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Amelioration of diabetes-induced neurobehavioral and neurochemical changes by melatonin and nicotinamide: Implication of oxidative stress–PARP pathway

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ABSTRACT

Diabetes associated hyperglycemia results in generation of reactive oxygen species which induces oxidative stress and initiate massive DNA damage leading to overactivation of poly (ADP-ribose) polymerase (PARP). In this study, we have elucidated the involvement of oxidative stress–PARP pathway using pharmacological interventions (melatonin, as an anti-oxidant and nicotinamide, as a PARP inhibitor) in diabetes-induced neurobehavioral and neurochemical alterations. Sprague–Dawley rats were rendered diabetic by a single intraperitoneal injection of streptozotocin. Behavioral and cognitive deficits were assessed after 8 weeks of diabetes induction using a functional observation battery, passive avoidance and rotarod test. Acetylcholinesterase activity was significantly decreased in hippocampus of diabetic rats as compared to control rats. Diabetic animals showed significant increase in malondialdehyde levels and reduction in NAD levels in hippocampus. Glutamate and GABA levels were also altered in hippocampus of the diabetic animals. Two week treatment with melatonin (3 and 10 mg/kg) and nicotinamide (300 and 1000 mg/kg) alone and in combination significantly improved the neurobehavioral parameters which were altered in diabetes. Neurotransmitter (glutamate and GABA) levels were improved by these interventions. Our results emphasize that simultaneous inhibition of oxidative stress–PARP overactivation cascade can be beneficial in treatment of diabetes associated CNS changes.

1. Introduction

Diabetes mellitus is a heterogeneous metabolic disorder characterized by hyperglycemia resulting from defective insulin secretion, resistance to insulin action or both. It is estimated that approximately 371 million people worldwide, within the age group of 20–79 years were affected by diabetes in 2011. By 2030, diabetes is predicted to affect 537 million people worldwide, which would be about 10% of the adult population. According to WHO, neurological and psychological complications are developed in 15–40% of diabetic patients (Bernstein et al., 2013; Guariguata, 2012). Hyperglycemia leads to severe diabetic complications, such as cardiovascular disease, retinopathy, nephropathy, peripheral and autonomic neuropathy through oxidative stress. Diabetes associated cognitive dysfunction has been recognized in the medical literature since 1922 (Richardson, 1991).

In diabetes, acute and chronic metabolic and vascular disturbances can impair the functional and structural integrity of the brain. Hyperglycemia activates multiple pathways such as polyol pathway, myo-inositol depletion, increased sorbitol accumulation disturbed Ca^{2+} homeostasis, increased advanced glycation end products, increased reactive oxygen species, and alteration of protein kinase C activity (Ryle et al., 1998; Levy et al., 1994; Knudsen et al., 1989; Flier et al., 1987; Vlassara et al., 1983). Cerebrovascular changes like reduced blood flow to the brain, impaired vascular reactivity and neurotropic changes like reduced level of IGF (insulin growth factor) indicate that these abnormalities precede functional cognitive impairments and apoptotic neuronal loss in hippocampus (Li et al., 2002, 2005). In humans, diabetes mellitus is associated with moderate impairments in cognitive function, a high risk of affective disorders, dementia and Alzheimer's disease (Brismar et al., 2007; Northam et al., 2006; Ristow, 2004; Sima, 2004; Ott et al., 1999; Biessels et al., 1994).

Animal models of diabetes, including the streptozotocin-induced diabetic rats, have been proved to be very useful to determine the underlying cause of CNS complications. Streptozotocin (STZ)-induced

Abbreviations: IGF, insulin growth factor; STZ, streptozotocin; PARP, poly (ADP-ribose) polymerase; AIF, apoptosis inducing factor; FOB, Functional Observational Battery; AChE, acetylcholinesterase; OPA, o-phthalaldehyde; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappa; B AP-1, activator protein-1; MDA, malondialdehyde.

