Dopamine, a neurotransmitter in the central nervous system

Dopamine (DA) is a major neurotransmitter within the mammalian central and peripheral nervous system (CNS). Changes in central dopamine neurotransmission are implicated in processes as diverse as muscle rigidity, hormonal regulation, thought disorder and cocaine addiction. Peripheral dopamine mediate changes in blood flow, glomerular filtration rate, sodium excretion and catecholamine release.

Dopamine containing neurons arise mainly from DA cell bodies in the substantia nigra and ventral tegmental area in mid-brain region (Tarazi, et al., 2001; 1996; Creese, et. al., 1997; Royh, et. al., 1991; Carlsson, 1993; Lookingland, et al., 1995). Dopaminergic system is organized into four major subsystems (i) the nigrostriatal system involving neurons projecting from the substantia nigra pars compacta to the caudate-putamen of the basal ganglia. This is the major DA system in the brain as it accounts for about 70% of the total DA in the brain, and its degeneration makes a major contribution to the pathophysiology of Parkinson’s disease; (ii) the mesolimbic system that originates in the midbrain tegmentum and projects to the nucleus accumbens septi and lateral septal nuclei of the basal forebrain as well as the amygdala, hippocampus, and the entorhinal cortex, all of which are considered components of the limbic system and so are of particular interest for the patho-physiology of idiopathic psychiatric disorders; (iii) the mesocortical system, which also arises from neuronal cell bodies in the tegmentum which project their axons to the cerebral cortex, particularly the medial prefrontal regions; (iv) the tuberinfundibular pathway, which is a neuroendocrinological pathway arising from the arcuate and other nuclei of the hypothalamus and ending in the median eminence of the inferior hypothalamus. DA released in this system exerts regulatory effects in the anterior pituitary and inhibits the release of prolactin. DA is involved in the control of both motor and emotional behavior. Despite the large number of crucial functions it performs, this chemical messenger is found in a relatively small number of brain cells. In
Fig. 1

Neuronal biochemical processes involving dopamine

1. Conversion of tyrosine to DOPA by tyrosine hydroxylase.
2. Conversion of DOPA to DA by aromatic L-amino acid decarboxylase.
3. Pooling of DA in a vesicle.
4. Exocytosis of a vesicle and DA-release into the synaptic cleft.
5. Activation of postsynaptic DA-receptors.
6. Activation of DA-autoreceptors.
7. Inhibition of tyrosine hydroxylase.
8. Reuptake of DA by the DA-transporter.
9. Metabolism of DA: conversion to 3-methoxytyramine by COMT.
10. Oxidation of MT to homovanillic acid by MAO.
11. Mitochondrion.
12. Mitochondrial oxidation of DA to DOPAC by MAO.
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.

**Biosynthesis of dopamine**

Dopamine is synthesized from the amino acid L-tyrosine (Fig: 1). L-tyrosine is hydroxylated by the enzyme tyrosine hydroxylase (TH) to give L-3, 4-dihydroxyphenylalanine (L-DOPA) which is the rate limiting step. L-DOPA is subsequently decarboxylated to dopamine by the enzyme aromatic L-amino acid decarboxylase. Therefore, it is not possible to enhance the levels of DA by providing L-tyrosine. The activity of tyrosine hydroxylase is regulated by several endogenous mechanisms. For example, the enzyme is activated by increased neuronal impulse flow, but is inactivated either by DA itself as an end-product inhibitor, or by activation of presynaptic DA receptors. On the other hand, the enzyme aromatic L-amino acid decarboxylase converts L-DOPA to DA instantaneously. Therefore, providing L-DOPA creates a possibility to enhance the formation of DA (Fig: 1).

**Dopamine reuptake and metabolism**

Dopamine exerts its functions mediated through various receptors and these actions are terminated to prevent continuous stimulation of the receptors. This inactivation is brought about by reuptake mechanisms and metabolism of DA. Reuptake of DA is accomplished by a high affinity carrier present in the membrane, the dopamine transporter (DAT) (Fig: 1). The dopamine transporter recycles extracellular DA by actively pumping it back into the nerve terminal. The dopamine content which is about 70 to 80 % in the striatal synaptic cleft is inactivated by this process. Drugs, such as cocaine, are able to block the action of the dopamine transporter, thereby sustaining the presence of dopamine in the synaptic cleft and its action on dopamine receptors. Part of the dopamine is inactivated by conversion to inactive compounds by metabolic enzymes, which are present both intra- and extraneuronally. Monoamine oxidase (MAO), aldehyde dehydrogenase (AD) and catechol-O-methyltransferase (COMT) are responsible for the metabolism of DA. Dopamine after reuptake may intraneuronally be deaminated by MAO to give dihydroxyphenyl acetaldehyde, which subsequently is converted to 3, 4-
Dopamine D₁ Receptor

Fig: 2 Signalling pathways of D₁ like receptors.

Gs: Stimulatory G proteins α subunits.
G?: Unknown or novel G protein α subunits with which the receptor may interact.
cAMP: cyclic Adenosine monophosphate
PLC: Phospholipase C
K⁺: Potassium ions
Na⁺: Sodium ions
H⁺: Hydrogen ions
dihydroxyphenylacetic acid (DOPAC) by AD. DOPAC is then methylated by COMT to give homovanillic acid (HVA). Extraneuronally, DA is metabolized by an alternative route in which it is first O-methylated to 3-methoxytyramine (3-MT) through the action of COMT and subsequently oxidized by MAO and AD to HVA.

