Diabetes mellitus is a major global health problem that affects more than 246 million people in the world, but by 2025, that number is estimated to reach 380 million (Sicree et al., 2006; Zimmet et al., 2001). It is an increasingly prevalent metabolic disorder in humans and is characterised by hyperglycemia (Kumar & Clarke, 2002; Dunne et al., 2004). The symptoms of diabetes mellitus result from abnormal glucose metabolism. The lack of insulin activity results in failure of transfer of glucose from the plasma into the cells. This situation is so called “starvation in the midst of plenty”. The pancreatic hormones have an important role in the regulation of glucose metabolism. The secretion of insulin by β-cells of the endocrine pancreas is regulated by glucose and other circulating nutrients. It is also modulated by several hormones and neurotransmitters, among which acetylcholine and GABA plays a prominent role. Hypoglycemia is a serious complication of diabetes and insulin therapy. Hypoglycemic brain injury occurs most frequently in patients attempting tight glucose control (Auer, 2004; Davis et al., 1998).

Increased incidence of hypoglycemia occurs when attempts are made to institute tight glycemic control using currently available regimens of subcutaneous insulin administration in diabetic patients (Cryer, 1994). Insulin and sulfonylurea therapy for diabetes mellitus carries the risk of hypoglycemic brain injury and this risk is a major impediment to optimal glucose regulation in diabetic patients (Davis et al., 1998). Tight blood glucose control reduces the risk of diabetes complications but also increases the risk of hypoglycemic episodes. Symptomatic hypoglycemia occurs frequently in insulin-treated patients and 36% of patients were found in one study to have experienced hypoglycemic coma in their lifetime (Pramming et al., 1991). Upto 10% of patients practicing conventional insulin therapy and 25% of those practicing intensive therapy suffer at least one episode of severe, temporarily disabling hypoglycaemia, often with seizure or coma, in a
given year (Cryer, 1994) and hypoglycemia causes recurrent and even persistent psychological morbidity in many diabetic patients. Speculation that an adaptation in the CNS exist in patients with diabetes, depending upon antecedent glycemia, appeared nearly a decade ago (Cryer, 2003). Amiel et al., (1988) observed that lower glucose concentrations were required to initiate epinephrine secretion following a period of intensified diabetes management with its attendant increase in hypoglycemia. Similar hormonal defects are induced in patients with diabetes (Hepburn et al., 1991; Dagogo et al., 1993) and nondiabetics (Davis & Shamoon, 1991; Heller & Cryer, 1991; Veneman et al., 1993), after an episode of hypoglycemia.

Glucose is the only fuel that neuronal tissue use for energy under normal circumstances (Sokoloff, 1981). Chronic changes in the antecedent level of glycemia (either sustained hyperglycemia or hypoglycemia) induce alterations in brain glucose metabolism in rodents (Boyle et al., 1994). Sensory and cognitive impairments have been documented in diabetic humans and animals, but the pathophysiology of diabetes in the central nervous system is poorly understood. It seems that glucose metabolism and energy homeostasis of the body are also 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). 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).

Depending upon its severity, hypoglycemia cause irritability, impaired concentration, focal neurological deficits, seizures, coma and with profound hypoglycemia, neuronal death (Auer 2004; McCrimmon & Sherwin, 1999; Ben-Ami et al., 1999). Symptoms of hypoglycemia result primarily from a lowered glucose level in the brain and its effects on the central and autonomic nervous systems (Charles & Goh, 2005). Declining glucose levels in the brain stimulate the autonomic nervous system, causing epinephrine and norepinephrine to be released
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from the adrenal medulla. Norepinephrine and acetylcholine from the sympathetic nervous system is also involved in glucose control. Symptoms occur as these hormones and neurotransmitters simultaneously stimulate α-cells in the pancreas to release glucagon, which consequently induces new glucose production in the liver (Cryer 2002 a, b, 2003). In this homeostatic mechanism, rising blood glucose levels shut down the neoglucogenesis activities of autonomic nervous system (McAulay et al., 2001, Charles & Goh, 2005). Recent studies indicate that neuronal NADPH oxidase is the primary source of neuronal oxidative stress after hypoglycemia and the rate of superoxide production is influenced by the blood glucose concentration achieved in the immediate posthypoglycemic period. Restoring blood glucose to 1–2 mM during the first hour after hypoglycemia resulted in less superoxide production and less neuronal death than restoration to higher glucose levels (>5 mM).

Hypoglycemia and brain

Hypoglycemia and glucose deprivation as its extreme expression are very interesting, because in the nervous system glucose is not only the main source of energy necessary for its functioning, but also a substance capable of preventing oxidative damage and reducing the damage to mitochondria caused by neurotoxicity (Delgado et al., 2000). A major concern of diabetic patients is that repeated episodes of hypoglycemia results in neuronal loss because of impaired fuel supply (McNay & Cotero, 2010). The incidence of severe hypoglycemia in patients with diabetes treated by intensive insulin therapy is two to six times higher as in conventionally treated patients with diabetes. Hypoglycemia-induced brain injury is a significant obstacle to optimal blood glucose control in diabetic patients (Sang et al., 2005). Disorders in the transport and metabolism of glucose are an important signal for triggering the apoptotic cascade (Moley & Mueckler, 2000). Glucose deprivation leads to rapid suppression of synaptic transmission (Sakurai et al., 2002; Gee et al., 2010). In particular, recurrent hypoglycemic
episodes during the night represent a relevant risk for the patient, because they are often not realized and lead to a deterioration in the awareness for subsequent hypoglycemic episodes. Recent data show that recurrent hypoglycemia not only affects neuroendocrine counter regulation but also autonomic and neuroglucopenic symptoms (Minna et al., 2005; Kale et al., 2006). Hyperglycemia and hypoinsulinemia could increase the neuronal damage produced by pathological events such as hypoxia, hypoglycemia (Messier & Gagnon, 1996).

Hypoglycemia-induced brain injury is a significant obstacle to optimal blood glucose control in diabetic patients. The progress of neuronal dysfunction and damage during energy deprivation is a complex process that includes presynaptic and postsynaptic mechanisms (Auer & Siesjo, 1988; Martin et al., 1994). As in brain injury associated with ischaemia and neurodegenerative conditions, altered neurotransmitter action appears to play a role in hypoglycemic brain injury (Aral et al., 1998; Auer & Seisjo, 1993). Two main events have been described when energy levels are reduced: an increased release of excitatory amino acids and a reduced concentration of intracellular ATP, which leads to diminished Na⁺/K⁺-ATPase activity (Benveniste et al., 1984; Hansen, 1985; Lees, 1991; Roettger & Lipton, 1996). Children and adults exposed to hypoglycemia develop long-term impairment of cognitive function (Hawdon, 1999; Karp, 1989; Ryan et al., 1985; Vannucci & Vannucci, 2001) and are at risk of epilepsy (Kaufman, 1998). Prolonged insulin-induced hypoglycemia causes widespread loss of neurons and permanent brain damage with irreversible coma. Severe hypoglycemia constitutes a medical emergency, involving seizures, coma and death. Studies suggest that suppressing seizures during hypoglycemia decrease subsequent neuronal damage and dysfunction (Abdelmalik et al., 2007). The only treatment for hypoglycemia is blood glucose repletion and there is no currently available intervention for preventing the neuronal death that develops after hypoglycemia is corrected.
Hypoglycemic coma induces a purely neuronal lesion of neo cortex and the hippocampus in rat brain (Wieloch et al., 1984). CT studies show that hypoglycemia predominantly affects cerebral gray matter in the brain. Analysis of regional cerebral blood flow (CBF) differences identified neuronal activation during hypoglycemia in bilateral medial prefrontal cortex (Auer & Siesjo, 1993). Hypoglycemic neuronal death is most pronounced in specific neuron populations: neurons in the hippocampal CA1, subiculum and dentate granule cell layer; cortical layers 2 and 3 of cerebral cortex and the dorsolateral striatum (Auer et al., 1989; Auer & Siesjo, 1993). The hippocampal neurons in particular are important for learning and memory. Patients who survive hypoglycemic coma are left with significant cognitive impairment (Kalimo & Olsson, 1980; Patrick & Campbell, 1990).

