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Neuropharmacology and analgesia

Curcumin restores diabetes induced neurochemical changes in the brain stem of Wistar rats

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ABSTRACT

Diabetes mellitus, when poorly controlled, leads to debilitating central nervous system (CNS) complications including cognitive deficits, somatosensory and motor dysfunction. The present study investigated curcumin's potential in modulating diabetes induced neurochemical changes in brainstem. Expression analysis of cholinergic, insulin receptor and GLUT-3 in the brainstem of streptozotocin (STZ) induced diabetic rats were studied. Radioreceptor binding assays, gene expression studies and immunohistochemical analysis were done in the brainstem of male Wistar rats. Our result showed that B_{max} of total muscarinic and muscarinic M_3 receptors were increased and muscarinic M_1 receptor was decreased in diabetic rats compared to control. mRNA level of muscarinic M_3, α_7-nicotinic acetylcholine, insulin receptors, acetylcholine esterase, choline acetyltransferase and GLUT-3 significantly increased and M_1 receptor decreased in the brainstem of diabetic rats. Curcumin and insulin treatment restored the alterations and maintained all parameters to near control. The results show that diabetes is associated with significant reduction in brainstem function coupled with altered cholinergic, insulin receptor and GLUT-3 gene expression. The present study indicates beneficial effect of curcumin in diabetic rats by regulating the cholinergic, insulin receptor and GLUT-3 in the brainstem similar to the responses obtained with insulin therapy.

1. Introduction

Diabetes mellitus is a major global health problem currently affecting more than 180 million people worldwide. In recent years it has become evident that diabetes causes significant CNS complications, resulting in important functional impairments (Mijnhout et al., 2006). Prolonged exposure to chronic hyperglycemia in diabetes leads to various complications, affecting the neurological, cardiovascular, renal, visual and auditory systems (Brownlee, 2001). Curcumin is a natural plant polyphenolic Q7 compound with powerful anti-oxidant, anti-diabetic, anti-inflammatory and anti-cancer properties and have a long tradition in folk medicine (Kumar et al. 2010a, 2010b).

Brainstem along with hypothalamus serves as the key center of the central nervous system regulating the body homeostasis. Brainstem reticular formation has been considered to play an important role in generating behavioral states as well as in the modulation of pain sensation (Zambreanu et al., 2005) and the reticular functions originate from interacting neuronal groups in the brainstem, including cholinergic, adrenergic and serotoninergic neurons (Steriade, 1996). In addition to glucose, insulin secretion from the endocrine pancreatic b cells is stimulated by several neuropeptide hormones and neurotransmitters, among which acetylcholine; the muscarinic cholinergic receptor agonist plays a prominent role (Ahren, 2000; Gilon and Henquin, 2001). Alterations in glucose transport and utilization are known to occur in the important regions of brain connected with learning and memory (McNay et al., 2000; Krebs and Parent, 2005). Insulin and insulin receptors in the brain suggest their functional involvement in brain cognition phenomena such as learning and memory. Insulin has been shown to exert a memory-enhancing action on both humans and experimental animals (Park et al., 2000; Kern et al., 2001). Also the brain glucose uptake is dependent on facilitative glucose transporters such as GLUT-3. The expression, regulation and activity of glucose transporters play an essential role in neuronal homeostasis, since glucose represents the primary energy source for the brain (Zhao et al., 2010).

Curcumin, the active compound in turmeric, because of its antioxidant and anti-inflammatory properties, has been demonstrated in the prevention and treatment of neurodegenerative disorders such as Alzheimer's disease and multiple sclerosis

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(Cole et al., 2007). Previous study showed that curcumin and turmeric treatment have countered the hyperglycemia-induced oxidative stress (Suryanarayana et al., 2005). This encouraged us to test the ability of curcumin in ameliorating STZ-induced dysfunctions in brainstem. Therefore, the present study was undertaken to investigate the effect of curcumin on cholinergic enzymes and receptors, insulin receptor, and GLUT-3 in STZ-induced diabetic model of rat. Present study will certainly explain the therapeutic possibilities of curcumin for diabetes associated neurological dysfunctions.

2. Materials and methods

2.1. Animals and chemicals

This study was conducted using protocols approved by the Institutional Animal Care and Use Committee in accordance with National Institutes of Health guidelines (Institute for Laboratory Animal Research and National Research Council, 2010). All animal care and procedures done is approved by Institutional and CPCSEA guidelines. All animals were housed in pairs, allowed standard rat diet and water *ad libitum*, and maintained on a 10-h light/14-h dark cycle. All efforts were made to minimize the number of animals used and their suffering. Adult male Wistar rats (initial body weight 180–250 g) were divided into four experimental groups: nondiabetic vehicle-treated control animals, untreated diabetic animals, insulin-treated diabetic animals and curcumin treated diabetic animals. Each group consisted of 6–8 animals.