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diabetes produces hippocampal apoptosis and astrogliosis, decreases proliferation rate in the dentate gyrus resulting in poor neurogenesis and reduces number of hilar neurons (Beauquis et al., 2006, 2008; Revsin et al., 2005; Saravia et al., 2004). Cognitive deficit might be connected to neurotoxic effects of hyperglycemia and changes in neurotransmission and neuronal functionality. The increased oxidative stress in diabetes produces oxidative damage in many regions of rat brain including the hippocampus (Kuhad and Chopra, 2008). Oxidative stress and related pathways have a major contributory role in the development of diabetic complications, whereas antioxidant therapy may protect or restore physiological function (Kuhad and Chopra, 2008). Melatonin as well as its metabolites are well established antioxidants (Galano et al., 2011, 2013). Melatonin has been found to be neuroprotective in streptozotocin-induced rat model of diabetes (Negi et al., 2010a, 2011; Saravia et al., 2004). Quenching of free radicals by melatonin is the central mechanism for counteracting oxidative stress-induced neurotoxicity (Rosales-Corral et al., 2012; Tuzcu and Baydas, 2006).

Hyperglycemia also causes overproduction of nitric oxide (NO) which reacts with superoxide resulting in the generation of highly reactive peroxynitrite which induces massive DNA damage. Downstream of this DNA damage, the enzyme poly (ADP-ribose) polymerase (PARP, EC 2.4.2.30) is activated which is a nuclear enzyme that acts as a nicksensor and facilitates DNA repair induced by oxidative stress, ionizing radiations and cytotoxic agents. However, in case of extensive DNA damage, PARP-1 overactivation induces a decrease in NAD^+ and ATP levels, leading to energy failure and cell necrosis. PARP overactivation may release apoptosis inducing factor (AIF) from the mitochondria and facilitates AIF translocation to the nucleus, thus activating a caspase-independent type of apoptosis (Chaitanya et al., 2010). Moreover, the beneficial effects of PARP inhibitors like 3-aminobenzamide and 5-aminoisoquinolinone have been shown in spinal cord trauma (Genovese et al., 2005). PARP inhibition using nicotinamide can be viewed as an important target to delineate manifestation induced by diabetes and its CNS complication. We have earlier demonstrated the protective effect of melatonin and nicotinamide and their combination in diabetic neuropathy (Negi et al., 2010b, 2011). However, their effects on diabetes-induced neurobehavioral and cognitive dysfunctions have not been investigated. Therefore, in the present study we investigated the neuroprotective role of melatonin and nicotinamide on neurobehavioral and neurochemical alterations occurring in diabetes.

2. Material and methods

2.1. Induction of diabetes and experimental design

The experiments were performed in accordance with regulations specified by the Institutional Animal Ethics Committee (IAEC), NIPER. Male Sprague–Dawley rats (250–270 g) were used for the study and were fed on a standard rat diet and water ad libitum. Diabetes was induced by streptozotocin at a dose of 55 mg/kg (i.p.). Blood samples were collected from tail vein ~72 h after STZ administration. Rats with plasma glucose level more than 250 mg/dl were considered diabetic and were further considered for study. The experimental groups were comprised of non-diabetic control group (ND), diabetic control rats (STZ-D), and diabetic rats treated with melatonin (D + M3 and D + M10, respectively, for melatonin 3 and 10 mg/kg, p.o.), nicotinamide (D + N300 and D + N1000, respectively, for nicotinamide 300 and 1000 mg/kg, p.o.) and a combination of melatonin 3 mg/kg and nicotinamide 300 mg/kg (D + M3 + N300). The experimental groups consisted of 6 animals each. The treatment was started 6 weeks after diabetes induction and continued for two weeks. The behavioral and biochemical experiments were performed 24 h after administration of the last dose.

