INTRODUCTION

The brain neurotransmitters' receptor activity and hormonal pathways control many physiological functions in the body. Y-aminobutyric acid, also known as GABA was discovered over 40 years ago as a key inhibitory neurotransmitter in the brain (Bazemore et al., 1957, Kmjevic & Phillis, 1963). Since then, evidence has accumulated that this amino acid may function as a neurotransmitter not only in the central nervous system but also in the peripheral nervous system, including the myenteric plexus (Amenta, 1986, Hills & Taniyama, 1987), major pelvic ganglia (Akasu et al., 1999), and sympathetic ganglia, encompassing the rat superior cervical ganglion (Bertilsson et al., 1976, Kasa et al., 1988, Wolff et al., 1986) and abdominal prevertebral ganglia (Parkman & Stapelfeldt, 1993). In the mammalian central nervous system (CNS), GABA is the most important inhibitory neurotransmitter occurring in 30-40% of all synapses. Three types of GABA receptors have been identified: GABA_A and GABA_C receptors are ligand-gated Cl⁻ channels, while GABA_B receptors are G protein coupled (Chebib & Johnston, 1999). GABA_A receptors are ligand-gated Cl⁻ channels that consist of a heteromeric mixture of protein subunits forming a pentameric structure, and GABA_B receptors couple to Ca²⁺ and K⁺ channels via G proteins and second messengers (Johnston, 1996). In the central nervous system, application of GABA reduces excitability by a combination of GABA_A and GABA_B receptor activation, leading to membrane repolarization, reduced Ca²⁺ influx, and suppression of neurotransmitter release.

Fast inhibitory neurotransmission in the mammalian central nervous system is mediated mainly by the GABA_A receptor, a ligand-gated chloride channel. The receptor complex presumably is composed of five protein subunits, each consisting of an extracellular N-terminal domain with a putative cysteine loop, four largely conserved transmembrane segments (TM), and a variable intracellular region between TM3 and TM4. This topology is characteristic for members of the superfamily of
ligand-gated ion channel receptors (Schofield, 1987). Several GABA<sub>A</sub> receptor subunits (α 1–6, β 1–3, γ 1–3, δ, ε, θ, π, and ρ 1–3) have been cloned from mammalian brain (Korpi, 2002; Mehta et al., 1999). Thus, the genetic diversity of multiple GABA<sub>A</sub> receptor subunits permits the assembly of a vast number of receptor heteromeric isoforms. Apparently, the subunit composition determines the pharmacological profile of the resulting receptor subtypes (Barnard et al., 1998). Mechanisms that modulate the stability and function of postsynaptic GABA<sub>A</sub> receptor subtypes and that are implicated in functional plasticity of inhibitory transmission in the brain are of special interest (Luscher & Keller, 2004).

GABA<sub>C</sub> receptors appear to be relatively simple ligand-gated Cl<sup>-</sup> channels with a distinctive pharmacology, in that they are not blocked by bicuculline and not modulated by barbiturates, benzodiazepines or neuroactive steroids. Compared with GABA<sub>A</sub> receptors, GABA<sub>C</sub> receptors are activated at lower concentrations of GABA and are less liable to desensitization. In addition, their channels open for a longer time. The pharmacology of these novel subtypes of GABA receptors are yet to be explored and may yield important therapeutic agents (Johnston, 1996).