Control: rats of this group received single intrafemoral injection of 0.1 M citrate buffer (vehicle), pH 4.5. Diabetic: diabetes was induced in non-fasted rats by a single intrafemoral vein injection of freshly prepared streptozotocin (STZ) (55 mg/kg body weight; Sigma, St. Louis, MO) dissolved in citrate buffer (0.1 M, pH 4.5.) under anesthesia (Hohenegger and Rudas, 1971; Arison et al., 1967). Rats were anesthetized using diethyl ether. Ether anesthesia was induced in a covered beaker and was maintained by a paper nose cone containing a pledget of ether moistened cotton. Diabetes was confirmed in rats by measuring the fasting serum glucose level (in the morning hours) three days after STZ injection. Blood glucose level above 250 mg/dl was considered as diabetic and other rats were excluded from the study. The insulin and curcumin treatment was started from fourth day after STZ injection. Diabetic rats treated with insulin: this group received subcutaneous injections (1 unit/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. Diabetic rats treated with curcumin: curcumin was given to this group of diabetic rats in the dosage of 60 mg/kg (Sigma, St. Louis, MO) suspended in 0.5% w/v sodium carboxymethyl cellulose via oral gavage in constant volume of 5 ml/kg body weight (Sharma et al., 2006). As per our preliminary studies, the present dosage of 60 mg/kg curcumin is standardised, and is found to possess significant potential in ameliorating diabetes related complications in other reports also (Zhao et al., 2008; Sharma et al., 2006). The treatments were given for a period of 14 days and were sacrificed on 15th day by decapitation. The brainstem was dissected out quickly over ice according to the modified procedure of Glowinski and Iversen (1966) and the tissues collected were stored at −80 °C until assayed.

2.2. Total muscarinic, muscarinic M_1 and M_3 receptor binding studies in the brainstem

Binding assay in brainstem was done according to the modified procedure of Yamamura and Synder (1981). Total muscarinic, and muscarinic M_1 receptor binding parameter assays were done using [^{3}H] QNB (L-[Benzilic-4,4′-3H] Sp. Activity 42 Ci/mmol, NEN Life Sciences Products Inc., Boston, USA) 0.1–2.5 nM and M_3 receptor using [^{3}H] DAMP (N-methyl-^{3}H) Sp. Activity 83 Ci/mmol, NEN Life Sciences Products Inc., Boston, USA) using 0.01–5 nM. The non-specific binding was determined using 100 μM atropine (Sigma, St. Louis, MO) for total muscarinic, pirenzepine (Sigma, St. Louis, MO) for muscarinic M_1 and 4-DAMP mustard (Sigma, St. Louis, MO) for M_3 receptor. Total incubation volume of 250 μl contains 200–250 μg protein concentrations. Tubes were incubated at 22 °C for 60 min and filtered rapidly through GF/C filters (Whatman, no. 1822-025). The filters were washed quickly by three successive washing with 5.0 ml of ice cold 50 mM Tri–HCl buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter (Wallac, Turku, Finland). The non-specific binding determined showed 10% in all our experiments.

2.3. Protein determination

The amount of protein was measured by the method of Lowry et al. (1951) using different concentrations of bovine serum albumin (20, 40, 60 and 80 μg/ml) as standard. The intensity of the purple blue color formed was proportional to the amount of protein, which was read in a spectrophotometer (Shimadzu UV-1700, Kyoto, Japan) at 660 nm.

2.4. Receptor data analysis

The receptor binding parameters were determined using Scatchard analysis (Scatchard, 1949). The specific binding was determined by subtracting non-specific binding from the total. The binding parameters, maximal binding (B_{max}) and equilibrium dissociation constant (K_d), were derived by linear regression analysis by plotting the specific binding of the radioligand on *X*-axis and bound/free on *Y*-axis using Sigma plot software (version 2.0, Jandel GmbH, Erkrath, Germany). The maximal binding is a measure of the total number of receptors present in the tissue and the equilibrium dissociation constant is the measure of the affinity of the receptors for the radioligand. The K_d is inversely related to receptor affinity.

2.5. Analysis of cholinergic enzymes, insulin receptor, GLUT-3, muscarinic M_1, M_3 and α_7 nicotinic acetylcholine receptor expression in the rat brainstem using real-time PCR

RNA was isolated from the brainstem of experimental rats using the Tri-reagent (MRC, USA). Total cDNA synthesis was performed using ABI PRISM cDNA archive kit in 0.2 ml microfuge tubes. The reaction mixture of 20 μl contained 0.2 μg total RNA, 10 × RT buffer, 25 × dNTP mixture, 10 × random primers, MultiScribe RT (50 U/μl) and RNase free water. The cDNA synthesis reactions were carried out at 25 °C for 10 min and 37 °C for 2 h using an Eppendorf personal cycler. Real-time PCR assays were performed in 96-well plates in ABI 7300 real-time PCR instrument (Applied Biosystems). The primers and probes were purchased from Applied Biosystems, Foster City, CA, USA. The TaqMan reaction mixture of 20 μl contained 25 ng of total RNA-derived cDNAs, 200 nM each of the forward primer, reverse primer and TaqMan probe for muscarinic M_1 receptor gene and endogenous control β-actin and 12.5 μl of Taqman 2X Universal PCR Master Mix (Applied Biosystems) and the volume was made up with RNAse free water. The following thermal cycling profile was used (40 cycles): 50 °C for 2 min, 95 °C for 10 min, 95 °C for 15 s and 60 °C for 1 min.

Fluorescence signals measured during amplification were considered positive if the fluorescence intensity was 20-fold greater than the standard deviation of the baseline fluorescence.

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