old rat brain were also studied. The molecular studies on the brain damage through 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 glutamate content in the brain regions cerebral cortex (CC), cerebellum (CB), hippocampus and pancreas of hypoglycaemic and diabetic adult and old rats.

2. To study glutamate and NMDA receptors alterations in the brain regions and pancreas of hypoglycaemic and diabetic adult and old rats.

3. To study NMDAR1, NMDA2B, mGluR5 glutamate receptor subtypes and GLAST glutamate transporter gene expression in the brain regions and pancreas of hypoglycaemic and diabetic adult and old rats.

4. To study the second messengers IP3, cGMP and cAMP in the brain regions and pancreas of hypoglycaemic and diabetic adult and old rats.

5. To study the expression of NMDAR1, NMDA2B mGluR5 and IP3 receptors using confocal microscope by immunofluorescent receptor specific antibodies in the brain slices of cerebral cortex, cerebellum and pancreatic islets of hypoglycaemic and diabetic adult and old rats.

6. To study the effect of glutamate and dopamine receptor antagonists on intracellular calcium release in pancreatic islets of rats in vitro using confocal microscope.

7. To study the behavioural changes in the hypoglycaemic and diabetic adult and old rats using rotarod test.