Dopamine receptors

Dopamine mediates its actions via membrane receptor proteins. DA receptors are found on postsynaptic neurons in brain regions that are DA-enriched. In addition, they reside presynaptically on DA neuronal cell bodies and dendrites in the midbrain as well as on their terminals in the forebrain. Dopamine receptors belong to a family of large peptides that are coupled to G-proteins which are modified by attached carbohydrate, lipid-ester or phosphate groups. The topologies of the five dopamine receptors are predicted to be the same as all the other G-protein-coupled receptors (Fig: 2 & 3). They are characterized by having seven hydrophobic transmembrane-spanning regions. The third intracytoplasmic loop is functionally critical and interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects (Tarazi, et al., 1996; Creese, et. al., 1997; Royh, et. al., 1991; Carlsson, 1993). In their putative transmembrane domains, the DA D1 and D5 receptors are 79% identical to each other, while they are only 40-45% identical to the DA D2, D3, and D4 receptors. Conversely, the DA D2, D3, and D4 receptors are between 75% and 51% identical to each other. They contain seven putative membrane-spanning helices which would form a narrow dihedral hydrophobic cleft surrounded by three extracellular and three intracellular loops. The receptor polypeptides are probably further anchored to the membranes through palmitoylation of a conserved Cys residue found in their carboxy tails, 347 in DA D1, the C-terminus in DA D2 like receptors. The dopamine receptors are glycosylated in their N-terminal domains. Dopamine D1 like subtypes have potential glycosylation sites in their first extra cytoplasmic loop.

Dopamine receptors are divided into two families on the presence or absence of ability of DA to stimulate adenylyl cyclase and produce the second-messenger molecule cyclic-AMP (cAMP) (Calne, 1979; Schwartz, et al 1992; Civelli, et al, 1993; O'Dowd,
Figure: 3 Signalling pathways of dopamine D₂ receptor.

Gi: Inhibitory G protein α subunits;
Gz, Go, G?: Unknown or novel G protein α subunits with which the receptor may interact.
cAMP: cyclic Adenosine monophosphate
AA: Amino Acid
PLC: Phospholipase C
K⁺: Potassium ions
Ca²⁺: Calcium ions
Na⁺: Sodium ions
H⁻: Hydrogen ions
1993; Jackson, et al. 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 (Fig. 2). Dopamine D2 like receptors inhibit the production of cAMP (Fig. 3). This pharmacological characterization is based on the ability of some DA agents to block adenylyl cyclase activity to inhibit the release of prolactin in vivo and in vitro in a cAMP-independent fashion (Seeman, 1980). Applications of recent technical advances in molecular genetics have greatly facilitated the isolation and characterization of novel DA receptors, DA D3, D4 and D5, with different anatomical localization from traditional DA D1 or DA D2 receptors. Based upon their pharmacological profiles, including their effects on different signal transduction cascades, these receptors are currently divided into two families: the DA D1-like family which includes dopamine D1 and D3 receptors. The DA D2-like family includes dopamine D2, D3 and D4 receptors (Grandy, et al., 1993; Shen, et al., 1993; Schwartz, et al., 1995). The genomic organizations of the DA receptors demonstrate that they are derived from the divergence of two gene families that mainly differ in the absence or the presence of introns in their coding sequences. Dopamine D1 like receptors genes do not contain introns in their coding regions, a characteristic shared with most G protein-coupled receptors. The genes encoding the dopamine D2 like receptors are interrupted by introns (Marc, et al., 1998). Furthermore, most of the introns in the DA D2-like receptor genes are located in similar positions.

Dopamine D1-like family

The dopamine D1 receptor is the most abundant DA receptor in the central nervous system. The DA D1-like receptors are characterized by a short third loop as in many receptors coupled to Gs protein (Civelli, et al., 1993; Gingrich & Canon, et al., 1993; O'Dowd, 1993). The dopamine D1-like receptors have short third intracellular loops and long carboxy terminal tails. The DA D1 like receptors are classified into dopamine D1 and D5. In the DA 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 can be 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 D3 receptor (41 amino acids). The amino acid sequence of this loop is divergent in the dopamine D3 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 any introns, encodes a protein that extends for 446 amino acids (Caron, et al., 1991). In humans dopamine D1 receptor gene has been localized to chromosome 5 (Kennedy, et al., 1990). The dopamine D1 receptors show characteristic ability to stimulate adenylyl cyclase and generate inositol 1, 4, 5-trisphosphate (IP3) and diacylglycerol via the activation of phospholipase C (Sibley, et al., 1990; Monsma, et al., 1990). Dopamine D1 receptors are highly expressed in basal ganglia followed by cerebral cortex, hypothalamus and thalamus. Dopamine D1 receptors mRNA is colocaled in striatal neurons of the basal ganglia with mRNA for dopamine receptor phosphor protein (DARPP-32; KD) which is a dopamine and cyclic-AMP-regulated phosphoprotein. Dopamine Receptor Phosphor Protein contributes to the actions of 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 D2 receptors**

The dopamine D2 receptor gene is intronless and encodes a protein that extends for 477 amino acids (George, et al., 1991). This protein has an overall 50% homology with DA D1 receptor and 80% if only the seven transmembrane segments are considered. The gene encoding the human dopamine D2 protein is located at the short arm of chromosome 4, the same region where the Huntington disease gene has been located (Gusella, 1989). Two dopamine D3 receptor pseudogenes having 154 amino acids have
been identified with 90% homology (Gusella, 1989). These pseudogenes, however, contain stop codons in their coding regions that prevent them from expressing functional receptors. The functions of these pseudogenes, which appear so far to be specific to humans, are not yet known (Allen, et al., 1991).

