LITERATURE REVIEW
2. LITERATURE REVIEW

2.1. BRAIN NEUROTRANSMITTER CHANGES DURING DIABETES

A significant increase in the catecholamine contents and activity of metabolising enzymes has been reported in experimental diabetes (Gupta et al., 1992). Norepinephrine (NE) has been reported to increase in several brain regions during diabetes (Tassava et al., 1992; Chen & Yang, 1991; Garris, 1990; Lackovic et al., 1990; Wesselmann et al., 1988; Chu et al., 1986; Fushimi et al., 1984; Oreland & Shaskan, 1983), but a significant decrease in NE has been reported in hypothalamus (Ohtani et al., 1997), pons and medulla (Ramakrishna & Namasiyayam, 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 & Namasiyayam, 1995). Streptozotocin-induced diabetes and acute insulin deficiency were demonstrated to result in increased content of EPI in the supra chiasmatic nucleus. In addition, a decreased turnover of dopamine in the ventromedial nucleus was found to be increased in the insulin treated diabetic animals (Oliver et al., 1989). These data indicate that experimental diabetes and acute insulin deficiency results in the rapid onset of detectable alterations in EPI and DA activity in specific hypothalamic nuclei. This can lead to the development of secondary neuroendocrine abnormalities known to occur in the diabetic condition. The DA content was increased in whole brain (Chen & Yang, 1991, Lackovic et al., 1990), corpus striatum (Chu et al., 1986), cerebral cortex and hypothalamus of diabetic rats (Ohtani et al., 1997; Tassava et al., 1992; Shimizu, 1991).

In case of 5-HT there are contradicting reports which state that 5-HT content is increased in the brain regions and hypothalamic nuclei (Chen & Yang, 1991, Lackovic et al., 1990; Bitar et al., 1987), but majority of reports suggest a decrease in brain 5-HT content during diabetes (Jackson & Paulo, 1999; Sandrini et al., 1997; Sumiyoshi et al., 1997; Thorre et al., 1997; Shimizu, 1991, Chu et al., 1986; Kulikov et al., 1986). Ohtani et al. (1997) have reported a significant decrease in extracellular concentrations of NE. 5-HT and their metabolites in the ventromedial hypothalamus (VMH). The ratio of MHPG/NE and 5-HIAA/5-HT were increased. A similar observation was reported by
Ding et al., (1992) with a decrease in 5-HT in entorhinal cortex (19%) and 5-HT turnover (5-HIAA/5-HT) that increased by 48%. Chu et al., (1986) has reported lower 5-HT levels in both hypothalamus and brain stem 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). It also produced a significant increase in 5-HIAA that was observed at 2, 3, 4 and 6 hours after insulin administration (Kwok & Juorio, 1987). There was no change in 5-HIAA content in the corpus striatum during diabetes (Chu et al., 1986).

2.2. HYPERGLYCAEMIC EFFECT BY 5-HT RECEPTOR AGONISTS

The 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A} receptor subclasses are the two main receptors that mediate a hyperglycaemic response when stimulated by their respective agonists.

2.2.1. Hyperglycaemia induced by 5-HT\textsubscript{1A} receptor stimulation

8-OH-DPAT has been reported as a selective agonist for 5-HT\textsubscript{1A} receptor. It binds and activates the 5-HT\textsubscript{1A} receptors and decreases plasma insulin and increases basal plasma glucose levels in several strains of rats and other species via a central mechanism of action (Laude et al., 1990; Bauhelal et al., 1990b; Chaouloff & Jeanrenard, 1988, Chaouloff & Jeanrenaud, 1987). The decrease in insulin is brought about by increased sympathetic activity that stimulates the adrenal gland to secrete EPI which in turn inhibits insulin secretion (Bauhelal & Mir, 1993, Bauhelal & Mir, 1990a). Both adrenal cortex and medulla can participate in glucose metabolism through their respective secretory products i.e., corticosteroids and catecholamines respectively. The fact that 8-OH-DPAT increases plasma EPI and that selective \(\alpha_2\) and \(\beta_2\) adrenoreceptor agonists suppress 8-OH-DPAT mediated hyperglycaemia supports the involvement of adrenal catecholamines in the metabolic effects of 8-OH-DPAT (Chaouloff et al. 1990a, Bugdy et al., 1989, Chaouloff & Jeanrenaud, 1987). The EPI releasing effect of 8-OH-DPAT are
blocked by both 5-HT<sub>1A</sub> and β-adrenoreceptor antagonist (-)-pindolol. However, selective β<sub>1</sub> or β<sub>2</sub>-adrenoreceptor antagonists do not block this effect (Chaouloff et al., 1990a).

