**Introduction**

Homeostasis of blood and cellular glucose is an important factor of body functioning as a whole and the nervous system in particular. Glucose is the principal source for energy production in the brain and undisturbed glucose metabolism is pivotally significant for normal function of this organ. 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. Glucose metabolism and energy homeostasis of the body are regulated by the nerve system and special glucose sensory neurons with action potential depending on the glucose level in the extracellular medium (Levin et al., 2004). The glucose excitable neurons elevate their activity with an increase in the external glucose concentration and the glucose suppressible neurons are activated with a decrease in its level. These specialized neurons use glucose and products of its intracellular metabolism for regulation of their activity and release of a neurotransmitter (Yang et al., 2004).

Diabetes mellitus is a common metabolic disorder resulting from defects in insulin secretion, insulin action, or both (Feldman, 1997). Hyperglycemia - high blood glucose level is associated with diabetes. Hypoglycemia - low level of blood glucose, is a relatively common episode primarily affecting diabetic patients receiving treatment with insulin or other hypoglycemic drugs and patients suffering from insulinoma (Cryer, 2004). The neurological consequences of diabetes mellitus in the central nervous system (CNS) are now receiving greater attention. Cognitive deficits, along with morphological and neurochemical alterations illustrate that the neurological complications of diabetes are not limited to peripheral neuropathies (Biessels et al., 1994). The central complications of hyperglycemia also include the potentiation of neuronal damage observed following hypoxic/ischemic events, as well as stroke (McCall, 1992). Glucose
utilization is decreased in the brain during diabetes (McCall, 1992), providing a potential mechanism for increased vulnerability to acute pathological events. Neuroendocrine dysfunction is also observed in diabetes (Mooradian, 1988).

Severe hypoglycemia is a serious complication of insulin therapy in diabetic patients exceeding insulin administration and hypoglycemic episodes are frequent in many people with type 1 diabetes mellitus and advanced type 2 diabetes mellitus (Cryer, 2004). Furthermore, the ability to sense a reduction in blood glucose levels and the counterregulatory mechanisms responsible for its correction are impaired in patients with diabetes, which make them susceptible of suffering from hypoglycemia (Becker & Ryan, 2000; Jones & Davis, 2003; Cryer, 2006). Diabetes mellitus and its most common treatment side effect, hypoglycemia, have multiple effects on the central nervous system. Transient neurological deficits associated with hypoglycemia is generalized with confusion, motor restlessness, hypotonia and generalized seizures (Lahat et al., 1995). The brain’s activities rely heavily on glucose for energy (Laughlin, 2004). The metabolization of glucose from the bloodstream allows each brain region to carry out its given functions (McNay et al., 2001). Regulation of glucose at the biochemical level affects every area of the brain and has impact from cellular to behavioral brain function. Intense glycemic control with low target ranges invariably carries a risk of inadvertent hypoglycemic episodes. Several studies have 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). Hypoglycemia impose alterations upon both the central (CNS) and peripheral (PNS) nervous systems. Hypoglycemia lead to brain damage and long-term cognitive impairment (Wieloch, 1985; Gazit et al., 2003). The hypoglycemic counter regulatory mechanisms are blunted irreversibly by disease duration or by acute episodes of prior stress (Ertl & Davis, 2004). Hypoglycemia affects all aspects of life, including employment, driving, recreational activities involving exercise and travel. Measures should be taken in
all of these situations to avoid the potentially dangerous side-effect of insulin therapy (Frier, 2008).

Studies suggest that acute or chronic hypoglycemia lead to neurological dysfunction and injury. Hypoglycemia-induced brain injury is a significant obstacle to optimal blood glucose control in diabetic patients. Prolonged insulin-induced hypoglycemia causes widespread loss of neurons and permanent brain damage with irreversible coma. As in brain injury associated with ischemia and neurodegenerative conditions, altered neurotransmitter action appears to play a role in hypoglycemic brain injury. Pathological studies in humans and animals show that hypoglycemia-induced neuronal death occurs preferentially in the hippocampus, superficial layers of the cortex and striatum (Auer, 2004). Because of the extensive neuronal loss, one of the neurological consequence associated with hypoglycemia is cognitive decline. According to clinical studies, significant learning and memory deficits correlate with the frequency of hypoglycemia 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 hypoglycemic coma (Rovet & Ehrlich, 1999; Hannonen et al., 2003) and cognitive impairment (Wredling et al., 1990; Langan et al., 1991). Hypoglycemic episodes in diabetic patients induce cognitive impairment in children (Naguib et al., 2009) and adults (Akyol et al., 2003; Carroll et al., 2003; Roberts et al., 2008) and in rodent models of hypoglycemia and type 1 diabetes hippocampal damage has been associated with impairment in learning and memory tests (Suh et al., 2003; Alvarez et al., 2009). During moderate hypoglycemia, the patient experiences impaired motor function, confusion and inappropriate behaviour but is still aware enough to take action, whereas severe hypoglycemia lead to disabling neurologic
impairment, coma or seizure (Cryer, 2002). Patients with recurrent severe hypoglycemia are exposed to convulsions or seizures which are mistakenly diagnosed as epileptic attacks (Frier, 2000).