**Glucose homeostasis and pancreas**

The endocrine cells of pancreas are arranged into small islands of cells called the islets of Langerhans. The interactive function of both the exocrine and the endocrine parts are particularly important for the normal functioning of the body. The endocrine cells produce indispensable hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide, which are crucial to the optimum functioning of body metabolism. The pancreas is well innervated by autonomic nerves rich in different types of neuropeptides including vasoactive intestinal polypeptide and neuropeptide Y; galanin, Calcitonin-gene-related-peptide, cholecystokinin and leucine-enkephalin (Adeghate et al., 2001). In addition to the presence of neuropeptides, neurotransmitters such as serotonin, GABA or neurotransmitter-regulating enzymes such as tyrosine hydroxylase and dopamine β hydroxylase have been identified in the pancreas (Adeghate & Donath 1991; Adeghate & Ponery 2001; Adeghate & Ponery 2002). The endocrine pancreas is richly innervated, but the abundance and organisation of these innervations are highly variable between species (Kobayashi & Fujita, 1969).
Most of the nerve fibers enter the pancreas along the arteries (Miller, 1981; Woods & Porte, 1974). Unmyelinated nerve fibers are found in the neighborhood of all islet cell types at the periphery and within the islet. At some distance from the islets, glial Schwann cells often form a thin sheet around nerve fibers on their travel toward and within the islet. In the vicinity of islet cells, however, it is not rare to see some nerve fibers lacking this glial protection and coming close to or ending blindly 20–30 nm from the endocrine cells (Bock, 1986; Fujita & Kobayashi, 1979; Legg, 1967; Radke & Stach, 1986; Shorr & Bloom, 1970; Watari, 1968).

The preganglionic fibers of the parasympathetic limb originate from perikarya located in the dorsal motor nucleus of the vagus (Berthoud et al., 1990; Berthoud & Powley, 1991; Chen et al., 1996) and possibly also in the nucleus ambiguus (Luiten et al., 1986) which are both under the control of the hypothalamus. They are organized in well separated branches traveling within the vagus nerves (cranial nerve X) and through the hepatic, gastric (Berthoud et al., 1990; Berthoud & Powley, 1991) and possibly celiac branches of the vagus (Kinami et al., 1997). They reach intrapancreatic ganglia that are dispersed in the exocrine tissue. These ganglia send unmyelinated postganglionic fibers toward the islets. Preganglionic vagal fibers release acetylcholine that binds to nicotinic receptors on intraganglionic neurons. Postganglionic vagal fibers release several neurotransmitters: acetylcholine, Vasoactive Intestinal Peptide (VIP), gastrin-releasing peptide (GRP), nitric oxide (NO) and pituitary adenylate cyclase-activating polypeptide (PACAP) (Havel et al., 1997, Ahren et al., 1999; Love & Szebeni, 1999; Wang et al., 1999; Ahren, 2000; Myojin et al., 2000). Cholinergic terminals are found in the neighborhood of all islet cell types at the periphery and within the islet (Van der Zee et al., 1992). The importance of the cholinergic innervation of the endocrine pancreas is attested by the presence of a 10-fold higher activity of choline acetyltransferase and acetylcholine esterase (the enzymes involved in the synthesis and the degradation of acetylcholine
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respectively) in the islets than in the surrounding exocrine tissue (Godfrey & Matschinsky, 1975). Cholinergic synapses with endocrine cells have been observed in some species (Golding & Pow, 1990).

Understanding the organisation of the pancreatic innervations permits correct interpretation of some experiments using different cholinergic antagonists. The stimulation of insulin release occurring upon electrical stimulation of vagal nerves in the dog is abolished by both nicotinic and muscarinic antagonists (Ahren & Taborsky Jr, 1986). In the perfused rat pancreas, nicotine produces an increase of insulin secretion that is blocked by atropine (Miller, 1980). These observations can be explained by the presence of nicotinic receptors on pancreatic ganglia and nerves (Stagner & Samols, 1986; Karlsson & Ahren, 1998; Kirchgessner & Liu, 1998) and muscarinic receptors on β-cells.

The overall effect of a parasympathetic stimulation is an increase of insulin secretion because postganglionic fibers contain various neurotransmitters in addition to the classic neurotransmitter acetylcholine. It is important to keep in mind that parasympathetic neurotransmission is the sum of various biological effects. VIP and PACAP stimulate insulin secretion by increasing cAMP levels (Ahren, 2000). They act on the same family of receptors and exert their action by two mechanisms, directly by stimulating β-cells through the PLC-PKC pathway (Ahren, 2000) and indirectly by activating intrapancreatic postganglionic nerves that stimulate insulin secretion (Karlsson & Ahren, 1998).

The stimulatory role of ACh in insulin secretion is well established. Studies from our laboratory have reported the regulatory role of mACHRs in glucose induced insulin secretion (Renuka et al., 2004, 2006). Muscarinic M1 receptor subtype antagonist, pirenzepine inhibits cholinergic mediated insulin secretion confirming the role of this receptor subtype in insulin synthesis and secretion (Iismaa et al., 2000). Most of the studies with ACh suggest muscarinic M3 receptor as the predominant cholinergic receptor subtype expressed by pancreatic β-cells and in pancreatic insulin and glucagon release (Gilon &
Henquin, 2001; Duttaroy et al., 2004). The presence of GABA and functional GABA<sub>A</sub> and GABA<sub>B</sub> receptors in pancreatic endocrine cells and their ability to modulate secretion of insulin and glucagon is well studied (Brice et al., 2002). Over-expression of glutamate decarboxylase (GAD65), the enzyme which regulate conversion of glutamate to GABA, in mouse β-cells leads to an inhibition of insulin release and glucose intolerance in vivo (Shi et al., 2000). Although these manoeuvres, which are likely to lead to a decrease in intracellular glutamate concentrations, are entirely compatible with an important role of glutamate in the b-cell (Maechler & Wollheim, 2000) it should be stressed that the product of glutamate breakdown, GABA itself act as an inhibitor of insulin secretion.

**Brain neurotransmitters and diabetes**

Diabetes mellitus is a metabolic disorder that either arrives during the early years of growth (Juvenile diabetes) or later in life called as maturity onset diabetes. It is observed as the body’s inability to effectively regulate the sugar balance which leads to severe complications such as hyperglycemia, obesity, neuropathy, nephropathy, retinopathy, cardiopathy, osteoporesis and coma leading to death. Pancreatic damage resulting in the dysfunction of α and β cells causes disordered glucose homeostasis.

Hyperglycemia during diabetes has been reported to cause degenerative changes in neurons of the central nervous system (Garris, 1990; Lackovic, et al., 1990; Bhattacharya & Saraswathi, 1991). Previous studies demonstrated adrenergic, serotonergic and dopaminergic receptor function alterations in the brain of diabetic rats (Abraham & Paulose, 1999; Padayatti & Paulose, 1999; Paulose et al., 1999, Eswar et al., 2007). The concentration of 5-HT, DA and NE increased in the brain regions of diabetic rats and accumulation of these monoamines is produced by inhibition of monoamine oxidase activity (Salkovic, et al., 1992). Norepinephrine has been reported to increase in several brain regions during diabetes. Ohtani et al., (1997) have reported a significant decrease
in extracellular concentrations of NE, 5HT and their metabolites in the ventro medial hypothalamus (VMH). Epinephrine (EPI) levels were significantly increased in the striatum, hippocampus and hypothalamus of diabetic rats and these changes were reversed to normal by insulin treatment (Ramakrishan & Namasivayam, 1995). Diabetes is reported to cause a high level of degeneration in neurons in different regions of the brain. Streptozotocin-induced diabetes and acute deficiency of insulin is reported to result in increased concentrations of EPI in the supra chiasmatic nucleus. It is also reported that β-adrenergic receptor populations were decreased in diabetes (Garris, 1995). 5-HT content in the brain is reported to be decreased during diabetes (Chu et al., 1986; Sumiyoshi et al., 1997; Jackson & Paulose, 1999). Sandrini et al (1997) reported reduced concentration of serotonin in the cerebral cortex and in the brain-stem of the rat during diabetes. Garris (1990) reported chronically elevated levels of NE in the brain regions of amygdala, hypothalamus and medulla of diabetic mice. This was proposed to be associated with the expression of the gene causing diabetes mellitus. Hyperglycemia is reported to alter the noradrenergic and cholinergic nerve components (Akria, et al., 1994) with decrease in the Na⁺ K⁺ ATPase activity in different brain regions (Gurcharan & Sukwinder, 1994). NE, DA and 5-HIAA are reported to be increased in the heart and adrenal gland of STZ induced diabetic rats. In the adrenal gland there was an initial reduction followed by a steady increase in the concentration of NE and EPI (Cao & Morrison, 2001). Studies of Gireesh et al., (2008a) showed that there is a decrease in total muscarinic and muscarinic M1 receptors during diabetes in the cerebral cortex. A decreased muscarinic M1 receptor gene expression in the hypothalamus, brainstem and pancreatic islets of diabetic rats was also demonstrated by Gireesh et al., (2008b). Changes in neurotransmitter concentration and in receptor binding have been described in rat brain regions (Bitar et al., 1986). Diabetes is reported to cause increased glutamate content and glutamate receptor activation in the brain regions is reported by Joseph et al (2007, 2008). Glutamate excitotoxicity causing increase
Ca\textsuperscript{2+} mediating neuronal dysfunction contributes to hyperglycemia induced cell death (Joseph et al., 2010).

