Literature Review

Diabetes mellitus is a chronic disease characterized by relative or absolute deficiency of insulin, resulting in glucose intolerance. Diabetes mellitus is a major global health problem that affects more than 185 million people around the world (Zimmet et al., 2001). The classic symptoms of diabetes mellitus results from abnormal glucose metabolism. The lack of insulin activity results in failure of transfer of glucose from the plasma into the cells. This situation so called “starvation in the midst of plenty”. The body responds as if it were in the fasting state, with stimulation of glucogenolysis, gluconeogenesis and lipolysis producing ketone bodies. The disease is an increasingly prevalent metabolic disorder in humans and is characterised by hyperglycemia (Dinne et al., 2004; Kumar et al., 2002). The number of diabetic patients is expected to reach 300 million by the year 2025. The pancreatic hormones have an important role in the regulation of glucose metabolism. The secretion of insulin by β-cells of the endocrine pancreas is regulated by glucose and other circulating nutrients. It is also modulated by several hormones and neurotransmitters, among which acetylcholine plays a prominent role.

The pancreas

The pancreas is a mixed gland, with a large exocrine and a much smaller endocrine gland. The endocrine cells are arranged into small islands of cells called the islets of Langerhans. The interactive function of both the exocrine and the endocrine parts are particularly important for the normal functioning of the body. The endocrine cells produce indispensable hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide, which are crucial to the optimum functioning of body metabolism. The pancreas is well innervated by autonomic nerves rich in different
types of neuropeptides including vasoactive intestinal polypeptide and neuropeptide Y; galanin, Calcitonin-gene-related-peptide, cholecystokinin and leucine-enkephaline (Adeghate et al., 2001). In addition to the presence of neuropeptides, neurotransmitters such as serotonin, GABA or neurotransmitter-regulating enzymes such as tyrosine hydroxylase and dopamine hydroxylase have been identified in the pancreas. (Adeghate & Donáth 1991; Adeghate & Ponery 2001; Adeghate & Ponery 2002).

**β-Cell function: physiology and pathophysiology**

Islets of Langerhans are microscopic organelles scattered diffusely throughout the pancreas. Each islet contains approximately 2000 cells, which include four types: α, β, δ and PP cells. The major secretory products of these cells are glucagon, insulin, somatostatin, and pancreatic polypeptide, respectively. The α-cell secretes glucagon primarily in response to hypoglycemia, but also to amino acids. The β-cell secretes insulin in response to elevated glucose levels and also responds to other substances such as glucagon and acetylcholine. Insulin responses to intravenous glucose are time-dependent and referred to as first- and second-phase responses. The δ-cell releases somatostatin in response to glucose. The PP cell releases pancreatic polypeptide in response to hypoglycemia and secretions. The functions of these hormones are distinctly different. Glucagon stimulates glycogenolysis in the liver to increase blood glucose levels. Insulin decreases hepatic glucose production and increases glucose entry into muscle and fat cells. Somatostatin inhibits the secretion of many hormones, including insulin and glucagon and likely is an intra islet paracrine regulator of α and β cells. The function of pancreatic polypeptide in humans remains unclear (Robertson & Harmon, 2006).
The endocrine pancreas is richly innervated, but the abundance and organisation of these innervations are highly variable between species (Kobayashi & Fujita, 1969). Most of the nerve fibers enter the pancreas along the arteries (Miller, 1981; Woods & Porte, 1974). Unmyelinated nerve fibers are found in the neighborhood of all islet cell types at the periphery and within the islet. At some distance from the islets, glial Schwann cells often form a thin sheet around nerve fibers on their travel toward and within the islet. In the vicinity of islet cells, however, it is not rare to see some nerve fibers lacking this glial protection and coming close to or ending blindly 20–30 nm from the endocrine cells (Legg, 1967; Watari, 1968; Kobayashi & Fujita, 1969; Shorr & Bloom, 1970; Fujita & Kobayashi, 1979; Bock, 1986; Radke & Stach, 1986a; Radke & Stach, 1986b).

The autonomic innervations of the endocrine pancreas have several origins. Classically, the autonomic nervous system uses two interconnected neurons to control effectors functions and is divided into two systems, the sympathetic and the parasympathetic nervous systems, according to the location of the preganglionic cell bodies. However, there are indications suggesting that these two systems are not always independent of each other, but display anatomical interactions (Berthoud & Powley, 1993) or share similar neurotransmitters (Verchere et al., 1996; Sheikh et al., 1988, Liu et al., 1998).

The parasympathetic innervation

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

Understanding the organisation of the pancreatic innervations permits correct interpretation of some experiments using different cholinergic antagonists. The stimulation of insulin release occurring upon electrical stimulation of vagal nerves in the dog is abolished by both nicotinic and muscarinic antagonists (Ahrén & Taborsky Jr, 1986). In the perfused rat pancreas, nicotine produces an increase of insulin secretion that is blocked by atropine (Miller, 1981). These observations can be explained by the presence of nicotinic receptors on pancreatic ganglia and nerves (Stagner & Samols, 1986; Karlsson & Ahrén, 1998; Kirchgessner & Liu, 1998) and muscarinic receptors on β-cells.
The overall effect of a parasympathetic stimulation is an increase of insulin secretion because postganglionic fibers contain various neurotransmitters in addition to the classic neurotransmitter acetylcholine. It is important to keep in mind that parasympathetic neurotransmission is the sum of various biological effects. VIP and PACAP stimulate insulin secretion by increasing cAMP levels (Ahrén, 2000). They act on the same family of receptors (Jian et al., 1999) and exert their action by two mechanisms, directly by stimulating β-cells through the PLC-PKC pathway (Ahrén, 2000) and indirectly by activating intrapancreatic postganglionic nerves that stimulate insulin secretion (Karlsson & Ahrén, 1998).

The sympathetic innervation

The sympathetic innervation of the pancreas originates from preganglionic perikarya located in the thoracic and upper lumbar segments of the spinal cord (Furuzawa et al., 1996). The myelinated axons of these cells traverse the ventral roots to form the white communicating rami of the thoracic and lumbar nerves that reach the paravertebral sympathetic chain (Chusid, 1979). Preganglionic fibers communicate with a nest of ganglion cells within the paravertebral sympathetic chain or pass through the sympathetic chain, travel through the splanchnic nerves and reach the celiac (Brunicardi et al., 1995; Furuzawa et al., 1996; Ahrén, 2000) and mesenteric ganglia (Furuzawa et al., 1996). Ganglia within the paravertebral sympathetic chain and the celiac and mesenteric ganglia, give off postganglionic fibers that eventually reach the pancreas. The existence of intrapancreatic sympathetic ganglia has also been reported (Liu et al., 1998). The preganglionic fibers release acetylcholine that acts on nicotinic receptors on intraganglionic neurons, whereas the postganglionic fibers release several neurotransmitters: norepinephrine, galanin, (Ahrén, 2000; Myojin et al., 2000). A rich supply of adrenergic nerves in close
proximity of the islet cells has been observed in several mammalian species (Radke & Stach, 1986c).

**Impact of Diabetes on Central nervous system**

The brain has not traditionally been considered a target for diabetic complications, but new research has shown that the disease does have particular effects on the CNS. These include impaired learning and memory, neurodegeneration and loss of synaptic plasticity. Most drug discovery efforts aimed at diabetes target insulin action in peripheral tissues. There is evidence that there is substantial overlap between the CNS circuits that regulate energy balance and those that regulate glucose levels, suggesting that their dysregulation could link obesity and diabetes. Some of the abnormalities demonstrated in experimental diabetic neuropathy include a decreased axonal transport, a reduced nerve conduction velocity, increase in resistance to ischemic conduction failure and impaired axon regeneration (Calcutt, 1994; Biro, 1997; Longo, 1986). Diabetes also leads to clinically relevant end-organ damage in the CNS as a result of both acute and chronic metabolic and vascular disturbances (McCall, 1992; Biessels *et al.*, 1994). The consequences of acute metabolic and vascular insults to the brain, such as hypoglycaemia and stroke, are well recognized and have been reviewed extensively. Moreover, recent epidemiological studies demonstrate an association between diabetes and vascular dementia as well as AD (Stewart. *et al.*, 1999; Ott *et al.*, 1999).

