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 et al., 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 glycaemic control using currently available regimens of subcutaneous insulin administration in diabetic patients (Cryer et al., 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 et al., 1994, DCCT, 1987, 1991), and hypoglycaemia causes recurrent and even persistent psychological morbidity in many diabetic patients (Cryer et al., 1994). Speculation that an adaptation in the CNS might exist in patients with diabetes, depending upon antecedent glycaemia, appeared nearly a decade ago (Cryer, 1985, 2003). Amiel et al. (1988) observed that lower glucose concentrations were required to initiate epinephrine secretion following a period of intensified diabetes management with its attendant increase in 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 & Shamoon, 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 (McCrimmon et al., 1997; Auer & Siesjo, 1988; 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 et al., 1999, 2002 a, b, 2003). In this homeostatic mechanism, rising blood glucose levels shut down the neoglucogenesis activities of autonomic nervous system (Cryer et al., 1997; Towler et al., 1993; McAulay et al., 2001, Charles et al., 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 may be 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 et al., 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 still 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, can have debilitating consequences, including seizures or coma or even death (Jane, 1999).
Hypoglycaemia and brain

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; Karen et al., 2006).
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 ascend 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, Auer et al., 1992). 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 neo cortex 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 glutamergic innervation, and evidence suggests that hypoglycaemic injury in these neurons is precipitated almost entirely by sustained glutamate receptor activation (excitotoxicity) (Auer et al., 1985b). The hippocampal neurons in particular are important for learning and memory, and patients who survive hypoglycaemic coma may be left with significant cognitive impairment (Patrick & Campbell, 1990; Kalimo & Olsson, 1980).

**Dopamine, a neurotransmitter in the central nervous system**

Dopamine (DA) is the predominant catecholamine neurotransmitter in the mammalian brain, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation. This catecholamine also plays multiple roles in the periphery as a modulator of cardiovascular function, catecholamine release, hormone secretion, vascular tone, renal function, and gastrointestinal motility (Missale et al., 1998).

Dopamine containing neurons arise mainly from DA cell bodies in the substantia nigra and ventral tegmental area in mid-brain region (Tarazi et al., 1997 a, b, 1998 a, b, 2001; Tepper, et. al., 1997; Royh, et. al., 1991; Carlsson, 1993; Lookingland et al., 1995). Dopaminergic system is organized into four major subsystems (i) the nigrostriatal system involving neurons projecting from the substantia nigra, pars compacta to the caudate-putamen of the basal ganglia. This is
the major DA system in the brain as it accounts for about 70% of the total DA in the brain, and its degeneration makes a major contribution to the pathophysiology of Parkinson's disease; (ii) the mesolimbic system that originates in the midbrain tegmentum and projects to the nucleus accumbens septi and lateral septal nuclei of the basal forebrain as well as the amygdala, hippocampus and the entorhinal cortex, all of which are considered components of the limbic system and so are of particular interest for the patho-physiology of idiopathic psychiatric disorders; (iii) the mesocortical system, which also arises from neuronal cell bodies in the tegmentum which project their axons to the cerebral cortex, particularly the medial prefrontal regions; (iv) the tuberinfundibular pathway, which is a neuroendocrinological pathway arising from the arcuate and other nuclei of the hypothalamus and ending in the median eminence of the inferior hypothalamus. DA released in this system exerts regulatory effects in the anterior pituitary and inhibits the release of prolactin. DA is involved in the control of both motor and emotional behaviour. Despite the large number of crucial functions it performs, this chemical messenger is found in a relatively small number of brain cells. In fact, while there are a total of 10 billion cells in the cerebral cortex alone, there are only one million dopaminergic cells in the entire brain (Missale et al., 1998).

**Biosynthesis of dopamine**

Dopamine is synthesized from the amino acid L-tyrosine. L-tyrosine is hydroxylated by the enzyme tyrosine hydroxylase (TH) to give L-3, 4-dihydroxyphenylalanine (L-DOPA) which is the rate limiting step. L-DOPA is subsequently decarboxylated to dopamine by the enzyme aromatic L-amino acid decarboxylase. Therefore, it is not possible to enhance the levels of DA by providing L-tyrosine. The activity of tyrosine hydroxylase is regulated by several endogenous
mechanisms. For example, the enzyme is activated by increased neuronal impulse flow, but is inactivated either by DA itself as an end-product inhibitor, or by activation of presynaptic DA receptors. On the other hand, the enzyme aromatic L-amino acid decarboxylase converts L-DOPA to DA instantaneously. Therefore, providing L-DOPA creates a possibility to enhance the formation of DA.

**Dopamine reuptake and metabolism**

Dopamine exerts its functions mediated through various receptors and these actions are terminated to prevent continuous stimulation of the receptors. This inactivation is brought about by reuptake mechanisms and metabolism of DA. Reuptake of DA is accomplished by a high affinity carrier present in the membrane, the dopamine transporter (DAT). The dopamine transporter recycles extracellular DA by actively pumping it back into the nerve terminal. The dopamine content which is about 70 to 80% in the striatal synaptic cleft is inactivated by this process. Drugs, such as cocaine, are able to block the action of the dopamine transporter, thereby sustaining the presence of dopamine in the synaptic cleft and its action on dopamine receptors. Part of the dopamine is inactivated by conversion to inactive compounds by metabolic enzymes, which are present both intra- and extraneuronally. Monoamine oxidase (MAO), aldehyde dehydrogenase (AD) and catechol-O-methyltransferase (COMT) are responsible for the metabolism of DA. Dopamine after reuptake is intraneuronally deaminated by MAO to give dihydroxyphenyl acetaldehyde, which subsequently is converted to 3, 4-dihydroxyphenylacetic acid (DOPAC) by AD. DOPAC is then methylated by COMT to give homovanillic acid (HVA). Extraneuronally, DA is metabolized by an alternative route in which it is first O-methylated to 3-methoxytyramine (3-MT) through the action of COMT and subsequently oxidized by MAO and AD to HVA.
Dopamine receptors