Dopamine D₃ 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 (Basbaum, et al., 1979). Dopamine D₃ receptors, like DA D₁ receptors, appear to interact with G-proteins and can stimulate adenylyl cyclase, with relatively high affinity for DA and DA D₁-selective agonists (George, et al., 1991).

**Dopamine D₃ 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 (Marc, et al., 1998; Sibley, 1999). It is generally believed that the membrane enclosed part of the amino-acid chain of G-protein coupled receptors is folded into seven α-helices. The transmembrane helices consist primarily of hydrophobic amino-acid residues. The unique feature of DA D₂ like receptors family is that they posses a bigger third cytoplasmic (intracellular) loop in common, which is thought to be the site where the G-protein couples (Marc, et al., 1998). Between the different dopamine receptors, the third loop also displays the greatest variability in amino-acid sequence. This may have consequences for their respective second messenger systems. The DA D₂-like receptors are coupled to Gi-protein and inhibit the formation of cyclic AMP. The DA D₂ receptors tertiary structure is stabilized by two cysteine disulphide bridges.

**Dopamine D₂ receptors**

The DA D₂ receptor gene encodes a protein that extends for 415 amino acids. Similar to other G-protein coupled receptors, the DA D₂ receptor has seven
transmembrane segments, but in contrast to DA D1-like receptors, the third cytoplasmic domain is long and the carboxy terminus is short. Unlike the DA D1-like receptor genes, the DA D2 receptor gene contains seven introns that are spliced out during mRNA transcription (Fischer, et al., 1989). The gene encoding this receptor was found to reside on q22-q23 of human chromosome 11 (Makam, et al., 1989). The dopamine D2 receptor was the first receptor to be cloned (Chrisre, 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, et al., 1991). The dopamine D2 receptors are highly expressed in basal ganglia, nucleus accumbens septi and ventral tegmental area (Schwartz, et al., 1991).

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 DA D2 receptor isoforms. The two isoforms derived from the same gene by alternative RNA splicing which occurs during the maturation of the DA D2 receptor pre-mRNA (Schwartz, et al., 1989). Dopamine D2 receptor isoforms (DA D2L and DA D2S) vary within each species by the presence or absence of a 29-amino acid sequence in the third cytoplasmic domain of the DA D2 receptor peptide chain. Both variants share the same distribution pattern; with the shorter form less abundantly transcribed in addition they appear to differ in their mode of regulation (Marc, et al., 1998). Pharmacologically, both isoforms exhibit nearly similar profiles in terms of their affinities to different DA D2-selective agents, and inhibit adenylyl cyclase activity. However, these isoforms display an opposite regulatory effect (Sibley, et al., 1994). These isoforms have the same pharmacological profile, even though a marginal difference in the affinity of some substituted response to dopamine treatment is reported: Dopamine induces the up-regulation of DA D2L isoform of dopamine D2 receptors (Mohell, et al., 1993; Castro,
When expressed in host cell lines, both isoforms inhibited adenylyl cyclase (Marc, et al., 1998; Sibley, 1999). However, the DA D2S receptor isoform displayed higher affinity than the DA D2L in this effect (Seeburg, et al., 1989, Marc, et al., 1998). The isoforms of DA D2 mediate a phosphatidylinositol-linked mobilization of intracellular calcium in mouse Ltk [-] fibroblasts. Protein kinase C (PKC), 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 D2 receptors**

Dopamine D3 receptor gene contains five introns and encodes a 446 amino acid protein (Schwartz, et al., 1990). The gene encoding this receptor resides on chromosome 3 (Giros, et al., 1990). The DA D3 receptors bear close structural and pharmacological similarities to the DA D2 receptors. Dopamine D3 mRNA occurs in longer and shorter spliced forms generated from the same gene (Schwartz, et al., 1991). Distribution of dopamine D3 receptor mRNA are distributed and expressed mainly in subcortical limbic regions including islands of Calleja, nucleus accumbs septi and olfactory tubercle, with low levels of expression in the basal ganglia (Marc, et al., 1998). D3 receptor mRNA has also been found in neurons of the cerebellum, which may regulate eye-movements (Lévesque, et al., 1992). The status of the DA D3 molecular entity as a functional receptor remains uncertain since it neither couples to G-proteins nor consistently transduces an effector mechanism (Schwartz, et al., 1990; Sokoloff, et al., 1992; Marc, et al., 1998). However, the structural similarity with DA D2 receptor raises the possibility that DA D3 receptor may also inhibit adenylyl cyclase activity in its normal cellular setting. More recent studies reported that DA D3 receptors might mediate positive regulatory influences of DA on production of the peptide neurotensin (Lévesque, et al., 1995; Marc, et al., 1998).

