Biomedicine & Pharmacotherapy 84 (2016) 559–568

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www.sciencedirect.com

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EM|consulte
www.em-consulte.com/en

Original article

Neuroprotective effects of quercetin on memory and anxiogenic-like behavior in diabetic rats: Role of ectonucleotidases and acetylcholinesterase activities

Roberto M. Maciel[a], Fabiano B. Carvalho[b,**], Ayodeji A. Olabiyi[b,d], Roberta Schmatz[b], Jessié M. Gutierres[b], Naiara Stefanello[b], Daniela Zanini[b], Michelle M. Rosa[b], Cinthia M. Andrade[a,b], Maribel A. Rubin[b], Maria Rosa Schetinger[b], Vera Maria Morsch[b], Cristiane C. Danesi[c], Sonia T.A. Lopes[a,*]

[a] *Programa de Pós-Graduação em Medicina Veterinária, Laboratório de Análises Clínicas Veterinária, Centro de Ciências Rurais, Universidade Federal de Santa Maria, Santa Maria/RS 97105-900, Brazil*
[b] *Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria/RS 97105-900, Brazil*
[c] *Programa de Pós-Graduação em Ciências Odontológicas, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil*
[d] *Department of Medical Biochemistry, Afe Babalola University, Ado Ekiti, P.M.B 5454. Ado Ekiti, Nigeria*

ARTICLE INFO

Article history:
Received 1 June 2016
Received in revised form 13 September 2016
Accepted 19 September 2016

Keywords:
Diabetes
Memory
Anxiety
Acetylcholinesterase
Ectonucleotidases
Quercetin

ABSTRACT

The present study investigated the protective effect of quercetin (Querc) on memory, anxiety-like behavior and impairment of ectonucleotidases and acetylcholinesterase (AChE) activities in brain of streptozotocin-induced diabetic rats (STZ-diabetes). The type 1 diabetes mellitus was induced by an intraperitoneal injection of 70 mg/kg of streptozotocin (STZ), diluted in 0.1 M sodium-citrate buffer (pH 4.5). Querc was dissolved in 25% ethanol and administered by gavage at the doses of 5, 25 and 50 mg/kg once a day during 40 days. The animals were distributed in eight groups of ten animals as follows: vehicle, Querc 5 mg/kg, Querc 25 mg/kg, Querc 50 mg/kg, diabetes, diabetes *plus* Querc 5 mg/kg, diabetes *plus* Querc 25 mg/kg and diabetes *plus* Querc 50 mg/kg. Querc was able to prevent the impairment of memory and the anxiogenic-like behavior induced by STZ-diabetes. In addition, Querc prevents the decrease in the NTPDase and increase in the adenosine deaminase (ADA) activities in SN from cerebral cortex of STZ-diabetes. STZ-diabetes increased the AChE activity in SN from cerebral cortex and hippocampus. Querc 50 mg/kg was more effective to prevent the increase in AChE activity in the brain of STZ-diabetes. Querc also prevented an increase in the malondialdehyde levels in all the brain structures. In conclusion, the present findings showed that Querc could prevent the impairment of the enzymes that regulate the purinergic and cholinergic extracellular signaling and improve the memory and anxiety-like behavior induced by STZ-diabetes.

1. Introduction

Diabetes mellitus (DM) consists a group of metabolic dysfunction characterized by hyperglycemia resulting from a defect in insulin secretion or insulin action [1]. Hyperglycemia persistent is indeed the causal link in the evolution of neuropathy and uncontrolled diabetes [2]. It has been shown that persistent hyperglycemia increases production of reactive oxygen species (ROS) for all tissues across of glucose auto-oxidation and protein glycosylation.

* Corresponding author at: Programa de Pós–Graduação em Medicina Veterinária, Laboratório de Análises Clínicas Veterinária, Centro de Ciências Rurais, Universidade Federal de Santa Maria, Santa Maria/RS 97105-900, Brazil.
** Corresponding author at: Programa de Pós Graduação em Ciências Biológicas: Bioquímica Toxicológica, Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria/RS 97105-900 Brazil.
E-mail addresses: fabiseco@yahoo.com.br (F.B. Carvalho), sonia@smail.ufsm.br (S.T.A. Lopes).

http://dx.doi.org/10.1016/j.biopha.2016.09.069
0753-3322/

The cholinergic systems provide diffuse innervations to practically all brain [3]. The broad cholinergic innervation acting via nicotinic acetylcholine receptors has been found to influence arousal, attention, sleep, fatigue, anxiety, central processing of pain, and a number of cognitive functions [4–6]. The enzyme acetylcholinesterase (AChE) is found in the cholinergic terminal and is the most efficient enzyme that rapidly hydrolyze the neurotransmitter acetylcholine (ACh) at cholinergic synapses as well as the neuromuscular junction [7]. In addition, the hippocampus and cortical regions of the brain are main sites for cholinergic transmission to monitor learning and memory processing, and seem to be more sensitive to oxidative damage [8] which contributes neuronal damage and establishing of cognitive deficit [9].