**Brain neurotransmitter changes during diabetes**

Neurotransmitters have been reported to show significant alterations during hyperglycemia resulting in altered functions causing neuronal degeneration. A significant increase in the catecholamine contents and activity of metabolising
enzymes has been reported in experimental diabetes (Gupta et al., 1992). Norepinephrine has been reported to increase in several brain regions during diabetes (Tassava et al., 1992; Chen & Yang, 1991), but a significant decrease in NE has been reported in hypothalamus (Ohtani et al., 1997) pons and medulla (Ramakrishna & Namasivayam, 1995). EPI levels were significantly increased in the striatum, hippocampus and hypothalamus of diabetic rats and these changes were reversed to normal by insulin treatment (Ramakrishna & Namasivayam, 1995). STZ-induced diabetes and acute insulin deficiency were demonstrated to result in increased content of EPI in the supra chiasmatic nucleus. In addition to this, a decreased turnover of dopamine in the ventromedial nucleus in diabetes was found to be reversed by insulin treatment (Oliver et al., 1989). These data indicate that experimental diabetes and acute insulin deficiency result in the rapid onset of detectable alterations in epinephrine and dopamine activity in specific hypothalamic nuclei. This lead to the development of secondary neuroendocrine abnormalities known to occur in the diabetes. The dopamine content was increased in whole brain, (Lackovic et al., 1990; Chen & Yang, 1991) corpus striatum (Chu et al., 1986) cerebral cortex and hypothalamus of diabetic rats (Tassava et al., 1992; Ohtani et al., 1997). The plasma dopamine content was decreased in diabetic rats (Eswar et al., 2006). Serotonin (5-HT) content is increased in the brain regions and hypothalamic nuclei (Lackovic et al., 1990; Chen & Yang, 1991) but there are reports suggesting a decrease in brain 5-HT content during diabetes (Sandrini et al., 1997; Sumiyoshi et al., 1997; Jackson & Paulose, 1999). Brain tryptophan was also reduced during diabetes (Jamnicky et al., 1991). Insulin treatment was reported to reverse this reduced tryptophan content to normal (Jamnicky et al., 1993).
**Acetylcholine**

Cholinergic system plays an important role in physiological and behavioural functions. Acetylcholine acts by binding to specific membrane receptors and is divided into muscarinic and nicotinic receptors. Cholinergic stimulation of pancreatic β-cells increases insulin secretion (Kaneto et al., 1967). These are mediated by muscarinic cholinergic, rather than nicotinic receptors (Stubbe & Steffens, 1993; Ahren et al., 1990) and is dependent on extracellular glucose concentration (Henquin et al., 1988). Acetylcholine stimulated insulin secretion coupling is mediated by complex mechanisms of signal transduction. It has been proposed that acetylcholine activates phospholipid turnover and thereby increases the intracellular calcium level. Normal β-cells' voltage-dependent sodium channels are important for membrane depolarisation. acetylcholine increases sodium influx into the cells (Henquin et al., 1988). Acetylcholine hyperpolarises the cell by increasing potassium permeability. Quist (1982) reported that carbachol causes Ca$^{2+}$-dependent stimulation of phosphate incorporation into phosphatidyl inositol phosphates in the canine heart. Cholinergic stimulation of phosphatidyl inositol phosphates synthesis is blocked by muscarinic antagonist atropine (Brown & Brown, 1983).

**Muscarinic receptors**

Muscarinic receptors are a family of G protein-coupled receptors that have a primary role in central cholinergic neurotransmission. Specific agonists, which activate postsynaptic muscarinic receptors, stimulate cholinergic signaling (Valentin et al., 2006). The muscarinic acetylcholine receptors are widely distributed throughout the body and subserve numerous vital functions in both the brain and autonomic nervous system (Hassal et al., 1993). Activation of muscarinic receptors in the periphery causes decrease in heart rate, relaxation of blood vessels, constriction in the
airways of the lung, increase in the secretions and motility of the various organs of the gastrointestinal tract, increase in the secretions of the lacrimal and sweat glands, and constriction in the iris sphincter and ciliary muscles of the eye (Wess, 1993). In the brain, muscarinic receptors participate in many important functions such as learning, memory and the control of posture.

Muscarinic receptors are members of a large family of plasma membrane receptors that transduce the intracellular signals via coupling to guanine nucleotide binding regulatory proteins (G proteins) (Nathanson, 1987; Bonner, 1989; Hulme et al., 1990). Molecular cloning studies have revealed the existence of five molecularly distinct mammalian muscarinic receptor proteins (Bonner, 1989; Hulme et al., 1990).

All mammalian muscarinic receptor genes share one common feature with several other members of G-protein receptor gene family i.e., their open reading frame contained within a single exon (Bonner et al., 1987). Like all other G protein coupled receptors, the muscarinic receptors are predicted to conform to a generic protein fold consisting of seven hydrophobic transmembrane helices joined by alternating intracellular and extracellular amino-terminal domain and a cytoplasmic carboxy-terminal domain. The five mammalian muscarinic receptors display a high degree of sequence identity sharing about 145 amino acids. Characteristically all muscarinic receptors contain a very large third cytoplasmic loop, which, except for the proximal portions, displays virtually no sequence identity among the different subtypes (Bonner, 1989). Agonist binding to muscarinic receptors is thought to trigger conformational changes within the helical bundle, which are then transmitted to the cytoplasmic face where the interaction with specific G proteins are known to occur. Site directed mutagenesis and receptor-modeling studies suggest that almost all G protein coupled receptors plays a pivotal role in mediating the conformational changes associated with receptor activation (Wess, 1993).
The ligand binding to muscarinic receptors is predicted to occur in a pocket formed by the ring like arrangement of the seven transmembrane domains (Wess et al., 1991; Hulme et al., 1990). Ligand binding appears to be initiated by ion-ion interaction between positively charged amino head present in virtually all muscarinic receptor ligands and a conserved Asp residue located in TM III. In addition a previous mutagenesis study has shown that replacement of the conserved TM III Asp residue in the rat muscarinic M1 receptor with Asn results in a receptor unable to bind to [³H] QNB.

Sequence analysis shows that the hydrophobic core of all muscarinic receptors contains a series of conserved Ser, Thr and Tyr residues, most of which do not occur in other G protein coupled receptors. Pharmacological analysis of mutant M3 muscarinic receptors showed that two Thr residues (Thr231 and Thr234) and four Tyr residues (Tyr148, Tyr506, Tyr529 and Tyr533) are important for high affinity acetylcholine binding (Wess et al., 1991). It has been shown that a Pro 201 to Ala mutant M3 muscarinic receptor exhibits affinities for both muscarinic agonists and antagonists 80-450 times less than those of the wild type (Wess et al., 1993).

In the periphery, among other effects, muscarinic receptors mediate smooth muscle contraction, glandular secretion and modulation of cardiac rate and force. In the CNS there is evidence that muscarinic receptors are involved in motor control, temperature regulation, cardiovascular regulation and memory. Interest in the classification of muscarinic receptors involved in functions at different locations has been heightened by the potential therapeutic application of selective agents in areas such as AD, Parkinson’s disease, asthma, analgesia, and disorders of intestinal motility, cardiac and urinary bladder function (Caulfield & Birdsall, 1998).
Classification

Muscarinic receptors are widely distributed throughout the central and peripheral nervous system. They have critical functions in learning and memory, attention and motor activity (Bonner, 1989; Weiner et al., 1990; Levey, 1993). The five muscarinic receptor subtypes are designated as M1 - M5. The odd-numbered receptors (M1, M3, and M5) couple to Gq/11, and thus activate PLC, which initiates the phosphatidyl inositol trisphosphate cascade. This leads to the dissociation of phosphatidyl 4, 5- bisphosphates (PIP2) into two components, i.e., IP\textsubscript{3} and DAG. IP\textsubscript{3} mediates Ca\textsuperscript{2+} release from the intracellular pool (endoplasmic reticulum), whereas DAG is responsible for activation of protein kinase C. On the other hand, PIP2 is required for the activation of several membrane protein, such as the “M current” channel and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger, and muscarinic receptor- dependent depletion of PIP2 inhibits the function of these proteins (Bonner et al., 1987; Caulfield & Birdsall, 1998; Bonner et al., 1988; Fuster et al., 2004; Suh & Hille, 2005; Winks et al., 2005; Meyer et al., 2001). The M1, M2 and M4 subtypes of macetylcholineRs are the predominant receptors in the CNS. These receptors activate a multitude of signaling pathways important for modulating neuronal excitability, synaptic plasticity and feedback regulation of acetylcholine release (Volpivelli et al., 2004).

