Glucose is the only fuel that neuronal tissue can use for energy under normal circumstances (Sokoloff, 1981). The brain can neither synthesize nor store more than a few minutes worth of glucose; thus a continuous systemic supply is essential for normal cerebral metabolism (Pardridge, 1983). Chronic changes in the antecedent level of glycaemia (either sustained hyperglycaemia or hypoglycaemia) induce alterations in brain glucose metabolism in rodents (McCall et al., 1982; Boyle et al., 1994). Diabetes mellitus is a major global health problem that affects more than 185 million people around the world (Amos et al., 1997; Zimmet, 1999; Zimmet et al., 2001). This disease is an increasingly prevalent metabolic disorder in humans and is characterised by hyperglycaemia (Kumar & Clarke, 2002; Dunne et al., 2004). The number of diabetic patients is expected to reach 300 million by the year 2025. The projected increase in the number of diabetic patients will strain the capabilities of healthcare providers the world over (Adeghate et al., 2006). 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 dopamine plays a prominent role.

There is an increased incidence of hypoglycaemia when attempts are made to institute tight glycemic control using currently available regimens of subcutaneous insulin administration in diabetic patients (Cryer, 1994). Tight blood glucose control can reduce the risk of diabetes complications but also increases the risk of hypoglycaemic episodes. Symptomatic hypoglycaemia occurs frequently in insulin-treated patients, and 36% of patients were found in one study to have experienced hypoglycaemic 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 hypoglycaemia causes recurrent and even persistent psychological morbidity in many diabetic patients (Cryer, 1994). Speculation that an adaptation in the CNS might exist in patients with diabetes, depending upon antecedent glycaemia, appeared nearly a decade ago (Cryer & Gerich, 1985; Cryer, 2003). Hypoglycaemia-induced brain injury is a significant obstacle to optimal blood glucose control in diabetic patients (Sang et al., 2005). 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 hypoglycaemia. Similar hormonal defects with unawareness of symptoms can be induced in patients with diabetes (Dagogo et al., 1993; Hepburn et al., 1991) and nondiabetics (Veneman et al., 1993; Heller & Cryer, 1991; Davis & Shamo, 1991), some after a solitary episode of hypoglycaemia.

Insulin and sulfonylurea therapy for diabetes mellitus carries the risk of hypoglycaemic brain injury and this risk is a major impediment to optimal glucose regulation in diabetic patients (Davis et al., 1998). Depending upon its severity, hypoglycaemia can cause irritability, impaired concentration, focal neurological deficits, seizures, coma and with profound hypoglycaemia, neuronal death (Auer & Siesjo, 1988; McCrimmon et al., 1997; Ben-Ami et al., 1999). Symptoms of hypoglycaemia result from the actions of hormones and neurotransmitters in the process of restoring blood glucose levels. Declining glucose levels in the brain stimulate the autonomic nervous system, causing epinephrine and norepinephrine to be released 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, 1999, 2002 a, b, 2003). In this homeostatic mechanism, rising blood glucose levels shut down the neoglucogenesis activities of autonomic nervous system (Cryer, 1997; Towler et al., 1993; McAulay et al., 2001, Charles & Goh, 2005). Recent studies indicate that neuronal NADPH oxidase is the primary source of neuronal oxidative stress after hypoglycaemia and the rate of superoxide production is influenced by the blood glucose concentration achieved in the immediate posthypoglycaemic period. Restoring blood glucose to 1–2 mM during the first hour after hypoglycaemia resulted in less superoxide production and less neuronal death than restoration to higher glucose levels (>5 mM). It is suggested that a gradual correction of blood glucose in patients with hypoglycaemic coma is preferable to more rapid correction and hyperglycaemia (Sang et al., 2007). Symptoms of hypoglycaemia result primarily from a lowered glucose level in the brain and its effects on the central and autonomic nervous systems (Charles & Goh, 2005). 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 and both local energy store and the supply of alternate sources are limited. The initiating events in hypoglycaemic encephalopathy are not understood completely. But brain injury appears to result from a number of processes that are initiated when blood glucose concentration decrease. Severe hypoglycaemia, whether in patients with type 1 or type 2 diabetes, have debilitating consequences, including seizures or coma or even death (Jane, 1999).

**Hypoglycaemia and brain**

Hypoglycaemia is a collection of symptoms brought about by an abnormally low plasma glucose level. The brain and other tissues require glucose in order to
function properly. Hypoglycaemia constitutes a unique metabolic brain insult (Auer, 2004). Glucose arrives in the central nervous system (CNS) through the specific brain capillary endothelial transporter, GLUT 1 (Pardridge et al., 1990), at a rate that is generally far in excess of the phosphorylation rate by hexokinase (Pardridge, 1983). Therefore, at euglycaemia, glucose transport is not rate-limiting for brain metabolism; but during an acute reduction in the glucose concentration, a level is reached where transport assumes a rate-limiting role. Beyond this critical point, hexokinase is not fully saturated and brain energy metabolism deteriorates. Among the ultimate consequences of neuroglycopenia are initial elevations in epinephrine and glucagon, which serve to increase systemic glucose production and restore glucose provision to the brain. Widespread regions of the brain have been shown to direct this hormonal response during acute CNS fuel deprivation (Frizzell et al., 1993). Maintaining cerebral normoglycaemia while inducing systemic hypoglycaemia greatly attenuates this counterregulatory hormone response (Frizzell et al., 1993, Biggers et al., 1989). The incidence of severe hypoglycaemia in patients with diabetes treated by intensive insulin therapy is two to six times higher as in conventionally treated patients with diabetes. In particular, recurrent hypoglycaemic 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 hypoglycaemic episodes. Recent data show that recurrent hypoglycaemia not only affects neuroendocrine counter regulation but also autonomic and neuroglucopenic symptoms (Minna et al., 2005; Kale et al., 2006).

Studies suggest that acute or chronic hypoglycaemia leads to neurological dysfunction and injury. 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). Two main events have been
described when energy levels are reduced: an increased release of excitatory amino acids (EAA) and a reduced concentration of intracellular ATP, which leads to diminished Na+/K+-ATPase activity (Benveniste et al., 1984; Erecinska & Silver, 1989; Hansen, 1985; Lees, 1991; Roettger & Lipton, 1996). It is well accepted that the excessive stimulation of EAA receptors associated with metabolic inhibition hampers the recovery of [Na+]i and [Ca2+]i loads and facilitates cell death (Rothman et al., 1987; Novelli et al., 1988; Monyer et al., 1989; Lees, 1991; Rose et al., 1998; Cebers et al., 1998). 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-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 (Aral et al., 1998; Auer, 1991; Auer & Seisjo, 1993). 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 culminate in cell death even after glucose normalization (Sang et al., 2003, 2004, 2005, 2007).

Clinical signs and symptoms of metabolic encephalopathies consist of a generalized depression of cerebral function, including consciousness. The effect on consciousness is a consequence of decreased integrative capacity of the neocortex (Jane, 1999). Arousal of the neocortex and other forebrain structures involved in cognition is mediated by specific brainstem nuclei and their projecting fiber tracts, which together constitute the ascending reticular activating system (ARAS).
Activating pathways ascends from the ARAS via thalamic synaptic relays to the neocortex. Metabolic encephalopathies result from alterations of brain chemistry at both neocortical and brainstem ARAS centers (Pulsinelli & Cooper, 1994). If the glucose supply to the brain is not maintained, there is a decrease in cerebral electrical activity, membrane breakdown and release of free fatty acids and altered amino acid metabolism, including increased production of glutamate which is one of the excitatory amino acid neurotransmitter found only in the central nervous system. It is believed to play a major role in the pathophysiology of hypoglycaemic brain injury.

There is increasing evidence that specific changes in mitochondrial function also play a major role in the early events leading to hypoglycaemic encephalopathy. Hypoglycaemic brain injury is a common and serious complication of insulin therapy. Not surprisingly, hypoglycaemic brain injury occurs most frequently in patients attempting tight glucose control (Davis et al., 1998). 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 hypoglycaemia is blood glucose repletion and there is no currently available intervention for preventing the neuronal death that develops after hypoglycaemia is corrected.

Hypoglycaemic coma induces a purely neuronal lesion of neocortex and the hippocampus in rat brain (Wieloch et al., 1984). CT studies show that hypoglycaemia predominantly affects cerebral gray matter in the brain. Analysis of regional cerebral blood flow (CBF) differences identified neuronal activation during hypoglycaemia in bilateral medial prefrontal cortex (Auer & Siesjo, 1993). Hypoglycaemic 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). These
neurons receive a rich glutamatergic innervation and evidence suggests that hypoglycaemic injury in these neurons is precipitated almost entirely by sustained glutamate receptor activation (excitotoxicity) (Auer et al., 1985). The hippocampal neurons in particular are important for learning and memory and patients who survive hypoglycaemic coma are left with significant cognitive impairment (Kalimo & Olsson, 1980; Patrick & Campbell, 1990).

**Ageing and the brain**

Ageing causes changes to the brain size, vasculature, and cognition (Peters, 2006). The brain shrinks with increasing age and there are changes occurring at the molecular level to morphology. The region specific changes in dendritic branching and spine density are more characteristic of the effects of ageing on neuronal morphology (Sara & Carol, 2006). The brains of individuals, who are cognitively normal, show age-related changes that include an overall reduction in brain volume and weight, which are associated with gyral atrophy and widening of the sulci of the cerebral cortex, and enlargement of the brain ventricles. Microscopically, there are increasing amounts of the age-related pigment, lipofuscin, granulovacuolar degeneration in neurons, hirano bodies, diffused deposits of beta-amyloid in parenchyma, neurofibrillary tangles in hippocampus and amygdala and sparse numbers of senile plaques in these regions and in other cortical areas of the brain (Anderton, 1997). Of these changes, neurofibrillary tangles and senile plaques are the neuropathological hallmark of Alzheimer's disease in which they are more abundant and widespread (Hof et al., 1996). Alzheimer's disease has therefore been regarded as accelerated brain ageing. Understanding the molecular basis of plaque and tangle formation is advancing greatly and is the main focus of research into the cellular and molecular changes observed in the ageing brain. The nature of the cognitive and
neurobiological alterations associated with age-related change is substantially different from that seen in the early stages of a dementing illness, such as Alzheimer's disease (Albert et al., 1997). The interplay between genetic and environmental factors determines the degree of pathological brain ageing and whether or not individuals develop dementia in later stages.