The extracellular nucleotide ATP and its nucleoside derivative adenosine are important signaling molecules involved in numerous physiological and pathological functions [10]. The ATP and extracellular adenosine levels are regulated by a cascade of cell-surface-bound enzymes named ectonucleotidases. The NTPDase is an enzyme that hydrolyses ATP and ADP into AMP, which is subsequently converted to adenosine by the enzyme 5′-nucleotidase [11,12]. Moreover, adenosine is cleaved by enzyme adenosine deaminase to inosine in the synaptic cleft [13,14]. Together, these enzymes constitute an organized enzymatic cascade for the regulation of nucleotide-mediated signaling, controlling rate, degradation, and nucleoside formation [15,16]. It has been described also the ectonucleotidases involvement on learning and memory process in rats [17–19].

The various purinergic receptor subtypes are said to be widely distributed throughout the central nervous system (CNS) and they control local network behaviors by regulating the balance between the release and effects of ATP and adenosine as well as ectonucleotidases activities on synaptic transmission [18,20–25]. Furthermore, the extracellular ATP and adenosine levels have been related to processes of learning and memory formation, since various evidences point to LTP and LTD and synaptic plasticity as a neural basis for cognitive processes [26–28]. Furthermore, adenosine receptor antagonists, such as caffeine has been shown to improve memory and induce anxiety in rats [29–33].

Antioxidants, antihyperglycemics and insulin sensitizing agents are reported to reduce cognitive dysfunction in diabetic condition [34–36]. However, at present, no specific treatments are available for the management and/or prevention of cognitive dysfunction in DM. Quercetin (Querc) is a flavonoid that possesses free radical scavenging properties and can protect from oxidative injury by its ability to modulate intracellular signals and promote cellular survival [37]. It has been shown that Querc is able to protect the memory loss in diabetic rats however studies involving the ectonucleotidases and AChE enzymes have not been described. Therefore, this study seeks to investigate the neuroprotective effects of quercetin on memory and anxiogenic-like behavior and ectonucleotidases and acetylcholinesterase activities in STZ induced diabetic rats.

2. Materials and methods

2.1. Chemical reagents

Quercetin (Querc, >95% purity, Q4951), 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB), streptozotocin, acetylthiocholine chloride, malondialdehyde tetrabutylammonium salt (MDA), 2-thiobarbituric acid (TBA), trizma base, nucleotides and Percoll reagent were obtained from Sigma Chemical Co (St. Louis, MO, USA). All the other chemicals used in this experiment were of highest purity.

2.2. Animals

Male *Wistar* rats (100 animals with 70–90 days old; 200–250 g) from the Central Animal House of the Federal University of Santa Maria were used in this study. The animals were maintained at a constant temperature (23 ± 1 °C), on a 12 h dark/light cycle with free access to food and water. All procedures were approved by the Animal Ethics Committee from the Federal University of Santa Maria (protocol number: 57/2010).

2.3. Experimental protocol

All animals were acclimatized for the period of 15 days, at five rats per cage, before the initiation of the experimental protocols as previously described [38–42]. The rats were randomly divided into eight groups: Vehicle, Querc 5 mg/kg, Querc 25 mg/kg, Querc 50 mg/kg, diabetic, diabetic *plus* Querc 5 mg/kg, diabetic *plus* Querc 25 mg/kg and diabetic *plus* Querc 50 mg/kg. Diabetes was induced by a single intraperitoneal injection of 70 mg/kg streptozotocin (STZ) diluted in 0.1 M sodium-citrate buffer (pH 4.5). STZ-treated rats received 5% of glucose instead of water for 24 h after diabetes induction in order to reduce death due to hypoglycemic shock. Blood samples were taken from the tail vein 48 h after STZ induction to measure glucose levels. Only animals with fasting glycaemia over 250 mg/dL were considered diabetic and used for the study. Ten days after diabetes induction, the treatments with vehicle or Querc were initiated. Querc was freshly prepared in 25% ethanol (vehicle) and was administered at between 3 and 4 p.m. once a day during 40 days, by gavage. The Querc doses were based on previous experimental doses used by our research group which reported beneficial effects [43–46] and was adjusted weekly based on individual weight. During the experiment the blood glucose levels were verified six times (15 days before, on the first day, 10, 20, 30, and 40 days after the beginning of treatment). A saline group was performed in control and STZ-diabetic rats to exclude possible effects of ethanol vehicle. The saline groups were not included in this study since there were not significant differences between vehicles saline and ethanol.

2.4. Behavioral parameters

2.4.1. Inhibitory avoidance task

After treatment with Querc (40 days), animals were subjected to training in a step-down inhibitory avoidance task as previously describe [17,47]. Twenty four hours later, the animals were subjected to the test in a step-down inhibitory avoidance task and recorded. Briefly, the inhibitory avoidance apparatus consisted of a 25 × 25 × 35 cm box with a grid floor whose left portion was covered by a 7 × 25 cm platform, 2.5 cm high was used. The rat was placed gently on the platform facing the rear left corner, and when the rat stepped down with all four paws on the grid, a 3-s 0.4 mA shock was applied to the grid. Retention test took place in the same apparatus 24 h later. Test step-down latency was taken as a measure of retention, and a cut-off time of 300 s was established.

2.4.2. Open field

Immediately after the inhibitory avoidance test session, the animals were transferred to an open-field measuring 56 × 40 × 30 cm, with the floor divided into 12 squares measuring 12 × 12 cm each. The open field session lasted for 5 min and during this time, an observer, who was not aware of the pharmacological treatments, recorded the number of crossing responses and rearing responses manually. This test was carried out to identify motor disabilities, which might influence inhibitory avoidance performance at testing.