**Brain neurotransmitters and hypoglycemia**

Glucose in brain, supplies energy essential for maintenance of the nervous system. During hypoglycemia, energy dependent mechanisms for restoring normal transmembrane gradients of Na\textsuperscript{+} and Ca\textsuperscript{2+} cannot operate because of the depletion of ATP and phosphocreatine associated with hypoglycemia. Excess Ca\textsuperscript{2+} influx activates cellular phospholipases and proteases, alters mitochondrial metabolism, triggers free radical formation, changes patterns of synaptic transmission, and eventually result in selective neuronal necrosis (Jane et al., 1999). Deficiency in glucose that results from hypoglycemic insults triggers neuronal injuries. Balance in ion homeostasis is disturbed, which in turn results in membrane depolarization and massive release of neurotransmitters, including glutamate. The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA-kainate (Choi, 1988). In addition, neurons impaired of energy metabolism appear to be highly sensitive to excitotoxicity (Monyer et al., 1992; Cebers et al., 1998).

Pyruvate derived from glucose is the major precursor of the acetyl group of acetylcholine. Inhibition of pyruvate oxidation results in reduced ACh synthesis both *in vitro* and *in vivo*. Incorporation of \textsuperscript{[14C]} choline into ACh in brain *in vivo* is decreased in rats with insulin-induced hypoglycemia. Hypoglycemia results in decreased synthesis of the neurotransmitter pool of ACh are supported by the observation that administration of the CNS cholinesterase inhibitor phystostigmine to hypoglycemic animals delays the onset of seizures and coma (Gibson & Blass, 1976).

Similar findings of an adverse effect of hypoglycemia on the synthesis of the amino acid neurotransmitters GABA and glutamate have also been reported. Utilization of amino acids such as glutamate and glutamine as alternative energy
substrates in moderate to severe hypoglycaemia results in accumulation of aspartate and ammonia in the brain. Hypoglycemia also produces a transient but substantial increase in extracellular concentrations of glutamate, GABA and dopamine, as measured using in vivo cerebral microdialysis (Butterworth, 1999). Studies reported that modulation of the GABAergic system in the ventromedial hypothalamus (VMH) alters both glucagon and sympathoadrenal, but not corticosterone, responses to hypoglycemia. GABAergic inhibitory tone within the VMH modulates glucose counter regulatory responses (Owen et al., 2006). Alterations of neurotransmission mediated by ACh, Glu, GABA and/or DA contribute to the neurological signs and symptoms that characterize moderate hypoglycemia.

**Cholinergic regulation of glucose homeostasis**

**Acetylcholine**

Cholinergic system plays an important role in physiological and behavioural functions mediated by acetylcholine. Acetylcholine acts by binding to specific membrane receptors and is divided into muscarinic and nicotinic receptors. ACh consists of choline; which is taken up by the cholinergic cells through a sodium-dependent choline uptake system and an acetyl group provided by acetyl-Coenzyme A (intracellularly produced by oxidative metabolism in mitochondria). The synthesis of ACh takes place in the axonal terminals and is catalyzed by the cytosolic enzyme choline acetyltransferase (ChAT) (Van Der Zee et al., 1999). Acetylcholine not only acts as a chemical signal at the neuromuscular junction, it plays a role in neural network formation. Acetylcholine, as a neurotransmitter mediates muscle contraction and glandular secretions via an extensive array of peripheral nerves, and in its capacity as a neuromodulator mediates consciousness by ultimately regulating the tone of activity patterns throughout the entire cerebral cortex.
Cholinergic Innervation

Cholinergic neurons form extensive networks with each other and with glutamatergic and GABAergic neurons. Cholinergic neurons also interconnect with other neuromodulator systems, such as networks using dopamine, norepinephrine, serotonin and histamine, to modulate sensory information relayed by glutamate synapses. Neuroplasticity is essential to the continuous updating these neural networks and some cholinergic axons are particularly plastic (Farris et al., 1995). At both central and peripheral sites, cholinergic receptor activation leads to cytoskeletal responses. Centrally, muscarinic acetylcholine receptors, which utilize G-protein mechanisms, far out number nicotinic acetylcholine receptors, which are ionotropic in nature. In the periphery, nicotinic acetylcholine receptors predominate. Despite marked differences in receptor mechanisms, the binding of acetylcholine to either muscarinic or nicotinic acetylcholine receptors triggers cytoskeletal protein interactions with other cytoskeletal proteins at central and peripheral sites (Woolf, 2006).

Cholinergic neurons comprise less than one percent of neurons in the nervous system, yet they appear to be involved in the most intriguing and enigmatic of neural functions, ranging from gross observable movement to consciousness. Cholinergic systems orchestrate activity across groups of muscle cells and in conjunction monoamines - dopamine, norepinephrine, serotonin and histamine neuronal assemblies in cortical fields. The end result is a unified action, either behavioral or cognitive, which because of inherent neuroplasticity can be honed through the learning process to become purposeful action (Woolf & Butcher, 2010).

With a few exceptions, cholinergic neurons assume a ventral or basal location. This ventral or basal location is consistent with the motor and “cognitive action” functions of cholinergic neurons. Mesopontine cholinergic neurons are located in the laterodorsal tegmental nucleus and the pedunculopontine tegmental
nucleus, although the boundaries of the pedunculopontine nucleus are unclear resulting in some cholinergic neurons invading surrounding regions. These cholinergic neurons innervate the spinal cord, brainstem, thalamus, hypothalamus, basal forebrain and medial frontal cortex (Woolf & Butcher, 1986; Rye et al., 1987). Mesopontine cholinergic neurons are intermingled with separate populations of glutamatergic and GABAergic cells (Wang & Morales, 2009). Mesopontine cholinergic neurons to the thalamus and basal forebrain play roles in higher cognitive functions, such as gating sensory input, attention and mediating level of consciousness. Cholinergic and GABAergic circuits in the mesopontine region have been shown to inhibit the startle response, a response that is necessary for gating sensory inputs (Bosch & Schmid, 2008). Some studies link mesopontine cholinergic cells with attention, but not all studies are in agreement (Rostron et al., 2009). In one such study, lesions of the pedunculopontine tegmental nucleus produced an increase in errors and latency times on a serial reaction time task testing attention (Inglis et al., 2001).

Brain sites capable of producing coma and neurotransmitter correlates of coma point to a role for cholinergic mesopontine neurons in consciousness. Damage to mesopontine areas containing cholinergic neurons produces coma (Parvizi & Damasio, 2003). Many anesthetics block central cholinergic receptors and overdose can lead to central anticholinergic syndrome and coma (Moos, 2007). The role of cholinergic systems in consciousness is additionally illustrated by the pharmacology of anesthetic drugs that markedly diminish conscious awareness. Many known anesthetic compounds target nicotinic acetylcholine receptors and GABA_A receptors (among other receptors). Muscarinic acetylcholine receptors and GABA_B receptors represent potential targets for future anesthetics (Sanders et al., 2008; Van Dort et al., 2008). The cholinergic basal forebrain plays a role in selective attention, learning, memory, perception and consciousness (Sarter et al., 2003). Specific regions of the basal forebrain play particular roles, even though each region takes a part in multiple functions and
there is overlap in function among the regions. Spatial learning, contextual learning, associative learning, and reorganization of sensory fields are some examples. Cholinergic septohippocampal pathway appears to contribute to its role in memory.