**Neuropathological changes associated with normal brain ageing**

The ageing brain shows selective neurochemical changes involving several neuronal cell populations. Ageing and its variants, such as Alzheimer’s disease (AD), viewed as the result of alterations in the levels of Abeta, metals, cholinesterase enzymes and neuronal gene expression (Lahiri, 2005). Neurofibrillary tangles and senile plaques are common neuropathological features in both normal brain ageing and Alzheimer's disease. Layer II of the entorhinal cortex is involved with neurofibrillary tangle formation in all of the cases, while the CA1 field of the hippocampus and the subiculum are less consistently affected. Neocortical area 20 is particularly prone to develop neurofibrillary tangles in intellectually preserved elders, whereas other neocortical areas are relatively spared. Substantial senile plaque formation is seen in the neocortex of non-demented cases. Mild cognitive impairment is correlated with neurofibrillary tangle densities in layer II of the entorhinal cortex, and clinically overt Alzheimer's disease with neurofibrillary tangle densities in area 20. In non-demented cases, there is an early development of neurofibrillary tangles in areas usually spared in the course of the degenerative process in younger individuals. These observations demonstrate that mesial and inferior temporal lobe structures are affected more frequently in normal brain ageing. In this respect, neurofibrillary tangle formation in area 20 represent a crucial step of the degenerative process because it precedes the emergence of the neuropsychological deficits characteristic of age related
disorders. In addition, this reveals age-related heterogeneity in the regional vulnerability of the brain region during normal brain ageing (Hof et al., 1996).

**Neural plasticity in the ageing brain**

Aged animals have alterations in the mechanisms of plasticity that contribute to cognitive functions. One functional alteration that could directly affect plasticity is reduced synapse number, which could make it more difficult to attain the sufficient amount of active synapses that is necessary for the network modification. An early electron microscopic investigation at the perforant path–granule cell synapse showed that aged rats have a 27% decrease in axodendritic synapse number in the middle molecular layer of the dentate gyrus compared with young rats (Bondareff & Geinisman, 1976). Moreover, spatial memory deficits have been shown to correlate with a reduction in perforated synapses at the medial perforant path–granule cell synapse (Geinisman et al., 1986). The total number of synaptic contacts per neuron was found to be diminished significantly in the middle and inner molecular layer of dentate gyrus of aged rats relative to young adults. Both perforated and non-perforated axospinous synapses showed age-dependent decreases in numbers (Geinisman et al., 1992). Cognitive functions that rely on the medial temporal lobe and prefrontal cortex, such as learning, memory and executive function show considerable age-related decline. Several neural mechanisms in these brain areas also seem to be vulnerable during the ageing process. Age-related changes in the medial temporal lobe and prefrontal cortex results in altered functional plasticity contribute to behavioural impairments in the absence of significant pathology (Burke & Barnes, 2006). The subtle changes in neuronal morphology, cell–cell interactions and gene expression that contribute to alterations in plasticity in aged animals disrupt the network dynamics of
aged neurons that ultimately contribute to selective behavioural impairments (Sara & Carol, 2006).

**Memory and ageing**

Memory is an organism's ability to store, retain, and subsequently retrieve information. Ageing affects memory by changing the way the brain stores information and recall the stored information. Studies comparing the effects of ageing on episodic memory, semantic memory, short-term memory and priming found that episodic memory is greatly impaired in normal ageing (Nilsson, 2003). These deficits are related to impairments seen in the ability to refresh recently processed information (Johnson et al., 2002). The ability to encode new memories of events or facts and working memory showed decline in both cross-sectional and longitudinal studies (Hedden & Gabrieli, 2004). In addition, older adults tend to be worse at remembering the source of their information for a particular item or fact (Johnson et al., 1993), a deficit that is related to declines in the ability to bind information together in memory (Mitchell et al., 2000). In contrast, implicit or procedural memory typically shows no decline with age (Fleischman et al., 2004), short-term memory shows little decline (Nilsson, 2003) and semantic knowledge, such as vocabulary improves with age (Verhaeghen, 2003). In addition, the enhancement seen in memory for emotional events is also maintained with age (Mather & Carstensen, 2005). Brain imaging studies have revealed that older adults are more likely to use both hemispheres when completing memory tasks than younger adults (Cabeza et al., 2002). In addition, older adults show a positive effect when remembering information, which seems to be a result of the increased focus on regulating emotion seen with age (Mather & Carstensen, 2005; Isaacowitz et al., 2006).
In normal ageing, cognitive functions remain unimpaired over the life span whereas sustained decline might represent a pathologic condition (Morris et al., 1991; Linn et al., 1995). Alzheimer’s disease (AD) is the most common cause of dementia demonstrating progressive decline in memory, language and visuospatial abilities. Distinguishing AD from normal ageing has been a recurring nosologic and diagnostic problem (Morris et al., 1991; Berg et al., 1982). However, memory loss is qualitatively different in normal ageing from the kind of memory loss associated with a diagnosis of Alzheimer's (Budson & Price, 2005). Recent research has identified a transitional state between the cognitive changes of normal ageing and AD; known as mild cognitive impairment (MCI). Many people who experience mild cognitive impairment are at a high risk of developing AD. Several studies have indicated that MCI individuals are at an increased risk for developing AD, ranging from 1% to 25% per year; 24% of MCI patients progressed to AD in 2 years and 20% more over 3 years, whereas a recent study indicated that the progression of MCI subjects was 55% in 4.5 years (Arnáiz & Almkvist, 2003). In neuropathologic studies, Gomez-Isla et al., (1996) reported specific neuronal loss in the entorhinal cortex in persons with very mild AD and no change in the same region in the cognitively intact elderly. These observations imply that AD and normal ageing are dichotomous.

**Glutamate Receptors**

Glutamate is the most prominent neurotransmitter in the body, being present in over 50% of nervous tissue. A large proportion of the glutamate present in the brain is produced by astrocytes through synthesis *de novo* (Hertz et al., 1999), but levels of glutamate in glial cells are lower than in neurons, 2–3 mM and 5–6 mM, respectively. During excitatory neurotransmission, glutamate-filled vesicles are docked at a specialized region of the presynaptic plasma membrane known as the active zone.
Packaging and storage of glutamate into glutamatergic neuronal vesicles requires Mg\(^{2+}\)/ATP-dependent vesicular glutamate uptake systems, which utilize an electrochemical proton gradient as a driving force. Substances that disturb the electrochemical gradient inhibit this glutamate uptake into vesicles. The concentration of glutamate in vesicle reaches as high as 20–100 mM (Nicholls & Attwell, 1990). In brain tissue, low concentrations of glutamate and aspartate perform as neurotransmitters, but at high concentration these amino acids act as neurotoxins.

It acts through both ligand gated ion channels (ionotropic receptors) and G-protein coupled (metabotropic) receptors. Activation of these receptors is responsible for basal excitatory synaptic transmission and many forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD), which are thought to underlie learning and memory. It appears however, that aspartate aminotransferase and glutaminase account for a majority of glutamate production in brain tissue (McGeer et al., 1987).

The ionotropic receptors themselves are ligand gated ion channels, i.e. on binding glutamate that has been released from a companion cell, charged ions such as Na\(^+\) and Ca\(^{2+}\) pass through a channel in the centre of the receptor complex. This flow of ions results in a depolarisation of the plasma membrane and the generation of an electrical current that is propagated down the processes (dendrites and axons) of the neuron to the next in line. Metabotropic glutamate (mGlu) receptors are G-protein coupled receptors (GPCRs) that have been subdivided into three groups, based on sequence similarity, pharmacology and intracellular signalling mechanisms. Group I mGlu receptors are coupled to PLC and intracellular calcium signalling, while group II and group III receptors are negatively coupled to adenylyl cyclase.

Glutamate functions as a fast excitatory transmitter in the mammalian brain. Glutamate triggers neuronal death when released in excessive concentrations by over
excitation of its receptors (Vizi, 2000). 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). The excitatory amino acid glutamate is the most prevalent transmitter in the brain; its effect on postsynaptic receptors is limited by uptake process (Erecinska, 1997) and by diffusion of glutamate from the cleft. The cellular uptake of Glu is driven by the electrochemical gradients of Na\(^+\) and K\(^+\) and is accompanied by voltage and pH changes. In nervous tissue, glutamate dehydrogenase (GDH) appears to function in both the synthesis and the catabolism of glutamate and perhaps in ammonia detoxification (Mavrothalassitis et al., 1988). The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA kainite (Choi, 1988). Hypoglycaemia is associated with increased glutamate release (Sandberg et al., 1986) and conversely, glutamate toxicity is augmented by hypoglycaemia (Novelli et al., 1988).

The majority of excitatory synapses are glutamatergic, in which glutamate transmits the signal through postsynaptic ionotropic [N-methyl-D-aspartic acid (NMDA), \(-\text{amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)}, \text{and kainate (KA)}\] and metabotropic receptors (Bettler & Mulle, 1995). Glutamate is a fast excitatory transmitter in the CNS and has been shown, with GABA, to interact primarily with receptors in the synaptic cleft (Dingledine et al., 1999). Studies have shown that both ionotropic glutamate receptors and glutamate transporters are involved in oxygen-glucose deprivation-induced necrotic cell death in hippocampal slice cultures (Bonde et al., 2005). The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA–kainate (Choi, 1988). The presence of G protein-coupled glutamate receptors
(metabotropic Glu receptors) has been described and since 1991 (Conn & Pin, 1997), eight receptors have been discovered and classified into three groups based on their linkage to second messenger systems and their pharmacology: group I acts via the phosphoinositol system and groups II and III inhibit adenyl cyclase. In addition, the stimulation of receptors of these three groups directly influences voltage-gated Ca\(^{2+}\) and K\(^{+}\) channels through their G proteins, but their physiological correlate has not yet defined.

There are several reports of presynaptic localization of Glu receptors and their involvement in transmitter release. The fact that NMDA releases glutamate (Pittaluga et al., 1996), DA (Kuo et al., 1998) and NE (Pittaluga & Raiteri, 1992) from axon terminals indicates that glutamate released is able to facilitate transmitter release via NMDA receptors (Barnes et al., 1994; Desai et al., 1994). Montague et al., (1994) suggested that glutamate and NE release from cortical synaptosomes was in correlation with NMDA-induced production of nitric oxide (NO), an endogenous chemical that is able to inhibit basal membrane transporters, thereby increasing the concentration and life-span of transmitters (e.g., glutamate and NE) released into the extracellular space. The inhibition of neuronal NO synthase by 7-nitroindazole protects against NMDA mediated excitotoxic lesions but not against those evoked by AMPA or KA (Schulz et al., 1995).

The most consistent age-related change in the glutamatergic system is the loss of glutamate receptors. Significant decreases in the mRNA level of glutamate receptors were found in the aged cerebral cortex (Carpenter et al., 1992). Among different glutamate receptors, NMDA receptors are preferentially altered in the aged brain. Decrease in NMDA binding was shown in both rodents and mammalian brain (Cohen & Muller, 1992; Wenk et al., 1991). mRNA level of both NR1 and NR2B subunits of the NMDA receptors have been shown to decrease preferentially in the
aged cerebral cortex, whereas no age-related change was observed in the NR2A subunit (Magnusson, 2000). The modification of subunit expression alters the receptor composition of NMDA receptor in the aged brain and lead to age-related changes in the binding properties of this receptor (Gallagher et al., 1996; Priestley et al., 1995) and/or physiological properties such as desensitization (Monyer et al., 1992). Binding studies revealed significant decrease in NMDA but not AMPA and kainate receptors (Tamaru et al., 1991). These findings support a significant loss of postsynaptic glutamatergic receptors, especially the NMDA subtype, in the aged brain.