Muscarinic M1 receptor

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

**Muscarinic M2 receptor**

Muscarinic receptor activation in guinea pig heart produces a reduction in force of contraction and a decrease in the rate of beating. These effects are probably the consequence of inhibition of voltage-gated Ca\(^{2+}\) channels and activation of inwardly rectifying K\(^+\) channels, respectively. Extensive studies with many antagonists have defined this response as being mediated by the M2 receptor (Caulfield, 1993). Muscarinic M2 receptors mediate both negative and positive ionotropic responses in the left atrium of the reserpinized rat, latter effect being insensitive to pertussis toxin (Kenakin & Boselli, 1990). Central cholinergic transmission is activated by inhibition of the presynaptic M2 acetylcholine autoreceptor using selective antagonists. The presynaptic M2 autoreceptor negatively influences the release of acetylcholine in several brain regions, including the striatum, hippocampus, and cerebral cortex (Billard et al., 1995; Kitaichi et al., 1999; Zhank et al., 2002). A direct consequence of brain M2 autoreceptor inhibition is an elevation of acetylcholine release in the synaptic cleft. Methoctramine and other M2 receptor
antagonists have been shown to enhance the release of acetylcholine in different brain structures (Stillman et al., 1993; Stillman et al., 1996).

**Muscarinic M3 receptor**

M3 muscarinic receptors are broadly expressed in the brain, although the expression level is not high, compared to those of the M1 and M2 receptors (Levey, 1993). Muscarinic M3 receptor is widely distributed in the peripheral autonomic organs with the highest expression found in the exocrine glands (Candell et al., 1990; Pedder et al., 1991; Kashihara et al., 1992; Matsui et al., 2000). Expression of the M3 receptor in the rat pancreatic islets and insulin secreting cell lines has been established (Lismaa, 2000). M3 receptor also triggers direct contractions of smooth muscle, however, it only represents a minor fraction of total muscarinic receptor population in smooth muscle. It is expressed in relatively low density throughout the brain. Studies using knock out mice for M3 receptors gave evidences for the primary importance of these receptors in the peripheral cholinergic system. In urinary bladder, pupillary muscles and intestinal smooth muscles the cholinergic contractions are mediated predominately through M3 receptors (Matsui et al., 2000).

**Muscarinic M4 receptor**

Muscarinic M4 receptor is known to be abundantly expressed in the striatum (Levey, 1993). Muscarinic M4 receptors act as inhibitory muscarinic autoreceptors in the mouse (Zhang et al., 2002). The neuroblastoma-glioma hybrid cell line NG108–15 expresses M4 mRNA and M4 receptors can be detected readily in radioligand binding assays (Lazareno et al., 1990). Inhibition of adenyl cyclase activity by muscarinic agonists in rat corpus striatum is mediated by M4 receptors (Caulfield, 1993; Olianas et al., 1996).
**Muscarnic M5 receptor**

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

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

**Signal transduction by muscarinic activation**

Gq-protein-coupled receptors (GqPCRs) are widely distributed in the CNS and play fundamental roles in a variety of neuronal processes. Their activation results in phosphatidyl inositol 4,5-bisphosphate (PIP2) hydrolysis and Ca²⁺ release from
intracellular stores via the PLC-inositol 1,4,5-trisphosphate (IP$_3$) signaling pathway. Because early GqPCR signaling events occur at the plasma membrane of neurons, they are influenced by changes in membrane potential (Billups et al., 2006). Muscarinic receptors, which are G protein coupled, stimulate signaling by first binding to G protein complex (αβγ) which provides specificity for coupling to an appropriate effector. The α subunit interacts with an effector protein or ion channel to stimulate or inhibit release of intracellular second messengers. Mutation analysis showed that the G protein is primarily but not exclusively acts through interaction with the third cytoplasmic loop. It is suggested that the short sequences, N terminal 16-21 and C terminal 19 amino acids of the loop play a key role in determining the specificity (Wess et al., 1989).

*Cyclic adenosine monophosphate*

Adenylate cyclase is either positively or negatively regulated by G protein coupled receptors resulting in an increase or decrease in the generation of the second messenger, Cyclic adenosine monophosphate (cAMP). The stimulation of muscarinic M2 and M4 receptors endogenously expressed in cell lines, results in the inhibition of adenylate cyclase. G protein reconstitution experiments have shown that M2 receptors inhibit adenylate cyclase through Gi and possibly through the pertussis toxin insensitive Gz. In neuroblastoma SK-N-SH cells which express endogenous muscarinic M3 receptors stimulate adenylate cyclase activity (Baumgold & Fishman, 1988). The muscarinic M1 receptor which ectopically expressed at physiological levels in A9L cells, was shown to stimulate adenylate cyclase through an IP$_3$ and Ca$^{2+}$ dependent mechanism (Felder et al., 1989). In contrast, M1 receptors stimulate adenylate cyclase in CHO cells predominantly through an IP$_3$ and Ca$^{2+}$ independent
mechanism that also contained a small Ca\(^{2+}\) dependent component (Gurwitz et al., 1994).

**Phospholipase C**

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

**Phospholipase A2**

Phospholipase A2 catalyzes the hydrolysis of membrane phospholipids to generate free arachidonic acid and the corresponding lysophospholipid. Muscarinic receptors have been shown to stimulate the release of arachidonic acid and its eicosanoid metabolites in a variety of tissues including heart, brain and muscle (Abdel-Latif, 1986). Ectopic transfection experiments indicate that the muscarinic M1, M3 or M5 receptors, but not M2 or M4 receptors are linked to phospholipase A2 activation (Felder et al., 1990; Liao et al., 1990). Muscarinic receptor stimulated release of arachidonic acid occurs predominantly through the activation of
phospholipase A2 and phosphatidylcholine serves as the primary substrate. Studies suggested that calcium influx, through voltage independent calcium channel activation and diacylglycerol, through PLC activation were essential for phospholipase A2 activation (Brooks et al., 1989; Felder et al., 1990). In ileal smooth muscle cells, carbachol stimulated phospholipase A2 itself caused calcium influx, implicating an amplification mechanism in phospholipase A2 regulation (Wang et al., 1993).

**Phospholipase D**

Muscarinic receptor stimulated phospholipase D has been reported in a number of cell types including canine synaptosomes (Qian & Drewes, 1989), rat astrocytoma cells (Martinson, 1990), human neuroblastoma cells (Sandmann & Wurtman, 1991) and rat parotid cells (Guillemain & Rossignol, 1992). Association of muscarinic subtypes with phospholipase D has been shown in human embryonic kidney cells transfected with the muscarinic M1-M4 receptors. In most cells studied, phospholipase C and phospholipase D are usually stimulated simultaneously following receptor activation (Liscovitch, 1991).

**Calcium influx and release from intracellular stores**

Muscarinic receptors typically stimulate biphasic increases in intracellular calcium in most cells. The transient phase represents the release of calcium from IP$_3$ sensitive intracellular Ca$^{2+}$ stores. Ca$^{2+}$ influx through Ca$^{2+}$ channels play a central role in the regulation of multiple signaling pathways activated by muscarinic receptors. In excitable cells such as neurons and muscle cells, Ca$^{2+}$ passes predominantly through voltage sensitive Ca$^{2+}$ channels. In non-excitable cells, such as fibroblasts and epithelial cells, Ca$^{2+}$ passes through a family of poorly characterised voltage - insensitive Ca$^{2+}$ channels (Fasolato et al., 1994). Voltage-independent Ca$^{2+}$
channels open in response to receptor activation and have been classified into (1) receptor operated Ca\(^{2+}\) channels which are second messenger independent (2) second messenger - operated Ca\(^{2+}\) channels and (3) depletion operated Ca\(^{2+}\) channels which open following IP\(_3\) mediated depletion of intracellular stores and provide a source of Ca\(^{2+}\) for refilling the stores.

**a7 nicotinic acetylcholine receptor**

The nicotinic acetylcholine receptor (nAChR), a key player in neuronal communication, converts neurotransmitter binding into membrane electrical depolarization. This protein combines binding sites for the neurotransmitter acetylcholine and a cationic transmembrane ion channel. The nAChR also binds the addictive drug nicotine. It mediates synaptic transmission at the junction between nerve and muscle cells and various types of nAChR are expressed in the brain. It is involved in several neurological pathologies. Several genes have been identified in rat and chick neural or sensory tissue that encode for neuronal nAChR subunits that are distinct from those in the muscle nAChR, providing for a multitude of potential subtypes of neuronal nAChRs. The wide distribution of the some of these transcripts in mammalian brain indicates that neuronal nAChRs represent a major neurotransmitter receptor superfamily related to other ligand gated ion channels including serotonin (5HT\(_3\)), GABA\(_A\), N-methyl-D-aspartate, and glycine. However, in contrast to these other ligand gated ion channels where established pharmacology rapidly segued into the molecular biology, the pharmacology of neuronal nAChRs has only started to emerge as a result of the rapid advances in the molecular biology of the nAChR family (Changeux et al., 1998).