**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 DA D4 receptor to the DA D2 and D3 receptors is about 41% and 39% respectively, but this homology increases to
56% for both receptors when only the transmembrane spanning segments are considered. The gene encoding the human DA D₄ protein is located at the tip of the short arm of chromosome 11 (Civelli, et al., 1992; Marc, et al., 1998). Dopamine D₄ receptor gene has been localized in brain regions like hippocampus and frontal cortex using specific histoprobes (Civelli, et al., 1994). The stimulation of DA D₄ receptor inhibits adenylyl cyclase activity and release arachidonic acid in brain neurons (Huff, et al., 1994, Marc, 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; Marc, et al., 1998). These are called the dopamine D₄ alleles which are represented as DA D₄₂, D₄₄ and D₄₇. These may contribute to the pathophysiology of certain neuropsychiatric disorders (Jackson & Westlind, 1994).

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

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

Insulin is involved in glucose disposal into skeletal muscles, inhibition of hepatic glucose production and inhibition of lipolysis in adipocytes. Intracerebroventricular infusion of insulin results in an increase in mRNA levels for the DA reuptake transporter (Figlewicz, *et al.*, 1998). Dopamine analogues are reported to inhibit glucose-stimulated insulin release from the endocrine pancreas (Fischer, *et al.*, 1984). Islets cells have been shown to contain the putative enzymes that synthesis dopamine like tyrosine hydroxylase and di-hydroxy phenylalanine decarboxylase (Gagliardino & Borelia, 2001). Dopamine and increased glucose stimulus reduced the insulin release from the pancreatic islets with no change in calcium efflux (Carpinelli, *et al.*, 1994). Acute L-DOPA-induced dopamine accumulation in pancreatic islets is reported to cause an inhibitory effect on glucose-stimulated insulin response resulting in an increased MAO activity (Lundquist, *et al.*, 1991; Lundquist, 1985).

Brain neurotransmitters and diabetes

Diabetes mellitus is a metabolic disorder that either arrives during the early years of growth (Juvenile diabetes) or later in life called as maturity onset diabetes. It is observed as the body’s inability to effectively regulate the sugar balance which leads to severe complications such as hyperglycemia, obesity, neuropathy, nephropathy, retinopathy, cardiopathy, osteoporosis and coma leading to death. Pancreatic damage resulting in the dysfunction of α and β cells causes disordered glucose homeostasis. In diabetic individuals the regulation of glucose levels by insulin is defective, either due to defective insulin production which is called as Insulin Dependent Diabetes Mellitus (IDDM) or due to insulin resistance that is termed as Non Insulin Dependent Diabetes Mellitus (NIDDM).

Diabetes mellitus has been reported to cause degenerative changes in neurons of the central nervous system (Bhattacharya & Saraswathi, 1991; Garris, 1990; Lackovic, *et al.* 1990). The concentration of 5-HT, DA NE increased in the brain regions of diabetic rats and accumulation of these monoamines is produced by inhibition of monoamine oxidase activity (Salkovic, *et al.*, 1990). Norepinephrine has been reported to increase in
several brain regions during diabetes. Ohtani, et al., (1997) have reported a significant decrease in extracellular concentrations of NE, 5HT and their metabolites in the ventromedial hypothalamus (VMH). Epinephrine (EPI) levels were significantly increased in the striatum, hippocampus and hypothalamus of diabetic rats and these changes were reversed to normal by insulin treatment (Ramakrishan & Namasivayam, 1995). Diabetes is reported to cause a high level of degeneration in neurons in different regions of the brain. Streptozocin-induced diabetes and acute deficiency of insulin is reported to result in increased concentrations of EPI in the supra chiasmatic nucleus. 5-hydroxytryptamine content in the brain is reported to be decreased during diabetes to be decreased (Jackson & Paulose, 1999; Chu, et al., 1986; Sumiyoshi, et al., 1997; Thorre, et al., 1997). Garris (1995) had reported chronically elevated levels of NE in the brain regions of amygdala, hypothalamus and medulla of diabetic mice. This was proposed to be associated with the expression of the gene causing diabetes mellitus. Hyperglycemia is reported to alter the noradrenergic and cholinergic nerve components (Akria, et al., 1994) with decrease in the Na⁺ K⁺ ATPase activity in different brain regions (Gurcharan, et al., 1994).

Norepinephrine, DA and 5-HIAA are reported to be increased in the heart and adrenal gland in STZ rats. In the heart the initial changes in short-term diabetes included an increase in NE concentration but did not persist in the longer term diabetic animals. In the adrenal gland there was an initial reduction followed by a steady increase in the concentration of NE and EPI (Sheen, et al., 2001).

**Dopamine and its receptor alterations during diabetes**

Dopamine is implicated in diabetes. Hyperglycemia 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 (Ho, et al., 1994). In experimental diabetes and insulin deficiency there is a rapid onset of detectable alterations in hypothalamic DA activity leading to secondary neuroendocrine abnormalities. Lim, et al., (1995) have described an increase in the striatal dopamine and decrease in its metabolites dihydroxyphenylacetic acid and homovanillic acid. Tyrosine hydroxylase is reported to be depleted in nigrostriatal neurons in the genetically diabetic rat causing
marked reduction mesolimbic dopamine system. Insulin treatment could not restore the decreased DA to controlled conditions, impairing the dopamine biosynthesis (Kamei & Saitoh, 1994). Dopamine uptake affinity and velocity in synaptosomes is decreased significantly during diabetes. The dopamine content was increased in cerebral cortex and hypothalamus of diabetic rats (Chen & Yang, 1991; Ohtani, et al., 1997; Tassava, et al., 1992; Shimizu, 1991). Diabetes is reported to cause increased 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 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 streptozotocin 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 D2 receptor density has been reported to be increased in the striatum of diabetic rats (Lozovsky, et al., 1981; Trulson & Hummel, 1983; Serri et al., 1985). Intracerebroventricular application of alloxan and streptozotocin in rat striatum is reported to have caused an alteration in dopamine receptors and increased dopamine content which had a similar effect to peripheral, diabetogenic administration of these drugs (Salkovic, et al., 1995). The affinity of striatal DA D1 receptors was significantly increased without changes in the number of binding sites, while the binding of dopamine D2 receptors was significantly increased without affecting its affinity in the diabetic rats (Ho, et al., 1994). Dopamine D1 receptors are reported to decrease in hyporesponsiveness (Kamei, et al., 1998). 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).
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 mobilization from the adipose tissue (Oliveira, et al., 1998). Participation of dopaminergic tone in the control of insulin secretion and hyperglycemia has been given little focus. These studies recently 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).