Dopamine mediates its actions via membrane receptor proteins. DA receptors are found on postsynaptic neurons in brain regions that are DA-enriched. In addition, they reside presynaptically on DA neuronal cell bodies and dendrites in the midbrain as well as on their terminals in the forebrain. Dopamine receptors belong to a family of large peptides that are coupled to G-proteins which are modified by attached carbohydrate, lipid-ester or phosphate groups. The topologies of the five dopamine receptors are predicted to be the same as all the other G-protein-coupled receptors. They are characterized by having seven hydrophobic transmembrane-spanning regions. The third intracytoplasmic loop is functionally critical and interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects (Tarazi et al., 1997 a, b, 1998 a, b; Tepper, et. al., 1997; Royh, et. al., 1991; Carlsson, 1993). In their putative transmembrane domains, the DA D₁ and D₅ receptors are 79% identical to each other, while they are only 40–45% identical to the DA D₂, D₃, and D₄ receptors. Conversely, the DA D₂, D₃, and D₄ receptors are between 75% and 51% identical to each other. They contain seven putative membrane-spanning helices which would form a narrow dihedral hydrophobic cleft surrounded by three extracellular and three intracellular loops. The receptor polypeptides are probably further anchored to the membranes through palmitoylation of a conserved Cys residue found in their carboxy tails, 347 in DA D₁, the C-terminus in DA D₂ like receptors. The dopamine receptors are glycosylated in their N-terminal domains. Dopamine D₁ like subtypes have potential glycosylation sites in their first extra cytoplasmic loop.

Dopamine receptors are divided into two families on the presence or absence of ability of DA to stimulate adenylyl cyclase and produce the second-messenger
molecule cyclic-AMP (cAMP) (Kebabian & Calne, 1979; Schwartz et al 1992; Civelli et al, 1993; O'Dowd, 1993; Jackson & Westlind, 1994; Ogawa, 1995; Strange, 1996). This classification is based on similarities in structure, pharmacology, function and distribution. Dopamine D1 like receptors are characterized initially as mediating the stimulation of cAMP production. Dopamine D2 like receptors inhibit the production of cAMP. This pharmacological characterization is based on the ability of some DA agents to block adenylyl cyclase activity to inhibit the release of prolactin in vivo and in vitro in a cAMP-independent fashion (Seeman, 1980). Applications of recent technical advances in molecular genetics have greatly facilitated the isolation and characterization of novel DA receptors, DA D3, D4 and D5, with different anatomical localization from traditional DA D1 or DA D2 receptors. Based upon their pharmacological profiles, including their effects on different signal transduction cascades, these receptors are currently divided into two families: the DA D1-like family which includes dopamine D1 and D3 receptors. The DA D2 like family includes dopamine D2, D3 and D4 receptors (Grandy et al., 1993; Sibley et al., 1993; Schwartz et al., 1992). The genomic organizations of the DA receptors demonstrate that they are derived from the divergence of two gene families that mainly differ in the absence or the presence of introns in their coding sequences. Dopamine D1 like receptors genes do not contain introns in their coding regions, a characteristic shared with most G protein-coupled receptors. The genes encoding the dopamine D2 like receptors are interrupted by introns (Gingrich & Marc, 1993). Furthermore, most of the introns in the DA D2-like receptor genes are located in similar positions.

Dopamine D1-like family

The DA D1 receptor is the most abundant DA receptor in the central nervous system. The DA D1 like receptors are characterized by a short third loop as in many
receptors coupled to Gs protein (Civelli et al., 1993; Gingrich & Canon, 1993; O'Dowd, 1993). The DA D_1 like receptors have short third intracellular loops and long carboxy terminal tails. The DA D_1 like receptors are classified into DA D_1 and D_5. In the DA D_1 and D_5 receptor third intracellular loop and the carboxy terminus are similar in size but divergent in their sequence. In contrast, the small cytoplasmic loops 1 and 2 are highly conserved so that any difference in the biology of these receptors can be probably related to the third cytoplasmic loop and the carboxy terminal tail (Civelli et al., 1993, Gingrich & Canon, 1993; O'Dowd, 1993). The external loop between transmembrane domain (TM) TM4 and TM5 is considerably different in the two receptor subtypes, being shorter (27 amino acids) in the D_1 receptor than in the D_5 receptor (41 amino acids). The amino acid sequence of this loop is divergent in the DA D_5 receptor (Marc et al., 1998).

**Dopamine D_1 receptor**

DA D_1 receptors are found at high levels in the typical dopamine regions of brain such as the neostriatum, substantia nigra, nucleus accumbens and olfactory tubercles. DA D_1 receptor seems to mediate important actions of dopamine to control movement, cognitive function and cardiovascular function. The DA D_1 receptor gene, which lacks introns, encodes a protein that extends for 446 amino acids (Dohlman et al., 1991). In humans DA D_1 receptor gene has been localized to chromosome 5 (Sunahara et al., 1990). The DA D_1 receptors show characteristic ability to stimulate adenylyl cyclase and generate inositol 1, 4, 5-trisphosphate (IP_3) and diacylglycerol via

The activation of phospholipase C (Sibley et al., 1990; Monsma et al., 1990). DA D_1 receptors are highly expressed in basal ganglia followed by cerebral cortex, hypothalamus and thalamus. DA D_1 receptors messenger ribonucleic acid
(mRNA) is co-localized in striatal neurons of the basal ganglia with mRNA for DA receptor phosphor protein (DARPP-32; KD) which is a dopamine and cAMP-regulated phosphoprotein. DA Receptor Phosphor Protein contributes to the actions of DA D₁ receptor (Hemmings & Greengard, 1986; Greengard et al., 1987). The DA D₁ receptors in the brain are linked to episodic memory, emotion, and cognition.

**Dopamine D₅ receptors**

The DA D₅ receptor gene is intronless and encodes a protein that extends for 47 amino acids (George et al., 1991). This protein has an overall 50% homology with DA D₁ receptor and 80% if only the seven transmembrane segments are considered. The gene encoding the human DA D₅ protein is located at the short arm of chromosome 4, the same region where the Huntington disease gene has been located (Gusella, 1989). Two DA D₅ receptor pseudogenes having 154 amino acids have been identified with 90% homology (Gusella, 1989). These pseudogenes, however, contain stop codons in their coding regions that prevent them from expressing functional receptors. The functions of these pseudogenes, which appear so far to be specific to humans, are not yet known (Allen et al., 1991).