2.2. Behavioral parameters

2.2.1. Functional observational battery (FOB)

This test was performed to evaluate the effect of diabetes on behavior and physiological function using scores as described elsewhere with slight modification as per Table 1. The open field activities and sensory responses were observed in open field cages for 5 min (Columbus Instruments, OH, USA). The body temperature was measured by inserting rectal probes (Harvard apparatus, MA, USA). The probe was left in place until a stable temperature was achieved. Most symptoms were evaluated by their presence or absence. Some parameters were rated at 5-point scale like spontaneous activity, excitability, and auditory response (Mattsson et al., 1996; Irwin, 1968).

2.2.2. Motor coordination test

Motor coordination was assessed using a rotarod apparatus (IITC Life Science, CA, USA). Rotating speed and cut off time were fixed at 15 rpm and 180 s respectively. Rats were acclimatized for two days and on third day test session were carried out. The time taken for the animal to drop off the rod, or the number of animals remaining on the rod over a set duration was measured. The incidence of ataxia i.e., the ability of the rat to fall was recorded in control, diabetic and drug treated animals (Sharma et al., 2011).

2.2.3. Passive avoidance test

It was carried out using Shuttle Box Apparatus (Columbus Instruments, OH, USA). The apparatus consists of a two compartment dark/light shuttle box with a guillotine door separating the compartments. The dark compartment had a stainless steel shock grid floor. During

Table 1
Behavioral parameters of functional observational battery (Rajput et al., 2009; Irwin, 1968).

Spontaneous activity level (home cage): (1 = no movement and asleep/2 = slight movement of head or body/3 = moderate movement in the cage/4 = active movement around cage/5 = rapid movements in the cage)
Spontaneous activity level (open field): (1 = no body movement/2 = little or sluggish movement/3 = little movement with exploratory activity/4 = walking with very little or no running/5 = exploratory movements, including walking and running/6 = highly active with darting/running)
Posture: (normal/flattened, pelvis flat on surface/pelvis dragging/hunched, back raised up)
Respiration: [1 = normal/2 = breathing either fast and shallow or slow (bradypnea)/3 = breathing very fast shallow or very shallow and labored in appearance/4 = wheezing or breathing with mouth open/5 = weak breathing (breathing very little)]
[Convulsion, tremors, fasciculation, tonus, clonus, vocalization, Straub's tail, writhing, retropulsion, diarrhea & defecation, piloerection, fur appearance, ptosis, exopthalmia (protrusion of eye ball), stereotypy, pinna reflex, extensor thrust reflex, palpebral reflex, visual placing, surface righting, aerial righting, pupil reaction, tail pinch response, and urination spots] – present/absent
Excitation: (1 = no resistance, easy to hold or prick up/2 = slight resistance/3 = squirming or moving around/4 = excited, squirming, twisting/5 = aggressive actions like biting, tail and throat rattling)
Salivation: (0 = dryness of mouth/1 = normal salivation/2 = wetness around the mouth and chin/3 = drooling of saliva)
Lacrimation: (0 = dryness of eye/1 = normal eye/2 = wetness around the eyes)
Muscle tone: (normal/increase/decrease)
Gait: [1 = normal/2 = slight abnormality (uncoordinated, staggering, wobbly gait, exaggerated, overcompensated, or splayed moving hind limbs)/3 = moderate abnormality (hind limb point outward from body, forelimbs, drag or show abnormal positioning and walking on toes)/4 = severe abnormality (no movement)]
Arousal: [1 = very low (stupor, coma, or prostrate)/2 = low (sluggish, only some movements)/3 = somewhat low (slightly sluggish, some exploratory movements)/4 = moderate (alert, exploratory behavior)]
Auditory response (1–5 scale), somatosensory response (1–5 scale), visual approach response (1–5 scale), olfactory response (1–5 scale) [1 = no reaction or response/2 = slight or sluggish reaction (flinch or startle as evidence of perception)/3 = obvious reaction (locomotor orientation as evidence of perception)/4 = clear reaction or response (more intense startle or locomotion)/5 = exaggerated reaction (may jump, bite, or attack)]