Plasma levels of corticosterone showed an increase with 8-OH-DPAT treatment in rats (Chaouloff et al., 1990d; Aulakh et al., 1988; Koenig et al., 1987). An increase in corticosterone can be a potential contributing factor for 8-OH-DPAT mediated hyperglycaemia. Administration of 8-OH-DPAT also produced a sustained fall in blood pressure and heart rate that were preceded by transient (<5 min) increase in blood pressure. All these symptoms were abolished in adrenalectomised rats (Bauhelal & Mir, 1993). At high concentrations, 8-OH-DPAT can bind to α<sub>2</sub>-adrenoreceptors and inhibit insulin release via a mechanism similar to that of clonidine inhibition of insulin from pancreatic β-cells (Fozord et al., 1987).

Corticotropin releasing factor (CRF) and ACTH release is stimulated by 5-HT and 8-OH-DPAT (Calogero et al., 1989). CRF is reported to stimulate adrenal medulla via a central site of action manifested by increase in efferent adrenal nerve activity leading to increased plasma EPI concentration and blood pressure (Brown et al., 1985). Also, intracerebroventricular injections of CRF have induced hyperglycaemia in rats and dogs (Brown et al., 1982). EPI release and hyperglycaemia is induced by several other selective 5-HT<sub>1A</sub> receptor agonists such as buspirone, ipsapirone and flesinoxan, thus implicating a key role for 5-HT<sub>1A</sub> receptors in the regulation of glucose metabolism (Chaouloff et al., 1990e).

2.2.2. Hyperglycaemia induced by 5-HT<sub>2A</sub> receptor stimulation

Administration of a selective 5-HT<sub>2A</sub> receptor agonist DOI produced a rapid increase in blood glucose level. Administration of DOI is also accompanied by an increase (1500%) in EPI concentration (Glennon, 1987). Pre-treatment of the animals with 5-HT<sub>2A</sub> receptor antagonists i.e., ketanserin and LY53857, were able to reverse the increase in sympathetic nerve discharge produced by DOI (Chaouloff et al., 1990b; Hoyer, 1988c; McCall & Hornis, 1988). These findings show that the central 5-HT<sub>2A</sub> receptors stimulates sympathetic nerve discharge which in turn increases EPI release from adrenal medulla similar to the 5-HT<sub>1A</sub> receptor activation.
Administration of the 5-HT₂₅ receptor agonist, α-methyl-5-HT, can also elicit hyperglycaemic effects which are blocked by 5-HT₂₅ antagonist ketanserin (Chaouloff et al., 1990b). α-methyl-5-HT was able to significantly suppress food intake by food-deprived rats and also inhibited 2-deoxy-D-glucose induced hyperphagia in rats. α-methyl-5-HT induced hyperphagia was antagonised by ketanserin (Yamada et al., 1997; Sugimoto et al., 1996). Intraperitoneal administration of 5-HT brought about hyperglycaemia mediated through a dose dependent increase in plasma EPI level. 5-HT induced hyperglycaemia was abolished by pre-treatment with ketanserin and also adrenodemedullation (Yamada et al., 1995). This suggests that the hyperglycaemic effects of 5-HT are closely related to the decrease of EPI from the adrenal gland, mediated by 5HT₂₅ receptors. 5-HT₂₅ receptors may also be partly involved in the pharmacological effects of induction of hyperglycaemia induced by the 5-HT₄ receptor agonist, 5-methoxytryptamine (Yamada et al., 1997).

2.3. FACTORS AFFECTING INSULIN REGULATION FROM PANCREATIC β-CELLS

2.3.1. Glucose

D-Glucose is the major physiological stimulus for insulin secretion. The mechanism of glucose induced insulin release is not completely understood. Phosphorylation of glucose to glucose-6-phosphate serves as the rate limiting step in glucose oxidation (Schuit, 1996). Glucokinase acts as a glucose sensor during this process. Glucokinase is also linked to the phosphate potential, [ATP]/([ADP][Pi]) (Sweet et al., 1996). An increased ATP/ADP ratio is believed to close K⁺-ATP channel at the plasma membrane, resulting in decreased K⁺ efflux and subsequent depolarisation of the β-cell (Dunne, 1991). Depolarisation activates voltage-dependent Ca²⁺ channels, causing an influx of extracellular Ca²⁺ (Liu et al., 1996). Although intracellular Ca²⁺ activates protein kinases such as Ca²⁺ and calmodulin dependent protein kinases(Breen & Aschcroft, 1997), it remains unclear how increase in intracellular Ca²⁺ leads to insulin release. Intracellular Ca²⁺ stores appears to regulate a novel plasma membrane current [Ca²⁺ release activated non-selective cation current, I_{CRAN}], whose activity may control glucose activated secretion. Lesions in these pathways leads 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 L-type Ca\(^{2+}\) channel, enabling an appropriate function of this channel in the insulin secretory process
(Arkhammar et al., 1994).

