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At the Cutting Edge

Role of curcumin in the prevention of cholinergic mediated cortical dysfunctions in streptozotocin-induced diabetic rats

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ABSTRACT

Diabetes exacerbates neuronal injury mediated through neurotransmitters deregulation in cerebral cortex. Our study analyzed the neuroprotective effect of curcumin to prevent cortical dysfunction associated with diabetes. Our study revealed decreased gene expression of muscarinic M1, insulin receptor, SOD, choline acetyl transferase and increased gene expression of muscarinic M3, α7-nicotinic acetylcholine receptor, acetylcholine esterase and GLUT3 in cerebral cortex of diabetic rats. Curcumin and insulin treatment reversed this altered parameters to near control. Immunohistochemistry studies of muscarinic M1 and M3 confirmed the gene expression at protein level. Decreased novel arm entry of diabetic rats in Y-maze test, improved in treatment group. These results suggest that cholinergic dysfunction, impaired glucose transport and oxidative stress contributes to learning and memory deficits in diabetes and further suggest that antioxidant curcumin has potential therapeutic role in preventing and/or delaying the diabetic complications associated with brain.

Contents

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1. Introduction

Prolonged exposure to chronic hyperglycaemia in diabetes leads to various complications, affecting the neurological, cardiovascular, renal and visual systems (Brownlee, 2001). Previous studies involving behavioural and electrophysiological analysis indicate that diabetes mellitus induces cognitive impairment and defects in long-term potentiation (Hasanein and Shahidi, 2010). People with diabetes apparently face a greater risk of Alzheimer's disease and depression (Biessels et al., 2006). Gold et al. (2007) reported that type 2 diabetes mellitus causes neuronal damage and memory impairments. Also, abnormalities in executive functions which are mediated by the frontal lobe are noted in patients with diabetes (Qiu et al., 2006). Oxidative stress is believed to play an important role in the development of complications in diabetes associated neuronal disorders (Osawa and Kato, 2005). Greater understanding of central nervous system (CNS) involvement could lead to new strategies to prevent or reverse the damage caused by diabetes mellitus.

Antioxidant agents from diet have a significant therapeutic influence on various neurodegenerative disorders associated with diabetes and oxidative stress (Ahmad et al., 2005; Ishrat et al., 2006). Curcumin, a yellow pigment from Curcuma longa, is a major component of turmeric and exhibits a powerful antioxidant, an anti-diabetic, anti-inflammatory and anti-cancer properties (Commandeur and Vermeulen, 1996; Surh et al., 2001; Miller et al., 2001). A number of experimental studies have demonstrated curcumin's antioxidant and neuroprotective potential (Bala et al., 2006; Kuhad and Chopra, 2007). Curcumin antagonise the deficit of glucose energy metabolism or oxidative stress related to cognitive impairment associated with diabetes. 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).

Diabetes is also found to be associated with changes in somatic sensations which involve the cerebral cortex, cerebellum and thalamus. The cholinergic innervation of the cerebral cortex has been extensively investigated because of its role in arousal, learning and memory (Bartus et al., 1985; McCormick, 1989; Olton et al., 1991; Voytko et al., 1994). Alterations in glucose transport and utilization are known to occur in the important regions of brain connected with learning and memory (Krebs and Parent, 2005; McNay et al., 2000). The brain glucose uptake is dependent on facilitative glucose transporters. GLUT3 is the main neuronal glucose transporter (Maher et al., 1993) abundant in the brain.

Cognitive deficits and neurophysiological and structural changes are reported in the brain during diabetes mellitus (Brands et al., 2003; Mijnhout et al., 2006). However, the mechanisms of these changes remain obscure. Factors that contribute to cognitive deficits as well as the protective factors that reduce the impact of diabetes on brain functions are not completely elucidated. Therefore, this study was designed to investigate the beneficial effect of curcumin, a neuroprotective agent, in modulating cholinergic, insulin receptors and GLUT3 in the cerebral cortex of streptozotocin (STZ)-induced diabetic rats. Present study will certainly enlighten novel therapeutic possibilities for diabetes associated neurological dysfunctions.