DA D₅ receptor mRNA expression is unique and limited to the hippocampus and parafascicular nucleus of the thalamus (Civelli et al., 1992). It is involved in the thalamic processing of painful stimuli (Giesler et al., 1979). DA D₅ receptors appear to interact with G-proteins and can stimulate adenylyl cyclase, with relatively high affinity for DA and DA D₁-selective agonists (George et al., 1991).
Dopamine D₂ like family

DA D₂ like receptors belong to the G-protein coupled receptors and has 400 amino acid residues. DAD₂-like receptors are characterized by a long extracellular amino terminus which has several glycosylation sites and a shorter carboxy terminal tail with putative phosphorylation sites. The function of sugar moieties is unclear (Marc et al., 1998; Sibley, 1999). It is generally believed that the membrane enclosed part of the amino-acid chain of G-protein coupled receptors is folded into seven α-helices. The transmembrane helices consist primarily of hydrophobic amino-acid residues. The unique feature of DA D₂ like receptors family is that they posses a bigger third cytoplasmic (intracellular) loop in common, which is thought to be the site where the G-protein couples (Marc et al., 1998). Between the different dopamine receptors, the third loop also displays the greatest variability in amino-acid sequence. This may have consequences for their respective second messenger systems. The DA D₂-like receptors are coupled to Gi-protein and inhibit the formation of cAMP. The DA D₂ receptors tertiary structure is stabilized by two cysteine disulphide bridges.

Dopamine D₂ receptors

The DA D₂ receptor gene encodes a protein that extends for 415 amino acids. Similar to other G-protein coupled receptors, the DA D₂ receptor has seven transmembrane segments, but in contrast to DA D₁-like receptors, the third cytoplasmic domain is long and the carboxy terminus is short. Unlike the DA D₁-like receptor genes, the DA D₂ receptor gene contains seven introns that are spliced out during mRNA transcription (Fischer et al., 1989). The gene encoding this receptor was found to reside on q22-q23 of human chromosome 11 (Makam et al., 1989). The DA D₂ receptor was the first receptor to be cloned (Chrisre et al., 1988). The DA D₂ receptors are involved in several signal transduction cascades, including inhibition of
cAMP production (Vallar & Meldelesi, 1989), inhibition of phosphoinositide turnover (Epelbaum et al., 1986), activation of potassium channels and potentiation of arachidonic acid release (Axelrod et al., 1991). The DA D2 receptors are highly expressed in basal ganglia, nucleus accumbens septi and ventral tegmental area (Schwartz et al., 1992).

The DA D2 receptor exists as two alternatively spliced isoforms differing in the insertion of a stretch of 29 amino acids in the third intracellular loop and are designated as DA D2S and DA D2L (Seeburg et al., 1989; Marc et al., 1998). Because this loop seems to play a central role in receptor coupling, the existence of a splicing mechanism at this level could imply functional diversity. However, in spite of the efforts of several groups, no obvious differences have emerged so far between the two DA D2 receptor isoforms. The two isoforms derived from the same gene by alternative RNA splicing which occurs during the maturation of the DA D2 receptor pre-mRNA (Schwartz et al., 1989). DA D2 receptor isoforms (DA D2L and DA D2S) vary within each species by the presence or absence of a 29-amino acid sequence in the third cytoplasmic domain of the DA D2 receptor peptide chain. Both variants share the same distribution pattern; with the shorter form less abundantly transcribed in addition they appear to differ in their mode of regulation (Marc et al., 1998). Pharmacologically, both isoforms exhibit nearly similar profiles in terms of their affinities to different DA D2-selective agents, and inhibit adenylyl cyclase activity. However, these isoforms display an opposite regulatory effect (Sibley et al., 1993). These isoforms have the same pharmacological profile, even though a marginal difference in the affinity of some substituted response to dopamine treatment is reported: Dopamine induces the up-regulation of DA D2L isoform of DA D2 receptors (Castro & Strange, 1993). When expressed in host cell lines, both isoforms inhibited adenylyl cyclase (Marc et al., 1998; Sibley, 1999). However, the DA D2S receptor
isoform displayed higher affinity than the DA D2L in this effect (Seeburg et al., 1993). The isoforms of DA D2 mediate a phosphatidylinositol-linked mobilization of intracellular calcium in mouse Ltk [-] fibroblasts. Protein kinase C (PKC), however, differentially modulates DA D2S and D2L-activated transmembrane signalling in this system with a selective inhibitory effect on the dopamine D2S-mediated response.

**Dopamine D3 receptors**

Dopamine D3 receptor gene contains five introns and encodes a 446 amino acid protein (Schwartz et al., 1992). The gene encoding this receptor resides on chromosome 3 (Giros et al., 1990). The DA D3 receptors bear close structural and pharmacological similarities to the DA D2 receptors. DA D3 mRNA occurs in longer and shorter spliced forms generated from the same gene (Schwartz et al., 1992). Distribution of DA D3 receptor mRNA are distributed and expressed mainly in subcortical limbic regions including islands of Calleja, nucleus accumbens septi and olfactory tubercle, with low levels of expression in the basal ganglia. D3 receptor mRNA has also been found in neurons of the cerebellum, which regulate eye-movements (Levesque et al., 1992). The status of the DA D3 molecular entity as a functional receptor remains uncertain since it neither couples to G-proteins nor consistently transduces an effector mechanism. However, the structural similarity with DA D2 receptor raises the possibility that DA D3 receptor also inhibit adenylyl cyclase activity in its normal cellular setting. More recent studies reported that DA D3 receptors mediate positive regulatory influences of DA on production of the peptide neurotensin (Schwartz et al., 1992; Sokoloff et al., 1990).
**Dopamine D₄ receptors**

DA D₄ receptor gene contains four introns and encodes a 387 amino acid protein (Van Tol *et al.*, 1991). The overall homology of the DA D₄ receptor to the DA D₂ and D₃ receptors is about 41% and 39% respectively, but this homology increases to 56% for both receptors when only the transmembrane spanning segments are considered. The gene encoding the human DA D₄ protein is located at the tip of the short arm of chromosome 11 (Civelli & Bunzow, 1993; Missale *et al.*, 1998). DA D₄ receptor gene has been localized in brain regions like hippocampus and frontal cortex using specific histoprobes. The stimulation of DA D₄ receptor inhibits adenylyl cyclase activity and release arachidonic acid in brain neurons (Huff *et al.*, 1994, Misalle *et al.*, 1998). In humans, DA D₄ receptor occurs in several genomic polymorphic variants that contain two to eleven repeats of a 48 base pair segment that is expressed in the third cytoplasmic domain (Van Tol *et al.*, 1992; Misalle *et al.*, 1998). These are called the DA D₄ alleles which are represented as DA D₄₂, D₄₄ and D₄₇. These may contribute to the pathophysiology of certain neuropsychiatric disorders (Jackson & Westlind, 1994).