2.3.2. 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.

2.3.3. Fatty acids

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

2.3.4. Substrates derived from nutrients

This may involve indirect reflex stimulation triggered by food intake or local islet
stimulation through the production of a metabolite common to several substrates like
pyruvate (Lisa et al., 1994), citrate, ATP (Tahani, 1979), NADH and NADPH (Iain 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. 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 G\(_{ai}\) are involved in regulating glucose induced insulin release
(Konrad et al., 1995). GTP analogues are also important regulators of insulin secretion
Glucose induced insulin secretion is accompanied by an increase in the islet content of cAMP (Rabinovitch et al., 1976).

2.3.5. 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 as well as a 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\(^{2+}\) influx through voltage dependent L-type Ca\(^{2+}\) channels, thereby elevating [Ca\(^{2+}\)] and accelerating exocytosis (Carina et al., 1993). Protein phosphorylation by Ca\(^{2+}\)/Calmodulin and cAMP dependent protein kinase play a positive role in insulin granule movement which results in potentiation of insulin release from the pancreatic β-cell (Hisatomi et al. 1996).

2.3.6. Somatostatin

This hormone 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\(^{2+}\) currents or adenylate cyclase activity (Renstrom et al., 1996).

2.3.7. Epinephrine and norepinephrine

These 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 (Renstrom et al. 1996; Porte, 1967). Epinephrine exerts opposite effects on peripheral glucose disposal and glucose stimulated insulin secretion (Avogaro et al. 1996).

2.3.8. Pancreastatin

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

2.3.9. 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).

2.3.10. 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).

2.3.11. 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). Among other functions galanin inhibits insulin release (Ahren et al., 1991), probably via activation of G-proteins by the mediation of activated galanin receptors (Renstrom et al., 1996). However, galanin receptors are not as effective as α\(_2\)-adrenergic receptors in activating G-proteins.

2.3.12. Macrophage migration inhibitory factor (MIF)

MIF, originally identified as cytokines secreted by T lymphocytes was found recently to be both a pituitary hormone and a mediator released by immune cells in response to glucocorticoid stimulation. Recently it has been demonstrated that insulin secreting β-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 β-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).

2.3.13. Other agents

Coenzyme Q10 improved insulin release and it may also have a blood glucose lowering effect (Conget et al., 1996). Inositol hexa bisphosphate stimulates non Ca2+ mediated and purine-Ca2+ mediated exocytosis of insulin by activation of protein kinase C (Efanov et al., 1997). Small GTP-ases of the rab 3A family expressed in insulin secreting cells are also involved in the control of insulin release in rat and hamster (Regazzi et al 1996).

2.4. ROLE OF NEUROTRANSMITTERS IN INSULIN REGULATION

2.4.1. 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-PO4. 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 α1, α2 and β-adrenergic receptors. α1 has three subclasses- α1A, α1B, α1C (Price et al. 1994) and α2 has α2A, α2B and α2C. (Hamamdzic et al., 1995). β-adrenergic receptors are subclassified into β1, β2 and β3 (Dohlman et al., 1991). EPI and NE bind to these receptors in a concentration dependant manner. At low concentration EPI and NE can bind and activate β-adrenergic receptors which in turn stimulate the insulin secretion from pancreatic islets and at high concentration they can bind to α2A receptors and inhibit insulin secretion (Lacey et al. 1991).
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 adenylate cyclase inhibits insulin secretion. $\beta_3$ adrenoceptors stimulation also results in enhanced insulin secretion (Alef et al., 1996).

2.4.2. Dopamine

High concentrations of dopamine in pancreatic islets can decrease glucose stimulated insulin secretion (Tabeuchi et al., 1990). L-DOPA, the precursor of dopamine had similar effect to that of dopamine (Lindstrom & Sehlin, 1983). Dopamine $D_3$ receptors are implicated in the control of blood glucose levels (Alster & Hillegaart, 1996). Dopamine ($D_1$) receptors have also been reported to be present on pancreatic $\beta$-cells (Tabeuchi et al., 1990). These clearly indicate the role of dopamine in the regulation of pancreatic function.

2.4.3. Acetylcholine

Acetylcholine is a principle transmitter of the parasympathetic system. Acetylcholine, through vagal and non-vagal muscarinic pathways (Greenberg & Pokol, 1994) increases insulin secretion via muscarinic receptors on pancreatic islet cells (Tassava et al., 1992).