Factors affecting insulin regulation from pancreatic β-cells

D-Glucose is the major physiological stimulus for insulin secretion. The mechanism of glucose induced insulin release is not completely understood. Phosphorylation of glucose to glucose-6-phosphate serves as the rate limiting step in glucose oxidation (Schuit, 1996). Glucokinase acts as a glucose sensor during this process. Glucokinase is also linked to the phosphate potential, $[\text{ATP}] / ([\text{ADP}][\text{Pi}])$ (Sweet et al., 1996). An increased ATP/ADP ratio is believed to close $\text{K}^+$-ATP channel at the plasma membrane, resulting in decreased $\text{K}^+$ efflux and subsequent depolarisation of the β-cell (Dunne, 1991). Depolarisation activates voltage-dependent $\text{Ca}^{2+}$ channels, causing an influx of extracellular $\text{Ca}^{2+}$ (Liu, et al., 1996). Although intracellular $\text{Ca}^{2+}$ activates protein kinases such as $\text{Ca}^{2+}$ and calmodulin dependent protein kinase (Breen & Aschcroft, 1997), it remains unclear how increase in intracellular $\text{Ca}^{2+}$ leads to insulin release. Intracellular $\text{Ca}^{2+}$ stores appear to regulate a novel plasma membrane current $[\text{Ca}^{2+}$ release activated non-selective cation current, $I_{\text{CRAN}}]$, whose activity may control glucose activated secretion. Lesions in these pathways lead to the pathogenesis of diabetes mellitus (Dukes, et al., 1997). Glucose induced insulin secretion is also partly dependent upon the activation of typical isoforms of protein kinase C (PKC) within the β-cell (Harris, et al., 1996). It is suggested that PKC may be tonically active and effective in the maintenance of the phosphorylated state of the voltage-gated $\text{Ca}^{2+}$ channel, enabling an appropriate function of this channel in the insulin secretory process (Arkhammar et al., 1994).
channel, enabling an appropriate function of this channel in the insulin secretory process (Arkhammar et al., 1994).

**Amino acids**

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

**Fatty acids**


**Substrates derived from nutrients**

Pyruvate, citrate, ATP, NADH and NADPH are derived from nutrients that are involved in the intake or local islet stimulation (Lisa, et al., 1994; Tahani, 1979; Lain, et al., 1994). Adenosine diphosphate acts as an intracellular regulator of insulin secretion. Mg' -ADP is required for the stimulation of K⁺-ATP channels in intact β-cells. There are other intracellular factors such as arachidonate, guanine nucleotides, small monomeric GTP-binding proteins such as rab 3A (Regazzi, et al., 1996) and the heterotrimeric GTP-binding protein Gαi are involved in regulating glucose induced insulin release (Konrad, et al., 1995). GTP analogues are also important regulators of insulin secretion (Lucia, et al., 1987). Glucose induced insulin secretion is accompanied by an increase in the islet content of cAMP (Rabinovitch, et al., 1976).
Glucagon

Glucagon is the hormone secreted by pancreatic α-cells. It has been shown that glucagon has a striking stimulation of insulin release in the absence of glucose (Sevi & Lillia, 1966). The presence of specific glucagon receptors on isolated rat pancreatic β-cells and subpopulation of α- and δ-cells shows the relevance of glucagon on regulation of insulin secretion (Kiefer, 1996). Intra-islet glucagon appears to be a paracrine regulator of cAMP in vitro (Schuit, 1996). Glucagon stimulates insulin release by elevating cAMP. The cAMP through activation of protein kinase A, increases Ca<sup>2+</sup> influx through voltage dependent L-type Ca<sup>2+</sup> channels, thereby elevating [Ca<sup>2+</sup>]<sub>i</sub> and accelerating exocytosis (Carina, et al., 1993). Protein phosphorylation by Ca<sup>2+</sup>/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, et al., 1996).

Somatostatin

Somatostatin is secreted by the pancreatic δ-cells of the islets of Langerhans. Somatostatin inhibits insulin release (Ahren, et al., 1981). Its action is dependent on the activation of G-proteins but not associated with the inhibition of the voltage dependent Ca<sup>2+</sup> currents or adenylyl cyclase activity (Renstrom, et al., 1996).