GABA has been implicated in cell growth during differentiation in the cultures in at least certain neuron types (Spoerri, 1982). GABA was reported to be present in the pancreas in comparable concentrations with those in the central nervous system during the early seventies (Briel et al., 1975; Okada et al., 1976). Prolonged binding to peripheral benzodiazepine receptors is hypothesized to cause human β-cells functional damage and apoptosis (Marselli et al., 2004). Cytokines produced by immune system cells infiltrating pancreatic islets are candidate mediators of islet β-cells destruction in autoimmune insulin-dependent diabetes mellitus. Peripheral benzodiazepine receptors constitute the aspecific mitochondrial permeability transition pore, and that it has been suggested to be involved in cytokine-induced cell death (Trincavelli et al., 2002). In the CNS, GABA affects
neuronal activity through both the ligand-gated GABA_A receptor channel and the G protein-coupled GABA_B receptor. In the mature nervous system, both receptor subtypes decrease neural excitability, whereas in most neurons during development, the GABA_A receptor increases neural excitability and raises cytosolic Ca^{2+} levels. GABA_B receptor activation depresses GABA_A receptor-mediated Ca^{2+} rises by both reducing the synaptic release of GABA and decreasing the postsynaptic Ca^{2+} responsiveness. Collectively, GABA_B receptors play an important inhibitory role regulating Ca^{2+} rises elicited by GABA_A receptor activation. Changes in cytosolic Ca^{2+} during early neural development would, in turn, profoundly affect a wide array of physiological processes, such as gene expression, neurite outgrowth, transmitter release and synaptogenesis (Obrietan & van der Pol, 1998).

The endocrine part of the pancreas plays a central role in blood-glucose regulation. GABA released from β-cells is considered as an inhibitor of insulin secretion in pancreatic islets and that the effect is principally due to direct suppression of exocytosis in which GABA_B receptors are said to play a role when activated (Braun et al., 2004). GABA has been proposed to function as a paracrine signaling molecule in islets of Langerhans and the Glucose inhibition of glucagon secretion from rat alpha-cells is mediated by GABA released from neighboring β-cells (Wendt et al., 2004). GAD_{65} and the second isoform of glutamate decarboxylase, GAD_{67}, catalyze the formation of the inhibitory neurotransmitter GABA from glutamate. Both GAD and GABA are present in pancreatic islets at concentrations similar to those encountered in classical GABAergic regions of the brain (Taniguchi et al., 1977). In pancreatic islets, both GAD and GABA selectively localize to β-cells (Michalik & Erecinska, 1992). GABA is associated with a vesicular compartment distinctly different from insulin secretory granules (Sorenson et al., 1991). Type I diabetes mellitus is caused by the selective autoimmune destruction of insulin-producing β-cells in pancreatic islets of Langerhans. One of the
most important autoantigens in type I diabetes is the 65-kD isoform of glutamate decarboxylase (GAD\textsubscript{65}). GAD\textsubscript{65}-reactive cytotoxic T-lymphocytes can mediate the autoimmune destruction of the \(\beta\)-cells (Yoon \textit{et al}., 1999). Peripheral benzodiazepine receptors are present in purified human pancreatic islets suggesting their role in the mechanisms of insulin release (Giusti \textit{et al}., 1997). PK 11195 \(1-(2\text{-chlorophenyl})\text{-N-methyl-N-(1-methylpropyl)-3-isoquinoline-carboxamide}\), a potent and selective ligand for peripheral benzodiazepine binding sites inhibits insulin release from rat pancreatic islets (Pujalte \textit{et al}., 2000). GABA\textsubscript{B} receptors play a role in the regulation of the endocrine pancreas with mechanisms probably involving direct activation or inhibition of voltage dependent \(\text{Ca}^{2+}\)-channels, cAMP generation and G-protein-mediated modulation of \(K_{\text{ATP}}\) channels (Brice \textit{et al}., 2002).

In a study of conversion of glutamate to GABA (Fernandez-Pascual \textit{et al}., 2004) L-glutamine is metabolized preferentially to GABA and L-aspartate. They accumulate in islets preventing its complete oxidation in the Krebs cycle, which accounts for its failure to stimulate insulin secretion.

