INTRODUCTION

Brain neurotransmitters through their receptors or hormonal pathway can regulate physiological functions in diabetes and cell proliferation. Serotonin, also known as 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter found in cardiovascular tissue, the peripheral nervous system, blood cells and the central nervous system. 5-HT has been implicated in the regulation of diverse physiological processes, including cellular growth and differentiation (Tecott *et al.*, 1995), neuronal development (Eaton *et al.*, 1995) and regulation of blood glucose concentration (Smith & Pogson, 1977). Since the early seventies, the hypothesis for a control of circulating glucose and insulin levels by 5-HT system has been the matter of numerous works. There are reports on reduction in central nervous system (CNS) 5-HT synthesis and turn over in chronically hyperglycaemic rats (Trulson *et al.*, 1978). Specific 5-HT receptor agonists and antagonists can modulate circulating levels of blood glucose in rodents. 5-HT promotes hyperglycaemia by a mechanism that may involve increased renal catecholamine release (Wozniak & Linnoila, 1991). There is a prevailing view that blood glucose is lowered by 5-HT and that this response can be suppressed by 5-HT receptor antagonists (Furman & Wilson, 1980). All these evidences show that 5-HT has a role in the regulation of insulin secretion.

Recent observations indicate that insulin can stimulate pancreatic β-cell growth *in vivo*. The level to which β-cell proliferation increased is related to the degree to which insulin biosynthesis and/or release is enhanced (Chick *et al.*, 1975). Pancreatic regeneration after pancreatectomy has been well documented in animal models to study β-cell proliferation (Pearson *et al.*, 1977). Various hormones and growth factors have been shown to affect the proliferation of the endocrine and exocrine cell types of pancreas. The addition of new β-cells would increase the total insulin secretary potential. Studies have shown that insulin secretion is modulated by

the central nervous system, through its sympathetic and parasympathetic division, although glucose and other substrates are generally thought to be the principal regulators of insulin secretion. The pancreatic islets are richly innervated by parasympathetic, sympathetic and sensory nerves (Miller, 1981). Several different neurotransmitters are stored within the terminals of these nerves - serotonin, acetylcholine, noradrenaline and several neuropeptides. Stimulation of the autonomic nerves and treatment with neurotransmitters affect islet hormone secretion.

Most of the neurons synthesizing 5-HT in CNS are located in raphe nuclei of brain stem, but serotonergic nerve terminals can be found in virtually every brain region. These 5-HT containing cells give rise to ascending and descending pathways that innervate large areas of the brain and spinal cord. These pathways largely mediate the varied roles of 5-HT in sensory, motor and autonomic functioning. Dorsal motor nucleus of brain stem is connected to the endocrine pancreas exclusively via vagal fibres and has a role in neurally mediated insulin release (Azmitia & Gannon, 1986)

The effect of 5-HT is mediated in different tissues by different subclasses of receptors, each of which are coded by a distinct gene and possesses distinct pharmacological properties and physiological functions. In addition to its role as a neurotransmitter, 5-HT also has been shown to play a role in cell proliferation (Seuwen & Poussegur, 1990). The mitogenic action of 5-HT was first identified in bovine aortic smooth muscle cells (Nemeck *et al.*, 1986). There is a synergistic effect of 5-HT with traditional protein growth factors such as platelet derived growth factor, fibroblast growth factor, epidermal growth factor and insulin like growth factor (Crowley *et al.*, 1994). In aortic smooth muscle cells, 5-HT induced mitogenesis was comparable with that of human platelet derived growth factor. 5-HT rapidly elevates superoxide formation, stimulates protein phosphorylation, and

enhances proliferation of bovine pulmonary artery smooth muscle cells (SMCs). 5-HT_{1A} receptor is coupled through Gi to the activation of ERK1 and ERK2 in CHO cells (Daniel *et al.*, 1996). 5-HT has been increasingly recognised to be a mitogen for both vascular and non-vascular cells. 5-HT rapidly induces tyrosine phosphorylation of GTPase activating protein (GAP), possibly activates p21ras and produces cellular hyperplasia and hypertrophy in bovine pulmonary artery. All agents that block transport of 5-HT and 5-HT receptor antagonists inhibit proliferative response.

