Literature Review

Diabetes mellitus is the most common endocrine disorder characterized by increased blood glucose levels resulting from defective insulin secretion, resistance to insulin action or both. Diabetes is associated with long term complications and affects eyes, kidneys, blood vessels, heart, and nerves (Gispen & Biessels, 2000; Northam et al., 2006; Ametov & Kulidzhanian, 2012.). The latest World Health Organization statistics have estimated that more than 346 million people worldwide have diabetes. This number is likely to more than double by 2030 without intervention. Almost 80% of diabetes deaths occur in low- and middle income countries.

There are two main forms of diabetes (Zimmet et al., 2001), type 1 and type 2 diabetes. In type 1 diabetes, the body does not produce insulin, and daily insulin injections are required. Type 1 diabetes is usually diagnosed during childhood or early adolescence and it affects about 1 in every 600 children. Type 2 diabetes is the result of failure to produce sufficient insulin and insulin resistance. Elevated blood glucose levels are managed with reduced food intake, increased physical activity, and eventually oral medications or insulin.

History of Diabetes

The earliest description of diabetes was documented in the writings of Hindu scholars as long as in 1500 BC. They had already described “a mysterious disease causing thirst, enormous urine output, and wasting away of the body with flies and ants attracted to the urine of people” The term diabetes was probably coined by Apollonius of Memphis around 250 BC, which literally meant “to go through” or siphon as the disease drained more fluid than a person could consume. Later on, the Latin word “mellitus” was added because it made the urine sweet (MacCracken & Puzzles 1997). Early research linked diabetes to glycogen metabolism, and the islet cells of pancreas were discovered by Paul Langerhans, a
young German medical student. In 1916, Sharpey-Shafer of Edinburgh suggested that a single chemical was missing from the pancreas and proposed its name as “insulin.” The term insulin originates from the word *Insel*, which is German for an islet or island. EL Scott and Nikolae Paulesco were successful in extracting insulin from the pancreas of experimental dogs. The key breakthrough, though, came from the Toronto University with the discovery of insulin in 1921 by FG Banting and CH Best (Bliss 1982).

**Epidemiology of Diabetes**

The global increase in the prevalence of diabetes is due to population growth, aging, urbanisation and an increase of obesity and physical inactivity. The primary determinants of the epidemic are the rapid epidemiological transition associated with changes in dietary patterns and decreased physical activity (Goldberg & Mather, 2012). The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. In globally estimated diabetes prevalence for 2011 is 366 million and is expected to affect 552 million people by 2030. The International Diabetes Federation (IDF) estimates that in 2010 the five countries with the largest numbers of people with diabetes are India, China, the United States, Russia and Brazil. The first national study on the prevalence of type 2 diabetes in India was done between 1972 and 1975 by the Indian Council Medical Research (ICMR, New Delhi) (Ahuja, 1979). India has the world's largest diabetes population, it is estimated that in India 50.8 million people living with diabetes followed by China with 43.2 million. Most familiar to the general public are type 1 diabetes (previously known as juvenile-onset, insulin dependent diabetes mellitus, IDDM), type 2 diabetes (formerly known as maturity-onset diabetes, noninsulin dependent diabetes mellitus, NIDDM) and gestational diabetes. Type 2 diabetes is by far the most common, accounting for 85–95% of all cases of diabetes.
Pancreas

Pancreas is an essential organ important for digestion and glucose homeostasis in higher organism. Malfunction of the pancreas results in several debilitating diseases such as diabetes, pancreatitis and pancreatic cancer. The mature pancreas of higher vertebrates and mammals comprises two major functional units: the exocrine pancreas, which is responsible for the production of digestive enzymes to be secreted into the gut lumen, and the endocrine pancreas, which has its role in the synthesis of several hormones with key regulatory functions in food uptake and metabolism. The exocrine portion constitutes the majority of the mass of the pancreas, and contains only two different cell types, the secretory acinar cells and the ductular cells. An acinar cell produces various digestive enzymes like amylase, proteases nuclease etc and duct cells that transport these enzymes into the intestine. The endocrine portion, which comprises only 1–2% of the total mass, contains five different cell types, which are organized into mixed functional assemblies referred to as the islets of Langerhans. 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, Joost, 2008). 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 1990; Adeghate & Ponery 2001).

The islets of Langerhans, which are embedded in the exocrine pancreatic tissue, are known to secrete three hormones: insulin, glucagon and somatostatin. In 1869, a medical student named Paul Langerhans described systems of cells in the pancreas which he thought were lymph glands. Islets constitute about 2% by weight of the adult human pancreas and are multicellular microorgans (Bonner-
Weir, 2005). In descending order of both cell number and cell mass, islet cell types are β (insulin secreting, 70–80%), α (glucagon secreting, 15–20%), PP (pancreatic polypeptide secreting, 15–20%), δ (somatostatin secreting, 5–10%), and the recently discovered ghrelin-secreting cells ghrelin, 1%; (Wierup et al., 2002).

**Glucagon**

Pancreatic α -cells were discovered in 1907 as histologically distinct cells from the β-cells of the islet of Langerhans (Lane 1909). Glucagon-containing cells constitute about 28% of the total number of endocrine cells in a normal pancreatic islet (Adeghate et al., 1997). They are located in the periphery of the islets in normal animals. However, in STZ-diabetic animals many glucagon-positive cells are seen scattered within the central portion of the islets (Adeghate & Ponery 2003).

The history of glucagon begins with that of insulin. In 1921, when F. Banting and C. Best tested their first pancreatic extracts in depancreatized dogs, they observed that insulin-induced hypoglycemia was preceded by a transient, rather mild hyperglycemia, and they thought that this unwanted effect was due to epinephrine release (Best 1972). Murlin et al., 1923 must be credited with the discovery of glucagon, because they suggested that the early hyperglycemic effect of the pancreatic extracts was due to a contaminant with glucogenic properties that they also proposed to call “glucagon” or the mobilizer of glucose. In a classical paper published in 1948. Sutherland and de Duve established the α-cells of the pancreas as being the source of glucagon. Glucagon-containing cells constitute about 28% of the total number of endocrine cells in a normal pancreatic islet (Adeghate & Donáth 1991; Quesada et al., 2008).

Glucagon is a 29 amino acid peptide hormone processed from proglucagon. Proglucagon is expressed in various tissues (e.g., brain, pancreas, and intestine) and is proteolytically processed into multiple peptide hormones in a
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tissue-specific fashion. Glucagon acts via a seven transmembrane G protein-coupled receptor consisting of 485 amino acids (Jelinek et al., 1993). To date, glucagon binding sites have been identified in multiple tissues, including liver, brain, pancreas, kidney, intestine, and adipose tissues (Burcelin et al., 1996; Christophe, 1996). Glucagon is released into the bloodstream when circulating glucose is low. The main physiological role of glucagon is to stimulate hepatic glucose output, thereby leading to increases in glycemia. This provides the major counter regulatory mechanism for insulin in maintaining glucose homeostasis \textit{in vivo}. There is ample evidence suggesting that glucagon plays an important role in initiating and maintaining hyperglycemic conditions in diabetic animals and humans. Insulin and glucagon are the key regulatory hormones for glucose homeostasis. The absolute levels and, even more so, the ratios of the two hormones are tightly regulated \textit{in vivo}, depending on nutritional status. It has been reported that the absolute levels of glucagon or the ratios of glucagon to insulin are often elevated in various forms of diabetes in both animal and human subjects (Burcelin et al., 1996; Semenchenko et al., 2012).

**Insulin**

Insulin positive cells are the most numerous cell types in the normal pancreas. They are located in both the central and peripheral parts of the islet and account for about 60 - 70% of the total cell population in an islet of Langerhans (Adeghate & Ponery 2003). The pancreatic 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 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; Radke & Stach, 1986).

The biological effects of insulin in classical insulin target tissues, such as skeletal muscle, fat and liver are glucose uptake, regulation of cell proliferation, gene expression and the suppression of hepatic glucose production. Insulin decreases hepatic glucose production and increases glucose entry into muscle and fat cells. In the post-absorptive state, the majority of total body glucose disposal takes place in insulin independent tissues. Approximately 50% of all glucose use occurs in the brain, which is insulin-independent (Grill, 1990). Another 25% of glucose disposal occurs in the splanchnic area (liver plus gastrointestinal tissues) which is also insulin independent. The remaining 25% of glucose use in the post-absorptive state takes place in insulin-dependent tissues, primarily muscle and to a lesser extent adipose tissue. Approximately 85% of endogenous glucose production is derived from the liver and the remaining 15% is produced by the kidney (Krysiak et al., 2012).