The a7 nicotinic receptor, also known as the a7 receptor, is a type of nicotinic acetylcholine receptor, consisting entirely of a7 subunits (Rang et al., 2003). As with
other nicotinic acetylcholine receptors, functional α7 receptors are pentameric (i.e., (α7)5 stoichiometry). It is located in the brain, where activation yields post- and presynaptic excitation (Rang et al., 2003), mainly by increased Ca\(^{2+}\) permeability. Neuronal nicotinic cholinergic receptors are crucial to acetylcholine neurotransmission in both the CNS and autonomic nervous system. However, in the CNS, these receptors are more often associated with modulation of release of several neurotransmitters including dopamine, norepinephrine, GABA and glutamate (Wonnacott, 1997; Girod & Role, 2001). In the CNS, nicotinic acetylcholine receptors mediate the release of glutamate (Reno et al., 2004; De Filippi et al., 2001; Rossi et al., 2003) and norepinephrine (O Leary & Leslie, 2003). Thus, these receptors significantly influence the activity within the CNS circuitry and deregulation of this activity could contribute to diabetes mellitus associated disorders involving the CNS. Abnormalities of nicotinic acetylcholine receptor function in the hippocampus lead to cognitive and memory impairments (Green et al., 2005; Levin et al., 2002) and sensory gating deficits (Adler et al., 1998).

**Insulin secretion regulating factors**

**Glucose**

Glucose is an important regulator of various β-cell processes including insulin biosynthesis and release. Glucose, over short intervals stimulates insulin biosynthesis at the level of translation (Permut et al., 1972). Studies have shown that preproinsulin mRNA levels rise 4-10 folds 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).
Phosphorylation of glucose to glucose-6-phosphate serves as the rate limiting step in glucose oxidation (Schuit, 1996). Glucokinase acts as sensor during this process. The entry of glucose into β-cells is followed by an acceleration of metabolism that generates one or several signals that close ATP-sensitive K⁺ channels in the plasma membrane. The resulting decrease in K⁺ conductance leads to depolarisation of the membrane with subsequent opening of voltage dependent Ca²⁺ channels. The rise in the cytoplasmic free Ca²⁺ eventually leads to the exocytosis of insulin containing granules (Dunne, 1991; Gembal et al., 1992). Glucose induced insulin secretion is also partly dependent upon the activation of typical isoforms of protein kinase C within the β-cell (Harris, 1996). It is suggested that PKC is tonically active and effective in the maintenance of the phosphorylated state of the voltage-gated L-type Ca²⁺ channel, enabling an appropriate function of this channel in the insulin secretory process (Arkhammar, 1994).

**Fatty acids**

Short chain fatty acids and their derivatives are highly active stimulators of insulin release in sheep (Horino et al., 1968). Exogenous saturated long chain fatty acids markedly potentiated glucose-induced insulin release and elevated long chain acyl-CoA esters in the clonal β-cell line (Prentki et al., 1992). A novel ester of succinic acid 1, 2, 3-tri-(methyl-succinyl) glycerol ester displayed stimulation of insulin release and biosynthetic activity in pancreatic islets of Goto-Kakizaki rats (Laghmich et al., 1997). A monomethyl ester of succinic acid along with D-glucose is required to maintain the β-cell response to D-glucose (Fernandez et al., 1996).
Amino acids

Amino acids act as potent stimulators of insulin release. L-Tryptophan, which is the precursor of 5-Hydroxytryptamine (5-HT) act as a stimulator of insulin release (Bird et al., 1980). L-Arginine also stimulates insulin release from pancreatic β-cells. Several in vitro studies have suggested the production of nitric oxides from islet nitric oxide system have a negative regulation of the L-arginine induced secretion of insulin in mice.

Glucagon

Glucagon is the hormone secreted by pancreatic α-cells. It has been shown that glucagon has a striking stimulatory effect on insulin release in the absence of glucose (Sevi, 1966). The presence of specific glucagon receptors on isolated rat pancreatic β-cells as well as a subpopulation of α- and δ-cells shows the relevance of glucagon on regulation of insulin secretion. Intra-islet glucagon appears to be a paracrine regulator of cAMP in vitro (Schuit, 1996). Glucagon stimulates insulin release by elevating cAMP. cAMP through activation of protein kinase A, increases Ca$^{2+}$ influx through voltage dependent L-type Ca$^{2+}$ channels, thereby elevating Ca$^{2+}$ and accelerating exocytosis (Carina, 1993). Protein phosphorylation by Ca$^{2+}$/Calmodulin and cAMP dependent protein kinase play a positive role in insulin granule movement which results in potentiation of insulin release from the pancreatic β-cell (Hisatomi, 1996).

Somatostatin

This hormone is secreted by the pancreatic δ-cells of the islets of Langerhans. Somatostatin inhibits insulin release. Its action is dependent on the activation of G-
proteins but not associated with the inhibition of the voltage dependent \( \text{Ca}^{2+} \) currents or adenylate cyclase activity (Renstrom et al., 1996).

**Pancreastatin**

Pancreastatin is known to be produced in islet \( \beta \)-cells and to inhibit insulin secretion. Pancreastatin is a modulator of the early changes in insulin secretion after increase of glucose concentration within the physiological range (Ahren et al., 1996). It is reported to increase \( \text{Ca}^{2+} \) in insulin secreting RINm5F cells independent of extracellular \( \text{Ca}^{2+} \) (Sanchez et al., 1992).

**Amylin**

Amylin is a 37-amino acid peptide hormone co-secreted with insulin from pancreatic \( \beta \)-cells. Amylin appears to control plasma glucose via several mechanisms that reduce the rate of glucose appearance in the plasma. Amylin limits nutrient inflow into the gut and nutrient flux from the gut to blood. It is predicted to modulate the flux of glucose from liver to blood by its ability to suppress glucagon secretion. Amylin is absolutely or relatively deficient in type I - diabetes and in insulin requiring type II - diabetes (Young, 1997). It inhibits insulin secretion via an autocrine effect within pancreatic islets. Amylin fibril formation in the pancreas cause islet cell dysfunction and cell death in type II - diabetes mellitus (Alfredo et al., 1994).

**Nerve growth factor**

Nerve growth factor (NGF) is a neurotropic growth factor that promotes neurite outgrowth during development. This growth factor is capable of modulating \( \beta \)-cell plasticity because it promotes neurite-like outgrowth in fetal and adult pancreatic \( \beta \)-cells from primary cultures (Vidaltamayo et al., 1996) and in RINm5F
and insulinoma cells (Polak et al., 1993). In adult rat β-cells, \textit{in vitro} NGF stimulates glucose induced insulin secretion. The presence of the high affinity receptor for NGF has been described in insulinoma cell lines as well as in foetal and adult β-cells. The adult β-cells synthesise and secrete NGF in response to increasing extra cellular glucose concentration (Vidalatamayo et al., 1996). The effect of NGF on insulin secretion is partly mediated by an increase in Ca\textsuperscript{2+} current through Ca\textsuperscript{2+} channels (Rosenbaum et al., 2001).

\textbf{Neuropeptides}

Immunocytochemistry has revealed the presence of three neuropeptides in the nerve terminals of pancreatic ganglia and islets of different species: Vasoactive intestinal peptide (VIP), gastrin releasing peptide (GRP) and pituitary adenylate cyclase activating polypeptide (PACAP).

\textbf{Gastrin releasing peptide}

Gastrin releasing peptide (GRP) consists of a 27 amino acid residue. It is localised to pancreatic nerves, including islet nerve terminals of several species. GRP released from the pancreas after vagal nerve activation and stimulates insulin secretion (Knuhtsen et al., 1987; Sundler & Bottcher, 1991). In islets, activation by GRP receptors is coupled to PLC and phospholipase D (Wahl et al., 1992; Gregersen & Ahren, 1996).

\textbf{Role of neurotransmitters in insulin regulation & secretion}

\textit{Acetylcholine}

Acetylcholine is one of the principal neurotransmitters of the parasympathetic system. Acetylcholine, through vagal muscarinic and non-vagal muscarinic pathways
(Greenberg & Pokol, 1994) increases insulin secretion (Tassava et al., 1992). They function through muscarinic receptors present on pancreatic islet cells (Ostenson et al., 1993). Acetylcholine agonist, carbachol, at low concentration (10^{-7} M) stimulated insulin secretion at 4 mM and 20 mM concentrations of glucose (Renuka et al., 2006).