**Muscarinic receptors**

Muscarinic receptors are a family of G protein-coupled receptors that have a primary role in central cholinergic neurotransmission. Specific agonists, which activate postsynaptic muscarinic receptors, stimulate cholinergic signaling (Valentin et al., 2006). These receptors are widely distributed throughout the body and subserve numerous vital functions in both the brain and autonomic nervous system (Hassal et al., 1993). In the brain, muscarinic receptors participate in many important functions such as learning, memory and the control of posture. In the periphery, among other effects, muscarinic receptors mediate smooth muscle contraction, glandular secretion and modulation of cardiac rate and force. In the CNS there is evidence that muscarinic receptors are involved in motor control, temperature regulation, cardiovascular regulation and memory. Interest in the classification of muscarinic receptors involved in functions at different locations has been heightened by the potential therapeutic application of selective agents in areas such as AD, Parkinson’s disease, asthma, analgesia and disorders of intestinal motility, cardiac and urinary bladder function (Caulfield & Birdsall, 1998).

**Classification of Muscarinic receptors**

Muscarinic receptors are G protein coupled receptors (Hulme et al., 1990). Molecular cloning studies have revealed the existence of five molecularly distinct mammalian muscarinic receptor proteins (Bonner, 1989). Muscarinic receptors, widely distributed throughout the central and peripheral nervous system have critical functions in learning and memory, attention and motor activity (Weiner et
Their effects depend on the receptor subtypes involved. Cholinergic stimulation of pancreatic β-cells increases insulin secretion. These are mediated by muscarinic cholinergic, rather than nicotinic receptors (Ahren et al., 1990; Stubbe & Steffens, 1993) and are dependent on extracellular glucose concentration (Henquin & Nenquin, 1988). Acetylcholine stimulated insulin secretion coupling is mediated by complex mechanisms of signal transduction. It has been proposed that acetylcholine activates phospholipid turnover and thereby increases the intracellular Ca\textsuperscript{2+} level.

The five muscarinic receptor (mAChR) subtypes are designated as M1 - M5. The odd-numbered receptors (M1, M3, and M5) couple to Gq/11 and thus activate PLC, which initiates the phosphatidyl inositol trisphosphate cascade. This leads to the dissociation of phosphatidyl 4, 5- bisphosphates (PIP2) into two components, i.e. IP\textsubscript{3} and DAG. IP\textsubscript{3} mediates Ca\textsuperscript{2+} release from the intracellular pool (endoplasmic reticulum), whereas DAG is responsible for activation of protein kinase C. On the other hand, PIP2 is required for the activation of several membrane protein, such as the “M current” channel and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger and muscarinic receptor-dependent depletion of PIP2 inhibits the function of these proteins (Meyer et al., 2001; Fuster et al., 2004; Suh & Hille, 2005; Winks et al., 2005). The M1, M2 and M4 subtypes of mAChRs are the predominant receptors in the CNS. These receptors activate a multitude of signaling pathways important for modulating neuronal excitability, synaptic plasticity and feedback regulation of acetylcholine release (Volpicelli & Levey, 2004). The activation of mAChRs has been reported to suppress GABA release in the rat subfornical organ (Xu et al., 2001). Presynaptic M3 receptors inhibited excitatory and inhibitory transmission to rat subthalamic neurons (Shen & Johnson, 2000). M1 and probably M3 receptors inhibited GABA release in neurons of the rat lateral amygdala, nucleus accumbens, and striatum (Sugita et al., 1991).

Muscarinic M1 receptor
Muscarinic M1 receptors are predominantly expressed in the forebrain, including the cerebral cortex, hippocampus and corpus striatum, where this subtype contributes by 50-60% to the total of the muscarinic receptors (Hamilton et al., 1997; Gerber et al., 2001; Miyakawa et al., 2001). The muscarinic M1 receptor subtype, which is also expressed in peripheral tissues, has been implicated in stress adaptive cardiovascular reflexes and central blood pressure control. Studies have shown that central administration of the muscarinic M1 specific antagonist pirenzepine lowered the blood pressure (Brezenoff & Xiao, 1986; Buccafusco, 1996). A putative overexpression of the M1 subtype in selected brain areas of spontaneously hypertensive rats has been reported (Scheucher et al., 1991). Muscarinic agonist depolarisation of rat isolated superior cervical ganglion is mediated through M1 receptors (Brown et al., 1980). Muscarinic M1 is one of the predominant muscarinic receptor subtypes expressed in pancreatic islets (Gilon & Henquin, 2001). Studies in pancreatic islets revealed that activation of muscarinic receptors is pertussis toxin insensitive and Gq mediated. Muscarinic M1 receptor number was decreased in the brainstem during pancreatic regeneration without any change in the affinity (Renuka et al., 2006).

**Muscarinic M2 receptor**

Muscarinic receptor activation in guinea pig heart produces a reduction in force of contraction and a decrease in the rate of beating. These effects are probably the consequence of inhibition of voltage-gated Ca\(^{2+}\) channels and activation of inwardly rectifying K\(^{+}\) channels, respectively. Extensive studies with many antagonists have defined this response as being mediated by the muscarinic M2 receptor (Caulfield, 1993). Muscarinic M2 receptors mediate both negative and positive ionotropic responses in the left atrium of the reserpinated rat, latter effect being insensitive to pertussis toxin (Kenakin & Boselli, 1990). Central cholinergic transmission is activated by inhibition of the presynaptic muscarinic M2 acetylcholine autoreceptor using selective antagonists. The presynaptic
muscarinic M2 autoreceptor negatively influences the release of acetylcholine in several brain regions, including the striatum, hippocampus, and cerebral cortex (Kitaichi et al., 1999; Zhank et al., 2002). A direct consequence of brain muscarinic M2 autoreceptor inhibition is an elevation of acetylcholine release in the synaptic cleft. Methoctramine and other muscarinic M2 receptor antagonists have been shown to enhance the release of acetylcholine in different brain structures (Stillman et al., 1996).

**Muscarinic M3 receptor**

Muscarinic M3 receptors are broadly expressed in the brain, although the expression level is not high, compared to those of the muscarinic M1 and M2 receptors (Levey, 1993). Muscarinic M3 receptor is widely distributed in the peripheral autonomic organs with the highest expression found in the exocrine glands (Kashihara et al., 1992; Matsui et al., 2000). Expression of the muscarinic M3 receptor in the rat pancreatic islets and insulin secreting cell lines has been established (Lismaa et al., 2000). Muscarinic M3 receptor also triggers direct contractions of smooth muscle; however, it only represents a minor fraction of total muscarinic receptor population in smooth muscle. It is expressed in relatively low density throughout the brain. Studies using knock out mice for muscarinic M3 receptors gave evidences for the primary importance of these receptors in the peripheral cholinergic system. In urinary bladder, pupillary muscles and intestinal smooth muscles the cholinergic contractions are mediated predominately through muscarinic M3 receptors (Matsui et al., 2000).

**Muscarinic M4 receptor**

Muscarinic M4 receptor is known to be abundantly expressed in the striatum (Levey, 1993). Muscarinic M4 receptors act as inhibitory muscarinic autoreceptors in the mouse (Zhang & Warren, 2002). The neuroblastoma-glioma hybrid cell line NG108–15 expresses M4 mRNA and M4 receptors can be
detected readily in radioligand binding assays (Lazareno et al., 1990). Inhibition of adenylyl cyclase activity by muscarinic agonists in rat corpus striatum is mediated by M4 receptors (Caulfield, 1993; Olianas et al., 1996).

**Muscarinic M5 receptor**

The muscarinic M5 receptor was the last muscarinic acetylcholine receptor cloned. Localisation studies have revealed that the M5R is abundantly expressed in dopamine-containing neurons of the substantia nigra par compacta, an area of the midbrain providing dopaminergic innervation to the striatum. Concordantly, oxotremorine-mediated dopamine release in the striatum was markedly decreased in M5R-deficient mice. More intriguingly, in M5R-deficient mice, acetylcholine induced dilation of cerebral arteries and arterioles was greatly attenuated (Yamada et al., 2001), suggesting that the M5 receptor is suitable target for the treatment of cerebrovascular ischemia. Muscarinic M5 receptor subtype is expressed at low levels in the brain (Hulme et al., 1990; Hosey, 1992).