Insulin is secreted from the pancreatic β cells in response to various stimuli like glucose, arginine, sulphonylureas though physiologically glucose is the major determinant. Various neural, endocrine and pharmacological agents can also exert stimulatory effect. Glucose is taken up by beta cells through GLUT-2 receptors. After entering the beta cell, glucose is oxidized by glucokinase, which acts as a glucose sensor (Ait-Lounis et al., 2010). Glucose concentration below 90 mg/dl does not cause any insulin release. At such sub-stimulatory glucose concentrations, K⁺ efflux through open K$_{ATP}$ channels keeps the pancreatic β cell membrane at a negative potential at which voltage-gated Ca$^{2+}$ channels are closed (Saisho et al., 2012). As there is increase in plasma glucose, glucose uptake and
metabolism by the pancreatic β cell is enhanced. Rise in ATP concentration result in closure of K<sub>ATP</sub> channels, leading to a membrane depolarization, opening of voltage-gated Ca<sup>2+</sup> channels, Ca<sup>2+</sup> influx, a rise in intracellular calcium concentration, and ultimately exocytosis of insulin granules (Pinho <i>et al.</i>, 2010).

The physiological effects of insulin are mediated by its cell surface receptor, a α<sub>2</sub>β<sub>2</sub> transmembrane glycoprotein with intrinsic protein tyrosine kinase activity (Ebina <i>et al.</i>, 1985). Binding of insulin to the extracellular α-chains results in auto-phosphorylation of specific tyrosine residues in the portion of the β chains: two in the juxtamembrane region, three in the kinase (catalytic) domain, and two in the C-terminal tail (Feener <i>et al.</i>, 1993; Kohanski, 1993; Saisho <i>et al.</i>, 2012). Autophosphorylation of Tyr1158, Tyr1162 and Tyr1163 diphosphate (AMP-PNP) in the activation loop (A-loop) of the kinase domain is critical for stimulation of kinase activity and function (Rosen <i>et al.</i>, 1983).

As recently as 10 years ago, the brain was described as “an insulin insensitive organ” in medical textbooks. Evidence for the presence of insulin and its receptors in the CNS has challenged that notion (Ferrannini <i>et al.</i>, 1999; Schulingkamp <i>et al.</i>, 2000). Insulin is readily transported in to the CNS across the blood–brain barrier by a saturable, insulin receptor-mediated transport process (Baskin <i>et al.</i>, 1987; Banks <i>et al.</i>, 1997). The raising of the peripheral insulin concentration acetely increases the concentration in the brain and CSF, whereas prolonged peripheral hyperinsulinemia downregulates blood–brain barrier insulin receptors and reduces insulin transport into the brain (Wallum <i>et al.</i>, 1987). Insulin receptors are located on the synapse of both neurons and astrocytes (Rulifson <i>et al.</i>, 2002). The localisation of insulin receptors in the hippocampus and medial temporal cortex in rats is consistent with evidence that insulin influences memory (Park <i>et al.</i>, 2000).
Diabetes and central nervous system

The brain is not usually thought to be a target of chronic diabetes complications, but new research has shown that the disease has 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 (Myers & Olson, 2012).

CNS complications can include stroke and possibly cognitive impairment (Lin et al., 2010). Many studies both in type I and type II diabetic subjects have found significant impairment of various neurophysiological parameters (Meneilly et al., 1993). These neuropsychological changes are often accompanied with objective electrophysiological evidence of delayed conduction velocity and data processing time in the central nervous system (Khardori et al 1986). Persistent blood glucose elevation contributes to atherosclerosis that impairs blood flow to the brain. Individuals with glycosylated haemoglobin (HbA1c) levels (a test that indicates blood glucose levels over the previous 3 months) greater than 7% are nearly three times as likely to have a stroke compared with people who have an HbA1c level less than 5% (Myint et al., 2007). Other CNS complications may result from changes in blood–brain barrier or transport functions of the cerebral microvasculature (Mooradian 1997). Such damage might be associated with vascular dementia. Studies also suggest that diabetics are at greater risk of depression than non-diabetics (Lin et al., 2010).

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; Donato, 2012). Norepinephrine (NE) 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). Epinephrine (EPI) levels were significantly increased in the striatum, hippocampus and hypothalamus of diabetic rats and these changes were reversed to normal by insulin treatment (Ramakrishna & Namasivayam, 1995). STZ induced diabetes and acute insulin deficiency were demonstrated to result in increased content of EPI in the suprachiasmatic 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 leads to development of secondary neuroendocrine abnormalities, known to occur in 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).
Role of neurotransmitters in insulin regulation & secretion

*Acetylcholine*

Acetylcholine (ACh) is one of the principal neurotransmitters of the parasympathetic system. Ach is a major neurotransmitter from autonomic nervous system, regulates the cholinergic stimulation of insulin secretion, through interactions with muscarinic receptors especially through vagal muscarinic and non-vagal muscarinic pathways (Greenberg & Pokol, 1994). Muscarinic M1 receptor subtype antagonist, pirenzepine inhibits cholinergic mediated insulin secretion (Iismaa et al., 2000). 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). Treatment with the dopamine precursor L-DOPA in patients with Parkinson’s disease reduces insulin secretion upon oral glucose tolerance test (Rosati et al., 1972). In vitro studies performed in isolated pancreatic islets suggested the participation of the D2R in insulin secretion (Rubi et al., 2005). 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) Isabel et al., 2010 reported that the D2R plays an essential role in β-cell proliferation and insulin secretion adds a novel participant to the list of growth factors and hormones that control the fundamental and multifactorial process of glucose homeostasis. 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 a major neurotransmitter in the CNS, where GABA produces fast inhibition in mature neurons primarily by activation of GABA A receptor (GABAAR), a hetero-pentameric Cl⁻ channel (Luscher & Keller, 2004). A large amount of GABA is also produced in the pancreatic islet (Okada et al., 1976, Sorenson et al., 1991). Pancreatic GABA is primarily produced by the β cell (Vincent et al., 1983), in which GABA is stored in synaptic like micro-vesicles that are distinct from insulin containing large-dense core vesicles (Reetz et al., 1991). In the pancreatic islet, GABA released from β-cells plays a critical role in the regulation of glucagon secretion from α-cells. Specifically, GABA activates GABA ARs in α-cells, sequentially leading to an influx of Cl⁻ and membrane hyperpolarization, and hence an inhibition of glucagon secretion. Studies demonstrated that GABA ARs are also expressed in the primary islet β-cells (Glassmeier et al., 1998; Xu et al., 2006) and insulin-secreting clonal β-cell lines (Dong et al., 2006). Unlike in mature neurons and α-cells, stimulation of GABA ARs in β-cells induces membrane depolarization, enhancing insulin secretion in the presence of physiological concentrations of glucose (Dong et al., 2006; Braun et al., 2010). Recent studies demonstrated that GABA, in cooperation with insulin, enhances the proliferation and survival of the β-cells through activation of the PI3-K/Akt pathway. Remarkably, GABA promotes β-cell regeneration and reverses diabetes in mouse models (Soltani et al., 2011). 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 (Sandrini *et al*., 1997; Jackson & Paulose, 1999; Deuschle, 2012). 5-HT is synthesized within β-cells (Richmond *et al*., 1996), it is stored together with insulin in their secretory β-granules (Ekholm *et al*., 1971), and it is co-released when pancreatic islets are stimulated with glucose (Smith *et al*., 1999). 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**

Epinephrine (EPI) and Norepinephrine (NE) are secreted by the adrenal medulla. 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). Both β2- and α2-adrenoceptors are expressed in the islets. Noradrenaline has been shown to stimulate insulin and glucagon secretion through the β2-adrenergic receptors (Kuo *et al*., 1973; Ahrén, 2000). At the same time, noradrenaline also interacts with α2-adrenoceptors, which results in the inhibition of insulin secretion and the stimulation of glucagon secretion (Ahren, 2000).
Therefore, catecholamines may affect insulin secretion both as stimulators through \( \beta_2 \)-adrenoceptors and as inhibitors through \( \alpha_2 \)-adrenoceptors (Ullrich & Wollheim, 1985). Studies reported that adrenaline did not inhibit insulin secretion in mice with a double knockout of \( \alpha_2A \) and \( \alpha_2C \) adrenoceptors, and that the inhibition of insulin secretion by adrenaline was partially reduced in mice with single knockout of these receptors (Peterhoff et al., 2003). Transgenic mice with \( \beta \)-cell-specific overexpression of \( \alpha_2A \) adrenoceptors displayed reduced glucose-stimulated insulin secretion and impaired glucose tolerance (Devedjian et al., 2000; Champaneri et al., 2012).