Epinephrine and norepinephrine

Epinephrine and norepinephrine are secreted by the adrenal medulla. Norepinephrine is the principal neurotransmitter of sympathetic nervous system. Epinephrine and norepinephrine inhibit insulin secretion, both in vivo and in vitro (Renstrom, et al., 1996; Porte, 1967). Epinephrine and norepinephrine exerts opposite effects on peripheral glucose disposal and glucose stimulated insulin secretion (Avogaro, et al., 1996).
Pancreastatin

Pancreastatin is known to be produced in islet β-cells and inhibits 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). Pancreastatin is reported to increase Ca$^{2+}$ in insulin secreting RINm5F cells, independent of extracellular calcium (Sanchez, et al., 1992).

Amylin

Amylin is a 37 amino acid peptide hormone co-secreted with insulin from pancreatic β-cells. Amylin appears to control plasma glucose via several mechanisms that reduce the rate of glucose appearance in the plasma. Amylin is absolutely or relatively deficient in type-I diabetes and in insulin requiring type-II diabetes (Young, 1997). Islet amyloid polypeptide (IAPP) or amylin inhibits insulin secretion via an autocrine effect within pancreatic islets. Amylin fibril formation in the pancreas may cause islet cell dysfunction and cell death in Type-II Diabetes mellitus (Alfredo, et al., 1994).

Adrenomedullin

Adrenomedullin is a novel hypotensive adrenal polypeptide isolated from a human pheochromocytoma and is structurally related to calcitonin gene related peptide and islet amyloid polypeptide. It has been suggested that besides being an adrenal hypotensive peptide, adrenomedullin may be a gut hormone with potential insulinotropic function (Mulder, et al., 1996).

Galanin

Galanin is a 29 amino acid neuropeptide localised in the intrinsic nervous system of the entire gastrointestinal tract and the pancreas of man and several animal species (Scheurink, et al., 1992). Galanin inhibits insulin release (Ahren, et al., 1991), probably
via activation of G-proteins by the mediation of activated galanin receptors. However, galanin receptors are not as effective as $\alpha_2$-adrenergic receptors in activating G-proteins (Renstrom, et al., 1996).

Macrophage migration inhibitory factor

Macrophage migration inhibitory factor (MIF) is a cytokine secreted by T lymphocytes. It was found recently to be both a pituitary hormone and a mediator released by immune cells in response to glucocorticoid stimulation. It has been demonstrated that insulin secreting $\beta$-cells of the islets of Langerhans expresses MIF and its production is regulated by glucose in a time and concentration dependent manner. MIF and insulin co-localise within the secretory granules of the pancreatic $\beta$-cells and once released, MIF appears to regulate insulin release in an autocrine fashion. MIF is therefore a glucose dependent islet cell product that regulates insulin secretion in a positive manner and may play an important role in carbohydrate metabolism (Waeber, et al., 1997).

Other agents

Coenzyme $Q_{10}$ improved insulin release and it may also have a blood glucose lowering effect (Conget, et al., 1996). Inositol hexa bisphosphate stimulates non Ca$^+$ mediated and purine-Ca$^{2+}$ mediated exocytosis of insulin by activation of protein kinase C (Efanov, et al., 1997). Insulin secretion and release in rats and hamsters are also reported to be controlled by small GTP-ases of the rab 3A family expressed in insulin secreting cell lines (Regazzi, et al., 1996).

ROLE OF NEUROTRANSMITTERS IN INSULIN REGULATION

Epinephrine and Norepinephrine

Epinephrine and norepinephrine has an antagonistic effect on insulin secretion and glucose uptake (Renstrom, et al., 1996; Porte, 1967). They also inhibit insulin - stimulated glycogenesis through inactivation of glycogen synthase and activation of
phosphorylase with consequent accumulation of glucose-6-phosphate. In addition, it has been reported that epinephrine enhances glycolysis through an increased activation of phosphofructokinase.

The adrenergic receptors are seven-pass transmembrane receptors that are coupled to G-proteins. Adrenergic receptors are mainly classified into $\alpha_1$, $\alpha_2$ and $\beta$-adrenergic receptors. $\alpha_1$ adrenergic receptor has three subclasses - $\alpha_{1A}$, $\alpha_{1B}$, $\alpha_{1C}$ (Price, et al., 1994) and $\alpha_2$ has $\alpha_{2A/D}$, $\alpha_{2B}$ and $\alpha_{2C}$ (Hamamdzic, et al., 1995). $\beta$-adrenergic receptors are subclassified into $\beta_1$, $\beta_2$ and $\beta_3$ (Dohlman, et al., 1991). Epinephrine and NE bind to these receptors in a concentration dependent manner. Epinephrine and NE at low concentrations can bind and activate $\beta$-adrenergic receptors which in turn stimulate the insulin secretion from pancreatic islets and at high concentration they can bind to $\alpha_{2A}$ receptors and inhibit insulin secretion (Lacey, et al., 1993). Previous studies had shown that in diabetic condition $\alpha_{2A}$ receptors are more activated which brought out the insulin inhibition and in turn hyperglycaemia (Lacey, et al., 1993). Rat islet cell membrane is equipped with $\alpha_{2A}$-adrenoceptors (Filipponi, et al., 1986) which are linked to adenylyl cyclase inhibiting insulin secretion. $\beta_3$ adrenoreceptors stimulation also results in enhanced insulin secretion (Alef, et al., 1996).

