

Introduction

Diabetes mellitus is a common metabolic disorder characterised by hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Feldman, 1997). Diabetes mellitus is known to be associated with neurological complications in both the peripheral nervous system (PNS) and the central nervous system (CNS) (Greene, 1999). Even though insulin secretion is mainly regulated by changes in circulating concentrations of glucose and other metabolic fuels, stimuli such as neurotransmitters and gastrointestinal hormones makes an important contribution to the overall regulation of pancreatic beta cell function. Controlling blood sugar is essential for avoiding long-term complications of diabetes like learning and memory deficit. Greater understanding of CNS involvement could lead to new strategies to prevent or reverse the damage caused by diabetes mellitus. Acetylcholine, a major neurotransmitter from the autonomic nervous system, regulates the cholinergic stimulation of insulin secretion, through interactions with muscarinic receptors (Satin & Kinard, 1998; Ahren, 2000; Gilon & Henquin, 2001). Dopamine in the CNS is involved in the control of both motor and emotional behaviour (Vallone et al., 2000) and peripherally modulates insulin secretion in the pancreatic islets (Nogueira et al., 1994). The autonomic nervous system plays a prominent role in the regulation of insulin secretion. It has been proposed that neuronal afferent signals delivered to the pancreatic β-cell through the vagus are responsible for the cephalic phase of insulin secretion. These effects are mediated by acetylcholine, which is released from nerve terminals and acts upon muscarinic cholinergic receptors in the β-cell plasma membrane (Sharp et al., 1974; Berthoud et al., 1980; Mathias et al., 1985; Ahren, 2000). Cholinergic agonist carbachol increases insulin secretion from isolated rat islets (Zawalich, 1989b). Carbachol stimulated insulin secretion is inhibited by atropine, a general muscarinic antagonist, confirming the role of
muscarinic receptors in cholinergic induced insulin secretion. Reverse transcription analysis of rat pancreatic islets indicated that muscarinic M1 and M3 are predominant receptors in the islets (Lismaa et al., 2000). Muscarinic M1 and M3 receptors function differentially regulate glucose induced insulin secretion (Renuka et al., 2006). Increased activity of muscarinic M1 and M3 receptor subtypes stimulate insulin secretion and islet cell proliferation during the regeneration of pancreas (Renuka et al., 2005) The muscarinic receptor stimulation by acetylcholine leads to activation of phospholipase C (PLC), which, in turn, hydrolyses phosphatidylinositol 4, 5-bisphosphate (PIP2) to produce inositol triphosphate (IP3) and diacylglycerol (DAG) (Best & Malaisse, 1983; Zawalich et al., 1989). In pancreatic β-cells, IP3 mobilises Ca\(^{2+}\) from intracellular stores, resulting in an elevation of the intracellular concentration of Ca\(^{2+}\) and allowing activation of Ca\(^{2+}\)/calmodulin. DAG on the other hand, activates PKC (Nishizuka, 1995; Renstrom et al., 1996). PKC, like Ca\(^{2+}\)/calmodulin, accelerates exocytosis of insulin granules (Nakano et al., 2002).