2.4.4. $\gamma$-Aminobutyric acid (GABA)

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 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 et al 1990). GABA through its receptors have been demonstrated to attenuate the glucagon and somatostatin secretion from pancreatic $\alpha$-cells and $\delta$-cells respectively.
GABA which is present in the cytoplasm and in synaptic-like microvesicles (Reetz et al., 1991) is co-released with insulin from \( \beta \)-cells in response to glucose. The released GABA inhibits islet \( \alpha \) and \( \delta \)-cell hormonal secretion in a paracrine manner. During diabetes the destruction of \( \beta \)-cells lead to decrease in GABA release resulting in the enhancement of glucagon secretion from \( \alpha \)-cells leading to hyperglycaemia. The brain GABAergic mechanisms also play an important role in glucose homeostasis. Inhibition of central GABA\(_A\) receptors increases 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.

2.4.5. Serotonin

The brain 5-HT content is decreased during diabetes (Jackson & Paulose, 1999; Sandrini et al. 1997; Sumiyoshi et al. 1997; Thorpe et al., 1997; Shimizu, 1991; Chu et al. 1986; Kulikov et al. 1986). This decrease is reported to be due to a decrease in uptake of tryptophan through the BBB (Madras et al., 1974; Fernstrom & Wurtman, 1972; Fernstrom & Wurtman, 1971) and a decrease in rate of 5-HT synthesis (Carndall et al. 1981). The turnover rate of 5-HT to 5-HIAA in diabetic rats was also reported to be lower (Sandrini et al., 1997; Kwok & Juorio, 1987). A decrease in brain 5-HT will lead to an up-regulation of 5-HT\(_{2A}\) receptors of cerebral cortex and brain stem which in turn can inhibit insulin secretion due to increased sympathetic activity (Jackson & Paulose, 1999).

2.5. CLASSIFICATION OF 5-HT RECEPTORS

Protein receptors that mediate the actions of 5-HT have existed in the membranes of a variety of animal cell types for millions of years. Their ancestry have been traced to be older than adrenoreceptors (Hen, 1992; Venter et al., 1988). It is likely that during such a long period of time the older receptors must have undergone mutations and during evolution a number of its variants or subclasses must have been formed. This undoubtedly is the case with 5-HT and NE receptors. Of all the neurotransmitter receptors 5-HT receptors have the largest number of variants or subclasses.
The 5-HT receptors can be classified into seven main classes (Peroutka, 1993). They comprise the 5-HT₁, 5-HT₂, 5-HT₃, 5-HT₄, 5-HT₅ and the recently cloned 5-HT₆ and 5-HT₇ but these receptors are yet to be fully characterised operationally and transductionally in intact tissues.

2.5.1. 5-HT₁ receptor family

5-HT₁ receptors were first identified as a high-affinity site for 5-HT in radioligand binding studies on brain homogenates using [³H]5-HT (Peroutka & Synder, 1979). Several subtypes of 5-HT₁ receptor family have been characterised. They are 5-HT₁A, 5-HT₁B and 5-HT₁D. These subtypes can be labelled in brain under appropriate conditions with [³H]8-OH-DPAT, [¹²⁵I]Cyanopindolol, [³H]Mesclergide and [¹²⁵I]GTLI respectively (Boulenguez et al., 1992; Bruinvels et al., 1991, Hoyer et al. 1985a; Hoyer et al. 1985b Gozlan et al., 1983).

2.5.1.1. 5-HT₁A receptors

5-HT₁A receptors are most widely distributed in the hippocampus and dorsal raphe (Radja et al., 1991, Marcinkiewicz et al., 1984). Many of these regions are components of pathways involved in the modulation of emotion and the limbic system. Their distribution is common to several mammals including humans (Pazos et al 1987a; Hoyer et al 1986a). The presence of high densities of 5-HT₁A receptors in raphe nuclei indicates that 5-HT can modulate the activity of serotonergic neurons. 5-HT₁A receptors are also present in the cerebral cortex, hypothalamus and the substantia gelatinosa of the spinal cord. The localisation of 5-HT₁A receptors in these areas also suggests that 5-HT₁A mechanisms could also be involved in the function of the hypothalamus and in the integrative function of the cerebral cortex.

The 5-HT₁A receptor was also reported to exist in two isoforms in rat brain regions, i.e., a high affinity 5-HT₁A receptor and a low affinity 5-HT₁A receptor. They are two independent 5-HT₁A receptor proteins rather than two inter-convertible stages of a single protein. These two isoforms can be labelled by high and low concentrations of [³H]8-OH-DPAT (Nenonene et al 1994).
A number of agonists show selectivity for 5-HT\textsubscript{1A} receptors e.g., 8-OH-DPAT, 5-CT, buspirone, ipsapirone, gepirone, 5-methyl-urapidil, flesinoxan and MDL72832 (Richardson & Hoyer, 1990). The most significant antagonists for 5-HT\textsubscript{1A} are NAN190 (Glennon et al. 1988), MDL73005 (Hibert & Moser, 1990), 5-F-8-OH-DPAT and (±)WAY100135, of these (±)WAY100135 has been described as a selective antagonist that is devoid of any partial agonist activity (Bill et al., 1993). [\textsuperscript{3}H]8-OH-DPAT has been used as the radioligand for 5-HT\textsubscript{1A} receptors (Gozlan et al. 1983).