**Dopamine**

Dopamine is reported to inhibit glucose stimulated insulin secretion from pancreatic islets (Tabeuchi et al., 1990). Eswar et al., (2006) reported that dopamine significantly stimulated insulin secretion at a concentration of 10^{-8} M in the presence of high glucose (20mM). Reports show that experimental diabetes and insulin deficiency result in the rapid onset of detectable alterations in dopaminergic activity in specific hypothalamic nuclei. The uptake affinity and velocity of dopamine in synaptosomes decreased significantly during diabetes. The dopamine content was increased in the cerebral cortex and hypothalamus of diabetic rats (Shiimzu, 1991; Tassava et al., 1992; Ohtani et al., 1997). The altered turnover ratio in the limbic forebrain is reported to cause enhanced spontaneous locomotor activity in diabetic rats (Kamei et al., 1994).

High concentrations of dopamine in pancreatic islets decrease glucose stimulated insulin secretion (Tabeuchi et al., 1990). L-DOPA, the precursor of dopamine had similar effect to that of dopamine (Lindstrom & Sehlin, 1983). Dopamine D3 receptors are implicated in the control of blood glucose levels (Alster & Hillegaart, 1996). Dopamine D1 receptors have also been reported to be present on pancreatic β-cells (Tabeuchi et al., 1990). These clearly indicate the role of dopamine in the regulation of pancreatic function.
Gamma-Aminobutyric acid

Gamma aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the CNS. GABA is reported to present in the endocrine pancreas at concentrations comparable with those found in CNS. 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). GABA through its receptors has been demonstrated to attenuate the glucagon and somatostatin secretion from pancreatic α–cells and δ-cells respectively (Gaskins, 1995). It is present in the cytoplasm and in synaptic-like microvesicles (Reetz, 1991) and is co-released with insulin from β-cells in response to glucose. The released GABA inhibits islet α- and β-cell hormonal secretion in a paracrine manner. During diabetes the destruction of β-cells will lead to decrease in GABA release resulting in the enhancement of glucagon secretion from α-cells leading to hyperglycemia. The brain GABAergic mechanisms also play an important role in glucose homeostasis. Inhibition of central GABA_A receptors increases plasma glucose concentration (Lang, 1995). GABA_A receptors in brainstem have a regulatory role in pancreatic regeneration (Kaimal et al., 2007) Thus, any impairment in the GABAergic mechanism in the CNS and/or in the pancreatic islets is important in the pathogenesis of diabetes.
Serotonin

Serotonin content is increased in the brain regions and hypothalamic nuclei (Lackovic et al., 1990; Chen & Yang, 1991), but there are reports suggesting a decrease in brain 5-HT content during diabetes (Sumiyoshi et al., 1997; Sandrini et al., 1997; Jackson & Paulose, 1999). Ohtani et al. (1997) have reported a significant decrease in extracellular concentrations of NE, 5-HT and their metabolites in the ventro medial hypothalamus (VHM). The ratio of 5-HIAA/5-HT was increased. A similar observation was reported by Ding et al. (1992) with a decrease in 5-HT in cortex (19%) and 5-HT turnover (5-HIAA/5-HT) that increased by 48%. Chu et al., (1986) has reported lower 5-HT levels in both hypothalamus and brainstem but not in corpus striatum. Insulin treatment brought about an increase in the cerebral concentration of 5-HIAA and accelerated the cerebral 5-HT turnover (Juszkiewicz, 1985). The 5-HIAA concentration was reported to be approximately twice as high as the controls regardless of duration of treatment. Brain tryptophan, the precursor of 5-HT, was also reduced in brain regions during diabetes (Jamnicky et al., 1991). Insulin treatment was reported to reverse this reduced tryptophan content to normal (Jamnicky et al., 1993). There was a significant increase in 5-HIAA observed at 2-6 hours after insulin administration (Kwok & Juorio, 1987).

Epinephrine and Norepinephrine

These are secreted by the adrenal medulla. Norepinephrine (NE) is a principal neurotransmitter of sympathetic nervous system. These hormones inhibit insulin secretion, both in vivo and in vitro (Porte, 1967; Renstrom et al., 1996). Epinephrine exerts opposite effects on peripheral glucose disposal and glucose stimulated insulin secretion (Avogaro 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 hypoglycemia, a 15 to 40 fold increase of epinephrine plays a pivotal role in increasing glucose production independently of glucagon (Gauthier et al., 1980). It is already known that, when used in high doses in vivo or in vitro, epinephrine reduces the insulin response to stimulators (Malaisse, 1972). In vitro studies with yohimbine – α₂-adrenergic receptor antagonist, showed that the insulin secretion from the pancreatic islets increased significantly suggesting that when the alpha 2-adrenergic receptors are blocked, it enhances islet cell proliferation and insulin secretion (Ani et al., 2006). EPI and NE have an antagonistic effect on insulin secretion and glucose uptake (Porte et al., 1966). 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. In humans, adrenaline stimulates lipolysis, ketogenesis, thermogenesis and glycolysis and raises plasma glucose concentrations by stimulating both glycogenolysis and gluconeogenesis. Adrenaline is, however, known to play a secondary role in the physiology of glucose counter-regulation. Indeed, it has been shown to play a critical role in one pathophysiological state, the altered glucose counter-regulation in patients with established insulin-dependent diabetes mellitus (Cryer, 1993). The inhibitory effect of EPI upon insulin secretion induced by glucose was reported by Coore and Randle, (1964), who incubated pancreatic tissue from the rabbit. As judged by Malaisse et al., (1967) the inhibitory effect of EPI on glucose-induced insulin secretion is mediated through the activation of α-adrenoreceptors.
Central muscarinic regulation of glucose homeostasis

The acetylcholine esterase inhibitor, soman induced marked and sustained hypertension in rats (Letienne et al., 1999). Stimulation of muscarinic receptors in the nucleus tractus solitarius (NTS) of the rat decreases arterial blood pressure and heart rate. Atropine injected into the NTS of rats produced a dose-dependent inhibition of cardiovascular response elicited by injection of acetylcholine into the same site. It is suggested that cholinergic mechanisms in the NTS are not involved in the tonic regulation of cardiovascular function or the baroreceptor reflex (Tsukamoto et al., 1994).

When carbachol, muscarine, bethanechol, methacholine, or neostigmine was injected into the third cerebral ventricle, it caused a dose-dependent increase in the hepatic venous plasma glucose concentration. However, in the case of 1, 1-dimethylphenyl-4-piperazinium iodide (DMPP) or nicotine, the level of hepatic venous glucose did not differ from that of the saline-treated control rats. The increase in glucose level caused by neostigmine was dose-dependently suppressed by co-administration of atropine. These facts suggest that cholinergic activation of muscarinic receptors in the CNS plays a role in increasing hepatic glucose output. Injection of neostigmine, an inhibitor of cholinesterase, into the ventricle resulted in the increase of not only glucose, but also glucagon, epinephrine, and norepinephrine in the hepatic venous plasma. Neostigmine-induced increments in glucose did not occur in adrenalectomized rats. This suggests that the secreted epinephrine acts directly on the liver to increase hepatic glucose output (Iguchi et al., 1986).

The injection of adrenaline and carbachol into the third cerebral ventricle resulted in a marked hyperglycemia associated with increased immunoreactive glucagon. Adrenaline-induced hyperglycemia was not affected by bilateral adrenalectomy, while carbachol-induced hyperglycemia was completely inhibited by
adrenalectomy. The injection of somatostatin with adrenaline into the third cerebral ventricle did not influence adrenaline-induced hyperglycemia, while carbachol-induced hyperglycemia was inhibited by co-administration with somatostatin (Iguchi et al., 1985).

Atropine injected into the third cerebral ventricle suppressed epinephrine secretion and dose-dependently inhibited hepatic venous hyperglycemia induced by neostigmine in intact rats. The neostigmine-induced glucagon secretion which occurs in adrenalectomised rats was suppressed by atropine. Atropine also prevented the neostigmine-induced hyperglycemia in adrenalectomised rats receiving constant somatostatin infusion through femoral vein. Phentolamine, propranolol and hexamethonium showed no significant inhibitory effect on neostigmine-induced hyperglycemia, epinephrine and glucagon secretion in intact rats, glucagon secretion in adrenalectomised rats, or hyperglycemia in adrenalectomised rats. These results suggest that neostigmine-induced epinephrine and glucagon secretion and increased hepatic glucose output stimulated by direct neural innervation to liver is mediated by central muscarinic receptor in fed rats (Iguchi et al., 1990).

Studies by Iguchi et al., (1992) suggest that the glucoregulatory hippocampal activity evoked by the acetylcholine esterase inhibitor, neostigmine transmitted to peripheral organs via the ventromedial hypothalamus. The ventromedial hypothalamus, lateral hypothalamus, paraventricular hypothalamus and median site of the lateral-preoptic area were involved in increasing the plasma levels of glucose and epinephrine by cholinergic stimulation (Honnura et al., 1992).