Studies of the muscarinic M5 receptor have been hampered both by the lack of selective ligands and of tissues or cell lines that endogenously express the native receptor protein. Immunoprecipitation and RT-PCR studies have shown that the M5 receptor is expressed at very low densities in the mammalian brain. However, *in situ* hybridisation studies have demonstrated that M5 transcripts are highly concentrated in the basal ganglia and are the only muscarinic receptor transcripts expressed on dopaminergic neurons in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) (Reever et al., 1997). Another potentially useful system is the eosinophilic leukemia cell line (EoL-1) where M5 receptors are induced on differentiation with interferon-γ (Mita et al., 1996).
Signal transduction by muscarinic activation

Gq-protein-coupled receptors (GqPCRs) are widely distributed in the CNS and play fundamental roles in a variety of neuronal processes. Their activation results in phosphatidylinositol 4,5-bisphosphate (PIP2) hydrolysis and Ca\(^{2+}\) release from intracellular stores via the PLC-inositol 1,4,5-trisphosphate (IP\(_3\)) signaling pathway. Because early GqPCR signaling events occur at the plasma membrane of neurons, they are influenced by changes in membrane potential (Billups et al., 2006). Muscarinic receptors, which are G protein coupled, stimulate signaling by first binding to G protein complex (\(\alpha\beta\gamma\)) which provides specificity for coupling to an appropriate effector. The \(\alpha\) subunit interacts with an effector protein or ion channel to stimulate or inhibit release of intracellular second messengers. Mutation analysis showed that the G protein is primarily but not exclusively acts through interaction with the third cytoplasmic loop. It is suggested that the short sequences, N terminal 16-21 and C terminal 19 amino acids of the loop play a key role in determining the specificity (Wess et al., 1989).

Cyclic adenosine monophosphate

Adenylate cyclase is either positively or negatively regulated by G protein coupled receptors resulting in an increase or decrease in the generation of the second messenger, Cyclic adenosine monophosphate (cAMP). The stimulation of muscarinic M2 and M4 receptors endogenously expressed in cell lines, results in the inhibition of adenylate cyclase. G protein reconstitution experiments have shown that muscarinic M2 receptors inhibit adenylate cyclase through Gi and possibly through the pertusis toxin insensitive Gz. In neuroblastoma SK-N-SH cells which express endogenous muscarinic M3 receptors stimulate adenylate cyclase activity (Baumgold & Fishman, 1988). The muscarinic M1 receptor which ectopically expressed at physiological levels in A9L cells, was shown to stimulate adenylate cyclase through an IP\(_3\) and Ca\(^{2+}\) dependent mechanism (Felder et al., 2000). In contrast, M1 receptors stimulate adenylate cyclase in CHO cells
predominantly through an IP$_3$ and Ca$^{2+}$ independent mechanism that also contained a small Ca$^{2+}$ dependent component (Gurwitz et al., 1994).

**Phospholipase C**

The family of PLC enzymes has been grouped into three classes, β, γ and δ (Rhee & Choi, 1992). PLC serves as the primary effector for the muscarinic M1 receptor that is coupled through Gq α subunits (Berstein et al., 1992). Muscarinic M1, M3 and M5 receptors stimulate the production of IP$_3$, independent of direct PLCβ and G protein interaction (Gusovsky et al., 1993). This alternate route for the generation of IP$_3$ involves the tyrosine kinase dependent phosphorylation of PLCγ, a mechanism normally stimulated by growth factors and their receptors (Meisenhelder et al., 1989). Expression studies revealed that the cloned muscarinic M2 receptor stimulates PLC through a pertussis toxin-sensitive G protein although with lower efficiency than muscarinic M1 or M3 receptors (Ashkenazi et al., 1987). Inhibition of PLC by an endogenously expressed muscarinic M2 receptor has been reported in FRTL5 cells suggesting that negative regulation occur in some cells (Bizzarri et al., 1990).

**Nicotinic Receptors**

The nicotinic acetylcholine receptor (nAChR), a key player in neuronal communication, converts neurotransmitter binding into membrane electrical depolarization. Nicotinic acetylcholine receptors that contain α7 subunits are prevalent in the mammalian brain and have received special attention because of their linkage to cognitive functions (Adams & Freedman, 1997; Levin & Simon, 1998). This protein combines binding sites for the neurotransmitter acetylcholine and a cationic transmembrane ion channel. It mediates synaptic transmission at the junction between nerve and muscle cells and various types of nAChR are expressed in the brain. It is involved in several neurological pathologies. The α7 nicotinic receptor, also known as the α7 receptor, is the predominant type of
nicotinic acetylcholine receptor in the brain, consisting entirely of α7 subunits (Rang et al., 2003). As with other nicotinic acetylcholine receptors, functional α7 receptors are pentameric i.e., (α7)5 stoichiometry. It is located in the brain, where activation yields post- and presynaptic excitation (Rang et al., 2003), mainly by increased Ca\(^{2+}\) permeability.

Neuronal nicotinic cholinergic receptors are crucial to acetylcholine neurotransmission in both the CNS and autonomic nervous system. However, in the CNS, these receptors are more often associated with modulation of release of several neurotransmitters including dopamine, norepinephrine, GABA and glutamate (Alkondon et al., 1999; Girod & Role, 2001). In the CNS, nicotinic acetylcholine receptors mediate the release of glutamate (De Filippi et al., 2001; Rossi et al., 2003; Reno et al., 2004) and norepinephrine (O Leary & Leslie, 2003). Thus, these receptors significantly influence the activity within the CNS circuitry and deregulation of this activity could contribute to disorders involving the CNS. Abnormalities of nicotinic acetylcholine receptor function in the hippocampus lead to cognitive and memory impairments (Green et al., 2005; Levin et al., 2002) and sensory gating deficits (Adler et al., 1998). Nicotinic acetylcholine receptors are involved with neuroplastic responses, such as dendritic growth in cholinceptive neurons (Torrao et al., 2003).

**GABA regulation of glucose homeostasis**

GABA is a major inhibitory neurotransmitter in the mammalian central nervous system (CNS) and is synthesized from glutamic acid by glutamic acid decarboxylase (GAD) (Gerber & Hare, 1979). There is significant ongoing work aimed at understanding the specific roles of synaptic and extrasynaptic GABA in regulating neural activity in both normal and pathological conditions. Precise GABAergic synaptic signaling is critical to the accurate transmission of information within neural circuits and even slight disruptions can produce hypersynchronous activity (Chagnac-Amitai & Connors, 1989). Moreover,
changes in ambient GABA can alter tonic inhibition and thus the overall synaptic tone of a brain region (Farrant & Nusser, 2005). There is an extensive literature showing that seizures can be provoked by blocking GABA synthesis with 3-mercaptoproprionic acid (MPA) \textit{in vivo} (Mares \textit{et al}., 1993). These studies were demonstrating the involvement of GABA in the prevention of the overstimulation of neuronal networks.

\textbf{GABA Receptors}

GABA mediates its actions \textit{via} three distinct receptors: the ionotropic GABA\textsubscript{A} and GABA\textsubscript{C} receptors and the metabotropic GABA\textsubscript{B} receptors (Bormann, 2000). In addition to its CNS functions, the GABAergic system is also present in peripheral tissues, including the gastrointestinal tract (Gilon \textit{et al}., 1990, 1991; Harty \textit{et al}., 1991; Krantis \textit{et al}., 1994). Ionotropic GABA receptors are the most important Cl\textsuperscript{−} channels in the central nervous system CNS and their expression has also been found in peripheral organs. These receptors mediate a fast inhibitory neurotransmission in the CNS (Akinci & Schofield, 1999). Ionotropic GABA receptors can be categorized into GABA\textsubscript{A} and GABA\textsubscript{C} receptors based on their subunit compositions and pharmacological properties (Bormann, 2000).