Acetylcholine
Acetylcholine is the neurotransmitter of the parasympathetic system. Cholinergic receptors are classified as ionotropic nicotinic receptor and metabotropic muscarinic receptor. Acetylcholine increases insulin secretion through muscarinic receptors in pancreatic islet cells (Tassava, et al., 1992; Greenberg & Pokol, 1994). Muscarinic receptors are classified as $M_1$, $M_2$, $M_3$, $M_4$ and $M_5$. They are G protein coupled receptors. They are characterized by having seven hydrophobic transmembrane-spanning regions that interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects. Expression studies have revealed the presence of $M_1$ and $M_3$ receptors in the pancreas. Acetylcholine is reported to be involved in the activation of glucose transport in the chromaffin cells. The cholinergic activation affecting this process is coupled with calmodulin and protein kinase C (Skar, et al., 2002).
\textbf{\gamma-Aminobutyric acid}

\gamma-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in central nervous system. GABA is reported to be present in the endocrine pancreas at concentrations comparable with those found in central nervous system. The highest concentration of GABA within the pancreatic islet is confined to \(\beta\)-cells (Sorenson, \textit{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 \(\beta\)-cells causing insulin-dependent diabetes mellitus (Baekkeskov, \textit{et al}., 1990). GABA through its receptors has been demonstrated to attenuate the glucagon and somatostatin secretion from pancreatic \(\alpha\)-cells and \(\delta\)-cells respectively (Gaskins, \textit{et al}., 1995). GABA which is present in the cytoplasm and in synaptic-like microvesicles is co-released with insulin from \(\beta\)-cells in response to glucose (Reetz, \textit{et al}., 1991). GABA inhibits islet \(\alpha\) and \(\delta\)-cell hormonal secretion in a paracrine manner. GABA release is decreased in diabetes resulting in the enhancement of glucagon secretion from \(\alpha\)-cells leading to hyperglycaemia. GABA is involved in the maintenance of glucose homeostasis and inhibition of central GABA\(_A\) receptors increasing the plasma glucose concentration (Lang, 1995). Thus, any impairment in the GABAergic mechanism in central nervous system and/or pancreatic islets is important in the pathogenesis of diabetes.

\textbf{Serotonin}

Brain serotonin content decreased during diabetes (Jackson \& Paulose, 1999). This decrease is reported to be due to a decrease in uptake of tryptophan through the blood brain barrier (BBB) (Madras, \textit{et al}., 1974; Fernstrom \& Wurtman, 1972; Fernstrom \& Wurtman, 1971) and a decrease in rate of 5-HT synthesis (Carndall, \textit{et al}., 1981). The turnover rate of 5-HT to 5-HIAA in diabetic rats was also reported to be lower (Sandrini, \textit{et al}., 1997; Kwok \& Juorio, 1987). A decrease in brain 5-HT will lead to an up regulation of 5-HT\(_{2A}\) receptors of cerebral cortex and brain stem which in turn can inhibit insulin secretion due to increased sympathetic activity (Jackson \& Paulose, 1999).
Central Nervous System regulation of pancreatic insulin secretion

The pancreas has innervations of nerves from the central nervous system. Three types of nerve endings are reported with in the pancreas. They are the sympathetic, parasympathetic and peptidergic nerves. The neurotransmitters found in these nerves are catecholamines, serotonin, acetylcholine and vascoactive intestinal polypeptides and cholecystokinin respectively. The nerve fibres enter the pancreas in association with the vascular supply. Adrenergic fibres innervate vessels, acini and islets whereas cholinergic nerves are found in the islets alone (Miller, 1981). The peptidergic nerves are present in both the exocrine and endocrine tissues of this gland and there is considerable interspecies variability as to which part receives a greater proportion of these fibres (Bishop, et al., 1980). The nerve terminals end approximately 20-30nm from the endocrine cells thus implying that neurotransmitters affect several cells by diffusing through the extracellular space (Miller, 1981). Acetylcholine infusion or in vivo stimulation of the vagus nerve increases insulin secretion from the pancreatic islets (Kaneto, et al., 1981). Vagotomy often has a little effect on the basal hormone secretion, but effect the release of hormones (Helman, et al., 1982). Intact nerve supply to the pancreas is supposed to be necessary for the islet growth and development (Smith and Davis 1983).

The vagus and splanchnic nerves travel via the pancreas and supply autonomic signals (Helman, et al., 1982). These nerves are related to the ventral hypothalamus which plays a pivotal role in the integration of neurohormonal functions (Oommura & Yoshimatsu, 1984). The ventro-medial hypothalamic nuclei are considered as the sympathetic centre and the stimulation of this area decreases insulin secretion (Helman, et al., 1982). Lesions in the ventro-medial hypothalamus resulted in behaviour alterations and morphological changes in the pancreatic islets (Sclafani, 1981). Ventrolateral hypothalamus is the parasympathetic centre, stimulation of which increases the circulating level of insulin (Helman, 1982). Lesions in the ventral lateral hypothalamus resulted in decreased body weight, food intake plasma levels and decrease in islet size (Powley & Opsahl, 1976).
The substantia nigra (SN) is one autonomic area in the central nervous system which plays an important role in controlling structure and activity of pancreatic islets. Lesions in the substantia nigra not only resulted in reduced size and number of islets cell populations but also decreased the content of insulin and glucagon in the pancreas (Smith and Davis 1983). Studies have focused on the existence of pathways between the SN and intermediolateral cells in the spinal cord and between the SN and hypothalamic paraventricular nucleus (Schmidt, et al., 1982). The hypothalamic paraventricular nucleus has direct connections with the dorsal vagal complex (Wright, et al., 1980; Swanson & Sawchenko, 1980). These reports underlined the role of SN in modulating the outflow of both sympathetic and parasympathetic signals that ultimately reach the pancreas.