Atropine in a dose-dependent manner suppressed the hyperglycemia induced by hippocampal administration of neostigmine, whereas hexamethonium had no significant effect. These observations suggest that the pathway for this experimental hyperglycemia involves, at least in part, the muscarinic cholinergic neurons in the
ventromedial hypothalamus (Iguchi et al., 1991). Takahashi et al., (1993) reported that neostigmine induced hyperglycemia affects not only the cholinergic system but also the noradrenergic and dopaminergic systems in the hypothalamus (Takahashi et al., 1993). Muscarinic cholinergic system is reported to participate in the HgCl$_2$-induced central hyperglycemic effect through the function of the adrenal medulla. Norepinephrine and dopamine content were found to be decreased suggesting that their neurons have hypothalamic glycoregulation (Takahashi et al., 1994).

Microinjections of carbachol or neostigmine into the ventromedial nucleus of the hypothalamus of fed, conscious rats produced marked increases in plasma glucose and lactate, which were suppressed or markedly reduced by previous adrenomedullation. The reports suggest that cholinergic synapses in the ventromedial hypothalamus participate in a central glucoregulatory system that increases hepatic glucose production mainly through a stimulation of adrenal medulla epinephrine secretion (Brito et al., 1993).

Neostigmine caused significant increases in serum glucose concentrations, hypothalamic noradrenergic and dopaminergic neuronal activities, and significantly suppressed hypothalamic serotonergic neuronal activity. All these responses to neostigmine were completely inhibited by the co-administration of atropine. These observations emphasize the important role of the interactions between cholinergic (muscarinic) and monoaminergic neurons in the brain (Gotoh & Smythe, 1992). In the ventromedial hypothalamic nucleus, lateral hypothalamus and paraventricular nucleus the cholinergic activity is increased after 2-D glucose administration (Takahashi et al., 1994 & 1996).

Central cholinergic-muscarinic activation with neostigmine stimulates sympathetic nervous activity in the liver, heart, pancreas and interscapular brown adipose tissue (Gotoh & Smythe, 1992). Histamine induction of CNS-mediated
Literature Review

Hyperglycemia involves neuronal transmission not only via H1 receptors but also, at least in part, by muscarinic cholinergic neurons (Nonogaki et al., 1993). The action of acetylcholine within the hypothalamus on the pancreatic hormone secretions is mediated to a large part through sympatho-adrenomedullary activity. However, a part of the decreased insulin response to glucose is mediated by direct innervation of the pancreas (Ishikawa et al., 1982).

Intravenous 2-D glucose induced a marked increase in plasma glucose that was not affected by intracerebroventricular administration. However, the hyperglycemia induced by intracerebroventricular 2-D glucose was significantly reduced by previous intracerebroventricular injection of atropine. Central cholinergic neurons participate in the complex neural events responsible for the hyperglycemic response to neurocytogluccopenia and to stressful situations (Brito et al., 2001). Intravenous administration of 2-D glucose caused neuroglycopenia and marked hyperglycemia. The cholinergic activity was increased after 2-D glucose administration (Takahashi et al., 1996).

**Dopamine, a neurotransmitter in the CNS**

Dopamine 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 dopamine cell bodies in the substantia nigra and ventral tegmental area in mid-brain region (Carlsson, 1993; Tarazi et al., 1997 a, b; 1998 a, b, 2001). 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 dopamine system in the brain as it accounts for about 70% of the total dopamine 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. Dopamine released in this system exerts regulatory effects in the anterior pituitary and inhibits the release of prolactin. Dopamine 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 dopamine 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 dopamine itself as an end-product inhibitor, or by activation of presynaptic dopamine receptors. On the other hand, the enzyme aromatic L-amino acid decarboxylase converts L-DOPA to dopamine instantaneously. Therefore, providing L-DOPA creates a possibility to enhance the formation of dopamine.

**Dopamine receptors**

Dopamine mediates its actions via membrane receptor proteins. Dopamine receptors are found on postsynaptic neurons in brain regions that are dopamine-enriched. In addition, they reside presynaptically on dopamine 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 (Carlsson, 1993; Tarazi et al., 1997 a, b, 1998 a, b). In their putative transmembrane domains, the dopamine D1 and D5 receptors are 79% identical to each other, while they are only 40–45% identical to the dopamine D2, D3, and D4 receptors. Conversely, the dopamine 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 dopamine D₁, the C-terminus in dopamine D₂ like receptors. The dopamine receptors are glycosylated in their N-terminal domains. Dopamine D₁ like subtypes has potential glycosylation sites in their first extra cytoplasmic loop.

Dopamine receptors are divided into two families on the presence or absence of ability of dopamine to stimulate adenylyl cyclase and produce the second-messenger molecule cyclic-AMP (Kebabin & 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 inhibits the production of cAMP. This pharmacological characterisation is based on the ability of some dopamine 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 characterisation of novel dopamine receptors, dopamine D₃, D₄ and D₅ with different anatomical localisation from traditional dopamine D₁ or dopamine D₂ receptors. Based upon their pharmacological profiles, including their effects on different signal transduction cascades, these receptors are currently divided into two families: the dopamine D₁ like family which includes dopamine D₁ and D₅ receptors. The dopamine D₂ like family includes dopamine D₂, D₃ and D₄ receptors (Schwartz et al., 1992; Grandy et al., 1993; Sibley et al., 1993). The genomic organisations of the dopamine 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 dopamine D2-like receptor genes are located in similar positions.

**Dopamine D1-like family**

The dopamine D1 receptor is the most abundant dopamine receptor in the CNS. The dopamine D1-like receptors are characterized by a short third loop as in many receptors coupled to Gs protein (Civelli *et al.*, 1993; Gingrich & Canon *et al.*, 1993; O’Dowd, 1993). The dopamine D1-like receptors have short third intracellular loops and long carboxy terminal tails. The dopamine D1-like receptors are classified into dopamine D1 and D5. In the dopamine D1 and D5 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 is probably related to the third cytoplasmic loop and the carboxy terminal tail (Civelli *et al.*, 1993, Gingrich & Canon *et al.*, 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 D1 receptor than in the D5 receptor (41 amino acids). The amino acid sequence of this loop is divergent in the dopamine D5 receptor (Marc *et al.*, 1998).

**Dopamine D1 receptor**

Dopamine D1 receptors are found at high levels in the typical dopamine regions of brain such as the neostriatum, substantia nigra, nucleus accumbens and olfactory tubercles. Dopamine D1 receptor seems to mediate important actions of
dopamine to control movement, cognitive function and cardiovascular function. The dopamine D1 receptor gene, which lacks introns, encodes a protein that extends for 446 amino acids (Dohlman et al., 1991). In humans dopamine D1 receptor gene has been localized to chromosome 5 (Sunahara et al., 1990). The dopamine D1 receptors show characteristic ability to stimulate adenylyl cyclase and generate inositol 1, 4, 5-trisphosphate (IP$_3$) and diacylglycerol via the activation of PLC (Monsma et al., 1990; Sibley et al., 1990). Dopamine D1 receptors are highly expressed in basal ganglia followed by cerebral cortex, hypothalamus and thalamus. Dopamine D1 receptors messenger ribonucleic acid (mRNA) is colocalized in striatal neurons of the basal ganglia with mRNA for dopamine receptor phosphor protein (DARPP-32; KD) which is a dopamine and cAMP-regulated phosphoprotein. Dopamine Receptor Phosphor Protein contributes to the actions of dopamine D1 receptor (Hemmings & Greengard, 1986; Greengard, et al., 1987). The dopamine D1 receptors in the brain are linked to episodic memory, emotion, and cognition.

**Dopamine D5 receptors**

The dopamine 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 dopamine D1 receptor and 80% if only the seven transmembrane segments are considered. The gene encoding the human dopamine D5 protein is located at the short arm of chromosome 4, the same region where the Huntington disease gene has been located. Two dopamine 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.
Dopamine 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). Dopamine D5 receptors appear to interact with G-proteins and can stimulate adenylyl cyclase, with relatively high affinity for dopamine and dopamine D1-selective agonists (George et al., 1991).