GABA\textsubscript{B} receptors mediate slow prolonged inhibition in the brain by activating postsynaptic G protein-coupled inwardly rectifying K\textsuperscript{+} channels (GIRKs) and inactivating presynaptic voltage-gated Ca\textsuperscript{2+} channels. GABA\textsubscript{B} receptors also inhibit adenylate cyclase, leading to diminished activity of PKA signaling pathways (Bowery, 2006). Structurally GABA\textsubscript{B} receptors are members of the class C family of G-protein coupled receptors (GPCR) and are encoded in vertebrates by two genes - GABA\textsubscript{B} receptor-1 (GABA\textsubscript{B}R1) and GABA\textsubscript{B}R2 respectively (Couve \textit{et al}., 2002; Bettler \textit{et al}., 2004).
GABA\textsubscript{A} Receptors

GABA\textsubscript{A} receptors are pentameric in structure, with the five subunits arranged like spokes of a wheel around a central Cl\textsuperscript{−} selective pore (Barnard, 2001). Nineteen GABA receptor subunits have been cloned from rats, which include \(\alpha\textsubscript{1}–6, \beta\textsubscript{1}–3, \gamma\textsubscript{1}–3, \rho\textsubscript{1}–3, \delta, \theta, \epsilon, \) and \(\pi\) (Whiting \textit{et al.}, 1999). The 19 subunits (\(\alpha\textsubscript{1}–6, \beta\textsubscript{1}–3, \gamma\textsubscript{1}–3, \delta, \epsilon, \theta, \pi, \rho\textsubscript{1}–2\)) are encoded by 19 distinct genes. Each subunit has four transmembrane segments, with both the amino and carboxy termini located extracellularly. These extracellular segments form the recognition sites (two per channel) for GABA and also, in some channel types, the recognition site (one per channel) for benzodiazepine-like allosteric modulators. The subunit composition determines both the biophysical properties of the receptor–channel complex and its pharmacology, most notably the sensitivity to benzodiazepines (BDZ) (Rudolph & Mohler 2004; Johnston, 2005). A typical benzodiazepine-sensitive GABA\textsubscript{A} receptor consists of two \(\alpha\textsubscript{1}, \alpha\textsubscript{2}, \alpha\textsubscript{3}, \) or \(\alpha\textsubscript{5}\) subunits, two \(\beta\textsubscript{2}\) or \(\beta\textsubscript{3}\) subunits (or one each) and a \(\gamma\textsubscript{2}\) subunit.

Classically, GABA\textsubscript{A} receptors have been recognized as mediating phasic inhibition through the generation of fast, transient, rapidly desensitizing currents (IPSCs) in postsynaptic neurons in response to synaptically released GABA. It has been recognized that GABA\textsubscript{A} receptors also contribute to tonic (extrasynaptic) inhibition, representing the Cl\textsuperscript{−} conductance activated at nonsynaptic sites in response to background concentrations of GABA (Farrant & Nusser, 2005). Phasic and tonic inhibitions are mediated by GABA\textsubscript{A} receptors with different subunit composition, GABA affinities and rates of desensitization. The most notable difference in subunit composition is that the receptors mediating tonic inhibition contain the \(\delta\) subunit, rather than the \(\gamma\) subunit characteristic of synaptic GABA\textsubscript{A} receptors (Nusser \textit{et al.}, 1998). Receptors containing \(\alpha\textsubscript{4}, \alpha\textsubscript{5}, \) or \(\alpha\textsubscript{6}\) are commonly found nonsynaptically. Pharmacologically, the most notable difference is that receptors with \(\alpha\textsubscript{4}, \alpha\textsubscript{6}, \) or \(\delta\) subunits are not potentiated by benzodiazepines or by nonbenzodiazepine benzodiazepine receptor agonists (such as zolpidem),
whereas those with $\alpha_1$, $\alpha_2$, $\alpha_3$, $\alpha_5$, or $\gamma_2$ subunits are benzodiazepine sensitive. The benzodiazepine-sensitive $\alpha$ subunits ($\alpha_1$, $\alpha_2$, $\alpha_3$, $\alpha_5$) differ from the insensitive ones ($\alpha_4$, $\alpha_6$) in possessing a histidine residue at position 101. Activation of GABA$_A$Rs, a Cl$^-$ ion channel, results in membrane hyperpolarization as a consequence of an inward Cl$^-$ flux (Kittler & Moss, 2003). In the CNS, GABA$_A$Rs are subject to modulation by their subunit composition, localization, number and phosphorylation states and variance of GABA concentration in the synaptic cleft (Chebib & Johnston, 1999; Mody & Pearce, 2004).

**GABA$_B$ Receptors**

The GABA$_B$ receptor is part of the class C of GPCRs that also includes the mGlu, the Ca$^{2+}$-sensing and the sweet and umami taste receptors among others (Pin et al., 2003). These receptors are dimers, either homodimers linked by a disulphide bond mGlu and Ca$^{2+}$-sensing receptors or heterodimers made of two similar, but distinct subunits, the GABA$_B$ and taste receptors. Indeed, the GABA$_B$ receptor was the first GPCR to be identified that requires two distinct subunits to function: the GABA$_{B1}$ and GABA$_{B2}$ subunits (Jones et al., 1998; White et al., 1998; Kaupmann et al., 2003). Although the GABA$_{B1}$ subunit was soon shown to bind all known GABA$_B$ ligands, both agonists and antagonists, this protein did not form a functional GABA$_B$ receptor when expressed alone (Kaupmann et al., 1997). Only when GABA$_{B1}$ was co-expressed with the homologous GABA$_{B2}$ subunit was a functional GABA$_B$ receptor observed, either in cell lines or in cultured neurons. The GABA$_B$ dimeric entity was confirmed in native tissue (Kaupmann et al., 1998). Indeed, both GABA$_{B1}$ and GABA$_{B2}$ mRNAs are co-localized in most brain regions. Second, both proteins are found in the same neurons, even in the same subcellular compartments as observed at the electron microscopic level. Moreover, co-immunoprecipitation of GABA$_{B1}$ with a GABA$_{B2}$ antibody could be demonstrated from brain membranes. Eventually, mice lacking either GABA$_{B1}$ or GABA$_{B2}$ share very similar phenotypes and none of the known

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GABA\textsubscript{B}-mediated responses could be measured in either mice (Prosser \textit{et al.}, 2001; Schuler \textit{et al.}, 2001).

GABA\textsubscript{B} receptors play an important role in maintaining excitatory–inhibitory balance in brain and alterations can lead to seizures (Hossein \textit{et al.}, 2008). GABA\textsubscript{B} receptors, the metabotropic receptors for GABA, are G protein-coupled receptors (GPCR) which regulate neuronal excitability both pre and postsynaptically. The action of GABA at presynaptic GABA\textsubscript{B} receptors is to reduce Ca\textsuperscript{2+} influx and thus inhibit neurotransmitter release (Takahashi \textit{et al.}, 1998). These receptors exist on GABAergic terminals (autoreceptors), or on terminals arising from cells containing other neurotransmitters, such as glutamate (heteroreceptors). Postsynaptically, GABA\textsubscript{B} receptors are responsible for the generation of the late inhibitory postsynaptic potential (IPSP), \textit{via} the opening of K\textsuperscript{+} channels and inhibit adenylate cyclase (Bettler \textit{et al.}, 1998). Abnormality in either of these functions could have consequences for the generation and/or prevention of epileptic seizures. Multiple laboratories have demonstrated altered expression of GABA\textsubscript{B}R1 and GABA\textsubscript{B}R2 in animal models for seizure disorders (Princivalle \textit{et al.}, 2003; Straessle \textit{et al.}, 2003). Han \textit{et al.} (2006) found that as a result of multiple seizures, there was a long-term decrease in GABA\textsubscript{B}R1 (15 days) and GABA\textsubscript{B}R2 (30 days) expression in rat hippocampus. Taken together, these animal studies suggest that the changes in the number GABA\textsubscript{B} receptors lead to epilepsy, due to changes in transmitter release (presynaptic) and inhibition (postsynaptic). Accordingly, GABA neurons have been alternately proposed to be highly vulnerable or relatively invulnerable after insults known to cause epilepsy (Ribak \textit{et al.}, 1979; Sloviter \textit{et al.}, 1987) and GABA-mediated inhibition is reportedly decreased, in animal models of epilepsy (Dalby \textit{et al.}, 2001).

\textbf{GABA\textsubscript{C} Receptors}

GABA\textsubscript{C} receptors, which are a subfamily of GABA\textsubscript{A} receptors, are members of the Cys-loop superfamily of ligand-gated ion channels (LGICs), an
important group of receptors involved in rapid synaptic transmission and whose malfunction can result in a variety of neurological disorders; hence, understanding their mechanism of action is of considerable pharmacological interest. GABA$_C$ receptors are mostly located in retinal neurons where they play a role in retinal signaling involved in diseases such as macromolecular degeneration (Bormann, 2000). The receptors are activated by the binding of GABA, the main inhibitory neurotransmitter in the central nervous system. GABA$_C$ receptors have distinct pharmacological properties from GABA$_A$ receptors, e.g., they are not inhibited by bicuculline, the classic GABA$_A$ receptor antagonist (Barnard et al., 1998; Chebib et al., 2000). Like all the LGICs belonging to the Cys-loop superfamily, GABA$_C$ receptors are composed of five subunits arranged in a pentagonal array around a central ion-permeant pore. Each subunit has an extracellular N-terminal domain (ECD), a transmembrane domain composed of four $\alpha$-helices, and an intracellular domain. Three subunits ($\rho_{1-3}$) have been identified; these can all form functional homomeric or heteromeric receptors (Enz, 2001).