**GLUTAMATE RECEPTORS**

Glutamate (Glu) 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). The excitatory amino acid Glu is the most prevalent transmitter in the brain; its effect on postsynaptic receptors is limited by uptake process (Erecinska, 1997) and by diffusion of Glu from the cleft. The removal of Glu from the extracellular fluid, limitation of its action occurs by uptake and by diffusion (Tong & Jahr, 1994). This is accomplished by a transporter in the plasma
membrane of both neurons and astrocytes (Brooks-Kayal et al., 1998; Gelagashvili & Schousboe, 1998). Electrophysiological evidence was obtained that the block of Glu transporters potentiates postsynaptic excitation of Glu receptors (Tong & Jahr, 1994). The cellular uptake of Glu is driven by the electrochemical gradients of Na\(^+\) and K\(^+\) and is accompanied by voltage and pH changes.

The majority of excitatory synapses are glutamergic, in which Glu transmits the signal through postsynaptic ionotropic [N-methyl-D-aspartic acid (NMDA), \(-\text{amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate (KA)}\) and metabotropic receptors (Bettler & Mulle, 1995). Glu 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). 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 adenylyl 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 Glu (Pittaluga et al., 1996), DA (Kuo et al., 1998) and NE (Pittaluga & Raiteri, 1992) from axon terminals indicates that Glu released is able to facilitate transmitter release via NMDA receptors (Barnes et al., 1994; Desai et al., 1994). Montague et al. (1994) suggested that Glu 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., Glu 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).

**NMDA receptors**

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 and Heinemann, 1994; McBain and Mayer, 1994; Danysz et al., 1995; Parsons et al., 1998). 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 memantine and (+)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., 1995).

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., 1998). 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 and Mayer, 1994).

In addition to NR1 and NR2, the NR3A subunit has recently been discovered. This receptor subunit, previously termed chi-I, 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 and Seeberg, 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 and 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 Mg\textsuperscript{2+} 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 (Danysz et al., 1995; Meldrum, 1985; Rogawski, 1993; Leeson and Iversen, 1994; Danysz et al., 1995; Besnard et al., 1996; Ishimaru and Toru, 1997; Parsons 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, osteoporesis and coma leading to death. Pancreatic damage resulting in the dysfunction of α and β 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 (Bhattacharya & Saraswathi, 1991; Garris, 1990; Lackovic et al., 1990). Our previous studies demonstrated adrenergic, serotonergic and DA D\textsubscript{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 et al., 1992). Norepinephrine has been reported to increase in several brain regions during diabetes. Ohtani et al., (1997) have reported a significant decrease in extracellular concentrations of NE, 5HT and their metabolites in the ventro medial hypothalamus (VMH). Epinephrine (EPI) levels were significantly increased in the striatum, hippocampus and hypothalamus of diabetic rats and these changes were reversed to normal by insulin treatment (Ramakrishan & Namasivayam, 1995). Diabetes is reported to cause a high level of degeneration in neurons in different regions of the brain. Streptozotocin -induced diabetes and acute deficiency of insulin is reported to result in increased concentrations of EPI in the supra chiasmatic nucleus. It is also reported that β-adrenergic receptor populations were decreased in diabetes (Garris, 1995). 5-HT content in the brain is reported to be decreased during diabetes (Jackson & Paulose, 1999; Chu et al., 1986; Sumiyoshi et al., 1997). 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⁺ K⁺ ATPase activity in different brain regions (Gurcharan et al., 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 (Morrison et al., 2001).
Dopamine and its receptor alterations during diabetes

Dopamine is implicated in diabetes. Hyperglycaemia in rats is reported to decrease dopaminergic activity in the striata suggesting the up regulation of dopamine receptors possibly due to the decreased DA metabolism (Hio et al., 1994). In experimental diabetes and insulin deficiency there is a rapid onset of detectable alterations in hypothalamic DA activity leading to secondary neuroendocrine abnormalities. Lim et al., (1994) have described an increase in the striatal DA and decrease in its metabolites dihydroxyphenylacetic acid and HVA. Tyrosine hydroxylase is reported to be depleted in nigrostriatal neurons in the genetically diabetic rat causing marked reduction in mesolimbic dopamine system. Insulin treatment could not restore the decreased DA to controlled conditions, impairing the dopamine biosynthesis (Kamei & Saitoh, 1994). Dopamine uptake affinity and velocity in synaptosomes is decreased significantly during diabetes. The DA content was increased in cerebral cortex and hypothalamus of diabetic rats (Chen & Yang, 1991; Ohtani et al., 1997; Tassava et al., 1992; Shimizu, 1991). Diabetes is reported to cause increased DA release with altered turnover ratio of DA metabolites from the mesolimbic systems. This resulted in the enhanced spontaneous locomotor activity which is suggested to be due to the up regulation of δ-opioid receptor-mediated functions (Kamei et al., 1994). The decrease in striatal DA transporter mRNA in experimental diabetes is suggested to be a possible cause for the disturbance in DA metabolism (Figlewicz et al., 1996). The DA turnover ratio in the limbic forebrain and midbrain in diabetic mice were significantly greater than those in non-diabetic mice (Kamei & Saitoh, 1996). Yawning behaviour in streptozotocin induced diabetes was significantly lowered when compared with their age-matched normal controls as a result of altered DA metabolism and decreased turnover to its metabolites (Heaton & Varrin, 1993).
DA receptors are reported to be increased in diabetes causing significant alterations in central dopaminergic system (Lozovsky et al., 1981). DA D₂ receptor density has been reported to be increased in the striatum of diabetic rats (Lozovsky et al., 1981; Trulson & Hummel, 1983; Serri et al., 1985). Intracerebroventricular application of alloxan and streptozotocin in rat striatum is reported to have caused an alteration in DA receptors and increased DA content which had a similar effect to peripheral, diabetogenic administration of these drugs (Salkovic et al., 1992). The affinity of striatal DA D₁ receptors was significantly increased without changes in the number of binding sites, while the binding of DA D₂ receptors was significantly increased without affecting its affinity in the diabetic rats (Hio et al., 1994). DA D₁ receptors are reported to decrease in hyporesponsiveness (Kamei et al., 1994). The increase in the central dopaminergic postsynaptic receptors has been related to decrease the locomotor and ambulatory activity in STZ-induced diabetic rats (Kobayashi et al., 1990; Shimomura et al., 1990). Recent studies from our laboratory reported DA D₂ receptor alterations in the brain and pancreas of STZ-induced diabetic rats (Eswar et al., 2007).