Dopamine plays an important role both centrally and peripherally. It also plays a major role in the regulation of appetite and growth hormone. Dopamine is synthesised from tyrosine, stored in vesicles in axon terminals and released when the neuron is depolarised. Dopamine interacts with specific membrane receptors to produce its effects. These effects are terminated by reuptake of dopamine into the presynaptic neuron by a dopamine transporter or by metabolic inactivation by monoamine oxidase B (MAO-B) or catechol-O-methyltransferase (COMT). The recent identification of five dopamine receptor subtypes provides a basis for understanding dopamine's central and peripheral actions. Dopamine receptors are classified into two major groups: dopamine D\(_1\) like and dopamine D\(_2\) like. Dopamine D\(_1\) like receptors consists of dopamine D\(_1\) and dopamine D\(_5\) receptors. Dopamine D\(_2\) like receptors consists of dopamine D\(_2\), dopamine D\(_3\) and dopamine D\(_4\) receptors. Stimulation of the dopamine D\(_1\) receptor give rise to increased production of cAMP. Dopamine D\(_2\) receptors inhibit cAMP production, but
activate the inositol phosphate second messenger system (Seeman, 1980). An imbalance between dopaminergic neurotransmission and dopamine receptors is known to be associated with the symptomatology of numerous neuropsychiatric disorders like schizophrenia, psychosis, mania and depression as well as neuropathological disorders like Parkinson's disease and Huntington's disease (Carlsson 1988, 1993; Bermanzohn & Siris 1992, Brown & Gershon 1993, Jakel & Maragos 2000, Kostrzewa & Segura-Aguilar 2003). Hyperglycaemia during diabetes is reported to damage dopaminergic functions. The progression of diabetes is associated with an impaired ability of the neurons in the CNS to release neurotransmitters resulting in behavioural changes (Broderick & Jacoby, 1989). The dopaminergic cells in particular are highly sensitive to excitotoxicity and oxidative stress when the energy metabolism is impaired (Callahan et al., 1998).

CAMP responsive element binding protein (CREB) is a protein that is a transcription factor. It binds to certain DNA sequences called CAMP response elements and thereby increases or decreases the transcription of the downstream genes (Lauren, 2005). In neuronal tissue, CREB regulation by nerve growth factor and insulin-like growth factor-1 is essential for neuronal plasticity, full axonal development, memory consolidation and neuroprotection (Spaulding, 1993; Shimomura, et al., 1998). The PLC activity decline in the brain is expected to affect DAG which is the principal molecular species of phosphoinositides in the nervous tissue (Whiting et al., 1979). Alterations in glucose utilisation are known to occur in the important regions of brain connected with learning and memory (Auer & Siesjo, 1993). The brain glucose uptake is ultimately dependent on facilitative glucose transporters. GLUT3 is the main neuronal glucose transporter (Kamal et al., 2000) abundant in the brain. Insulin receptor in peripheral tissues participates mainly in glucose metabolism; however its role in the CNS appears not to be related to glucose metabolism but to other neuronal activities such as memory (Zhao et al., 1999). Recently, much evidence has been presented
regarding the role of brain insulin or insulin receptors in memory formation (Frolich 
 et al., 1998).

Nutritional therapy is a major key in controlling diabetes. Antioxidant agents from diet have a significant therapeutic influence on various neurodegenerative disorders associated with diabetes and oxidative stress. Curcumin, a yellow pigment from Curcuma longa, is a major component of turmeric and exhibits powerful anti-oxidant, anti-diabetic, anti-inflammatory and anti-cancer properties (Commandeur & Vermeulen, 1996; Miller, 2001; Surh et al., 2001). A number of experimental studies have demonstrated curcumin's antioxidant and neuroprotective potential (Bala et al., 2006; Kuhad & Chopra, 2007). Also, curcumin modulates the expression of various molecular targets, such as transcription factors, enzymes, cytokines, cell cycle proteins, receptors and adhesion molecules (Shishodia et al., 2005). Curcumin antagonise the deficit of glucose energy metabolism or oxidative stress related to cognitive impairment associated with diabetes.