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 \textit{in vivo} or \textit{in vitro}, EPI reduces the insulin response to stimulators (Malaisse, 1972). \textit{In vitro} studies with yohimbine – \( \alpha_2 \)-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 phospho-fructokinase. 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, 1997). The inhibitory effect of EPI upon insulin secretion induced by glucose was reported by Coore & 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.

**Glutamate**

A growing body of evidence suggests that glutamate, the major excitatory neurotransmitter in the central nervous system, acts as a signaling molecule in peripheral tissues (Gill & Pulido, 2001; Skerry & Genever, 2001; Hinoi et al., 2004). In the cells of the endocrine pancreas, glutamate is stored in glucagon or insulin containing granules (Yamada et al., 2001; Hoy et al., 2002; Hayashi et al., 2003) and, once secreted, might act extracellularly to regulate hormone secretion (Moriyama &Hayashi, 2003; Uehara et al., 2004). In addition, glutamate has been implicated as a putative intracellular messenger coupling glucose metabolism to insulin secretion in cells (Maechler et al., 2002; Becerril Ángeles et al., 2010). The molecular mechanisms underlying the action of glutamate in the endocrine pancreas are only partially elucidated. The role of L-glutamate in insulin secretion has been robustly challenged (MacDonald & Fahien, 2000; Bertr et al., 2002). An increase in intracellular L-glutamate concentration on addition of glucose (16.7 mmol/l) in rat islets was not observed in a key study (MacDonald & Fahien, 2000). Incubation with L glutamine (10 mmol/l) increased the L-glutamate concentration 10-fold but did not stimulate insulin release, leading the authors to cast doubt on the proposed role of L-glutamate. In a separate study, it was demonstrated that, on incubation with glucose, a significant increase in L-glutamate concentration occurred in depolarized mouse and rat islets (Bertr et al., 2002).
Glutamate Receptors

Glutamate is the most prominent neurotransmitter in the body, being present in over 50% of nervous tissue. A large proportion of the glutamate present in the brain is produced by astrocytes through synthesis de novo (Hertz et al., 1999), but levels of glutamate in glial cells are lower than in neurons, 2–3 mM and 5–6 mM, respectively. During excitatory neurotransmission, glutamate-filled vesicles are docked at a specialized region of the presynaptic plasma membrane known as the active zone. Packaging and storage of glutamate into glutamatergic neuronal vesicles requires Mg²⁺/ATP-dependent vesicular glutamate uptake systems, which utilize an electrochemical proton gradient as a driving force. Substances that disturb the electrochemical gradient inhibit this glutamate uptake into vesicles. The concentration of glutamate in vesicle reaches as high as 20–100 mM (Nicholls & Attwell, 1990). In brain tissue, low concentrations of glutamate and aspartate perform as neurotransmitters, but at high concentration these amino acids act as neurotoxins.

Glutamate receptors are divided into two main groups: the fast-acting ligand-gated ionotropic channels and the slower-acting metabotropic receptors. The ionotropic receptors are cation-specific ion channels, and are subdivided into three groups: α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), kainite (KA) and N-methyl-D-aspartate (NMDA) receptor channels. Metabotropic glutamate (mGlu) receptors are G-protein coupled receptors (GPCRs) that have been subdivided into three groups, based on sequence similarity, pharmacology and intracellular signaling mechanisms. Group I mGlu receptors are coupled to PLC and intracellular calcium signaling, while group II and group III receptors are negatively coupled to adenylyl cyclase. Activation of these receptors is responsible for basal excitatory synaptic transmission and many forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD), which are thought to underlie learning and memory. It appears however, that aspartate aminotransferase and glutaminase account for a majority of glutamate production in brain tissue (McGeer et al., 1987).
Glutamate functions as a fast excitatory transmitter in the mammalian brain. Glutamate triggers neuronal death when released in excessive concentrations by over excitation of its receptors (Vizi, 2000). Glutamate receptor activation and excitotoxicity has long been recognized as an upstream event in this cascade (Wieloch, 1985). In brain, glutamate accumulation is reported to cause neuronal degeneration (Berman & Murray, 1996; Budd & Nicholas, 1996; Atlante et al., 1997). The excitatory amino acid glutamate is the most prevalent transmitter in the brain; its effect on postsynaptic receptors is limited by uptake process (Erecinska, 1997) and by diffusion of glutamate from the cleft. The cellular uptake of Glu is driven by the electrochemical gradients of Na\(^+\) and K\(^+\) and is accompanied by voltage and pH changes. In nervous tissue, glutamate dehydrogenase (GDH) appears to function in both the synthesis and the catabolism of glutamate and perhaps in ammonia detoxification (Mavrothalassitis et al., 1988). The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA kainite (Choi, 1988). Hypoglycemia is associated with increased glutamate release (Sandberg et al., 1986; Becerril Ángeles et al., 2010) and conversely, glutamate toxicity is augmented by hypoglycemia (Novelli et al., 1988).

The majority of excitatory synapses are glutamatergic, in which glutamate transmits the signal through postsynaptic ionotropic NMDA, AMPA, and KA and metabotropic receptors (Bettler & Mulle, 1995). Glutamate is a fast excitatory transmitter in the CNS and has been shown, with GABA, to interact primarily with receptors in the synaptic cleft (Dingledine et al., 1999). Studies have shown that both ionotropic glutamate receptors and glutamate transporters are involved in oxygen-glucose deprivation-induced necrotic cell death in hippocampal slice cultures (Bonde et al., 2005). The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA–kainate (Choi, 1988). The presence of G protein-coupled glutamate receptors has been described and since 1991 (Conn & Pin, 1997), eight receptors
have been discovered and classified into three groups based on their linkage to second messenger systems and their pharmacology: group I acts via the phosphoinositol system and groups II and III inhibit adenylyl cyclase. In addition, the stimulation of receptors of these three groups directly influences voltage-gated Ca\(^{2+}\) and K\(^+\) channels through their G proteins, but their physiological correlate has not yet defined (Rondón et al., 2010).

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

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

**NMDA receptors**

NMDA receptors are excitatory receptors in neurons that play a fundamental role in neuronal development, synaptic transmission, and synaptic plasticity. The discovery of potent and selective agonists and antagonists has resulted in extensive information on the NMDA receptor-channel complex (Wood et al., 1990). It consists of four domains: (1) the transmitter recognition site with which NMDA and L-glutamate interact; (2) a cation binding site located inside the channel where Mg$^{2+}$ can bind and block transmembrane ion fluxes; (3) a PCP binding site that requires agonist binding to the transmitter recognition site, interacts with the cation binding site and at which a number of dissociative anesthetics PCP and ketamine, opiate N-allylnormetazocine (SKF-10047) and MK-801 bind and function as open channel blockers; and (4) a glycine binding site that appears to allosterically modulate the interaction between the transmitter recognition site and the PCP binding site. NMDA is allosterically modulated by glycine, a co-agonist whose presence is an absolute requirement for receptor activation (Russo et al., 2008).

NMDA receptors form heterotetrameric channels containing two obligatory glycine-binding NR1 subunits and two other subunits, either from the NR2 subfamily (2A, 2B, 2C, 2D) or the NR3 subfamily (3A or 3B) (Premkumar & Auerbach 1997; Laube et al., 1998; Furukawa et al., 2005; Ulbrich et al., 2007). The non-NR1 subunits confer onto the receptor distinct physiological properties that lead to a diversity of NMDA receptor function (Cull-Candy & Leszkiewicz 2004). NR2 subunits bind glutamate and form NR1/NR2 receptors that require
glycine and glutamate for activation (Johnson & Ascher 1987). NR3 subunits bind glycine and NR1/NR3 receptors are gated by glycine alone; however, excitatory glycine receptors have not yet been observed in neurons expressing the NR3 subunit (Chatterton et al., 2002, Yao & Mayer 2006; Ai et al., 2010). Molecular cloning has identified to date cDNAs encoding NR1 and NR2A, B, C, D subunits of the NMDA receptor, the deduced amino acid sequences of which are 18% belonging to NR1 and NR2, 55% belonging to NR2A and NR2C or 70% belonging to NR2A and NR2B are identical. Site-directed mutagenesis has revealed that the NR2 subunit carries the binding site for glutamate within the N-terminal domain and the extracellular loop between membrane segments M3 and M4; whereas the homologous domains of the NR1 subunit carry the binding site for the co-agonist glycine. It has been shown that the combination of NR1 with different NR2 subunits results in diverse electrophysiological and pharmacological responses. NR1 and NR2A are ubiquitous, NR2B occurs in the forebrain, NR2C in the cerebellum, with NR2D being the rarest. There is a binding place in the channel pore for Mg2+, and at resting membrane potential, Mg2+ is attached to this binding site, blocking ion flow through the channel (Cull-Candy et al., 2001, Kalia et al., 2008, Paoletti & Neyton, 2007)