The 5-HT\textsubscript{1A} receptor is coded by a single intron-less mRNA. The human 5-HT\textsubscript{1A} receptor gene was first identified by screening a human library with probes for the \(\beta_2\)-adrenoreceptor isolated from clone G21 (Kobilka et al., 1987). G21 is intron-less and the corresponding protein has 421 amino acids with 7 transmembrane domains. The rat 5-HT\textsubscript{1A} receptor has also been cloned (Albert et al., 1990) and the receptor has 99\% sequence homology with the human equivalent in the putative trans-membrane domains. The G-protein coupling to 5-HT\textsubscript{1A} receptor appears to mediate both stimulation and inhibition of adenylate cyclase activity (Shenker et al., 1987, Shenker et al., 1985, Shenker et al 1983). The 5-HT\textsubscript{1A} may be coupling to at least two different G-proteins (G\(_{i}\) and G\(_{o}\)) in the same tissue or alternatively, inhibition and stimulation of adenylate cyclase are mediated by two closely related receptors, which are difficult to distinguish pharmacologically. Transduction system other than adenylate cyclase has been described for 5-HT\textsubscript{1A} receptors. Andrade et al (1986) reported the presence of pertussis toxin-sensitive G-protein that couples 5-HT\textsubscript{1A} receptors in hippocampal pyramidal cells to a K\textsuperscript{+} channel. Activation of the receptor leads to channel opening and hyperpolarisation.

In HeLa cells, it has been reported that 5-HT\textsubscript{1A} receptors mediate sodium-dependent potassium transport and Na\textsuperscript{+}/K\textsuperscript{−} ATPase activity (Raymond et al. 1991, Middleton et al., 1990; Fargin et al 1989; Raymond et al 1989). Eventhough there are reports of different second messenger coupling for 5-HT\textsubscript{1A} receptor, at present it appears that this receptor like the other members of 5-HT\textsubscript{1} family negatively couples to adenylate cyclase via \(\alpha_2\) adrenergic receptor. There are also reports that some isoforms of cyclase (type-II and IV) which are present in the brain can be activated by \(\beta/\gamma\)-subunits (Tang & Gillman, 1991). This has been demonstrated by injecting Xenopus oocytes with mRNAs
for the 5-HT$_{1A}$ receptor in combination with adenylate cyclase type-II and cystic fibrosis transmembrane conductance regulator gene (Uezono et al., 1993). Activation of 5-HT$_{1A}$ receptor leads to cAMP production via protein kinase A, which stimulates the cystic fibrosis transmembrane conductance regulator leading to chloride channel activation.

2.5.1.2. 5-HT$_{1B}$ receptors

High densities of 5-HT$_{1B}$ receptors are found in the globus pallidus and pars reticulata of the substantia nigra (Pazos & Palacios, 1985). In addition, the terminal autoreceptors of rat cortex has also been identified as 5-HT$_{1B}$ receptors (Middlemiss, 1986; Middlemiss, 1985; Middlemiss, 1984). Functionally the 5-HT$_{1B}$ receptors in vena cava appear to mediate NE release (Gothert et al., 1986b). The 5-HT$_{1B}$ receptors are also associated with DNA synthesis in hamster fibroblast (Seuwen et al. 1988). 5-HT$_{1B}$ receptors mediate hyperlocomotor activity produced by the 5-HT$_{1B}$ agonist RU 24969 (Lucki, 1992). Activation of 5-HT$_{1B}$ receptors also mediate hypophagia (Kennet & Curzon, 1988a).

Some of the indol β-adrenoreceptor antagonists such as SDZ 21009 and cyanopindolol act as 5-HT$_{1B}$ antagonists. 5-HT$_{1B}$ receptor binding can be performed with $[^{3}H]$5-HT in the presence of blocking concentrations of 5-HT$_{1A}$ and 5-HT$_{2C}$ receptor ligands (Peroutka et al., 1988) or with $[^{125}I]$Iodocyanopindolol in the presence of 30μM isoprenaline to avoid β-adrenoreceptor binding (Hoyer et al., 1985a).

The rat 5-HT$_{1B}$ receptor gene is intron-less, encoding a 386-amino acid protein, and has 96% homology in the TMR with the equivalent human clone (Adham et al. 1992, Voigt et al. 1991). The 5-HT$_{1B}$ receptors are negatively coupled to adenylate cyclase (Bauhelal et al. 1988).

