INTRODUCTION
1. INTRODUCTION

Diabetes is a metabolic disorder associated with insulin deficiency, which not only affects the metabolism of carbohydrates but is also associated with central as well as peripheral neuropathy. There are various factors, which regulate insulin secretion from the pancreatic β-cells, such as metabolic substrates, hormones and neurotransmitters. The central nervous system (CNS) neurotransmitters such as norepinephrine and serotonin play an important role in glucose homeostasis. These neurotransmitters mediate rapid intracellular communication within the nervous system by interacting with cell surface receptors. These receptors often trigger second messenger signalling pathways. Although neurotransmitter receptors by definition have been restricted to the nervous system, these receptors and second messenger systems have been observed in both neural and non-neural cells (Julius et al., 1989).

Since the early seventies, the hypothesis for a control of circulating glucose and insulin levels by serotonin (5-HT) systems has been the matter of numerous works. There are reports on reductions in central nervous system (CNS) 5-hydroxytryptamine synthesis and turn over in chronically hyperglycaemic rats (Bellush & Reid, 1991, Trulson et al., 1986). It has been well documented that long term hyperglycaemia in diabetic animals can lead to decreased functions of central 5-HT neurons leading to reduced brain tryptophan, 5-HT and 5-hydroxy indole acetic acid (5-HIAA). This decrease in brain 5-HT is due to the reduced uptake of tryptophan into the brain. One of the main determinants of brain tryptophan content is the circulating insulin level. An increase in the level of insulin can result in decreased plasma concentrations of large neutral amino acids, which compete with tryptophan for uptake into the brain. Streptozotocin (STZ) selectively destroys pancreatic β-cells and causes hypoinsulinemia leading to hyperglycaemia.

The effect of serotonin is mediated in different tissues by different subclasses of serotonin (5-HT) receptors, each of which are coded by a distinct gene and possess distinct pharmacological properties and physiological functions (Hoyer et al., 1991). Moreover, serotonin receptor subtypes couple to different intracellular signalling systems. In neurons that express the 5-HT2A receptors, receptor activation is likely to generate inositol polyphosphates that release intracellular calcium ions (Hoyer & Shoeffeter, 1988b). The
5-HT<sub>1A</sub> receptors mediate inhibition of adenylate cyclase activity through G-protein coupling (Shenker et al., 1987; Shenker et al., 1985; Shenker et al., 1983). Serotonin receptor subtypes, including 5-HT<sub>1A</sub>, 1b, 1c, 5-HT<sub>2</sub> and 5-HT<sub>3</sub>, have been defined on the basis of their pharmacological properties (Hoyer & Shoelfeter, 1988b). The existence of multiple receptor subtypes provides one mechanism by which a single neurotransmitter can elicit distinct cellular responses. The association of individual receptor subtypes with different G proteins and different signalling systems can achieve the variation in cellular response. Further flexibility is provided by the ability of distinct receptors for the same ligand to activate or inhibit the same second messenger system (Chung et al., 1988).

Neurotransmitter changes in the hypothalamus lead to impairment of the hypothalamic-pituitary-end organ axis. The secretion of adrenocorticotropic hormone, growth hormone, prolactin, thyroid stimulating hormone and the gonadotropins by the pituitary is governed by releasing factors from the hypothalamus. Regulation of the release factors from the hypothalamus involves complex neural circuit in which the serotonergic neurons represent one link in the control mechanism (Kruilich, 1979). Hypothalmo-pituitary-thyroid-response, that is, the secretion of thyroid stimulating hormone (TSH) is directly controlled by two factors, a negative feedback signal indicating serum thyroid status and a stimulatory factor, thyrotropin releasing hormone (TRH) secreted by the hypothalamus (Chen & Ramirez, 1981). The postulate that 5-HT neurons stimulate TSH secretion in rats is supported by the observation that injection of 5-HT into the third ventricle caused rapid increase in serum TSH and the effect was completely reversed by pretreatment of rats with cyproheptadine, a serotonin receptor antagonist. Dakshinamurti et al., (1985) have reported that pyridoxine-deficiency can lead to hypothyroidism accompanied by decreased level of hypothalamic serotonin without any change in brain norepinephrine and dopamine. This decreased serotonin reduces the synthesis and release of TSH from the pituitary through TRH secretion (Dakshinamurti et al., 1986). Smythe et al., (1982) have also reported that TSH release is controlled by 5-HT neuronal activity.