Similar to GABA$_A$ receptors, they possess a high permeability to $\text{Cl}^-$, but in contrast to GABA$_A$ channels, they are insensitive to bicuculline, barbiturates and benzodiazepines (Polenzani et al., 1991). The activity of GABA$_C$ receptors is regulated by extracellular agents, such as Zn$^{2+}$, H$^+$, Ca$^{2+}$ (Ouyang et al., 2007; Kaneda et al., 1997) and also by intracellular factors, such as Ca$^{2+}$, phosphatases and kinases (Feigenspan & Bormann 1994b; Kusama et al., 1995). Protein phosphorylation is postulated to be an important physiological mechanism for regulating GABA mediated synaptic inhibition (Moss & Smart, 1996).

**Glutamic Acid Decarboxylase (GAD)**

GABA the main inhibitory neurotransmitter in the brain is synthesized by GAD. GAD exists in two isoforms termed GAD65 and GAD67 due to their molecular weights of 65 and 67 kDa, respectively. These enzymes are the products of two independently regulated genes sharing 65% sequence homology in rats
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(Erlander et al., 1991; Bu et al., 1992). Most GABAergic interneurons express both subtypes of GAD (Houser & Esclapez 1994) which are simultaneously detectable in the rat brain as early as embryonic day 17 (Dupuy & Houser, 1996). GAD67 is found in axonal regions as well as in neuronal cell bodies, whereas GAD65 is mainly associated with synaptic terminals (Kaufman et al., 1991). Therefore it has been suggested that GAD67 mostly provides a pool of GABA for general metabolic activity while GABA synthesized by GAD65 is likely to be more involved in synaptic transmission (Martin & Rimvall, 1993). Mice lacking GAD65 are vital and do not exhibit changes in their brain GABA content though they have an increased susceptibility to seizures (Asada et al., 1996; Kash et al., 1997). During embryogenesis the mRNA coding for GAD67 is regulated by alternative splicing (Bond et al., 1990; Szabo et al., 1994). At least two additional transcripts exist, I-80 and I-86 (summarized as EGAD), distinguished by insertions of 80 or 86 base pair (bp) in GAD67 mRNA, respectively. The two inserts are identical with exception of the 6 bp at the 3'-end of the larger fragment containing an overlapping stop-start codon. The complete coding region of embryonic GAD messages comprises 1,860 (80 bp insert) and 1,866 bp (86 bp insert). Both embryonic transcripts code for a short enzymatically inactive GAD protein of 25 kDa (GAD25) which corresponds to the amino-terminal regulatory region of GAD67 and therefore has putative regulatory functions. Termination-reinitiation at the stop-start codon of I-80 additionally produces an enzymatically active protein of 44 kDa (GAD44) corresponding to the carboxy-terminal catalytic domain of GAD67 that contains the pyridoxal phosphate cofactor binding site (Szabo et al., 1994).

The early finding that baby food deficient in vitamin B6, a cofactor of the GABA-synthesizing enzyme GAD caused vitamin-reversible seizures provided one of the first clues that seizures might be caused by reduced synthesis of GABA (Bankier et al., 1983). Accordingly, GABA neurons have been alternately proposed to be highly vulnerable or relatively invulnerable after insults known to
cause epilepsy (Ribak et al., 1979; Sloviter, 1987) and GABA-mediated inhibition is reportedly altered, in a variety of constantly compared but greatly dissimilar animal models (Dalby & Mody, 2001). The use of GAD immunocytochemistry in kindled and control tissue was used to allow direct anatomic confirmation for measuring changes in GAD-immunoreactivity (GAD-IR) which would represent GABA synthesis for release by the recurrent inhibitory system of the fascia dentata. Immediately after the last kindled seizure, optically detected GAD-IR puncta densities were significantly reduced in stratum granulosum at 3 or 7 days after the last kindled seizure (Babb et al., 1989). GAD65-/- mice develop spontaneous seizures that result in increased mortality. Seizures can be precipitated by fear or mild stress. Seizure susceptibility is dramatically increased in GAD65 mice backcrossed into a second genetic background, the nonobese diabetic strain of mice enabling electroencephalogram analysis of the seizures. The generally higher basal brain GABA levels in this backcross are significantly decreased by the GAD65 mutation, suggesting that the relative contribution of GABA synthesized by GAD65 to total brain GABA levels is genetically determined (Kash et al., 1997).

Glutamate decarboxylase, the primary enzyme that is involved in the synthesis of GABA, has been identified as an early target antigen of the T-lymphocyte mediated destruction of pancreatic β-cells causing insulin-dependent diabetes mellitus (Baekkeskov et al., 1990). GABA through its receptors has been demonstrated to attenuate the glucagon and somatostatin secretion from pancreatic α-cells and δ-cells respectively (Gaskins, 1995). It is present in the cytoplasm and in synaptic-like microvesicles (Reetz, 1991) and is co-released with insulin from β-cells in response to glucose. The released GABA inhibits islet α- and β-cell hormonal secretion in a paracrine manner. During diabetes the destruction of β-cells will lead to decrease in GABA release resulting in the enhancement of glucagon secretion from α-cells leading to hyperglycemia. In patients with diabetes, an oral glucose load induced a paradoxical rise in glucagon secretion,
which could be normalized with optimal administration of insulin, suggesting that
the dysfunctional regulation of pancreatic $\alpha$–cells in diabetes is related to insulin
deficiency and an anomalous internal environment of the islets (Greenbaum et al.,
1991; Hamaguchi et al., 1991). However, this defect is undefined because of an
inadequate understanding of the mechanisms underlying suppression of glucagon
by insulin in response to hyperglycemia. Secretion of glucagon from $\alpha$– cells is
regulated by various factors, including glucose, zinc, and the chemical transmitter
gammaaminobutyric acid (GABA) (Pipeleers et al., 1985; Ishihara et al., 2003).
The role of GABA and the $\alpha$ type GABA receptor (GABA$\alpha R$) in the regulation of
glucagon release has been demonstrated (Braun et al., 2004; Rorsman et al.,
1989). Pancreatic $\beta$ -cells contain high concentrations of GABA and GAD
(Taniguchi et al., 1979). GABA is localized in “synaptic”- like microvesicles
within $\beta$ - cells that are distinct from the insulincontaining large dense core
vesicles, suggesting that exocytosis of pancreatic GABA is similar to the process
found in neurons (Reetz et al., 1991; Sorenson et al., 1991). Functional GABA$\alpha$Rs
are expressed in the $\alpha$ cells (Hales & Tyndale, 1994). It has been proposed that,
during hyperglycemia, GABA is co-released with insulin from the b cells and acts
on GABA$\alpha$Rs on the $\alpha$ cells to reduce their secretion of glucagon (Rorsman et al.,
1989).

The brain GABAergic mechanisms also play an important role in glucose
homeostasis. Inhibition of central GABA$\alpha$ receptors increases plasma glucose
concentration (Lang, 1995). GABA$\alpha$ receptors in brainstem have a regulatory role
in pancreatic regeneration (Kaimal et al., 2007). It is known that in ischemia, the
released glutamate activates glutamate receptors, particularly of the NMDA type,
increases the intracellular concentration of $\text{Ca}^{2+}$, and triggers a long-lasting
potentiation of NMDA-receptor-gated currents (Szatkowski & Attwell, 1994). The
massive amount of GABA released simultaneously in the hippocampus is an
important protective mechanism against the excessive release of excitatory amino
acids, counteracting the harmful effects that lead to neuronal death. The release of
GABA limit excitation and prevent this excitation from reaching neurotoxic levels. Activation of GABA\textsubscript{A} receptors increases C1\textsuperscript{−} conductance, inducing hyperpolarization and reducing cell excitability (Sivilotti & Nistri, 1991). Enhanced GABA release increases extracellular GABA levels, thus contributing to the maintenance of homeostasis in the hippocampus upon impending hyperexcitation. Moreover, hippocampal GABAergic neurons are more resistant than excitatory aminoacid neurons to transient ischemia (Matsumoto \textit{et al.}, 1991). The augmentation of inhibitory mechanisms has important neuroprotective effects. To date, drugs that enhance GABAergic systems provide significant neuronal protection when used before or after insults. Thus, any impairment in the GABAergic mechanism in the CNS and/or in the PNS is important in the pathogenesis of hypoglycemia and diabetes.