2.5.1.3. 5-HT$_{1D}$ receptors

The 5-HT$_{1D}$ receptors have been found to exist in brain of a wide range of non-rodent mammalian species including guinea pig, rabbit, dog, pig, calf and human (Maura et al., 1993; Beer et al., 1992). The 5-HT$_{1B}$ sites appear to be absent in these species and
5-HT1D receptor reflects the distribution and function of 5-HT1D receptors found in rodents. Radioreceptor assay is done using [3H]5-HT in presence of 100mM 8-OH DPAT and mesulergine to block 5-HT1A/5-HT1C binding, but these binding conditions are not homogenous and includes 5-HT1E receptors (Beer et al., 1992; Sumner & Humphrey, 1989; Hoyer & Neigt, 1988a). Activation of 5-HT1D receptors leads to inhibition of forskolin-stimulated adenylate cyclase activity in calf and guinea pig substantia nigra (Waeber et al., 1989; Hoyer & Shoefelter, 1988b). In addition, most studies performed with cells transfected with 5-HT1D receptors (both 5-HT1DA and 5-HT1DB) show that these receptors are negatively coupled to adenylate cyclase.

2.5.1.4. 5-HT1E receptors

5-HT1E is present in human frontal cortex and other brain regions similar to 5-HT1D receptor in varying relative proportions (Beer et al., 1992, Lowther et al. 1992, Leonhardt et al., 1989). The function of 5-HT1E receptor is not clearly known, although it appears to be coupled negatively to adenylate cyclase. The receptor consists of a single protein of 365 amino acids.

2.5.1.5. 5-HT1F receptors

The mRNA for 5-HT1F is concentrated in the dorsal raphe, hippocampus and cortex (Adham et al., 1993) but is not found in kidney, liver, spleen, heart and pancreas. In NIH3T3 cells, the transfected 5-HT1F receptor clones show negative coupling to adenylate cyclase like other 5-HT1 receptors. The intron-less gene for 5-HT1F receptor has a long open reading frame encoding a protein of 366 (human and rat) or 367 (mouse) amino acids in length (Adham et al., 1993, Lovenberg et al. 1993; Almaiky et al., 1992).

2.5.1.6. 5-HT1-like receptors

5-HT1-like receptors are a group of related receptors that have not yet been positively equated with any of the 5-HT1-binding site subtypes, identified in the CNS. These receptors mediate a number of functions like smooth muscle contraction and decreased EPI release from sympathetic nerves.
2.5.2. 5-HT\textsubscript{2} receptor family

The 5-HT\textsubscript{2} subclass have been further classified into 5-HT\textsubscript{2A}, 5-HT\textsubscript{2B} and 5-HT\textsubscript{2C} receptors, each of these receptors have been cloned and consists of a G-protein coupled single protein with seven trans-membrane domains (458 to 471 amino acids). All these subtypes mediate their effects through activation of phosphoinositide metabolism.

2.5.2.1. 5-HT\textsubscript{2A} receptor

5-HT\textsubscript{2A} receptors are widely distributed in peripheral tissues and cortex (Pazos et al., 1987b; Bardley et al., 1986a; Hoyer et al. 1986b; Pazos & Palacios, 1985). The effects mediated by these receptors include contractile response of vascular, bronchial, uterine and urinary smooth muscles. The 5-HT\textsubscript{2A} receptors also mediate platelet aggregation and increased capillary permeability. In the neocortex, these sites are mainly concentrated in laminae I and IV (rat) and III and V (human). In addition, 5-HT\textsubscript{2A} receptor distribution is found in the claustrum, some components of limbic system, particularly the olfactory nuclei and parts of basal ganglia.

Leysen et al. (1982) have reported that \[^{[3]H}\] ketanserin can be used as a selective ligand for the 5-HT\textsubscript{2A} receptors. Two subtypes of 5-HT\textsubscript{2A} receptors have been proposed and labelled by \[^{[3]H}\]DOB and \[^{[3]H}\] ketanserin (Peroutka et al. 1988). The 5-HT\textsubscript{2A} receptor polypeptide contains seven transmembrane regions and the amino acid sequence within the transmembrane regions is 80% identical with that of the 5-HT\textsubscript{2C} receptor. The 5-HT\textsubscript{2A} receptors are linked to phosphatidylinositol turnover. The receptors are coupled to phospholipase C, and inositol phospholipid hydrolysis and Ca\textsuperscript{2+} mobilisation are involved in the post-receptor events (Conn & Sanders-Bush, 1984a).