During normal development, NMDA receptor-mediated calcium fluxes are necessary for the formation and plasticity of synaptic connections, but also to initiate apoptotic pathways that mediate physiologic synaptic pruning. Disorders at this level have been implicated with cognitive disabilities and mood disorders (Pilpel et al., 2009; Gardiner, 2010) Ongoing NMDA receptor signalling remains critical in adult, and malfunctions underlie many brain disorders: over activation of NMDA receptors can cause excitotoxic neurodegeneration; insufficient activation underlies some forms of psychoses and cognitive deficits; and anomalous plasticity has been implicated in several forms of addiction, neuropathic pain, and behavioral disorders (Pittenger et al., 2007; Kalivas et al., 2009). Normal functioning of the NMDA receptor complex depends on a dynamic
equilibrium among various domain components. Loss of equilibrium during membrane perturbation causes the entire system to malfunction and result in abnormal levels of glutamate in the synaptic cleft (Olney, 1989). An important consequence of NMDA receptor activation is the influx of Ca$^{2+}$ into neurons (MacDermott et al., 1986; Murphy & Miller, 1988; Holopainen et al., 1989, 1990). Collective evidence suggests that when the membrane is depolarized, the Mg$^{2+}$ block is relieved and the receptor can be activated by glutamate. Activation of the NMDA receptor therefore requires the association of two synaptic events: membrane depolarization and glutamate release. This associative property provides the logic for the role of the NMDA receptor in sensory integration, memory function, coordination and programming of motor activity (Collingridge & Bliss, 1987; Lam et al., 2010) associated with synaptogenesis and synaptic plasticity.

The NMDA channel is blocked in a use and voltage dependent manner by Mg$^{2+}$. This means that NMDA receptors are activated only after depolarization of the postsynaptic membrane by, for example, AMPA receptor activation, which relieves the voltage dependent blockade by Mg$^{2+}$. This biophysical property and their high Ca$^{2+}$ permeability render NMDA receptors inherently suitable for their role in mediating synaptic plasticity underlying learning processes and development (Collingridge & Singer, 1990; Danysz et al., 1995; Russo et al., 2008). Similar to Mg$^{2+}$, uncompetitive NMDA receptor antagonists such as ketamine, dextromethorphan, memantine, phencyclidine (PCP) and (+) MK-801 [(+)-5-methyl-10, 11-dihydro-5H-dibenzocyclohepten-5, 10-imine maleate] block the NMDA channel in the open state, although the blocking kinetics and voltage of this effect depend on the antagonist (Rogawski, 1993; Parsons et al., 1998b).

To date, two major subunit families, designated NR1 and NR2, have been cloned. Various heteromeric NMDA receptor channels formed by combinations of NR1 and NR2 subunits are known to differ in gating properties, magnesium sensitivity, and pharmacological profile (Sucher et al., 1996; Parsons et al.,
The heteromeric assembly of NR1 and NR2C subunits, for instance, has much lower sensitivity to Mg$^{2+}$ but increased sensitivity to glycine and very restricted distribution in the brain (Lam et al., 2010). In situ hybridization has revealed overlapping but different expression profiles for NR2 mRNA. For example, NR2A mRNA is distributed ubiquitously like NR1, with the highest densities occurring in hippocampal regions and NR2B is expressed predominantly in forebrain but not in cerebellum, where NR2C predominates; NR2D is localized mainly in the brainstem (Moriyoshi et al., 1991; Monyer et al., 1992; Nakanishi, 1992; McBain & Mayer, 1994).

In addition to NR1 and NR2, the NR3A subunit has recently been discovered. This receptor subunit, previously termed chi-1, or NMDAR-L, is a relatively recently identified member of a new class in the ionotropic glutamate receptor family. It attenuates NMDA receptor currents when co-expressed with NR1/NR2 subunits in Xenopus oocytes but has no effect when tested with non-NMDA receptors or when expressed alone (Ciabarra et al., 1995; Sucher et al., 1995; Das et al., 1998; Lam et al., 2010). Highest levels are present in the spinal cord, brainstem, hypothalamus, thalamus, CA1 field of the hippocampus and amygdala and this distribution remains the same throughout life. Genetic knockout of NR3A in mice results in enhanced NMDA responses and increased dendritic spines in early postnatal cortical neurons, suggesting that NR3A is involved in the development of synaptic elements by modulating NMDA receptor activity (Das et al., 1998).

**AMPA receptors**

AMPA receptors are also widely expressed in the mammalian CNS and mediate fast excitatory neurotransmission in response to glutamate binding (Palmer et al., 2005). The cloning of the first AMPA receptor subunit in 1989 enabled the structural analysis of AMPA receptors and a detailed characterization of their physiology and pharmacology (Hollmann et al., 1989; Ai et al., 2010). AMPA receptor composed of different combinations of GluR1, GluR2, GluR3,
and GluR4 subunits. These subunits have a modular organization (Mayer, 2006; Sobolevsky et al., 2009). There is a large extracellular amino terminal domain that is involved in receptor assembly. All AMPA receptors are tetrameric combinations of the four subunits. While homomeric receptors are functional, native AMPA receptors are believed to be heteromers. Upon forming a tetrameric complex of GluR1-4s, AMPA receptors mediate fast excitatory neurotransmission that can be blocked by specific quinoxalinediones including 6-nitro-7-sulphamobezo (f) quinoxaline-2, 3-dione (NBQX), a potent and selective AMPA receptor antagonist. The AMPA receptors are permeable to Na\(^+\) and K\(^+\) but their Ca\(^{2+}\) permeability is variable. GluR2 subunit of AMPARs undergoes RNA editing at Q/R site in second transmembrane domain replacing glutamine to arginine during post transcriptional modification (Van Den Bosch et al., 2000; Van Damme et al., 2002; Duncan, 2009). GluR2 subunit is considered as the determinant of Ca\(^{2+}\) permeability of AMPA receptors (Van Damme et al., 2002; Kawahara & Kwak, 2005; Duncan, 2009). It was suggested that the lack of GluR2 subunits or unedited form of GluR2 makes the AMPA receptors permeable to Ca\(^{2+}\) ions. Some earlier studies have reported that the motor neurons possess unedited form of GluR2 or completely lack GluR2 subunit which makes the AMPA receptor highly permeable to Ca\(^{2+}\) ions thus increasing their vulnerability to excitotoxicity (Williams et al., 1997; Bar-Peled et al., 1999). In the mature hippocampus, most AMPARs are composed of GluR1–GluR2 or GluR2–GluR3 combinations (Wenthold et al., 1996), whereas GluR4-containing AMPARs are expressed mainly in early postnatal development (Zhu et al., 2000).

The release of even small and brief concentrations of glutamate into the synaptic cleft generates robust excitatory postsynaptic potentials (EPSPs). AMPA-mediated currents generate a fast upstroke and rapid current decay while NMDA-receptor activation provides a more prolonged phase of depolarization that can last several hundred milliseconds. EPSP generation is hypothesized to be controlled by AMPA receptor de/activation while the longer pharmacokinetics of NMDA
receptor sensitization provides ample opportunity for spatial and temporal summation at numerous postsynaptic inputs. The higher affinity of glutamate for NMDA-to-AMPA receptors likely explains these pharmacokinetic differences, as prolonged receptor activation is often the result of slower dissociation of agonist and receptor (Ai et al., 2010).

AMPA receptor trafficking has been widely studied, especially its intracellular cycling and its potential physiological sequelae. Like all membrane receptors, AMPA receptors are synthesized in the soma and transported to the cell surface via the secretory pathway involving multiple membrane sorting steps and cytoskeleton transport proteins (Kennedy & Ehlers, 2006; Kapitein et al., 2010). Dendritic AMPA receptor localization to synapses is regulated via two mechanisms: (1) exocytic and endocytic trafficking and recycling, respectively, in the secretory pathway and (2) membrane diffusion from extra-synaptic to synaptic localizations (Groc & Choquet, 2006; Newpher & Ehlers, 2008; Wang et al., 2008; Hoogenraad et al., 2010).