**NMDA receptors**

The discovery of potent and selective agonists and antagonists has resulted in extensive information on the NMDA receptor-channel complex (Wood et al., 1990). It consists of four domains:- (1) the transmitter recognition site with which NMDA and L-glutamate interact; (2) a cation binding site located inside the channel where Mg$^{2+}$ can bind and block transmembrane ion fluxes; (3) a PCP binding site that requires agonist binding to the transmitter recognition site, interacts with the cation binding site and at which a number of dissociative anesthetics PCP and ketamine, opiate N-allylnormetazocine (SKF-10047) and MK-801 bind and function as open channel blockers; and (4) a glycine binding site that appears to allosterically modulate the interaction between the transmitter recognition site and the PCP binding site. NMDA is allosterically modulated by glycine, a co-agonist whose presence is an absolute requirement for receptor activation. Molecular cloning has identified to date cDNAs encoding NR1 and NR2A, B, C, D subunits of the NMDA receptor, the deduced amino acid sequences of which are 18% belonging to NR1 and NR2, 55% belonging to NR2A and NR2C or 70% belonging to NR2A and NR2B are identical. Site-directed mutagenesis has revealed that the NR2 subunit carries the binding site for
glutamate within the N-terminal domain and the extracellular loop between membrane segments M3 and M4; whereas the homologous domains of the NR1 subunit carry the binding site for the co-agonist glycine.

Normal functioning of the NMDA receptor complex depends on a dynamic equilibrium among various domain components. Loss of equilibrium during membrane perturbation cause the entire system to malfunction and result in abnormal levels of glutamate in the synaptic cleft (Olney, 1989). An important consequence of NMDA receptor activation is the influx of Ca\(^{2+}\) into neurons (MacDermott et al., 1986; Murphy & Miller, 1988; Holopainen et al., 1989, 1990). Collective evidence suggests that when the membrane is depolarized, the Mg\(^{2+}\) block is relieved and the receptor can be activated by glutamate. Activation of the NMDA receptor therefore requires the association of two synaptic events: membrane depolarization and glutamate release. This associative property provides the logic for the role of the NMDA receptor in sensory integration, memory function, coordination and programming of motor activity (Collingridge & Bliss, 1987) associated with synaptogenesis and synaptic plasticity.

Activation of NMDA receptors results in the opening of an ion channel that is nonselective to cations. This allows flow of Na\(^+\) and small amounts of Ca\(^{2+}\) ions into the cell and K\(^+\) out of the cell (Dingledine et al., 1999; Liu & Zhang, 2000; Cull-Candy et al., 2001; Paoletti & Neyton, 2007). Calcium flux through NMDARs is thought to play a critical role in synaptic plasticity, a cellular mechanism for learning and memory. The NMDA receptor is distinct in that it is both ligand-gated and voltage-dependent. NMDA sensitive ionotropic glutamate receptors probably consist of tetrameric and heteromeric subunit assemblies that have different physiological and pharmacological properties. They are differentially distributed throughout the CNS (Seeburg, 1993; Hollmann & Heinemann, 1994; McBain & Mayer, 1994; Danysz et
al., 1995; Parsons et al., 1998a). NMDA receptors are probably heteromeric assemblies of four subunits. Each subunit has four hydrophobic regions, although only three form membrane-spanning domains - TM1, TM2, and TM4. TM2 makes a hairpin bend within the membrane and forms the channel pore; the "TM" terminology is therefore inappropriate. Functional NMDA receptor complexes are formed by combinations of NR1 and NR2 subunits, which contain glutamate recognition sites. Alternative splicing at three exons, one in the amino-terminal domain (N1) and two in the carboxyl-terminal domain (C1 and C2), generates eight isoforms for the NR1 subfamily. All heteromeric and homomeric NMDA receptor subtype complexes are permeant to Ca\(^{2+}\), Na\(^{+}\), and K\(^{+}\). The open NMDA channel is blocked by Mg\(^{2+}\) and uncompetitive NMDA receptor antagonists, such as (+)MK-801, in a voltage-dependent manner. The speed and voltage observed in this effect depend on the antagonist affinity and the subunit composition. In addition, most NMDA receptors are influenced by Zn\(^{2+}\) ions in a voltage-dependent manner, as well as by oxidation/reduction and pH.

The NMDA channel is blocked in a use- and voltage-dependent manner by Mg\(^{2+}\). This means that NMDA receptors are activated only after depolarization of the postsynaptic membrane by, for example, AMPA receptor activation, which relieves the voltage dependent blockade by Mg\(^{2+}\). This biophysical property and their high Ca\(^{2+}\) permeability render NMDA receptors inherently suitable for their role in mediating synaptic plasticity underlying learning processes and development (Collingridge & Singer, 1990; Danysz et al., 1995). Similar to Mg\(^{2+}\), uncompetitive NMDA receptor antagonists such as ketamine, dextromethorphan, memantine, phencyclidine (PCP) and (+)MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate] block the NMDA channel in the open state,
although the blocking kinetics and voltage of this effect depend on the antagonist (Rogawski, 1993; Parsons et al., 1998b).

To date, two major subunit families, designated NR1 and NR2, have been cloned. Various heteromeric NMDA receptor channels formed by combinations of NR1 and NR2 subunits are known to differ in gating properties, magnesium sensitivity, and pharmacological profile (Sucher et al., 1996; Parsons et al., 1998b). The heteromeric assembly of NR1 and NR2C subunits, for instance, has much lower sensitivity to Mg$^{2+}$ but increased sensitivity to glycine and very restricted distribution in the brain. In situ hybridization has revealed overlapping but different expression profiles for NR2 mRNA. For example, NR2A mRNA is distributed ubiquitously like NR1, with the highest densities occurring in hippocampal regions and NR2B is expressed predominantly in forebrain but not in cerebellum, where NR2C predominates; NR2D is localized mainly in the brainstem (Moriyoshi et al., 1991; Monyer et al., 1992; Nakanishi, 1992; McBain & Mayer, 1994).

In addition to NR1 and NR2, the NR3A subunit has recently been discovered. This receptor subunit, previously termed chi-1, or NMDAR-L, is a relatively recently identified member of a new class in the ionotropic glutamate receptor family. It attenuates NMDA receptor currents when coexpressed with NR1/NR2 subunits in Xenopus oocytes but has no effect when tested with non-NMDA receptors or when expressed alone (Ciabarra et al., 1995; Sucher et al., 1995; Das et al., 1998). Highest levels are present in the spinal cord, brainstem, hypothalamus, thalamus, CA1 field of the hippocampus and amygdala and this distribution remains the same throughout life. Genetic knockout of NR3A in mice results in enhanced NMDA responses and increased dendritic spines in early postnatal cortical neurons, suggesting that NR3A is involved in the development of synaptic elements by modulating NMDA receptor activity (Das et al., 1998).
The highest levels of NR1 mRNA in the adult rat and mouse CNS are in the olfactory bulb and the lowest levels are expressed in the spinal cord. Intermediate levels were found in frontal cortex, hippocampus, cerebellum, and whole brain (Franklin et al., 1993; Akazawa et al., 1994). Similar findings have been reported with antibodies to NR1 subunits (Petralia et al., 1994; Benke et al., 1995). mRNA for double-splice variants in the C1/C2 regions, such as NR1011 (NR1A), show an almost complementary pattern with respect to those lacking both of these inserts, such as NR1100 (NR1B). NR1A mRNA are more concentrated in rostral structures such as cortex, caudate and hippocampus, whereas NR1B mRNA are principally found in more caudal regions such as thalamus, colliculi, locus coeruleus and cerebellum (Laurie & Seeburg, 1994; Paupard et al., 1997). Others reported that the predominant splice variants in cortex and hippocampus were NR1A without N1 insert, whereas in the cerebellum the major variant was NR1B, containing N1 (Zhong et al., 1994). In the hippocampus, NR1A mRNA shows high levels in all regions and is expressed more intensely in CA3 pyramidal neurons (Paupard et al., 1997). mRNA for NR1A and NR1B splice forms is found nearly homogeneously throughout the adult CNS, whereas NR1A and NR1B mRNA is scarce, being detected only at very low levels in postnatal cortex and hippocampus (Laurie & Seeburg, 1994; Paupard et al., 1997). The predominant splice variant in cultured cortical neurons is NR1A (Zhong et al., 1994).

In developing rats, NR1 mRNA levels in cortex and hippocampus increased nearly three-fold from postnatal day 3 to day 15 and approximately doubled from day 15 to day 67 (Franklin et al., 1993; Nowicka & Kaczmarek, 1996). In contrast, cerebellum and brainstem showed no change in NMDAR1 mRNA levels between postnatal days 3 and 15 but levels also doubled from day 15 to day 67 (Franklin et al., 1993). Similar results were reported by a different group, although levels in the
hippocampus peaked at postnatal day 10 and declined thereafter (Pujic et al., 1993). In the hippocampus, NR1A mRNAs dominate at birth and exhibit mature patterns of labeling, with high levels of expression in the CA1 and CA3 regions and the dentate gyrus. In contrast, NR1B mRNAs are initially expressed at lower uniform levels but levels increase more in the CA3 region than in the CA1 region or the dentate gyrus in the second and third postnatal weeks (Paupard et al., 1997).

The NMDA receptor antagonists have potential therapeutic applications. NMDA receptors are involved in learning and other forms of plasticity, such as drug dependence and addiction, chronic pain and CNS development, as well as in normal or disturbed synaptic transmission in some areas of the CNS. Activation of NMDA receptors depends not only on the level of synaptic activity but also on other factors, such as agonist affinity, gating kinetics and Mg²⁺ sensitivity. The role of NMDA receptors in various processes depends on the subtype composition and area of the CNS involved. In animals, most NMDA receptor antagonists produce impairment of learning when given at sufficiently high doses before the association phase but not when administered after this phase or during retrieval (Rogawski, 1993; Leeson & Iversen, 1994; Danysz et al., 1995; Avenet et al., 1996; Parsons et al., 1998a).

