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APOCYNIN PROTECTS AGAINST NEUROLOGICAL DAMAGE INDUCED BY QUINOLINIC ACID BY AN INCREASE IN GLUTATHIONE SYNTHESIS AND Nrf2 LEVELS

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Abstract—Apocynin (APO) is a well-known NADPH oxidase (NOX) inhibitor. However, several studies have reported its ability to increase glutathione (GSH) levels. Due to GSH is a major non-enzymatic antioxidant in brain, the aim of this study was to evaluate, in the striatum of control and quinolinic acid (QUIN) injected rats, the effect of APO administration on: (1) GSH levels, (2) activity of some enzymes involved in the GSH metabolism, and (3) nuclear factor erythroid-2-related factor 2 (Nrf2) mRNA levels. Animals received QUIN 240 nmol in right striatum and APO (5 mg/kg, i.p.), 30 min before and 60 min after intrastriatal injection. APO treatment prevented the QUIN-induced histological damage to the striatum. In control rats, APO treatment increased GSH and Nrf2 mRNA levels and the activities of gamma-glutamylcysteine ligase (γ-GCL), glutathione-S-transferase (GST) and glutathione peroxidase (GPx). On the other hand, APO treatment prevented the QUIN-induced decrease in GSH and Nrf2 levels, and in γ-GCL and GPx activities. These data indicate that APO is able to increase GSH levels and the activity of proteins involved in its metabolism, which could be associated with its ability to increase the Nrf2 mRNA levels. © **2017 IBRO. Published by Elsevier Ltd. All rights reserved.**

Keywords: glutathione, apocynin, Nrf2 factor, quinolinic acid.

INTRODUCTION

Quinolinic acid (QUIN) is a well-known glutamate agonist acting on the N-methyl-D-aspartate receptor (NMDAr) capable of producing excitotoxic damage (Beal et al., 1986; Schwarcz et al., 1983; Stone, 1993, 2001). Moreover, QUIN induces progressive mitochondrial alteration, with the consequent reduction of adenosine triphosphate (ATP) levels and further neurodegeneration (Bordelon et al., 1997; Ribeiro et al., 2006; Schuck et al., 2007). Oxidative stress has also been involved as a mechanism of QUIN toxicity (Pérez-De La Cruz et al., 2012; Santana-Martínez et al., 2014), since the increase in some oxidative damage markers has been reported, as well as alterations in endogenous antioxidant systems (Rodríguez-Martínez et al., 2000; Leipnitz et al., 2005). Additionally, it has been reported in this model that the activation of some pro-oxidant enzymes such as nitric oxide synthase (NOS) or NADPH oxidase (NOX) participate in the induction of neuronal damage due to excitotoxic-prooxidant conditions, and the use of inhibitors of these enzymes or antioxidant compound generate a protective effect (Aguilera et al., 2007; Maldonado et al., 2010; Santana-Martínez et al., 2014). On the other hand, in different neurological disorders such as Alzheimer's disease, acquired immunodeficiency syndrome (AIDS)-dementia complex, hepatic encephalopathy or Huntington disease, it has been found an increase in QUIN levels (Sardar et al., 1995; Heyes et al., 1996; Walsh et al., 2002; Guidetti et al., 2004; Guillemin et al., 2005), suggesting that the alterations in the kynurenine pathway could be related to the physiopathology of these diseases (Maddison and Giordini, 2015). Due to this, QUIN infusion into the rat striatum has been used as an experimental model to

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Abbreviations: APO, Apocynin; ATP, adenosine triphosphate; CDNB, 1-choloro-2,4-dinitrobenzene; EDTA, ethylenediamine tetraacetic acid; GPx, glutathione peroxidase; GSH, glutathione; GST, glutathione-S-transferase; NDA, 2,3-naphthalenedicarboxaldehyde; NOX, NADPH oxidase; Nrf2, erythroid-2-related factor 2; OPA, *o*-phthalaldehyde; PMSF, *p*-formaldehyde, phenylmethylsulfonyl fluoride; QUIN, quinolinic acid; γ-GCL, gamma-glutamylcysteine ligase.

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study some mechanisms involved in neurodegeneration process.

Apocynin (APO: 4-hydroxy-3-methoxyacetophenone) is a compound isolated from the root of the medicinal plant *Picrorhiza Kurroa* (*Scrophulariaceae*), a native plant grown in the mountains of India, Nepal, Tibet, and Pakistan (Stefanska and Pawliczak, 2008). It has been reported that APO have anti-inflammatory (Engels et al., 1992; reviewed in Stefanska and Pawliczak, 2008), and antioxidant (Heumüller et al., 2008) properties.

