INTRODUCTION

Diabetes mellitus is the metabolic disorder associated with inability of the pancreatic β-cells to secrete sufficient insulin or dysfunction of insulin receptors in target tissues. Glucose homeostasis is tightly controlled by insulin. The capacity of β-cells to proliferate plays a fundamental role in determining the onset and severity of carbohydrate intolerance in diabetes (Wang et al., 1994). The β-cell mass reduction is a critical event in the development of insulin dependent diabetes mellitus. The acute onset of the disease is preceded by a period of progressive destruction of the pancreatic islets (Swenne, 1992).

The new concept is that β-cell mass is dynamic and increases and decreases both in function and mass to maintain the glycaemic level within a narrow physiological range. The changes in mass can be in both number and individual volume of the β-cells. When the mass cannot increase adequately, diabetes ensues (Bonner-Weir, 2000).

The two mechanisms of β-cell formation from the embryo, neogenesis, or differentiation from ductal precursor cells and replication of a differentiated β-cell are maintained postnatally even in the adult. Experimentally, increased proliferation of differentiated β-cells is seen in a number of models including partially pancreatectomised rats (Bonner-Weir, 2000).

The pancreatic islets are richly innervated by parasympathetic, sympathetic and sensory nerves. Several neurotransmitters- acetylcholine, norepinephrine, and neuropeptides are stored within the terminals. Stimulation of autonomic neurotransmitters and treatment with neurotransmitters affect insulin secretion. The facilitator action of vagal nerves and splanchnic inhibitory modulation of insulin release has been demonstrated. The cholinergic nerve fibres innervating the islets are of postganglionic origin and emanate from the intrapancreatic ganglia. These ganglia are controlled by the preganglionic fibres, originating primarily in the dorsal motor
nucleus of the vagus. They enter the pancreas along the vessels and terminate at intrapancreatic ganglia, from which the postganglionic nerves pass to the islets, these nerves penetrate the islets to terminate close to the endocrine cells. The postganglionic nerve fibres innervating the islets release acetylcholine, which directly stimulates insulin secretion from the islet β-cells through activation of muscarinic receptors (Ahren, 2000).

Regeneration is a complex interplay of several factors - growth factors, hormones and neurotransmitters. Nutrients including glucose are reported to stimulate β-cell replication (Swenne, 1982; Hellerstrom et al., 1985). The stimulatory effect of growth hormone on insulin production and β-cell replication are well documented (Swenne et al., 1987; Nielsen, 1986, Sjoholm et al., 2000). \textit{In vitro} and \textit{in vivo} studies have established the role of insulin in β-cell replication (Chick et al., 1973). Insulin interacts with type I IGF receptor and stimulates β-cell proliferation.

Parasympathetic activity plays an important role in insulin secretion from pancreatic β-cells. Cholinergic agonist carbachol increases insulin secretion from isolated rat islets (Zawalich & Zawalich, 2002). The muscarinic receptor stimulation by acetylcholine (ACh) leads to activation of phospholipase C, which, in turn, hydrolyzes phosphatidylinositol 4, 5-bisphosphate (PIP2) to produce IP3 and diacylglycerol (Best & Malaisse, 1983; Zawalich et al., 1989). In pancreatic β-cells, IP3 mobilizes Ca²⁺ from intracellular stores, resulting in an elevation of the intracellular concentration of Ca²⁺ and allowing activation of Ca²⁺/calmodulin. Diacylglycerol on the other hand, activates PKC (Nishizuka, 1995; Renstrom et al., 1996). PKC, like Ca²⁺/calmodulin, accelerates exocytosis of insulin granules (Nanko et al., 2002).

The mitogenic effect of acetylcholine has been studied in different cell types. Muscarinic acetylcholine receptors activate many downstream signaling pathways, some of which can lead to mitogen activated protein kinase (MAPK) phosphorylation.
Mitogen activated protein kinases play a role in regulating cell growth, differentiation and synaptic plasticity. Both Gi and Gq coupled muscarinic receptors have been shown to activate MAPK in various systems. Muscarinic M3 receptors activate MAPK in the oligodendrocyte progenitors (Ragheb et al., 2001). The involvement of M1 receptors has been reported in muscarinic activation of MAPK in PC12 cells (Berkeley et al., 2000). Acetylcholine analogue carbachol stimulated DNA synthesis via muscarinic receptors in primary astrocytes derived from perinatal rat brain (Ashkenazi, 1989). Carbachol is also mitogenic in certain brain derived astrocytoma and neuroblastoma, as well as in Chinese hamster ovary (CHO) cells expressing recombinant muscarinic receptors (Ashkenazi, 1989). Proliferation experiments with subtype specific antagonists in astrocytes suggest that cell proliferation is induced by the activation of muscarinic M3 receptors (Guizzetti, 1996, 2002).

The present work is an attempt to understand the role of acetylcholine muscarinic M1 and M3 receptors during pancreatic regeneration and insulin secretion. The work focuses on the changes in the muscarinic M1 and M3 receptors in brain and pancreas during pancreatic regeneration. The effect of these receptor subtypes on insulin secretion and pancreatic β-cell proliferation were studied in vitro using rat primary pancreatic islet culture. Muscarinic M1 and M3 receptor kinetics and gene expression studies during pancreatic regeneration and insulin secretion will help to elucidate the role of acetylcholine functional regulation of pancreatic β-cell proliferation and insulin secretion.
OBJECTIVES OF THE PRESENT STUDY ARE:

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 cholinergic activity using acetylcholine esterase assay in the brain regions - cerebral cortex (CC), brain stem (BS), cerebellum (CB) corpus striatum (CS) and hypothalamus (Hypo) during pancreatic regeneration in rats.
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 total muscarinic, M1 and M3 receptor kinetic parameters in CC, BS, CS and Hypo during pancreatic regeneration.
6. To study the muscarinic M1 and M3 receptor kinetic parameters in the pancreatic islets of experimental rats.
7. To study the expression of muscarinic M1 and M3 receptor mRNA in the brain regions during pancreatic regeneration.
8. To study the effect of acetylcholine, muscarinic M1 and M3 receptor ligands in insulin secretion using rat primary islet culture.
9. To study the effect of acetylcholine, muscarinic M1 and M3 receptor ligands in DNA synthesis using rat primary islet culture.