There is complex nature of GABAergic neurons and \(\beta\)-cells GABA in regulation of islet function. The mammalian pancreas, like the gut wall, has an intrinsic nervous system consisting of ganglia, interconnecting intrinsic nerve fibers, and extrinsic parasympathetic and sympathetic nerves (Berthoud \textit{et al}., 1991, 2001). Pancreatic ganglion neurons contain GABA\textsubscript{A} receptors. Exogenously added GABA acts through GABA\textsubscript{A} receptors to cause depolarization, inhibiting excitatory postsynaptic field potentials (fEPSPs). Ganglionic glial cells store and can release endogenous GABA. The presence of GABA in glial cells and the absence of GABA immunoreactivity in ganglion neurons and nerve fibers and endings suggest that GABA in pancreatic ganglia functions as a paracrine messenger molecule rather than as a neurotransmitter substance (Sha \textit{et al}., 2001). New studies provide evidence demonstrating the presence of GABAergic nerve cell bodies at the periphery of islets.
with numerous GABA-containing processes extending into the islet mantle. This close association between GABAergic neurons and islet α and δ-cells strongly suggests that GABA inhibition of somatostatin and glucagon secretion is mediated by these neurons (Sorenson et al., 1991). In mammalian peripheral sympathetic ganglia GABA acts presynaptically to facilitate cholinergic transmission and postsynaptically to depolarize membrane potential. Endogenous GABA released from ganglionic glial cells acts on pancreatic ganglion neurons through GABA_A receptors (Sha et al., 2001). The mammalian pancreas, like the gut wall, has an intrinsic nervous system consisting of ganglia, interconnecting intrinsic nerve fibers, and extrinsic parasympathetic and sympathetic nerves.

The natural source for new pancreatic β-cells is an important issue both for understanding the pathogenesis of diabetes, and for possibly curing diabetes by increasing the number of β-cells. Transplantation of pancreatic islets can now be applied successfully to treat diabetes, but its widespread use is hampered by a shortage of donor organs. Since insulin-producing β-cells cannot be expanded significantly in vitro, efforts are under way to identify stem or progenitor cells that potentially could be grown and differentiated into β-cells in vitro. Such cells could provide an ample supply of transplantable tissue. Current research in this field focuses mainly on pluripotent embryonic stem cells and on pancreas-specific adult progenitor cells. β-cell replication is the only source for new β-cells without contributions from stem cells or other non-β-cells. The pancreatic gland has an enormous potential for growth and regeneration, mainly in rodents. Animal models of pancreatic regeneration can be easily established in weanling rats. There are no reports that the human pancreas shows proliferative properties after partial pancreatectomy, but research in this field has been scarce.

Partial pancreatectomy is an established model to study the pancreatic regeneration. In the present study, we have investigated the changes in the brain and
pancreas- GABA, GABA receptor subtypes and their gene expression during pancreatic regeneration. Also, the effect of GABA, its receptor agonists and antagonists in presence of growth factors on DNA synthesis and insulin secretion were studied in vitro.
OBJECTIVES OF THE PRESENT STUDY

1. To induce regeneration of pancreatic tissue 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 GABA content in various rat brain regions – brain stem, cerebellum and hypothalamus during pancreatic regeneration.
4. To study the changes in GABA content in the pancreas of experimental rats during pancreatic regeneration.
5. To study the GABA, GABA$_A$ and GABA$_B$ receptor alterations during pancreatic regeneration in brain stem, hypothalamus and cerebellum of sham and experimental rats.
6. To study the GABA, GABA$_A$ and GABA$_B$ receptor alterations during pancreatic regeneration in pancreas of different experimental rat groups.
7. To study the alterations in the GABA receptor subtypes gene expression during pancreatic regeneration in brain stem, hypothalamus, cerebellum and pancreas of sham and experimental rats.
8. To study the effect of GABA, GABA$_A$ receptor agonist muscimol and GABA$_B$ receptor agonist baclofen on insulin secretion in isolated rat pancreatic islets.
9. To study the effect of GABA, GABA$_A$ receptor agonist muscimol and GABA$_B$ receptor agonist- baclofen on DNA synthesis in rats in vitro.