The anti-inflammatory properties of APO have been associated with its ability to inhibit NOX family (Simons et al., 1990; Qin et al., 2007; Maldonado et al., 2010). NOX is a proteic complex that can be assembled in response to several stimuli and is the responsible for superoxide anion production taking up electrons from NADPH and transferring them onto molecular oxygen. Superoxide anion can reacts with nitric oxide to form the strong oxidant agent peroxynitrite, or two superoxide anions can react to form H_2O_2; if sufficient H_2O_2 is produced, it can diffuses into the cell (Reviewed in 't Hart et al., 2014). Although the mechanism by which APO inhibit NOX is not completely known, this involves the impairment of the intracellular translocation of two cytosolic components (p47phox and p67phox subunits) to the subunits present in the cell membrane (gp91phox and p22phox) blocking the formation of active NOX complex (Simons et al., 1990; Stolk et al., 1994). The inhibitory effect occurs only after a lag time and it appears to involve the activation of APO via its enzymatic oxidation catalyzed by myeloperoxidase in presence of H_2O_2 and free thiols groups, leading to its dimerization (Ximenes et al., 2007; Stefanska and Pawliczak, 2008; 't Hart et al., 2014).

On the other hand, Heumüller et al. (2008) reported that APO acts as an antioxidant by scavenge H_2O_2 but not superoxide anion without NOX inhibition in endothelial and vascular smooth muscle cells. Moreover, APO was able to increase the renal superoxide dismutase activity and to prevent the nephrotoxic effect and oxidative damage induced by gentamicin (Abdelrahman, 2017).

Finally, several studies have reported that APO increases glutathione (GSH) levels in human alveolar epithelial cells (Lapperre et al., 1999), in the cortex of rats submitted to cerebral ischemia (Connell et al., 2011), in the heart of rats treated with endothelin 1 (an oxidative stress inductor) (Kleniewska et al., 2013) and in the kidney of Zucker diabetic fatty rat, a well established model of diabetes type 2 (Winiarska et al., 2014). In this work, this hypothesis was studied.

GSH is a tripeptide composed of γ-glutamate, cysteine, and glycine and is the most abundant nonprotein thiol in cells. It is synthesized into the cells via two reactions: (1) the γ-glutamylcysteine ligase (γ-GCL) catalyzes the reaction of glutamate with cysteine to produce a dipeptide, γ-glutamylcysteine; and (2) the GSH synthetase catalyzes the reaction of dipeptide with glycine. Cysteine is the rate-limiting substrate (reviewed in Aoyama and Nakaki, 2015). Because GSH is the main antioxidant in the central nervous system, the aim of this work was to study the effect of APO on GSH levels and in the activity of some enzymes involved in GSH metabolism: γ-GCL, glutathione-S-transferase (GST) and glutathione peroxidase (GPx) in a neurodegeneration model induced by QUIN in the rat striatum. Additionally, the nuclear factor erythroid-2-related factor 2 (Nrf2) participation was also evaluated.

EXPERIMENTAL PROCEDURES

Chemicals

APO, QUIN, *o*-phthalaldehyde (OPA), β-Nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), glutathione reductase, GSH, hydrogen peroxide (H_2O_2), L-cysteine, L-serine, ATP, cresyl violet acetate, sodium azide, 1-choloro-2,4-dinitrobenzene (CDNB), dithiothreitol (DTT), bovine serum albumin (BSA), *p*-formaldehyde, phenylmethylsulfonyl fluoride (PMSF), ethylenediamine tetraacetic acid (EDTA), 2,3-naphthalenedicarboxaldehyde (NDA), protease and phosphatase inhibitors, boric acid, and sulfosalicylic acid were obtained from Sigma–Aldrich (St. Louis, MO, USA). Glutamic acid was from JT Baker (Center Valley, PA, USA). Magnesium chloride hexahydrate ($MgCl_2 * 6H_2O$), eosin, and orthophosphoric acid (H_3PO_4) were obtained from Golden Bell Reagent (Guadalajara, Jal., Mexico). TaqMan Master 5× was from Applied Biosystem (Foster City, CA, USA). All other reagents were obtained from other known commercial sources. Deionized water from a Milli-Q system (Millipore, Bedford, MA, USA) was used for preparation of solutions.

Animals

Male Wistar rats (280–320 g) were used in this study. In the development of all experiments, animals were housed in acrylic box cages with access to standard commercial rat chow diet (Laboratory rodent diet 5001; PMI Feeds Inc., Richmond, IN, USA) and water *ad libitum*. Animals were maintained under standard environmental conditions of temperature (25 ± 3 °C), humidity (50 ± 10%), and lighting (12-h light/dark cycles). All experimental manipulations were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Local Guidelines on the Ethical Use of Animals from the Health Ministry of Mexico. During the experiments, all efforts were made to minimize animal suffering. The study was approved by Ethics Committee of Animals Research from Instituto Nacional de Neurología y Neurocirugía *Manuel Velasco Suárez*.

Experimental design

The animals were randomly divided into four groups ($n = 6$), as follows: (1) SHAM group treated with instrastriatal and i.p. injections of 0.9% isotonic saline solution (ISS, 300 mOsm) (as vehicle of QUIN and APO solution); (2) APO group treated with an i.p. injection of APO plus an intrastriatal injection of ISS; (3) QUIN group treated with an i.p. injection of ISS plus an intrastriatal injection of QUIN; and (4) QUIN + APO group treated with an i.p. injection of APO plus an intrastriatal injection of QUIN.

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