**Functional Effects Mediated via the NMDA Receptor Signal Transduction**

The NMDA class of glutamate receptors has a critical role in the induction of long-term potentiation (LTP), a synaptic modification that encode some forms of long-term memory. However, NMDA receptor antagonists disrupt a variety of mental processes (Caramanos & Shapiro, 1994; Javitt et al., 1996) that are not dependent on long-term memory. They interfere with working memory (Krystal et al., 1994; Adler et al., 1998) a short-lasting form of memory that is maintained by neuronal activity rather than by synaptic modification. This suggests that there are unknown functions
of the NMDA-receptor channel. Working memory is stored by the maintained firing of a memory-specific subset of neurons in networks of the prefrontal cortex (Funahashi et al., 1989). Firing is thought to be maintained by a reverberatory process (Amit et al., 1994) in which active neurons selectively excites each other through recurrent connections. The NMDA receptor in the forebrain is thought to modulate some forms of memory formation, with the NR2B subunit being particularly relevant to this process.

**Metabotropic glutamate receptor**

The metabotropic glutamate receptors or mGluRs are a type of glutamate receptor which are active through an indirect metabotropic process. They are members of the group C family of G-protein-coupled receptors, or GPCRs (Bonsi et al., 2005). Like all glutamate receptors, mGluRs bind to glutamate, an amino acid that functions as an excitatory neurotransmitter. The mGluRs perform a variety of functions in the central and peripheral nervous systems: they are involved in learning, memory, anxiety, and the perception of pain (Ohashi et al., 2002). They are found in pre- and postsynaptic neurons in synapses of the hippocampus, cerebellum (Hinoi et al., 2001) and the cerebral cortex, as well as other parts of the brain and in peripheral tissues (Chu & Hablitz, 2000). Like other metabotropic receptors, mGluRs have seven transmembrane domains that span the cell membrane (Platt, 2007). Unlike ionotropic receptors, metabotropic glutamate receptors are not ion channels. They activate biochemical cascades, leading to the modification of other proteins, as for example ion channels. This can lead to changes in the synapse's excitability, for example by presynaptic inhibition of neurotransmission (Sladeczek et al., 1992), or modulation and even induction of postsynaptic responses (Chu & Hablitz, 2000; Endoh, 2004; Bonsi et al., 2005; Platt, 2007).
Eight different types of mGluRs, labeled mGluR1 to mGluR8 are divided into groups I, II, and III (Chu & Hablitz, 2000; Hinoi et al., 2001; Endoh, 2004; Bonsi et al., 2005). Receptor types are grouped based on receptor structure and physiological activity (Ohashi et al., 2002). The mGluRs are further divided into subtypes, such as mGluR7a and mGluR7b. The mGluRs in group I, including mGluR1 and mGluR5, are stimulated strongly by the excitatory amino acid analog L-quisqualic acid (Chu & Hablitz, 2000; Bates et al., 2002) Stimulating the receptors causes the associated enzyme phospholipase C to hydrolyze phosphoinositide phospholipids in the cell's plasma membrane (Chu & Hablitz, 2000; Endoh, 2004; Bonsi et al., 2005). This leads to the formation of inositol 1,4,5-trisphosphate (IP3) and diacyl glycerol. Due to its hydrophilic character IP3 can travel to the endoplasmic reticulum where it induces, via fixation on its receptor, the opening of calcium channels increasing in this way the cytosolic calcium concentrations. The lipophilic diacylglycerol remains in the membrane acting as a cofactor for the activation of protein kinase C. These receptors are also associated with Na+ and K+ channels. Their action can be excitatory, increasing conductance, causing more glutamate to be released from the presynaptic cell, but they also increase inhibitory postsynaptic potentials, or IPSPs (Chu & Hablitz, 2000). They can also inhibit glutamate release and can modulate voltage-dependent calcium channels (Endoh, 2004). Group I mGluRs, but not other groups, are activated by 3,5-dihydroxyphenylglycine (DHPG) (Shigemoto et al., 1997) a fact which is useful to experimenters because it allows them to isolate and identify them. The receptors in group II, including mGluRs 2 and 3, and group III, including mGluRs 4, 6, 7, and 8 prevent the formation of cyclic adenosine monophosphate, or cAMP, by activating a G protein that inhibits the enzyme adenylyl cyclase, which forms cAMP from ATP (Chu & Hablitz, 2000; Hinoi et al., 2001; Medical Research Council, 2003; Bonsi et al., 2005). These receptors are involved in presynaptic inhibition (Endoh,
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and do not appear to affect postsynaptic membrane potential by themselves. Receptors in groups II and III reduce the activity of postsynaptic potentials, both excitatory and inhibitory, in the cortex (Chu & Hablitz, 2000). Different types of mGluRs are distributed differently in cells. One study found that Group I mGluRs are mostly located on postsynaptic parts of cells while groups II and III are mostly located on presynaptic elements (Shigemoto et al., 1997), though they have been found on both pre- and postsynaptic membranes (Endoh, 2004). Also, different mGluR subtypes are found predominantly in different parts of the body. mGluR4 is located only in the brain, in locations such as the thalamus, hypothalamus and caudate nucleus (InterPro, 2008). All mGluRs except mGluR6 are thought to exist in the hippocampus and entorhinal cortex (Shigemoto et al., 1997).

Metabotropic glutamate receptors are known to act as modulators of other receptors. For example, group I mGluRs are known to increase the activity of N-methyl-D-aspartate receptors (Skeberdis et al., 2001; Lea et al., 2002), a type of ion channel-linked receptor that is central in a neurotoxic process called excitotoxicity. It has been suggested that mGluRs act as regulators of neurons' vulnerability to excitotoxicity through their modulation of NMDARs, the receptor most involved in that process (Baskys & Blaabjerg, 2005). Excessive amounts of N-methyl-D-aspartate, an agonist for NMDARs, has been found to cause more damage to neurons in the presence of group I mGluR agonists (Bruno et al., 1995). Metabotropic glutamate receptors are also thought to affect dopaminergic and adrenergic neurotransmission (Wang & Brownell, 2007). Like other glutamate receptors, mGluRs have been shown to be involved in synaptic plasticity (Endoh, 2004; Bonsi et al., 2005) and in neurotoxicity and neuroprotection (Siliprandi et al., 1992; Baskys et al., 2005). They participate in long term potentiation and long term depression, and
Glutamate mediated excitotoxic cell death

Excitotoxicity is the pathological process by which nerve cells are damaged and killed by glutamate and similar substances. Evidence is gathering that excitatory amino acid (EAA) neurotransmission contribute to neuronal ischemic injury during conditions of metabolic stress (Olney et al., 1973; Choi, 1988). Excessive synaptic accumulation of glutamate can cause neuronal overactivation, precipitating a cascade of cellular events that lead ultimately to cell death, a phenomenon termed glutamate excitotoxicity. This occurs when receptors for the excitatory neurotransmitter glutamate such as the NMDA receptor and AMPA receptor are overactivated. Glutamate is a prime example of an excitotoxin in the brain and it is also the major excitatory neurotransmitter in the mammalian CNS (Temple et al., 2001). During normal conditions, glutamate concentration can be increased up to 1mM in the synaptic cleft, which is rapidly decreased in the lapse of milliseconds. When the glutamate concentration around the synaptic cleft cannot be decreased or reaches higher levels, the neuron kills itself by a process called apoptosis. Glutamate receptors, including the NMDA subtype and several non-NMDA subtypes, are transiently overexpressed in neonates and infants, in as much as EAAAs play a critical role in the development of the central nervous system (McDonald et al., 1990). Hardingham et al., (2002) noted that extrasynaptic NMDA receptor activation, triggered by both glutamate exposure or hypoxic/ischemic conditions, activate a CREB (cAMP response element binding protein) shut-off, which in turn, caused loss of mitochondrial membrane potential and apoptosis. Excitotoxins like NMDA and kainic acid which bind to these receptors, as well as pathologically high levels of
glutamate, cause excitotoxicity by allowing high levels of Ca\(^{2+}\) (Manev et al., 1989) to enter the cell. Ca\(^{2+}\) influx into cells activates a number of enzymes, including phospholipases, endonucleases, and proteases such as calpain. These enzymes go on to damage cell structures such as components of the cytoskeleton, membrane and DNA. Reports suggest that calcium influx through NMDA receptors is involved in ROS production and neuronal damage resulting from moderate energy depletion (Hernández-Fonseca et al., 2008). Excitotoxicity is involved in spinal cord injury, stroke, traumatic brain injury and neurodegenerative diseases of the central nervous system such as Multiple sclerosis, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alcoholism and Huntington's disease (Kim et al., 2002) and neurological disorders such as ischemia, cerebral trauma and some chronic neurodegenerative diseases. An excess of glutamate release, or a deficiency in its clearance from the synaptic cleft, which depends mainly on its transport by high affinity carriers, are potential sources for the accumulation of extracellular glutamate.

**Glutamate transporters**

The concentration of glutamate is regulated to ensure neurotransmission with a high temporal and local resolution. Neuronal damage is associated with excitotoxicity, a type of cell death triggered by the overactivation of glutamate receptors and the loss of calcium homeostasis. The removal of glutamate from the extracellular fluid occurs by uptake and by diffusion (Tong & Jahr, 1994). Failure of glutamate clearance lead to neuronal damage, named excitotoxic damage, due to the prolonged activation of glutamate receptors. Extracellular glutamate must be cleared quickly, perhaps within 1ms, to maintain glutamate below toxic levels (Trotti et al., 1998). Glutamate transporters represent the only significant mechanism for the uptake
of extracellular glutamate, and their importance for the long-term maintenance of low non-toxic glutamate concentrations is well documented (Danbolt, 2001).

Excitatory Amino Acid Transporters (EAAT), formerly known as glutamate transporters, belong to the family of neurotransmitter transporters. They serve to terminate the excitatory neurotransmitter signal by removal of glutamate from the neuronal synapse into glia cells. EAATs are membrane-bound pumps that resemble ion channels (Ganel & Rothstein, 1999). In humans as well as in rodents, five subtypes have been identified and named EAAT1-5. Subtypes EAAT1-3 are found in membranes of glial cells (astrocytes, microglia, and oligodendrocytes) as well as in endothelial cells, whereas EAAT4 is located on neurons (Anderson & Swanson, 2000). Finally, EAAT5 is only found in the retina where it is principally localised to photoreceptors and bipolar neurons in the retina (Pow & Barnett, 2000). In rodents, the orthologs for EAAT1-3 are named GLAST, GLT1 and EAAC1 respectively (Shigeri et al., 2004).

When glutamate is taken up into glia cells by the EAATs, it is not reused directly but converted to glutamine and stored vesicles. Subsequently these vesicle are released from Glia cells and glutamine transported back into the presynaptic neuron, converted back into glutamate and store into vesicles by action of the VGLUTs (Pow & Robinson, 1994; Shigeri et al., 2004). This process is named the glutamate-glutamine cycle. Given that glutamate transporters provide the main route by which glutamate is cleared, it is logically predicted that an aberration in transporter expression and function lead to toxic glutamate levels and thus promote neuronal degeneration (Tanaka et al., 1997). Recent studies have suggested the involvement of the glutamate transporters in radiation induced neurotoxicity (Martha et al., 2009). Studies in brain autopsy specimens of HIV-1-infected patients have shown that the
expression of EAAT-2 by activated microglia exert a compensatory effect that protects neurons from glutamate neurotoxicity (Xing et al., 2009).