The CNS neurotransmitters play an important role in the regulation of glucose homeostasis. 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 hyperglycemia resulting in altered functions causing neuronal degeneration (Bhardwaj et al., 1999). Chronic hyperglycemia during diabetes mellitus is a major initiator of diabetic micro-vascular complications like retinopathy, neuropathy and nephropathy (Sheetz & King, 2002). Impairment of dopaminergic and glutamatergic function is reported in brain regions of hypoglycemic and hyperglycemic rats (Robinson et al., 2009, Joseph et al., 2007, 2008, 2010) thereby contributing to neuronal damage. Severe hypoglycemia is frequently associated with seizures. Brain regions are prone to develop seizures and seizure-induced damage. Repeated hypoglycemic episodes have frequent memory problems, suggesting impaired hippocampal function (Kirchner et al., 2006).

Acetylcholine (ACh) is an excitatory neurotransmitter in both the PNS and CNS which functions as a neuromodulator. In the peripheral nervous system, acetylcholine activates muscles and is a major neurotransmitter in the autonomic nervous system. In the central nervous system, acetylcholine and the associated neurons form a neurotransmitter system, the cholinergic system, which tends to cause excitatory actions. ACh is involved with synaptic plasticity, specifically in learning and short-term memory. Acetylcholine released by cholinergic neurons from presynaptic neurons into the synaptic cleft acts as a ligand for Nicotinic Acetylcholine receptors, which are ligand gated Na+ ion channels. Ligand binding opens the channel causing depolarization and increases the probability of an action potential firing, occurring once the threshold is reached (Connors & Long, 2004).
The function of acetylcholine is mediated through nicotinic and muscarinic acetylcholine receptors (AChRs). Central mAChRs contribute to the regulation of GABAergic transmission and the activation of mAChRs enhanced GABA release in the rat globus pallidus (Kayadjianian et al., 1997) and substantia gelatinosa is reported (Baba et al., 1998). The muscarinic AChRs (mAChRs) are mediators of multiple cellular functions. mAChRs are expressed throughout the entire central nervous system of vertebrates (Levey, 1993) and are thought to be involved in many brain functions such as learning and memory. They are frequently found at presynaptic sites and their activation results in the modulation of transmitter release. The activation of neuronal nicotinic AChRs (nAChRs) results in fast excitatory synaptic transmission or the enhancement of the release of transmitters including glutamate, GABA and ACh (De Filippi et al., 2001). Studies have shown that cholinergic transmission is more sensitive to hypoxia and to low glucose concentrations than is axonal conduction (Dolivo et al., 1974).

GABA, the most important inhibitory neurotransmitter in the mammalian central nervous system, acts through 2 classes of receptors; GABA_\text{A} receptors are ligand-operated ion channels and GABA_\text{B} are the G-protein-coupled metabotropic receptors. Impairment of GABAergic transmission by genetic mutations or application of GABA receptor antagonists are reported to induced seizures. Dysfunction of the GABAergic system has a fundamental role in the propagation of seizures. Indeed mutant mice lacking the enzyme glutamate decarboxylase (GAD) or certain subunits of GABA_\text{A} receptors are prone to spontaneous seizures.

GABAergic neurons release GABA, which is one of two neuroinhibitors in the CNS, the other being Glycine. GABA has a homologous function to ACh, gating anion channels that allow Cl\(^{-}\) ions to enter the post synaptic neuron. Cl\(^{-}\) causes hyperpolarization within the neuron, decreasing the probability of an action potential firing as the voltage becomes negative. Despite our advances in the treatment of diabetes, hypoglycemic 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 hypoglycemia. 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 hypoglycemia cause transient or persistent alteration of cognitive functions and result in seizures or coma. The pathogenesis of hypoglycemia induced cognitive and functional deficit is largely unknown, but mechanisms that could result in damage to cells of the CNS include deregulation in neurotransmitter signaling. To understand the effects of hypoglycemia 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 hypoglycemia and to identify the mechanisms involved in hypoglycemia induced cell or tissue damage in brain. Identification of neuronal damage caused by neurotransmitter variations provides a better understanding of the neurochemical mechanisms responsible for hypoglycemia associated deficit in cognitive and behavioural response. The reports so far stated did not attempt to emphasis the functional role of muscarinic, nicotinic and GABAergic receptor subtypes in hypoglycemic and hyperglycemic brain.