**Dopamine D<sub>2</sub> like family**

Dopamine D2 like receptors belong to the G-protein coupled receptors and has 400 amino acid residues. Dopamine D2-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 (Marie et al., 1996; 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 dopamine D<sub>2</sub> 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 (Marie et al., 1996). Between the different dopamine receptors, the third loop also displays the greatest variability in amino-acid sequence. This has consequences for their respective second messenger systems. The dopamine D2-like receptors are coupled to Gi-protein and inhibit the formation of cAMP. The dopamine D2 receptors tertiary structure is stabilized by two cysteine disulphide bridges.
**Dopamine D2 receptors**

The dopamine D2 receptor gene encodes a protein that extends for 415 amino acids. Similar to other G-protein coupled receptors, the dopamine D2 receptor has seven transmembrane segments, but in contrast to dopamine D1-like receptors, the third cytoplasmic domain is long and the carboxy terminus is short. Unlike the dopamine D1-like receptor genes, the dopamine 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 dopamine D2 receptor was the first receptor to be cloned (Bunzow *et al*., 1988). The dopamine D2 receptors are involved in several signal transduction cascades, including inhibition of cAMP production (Vallar & Meldolesi, 1989), inhibition of phosphoinositide turnover (Epelbaum J *et al*., 1986), activation of potassium channels and potentiation of arachidonic acid release (Axelrod, 1991). The dopamine D2 receptors are highly expressed in basal ganglia, nucleus accumbens septi and ventral tegmental area (Schwartz *et al*., 1998).

The dopamine 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 dopamine D2S and dopamine 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 dopamine D2 receptor isoforms. The two isoforms derived from the same gene by alternative RNA splicing which occurs during the maturation of the dopamine D2 receptor pre-mRNA (Schwartz *et al*., 1989a). Dopamine D2 receptor isoforms (dopamine D2L and dopamine D2S) vary within each species by the presence or absence of a 29-amino acid sequence in the third
cytoplasmic domain of the dopamine 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 dopamine 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 dopamine D2L isoform of dopamine D2 receptors (Castro & Strange, 1993). When expressed in host cell lines, both isoforms inhibited adenylyl cyclase (Marc et al., 1998; Sibley, 1999). However, the dopamine D2S receptor isoform displayed higher affinity than the dopamine D2L in this effect (Seeburg et al., 1993). The isoforms of dopamine D2 mediate a phosphatidylinositol-linked mobilisation of intracellular Ca²⁺ in mouse Ltk fibroblasts. Protein kinase C, however, differentially modulates dopamine 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 dopamine D3 receptors bear close structural and pharmacological similarities to the dopamine D2 receptors. Dopamine D3 mRNA occurs in longer and shorter spliced forms generated from the same gene (Schwartz et al., 1992). Distribution of dopamine 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 dopamine 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 dopamine D2 receptor raises the possibility that dopamine D3 receptor also inhibit adenylyl cyclase activity in its normal cellular setting. More recent studies reported that dopamine D3 receptors mediate positive regulatory influences of dopamine on production of the peptide neurotensin (Sokoloff et al., 1990; Schwartz et al., 1992).

**Dopamine D4 receptors**

Dopamine D4 receptor gene contains four introns and encodes a 387 amino acid protein (Van Tol et al., 1991). The overall homology of the dopamine D4 receptor to the dopamine 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 dopamine D4 protein is located at the tip of the short arm of chromosome 11 (Civelli & Bunzow, 1993; Missalle et al., 1998). Dopamine D4 receptor gene has been localized in brain regions like hippocampus and frontal cortex using specific histoprobes. The stimulation of dopamine D4 receptor inhibits adenylyl cyclase activity and release arachidonic acid in brain neurons (Misalle et al., 1998). In humans, dopamine 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 dopamine D4 alleles.
which are represented as dopamine D4.2, D4.4 and D4.7. This contributes to the pathophysiology of certain neuropsychiatric disorders (Jackson & Westlind, 1994).

**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 dopamine metabolism (Hio et al., 1994). In experimental diabetes and insulin deficiency there is a rapid onset of detectable alterations in hypothalamic dopamine activity leading to secondary neuroendocrine abnormalities. Lim et al. (1994) have described an increase in the striatal dopamine 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 dopamine to controlled conditions, impairing the dopamine biosynthesis (Kamei & Saitoh, 1994). Dopamine uptake affinity and velocity in synaptosomes is decreased significantly during diabetes. The dopamine content was increased in cerebral cortex and hypothalamus of diabetic rats (Chen & Yang, 1991; Tassava et al., 1992; Ohtani et al., 1997). Diabetes is reported to cause increased dopamine release with altered turnover ratio of dopamine 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 dopamine transporter mRNA in experimental diabetes is suggested to be a possible cause for the disturbance in dopamine metabolism (Figlewicz et al., 1996). The dopamine 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 STZ
induced diabetes was significantly lowered when compared with their age-matched normal controls as a result of altered dopamine metabolism and decreased turnover to its metabolites (Heaton & Varrin, 1993).

Dopamine receptors are reported to be increased in diabetes causing significant alterations in central dopaminergic system (Lozovsky et al., 1981). Dopamine D<sub>2</sub> receptor density has been reported to be increased in the striatum of diabetic rats (Lozovsky et al., 1981; Trulson & Hummel, 1983). Intracerebroventricular application of alloxan and STZ in rat striatum is reported to have caused an alteration in dopamine receptors and increased dopamine content which had a similar effect to peripheral, diabetogenic administration of these drugs (Salkovic et al., 1992). The affinity of striatal dopamine D<sub>1</sub> receptors was significantly increased without changes in the number of binding sites, while the binding of dopamine D<sub>2</sub> receptors was significantly increased without affecting its affinity in the diabetic rats (Hio et al., 1994). Dopamine D<sub>1</sub> 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). Studies from our laboratory reported dopamine D<sub>2</sub> receptor alterations in the brain and pancreas of STZ-induced diabetic rats (Eswar et al., 2007).

Diabetes mellitus causes a condition called as neurocytoglucopenia 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 mobilisation from the adipose tissue (Oliveira et al., 1998). Participation of dopaminergic tone in the control of insulin secretion and hyperglycaemia has been given little focus. 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).

Alterations of glucose transport during diabetes

In diabetes mellitus apart from raised blood glucose levels, disturbances in the metabolism of a number of other biomolecules such as glycogen, lipids, proteins and glycoproteins have also been reported (Randle et al., 1963; Williamson et al., 1968). Treatment with insulin generally rectifies these disturbances in diabetic state as it increases the peripheral utilisation of glucose by influencing key enzymes of glucose metabolic pathways (Exton et al., 1966; Lenzen et al., 1990). The liver plays a major role in insulin-regulated glucose homoeostasis through the balance between glucose utilisation and glucose production, both processes being tightly coordinated (Nevado et al., 2006). It has been shown that glucose uptake and release required a family of membrane facilitated-diffusion glucose transporters which are expressed in a tissue-specific manner. In muscle and fat, GLUT4 is the main isoform of glucose transporters (Burant et al., 1991). In adipose tissue the concentrations of GLUT4 protein and mRNA are markedly decreased after 2-3 weeks of diabetes and they are restored by insulin therapy (Berger et al., 1989; Garvey et al., 1989), whereas in skeletal muscle the concentrations of GLUT4 protein and mRNA are marginally altered (Garvey et al., 1989; Bourey et al., 1990). In liver, GLUT2 is the main isoform of glucose transporters (Thorens et al., 1988). Much less information is available concerning the expression of GLUT2 in liver of diabetic rats and the results are somewhat contradictory.
Insulin and the brain

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

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

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

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

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

India has a rich history of using plants for medicinal purposes. Turmeric (Curcuma longa L.) is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases (Ammon & Wahl, 1991; Eigner & Scholz, 1999). *C. longa* L., botanically related to ginger (Zingiberaceae family), is a perennial plant having a short stem with large oblong leaves and bears ovate, pyriform or oblong rhizomes, which are often branched and brownish-yellow in colour. Turmeric is used as a food additive (spice), preservative and colouring agent in Asian countries, including China and South East Asia. It is also considered as auspicious and is a part of religious rituals. In old Hindu medicine, it is extensively used for the treatment of sprains and swelling caused by injury1. In recent times, traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis (Ammon *et al.*, 1992). The colouring principle of turmeric is the main component of this plant and is responsible for the antiinflammatory property. Turmeric was described as *C. longa* by Linnaeus and its taxonomic position is as follows:

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<thead>
<tr>
<th>Class</th>
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<tr>
<td>Subclass</td>
<td>Commelinids</td>
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<tr>
<td>Order</td>
<td>Zingiberales</td>
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<tr>
<td>Family</td>
<td>Zingiberaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Curcuma</td>
</tr>
<tr>
<td>Species</td>
<td>Curcuma longa</td>
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**Medicinal Properties:**

- anti-oxidant;
- arthritis: anti-inflammatory effects, possibly inhibits something in the pathway of Cox-2 but not Cox-2 itself; not only does it not cause ulcers but is
Literature Review

currently being used experimentally as a treatment for ulcers in western countries.
• anti-Alzheimer’s: inhibits formation of, and breaks down, Amyloid-beta oligomers (fibres) and aggregates in rodents;
• anti-platelet;
• anti-cancer effects: causes apoptosis in various cancer cell types including skin, colon, forestomach, duodenum and ovary in the laboratory; we await clinical trials in humans;
• anti: -viral, -fungal, -bacterial effects (inhibits Helicobacter Pylori);
• inhibits NFkappaB, 5-lipoxygenase, glutathione S-transferase and cytochrome P-450;
• Anti-diabetic effects in rodents; we await clinical trials in humans.