A physiological role for AMPA receptor trafficking and surface diffusion has been hypothesized in learning and memory. An LTP-like strengthening of neocortical synapses occurs after sensory stimulation in vivo (Holtmaat & Svoboda, 2009; Kessels & Malinow, 2009), and this process appears dependent on AMPA receptor number, localization and facilitation at synapses (Takahashi et al., 2003). In the brain, soon after birth, most excitatory synapses in the hippocampus (Hsia et al., 1998; Petralia et al., 1999) and other brain regions (Wu et al., 1996; Isaac et al., 1997; Losi et al., 2002) contain only NMDARs, whereas the prevalence of AMPARs increases gradually over the course of postnatal development. In fact, the delivery of AMPARs into synapses is a regulated process that depends on NMDAR activation and underlies some forms of synaptic plasticity in early postnatal development (Zhu et al., 2000) and in mature neurons (Sheng, & Lee 2001; Song, & Huganir 2002). Synaptic plasticity is thought to underlie higher cognitive functions, such as learning and memory (Elgersma et
al., 1999, Martin et al., 2000; Ai et al., 2010), and is also critical for neural development (Cline et al., 1998). Learning in the hippocampus also appears to be regulated by AMPA receptor dynamics (Whitlock et al., 2006) as evidenced by the recruitment of AMPA receptors to mushroom shaped dendritic spines in the CA1 region of the hippocampus 24 h after fear conditioning (Matsuo et al., 2008). Stress hormones have recently been recognized to play a role in AMPA receptor trafficking (Groc et al., 2008; Krugers et al., 2010; Yuen et al., 2011), and may provide a mechanism for the dose dependent facilitative and suppressive effects of corticosteroid hormones on synaptic plasticity and cognition (Martin et al., 2009). Further complexity in the regulation of ionotropic glutamatergic neurotransmission is provided by molecular variability at the transcriptional and post-transcriptional level. RNA editing of AMPA and kainate receptor subunits (Higuchi et al., 1993) and alternative splicing of mRNA transcripts (Sommer et al., 1990) modulate second messenger cascades critical for downstream intracellular effects.

**Glutamate receptors in pancreas**

Large evidence shows that pancreatic islets cells, particularly insulin secreting β cells, share with neurones common characteristics; they contain tyrosine hydroxylase (Teitelman & Lee, 1987), neurone specific enolase (Polak et al., 1984), Go protein (Terashima et al., 1987), glutamic acid decarboxylase, high levels of GABA (Okada, 1986) as well as synaptic-like microvesicles (Reetz et al., 1991). Coexpression of kainate KA1 or KA2 receptor subunits with GluR5–7 has not been observed in these cells, which suggests that the expression of functional kainate receptors is negligible (Morley et al., 2000; Molnar et al., 1995; Inagaki et al., 1995). Some laboratories have detected the expression of NMDA receptors in islet cells (Molnar et al., 1995; Inagaki et al., 1995; Ai et al., 2010) although neither NMDA evoked ion transport nor coexpression of NR1 and NR2A–D receptor subunits has been observed by other laboratories (Weaver et al., 1996; Morley et al., 2000) AMPA and kainate, agonists of AMPA receptors, each
stimulate insulin secretion from perfused or isolated islets or clonal islet cells in the presence of high levels of glucose (Bertrand et al., 1992, 1995).

The differential distribution of the GluR subunits in the pancreas has already been described (Molnar et al., 1995; Weaver et al., 1996). Liu et al., (1997) showed that GluR 1 and GluR 4 were mainly localized to insulin-secreting cells in the central mass of the pancreatic islet, it appears that insulin and non–insulin-secreting cells express different AMPA receptor subunits, which may be used to mediate their hormone secretion, as was suggested earlier by Bertrand et al., (1992, 1993). Weaver et al., (1996) reported that the AMPA receptors were located in the, α, β and PP cells but were generally absent from the δ cells, whereas kainate receptors were expressed in α and δ cells although they were not found in β or PP cells. These observations add to the evidence that these receptors may be involved in the regulation of hormone secretion (Barb et al., 1996; Cho et al., 2010). Studies of Weaver et al., (1996) show that glutamate depolarizes islet cells when glutamate serum levels are elevated. Intracellular Ca$^{2+}$ measurements and electrophysiological recordings showed that kainate, AMPA and NMDA elicited increases in Ca$^{2+}$ in single β-pancreatic cells and depolarized them. In addition, kainate and AMPA stimulated the release of insulin whereas NMDA did not (Hollmann & Heinemann, 1994; Rondón et al., 2010). This stimulatory effect was dependent on the glucose concentration: Glutamate stimulated insulin release in the presence of a glucose concentration of 8.3 mM but not in the presence of a low concentration (2.8 mM).

**Glutamate transporters**

Neuronal and glial glutamate transporters in the mammalian central nervous system remove the neurotransmitter glutamate from the synaptic cleft. After the release glutamate has been released to the synaptic cleft, it can be either inactivated by enzymatic degradation or transport back to the neuron or back to the glial cells by active transport. According to the structure and site of action, glutamate transporters as other neurotransmitter transporters, can be divided in to
two sub families: the plasma membrane transporters (EAATs) and vesicular transporters (VGLUTs) (Li et al., 2005). Excitatory amino acid transporters (EAAT), formerly known as glutamate transporters, belongs to the family of neurotransmitter transporters. The removal of glutamate from the extracellular milieu is achieved by a family of excitatory amino acid transporters 1 to 5 (EAAT1 to 5) that are localized in glial cells and neurons (Rothstein et al., 1994; Chaudhry et al., 1995). They serve to terminate the excitatory neurotransmitter signal by removal of glutamate from the neuronal synapse into glia cells. To date, 5 high-affinity, sodium-dependent glutamate transporters have been cloned from mammalian and human tissue: astrocyte-specific glutamate transporter (GLAST [excitatory amino acid transporter 1 (EAAT1)]), glutamate transporter 1 (GLT-1 [excitatory amino acid transporter 2 (EAAT2)]), excitatory amino acid carrier 1 (EAAC1 [excitatory amino acid transporter 3 (EAAT3)]), excitatory amino acid transporter 4 (EAAT4), and excitatory amino acid transporter 5 (EAAT5) (Arriza et al., 1994, Fairman et al., 1995). Immunohistochemical studies have revealed that EAAT 1 and EAAT 2 are localized primarily in astrocytes (Lehre et al., 1995), while EAAT 3 and EAAT 4 are distributed in neuronal membranes (Kanai & Hediger 1992; Anderson & Swanson, 2000; Li et al., 2005). The neuronal transporters EAAT3 and EAAT4 appear to be localized to plasma membranes in a perisynaptic distribution. The greatest density of these transporter proteins appears to be at the edge of postsynaptic densities, rather than within the synaptic cleft. EAAT5 is only found in the retina where it is principally localised to photoreceptors and bipolar neurons in the retina (Pow & Barnett, 2000; Hawkins, 2009). In rodents, the orthologs for EAAT1-3 are named GLAST, GLT1 and EAAC1 respectively (Shigeri et al., 2004).

EAATs are membrane bound pumps that resemble ion channels (Ganel & Rothstein, 1999). The uptake of one glutamate molecule is coupled to the uptake of three Na\(^+\) ions and one proton, and the extrusion of one K\(^+\) ion (Zerangue & Kavanaugh, 1996; Levy et al., 1998; Hawkins, 2009). The large electrochemical
gradients for Na\(^+\) and K\(^+\) provide the driving force for glutamate uptake against its gradient (Zerangue & Kavanaugh, 1996). The proton has been hypothesized to play a neuroprotective role during ischemia by shutting down reverse uptake of glutamate (Billups et al., 1996), but the exact mechanism by which the proton reduces reverse transport is unclear.

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

**Oxidative stress and diabetes**

Oxidative stress and oxidative damage to the tissue are common end points of chronic diseases, such as atherosclerosis, diabetes and rheumatoid arthritis (John et al., 1999). Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications (Moussa 2008). During diabetes, persistent hyperglycemia causes increased production of free radicals, especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation. The increase in the level of ROS in diabetes could be due to their increased production and/or decreased destruction by nonenzymic and
enzymic catalase (CAT), reduced glutathione (GSH), and superoxide dismutase (SOD) antioxidants. The level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes (Lipinski, 2001). Oxidative damage to various brain regions constitutes into the long term complications, morphological abnormalities and memory impairments (Fukui et al., 2001). In the study of Ha & Lee, 2000 it was shown that oxidative stress is one of the important mediators of vascular complications in diabetes including nephropathy. In the central nervous system, oxidative stress signifies an important pathway that leads to the damage of both neuronal and vascular cells (Root-Bernstein et al., 2002).