Recent studies have established a functional correlation of serotonergic and adrenergic function in the brain regions with insulin secretion in diabetic rats (Vahabzadeh et al., 1995). Administration of 5-HT<sub>1A</sub> agonist 8-OH-DPAT to conscious rats caused an
increase in blood glucose level. This increase in blood glucose is due to inhibition of insulin secretion by increased circulating EPI (Chaouloff et al., 1990a; Chaouloff et al., 1990d; Chaouloff & Jeanrenaud, 1987). The increase in EPI is brought about by increased sympathetic stimulation. This increase can lead to increased sympatho-medullary stimulation thereby inhibiting insulin release (Bauhelal & Mir, 1993, Bauhelal & Mir, 1990a; Chaouloff et al., 1990d). Also, studies have shown that Gi protein in the liver has been decreased in diabetes which will increase gluconeogenesis and glycogenolysis thereby causing hyperglycaemia (Pennington, 1987). Serotonergic control is suggested to exert different effects on insulin secretion according to the activation of different receptor subclasses (Pontiroli et al., 1975). In addition to this mechanism, the secretion of insulin is dependent on the turnover ratio of endogenous 5-hydroxy tryptophan (5-HTP) to 5-HT in the pancreatic islets (Jance et al., 1980).

The reports so far stated does not explain the complete mechanism and the subclass of 5-HT receptors whose expression regulate insulin secretion in a diabetic state. Also, there is no report of a direct regulation of insulin secretion by 5-HT from the pancreatic islets even though there are reports stating that the pancreatic islets is a rich source of 5-HT (Bird et al., 1980). Therefore, in the present study the mechanism by which 5-HT and its receptors regulate insulin secretion from pancreatic β-cells was investigated. Our results led to the following hypotheses by which 5-HT and its receptors regulate the insulin secretion.

**Central nervous system control of insulin secretion:**- This pathway is triggered by a decrease in brain 5-HT content brought about by a decrease in transport of tryptophan across the blood-brain-barrier (BBB). This transport of tryptophan across the BBB depends on the circulating insulin and tryptophan levels. There are a number of reports which state that a tryptophan deficient diet can lead to decreased circulating tryptophan and decreased uptake of it into the brain leading to decreased brain 5-HT synthesis (Fernstrom & Fernstrom, 1995; Fernstrom, 1991, Fernstrom, 1979; Biggio et al., 1974). This decreased brain 5-HT stimulates the over expression of 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors in the brain region, which lead to sympathetic stimulation that inhibits insulin release. Once the circulating insulin content is reduced, it leads to an
increase in large neutral amino acids, which compete with tryptophan for uptake into brain.

> **Peripheral control of insulin secretion:** This regulation occurs directly within the pancreatic β-cells. During diabetes the amount of 5-HT within the pancreatic islets increases. This excess 5-HT binds and down regulates the nuclear receptors and may directly alter the transcription of insulin gene from the β-cells.
SEROTONERGIC SYSTEM AND INSULIN REGULATION IN DIABETES MELLITUS

DECREASED CIRCULATING INSULIN

DECREASED TRYPTOPHAN TRANSPORT ACROSS BLOOD - BRAIN - BARRIER

DECREASED 5-HT

INCREASED 5-HT1A & 5-HT2A RECEPTORS

ADRENAL MEDULLA

SYMPATHETIC STIMULATION

EPINEPHRINE

HYPERGLYCEMIA

DECREASED INSULIN SECRETION

↑ 5-HT UPTAKE

(+)

↑ 5-HT TRANSPORTERS

(+)

(-)

(-)
1.1 MAJOR OBJECTIVES

1. To study the changes in monoamines and their metabolites in cerebral cortex (CC), brain stem (BS), hypothalamus (Hypo) and pancreas by high performance liquid chromatography (HPLC) in control, diabetic and diabetic rats treated with insulin, tryptophan alone and in combination with insulin.

2. To study the role of brain serotonin and its receptors in insulin secretion from pancreatic β-cells.

3. To study the kinetic parameters of serotonin receptors with special emphasis on 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors in brain regions and pancreatic islets of control, diabetic, diabetic + insulin, diabetic + tryptophan and diabetic + insulin + tryptophan treated rats.

4. To study alterations in G protein of 5-HT$_{1A}$ receptors using Gpp[NH]p.

5. To establish a functional correlation of brain 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors and insulin secretion during diabetes.

6. To study insulin secretion, in vitro, from isolated pancreatic β-cells in presence of 5-HT.

7. To study the role of tryptophan on brain 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors in diabetic state.

8. To establish the role and mechanism of peripheral 5-HT in regulating insulin release by its binding to nuclear proteins.

9. To study the expression of 5-HT$_{2A}$ receptor during diabetes and in diabetic rats treated with insulin, tryptophan and insulin + tryptophan by RT-PCR technique.