Vitamin D₃ is either synthesised in the epidermis from 7-dehydrocholesterol by the absorption of ultraviolet light, or obtained from the diet in a limited number of foods such as eggs, fish oils and fortified milk. The biological actions of Vitamin D₃ are mediated through binding to the vitamin D receptor (VDR), a member of the nuclear steroid hormone receptor family. An increased prevalence of diabetes has been described in vitamin D-deficient individuals (Chiu et al., 2004). Insulin synthesis and secretion has been shown to be impaired in β cells in vitamin D-deficient animals. Immunohistochemistry showed the presence of VDR in human pituitary gland (Perez-Fernandez et al., 1997), suggesting a possible role of Vitamin D in regulation of the brain endocrine system. It is of particular importance that VDR and catalytic enzymes are colocalised in the brain, supporting an autocrine/paracrine function for Vitamin D. These findings support a functional role for Vitamin D in the human brain (McGrath et al., 2001).
Approaches to the control and prevention of hyperglycemia are central to the management of diabetes mellitus (Herman & Crofford, 1997). The development of new dietary adjuncts and novel antidiabetic agents, which reinstate a normal metabolic environment and thereby reducing the long term complications associated with diabetes, is required. Such agents would both ideally stimulate the secretion and improve the action of insulin (Bailey & Flatt, 1995). Diabetes mellitus is associated with cognitive deficits and neurophysiological and structural changes in the brain (Brands et al., 2003; Mijnhout et al., 2006). However, the action mechanisms of this remain obscure. Factors that contribute to cognitive deficits as well as the protective factors that reduce the impact of diabetes on brain functions are still an enigma. The present study was designed to investigate the beneficial effect of curcumin and Vitamin D3 on impairment in the functional role of cholinergic, dopaminergic, insulin, Vitamin D receptor, GLUT3, PLC and CREB expression in the brain regions and pancreas of streptozotocin (STZ)-induced diabetic rats. Also, interaction of curcumin and Vitamin D3 with pancreatic muscarinic receptors and vesicular acetylcholine transporters were studied thereby, evaluating the therapeutic role of curcumin and Vitamin D3 in regulating insulin synthesis and release. Behavioural studies were conducted to evaluate the motor function and cognitive deficit in control and experimental rats. Our present study on curcumin and Vitamin D3 dependent regulation of cholinergic, dopaminergic, insulin and VDR in CNS and pancreas will certainly enlighten novel therapeutic possibilities for diabetes treatment.
OBJECTIVES OF THE PRESENT STUDY

1. To study the anti-hyperglycemic activity of curcumin and Vitamin D$_3$ in STZ-induced diabetic animal model.

2. To measure the circulating insulin and T3 concentration of control, diabetic, insulin, curcumin and Vitamin D$_3$ treated diabetic rats.

3. To study the behavioural changes in control and experimental rats using Y-maze, rotarod test, grid walk and beam walk test.

4. To study the total muscarinic, muscarinic M1 and muscarinic M3 receptor subtypes binding parameters in cerebral cortex, cerebellum, brain stem, corpus striatum, hippocampus and pancreas of control, diabetic, insulin, curcumin and Vitamin D$_3$ treated diabetic rats.

5. To study the total dopamine binding parameters in cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus of control, diabetic, insulin, curcumin and Vitamin D$_3$ treated diabetic rats.

6. To study the expression of acetylcholine esterase, choline acetyltransferase, muscarinic M1, muscarinic M3, α7 nicotinic acetylcholine, dopamine D1, dopamine D2, insulin and VDR gene expression in the cerebral cortex, cerebellum, brain stem, corpus striatum, hippocampus, hypothalamus and pancreas of control, diabetic, insulin, curcumin and Vitamin D$_3$ treated diabetic rats using Real Time PCR.

7. To study the gene expression status of GLUT2/GLUT3, PLC, CREB and superoxide dismutase in the cerebral cortex, cerebellum, brain stem,
corpus striatum, hippocampus, hypothalamus and pancreas of control, diabetic, insulin, curcumin and Vitamin D$_3$ treated diabetic rats using Real Time PCR.

8. To study the localisation and expression status of muscarinic M1, muscarinic M3, $\alpha_7$ nicotinic acetylcholine receptor ($\alpha_7$ nAchR), in the brain slices of cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus of control, diabetic, insulin, curcumin and Vitamin D$_3$ treated diabetic rats using specific antibodies in confocal microscope.

9. To study the localisation and expression status of acetylcholine esterase, muscarinic M1, muscarinic M3, vesicular acetylcholine transporter in the pancreas of control, diabetic, insulin, curcumin and Vitamin D$_3$ treated diabetic rats using specific antibodies in confocal microscope.