Oxidants are generated as a result of normal intracellular metabolism in mitochondria and peroxisomes, as well as from a variety of cytosolic enzyme systems. In addition, a number of external agents can trigger ROS production. A sophisticated enzymatic and nonenzymatic antioxidant defense system including catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) counteracts and regulates overall ROS levels to maintain physiological homeostasis. Lowering ROS levels below the homeostatic set point may interrupt the physiological role of oxidants in cellular proliferation and host defence. Similarly, increased ROS may also be detrimental and lead to cell death or to acceleration in ageing and age-related diseases. Because of their ability to directly oxidize and damage DNA, protein, and lipid, ROS are believed to play a key direct role in the pathogenesis of late diabetic complications (Rosen et al., 2001). In addition to these effects, a rise in ROS levels may also constitute a stress signal that activates specific redoxsensitive signalling pathways. Once activated, these diverse signalling pathways may have either damaging or potentially protective functions (Toren & Nikki, 2000). The primary ROS produced in the course of oxygen metabolism is superoxide, which is a highly reactive, cytotoxic ROS. Superoxide is dismutated to a far less reactive product, hydrogen peroxide (H$_2$O$_2$), by a family of metalloenzymes known as SOD (Brownlee, 2001). The ubiquitous
SOD catalyzes the disproportionation of superoxide to molecular oxygen and peroxide and thus is critical for protecting the cell against the toxic products of aerobic respiration.

Recently, pancreatic β-cells emerged as a target of oxidative stress-mediated tissue damage (Evans et al., 2003; Drews et al., 2010). Extracellular hyperglycemia causes intracellular hyperglycemia in β-cells, leading to the induction of ROS in pancreatic islets of diabetic animals. Indeed, it was shown that expression of oxidative stress markers such as 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 4-hydroxy-2, 3-nonenal (4-HNE) are increased in islets under diabetic conditions (Gorogawa et al., 2002). In addition, due to the relatively low expression of antioxidant enzymes such as catalase and glutathione peroxidase (Tiedge et al., 1997) β-cells are rather vulnerable to oxidative stress. Thus, it is likely that oxidative stress plays a major role in β-cell deterioration in type 2 diabetes. There are several sources of ROS productions in cells: the non-enzymatic glycosylation reaction (Matsuoka et al., 1997).

Hyperglycemia, defining established diabetes, can induce oxidative stress by various mechanisms; excessive levels of glucose reaching the mitochondria lead to an overdrive of the electron transport chain, resulting in overproduction of superoxide anions normally scavenged by mitochondrial SOD. When the latter fails oxidative stress develops and it was recently proposed that this mechanism is responsible for the activation of all major pathways underlying the different components of vascular diabetic complications (glycation, PKC activation, sorbitol pathway) (Nishikawa et al., 2000). The in vitro supplementation of SOD like drugs corrected most of these defects, supporting the importance of these mechanisms (Yamagishi et al., 2001; Recchioni et al., 2002). It has also been proposed that uncoupling mitochondrial NOS by hyperglycemia would be involved (Brodsky et al., 2002).
Glutamate mediated excitotoxic cell death

Glutamate is the principal excitatory neurotransmitter in the CNS, but it is also a potent neurotoxin that can kill nerve cells. Excessive glutamate signaling can lead to excitotoxicity, a phenomenon whereby overactivation of glutamate receptors (GluRs) initiates cell demise. Excitotoxic cell death was first described during the late 1950s in retinal neurons (Lucas & Newhouse, 1957; Carozzi & Ceresa, 2012) and was found later to occur in virtually all neurons that express GluRs (Olney et al., 1969). Thereafter, glutamate excitotoxicity has been implicated in acute injury to the CNS and in chronic neurodegenerative disorders (Choi, 1988; Lee et al., 1999). Activation of NMDA, AMPA, kainate and metabotropic receptor subtypes by glutamate (Paoletti, 2011), the most ubiquitous cerebral neurotransmitter, leads to an increase in the levels of free intracellular calcium (Coyle & Puttfarcken, 1993; Pivovarova & Andrews, 2011). Such events can cause prolonged depolarization and subsequent ionic imbalance, ATP depletion and increases in intracellular free calcium levels that together culminate in cerebral edema, raised intracranial pressure (ICP), vascular compression and brain herniation, an often fatal complication of severe head injury (Lau & Tymianski, 2010).

Over activation at NMDA receptors triggers an excessive entry of Ca\textsuperscript{2+}, initiating a series of cytoplasmic and nuclear processes that promote neuronal cell death. For instance, Ca\textsuperscript{2+} activated proteolytic enzymes, like calpains, can degrade essential proteins (Dong et al., 2009). Neuronal activity can lead to a marked increase in the concentration of cytosolic Ca\textsuperscript{2+}, which then functions as a second messenger that mediates a wide range of cellular responses (Blaustein 1988; Tymianski & Tator 1996; Mallick et al., 2007). Excessive influx of extracellular Ca\textsuperscript{2+} together with any Ca\textsuperscript{2+} release triggered from intracellular stores can elevate neuronal cytosolic free Ca\textsuperscript{2+} concentrations to levels that exceed the capacity of intracellular Ca\textsuperscript{2+} regulatory mechanisms and can lead to metabolic derangements such as the formation of free radicals and cell death (Choi, 1988; Sattler &
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Tymianski, 2000). Although cellular Ca\(^{2+}\) overload is unlikely to be a common pathway mediating all forms of neuronal cell death, several lines of evidence support a close relationship between excessive Ca\(^{2+}\) influx and neuronal injury in the adult mammalian nervous system (Choi 1988; Tymianski & Tator, 1996; Carozzi & Ceresa, 2012).

AMPA receptors are generally heteromers of subunits encoded by four genes, GluR1-4 (GluR-A-D) and exhibit a higher affinity to AMPA and glutamate compared to kainite (Hollmann & Heinemann 1994). The mRNAs for GluR1, 2, and 3 are expressed broadly throughout the CNS, whereas GluR4 shows a more restricted spatial and temporal expression pattern (Monyer et al., 1991). AMPA receptor subunits have four hydrophobic membrane domains with an extracellular N-terminal domain and a cytoplasmically disposed C-terminal tail (Hollmann & Heinemann 1994). Due to its influence on receptor Ca\(^{2+}\) permeability, the GluR2(R) subunit has attracted significant interest in studies of excitotoxicity. GluR2 mRNA is widely expressed in mammalian neurons that are highly vulnerable to excitotoxic damage, such as hippocampal pyramidal and granule neurons as well as cortical neurons (Kondo et al., 1997), rendering the majority of these AMPA receptors Ca\(^{2+}\) impermeable. Despite this generally low Ca\(^{2+}\) permeability, AMPA receptor toxicity is likely to be, at least in part, mediated by Ca\(^{2+}\) ions. Neurons expressing Ca\(^{2+}\) permeable AMPA gated channels can be identified by kainite induced Ca\(^{2+}\) uptake and have been shown to be present in many regions of the brain, though low levels (8–15%) (Turetsky et al., 1994; Sattler et al., 1998; Carozzi & Ceresa, 2012). This subpopulation of neurons was selectively destroyed in a Ca\(^{2+}\) dependent manner after AMPA or kainite exposures (Turetsky et al., 1994). In addition, Brorson et al., (1994) reported that Ca\(^{2+}\) entry via Ca\(^{2+}\) permeable AMPA/KA receptors was sufficient to induce excitotoxicity in cerebellar Purkinje cells. Surprisingly, GluR2 containing AMPA receptors do show low Ca\(^{2+}\) and other divalent cation permeability, especially in
cells expressing low levels of GluR2 relative to other AMPA receptor subunits (Jonas et al., 1994; Geiger et al., 1995; Mallick et al., 2007).

In the retina, glutamate is the primary excitatory transmitter in the vertical pathway from photoreceptors to ganglion cells (Lucas & Newhouse, 1957; Choi, 1988; Ng et al., 2004). Several recent studies have shown that a significant increase in glutamate in the retina is associated with the development of diabetic retinopathy (DR), a disease characterized by neurodegeneration and vasculopathy (Li & Puro, 2002; Diederen et al., 2006). Although the detailed mechanism remains unknown, it may be of critical importance for neuroprotection to remove excess glutamate from the extracellular space in the retina.

**Diabetes and apoptosis**

Apoptosis is a coordinated series of events for the programmed execution of cell death, and plays an important role in the maintenance of tissue homeostasis. A host of physical, chemical and biological factors can trigger apoptotic death by activating complex yet tightly controlled intracellular signal transduction pathways. The extrinsic pathway is activated upon ligation of the cell surface death receptors, which in turn activates downstream effector mechanisms orchestrated by the caspase family of cysteine proteases (Green, 2004).