Diabetes mellitus causes a condition called as neurocytogluicopenia where the increased glucose results in an increased sympathetic outflow into the liver, pancreas, adrenal medulla, adipose tissue and the circulation. This causes an increased hepatic glucose production, inhibition of insulin secretion and free fatty acid mobilization from the adipose tissue. Participation of dopaminergic tone in the control of insulin secretion and hyperglycaemia has been given little focus. Recent studies have shown that dopamine agonists play an important role in lowering the elevated shift in the sympathetic tone as a result of increased glucose levels and stimulate the parasympathetic tone which increases the insulin response (Oliveira et al., 1998).
**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 may result in selective neuronal necrosis (Jane et al., 1999). Deficiency in glucose that results from hypoglycaemic insults can 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 (Simon et al., 1984; 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 glutamergic terminals can prevent hypoglycaemic neuronal death (Wieloch et al., 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 et al., 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 and glucagon and 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. Karen et al., (2006) reported increased phenylethanolamine N-methyltransferase, tyrosine hydroxylase (TH), DBH protein in
diabetic rats exposed to hyperinsulinemic-hypoglycaemia. 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 (Minano et al., 1982).

Pyruvate derived from glucose is the major precursor of the acetyl group of. 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,
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.

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. IDDM patients with hypoglycaemia unawareness exhibited more profound cognitive dysfunction during acute hypoglycaemia which persisted for longer following blood glucose recovery (Gold et al., 1995). Severe hypoglycaemia with cognitive dysfunction is three times more common in intensively, rather than conventionally, treated IDDM (Maran et al., 1995). Severe hypoglycaemia causes pronounced neuroglycopenia that results in a profound degree of cognitive dysfunction and rarely can cause permanent neurological impairment (Mark et al., 2000). 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). Several antidepressants increase DA levels in the PFC (Tanda et al., 1994), and raising the DA level in patients with Parkinson's disease with L-3,4-dihydroxyphenylalanine improves their working memory deficit (Lange et al., 1992). These findings suggest that a reduced dopaminergic transmission in the PFC is responsible for the working memory deficits in the neuropsychiatric disorders.

Acute stress increases dopamine release and metabolism in a number of brain areas (Zangen et al., 1999). Dopaminergic innervation of the medial and dorsolateral PFC appears to be particularly vulnerable to stress and relatively low intensity levels of stress are capable of promoting significant responses. The prefrontal dopaminergic neurons have a number of higher functions including attention and working memory and the acquisition of coping patterns in response to stress (Castellano et al., 1999). Amphetamines and cocaine agonise these receptors and have a similar effect as stress, resulting in symptoms such as anxiety, panic, hypervigilence, exaggerated startle
reflexes and paranoia (Horger et al., 1999). 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 may be 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).

Although the mechanism responsible for cognitive deficits in stress-related neuropsychiatric disorders has been obscure, PFC dopaminergic dysfunction is thought to be involved. In animals, the mesoprefrontal dopaminergic system is particularly vulnerable to stress and chronic stress induces working memory impairment. Chronic stress induces working memory impairment through a D1 receptor-mediated hypodopaminergic mechanism in the PFC (Mizoguchi et al., 2000). The neurochemical studies on the dopaminergic neuronal activity in the PFC of the stressed rats revealed that the hyperdopaminergic mechanism is behind the acute stress-induced cognitive deficits (Armsten & Goldman-Rakic, 1998; Mizoguchi et al., 2000).

**Effect of glucose on brain dopamine and its receptors**

Dopamine has two distinct pathways that connect the striatum to the basal ganglia output nuclei - a direct pathway originating from neurons bearing DA D1 receptors and an indirect pathway originating from neurons expressing DA D2
receptors. Intrastriatal injection of selective DA D1, DA D2 or general DA agonists, in freely-moving rats reduced glucose utilization (Orzi et al., 2001). Glucose modulates substantia nigra (SN) DA neuronal activity and its release by acting on an ATP-sensitive potassium channel (K_{ATP}) (Levin, 2000). Changing SN glucose levels is reported to affect activities of (K_{ATP}) channel and DA neurons. Glucose modulates the motor activity involved in food intake. In experimental rats food deprivation cause a decrease in the activity of striatal DAT (Figlewicz et al., 1998). DA D1 receptor binding significantly increased in the accumbens and DA D2 binding decreased in the dorsal striatum as a result of excessive intake of sugar because palatable food stimulates the neural system. DA antagonists are reported to effectively modulate brain energy metabolism and release of DA thus effecting cerebral glucose utilisation (Walker et al., 1999). Stimulation or blockade of DA D3 receptors in cerebral cortex alters local glucose utilisation producing a unique pattern suggestive of potential antipsychotic activity (Levant et al., 1998).

**Effect of dopamine on blood glucose levels**

DA and its agonists have been reported to affect the blood glucose levels. Increase in glucose level has been suggested to be due to sympathoadrenal activation. Plasma glucose levels are reported to be under separate serotonergic and dopaminergic control exerted via 5-HT_{1A} and DA D3 receptors respectively (HiIlegaart et al., 1996). DA D3 receptor agonist, 7-OH DPAT, injection caused an increase in blood glucose level and decreased plasma insulin content showing the involvement of this receptor in glucose homeostasis. Evidences show that DA D2 receptor-mediated increase in plasma glucose is via sympathoadrenal activation (Saller & Kreamer, 1991). DA analogues like lergotrile, pergolide, bromocriptine (BRC), d-amphetamine and apomorphine when injected has reported to cause
hyperglycaemia in rats (Fischer et al., 1984). In contrary obese diabetic rats treated with a combination of dopaminergic receptor agonists SKF/38393 and BRC is reported to reduce hyperglycaemia (Cincotta et al., 1999).