2.5.2.2. 5-HT\textsubscript{2B} receptor

When functionally expressed in COS cells, the 5-HT\textsubscript{2B} receptors display high affinity for \[^{[3]H}\]5-HT and \[^{[32]}\]DOI. The receptor is coupled to phospholipase C. The human receptor protein is 80% homologous to the rat receptor and the intron/exon distribution in the gene is conserved in both species (Foguet et al., 1992).
2.5.2.3. \textit{5-HT}_{2c} receptor

The \textit{5-HT}_{2c} receptors are distributed throughout the choroid plexus of all mammals (Yagaloff \& Hartig, 1985). The \textit{5-HT}_{2c} transcripts are also found in significant densities in the olfactory nucleus, cingulate cortex and subthalamic nucleus (Mengod \textit{et al.}, 1990). The gene for \textit{5-HT}_{2c} has introns and it is possible that different gene products can occur due to alternate splicing. The protein sequence consists of 460 amino acids. The mouse and human homologues have been cloned and show 98\% homology in the transmembrane regions (Yu \textit{et al.}, 1991).

2.5.3. \textit{5-HT}_{3} receptor

\textit{5-HT}_{3} receptors are found exclusively associated with neurons of both central and peripheral origin and in a variety of neuronally derived cell lines such as NIE-115, NCB-20 and N18 cells (Peters \textit{et al.} 1991). In the brain, the highest densities of \textit{5-HT}_{3} receptors are found in discrete nuclei of the lower brain stem and the substantia gelatinosa at all levels of the spinal cord (Pratt \textit{et al.}, 1990). \textit{5-HT}_{3} receptor is a ligand-gated ion channel receptor. The activation of this receptor triggers a rapid depolarisation and a rapid influx of Ca\textsuperscript{2+} into the cytosol from the extracellular environment (Peters \textit{et al.} 1991).

2.5.4. Other \textit{5-HT} receptors

The \textit{5-HT}_{4} receptors are located on nerve cells where they mediate inhibition of voltage-activated potassium channels via stimulation of a cAMP-dependent protein kinase (Fagni \textit{et al.}, 1992). The \textit{5-HT}_{5} receptors are further classified into \textit{5-HT}_{5A} and \textit{5-HT}_{5B} receptors. Both these receptors show pharmacological properties similar to \textit{5-HT}_{1} receptors (Matthes \textit{et al.}, 1993). The \textit{5-HT}_{6} receptor has been cloned and belongs to a G-protein coupled receptor family. The receptor consists of 436 amino acids and has 36\% homology in the transmembrane region with that of various \textit{5-HT}_{1} and \textit{5-HT}_{2} receptors (Rout \textit{et al.}, 1993a). The \textit{5-HT}_{7} receptor is positively linked to adenylate cyclase and is predominantly expressed in rat hypothalamus and to a lesser extent in other brain regions (Lovenberg \textit{et al.} 1993, Rout \textit{et al.}, 1993b).
2.6. EFFECT OF 5-HT ON BLOOD GLUCOSE LEVEL

There are conflicting reports about the effect of 5-HT on blood glucose level. It has been reported that 5-HT can induce both hypoglycaemia and hyperglycaemia (Sugimoto et al., 1990; Itaya & Itoh, 1979). In normal mice, 5-HT induced a dose-dependent hypoglycaemia and an increase in serum insulin level. 5-HT also inhibited glucose-induced hyperglycaemia and increased glucose-stimulated insulin release. But in STZ—induced diabetic mice, 5-HT changed neither the glucose nor insulin levels (Sugimoto et al., 1990). Itaya & Itoh, (1979) reported an increase in plasma cAMP and glucose after i.p. administration of 5-HT.

2.7. 5-HT IS PRESENT WITHIN THE PANCREATIC ISLETS

Islet monoamines are located in the insulin storage granules (Bird et al. 1980; Jain-Etcheverry & Zieher, 1968; Falck & Hellmann, 1963). Consequently the role of intracellular islet monoamines in the regulation of insulin secretion has been the subject of many investigations. Pharmacological manipulations of pancreatic islet serotonin and dopamine content in in vitro and in vivo systems has resulted in evidence for monoaminergic inhibition (Feldman et al., 1972a; Feldman & Leboritz, 1972b) and stimulation of insulin secretion (Telib et al., 1968). Several amino acids are able to stimulate insulin release in the presence of glucose. 5-HTP is readily taken up into the islet in the presence of glucose and stimulates insulin secretion. But the enzyme 5-hydroxytryptophan decarboxylase readily converts it into 5-HT that inhibits insulin secretion (Sundler et al., 1990; Lindstrom & Sehlin, 1983). When 5-HTP was tested in conjunction with a decarboxylase inhibitor, the glucose stimulated insulin release from rabbit pancreas was significantly enhanced (Gylfe et al. 1973). Tryptophan, a precursor of 5-HTP, has a stimulating effect on insulin release from hamster pancreas. The presence of monoamine oxidase enzyme that catabolises5-HT within the β-cells shows an effective 5-HT metabolism within the islets (Pizzinat et al. 1999; Feldman & Chapman, 1975). 5-HT can also act as a marker for insulin secretion. 5-HT is taken up into insulin granules and co-released with insulin on stimulation of pancreatic β-cells by glucose (Zhou & Misler, 1996). All these evidences show the presence of 5-HT within the pancreatic islets.
and has a role in the regulation of insulin secretion from the β-cells. An increase in the level of EPI was also noted in diabetic islets. However, in total pancreas the NE and EPI contents were same in diabetic and non-diabetic rats (Ostenson et al. 1993).