Signal transduction through Second Messengers

Inositol 1,4,5-trisphosphate (IP3)

Many biological stimuli, such as neurotransmitters, hormones and growth factors, activate the hydrolysis of phosphatidyl inositol 4,5-bisphosphate (PIP2) in the plasma membrane which is hydrolyzed by phospholipase C (PLC) to produce IP3 and diacylglycerol (DAG). The IP3 mediates Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores by binding to IP3 receptors (IP3R). IP3R are the IP3 gated intracellular Ca\(^{2+}\) channels that are mainly present in the endoplasmic reticulum (ER) membrane. The IP3 induced Ca\(^{2+}\) signaling plays a crucial role in the control of diverse physiological processes such as contraction, secretion, gene expression and synaptic plasticity (Berridge, 1993). In response to many stimuli such as neurotransmitters, hormones and growth factors, PIP2 in the plasma membrane is hydrolyzed by PLC to produce IP3 and DAG. IP3 plays a dominant role as a second messenger molecule for the release of Ca\(^{2+}\) from intracellular stores, while DAG activates protein kinase C (PKC).

In mammalian cells, there are three IP3R subtypes- IP3R1, IP3R2 and IP3R3 which are expressed to varying degrees in individual cell types (Wojcikiewicz, 1995; Taylor et al., 1999) and form homotetrameric or heterotetrameric channels (Monkawa et al., 1995). In previous studies, a plasmid vector containing full-length rat IP3R3 linked to green fluorescent protein GFP-IP3R3 was constructed and visualized the distribution of GFP-IP3R3 was constructed in living cells (Morita et al., 2002; 2004). The confocal images obtained in these studies provided strong evidence that IP3Rs are distributed preferentially on the ER network. Furthermore, Morita et al., (2004) demonstrated that the expressed GFP-IP3R3 acts as a functional IP3-induced Ca\(^{2+}\)
channel. Frequently, IP3Rs are not uniformly distributed over the membrane but rather form discrete clusters (Bootman et al., 1997). The clustered distribution of IP3Rs has been predicted to be important in controlling elementary Ca^{2+} release events, such as Ca^{2+} puffs and blips, which act as triggers to induce the spatiotemporal patterns of global Ca^{2+} signals, such as waves and oscillations (Thomas et al., 1998; Swillens et al., 1999; Shuai & Jung, 2003). Tateishi et al., (2005) reported that GFP-IP3R1 expressed in COS-7 cells aggregates into clusters on the ER network after agonist stimulation. They concluded that IP3R clustering is induced by its IP3-induced conformational change to the open state, not by Ca^{2+} release itself, because IP3R1 mutants that do not undergo an IP3 induced conformational change failed to form clusters. However, their results are inconsistent with studies by other groups (Wilson et al., 1998; Chalmers et al., 2006), which suggested that IP3R clustering is dependent on the continuous elevation of intracellular Ca^{2+} concentration. Thus, the precise mechanism underlying IP3R clustering remains controversial. Studies by Tojyo et al., (2008) have shown that IP3 binding to IP3R, not the increase in Ca^{2+}, is absolutely critical for IP3R clustering. They also found that depletion of intracellular Ca^{2+} stores facilitates the generation of agonist-induced IP3R clustering.

Group I mGluRs (mGluR1/5 subtypes) are also demonstrated to mainly affect intracellular Ca^{2+} mobilization (Conn & Pin, 1997; Bordi & Ugolini, 1999). To sequentially facilitate intracellular Ca^{2+} release, group I receptors activate the membrane-bound phospholipase C (PLC), which stimulates phosphoinositide turnover by hydrolyzing PIP2 to IP3 and diacylglycerol. IP3 then causes the release of Ca^{2+} from intracellular Ca^{2+} stores (such as endoplasmic reticulum) by binding to specific IP3 receptors on the membrane of Ca^{2+} stores (Berridge, 1993). Altered Ca^{2+} levels could then engage in the modulation of broad cellular activities.
Cyclic Guanosine Monophosphate (cGMP)

cGMP generation has been associated with neurotransmission (Hofmann et al., 2000), vascular smooth muscle relaxation (Fiscus et al., 1985) and inhibition of aldosterone release from adrenal glomerulosa cell suspension (Matsuoka et al., 1985). The most extensively studied cGMP signal transduction pathway is that triggered by nitric oxide (NO) (Bredt & Snyder, 1990). cGMP effects are primarily mediated by the activation of cGMP-dependent protein kinases (PKGs). Two distinct mammalian PKGs- PKG-I and PKG-II- have been identified, as well as two splice variants of PKG-I - PKG-Iα and -Iβ. In the brain, PKG-I is highly expressed in cerebellar Purkinje cells and to a lesser extent, in striatal medium spiny neurons (De Camilli et al., 1984). PKG-II is a membrane-associated protein that is expressed throughout the brain (de Vente et al., 2001). The effects produced by the cGMP signaling pathway modulate drug-induced neural plasticity leading to behavioural alterations (Jouvert et al., 2004).

Activation of the NMDA receptor increases cAMP in the CA1 region of the hippocampus; this increase is mediated through Ca²⁺ calmodulin-dependent adenylyl cyclase (Chetkovich & Sweatt, 1993). The influx of Ca²⁺ also stimulates Ca²⁺ calmodulin-dependent nitric-oxide synthase (NOS) type to produce NO, which stimulates guanylyl cyclase to produce cGMP (Garthwaite, 1991; 2005). Cyclic nucleotide pathways cross talk to modulate each other’s synthesis, degradation and actions. Increased cGMP increase the activity of cGMP stimulated PDE2 to enhance hydrolysis of cAMP, or it inhibits the PDE3 family and decreases the hydrolysis of cAMP (Pelligrino & Wang, 1998). cAMP and cGMP are involved in NMDA receptor-mediated signaling in cerebral cortical and hippocampal neuronal cultures. The influx of Ca²⁺ via the NMDA receptor stimulates calcium/calmodulin dependent adenylyl cyclase, leading to production of cAMP. This increase in cAMP seems to be
tightly regulated by PDE4. The Ca\textsuperscript{2+} influx also stimulates the production of NO and subsequent activation of guanylyl cyclase, leading to cGMP production (Suvarna & O'Donnell, 2002).

**Cyclic Adenosine Monophosphate (cAMP)**

The second messenger concept of signaling was born with the discovery of cAMP and its ability to influence metabolism, cell shape and gene transcription (Sutherland, 1972) via reversible protein phosphorylations. cAMP is produced from ATP adenylyl cyclase (AC) in response to a variety of extracellular signals such as hormones, growth factors and neurotransmitters. Elevated levels of cAMP in the cell lead to activation of different cAMP targets. It was long thought that the only target of cAMP was the cAMP-dependent protein kinase (cAPK), which has become a model of protein kinase structure and regulation (Francis & Corbin, 1999; Canaves & Taylor, 2002). In recent years it has become clear that not all effects of cAMP are mediated by a general activation of cAPK (Dremier et al., 1997). Several cAMP binding proteins have been described: cAPK (Walsh et al., 1968), the cAMP receptor of *Dictyostelium discoideum*, which participates in the regulation of development (Klein et al., 1997), cyclic nucleotide gated channels involved in transduction of olfactory and visual signals (Kaupp et al., 1989; Goulding et al., 1992) and the cAMP-activated guanine exchange factors Epac 1,2 which specifically activate the monomeric G protein Rap (Kawasaki et al., 1998).

**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 hyperglycaemia, obesity, neuropathy, nephropathy, retinopathy, cardiopathy, osteoporosis and coma leading to death. Pancreatic damage resulting in the dysfunction of $\alpha$ and $\beta$ cells causes disordered glucose homeostasis. In diabetic individuals the regulation of glucose levels by insulin is defective, either due to defective insulin production which is called as Insulin Dependent Diabetes Mellitus (IDDM) or due to insulin resistance that is termed as Non Insulin Dependent Diabetes Mellitus (NIDDM).

Diabetes mellitus has been reported to cause degenerative changes in neurons of the central nervous system (Garris, 1990; Lackovic et al., 1990; Bhattacharya & Saraswathi, 1991). Our previous studies demonstrated adrenergic, serotonergic and dopamine D$_2$ 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 & Lackovic, 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 (Ramakrishna & Namasiyavam, 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 $\beta$-adrenergic receptor populations were decreased in diabetes (Garris, 1995). 5-HT content in the brain is reported to be decreased during diabetes (Jackson & Paulose, 1999; Chu et al., 1986; Sumiyoshi et
Garris, (1995) 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. Hyperglycaemia is reported to alter the noradrenergic and cholinergic nerve components (Akria et al., 1994) with decrease in the Na\textsuperscript{+} - K\textsuperscript{+} 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 in STZ rats. In the heart the initial changes in short-term diabetes included an increase in NE concentration but did not persist in the long term diabetic animals. 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).

**Brain neurotransmitters and hypoglycaemia**

Glucose in brain, supplies energy essential for maintenance of the nervous system. During hypoglycaemia, energy dependent mechanisms for restoring normal transmembrane gradients of sodium and calcium cannot operate because of the depletion of ATP and phosphocreatine associated with hypoglycaemia. Excess calcium 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, 1999). Deficiency in glucose that results from hypoglycaemic insults trigger neuronal injuries. Balance in ion homeostasis is disturbed, which in turn results in membrane depolarization and massive release of neurotransmitters, including glutamate (Siesjo, 1978; Erecinska &
Silver, 1989). 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 (Wieloch, 1985; Monyer et al., 1992; Cebers et al., 1998).

Hypoglycaemia causes several-fold elevations in brain extracellular glutamate and aspartate concentrations, and ablation of presynaptic glutamatergic terminals prevent hypoglycaemic neuronal death (Wieloch, 1985; Butcher et al., 1987). Pretreatment with glutamate receptor antagonists can also reduce hypoglycaemic neuronal death (Wieloch, 1985), but these agents are less effective when administered after hypoglycaemia has occurred (Nellgard & Wieloch, 1992). An additional limitation to the use of glutamate receptor antagonists in clinical settings is that these agents are themselves neurotoxic (Olney, 1989). Hypoglycaemic neuronal death is not a direct and immediate consequence of low-energy substrate but results instead from a cascade of events precipitated by the lack of substrate. Sustained activation of glutamate receptors has been established as a necessary upstream event in this cascade (Auer & Siesjo, 1993). 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; Sang et al., 2005). It is reported that moderate prolonged hypoglycaemia results in reduced cardiac vagal outflow in both diabetic patients and nondiabetic subjects (Minna et al., 2005).