**Glutamate receptors in diabetes and hypoglycaemia**

Neurodegeneration results from over activation of NMDA receptors (Rothman & Olney, 1995), 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. NMDA antagonists were screened against animal models of epilepsy (Avoli & Oliver, 1987), ischemia (Aitken et al., 1988; Ford et al., 1989; Rod and Auer, 1989), and hypoglycaemia (Wieloch, 1985).

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 glutamergic 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 may 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).

**MDH and GDH in diabetes and hypoglycaemia**

Diabetes is characterized by chronic hyperglycaemia that produces dysregulation of cellular metabolism. The molecular and functional basis of hypoglycaemia and hyperglycaemia to a certain extend can be elucidated by studying the changes in the metabolic enzymes. There is increasing evidence that specific changes in mitochondrial function may play a significant role in the early events leading to hypoglycaemic encephalopathy. Decreased fluxes of substrate through the tricarboxylic acid cycle results in decreased availability of reducing equivalents in mitochondria. As a result, there is incomplete reduction of molecular oxygen within mitochondria and increased formation of oxygen free radicals, which damage both mitochondrial membranes and mitochondrial DNA. Fragmentation of mitochondrial DNA interferes with synthesis of electron transport chain enzymes, such as subunits of cytochrome oxidase and nicotinamide adenine dinucleotide (NADH)-dehydrogenase that are coded for by the mitochondrial genome. Thus, the ability of the cell to restore ATP levels is impaired (Jane, 1999).

MDH is an enzyme directly involved in glucose metabolism. It catalyses the interconversion of L-malate and oxaloacetate using nicotinamide adenine dinucleotide (NAD) as coenzyme. Since MDH has been shown to play a role in the regulation of cytosolic \([\text{NAD}^+] / \text{NADH}\) redox state, it is possible that differences in this enzyme could cause differences in metabolic pathway. The mitochondrial enzyme
in addition to its role in the other half of the malate shuttle, it is also a necessary component of the TCA cycle. The simple Malate dehydrogenases occur in virtually all eukaryotic cells as isoenzymes identified as mitochondrial (m-MDH) and soluble or cytoplasmic (s-MDH) according to their cellular location. (Delbruk, 1959). In hypoglycaemia, gluconeogenesis is altered. Experimentally induced diabetes has shown to cause changes in the activity of metabolic enzymes altering the glucose metabolism (Chang et al., 1977; Kazmi et al., 1985; Tanaka et al., 1988; Belfiore et al., 1974).

GDH catalyzes reversible oxidative deamination of L-glutamate to α-ketoglutarate. Enzyme activity is regulated by several allosteric effectors. Transamination between this α-amino acid and α-keto acid determines the amount of this amino acid in the brain (Wurdig & Kugler, 1991). The importance of GDH in glucose homeostasis is also evident from recent findings that mutations in the GLUD1 gene, which encodes GDH, cause hyperinsulinism /hyperammonemia (HI/HA) syndrome (Smith et al., 2001; Stanley et al., 1998 & 2000; Tanizawa et al., 2002; Weinzimer et al., 1997; Zammarchi et al., 1996). Insulin induced hypoglycaemic coma in animals was associated with inhibition of glycolysis and glycogenolysis and decreased activities of succinate dehydrogenase and GDH in the cerebral hemispheres and brainstem (Telushkin et al., 2006).

**Glucose uptake by pancreatic islets**

Signal transduction in the pancreatic β-cell and thereby the insulin secretory process is regulated by a sophisticated interplay between glucose and a plethora of additional factors including other nutrients, neurotransmitters, islet generated factors
and systemic growth factors. The coupling of glucose metabolism to electrical activity remains central in all models of β-cell stimulus-secretion coupling. The resting membrane potential of the β-cell is set by the ATP-sensitive potassium (K\textsubscript{ATP}) channel (Ashcroft & Rorsman, 1990). Incubation of the pancreatic β-cell with stimulatory glucose concentrations leads to the activation of a cascade of reactions, which ends in the exocytosis of stored insulin. This complex of processes starts with the uptake of glucose by the β-cell high-κ\textsubscript{m}/low affinity glucose transporter GLUT2 and proceeds with the conversion of glucose into glucose-6-phosphate by the β-cell isoform of glucokinase (Matschinsky, 1996). Metabolism of glucose in glycolysis and the Krebs cycle results in the generation of ATP. Elevation in the ATP/ADP ratio leads to closure of the K\textsubscript{ATP}, which in turn results in depolarization of the plasma membrane. The subsequent opening of voltage-gated L-type Ca\textsuperscript{2+} channels leads to an increase in the cytoplasmic free Ca\textsuperscript{2+} concentration, [Ca\textsuperscript{2+}]\textsubscript{i}, which promotes insulin secretion (Berggren & Larsson, 1994).

Glucose is transported into the β-cell cell by facilitated diffusion through a glucose transporter; elevated concentrations of glucose in extracellular fluid lead to elevated concentrations of glucose within the β-cell. Elevated concentrations of glucose within the β-cell cell ultimately leads to membrane depolarization and an influx of extracellular calcium. The resulting increase in intracellular calcium is thought to be one of the primary triggers for exocytosis of insulin-containing secretory granules. The mechanisms by which elevated glucose levels within the β-cell cell cause depolarization is not clearly established, but seems to result from metabolism of glucose and other fuel molecules within the cell, perhaps sensed as an alteration of ATP:ADP ratio and transduced into alterations in membrane conductance. An increased level of glucose within β cells also appears to activate calcium-independent pathways that participate in insulin secretion.
Factors affecting insulin regulation from pancreatic β-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. Glucokinase is also linked to the phosphate potential, [ATP]/([ADP][Pi]) (Sweet et al., 1996). 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 β-cell (Dunne, 1991). Depolarisation activates voltage-dependent Ca^{2+} channels, causing an influx of extracellular Ca^{2+} (Liu et al., 1996). 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 may control glucose activated secretion. Lesions in these pathways lead to the pathogenesis of diabetes mellitus (Dukes et al., 1997). Glucose induced insulin secretion is also partly dependent upon the activation of typical isoforms of protein kinase C (PKC) within the β-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. 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 can bind and activate β-adrenergic receptors which in turn stimulate the insulin secretion from pancreatic islets and at high concentration they can bind to α2A receptors and inhibit insulin secretion. Previous studies had shown that in diabetic condition α2A 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 α2A-adrenoceptors (Filipponi et al., 1986) which are linked to adenylyl cyclase inhibiting insulin secretion. β3 adrenoreceptors stimulation also results in enhanced insulin secretion (Alef et al., 1996).