Phospholipase C (PLC) is a key enzyme in phosphatidyl inositol turnover and generates two second messengers, inositol 1,4,5-bisphosphate (IP3) and diacylglycerol (DAG) from phosphatidyl inositol 4,5-bisphosphate [PI(4,5)P2] in response to activation of receptors by hormones, neurotransmitters, growth factors, and other molecules. IP3 induces calcium mobilization and DAG induces activation of protein kinase C. PI(4,5)P2 is a substrate for PLC, and PI 3- kinase. In addition, PI(4,5)P2 directly regulates a variety of cellular functions, including cytoskeletal reorganization, exocytosis and channel activity. Therefore, strict regulation of PI(4,5)P2 levels by PLC or other converting enzymes is necessary for homeostasis (Fukami, 2002). Disruption of the cyclic AMP response element binding (CREB) protein expression and its activity is associated with
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hypoglycemia and reduced expression of gluconeogenic enzymes (Herzig et al., 2001). Insulin administration to rodents is the most common experimental model of hypoglycemia (Auer et al., 1985). The development of hypoglycemia is associated with a decrease in the glucose inflow to the brain, which cause convulsions, coma (Shah et al., 1984) and even death. Therefore, studies on damages of the central nerve system under conditions of hypoglycemia are very important for clinical applications.

In the present study, a detailed investigation on the alterations of muscarinic M1, M3, α7 nicotinic acetylcholine receptor (α7 nAchR), GABA receptors and its subtypes; GABA \( \text{A}_{\alpha 1} \) and GABA \( \text{B} \) in the brain regions of streptozotocin induced diabetic and insulin induced hypoglycemic rats were carried out. Gene expression of acetylcholine esterase (AChE), choline acetyltransferase (ChAT), GAD, GLUT3, Insulin receptor, superoxide dismutase (SOD), Bax protein, Phospholipase C and CREB in hypoglycemic and hyperglycemic rat brain were studied. Muscarinic M1, M3 receptors, AChE, ChAT, GABA \( \text{A}_{\alpha 1} \), GABA \( \text{B} \), GAD, Insulin receptor, SOD, Bax protein and Phospholipase C expression in pancreas was also carried out. The molecular studies on the CNS and PNS damage will elucidate the therapeutic role in the corrective measures of the damage to the brain during hypoglycemia and hyperglycemia.
OBJECTIVES OF THE PRESENT STUDY

1. To measure the circulating insulin level in streptozotocin induced diabetic and insulin induced hypoglycemic rats.

2. To study the behavioural changes in diabetic and hypoglycemic rats using Y-maze and grid walk.

3. To study the total muscarinic, muscarinic M1 and muscarinic M3 receptor subtypes binding parameters in cerebral cortex, cerebellum, brainstem, corpus striatum, hippocampus and pancreas of diabetic and hypoglycemic rats.

4. To study the GABA binding parameters in cerebral cortex, cerebellum, brain stem, corpus striatum, hippocampus and pancreas of diabetic and hypoglycemic rats.

5. To study the expression of AChE, ChAT, muscarinic M1, muscarinic M3, α7 nAChR, GABA<sub>α1</sub>, GABA<sub>B</sub>, GAD, insulin receptor gene expression in the cerebral cortex, cerebellum, brainstem, corpus striatum, hippocampus of diabetic and hypoglycemic rats using Real Time PCR.

6. To study the gene expression status of GLUT3, superoxide dismutase, Bax, phospholipase C, CREB in the cerebral cortex, cerebellum, brainstem, corpus striatum and hippocampus of diabetic and hypoglycemic rats using Real Time PCR.
7. To study the expression of acetylcholine esterase, choline acetyltransferase, muscarinic M1, muscarinic M3, GABA\textsubscript{A}\text{R1}, GABA\textsubscript{B}\text{R}, GAD, insulin receptor, superoxide dismutase, Bax protein and phospholipase C in the pancreas of diabetic and hypoglycemic rats using Real Time PCR.

8. To study the localisation and expression status of muscarinic M1, muscarinic M3, \(\alpha\)7 nicotinic acetylcholine receptor and GABA\textsubscript{A}\text{R1} receptors in the brain slices of cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus of diabetic and hypoglycemic rats using specific antibodies in confocal microscope.

9. Immunocytochemical studies to analyse the localisation and expression status of muscarinic M1, muscarinic M3 and GABA\textsubscript{A}\text{R1} receptors in the pancreas of diabetic and hypoglycemic rats using specific antibodies in confocal microscope.