2. Materials and methods

Bio chemicals used in the present study were purchased from Sigma Chemical Co., St. Louis, USA. All other reagents of analytical grade were purchased locally. Quinuclidinyl benzilate, L-[benzilic-4,4′-3H], ([^{3}H] QNB)(sp. activity 42 Ci/mmol) and 4-DAMP, [N-methyl-3H] (sp. activity 83 Ci/mmol) were purchased from NEN Life Sciences Products Inc., Boston, USA. Streptozotocin, pirenzepine, 4-DAMP mustard, and curcumin from Sigma Chemical Co., USA. Tri-reagent kit was purchased from MRC, USA. Real-time PCR Taqman probe assays on demand were from Applied Biosystems, Foster City, CA, USA.

Male adult Wistar rats of 180–240 g body weight were used for all experiments. They were housed in separate cages under 12 h light and 12 h dark periods. Rats had free access to standard food and water *ad libitum*. All animal care and procedures were done in accordance with the Institutional and National Institute of Health guidelines. Diabetes was induced in rats by single intrafemoral vein injection of STZ freshly dissolved in 0.1 M citrate buffer, pH 4.5, under anaesthesia (Junod et al., 1969). STZ was given at a dose of 55 mg/kg body weight (Hohenegger and Rudas, 1971; Arison et al., 1967). Animals were divided into the following groups: (I) control, (ii) diabetic, (iii) insulin-treated diabetic and (iv) curcumin-treated diabetic rats. Each group consisted of 6–8 animals. The insulin-treated diabetic group received subcutaneous injections (1 U/kg body weight) of Lente and Plain insulin (Boots India) daily during the entire period of the experiment. The last injection was given 24 h before sacrificing the rats. Curcumin-treated groups received 60 mg/kg suspension of curcumin orally for the entire period of the experiment. Curcumin was suspended in 0.5% (w/v) sodium carboxymethylcellulose immediately before administration in constant volume of 5 ml/kg body weight (Sharma et al., 2006). Rats were sacrificed on 15th day by decapitation. The cerebral cortex was dissected out quickly over ice according to the procedure of Glowinski and Iversen (1966) and the tissues collected were stored at −80 °C until assayed.

2.1. Estimation of blood glucose

Glucose was measured by GOD-POD glucose estimation kit (Biolab Diagnostics Pvt Ltd). Blood samples were collected from the tail vein at 0 h (before the start of the experiment), 3rd, 6th, 10th and 14th day and the glucose levels were estimated subsequently. Along with this blood samples were collected 3 h after the administration of morning dose of insulin and curcumin. The results were expressed in terms of milligram per decilitre of blood.

2.2. Y-maze test

For Y-maze test, the experimental rats were retained for five weeks and the rats were subjected to training and test on day time to assess short-term and spatial memory performance in Y-maze as per Nitta et al. (2002). The Y-maze was made of grey wood, covered with black paper, and consisted of three arms with an angle of 120° between each of the arms. Each arm was 8 cm width × 30 cm length × 15 cm height. The three identical arms were randomly designated: start arm, in which the rat started to explore (always open); novel arm, which was blocked at the 1st trial, but open at the 2nd trial; and the other arm (always open). The maze was placed in a separate room with enough light. The floor of the maze was covered with sawdust, which was mixed after each individual trial in order to eliminate olfactory stimuli. Visual cues were placed on the walls of the maze.

The Y-maze test consisted of two trials separated by an inter-trial interval (ITI). The first trial (training) was of 10 min duration and allowed the rat to explore only two arms (start arm and the other arm) of the maze, with the third arm (novel arm) blocked. After a 1 h ITI (Ma et al., 2007), the second trial (retention) was conducted, during which all three arms were accessible and novelty vs. familiarity was analyzed through comparing behaviour in all three arms. For the second trial, the rat was placed back in the maze in the same starting arm, with free access to all three arms for 5 min. The time spent in each arm was analyzed. Data was expressed