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. 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., 2006 a, 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). 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 (Paulose, 2004; Renuka et al., 2004, 2005 & 2006).
**γ-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 (Kaimal et al., 2007). Also, we reported the role of GABA in hepatocyte proliferation (Biju & Paulose, 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 GABA_A 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) (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 (Sandrini et al., 1997; Kwok & Juorio, 1987). A decrease in brain 5-HT will lead to an up regulation of 5-HT$_{2A}$ receptors of cerebral cortex and brain stem which in turn can 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. (Mohan et al., 2005 a, b & 2005).

Dopamine in Pancreatic glucose uptake and Insulin Secretion

Glucose uptake is the initial step in glucose-stimulated insulin secretion (GSIS) by pancreatic β-cells (Guillam et al., 2000). In the pancreatic islets of Langerhans, glucose uptake by β-cells initiates a cascade of cellular events resulting in insulin secretion. A key response leading to insulin release is the change in transmembrane potential associated with the opening and closing of ion channels. Glucose uptake and metabolism increases the ratio of ATP/ADP, leading to the blockade of ATP-sensitive potassium (K'-ATP) channels. Inhibition of these channels results in cell membrane depolarization and subsequent activation of voltage-gated Ca$^{2+}$ (CaV) channels. Influx of extracellular Ca$^{2+}$ causes through (CaV) channels oscillatory elevations in [Ca$^{2+}$]$_i$, fusion of insulin-containing vesicles with the cell membrane, and insulin release (Rorsman & Renstrom, 2003). This entire process is
suppressed or terminated by the opening of voltage-gated K+ (KV) channels (MacDonald et al., 2001). The integrated process of channel gating is critical for the coordination of insulin release and thus the consequent maintenance of proper plasma glucose levels.

Insulin is involved in glucose disposal into skeletal muscles, inhibition of hepatic glucose production and inhibition of lipolysis in adipocytes. Intracerebroventricular infusion of insulin results in an increase in mRNA levels for the DA reuptake transporter (Figlewicz et al., 1998). Dopamine analogues are reported to inhibit glucose-stimulated insulin release from the endocrine pancreas (Fischer et al., 1984). Islets cells have been shown to contain the putative enzymes that synthesis dopamine like tyrosine hydroxylase and di-hydroxy phenylalanine decarboxylase. DA and increased glucose stimulus reduced the insulin release from the pancreatic islets with no change in calcium efflux (Nogueira et al., 1994). Acute L-DOPA-induced dopamine accumulation in pancreatic islets is reported to cause an inhibitory effect on glucose-stimulated insulin response resulting in an increased MAO activity (Lundquist et al., 1991, Lundquist, 1985). Our recent studies reported the role of DA in insulin secretion mediated through DA D2 receptors (Eswar et al., 2006).

DA is involved in the control of food intake, energy expenditure, glucose and lipid metabolism, blood pressure and insulin release. DA D2 receptor neurotransmission is diminished in the brains of obese animal models and humans. DA D2 receptor activation facilitates glucose metabolism, lowers blood pressure and stimulates resting energy expenditure in non-diabetic obese individuals. Long term treatment with DA D2 receptor agonists improves metabolic control in obese humans with type 2 diabetes. Effects of DA on insulin secretion in general and on pancreatic
β-cell function in particular have been poorly studied. Insulin exocytosis from the β-
cell is primarily controlled by metabolism-secretion coupling. First, glucose
equilibrates across the plasma membrane and is phosphorylated by glucokinase,
initiating glycolysis (Matschinsky, 1996). Subsequently, mitochondrial metabolism
generates ATP, which promotes the closure of ATP-sensitive potassium channels and
as a consequence, depolarization of the plasma membrane (Rorsman et al., 1996). This
leads to calcium influx through voltage-gated calcium channels and a rise in cytosolic
calcium, triggering insulin exocytosis (Lang, 1999). Additional signals participating in
the amplifying pathway (Henquin, 2000) are necessary to reproduce the sustained
secretion elicited by glucose. Insulin secretion evoked by glucose metabolism can be
further modulated by parasympathetic and sympathetic neurotransmitters (Ahren,
2000).

The role and the peripheral mechanism of action of central dopamine on basal
pancreatic exocrine secretion in conscious rats revealed that central dopamine
inhibited pancreatic exocrine secretion via DA D₁ like receptors and that the inhibitory
effect is mediated via sympathetic nerves, especially α-adrenoceptors. Presence of
dopamine is reported in peripheral tissues (Hakanson et al., 1989). Dihydroxy phenyl
acetic acid decarboxylase (DDC), DBH and aromatic L-amino decarboxylase (AAD)
are present in endocrine cells of adult rats (Gagliardino et al. 1997; Yamada et al.,
1999; Kampe et al., 1995). As dihydroxy phenyl acetic acid decarboxylase and DBH
are enzymes specifically involved in catecholamine synthesis and insular cells are
reported to possess the capacity to synthesise these amines. Thus, endogenously
synthesised islet catecholamines have been suggested to participate in paracrine
regulation of insulin secretion. Secretory granules of pancreatic β-cells have the
ability to store (Ahren & Lundquist, 1985) substantial amounts of calcium, dopamine
and serotonin. L-3, 4-dihydroxyphenylalanine is rapidly converted in islet beta-cells to dopamine. Acute L-DOPA-induced dopamine accumulation in pancreatic islets is accompanied by rapid changes in MAO activity, concomitant with an inhibitory effect on glucose-stimulated insulin response (Ahren & Lundquist, 1985). It is reported that increased hydrogen peroxide production, following increased MAO activity, augment the inhibitory effect of dopamine accumulation on insulin release (Lundquist et al., 1991). Dopamine is reported to suppress the somatostatin secretion predominantly through activation of dopaminergic receptors, whereas it suppresses insulin release through an alpha adrenergic mechanism and stimulates glucagon release through a β-adrenergic mechanism (Malaisse et al., 1992). There has not been any detailed study on the distribution of dopamine receptor subtypes in the pancreatic islets or the pancreas except for these studies.