Clinically, hypoglycaemia results in depression of CNS function, with rostral brain regions being affected before more caudally situated regions. For example, in severe hypoglycaemia associated with isoelectric EEG tracings, cerebral cortical
activity is absent but medullary function persists, as indicated by the maintenance of effective respiratory and cardiovascular activity. Reduced synthesis of neurotransmitters rather than a global cerebral energy deficit explains the neurological symptoms and EEG changes in moderate hypoglycaemia (Butterworth, 1983; 1999). The physiologic disturbances associated with acute hypoglycaemia result in a stress response, with release of catecholamines, glucagon, subsequent lipolysis and glycogenolysis in an attempt to increase substrate availability for normal metabolic processes (Jane, 1999). Protection against epinephrine defects, both without and with antecedent hypoglycaemia in diabetes, is associated with enhancement of adrenal catecholamine-synthesizing enzyme levels. It is reported that 2-D Glucose augmented the turnover of NE, DA and 5-HT under the fasted condition. Insulin perfusion within the medial hypothalamic sites evoked a significant increase in the synthesis and release of DA from the sated rat, but did not alter its turnover. However, in the interval following insulin perfusion, DA and 5-HT turnover were enhanced while the efflux of 5-HT was suppressed.

Pyruvate derived from glucose is the major precursor of the acetyl group. Inhibition of pyruvate oxidation results in reduced ACh synthesis both in vitro and in vivo. Incorporation of [14C]choline into ACh in brain in vivo is decreased in rats with insulin-induced hypoglycaemia. Hypoglycaemia results in decreased synthesis of the neurotransmitter pool of ACh are supported by the observation that administration of the CNS cholinesterase inhibitor physostigmine to hypoglycaemic animals delays the onset of seizures and coma (Gibson & Blass, 1976). It is also reported that the extracellular concentrations of acetylcholine both in the hippocampus and striatum did not change during hypoglycaemia. Changes of hippocampal cholinergic release is not involved in the mechanism of cognitive impairment during hypoglycaemia (Hiroyuki et al., 2006).
Similar findings of an adverse effect of hypoglycaemia 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. Hypoglycaemia also produces a transient but substantial increase in extracellular concentrations of glutamate, GABA and dopamine, as measured using in vivo cerebral microdialysis (Butcher et al., 1987; Butterworth, 1983, 1999). Studies reported that modulation of the GABAergic system in the ventromedial hypothalamus (VMH) alters both glucagon and sympathoadrenal, but not corticosterone, responses to hypoglycaemia. GABAergic inhibitory tone within the VMH modulates glucose counterregulatory 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 hypoglycaemia. Enhanced glutamate receptor subtype activity in the cerebellum (Joseph et al., 2007) and cerebral cortex (Joseph et al., 2008) in insulin induced hypoglycaemic and streptozotocin induced diabetic rats were reported by studies from our laboratory. Dopaminergic dysfunction in hippocampus during hypoglycaemia and hyperglycaemia contributing to cognitive and memory deficits was recently reported (Robinson et al., 2009).

Hypoglycaemia results in cognitive dysfunction. Wredling et al., (1990) reported permanent neuropsychological impairment after recurrent episodes of severe hypoglycaemia in diabetic patients. Severe deterioration in cognitive function and personality in patients with long-standing diabetes as a complication of a consequence of insulin treatment is reported (Gold et al., 1995). IDDM patients with hypoglycaemia unawareness exhibited more profound cognitive dysfunction during acute hypoglycaemia which persisted for longer following blood glucose recovery.
Severe hypoglycaemia with cognitive dysfunction is three times more common in intensively, rather than conventionally, treated IDDM (Maran et al., 1995). In the insulin treated diabetic patients exposed to a spontaneous episode of severe hypoglycaemia, the cognitive decrements and altered mood states noted is persistent and is the consequence of previous exposure to recurrent episodes of severe hypoglycaemia (Strachan et al., 2000). Recurrent hypoglycaemia significantly diminished cognitive performance in both diabetic and nondiabetic animals. The diabetic hippocampus adapt to high circulating glucose, with increased susceptibility to reductions in glucose availability. RH diminishes ability to meet the demands of a relatively demanding cognitive challenge during hypoglycaemia (McNay, 2005). Recurrent hypoglycaemia markedly affects hippocampally dependent spatial working memory task. This is accompanied by alterations within the hippocampus, including both ECF glucose and lactate levels during cognitive testing and electrophysiological function. The impact of recurrent hypoglycaemia on cognition is multifaceted and includes both metabolic and electrophysiological components (McNay et al., 2006).

Exposure to stress is known to precipitate or exacerbate many neuropsychiatric disorders such as depression, Parkinson's disease, schizophrenia, and others (Schwab & Zieper, 1965; Mazure, 1995). All these disorders involve a working memory deficit caused by prefrontal cortical (PFC) dysfunction (Mattes, 1980; Weinberger et al., 1986; Deutch, 1993; Fibiger, 1995). NMDA and opiate receptors are plentiful in this area and stress-induced innervation of the fronto-cortical neurons is prevented if these receptors are selectively blocked. This increase of DA from the dendrites of dopamine neurons is due to an alteration in GABA regulation of the dopamine neurons. As with noradrenergic systems, single or repeated exposures to stress potentiates the capacity of a subsequent stressor to increase DA function in the forebrain without altering basal DA turnover, suggesting that the receptors have been
hyper-sensitized (Basso et al., 1999). DA neurons are vulnerable to metabolic stress (Callahan et al., 1998). The maintenance of normal energy metabolism in T1DM during hypoglycaemia effect glucose sensing in the brain and contribute to hypoglycaemia-associated autonomic failure (Bischof et al., 2006).

**Ageing and diabetes**

Diabetes mellitus is a major global health problem that affects more than 185 million people around the world (Amos et al., 1997; Zimmet, 1999; Zimmet et al., 2001). The disease is an increasingly prevalent metabolic disorder in humans and is characterised by hyperglycemia (Kumar & Clark, 2002; Dunne et al., 2004). The number of diabetic patients is expected to reach 300 million by the year 2025. The projected increase in the number of diabetic patients will strain the capabilities of healthcare providers the world over (Adeghate et al., 2006). 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 plays a prominent role. The progression of diabetes is associated with an impaired ability of the neurons in the CNS to release neurotransmitters (Broderick & Jacoby, 1989). Numerous neurochemical studies using both animals and humans have revealed age-related changes in neurotransmitter enzyme activities and receptor binding (McGeer & McGeer, 1982; Hepler et al., 1985; Smith, 1988). Neurotransmitters show significant alterations during hyperglycaemia and causes degenerative changes in neurons of the central nervous system (Garris, 1990; Lackovic et al., 1990; Bhardwaj, et al., 1999). Studies on STZ-induced diabetic rat models have shown similar results which exhibits morphological, behavioural and electrophysiological alterations on diabetes (Jakobsen et al., 1987; Biessels et al., 1989).
Learning and memory deficits are associated with Type I and Type II diabetes mellitus (Gispen & Biessels, 2000) and brain morphological abnormalities have been found in diabetic patients, mainly in the cortical area (Dejgaard et al., 1991). STZ-induced diabetes results in structural alterations of mAChRs in the brain (Latifpour et al., 1991) which in turn alters cholinergic nerve components (Akria et al., 1994) with decrease in the Na+, K+-ATPase activity (Gurcharan & Sukwinder, 1994). Studies of Latifpour and McNeill, (1984) on long-term STZ-induced diabetes reported large reduction in muscarinic receptor densities as compared with their age-matched controls. Ageing and diabetes are intimately related at a molecular level and hence diabetes is able to provide the link between disease treatment and the prevention of age-related diseases. If specific molecular pathways controlling the rate of ageing are modulated genetically, then perhaps they are modulated pharmacologically (Geesaman, 2006). These insights ultimately have an important impact on the discovery and development of drugs to both treat and prevent a wide range of diseases.

**Ageing and hypoglycaemia**

The increasing proportion of elderly persons in the global population, and the implications of this trend in terms of increasing rates of chronic diseases such as diabetes mellitus, continue to be a cause for concern for clinicians and healthcare policy makers. The diabetes of the elderly subjects has two forms: diabetes of long duration, manifesting itself in younger or medium ages and senile diabetes, appearing above the age of 65 years. The diagnosis and treatment of diabetes in the elderly is challenging, as age-related changes alter the clinical presentation of diabetic symptoms. There are numerous reasons to maintain blood glucose levels below 11.1 nmol/L (200 mg/dl) in older persons and there are a number of changes often seen
with advancing age that persons and there are a number of changes often seen with advancing age that interfere with the management of diabetes mellitus, e.g. hypodipsia, anorexia, visual disturbance, altered renal and hepatic function, depression, impaired basoreceptor response and multiple medications (Morley & Perry, 1991). Combination therapy of insulin with oral hypoglycaemic agents is not recommended in this group of patients. Combination therapy of insulin with oral hypoglycaemic agents is not recommended in this group of patients. The decline in cognitive function, especially on challenging tasks, associated with aging is well known and relatively well-characterised. Recent evidence has provided strong support for the view that reduced ability to provide and regulate fuel supply, i.e., glucose, to the aged brain is a major cause of such decline (McNay, 2005). Inability to regulate glucose also defines diabetes and both diabetes and the recurrent hypoglycemia seen in intensively insulin-treated diabetic patients also affect cognition. As type 2 diabetes progresses in older persons, polypharmacy intensification is required to achieve adequate glycaemic control with the attendant increased risk of adverse effects as a result of age-related changes in drug metabolism. The recently available oral glucose lowering agents in the market along with the newer types of insulin are used in elderly diabetic patients. The effect of aging on metabolism and drug elimination kinetics must, however, be taken into consideration. In particular, it should be borne in mind that the risk of hypoglycemia is more deleterious in the elderly and should be avoided (Oiknine & Mooradian, 2003). A better compliance is obtained, being a fundamental aspect in the elderly diabetics and a reduction of the number and severity of the hypoglycemia, which are the most important aspects in the elderly diabetes (Motta et al., 2008). The recent introduction of the incretins, a group of intestinal peptides that enhance insulin secretion after ingestion of food, as novel oral antihyperglycaemic treatments may prove significant in older persons (Abbatecola et al., 2008).
Glutamate receptors in diabetes and hypoglycaemia

Neurodegeneration results from over activation of NMDA receptors causing excitotoxicity proposed to be responsible for certain neurological diseases. 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. Diabetes mellitus induces cognitive impairment and defects of long-term potentiation in the hippocampus as indicated by behavioural and electrophysiological analysis. Considered to be an important mechanism of learning and memory in mammals, long-term potentiation is known to require regulation of the glutamate receptor properties. According to many studies, defects of long-term potentiation in the hippocampus of diabetic animals are due to abnormal glutamate receptors. Earlier studies explained that changes in glutamate receptors account for modifications of long-term potentiation in various models of diabetes mellitus. Deficits in long-term potentiation during chronic diabetes arise from dysfunction of the NMDA subtype of glutamate receptors in early stages of the disease (Trudeau et al., 2004). Previous studies demonstrated that disruption of glutamate homeostasis occurs in the diabetic retina (Qing & Donald, 2002). Binding properties of brain glutamate receptors of STZ induced rats and the possible role of AMPA receptors in cognitive deficits during diabetes is reported by Gagne et al., (1997). Altered glutamatergic neurotransmission and calcium homeostasis contribute to retinal neural cell dysfunction and apoptosis in diabetic retinopathy. Elevated glucose is reported to change the expression of ionotropic Glutamate receptor subunits and impairs calcium homeostasis in retinal neural cells (Ana et al., 2006). It is suggested that enzymes of the glutamate system respond differently towards diabetes or deprivation of food and diabetes affect the glutamate uptake system in glial cells (Galanopoulos et al., 1988). Recent studies suggest that glutamate plays a
pivotal role in the processing of sensory information in the spinal cords of patients with diabetic neuropathy. Abnormal expression of multiple glutamate receptors is involved in the development of diabetic neuropathy (Tomiyama et al., 2005).