Cell death can occur by necrosis or apoptosis, with these two mechanisms having distinct histological and biochemical markers (Kanduc et al., 2002; Ueda et al., 2004). In contrast to necrosis, apoptosis involves a cascade of intracellular events that ultimately culminates in cell destruction (Green & Droemer, 2004). This process involves caspases, cysteine-dependent, aspartate-specific proteases, that exist in an inactivated state that, when activated, initiate the death program. The intrinsic apoptotic pathway can be initiated by external signals or internal changes, such as release of cytochrome $c$ from mitochondria; indeed, mitochondrion is a very important component of this cascade (Hengartner et al., 2000). Release of apoptogenic factors from mitochondria can be induced by
distinct factors, including members of the Bcl-2 protein family. The balance between proapoptotic and antiapoptotic members of this family has a crucial role in determining the integrity of the mitochondria and, hence, cell death (Kanduc et al., 2002). The extrinsic cell-death pathway involves activation of extracellular death receptors, which belong to the TNF receptor superfamily (Wajant, 2003). Binding of the appropriate ligand to one of these receptors results in receptor aggregation and recruitment of FADD (Fas-associated death domain) and procaspase 8 (also called FLICE or MACH-1). Procaspase 8 can then be activated by self-cleavage or cleavage by another caspase 8 molecule (Kaufmann & Earnshaw, 2000). Activated caspase 8, functioning as an initiator caspase, activates downstream executioner caspases that cleave cell death substrates or directly induces apoptosis (Muzio et al., 1998). Caspases have a pivotal role in the progression of a variety of neurologic disorders. Caspase 8 is the apical protease in the extrinsic apoptotic pathway activated at the plasma membrane by various TNF family death receptors (Ashkenazi & Dixit, 1998; Su et al., 2012).

Caspases are evolutionarily conserved cysteine-aspartyl specific proteases that play a key role in apoptosis. In mammals, there are over 14 caspases, of which some are involved in apoptosis and others in cytokine activation (Woo et al., 2000; Creagh et al., 2003; Shi, 2004). The processes that lead to diabetic embryopathy in the embryos of diabetic mothers are not well understood. However, it is clear that caspase-3 and 6 activity and Bax levels are increased, implying alterations in the apoptotic processes (Gareskog et al., 2007; Yang et al., 2008). However, knowledge is limited regarding the processes that account for this increased apoptosis. As a key upstream regulator, caspase 8 is also activated by maternal diabetes (Toder et al., 2002).

Oxidative stress has been cited as another critical mediator of cell death, and may either trigger or modulate apoptosis (Oyarzún et al., 2011). A role for oxidative stress in apoptosis has been shaped by several independent observations. For many years, direct treatment of cells with oxidants such as hydrogen peroxide
or redoxactive quinones was thought to exclusively cause necrosis, but more recent studies have shown that lower doses of these agents can trigger apoptosis (Hampton & Orrenius, 1997).

**Inositol 1, 4, 5-trisphosphate (IP3)**

IP3 is a ubiquitous second messenger that functions by binding to receptors (IP3Rs) on the ER membrane to cause liberation of sequestered Ca\(^{2+}\) (Berridge, 1997, Foskett *et al.*, 2007). The resultant cytosolic Ca\(^{2+}\) transients serve numerous signaling functions in neurons, including modulation of membrane excitability synaptic plasticity and gene expression (Fujii *et al.*, 2000; Yamamoto *et al.*, 2002; Stutzmann *et al.*, 2003). Many biological stimuli, such as neurotransmitters, hormones and growth factors, activate the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) in the plasma membrane which is hydrolyzed by phospholipase C (PLC) to produce IP3 and diacylglycerol (DAG). The IP3 mediates Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores by binding to IP3R. IP3R are the IP3 gated intracellular Ca\(^{2+}\) channels that are mainly present in the endoplasmic reticulum (ER) membrane. The IP3 induced Ca\(^{2+}\) signaling plays a crucial role in the control of diverse physiological processes such as contraction, secretion, gene expression and synaptic plasticity (Berridge, 1993). In mammalian cells, there are three IP3R subtypes- IP3R1, IP3R2 and IP3R3 which are expressed to varying degrees in individual cell types (Wojcikiewicz, 1995; Taylor *et al.*, 2002) and form homotetrameric or heterotetrameric channels (Bosanac *et al.*, 2004).

Group I mGluRs (mGluR1/5 subtypes) are also demonstrated to mainly affect intracellular Ca\(^{2+}\) mobilization (Bordi & Ugolini, 1999). To sequentially facilitate intracellular Ca\(^{2+}\) release, group I receptors activate the membrane bound phospholipase C (PLC), which stimulates phosphoinositide turnover by hydrolyzing PIP2 to IP3 and diacylglycerol. IP3 then causes the release of Ca\(^{2+}\) from intracellular Ca\(^{2+}\) stores (such as endoplasmic reticulum) by binding to
specific IP3 receptors on the membrane of Ca\textsuperscript{2+} stores. Altered Ca\textsuperscript{2+} levels could then engage in the modulation of broad cellular activities. The neuronal intracellular calcium has an important role in the regulation of synaptic plasticity (Barbara, 2002). Moreover, disruptions in this pathway are implicated in neurodegenerative disorders (Mattson et al., 2000; LaFerla, 2002). Therefore, factors that modulate or disrupt IP3 mediated Ca\textsuperscript{2+} signaling are expected to exert powerful physiological and possibly pathological effects on the nervous system.

Insulin secretion is largely a Ca\textsuperscript{2+} dependent process and restricted increases in intracellular Ca\textsuperscript{2+} have been related to impairment of glucose-stimulated insulin release (Boschero et al., 1990). Several lines of evidence point to IP3 playing an important role in insulin secretion. IP3 was shown to mobilize intracellular Ca\textsuperscript{2+} in permeabilized insulin-secreting cells and IP3 production correlated with Ca\textsuperscript{2+} mobilization in intact cells (Biden et al., 1984; Gromada et al., 1996). In the mouse anx7 (1/2) phenotype, where there is a profound reduction in IP3R expression and Ca\textsuperscript{2+} mobilization in islets, there is also defective insulin secretion (Srivastava et al., 1999; Ye et al., 2011).

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

Cytosolic Ca\textsuperscript{2+} is a focal point of many signal transduction pathways and modulates a diverse array of cellular activities ranging from fertilization to cell death (Berridge et al., 2000). In most cell types, the major internal [Ca\textsuperscript{2+}] stores are the endoplasmic reticulum/sarcoplasmic reticulum (ER/SR). One mechanism for mobilizing such stores involves the phosphoinositide pathway. The binding of many hormones to specific receptors on the plasma membrane leads to the activation of an enzyme (phosphoinositidase C) that catalyses the hydrolysis of phospholipids to produce the intracellular messenger inositol 1,4,5-trisphosphate (IP3) (Luciani et al., 2009). Although derived from a lipid, IP3 is water soluble and diffuses into the cell interior where it encounters IP3 receptors (IP3Rs) on the ER/SR. The binding of IP3 changes the conformation of IP3Rs such that an
integral channel is opened, thus allowing the Ca\textsuperscript{2+} stored at high concentrations in the ER/SR to enter the cytoplasm (Ye et al., 2011). A critical feature of IP3Rs is that their opening is regulated by the cytosolic Ca\textsuperscript{2+} concentration. This sensitivity to cytosolic Ca\textsuperscript{2+} allows them to act as Ca\textsuperscript{2+} induced calcium release channels that promote the rapid amplification of smaller trigger events.

**Curcumin**

Curcumin is the principal curcuminoid of the popular Indian spice turmeric. Turmeric is a spice which is obtained from rhizomes of plant *Curcuma longa*, which is a member of the ginger family (Zingiberaceae). *C. longa* 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. The origin of the plant is not certain, but it is thought to be originated from south eastern Asia, most probably from India. The plant is cultivated in all parts of India (Kapoor, 2000). India produces most of the world supply (Leung & Foster, 1996), but turmeric is cultivated also in southern China, Taiwan, Japan, Burma, and Indonesia (Yen, 1992) as well as throughout the African continent (Iwu, 1993; Stefanska, 2012). The commercially available material (i.e. turmeric powder) in Europe is obtained mainly from India and somewhat from other south eastern Asian countries (Murugananthi et al., 2008). *C.longa* 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 injury. 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., 1991; Meng et al., 2012).