Curcumin and Alzheimer’s Disease (AD)

Recently curcumin has been proposed as a potential remedy against brain ageing and neurodegenerative disorders (Cole et al., 2007), and it has been evaluated in a pilot clinical trial in AD patients, with encouraging preliminary results (Baum L. et al., 2008). Curcumin is highly lipophilic and cross the blood-brain barrier. Although its bioavailability is very low, since the drug is rapidly metabolized by conjugation, curcumin reach brain in a sufficient concentration to activate signal transduction events and to decrease Amyloid β aggregation (Yang et al., 2005). Epidemiological studies suggested that curcumin, one of the most prevalent nutritional and medical compounds used by the Indian population, is responsible for the significantly reduced (4.4-fold) prevalence of AD in India compared to United States (Chandra et al., 2001). Furthermore elderly Singaporeans who eat curry with turmeric had higher Mini-Mental State Examination scores than those who did not.
Vitamin D₃

Several forms of Vitamin D are distinguished, namely Vitamin D₁, D₂, D₃ and D₄. Of these, the most important for humans is D₃. The action of UV-B rays with a wavelength of 290–315 nm on the skin results in photochemical synthesis of previtamin D₃ from the provitamin 7-dehydrocholesterol, which in turn is formed in the liver from cholesterol. Over a period of two to three days, a thermally induced change in molecular structure (isomerisation) then results in the conversion of previtamin D₃ to vitamin D₃ (cholecalciferol) in a process that does not require sunlight. Vitamin D₃ then undergoes enzymatic conversion in the liver and the kidneys to the active substance 1,25-dihydroxycholecalciferol, also known as 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃). 1,25-dihydroxyvitamin D₃ is the active form of the Vitamin in human beings (Holick, 1987; Haussler et al., 1998).

Vitamin D receptor

Certain VDR gene polymorphisms are associated with type 1 diabetes (Mathieu et al., 1994; Ambrosio et al., 1998). Vitamin D receptors are activated when certain mediator substances, or ligands, dock at them. This ligand function can be
exerted not only by vitamin D compounds, but also by steroid hormones, thyroid hormones and Vitamin A1 acid. By binding to the receptor, these ligands regulate the metabolism of Ca$^{2+}$ and phosphate, and thus also of bone and control cell replication and differentiation. This occurs via an influence on the synthesis of certain regulatory proteins. When a VDR is activated by binding of a ligand, it exerts its action as a transcription factor. This means that it binds to specific sites on DNA (deoxyribonucleic acid), the molecule in the cell nucleus that bears genetic information and thereby initiates the synthesis of certain regulatory proteins.

**Vitamin D and diabetes**

The discovery of receptors for 1,25(OH)2D3, the activated form of vitamin D, in tissues with no direct role in calcium and bone metabolism (e.g. pancreatic beta cells and cells of the immune system) has broadened our view of the physiological role of this molecule (Holick, 1987; Haussler *et al*., 1998). An increased prevalence of type 2 diabetes has been described in Vitamin D-deficient individuals (Boucher *et al*., 1995; Chiu *et al*., 2004) and insulin synthesis and secretion have been shown to be impaired in beta cells from Vitamin D-deficient animals. Glucose tolerance is restored when vitamin D levels return to normal. The identification of receptors for 1,25(OH)2D3 in cells of the immune system led to experiments in animal models of type 1 diabetes in which the administration of high doses of 1,25(OH)2D3 was shown to prevent type 1 diabetes (Mathieu *et al*., 1994; Boucher *et al*., 1995), mainly through immune regulation. It has been demonstrated that 1, 25(OH) 2D3 is one of the most powerful blockers of dendritic cell differentiation and that it directly blocks IL-12 secretion (Ambrosio *et al*., 1998). Lymphocyte proliferation is inhibited and regulator cell development is enhanced (Halteren *et al*., 2002). This review provides an overview of the data available on the role of Vitamin D in type 1 and type 2 diabetes
and discusses possible applications of the molecule or its synthetic analogues (Bouillon et al., 2003) in clinical disease. The terminology used in many papers to describe vitamin D and its metabolites is confusing, with misnomers leading to misunderstanding and over-interpretation of data. In this review the term vitamin D refers to the product that is in food (vitamins D2 and D3) and is synthesised in the skin under the influence of UVB radiation (vitamin D3), whereas the metabolically active molecule is referred to as 1,25 (OH)2D3.

**Vitamin D3 and Central nervous system**

Regulatory effect of vitamin D on NGF and GDNF suggests that it is a potent neuroprotective agent (Kalueff & Tuohimaa, 2007). The active form of vitamin D, 1,25(OH)2D would appear to provide some protection against excitatory neurotransmitters such as glutamate (Ibi et al., 2001). Vitamin D also protect the brain against reactive oxygen species via up regulation of antioxidant molecules, such as glutathione, in non-neuronal cells (Garcion et al., 1999). Vitamin D suppress macrophage activity in the brain after lipopolysaccharide-induced brain inflammation (Garcion et al., 1998). Inflammatory mechanisms induced by experimental autoimmune encephalitis (EAE) are also diminished by this vitamin (Nataf et al., 1996). It has been shown in vitro that activated microglia metabolise 25(OH) D and produce the biologically active 1, 25(OH) 2D (Neveu et al., 1994). Thus, non-neuronal cells in the brain mediate anti-inflammatory effects of vitamin D via its local synthesis. Vitamin D has also been shown to preserve dopamine and serotonin content in the brains of animals repeatedly administered with neurotoxic doses of methamphetamine (Cass et al., 2006).

Similar to the benefits of traditional antioxidant nutrients, Vitamin D3 inhibits inducible nitric oxide synthase (Garcion et al., 1997), an enzyme that is up regulated
during ischemic events and in patients with Alzheimer’s and Parkinson’s disease. Vitamin D₃ also enhances innate antioxidant pathways. The hormone upregulates gamma glutamyl transpeptidase (Baas et al., 2000) and subsequently increases glutathione. Glutathione is an innate antioxidant which protects oligodendrocytes and the integrity of the nerve conduction pathway critical to mental processing.

Vitamin D has been detected in the cerebrospinal fluid and this hormone has been shown to cross the blood-brain barrier (Balabanova et al., 1984; Gascon-Barre & Huet., 1983; Pardridge, Sakiyama, & Coty, 1985). The presence of VDR in the limbic system, cortex, cerebellum of rodents and humans (Eyles et al., 2005; Langub et al., 2001; Musiol et al., 1992; Walbert, Jirikowski, & Prufer, 2001) support a functional role for Vitamin D in the regulation of behaviour and cognitive functions. It is also consistent with the distribution of other neurosteroids (Prufer & Jirikowski., 1997). VDR is found in the olfactory, visual and auditory sensory systems (Glaser et al., 1999; Prufer et al., 1999; Zou et al., 2008), suggesting that the somatosensory system is also a target of 1,25(OH)₂D. VDR like immunoreactivity was found in the nucleus vestibularis, which extends its efferents to cerebellar Purkinje cells and the thalamic part of the vestibular system, nucleus ventrolateralis, suggesting that the vestibular system is also a target of VD (Prufer et al., 1999). Expression of VDR in motor neurons (Prufer et al., 1999) suggests its role in regulation of motor functions. A putative receptor for Vitamin D has been detected in chick brain (Jia & Nemere, 1999), allowing speculation that Vitamin D could act like other neuroactive hormones in modulating neuronal activity and neurotransmitter receptors (Zakon, 1998; Rupprecht & Holsboer, 1999). It is of particular importance that VDR and catalytic enzymes are colocalized in the brain (Baulieu, 1998; Melcangi & Panzica, 2001), supporting an autocrine/paracrine function for Vitamin D. These findings support a functional role for Vitamin D₃ in the human brain (McGrath et al., 2001).
Vitamin D₃
Molecular formula C₂₇H₄₈O
Molar mass 384.64 g/mol