Dysfunction of pancreatic islets plays an important role in the etiology of diabetes as chronic hyperglycaemia impairs islet function. It has been proposed that chronic hyperglycaemia resulting from peripheral insulin resistance impair secretogogue-induced insulin release. Dopamine agonists influence central circadian neuroendocrine activities regulating metabolism to reduce insulin secretion (Lang et al., 1998). Timed dopaminergic stimulation is reported to normalize the circadian rhythm of corticosterone release in obese insulin resistant animals (Lang et al., 1998). It has been reported that administration of dopamine receptor agonists, bromocriptine and/or SKF38393 in diabetic rats decreased insulin resistance, increased secretion of insulin from the islet cells and normalized the daily corticosterone rhythm. Dopamine receptor agonists are suggested to improve the decreased regulatory mechanisms in the hypothalamic-neuroendocrine system during diabetes and reduce β-cell toxicity.
Hyperglycaemia causes functional deficits in the CNS aminergic neurons which are too subtle and take a longer time to manifest. Reports emphasized that treatment of gastric stasis in diabetic patients using dopamine blocker metoclopramide resulted in increased frequency and severity of dopamine associated tardive dyskinesia (Casey et al., 1991). Tardive dyskinesia due to DA supersensitivity in antipsychotic drug treated animals can be corrected by Prolyl-leucyl-glycinamide (PLG) (Chiu et al., 1981). Also, diabetes caused a shift in the CNS resulting in an increased sympathetic tone that resulted in a decreased insulin secretion. Recently the presence of DA in the adrenal medulla is being stated to draw importance as it is necessary to control secretions of NE and EPI. Both dopamine (Salamone et al., 2003) and insulin (Oomura & Kita, 1981; Havrankova et al., 1981; Bruning et al., 2000) actions in the brain modulate appetite and feeding behaviours. Interestingly, treatment with L-dopa alters insulin secretion in patients with Parkinson disease (Rosati, 1976). Moreover, antipsychotic (neuroleptic) drugs blocking dopamine receptors may cause hyperinsulinemia (Sowell et al., 2002), hypoglycaemia (Budman & Gayer, 2001), increase appetite, and obesity (Pijl, 2003, Ananth et al., 2004) and are associated with diabetes (Pijl, 2003; Marder et al., 2004; Citrome, 2004). Dopamine action on beta cells have relevant implications for the study of obesity and diabetes, in particular in situations where dopamine transmission is altered (Blanca et al., 2005). Dopamine regulates pancreatic insulin secretion in a concentration dependent manner (Eswar et al., 2006). But the molecular mechanism is not well studied in detail.

**Glutamate in Pancreatic Glucose uptake and Insulin secretion**

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. Weaver et al., (1996) and Inagaki et al., (1995) 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 cell. 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 glutamergic 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; Malaisse, 1973; Malaisse & Pipeleers, 1974). Although several studies have indicated that glucose alters the state of $Ca^{2+}$ in the pancreatic islets, the nature of the changes and the mechanisms by which they occur are poorly understood (Taljedal, 1976).

**Electrophysiological changes during diabetes and hypoglycaemia**

Neuroelectrophysiological recordings represent a non-invasive and reproducible method of detecting central and peripheral nervous system alterations in diabetes mellitus (Morano et al., 1996). Diabetes mellitus is associated with chronic
complications such as nephropathy, angiopathy, retinopathy and peripheral neuropathy. In diabetic patients, hyperglycaemia precipitate seizures and in experimental diabetes, indications for an increased neuronal excitability have been found (Anderson et al., 2006). Neurophysiological alterations have also been described in animal models of diabetes, in particular in rats. Cerebral metabolic (Knudsen et al., 1989; Kumar & Menon, 1993) and vascular (Duckrow et al., 1987; Jakobsen et al., 1990) disturbances have been demonstrated within weeks after diabetes induction. EEG recordings showed changes in the brain activity of 14 day diabetic rats compared to control rats (Gireesh, 2007). It is reported that metabolic control influences the EEG and improvement of glucose metabolism is an important factor in avoiding EEG abnormalities in young diabetic patients (Hauser et al., 1995). The degree of metabolic control had no effect on the electroencephalographic findings during the early years of diabetes, but previous severe hypoglycaemia, young age, and early onset seem to be important risk factors for electroencephalographic abnormalities (Soltesz & Acsadi, 1989).

EEG at the time of diagnosis of IDDM is reported to be useful in identifying those patients at increased risk for coma and/or convulsion as a result of hypoglycaemia. (Tupola et al., 1998). As blood glucose concentrations fall below 2 mm, EEG initially shows increased amplitude and decreased frequency, followed by decreased amplitude and frequency as blood glucose concentrations approach 1 mm. Below 1 mm blood glucose concentrations, brain ATP levels become depleted (Siesjo, 1978). As hypoglycaemia progresses below 1 mm, the EEG becomes isoelectric and neuronal cell death ensues (Butterworth, 1999). Hypoglycaemia only causes neuronal death when the EEG becomes flat. This usually occurs after glucose levels have fallen below 1 mM (18 mg/dl) for some period, depending on body glycogen reserves. At
the time that abrupt brain energy failure occurs, the excitatory amino acid aspartate is massively released into the limited brain extracellular space and floods the excitatory amino acid receptors located on neuronal dendrites. Calcium fluxes occur and membrane breaks in the cell lead rapidly to neuronal necrosis (Auer, 2004). Recurrent severe hypoglycaemia and poor metabolic control are risk factors for EEG abnormalities in adolescents with type 1 diabetes receiving multiple insulin injection therapy treatment (Hyllienmark et al., 2005).

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. Glutamate receptor activation and excitotoxicity has long been recognized as an upstream event in this cascade. 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 DA through DA D₁ and DA D₂ receptors during hyperglycaemia and hypoglycaemia 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 hyperglycaemic effect on brain cellular function of dopamine through DA D₁ and DA D₂ receptors and glutamate through NMDA receptors. EEG recording in hypoglycaemic and hyperglycaemic will be carried out to measure brain activity. In vitro studies will be done to confirm the receptor subtypes functional regulation on glucose uptake and insulin secretion.