Studies reported that neurons impaired of energy metabolism are highly sensitive to excitotoxicity (Simon et al., 1984; Wieloch, 1985; Monyer et al., 1989; Cebers et al., 1998). Pathophysiological mechanisms responsible for neuronal cell death in hypoglycaemia include the involvement of glutamate excitotoxicity. Hypoglycaemia specifically increases the sensitivity of NMDA receptors to activation by glutamate, which result in a lower threshold for glutamate induced excitotoxicity (Jane, 1999). Severe and prolonged hypoglycaemia results in increased release of glutamate in the brain, leading to membrane depolarization. This is followed by cerebral energy failure and neuronal cell death. Glutamate neurotoxicity is thus implicated in the pathogenesis of hypoglycaemia induced neuronal death and Ca\(^{2+}\) calmodulin-dependent protein kinase II appears to be one of the intracellular targets for glutamate neurotoxicity in hypoglycaemia (Hu et al., 1995). Hypoglycaemia causes several-fold elevations in brain extracellular glutamate concentrations and pretreatment with glutamate receptor antagonists prevent hypoglycaemic neuronal death (Nellgard & Wieloch, 1992; Sandberg et al., 1986; Wieloch, 1985).

**Inositol 1, 4, 5-trisphosphate (IP3) and activation of calcium release**

Cytosolic Ca\(^{2+}\) is a focal point of many signal transduction pathways and modulates a diverse array of cellular activities ranging from fertilization to cell death (Berridge et al., 2000). In most cell types, the major internal [Ca\(^{2+}\)] stores are the endoplasmic reticulum/sarcoplasmic reticulum (ER/SR). One mechanism for mobilizing such stores involves the phosphoinositide pathway. The binding of many hormones to specific receptors on the plasma membrane leads to the activation of an
enzyme (phosphoinositidase C) that catalyses the hydrolysis of phospholipids to produce the intracellular messenger inositol 1,4,5-trisphosphate (IP3). Although derived from a lipid, IP3 is water soluble and diffuses into the cell interior where it encounters IP3 receptors (IP3Rs) on the ER/SR. The binding of IP3 changes the conformation of IP3Rs such that an integral channel is opened, thus allowing the \([Ca^{2+}]\) stored at high concentrations in the ER/SR to enter the cytoplasm. A critical feature of IP3Rs is that their opening is regulated by the cytosolic \(Ca^{2+}\) concentration. This sensitivity to cytosolic \([Ca^{2+}]\) allows them to act as \(Ca^{2+}\)-induced calcium release (CICR) channels that promote the rapid amplification of smaller trigger events.

**Factors affecting insulin regulation from pancreatic \(\beta\)-cells**

D-Glucose is the major physiological stimulus for insulin secretion. The mechanism of glucose induced insulin release is not completely understood. Phosphorylation of glucose to glucose-6-phosphate serves as the rate limiting step in glucose oxidation (Schuit, 1996). Glucokinase acts as a glucose sensor during this process. An increased ATP/ADP ratio is believed to close \(K^{+}\)-ATP channel at the plasma membrane, resulting in decreased \(K^{+}\) efflux and subsequent depolarisation of the \(\beta\)-cell (Dunne, 1991). Depolarisation activates voltage-dependent \(Ca^{2+}\) channels, causing an influx of extracellular \(Ca^{2+}\) (Liu et al., 1998). Although intracellular \(Ca^{2+}\) activates protein kinases such as \(Ca^{2+}\) and calmodulin dependent protein kinase (Breen & Aschcroft, 1997), it remains unclear how increase in intracellular \(Ca^{2+}\) leads to insulin release. Intracellular \(Ca^{2+}\) stores appear to regulate a novel plasma membrane current \([Ca^{2+}\ release activated non-selective cation current\], whose activity control glucose activated secretion. Glucose induced insulin secretion is also partly dependent upon the activation of typical isoforms of PKC within the \(\beta\)-cell (Harris et al., 1996). It is suggested that PKC is tonically active and effective in the
maintenance of the phosphorylated state of the voltage-gated L-type Ca\(^{2+}\) channel, enabling an appropriate function of this channel in the insulin secretory process (Arkhammar et al., 1994). Glucose is an important regulator of various β-cell processes including insulin biosynthesis and release. Glucose, over short intervals stimulates insulin biosynthesis at the level of translation (Permut & Kipnis, 1972). Studies have shown that preproinsulin mRNA levels rise 4-10 fold in response to glucose stimulation. Studies of insulin gene expression in primary cultures of rat islets transfected Insulin I gene 5'-flanking sequence suggested that metabolic signal from glucose influx is transmitted through the insulin enhancer (German et al., 1990).

**ROLE OF NEUROTRANSMITTERS IN INSULIN REGULATION**

**Epinephrine and Norepinephrine**

Epinephrine and norepinephrine has an antagonistic effect on insulin secretion and glucose uptake (Renstrom et al., 1996; Porte, 1967). They also inhibit insulin-stimulated glycogenesis through inactivation of glycogen synthase and activation of phosphorylase with consequent accumulation of glucose-6-phosphate. In addition, it has been reported that epinephrine enhances glycolysis through an increased activation of phosphofructokinase. EPI and NE at low concentrations bind and activate β-adrenergic receptors which in turn stimulate the insulin secretion from pancreatic islets and at high concentration they can bind to α\(_{2A}\) receptors and inhibit insulin secretion (Lacey et al., 1993). Previous studies had shown that in diabetic condition α\(_{2A}\) receptors are more activated which brought out the insulin inhibition and in turn hyperglycaemia (Lacey et al., 1993). Rat islet cell membrane is equipped with α\(_{2A}\)-adrenoceptors (Filipponi et al., 1986) which are linked to adenylyl cyclase inhibiting insulin secretion.
NE and EPI, the flight and fright hormones are released in all stress conditions and are the main regulators of glucose turnover in strenuous exercise (Simartirkis et al., 1990). In severe insulin induced hypoglycaemia, a 15 to 40-fold increase of epinephrine plays a pivotal role in increasing glucose production independently of glucagon (Gauthier et al., 1980). In humans, adrenaline stimulates lipolysis, ketogenesis, thermogenesis and glycolysis and raises plasma glucose concentrations by stimulating both glycogenolysis and gluconeogenesis. It is already known that, when used in high doses in vivo or in vitro, EPI reduces the insulin response to stimulators (Malaisse, 1972). In vitro studies with yohimbine showed that the insulin secretion from the pancreatic islets increased significantly suggesting that when the α₂-adrenergic receptors are blocked, it enhances islet cell proliferation and insulin secretion. Our previous studies demonstrated the role of α and β-adrenergic receptors in the insulin secretion (Ani et al., 2006a; b; c). We also reported the effect of NE in DA mediated insulin secretion (Eswar et al., 2006).

**Acetylcholine**

Acetylcholine is the neurotransmitter of the parasympathetic system. Cholinergic receptors are classified as ionotropic nicotinic receptor and metabotropic muscarinic receptor. Acetylcholine increases insulin secretion through muscarinic receptors in pancreatic islet cells (Tassava et al., 1992; Greenberg & Pokol, 1994). Muscarinic receptors are classified as M₁, M₂, M₃, M₄ and M₅. They are G protein coupled receptors. They are characterized by having seven hydrophobic transmembrane-spanning regions that interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects. Expression studies have revealed the presence of M₁ and M₃ receptors in the pancreas. Acetylcholine is reported to be involved in the activation of glucose transport in the chromaffin cells. The cholinergic activation affecting this process is coupled with calmodulin and
protein kinase C (Serck-Hanssen et al., 2002). It is reported that the role of acetylcholine in insulin secretion is mediated through M₁ and M₃ receptors (Renuka et al., 2004; 2005; 2006).

\[ \gamma \text{-Aminobutyric acid} \]
Gamma aminobutyric acid (GABA) is the major inhibitory neurotransmitter in central nervous system. GABA is reported to be present in the endocrine pancreas at concentrations comparable with those found in central nervous system. The highest concentration of GABA within the pancreatic islet is confined to β-cells (Sorenson et al., 1991). 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). The brain GABAergic mechanisms also play an important role in glucose homeostasis. Recent studies reported the regulatory role of GABA during pancreatic regeneration (Balaram et al., 2007; 2008). Also, we reported the role of GABA in hepatocyte proliferation (Biju et al., 2002). GABA through its receptors has been demonstrated to attenuate the glucagon and somatostatin secretion from pancreatic α-cells and δ-cells respectively (Gaskins et al., 1995). GABA which is present in the cytoplasm and in synaptic-like microvesicles is co-released with insulin from β-cells in response to glucose (Reetz et al., 1991). GABA inhibits islet α and δ-cell hormonal secretion in a paracrine manner. GABA release is decreased in diabetes resulting in the enhancement of glucagon secretion from α-cells leading to hyperglycaemia. GABA is involved in the maintenance of glucose homeostasis and inhibition of central GABAₐ receptors increasing the plasma glucose concentration (Lang, 1995). Thus, any impairment in the GABAergic mechanism in central nervous system and/or pancreatic islets is important in the pathogenesis of metabolic stress.
**Serotonin**

Brain serotonin content decreased during diabetes (Jackson & Paulose, 1999). This decrease is reported to be due to a decrease in uptake of tryptophan through the blood brain barrier (BBB) (Fernstrom & Wurtman, 1971; Fernstrom & Wurtman, 1972; Madras et al., 1974) and a decrease in rate of 5-HT synthesis (Carndall et al., 1981). The turnover rate of 5-HT to 5-HIAA in diabetic rats was also reported to be lower (Kwok & Juorio, 1987; Sandrini et al., 1997). A decrease in brain 5-HT lead to an up regulation of 5-HT2A receptors of cerebral cortex and brain stem which in turn inhibit insulin secretion due to increased sympathetic activity (Jackson & Paulose, 1999). Our earlier studies reported the role of serotonin in cell proliferation (Sudha & Paulose, 1998). The functional regulation of brain 5-HT during pancreatic regeneration is also reported (Mohanan et al., 2005a, b; 2006).