5-HT receptors can be classified into seven classes from 5-HT_1 to 5-HT_7 based upon their pharmacological profiles, cDNA-deduced primary sequences and signal transduction mechanisms of receptors (Hoyer *et al.*, 1994). The 5-HT_{1A} receptor was reported to exist in two isoforms in rat brain regions, i.e., a high affinity 5-HT_{1A} receptor and a low affinity 5-HT_{1A} receptor. These two isoforms can be labelled by high and low concentrations of [^{3}H]8-OH DPAT (Nenonene *et al.*, 1994). 8-Hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) is a 5-HT_{1A} receptor-selective agonist that has recently been reported to trigger adrenal catecholamine release and hyperglycaemia by the activation of 5-HT_{1A} receptors (Chauloff & Jeanrenaud, 1987). The EPI releasing effect of 8-OH DPAT are blocked by both 5HT_{1A} and β-adrenoreceptor antagonist (-)-pindolol (Chauloff *et al.*, 1990b). Neurotransmitters that elevate cAMP inhibit cell proliferation. 5-HT_{1A} receptor agonists promote adenylyl cyclase activity by activation of stimulatory G proteins (Gs), inhibit proliferation (Fanburg & Lee, 1997). Neurite outgrowth is inhibited by micromolar amounts of 5-HT added to dissociated cultures of cortical or raphe neurons. 5-HT_1 agonists could potentially be providing long term repression of G protein coupled receptors and other MAP kinase-responsive genes. There are also reports regarding 5-HT_{1A} receptor mediated stimulation of cell division in different cell types. The human 5-HT_{1A} receptor expressed in Chinese hamster ovary cells

promotes activation of ERK1 and ERK2 (Daniel *et al.*, 1996). 5-HT_1 mediates inhibitory signaling pathways primarily in neuroendocrine cells, and stimulatory pathways are mostly restricted to mesenchymal and/or immune cells, this cell specificity appears not to be absolute. In hippocampal membranes, the 5-HT_1 receptor mediates increased rather than reduced cAMP levels, presumably via activation of adenylyl cyclase type II (ACII). Furthermore, an inhibitory signal, such as a decrease in cAMP, might lead to a stimulation of cell proliferation in mesenchymal cells. Inhibitory effects of 5-HT have been linked to activation of 5-HT_{1A} receptors.

5-HT_{2C} receptor is one of the three closely related receptor subtypes in 5-HT_2 receptor family. Administration of 5-HT_{2C} receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) triggers adrenal catecholamine release and hyperglycaemia. The hyperglycaemic effect of DOI administration is mediated by centrally located 5-HT_{2C} receptors and, in turn, adrenal epinephrine release due to increase in sympathetic nerve discharge (Welch & Saphier, 1994). This suggests that insulin release is inhibited by 5-HT_{2C} receptor agonist and the activation of 5-HT receptors may affect glycaemia. The 5-HT_2 receptor subtype of 5-HT has been shown to mediate cell growth in fibroblasts. 5-HT enhances EGF-stimulated DNA synthesis of mature rat hepatocytes in primary culture and this effect of 5-HT is suggested to be mediated by the 5-HT_2 receptors. *In vivo* studies indicated that the 5-HT_2 receptors were activated in the regenerating rat liver during DNA synthetic phase. These receptors are coupled to phosphoinositide turnover and diacylglycerol formation, which activates protein kinase C, an important second messenger for cell division. The 5-HT_{2C} receptors activate phospholipase. This receptor functions as a protooncogene when expressed in NIH 3T3 fibroblasts (Julius *et al.*, 1989). The

presence of 5-HT_{2C} receptors may initiate tumorigenesis by facilitating the growth of fibroblasts in the mouse.

Several studies have described the role of 5-HT_{1A} and 5-HT_{2C} receptors in neuroendocrine regulation and cell proliferation. The involvement of these receptors in the regulation of catecholamine release by facilitating sympathetic system has been examined. However, there have not been many studies examining the role of central 5-HT_{1A} and 5-HT_{2C} receptors and their relationship between sympathoadrenal secretions and insulin secretion during pancreatic regeneration. In the present study, the changes in the brain and pancreatic 5-HT, its receptor subtypes, and their gene expression were investigated during pancreatic regeneration in rats. The work focuses on the role of 5-HT_{1A} and 5-HT_{2C} receptor changes and their regulatory role in pancreatic islet cell proliferation.

OBJECTIVES OF THE PRESENT STUDY

1. To induce pancreatic regeneration by partial pancreatectomy in weanling rats.
2. To study the DNA synthesis by [^{3}H]thymidine incorporation during pancreatic regeneration.
3. To study the changes in 5-HT content in various rat brain regions – cerebral cortex (CC), brain stem (BS) and hypothalamus (Hypo) during pancreatic regeneration using High Performance Liquid Chromatography.
4. To study the changes in epinephrine and norepinephrine content in plasma and adrenals during pancreatic regeneration using High Performance Liquid Chromatography.
5. To study the 5-HT content in plasma and pancreas of experimental rats.
6. To study the 5-HT_{1A} and 5-HT_{2C} receptor changes in CC, BS, Hypo and in the pancreas of different experimental groups of rats.
7. To study the effect of 5-HT, 5-HT_{1A} and 5-HT_{2C} receptor ligands in insulin secretion using rat primary islet culture.
8. To study the effect of 5-HT, 5-HT_{1A} and 5-HT_{2C} receptor ligands in DNA synthesis using rat primary islet culture.
9. To study the gene expression of 5-HT_{1A} and 5-HT_{2C} receptors in the brain and pancreas of different experimental groups of rats.