The central vagal connection with dopaminergic innervation is reported to reach the pancreatic islets through the parahypothalamic ventricular (PHV) nucleus while adrenergic and serotonergic innervations reach the pancreas through the brain stem (Smith and Davis 1983; Lowey et al., 1994).

Effect of dopamine on blood glucose levels

Dopamine and its agonists have been reported to affect the blood glucose levels. Increase in glucose level has been suggested to be due to sympathoadrenal activation. Plasma glucose levels are reported to be under separate serotonergic and dopaminergic control exerted via 5-HT$_1A$ and DA D$_3$ receptors respectively (Hillegaart, et al., 1996). Dopamine D$_3$ receptor agonist, 7-OH DPAT, injection caused an increase in blood glucose level and decreased plasma insulin content showing the involvement of this receptor in glucose homeostasis. Evidences show that DA D$_2$ receptor-mediated increase in plasma glucose is via sympathoadrenal activation (Saller & Kreamer, 1991). Dopamine analogues like lergotrile, pergolide, bromocriptine, d-amphetamine and apomorphine when injected has reported to cause hyperglycemia in rats (Fischer, et al., 1984). In contrary obese diabetic rats treated with a combination of dopaminergic receptor agonists SKF/38393 and bromocriptine (BC) is reported to reduce hyperglycemia (Cincotta, et al., 1999).
Pancreatic dopamine and insulin secretion

The role and the peripheral mechanism of action of central dopamine on basal pancreatic exocrine secretion in conscious rats revealed that central dopamine inhibited pancreatic exocrine secretion via DA D₁-like receptors and that the inhibitory effect is mediated via sympathetic nerves, especially α-adrenoceptors (Miyasaka, et al., 1998). Presence of dopamine is reported in peripheral tissues (Hakanson, et al., 1989). Dihydroxy phenyl acetic acid decarboxylase (DDC), dopamine β hydroxylase (DBH) and aromatic L-amino decarboxylase (AAD) are present in endocrine cells of adult rats (Takayanagi, Watanabe, 1996; Gagliardino, et al. 1997; Yamada, et al., 1999; Kampe, et al., 1995). As dihydroxy phenyl acetic acid decarboxylase and DBH are enzymes specifically involved in catecholamine synthesis and insular cells are reported to possess the capacity to synthesise these amines. Thus, endogenously-synthesised islet catecholamines have been suggested to participate in paracrine regulation of insulin secretion. Secretory granules of pancreatic β-cells have the ability to store (Ahren & Lundquist, 1985) substantial amounts of calcium, dopamine and serotonin. L-3, 4-dihydroxyphenylalanine is rapidly converted in islet beta-cells to dopamine. Acute L-DOPA-induced dopamine accumulation in pancreatic islets is accompanied by rapid changes in MAO activity, concomitant with an inhibitory effect on glucose-stimulated insulin response (Ahren & Lundquist, 1985). It is reported that increased hydrogen peroxide production, following increased MAO activity, may possibly augment the inhibitory effect of dopamine accumulation on insulin release (Lundquist, et al., 1991). Dopamine is reported to suppress the somatostatin secretion predominantly through activation of dopaminergic receptors, whereas it suppresses insulin release through an alpha adrenergic mechanism and stimulates glucagon release through a β-adrenergic mechanism (Malaisse, et al., 1992). There has not been any detailed study on the distribution of dopamine receptor subtypes in the pancreatic islets or the pancreas except for these studies. Sympathetic α₁ receptors and dopamine D₁ are reported to be distributed on the beta cells while β₂ receptors are located on the D cells and dopamine D₂ receptors in the beta neurons (Imamura, et al., 1990).
Dysfunction of pancreatic islets plays an important role in the etiology of diabetes as chronic hyperglycemia impairs islet function. It has been proposed that chronic hyperglycemia resulting from peripheral insulin resistance may impair secretogogue-induced insulin release. Dopamine agonists influence central circadian neuroendocrine activities regulating metabolism to reduce insulin secretion (Lang, et al., 1998). Timed dopaminergic stimulation is reported to normalize the circadian rhythm of corticosterone release in obese insulin resistant animals (Lang, et al., 1998). It has been reported that administration of dopamine receptor agonists, bromocriptine and/or SKF38393 in diabetic rats decreased insulin resistance, increased secretion of insulin from the islet cells and normalized the daily corticosterone rhythm. Dopamine receptor agonists are suggested to improve the decreased regulatory mechanisms in the hypothalamic-neuroendocrine system during diabetes and reduce β-cell toxicity.

Hyperglycemia causes functional deficits in the CNS aminergic neurons which are too subtle and take a longer time to manifest. Reports emphasized that treatment of gastric stasis in diabetic patients using dopamine blocker metoclopramide resulted in increased frequency and severity of dopamine associated tardive dyskinesia. Also, diabetes caused a shift in the CNS resulting in an increased sympathetic tone that resulted in a decreased insulin secretion. Recently the presence of DA in the adrenal medulla is being stated to draw importance as it is necessary to control secretions of NE and EPI. Dopamine regulates pancreatic insulin secretion in a concentration dependent manner (Zern, et al., 1980). But the molecular mechanism is not well studied in detail. Further studies on the involvement of dopamine will lead to improved therapeutic strategies to treat diabetes.