**Central glutamatergic regulation of glucose homeostasis**

Although the role of glutamate as a signaling molecule is well established in the central nervous system, a similar role in the periphery has only recently been suggested. Inagaki et al., (1995) and Weaver et al., (1996) have detected functional glutamate receptors in the pancreatic islets of Langerhans. Pancreas is composed of four major cell types: the insulin-secreting β-cell, the glucagon-secreting α-cell, the pancreatic polypeptide-secreting PP cell and the somatostatin-secreting delta cells. The electrically excitable β-cells are stimulated to secrete insulin in response to changes in serum glucose concentrations. Secretion of insulin and the three other major peptide hormones found in islets is also believed to be affected by other metabolic and neuronal signals (Boyd, 1992; Ashcroft et al., 1994). Bertrand et al., (1992; 1993) have shown that AMPA receptor agonists can potentiate both insulin and glucagon secretion from a perfused pancreas preparation and that oral or intravenous
glutamate can increase insulin secretion and glucose tolerance *in vivo* (Bertrand *et al*., 1995).

The precise role of a glutamatergic signaling system in islet physiology or pathology is not completely understood. Glutamate also subserves communication between islets and the central nervous system. Glucose-stimulated insulin release is Ca$^{2+}$-dependent, perhaps because Ca$^{2+}$ couples the process of stimulus recognition to that of insulin discharge (Douglas, 1968; Milner & Hales, 1970; Matthews, 1970; Malaisse, 1973; Malaisse *et al*., 1974). Although several studies have indicated that glucose alters the state of Ca$^{2+}$ in the pancreatic cells, the nature of the changes and the mechanisms by which they occur are poorly understood (Hellman *et al*., 1976).

**Triiodothyronine (T3) regulation on diabetes and ageing**

Diabetes mellitus and thyroid diseases are the two common endocrinopathies seen in the adult population. Insulin and thyroid hormones being intimately involved in cellular metabolism and excess/deficit of either of these hormones could result in the functional derangement of the other. In euthyroid individuals with diabetes mellitus, the serum T3 levels, basal TSH levels and TSH response to thyrotropin releasing hormone (TRH) is influenced by the glycemic status (Schlienger *et al*., 1982). Alterations in serum T3 levels have been described in association with energy deprivation (Vagenakis *et al*., 1975; Eisenstein *et al*., 1978) wasting illnesses (Burke & Eastman, 1974), the neonatal period (Larsen, 1972) and the use of such drugs as propylthiouracil (Oppenheimer *et al*., 1972), dexamethasone (Chopra *et al*., 1975) and propranolol (Roszkowska *et al*., 1974; Tevaarwerk & Boyd, 1977; Tevaarwerk *et al*., 1978). An increase in the serum T3 level has been reported in response to long-term growth hormone administration in growth-hormone-deficient children (Sato *et al*., 1977). Fasting appears to inhibit 5'-monodeiodination, causing a decrease in the rate
of conversion of T4 to T3 and an increase in the reverse T Concentration. Poorly controlled diabetes, both Type 1 and Type 2, induce a “Low T3 state” characterized by low serum total and free T3 levels, increase in reverse T3 (rT3) but near normal serum T4 and TSH concentrations. Low serum T3 is due to reduced peripheral conversion of thyroxine (T4) to tri-iodothyronine (T3) via 5’ monodeiodination reaction. Studies indicate that long term diabetic control determines the plasma T3 levels. TSH responses and low T3 state normalized with improvement in glycaemic status but even with good diabetes control, the normal nocturnal TSH peak is not restored in C-peptide negative patients i.e., those with totally absent pancreatic β cell function (Ciro et al., 1997). Studies show decreased insulin secretion (Ahren et al., 1985) as well as normal or increased levels of insulin is reported in the peripheral and portal circulation in hyperthyroidism (Dimitriadis et al., 1985). Long term thyrotoxicosis has been shown to cause beta cell dysfunction resulting in reduced pancreatic insulin content, poor insulin response to glucose and decreased rate of insulin secretion (Bech et al., 1996).

In hyperthyroidism, the endogenous glucose production is greatly increased by a variety of mechanisms: (a) an increase in the availability of gluconeogenic precursors in the form of lactate, glutamine and alanine from skeletal muscles and glycerol from adipose tissue, (b) an increase in the concentration of plasma FFA stimulating hepatic gluconeogenesis (Dimitriadis & Raptis, 2001); (c) an increase in glycogenolysis due to inhibition of glycogen synthesis resulting in hepatic glucose output even in fed state (Holness & Sugden, 1987); (d) an upregulation of GLUT-2 glucose transporters protein expression in the hepatocyte plasma membrane. This permits increased glucose efflux to occur without intracellular glucose accumulation which would limit hepatic glucose production (Mokuno et al., 1990); and (e) an increased secretion and exaggerated effects of glucagon and adrenaline on liver cells
In skeletal muscle, there is a preferential increase in glucose uptake and lactate formation relative to glucose oxidation and storage in hyperthyroid state. This is due to increase in both basal and insulin stimulated GLUT1 and GLUT-4 transporters (Haber et al., 1995), increased responsiveness of glycogenolysis to beta adrenergic stimulation (Dimitriadis & Raptis, 2001), increased activity of hexokinase and 5-phosphofructokinase and decreased sensitivity of glycogen synthesis to insulin (Dimitriadis et al., 1997). In hypothyroidism, the synthesis and release of insulin is decreased (Ahren et al., 1985). The rate of hepatic glucose output is decreased probably due to reduced gluconeogenesis. A post receptor defect has been proposed to explain the decrease in insulin stimulated glucose utilization in peripheral tissues (Dimitriadis & Raptis, 2001). A reduced secretion of thyroid hormones with age has been documented in humans and animals with no substantial increase in TSH secretion, which is indicative of an age-related impairment of the pituitary sensitivity to the negative control exerted by thyroid hormones. Studies in young animals of both sexes showed an inverse correlation between the density of pituitary T3 receptors and plasma TSH whereas in old animals an age-related impairment of T3 action was reported on the thyrotrophs or changes pertaining to others factors modulating TSH secretion (Donda et al., 1987).

Calcium imaging

The Langerhans’ islet is another example of the presence of peripheral glutamatergic systems (Satin & Kinard, 1998). Intracellular free Ca\(^{2+}\) concentration plays a pivotal role in the regulation of various cellular functions as an intracellular messenger system. After stimulation of islets with AMPA or kainate, intracellular Ca\(^{2+}\) increased by way of activation of voltage-gated Ca\(^{2+}\) channels (Inagaki et al., 1995; Weaver et al., 1999), resulting in an elevated level of insulin secretion through
increased exocytosis of insulin granules in β-cells (Bertrand et al., 1992). Since the development of digital video imaging of Ca⁡^{2+} novel findings including Ca⁡^{2+} oscillations (Berridge & Galione, 1988; Berridge, 1991) and Ca⁡^{2+} waves (Berridge, 1993) have been described in many different cultured cell types. Ca⁡^{2+} spots were reported as an elementary Ca⁡^{2+} influx event through mechanosensitive channels directly coupled with the initial step in mechanotransduction in cultured endothelial (Ohata et al., 2001a, b; Tanaka & Takamatsu, 2001) and cultured lens epithelial cells (Ohata et al., 2001b, c). The Ca⁡^{2+} spots, which develop sporadically, exhibit a spatiotemporal pattern distinct from Ca⁡^{2+} sparks, the elementary Ca⁡^{2+} release events from intracellular stores (Cheng et al., 1993; Nelson et al., 1995).

Cerebellar dysfunction and hypoglycaemia

The cerebellum is known to be resistant to hypoglycaemia, and selective cerebellar dysfunction caused by hypoglycaemia has not been reported. In a case of episodic bilateral cerebellar dysfunction caused by hypoglycaemia, quantitative dynamic PET study demonstrated decreased glucose uptake-to-utilization ratio and increased leak of glucose in the cerebellum indicating that cerebellum is not invariably resistant to hypoglycaemia (Kim et al., 2005). Motor learning, a process by which an animal learns to perform a motor skill more accurately and efficiently through practice, plays an essential role in human life. Unlike explicit memory such as recognition memory and spatial memory, motor learning is characterized by slow development, without the requirement of conscious recall, and in general being lifetime-lasting (Llinas & Welsh, 1993; Tulving & Markowitsch, 1998; Eichenbaum, 2000). Based on the role of the cerebellum in motor activities such as fine motor movement and motor coordination as well as the computational network within the neural circuitries, cerebellar motor learning was first postulated by Albus, (1971) and
Marr, (1969). It is well accepted that motor learning undergoes a typical form of use-dependent plasticity in the brain (Kleim et al., 2003; Butefisch et al., 2004). At the same time, it has been well established that the NMDA receptor (NMDAR) plays a central role in synaptic plasticity (Nakanishi, 1992). Accumulating evidence has indicated that NMDAR also plays a role in motor learning (Takehara et al., 2004; Llansola et al., 2005; Dang et al., 2006). It was reported recently that NMDA receptors in cerebellar granule cells helped in voluntary motor training on motor learning in the mouse (Jiao et al., 2008).

Hypoglycaemic brain injury is a common and serious complication of insulin therapy and occurs most frequently in patients attempting tight glucose control (Davis et al., 1998). Neuronal death resulting from hypoglycaemia is the result of a series of events triggered by reduced glucose availability and the normalization of blood glucose levels does not necessarily block or reverse this cell death process once it has begun. Elimination of hypoglycaemia from the lives of people with diabetes and long term maintenance of euglycaemia will undoubtedly require glucose-regulated insulin replacement or secretion. Pending that ultimate goal, there is a critical need to develop therapeutic approaches that minimize both hyper- and hypoglycaemia. The only treatment for hypoglycaemia is blood glucose repletion and there is no currently available intervention for preventing the neuronal death that develops after hypoglycaemia is corrected. Recurrent hypoglycaemia in IDDM has become even more a major focus of research and clinical interest. The brain regions most vulnerable to hypoglycaemia are important for learning and memory. Accordingly, patients who recover from severe hypoglycaemia are left with difficulties in cognition, particularly short-term memory, out of proportion to gross motor disability (Langan et al., 1991). The preservation of neuron cell bodies is not always accompanied by normal synaptic activity and function (Li et al., 2003). Several lines of evidence
suggest that dopamine is associated with mechanisms underlying the neurobiologic response to metabolic stress.

Studies on the functional regulation of glutamate through NMDA receptors during diabetes and hypoglycaemia during adult and old will lead to a better understanding of the cognitive and memory function due to neuronal damage in the brain. The present study will be carried out to elucidate hypoglycaemic and diabetic effect on brain cellular function of glutamate through NMDA receptors.