Turmeric is one of most essential spices all over the world with a long and distinguished human use particularly in the Eastern civilization (Ravindran, 2007). The other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin. The curcuminoids are polyphenols and are responsible for
the yellow colour of turmeric. Turmeric consists of 3-5% curcuminoids. Curcumin is the most important fraction which is responsible for the biological activities of turmeric. The melting point of curcumin is 184°C. It is soluble in ethanol and acetone, but insoluble in water (Joe et al., 2004). Curcumin exists in solution as ketoenol tautomers (Payton et al., 2007). Because of its biological activities, a large number of studies have been presented on curcumin. According to these studies, curcumin exhibits antiinflammatory (Chainani-Wu, 2003; Stefanska, 2012) antioxidant (Masuda et al., 1993, Cohly et al., 1998) anticarcinogenic (Frank et al., 2003) antiviral (Suai et al., 1993) antimicrobial activity (Mahady et al., 2002, Han & Yang 2005) beside these, curcumin has a variety of potentially therapeutic properties, such as antineoplastic, antiapoptotic, antiangiogenic, cytotoxic, immunomodulatory, (Strimpakos & Sharma, 2008; Meng et al., 2012) and antithrombotic, wound healing, antidiabetogenic, antistressor and antilithogenic actions (Chainani-Wu, 2003; Rungseesantivanon et al., 2010; Stefanska, 2012). The colouring principle of turmeric is the main component of this plant and is responsible for the anti inflammatory property. Turmeric was described as *C. longa* by Linnaeus and its taxonomic position is as follows:

<table>
<thead>
<tr>
<th>Class</th>
<th>Liliopsida</th>
</tr>
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<tbody>
<tr>
<td>Subclass</td>
<td>Commelinids</td>
</tr>
<tr>
<td>Order</td>
<td>Zingiberales</td>
</tr>
<tr>
<td>Family</td>
<td>Zingiberaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Curcuma</td>
</tr>
<tr>
<td>Species</td>
<td><em>Curcuma longa</em></td>
</tr>
</tbody>
</table>
Structure of curcumin

Chemical Formula: C_{21}H_{20}O_{6}
Molecular Weight: 368.38

Curcumin and neurodegenerative diseases

Curcumin exhibits antioxidant, anti-inflammatory, antirheumatic antimicrobial and anti cancer activities (Ammon & Wahl, 1991; Rao et al., 1995; Ruby et al., 1995; Zhao et al., 2012) as well as nephroprotective activity, therapeutic activity against myocardial infarction, skin diseases and cystic fibrosis (Aggarwal et al., 2003; Limtrakul et al., 1997; Dikshit et al., 1995). Studies revealed that curcumin mediates its anti-inflammatory and antioxidant effects by downregulation of nuclear factor-kB (NF-kB) and modulation of several important molecular targets, including, enzymes COX-2 (cyclooxygenase-2), iNOS (inducible nitric oxide synthases), and cytokines TNF α (Tumor necrosis factoralpha), IL-1b (Interleukin-1 beta), IL-6 (Interleukin-6) and chemokines (Hong et al., 2004; Kim et al., 2007; Kunnumakkara et al., 2008; Moon et al., 2008).

Brain is perhaps the most sensitive organ to oxidative damages (Halliwell, 1992). This organ consumes 20% of the body’s oxygen despite accounting for only 2% of the total body weight (Smith et al., 2007). Oxidative stress, which is due to the highly oxidative intracellular environment of the neurons and glial cells, has been shown to increase with both normal brain ageing as well as with brain injuries (Lu et al., 2004; Zhao et al., 2012). Administration of curcumin
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significantly reduced the progression of kindling and also attenuated the oxidative stress in mice; therefore it could be a candidate to control development of seizure and oxidative stress during epilepsy (Guangwei et al., 2010). Curcumin have been described to ability for scavenge oxygen derived free radicals that it has been implicated its potential as a neuroprotective agent (Sharma et al., 2009; Rungseesantivanon et al., 2010). Cerebral edema, a cause of increased intracranial pressure after acute brain injury, was significantly controlled by pretreatment as well as post treatment with curcumin (Thiyagarajan & Sharma, 2004). Curcumin has potential to increase the cholinergic activity of neurons in streptozotocin-induced dementia in rats (Awasthi et al., 2010)

AD is the most common form of progressive neurodegenerative dementia in the elderly population, and after heart disease, cancer and stroke AD is the fourth common cause of death in western countries (Selkoe, 2001; Zhao et al., 2012). AD is induced by different causes including genetics, oxidative stress, head trauma, inflammation and environmental factors (Butterfield, 2005; Zhu et al., 2005). AD is associated with impairment in working memory (Germano & Kinsella, 2005), visuoception, attention and semantic memory (Bolla, 1992). Oxidative stress and extracellular beta-amyloid (Aβ) deposits is known to contribute to the etiology of AD (Zhu et al., 2005; Hardy & Higgins, 1992). Oxidative damage to lipid and protein can lead to structural and functional disruption of the cell membrane, inactivation of enzymes, and finally caused cell death (Ashok & Ali, 1992; Meng et al., 2012).

Vitamin D₃

Most vertebrates synthesise vitamin D in their skin under the influence of UV light (Holick & Clark, 1978; Holick, 2003). An efficient sun exposure of the face and hands to the sun for 2 h/week is probably sufficient to maintain normal levels. There are two forms of vitamin D, vitamin D₃ (cholecalciferol), which is produced from the conversion of 7-dehydrocholesterol in the epidermis and
dermis in humans, and vitamin D$_2$ (ergocalciferol) which is produced in mushrooms and yeast (Langer et al., 2012). The chemical difference between vitamin D2 and D3 is in the side chain; in contrast to vitamin D$_3$, vitamin D$_2$ has a double bond between carbons 22 and 23 and a methyl group on carbon 24. Vitamin D can be obtained from dietary sources of vegetable (vitamin D$_2$, also known as ergocalciferol) or animal origin (vitamin D$_3$, also known as cholecalciferol). The best food sources are fatty fish or their liver oils; however, small amounts are also found in butter, cream and egg yolk. One of the major biological functions of vitamin D is to maintain calcium homeostasis (Norman et al., 1982; Zitman-Gal et al., 2012) which impacts on cellular metabolic processes and neuromuscular functions. Vitamin D affects intestinal calcium absorption by increasing the expression of the epithelial calcium channel protein, which in turn enhances the transport of calcium through the cytosol and across the basolateral membrane of the enterocyte (Langer et al., 2012).

**Structure of Vitamin D$_3$**

![Structure of Vitamin D$_3$](image)

**Molecular formula** C$_{27}$H$_{44}$O  
**Molar mass** 384.64 g/mol

**Vitamin D receptor**

1,25(OH)$_2$D$_3$ form of vitamin D is metabolically active, and this molecule exerts its effects by activating the nuclear vitamin D receptor (VDR)
The VDR is a member of the nuclear receptor superfamily (Buell & Dawson-Hughes, 2008; Brown & Slatopolsky, 2008) of ligand activated transcription factors, which also includes the thyroid hormone receptor, the retinoic acid receptor and the peroxisome proliferator activated receptor. The VDR regulates gene transcription both positively and negatively by binding to hexameric core binding motifs in the promoter regions of target genes, designated vitamin D response elements (Zitman-Gal et al., 2012). 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 $\text{Ca}^{2+}$ 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. VDR is expressed in most brain areas. Vitamin D$_3$ has been detected in the cerebrospinal fluid, and this hormone has been shown to cross the blood-brain barrier (Balabanova et al., 1984). The presence of VDR in the limbic system, cortex, cerebellum of rodents and humans (Musiol et al., 1992; Eyles et al., 2005) support a functional role for Vitamin D$_3$ in the regulation of behaviour and cognitive functions.

**Vitamin D and diabetes**

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 (Kadowaki & Norman, 1984; Tanaka et al., 1984; Langer et al., 2012). This impairment is primarily caused by the direct effect of
vitamin D deficiency on the beta cell. Glucose tolerance is restored when vitamin D levels return to normal (Brazdilova et al., 2012). β cells of endocrine pancreas are among the nonclassical target tissues for the action of 1α,25(OH)2D3. Studies revealed the presence of receptor protein for 1α, 25(OH)2D3 in chick pancreas (Christakos & Norman, 1981; Pike, 1981) and the presence of immunoreactive vitamin D-dependent calcium binding protein (CaBP) in pancreas (Morrissey et al., 1975). 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; Eichhorn et al., 2012), 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). Evidence suggests that Vitamin D3 has potential benefits with respect to diabetes. Cholecalciferol has been shown lower blood pressure (Vianna et al., 1992) and may have a role on normal pancreatic function and treatment of diabetes (Bland et al., 2004; Magge et al., 2012).