**Insulin and Insulin receptors in the brain**

Two decades ago both insulin and its receptors were discovered in the brain (Havrankova \textit{et al.}, 1978). Moreover, contrary to old assumptions, it is now known that insulin is actively transported across the blood–brain barrier and it is produced locally in the brain (Schwartz \textit{et al.}, 1998). Concentrations of insulin receptors in the brain are particularly high in neurons, with abundant insulin receptor protein in both cell bodies and synapses (Zhao \textit{et al.}, 1999).

These findings have raised questions about the physiological role of insulin in the brain. Some suggest that, as in peripheral tissues, insulin mainly acts by mediating cerebral glucose uptake (Hoyer, 1998), but this opinion is not shared by others. Insulin and insulin receptors appear to play a modulatory role in certain behaviours, such as feeding behaviour, learning and memory (Wickelgren, 1998; Kumagai, 1999). For example, after training in a water maze, insulin receptor mRNA levels were increased in the hippocampus of rats, in parallel with accumulation of insulin receptor protein. Moreover, intracerebroventricular
administration of insulin facilitated retention of a passive-avoidance task in rats (Park et al., 2000).

The complexity of the mechanisms underlying these behavioural findings is only now starting to be appreciated (Fernandes et al., 1999). When applied to brain slices, insulin inhibits the spontaneous firing rate of hippocampal pyramidal neurons and the frequency of AMPA-receptor mediated miniature EPSCs of cerebellar Purkinje neurons. In addition, insulin attenuates the amplitude of AMPA-receptor-mediated currents in cerebellar Purkinje neurons (Palovcik et al., 1984), through the stimulation of clathrin-dependent receptor internalisation, a phenomenon that appears to have links with cerebellar LTD (Wang et al., 2000). These same authors have reported no effect of insulin on NMDA-receptor-mediated currents in cerebellar Purkinje neurons. Conversely, in hippocampal slices insulin has been shown to increase NMDA-receptor mediated EPSPs (Liu et al., 1995). These different findings are possibly due to variations in insulin signalling in different brain regions. Insulin thus appears to play a modulatory role in synaptic transmission in the brain. However, studies of its involvement in behaviour and synaptic transmission have so far mainly examined its effects after local (for example, intracerebroventricular) administration or ex vivo. The challenge for future studies will be to determine whether systemic insulin also has neuromodulatory effects under physiological conditions and to dissociate these effects from the associated effects of insulin on peripheral and central glucose homeostasis.

Altering neuronal glucose transport during diabetes and hypoglycemia

Under physiological conditions, maintenance of normal cerebral functions depends almost entirely on the availability of glucose for the supply of ATP (Anderson & Swanson, 2000). Since the brain cannot store the significant carbohydrates, a steady glucose supply is required from the blood. Thus glucose transport into the brain is critical for the maintenance of brain metabolism.
Clinical and experimental studies have revealed that altered glucose status is an important factor controlling learning and memory processes (Messier & Gagnon, 1996). Although under basal conditions the rate of glucose transport is not the rate-limiting step for glycolysis in the central nervous system hypoglycemia or hyperglycemia is known to change the glucose transport system in the brain (Devivo, et al., 1991) suggesting that there should be glucose-regulatable mechanisms associated with the transport of glucose. The brain is dependent on a steady supply of glucose (Erecinska & Silver, 1994).

Neuronal glucose uptake relies on the glucose transporter isoforms GLUT1 (Maher et al., 1994), GLUT3 (Olson & Pessin, 1996), and GLUT8 (Piroli et al., 2002; Sankar et al., 2002) on the plasma membrane. Due to its relative abundance in the brain, GLUT3 is often considered the neuronal glucose transporter (Maher et al., 1994). GLUT3 is a major facultative glucose transporter expressed in neurons, though there is evidence for expression in neurons of GLUT1, GLUT2, GLUT4, GLUT5 and GLUT8 (Choeiri et al., 2002). Classically, neurons are considered to be insulin insensitive, that is, glucose uptake is not significantly increased in response to insulin stimulation (Heidenreich et al., 1989) as is regularly observed in muscle and fat cells (Watson & Pessin, 2001). Insulin treatment dramatically increased the translocation of GLUT 3 to the plasma membrane, and insulin pre-treatment potentiated a KCl stimulated glucose uptake (Uemura & Greenlee, 2006).

Although neurons have insulin receptors (Schulingkamp et al., 2000), they do not respond to insulin alone by increasing glucose uptake. In insulin-sensitive cells, insulin increases glucose uptake by promoting two critical processes: translocation of vesicles containing the glucose transporter isoform GLUT4 to the plasma membrane and their fusion with the plasma membrane (Czech & Corvera, 1999; Olson & Pessin, 1996. In neurons, fusion of GLUT3 with the plasma membrane is induced by membrane depolarization, resulting in a marked increase in glucose uptake (Uemura & Greenlee, 2001). It is well known that nerve cells in
the hippocampus, striatum, piriform and neocortical regions are susceptible to ischaemic injury and stress during glucose deprivation (Thilmann et al., 1986). GLUT3 is probably also a stress-induced protein, which may protect the damaged nerve cell from energy depletion. This indicates that glucose starvation effectively activates the transcriptional rate of the GLUT3 gene in vulnerable neurons (Nagamatsu et al., 1993). Many studies have been done describing changes subcellular localization of glucose transporters in response to a variety of different stimuli including insulin, IGF-1, and glucose deprivation (Fladeby et al., 2003; Shin et al., 2004; Watson and Pessin, 2001).

**Oxidative stress and neuronal damage during diabetes and hypoglycemia**

Oxidative stress is known to be present in different pathological conditions in the CNS such as ischemia and various neurodegenerative diseases (Margaill et al., 2005; Halliwell, 2006). The deleterious actions of diabetes and stress increase oxidative stress in the brain, leading to increases in neuronal vulnerability. The presence of oxidative stress during hypoglycemia has been recently suggested (Patocková et al., 2003; Singh et al., 2004; Suh et al., 2007), although its temporality and regional distribution in brain have not been explored in detail. Oxidative stress has been suggested as a mechanism contributing to neuronal death induced by hypoglycemia and an early production of reactive species (RS) during the hypoglycemic episode has been observed. Early activation of calcium-dependent ROS producing pathways is involved in neuronal death associated with glucose deprivation (Paramo et al., 2010). Recent studies show that oxidative stress develops mainly after the isoelectric period during the glucose perfusion phase and that its presence is related to the subsequent death of neurons (Suh et al., 2007). Diabetes has been reported to increase superoxide dismutase (SOD) activity within the brain which in turn makes the brain more vulnerable to damage (Kadekar et al., 1988). During prolonged periods of stress, exhaustion of neuronal defense mechanisms, such as anti-oxidant enzymes, reported to increase
neuronal vulnerability to the point where neuronal adaptation shifts from neuronal plasticity towards neuronal damage (Reagan et al., 2000).

In the central nervous system, programmed cell death or apoptosis is considered to be an important phenomenon that is related to neuron vulnerability to stress condition. Bax is a protein, identified as regulating molecules for programmed cell death. A possible relationship between the localization and expression of Bax protein and the cell vulnerability in central nervous system is reported (Hara et al., 2004).

**The cAMP responsive element binding protein (CREB)**

The cAMP responsive element binding protein (CREB) is a nuclear protein that modulates the transcription of genes with cAMP responsive elements in their promoters. Increases in the concentration of either Ca\(^{2+}\) or cAMP trigger the phosphorylation and activation of CREB. This transcription factor is a component of intracellular signaling events that regulate a wide range of biological functions, from spermatogenesis to circadian rhythms and memory. Byrne (1993) reported the role of CREB in cellular events underlying long-term but not short-term memory. Genetic and pharmacological studies in mice and rats demonstrate that CREB is required for a variety of complex forms of memory, including spatial and social learning, thus indicating that CREB may be a universal modulator of processes required for memory formation (Silva, 1998).

The present study was carried out to elucidate hypoglycemic and hyperglycemic effect on brain cholinergic and GABAergic receptors functional regulation, alterations in insulin receptor, GLUT3, pro-apoptotic protein - Bax and the changes at second messenger level by analyzing the expressional changes in second messenger enzyme; phospholipase C and changes at transcription level using transcription factor, CREB in the brain regions and pancreas of experimental rats. Studies on the functional regulation of ACh and GABA through their receptor subtypes during hyperglycemia and hypoglycemia will lead to a better
understanding of the cognitive and memory dysfunction due to neuronal damage in the brain.