2.8. ROLE OF TRYPTOPHAN IN THE PHYSIOLOGICAL REGULATION OF BRAIN SEROTONIN

Administration of L-tryptophan which is a precursor of 5-HT can increase the brain 5-HT content during diabetes. The rate limiting enzyme, tryptophan hydroxylase is usually not saturated with tryptophan. Any process that increases the brain tryptophan leads to an increase in brain 5-HT content (Curzon & Mursden, 1975, Friedman et al. 1972; Ecclestron et al. 1965). Administration of an amino acid mixture, containing all essential amino acids but not tryptophan caused a parallel depletion of total and free serum tryptophan and thereby decrease in brain tryptophan and serotonin (Biggo et al. 1975). An intraperitoneal administration of 50-100mg/kg L-tryptophan brought an increase in hypothalamic tryptophan (286%), 5-HT (23%) and 5-HIAA (20%) after 30min. Rest of the brain also showed an increase in tryptophan (256%), 5-HT (29%) and 5-HIAA (12%) after 30min. Administration of 100mg/kg i.p p-chlorophenylalanine (p-CPA) which is a tryptophan hydroxylase inhibitor did not bring any increase in brain 5-HT after administration of L-tryptophan. Hutson et al. (1985) have reported a similar increase in 5-HT and 5-HIAA in the cerebrospinal fluid (CSF) as seen in brain after i.p. administration of tryptophan.

2.9. INSULIN IS A MAJOR DETERMINANT FOR THE TRANSPORT OF TRYPTOPHAN ACROSS THE BLOOD-BRAIN-BARRIER

The major determinant of brain tryptophan concentration is insulin as this can result in decreased plasma concentration of large neutral amino acids (valine, leucine, and isoleucine). These amino acids compete with tryptophan for uptake into the brain across the BBB (Madras et al., 1974; Fernstrom & Wurtman, 1972; Fernstrom & Wurtman, 1971). Trulson & Mackenzie, (1978) have reported that after 4 weeks of administration of streptozotocin the brain tryptophan content was decreased by 27%. Insulin administration
was able to bring back the brain tryptophan and 5-HIAA levels to normal. Tryptophan uptake across the BBB is increased in the presence of insulin. Insulin enhances the uptake of branched chain amino acids thereby decreasing their plasma concentration. Since these amino acids compete with tryptophan for transport into brain, there is a resultant increase in brain tryptophan (Curzon & Mursden, 1975).

There are also contradicting reports that during diabetes there is an increase in brain tryptophan uptake (Demontis et al 1977). They state that during diabetes there is an increase in lipolysis. The free fatty acids will tend to bind to plasma albumin. This will increase the free plasma tryptophan and thereby increases the chances for tryptophan uptake into brain. From the above reports we can conclude that the effect of insulin on brain tryptophan is not direct, but mediated via insulin induced changes in serum tryptophan to other competing amino acids ratio.

**2.10. DIET CAN INFLUENCE BRAIN SEROTONIN SYNTHESIS**

Tryptophan is transported into the brain by a competitive carrier system that is shared by large neutral amino acids such as tyrosine, phenylalanine, leucine, isoleucine and valine. Physiological variations in the plasma neutral amino acid pattern, either as a change in plasma tryptophan or in the plasma concentration of one or more of its competitors directly alters this competitive process. This variation in tryptophan uptake influences brain tryptophan level and thus serotonin synthesis (Fernstrom & Fernstrom, 1995; Fernstrom, 1991, Fernstrom, 1979; Biggio et al 1974). It is not only tryptophan that is influenced by the diet but other amino acids such as tyrosine, which is the precursor for DA and NE, is also influenced by diet. The same process is applicable for the uptake of choline, the precursor of acetylcholine (Fernstrom, 1994, Fernstrom, 1977; Wurtman & Fernstrom, 1975). A similar observation was reported by DeMarte & Enesco, (1985). They maintained a group of mice for 78 weeks on tryptophan restricted, protein restricted and control diet. They found that brain 5-HT levels were significantly reduced only in mice on the tryptophan-restricted diet, but not in mice on the protein restricted diet.