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Protective effect of L-kynurenine and probenecid on 6-hydroxydopamine-induced striatal toxicity in rats: Implications of modulating kynurenate as a protective strategy

Daniela Silva-Adaya [a,1], Verónica Pérez-De La Cruz [a], Juana Villeda-Hernández [b], Paul Carrillo-Mora [a], Irma Gabriela González-Herrera [a], Esperanza García [c], Laura Colín-Barenque [d], José Pedraza-Chaverrí [e], Abel Santamaría [a,*]

[a] Laboratorio de Aminoácidos Excitadores, Instituto Nacional de Neurología y Neurocirugía, Mexico City 14269, Mexico
[b] Laboratorio de Enfermedades Neurodegenerativas, Instituto Nacional de Neurología y Neurocirugía, Mexico City 14269, Mexico
[c] Departamento de Neuroinmunología, Instituto Nacional de Neurología y Neurocirugía, Mexico City 14269, Mexico
[d] Laboratorio de Neuromorfologia, Facultad de Estudios Superiores Iztacala, UNAM, Tlalnepantla 54090, Mexico
[e] Departamento de Biología, Facultad de Química, Universidad Nacional Autónoma de Mexico, Mexico D.F. 04510, Mexico

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ABSTRACT

The neuroactive metabolite at the kynunerine pathway, kynurenic acid (KYNA), is a well-known competitive antagonist at the co-agonist glycine site of the N-methyl-D-aspartate receptor (NMDAr), and also decreases the extracellular levels of glutamate by blocking α7-nicotinic acetylcholine receptor (α7-nAchr) located on glutamatergic terminals. KYNA has been often reported to be neuroprotective in different neurotoxic models. The systemic administration of L-kynurenine (L-KYN) – the precursor of KYNA – together with probenecid (PROB) – an inhibitor of organic acids transport – to rodents increases KYNA levels in the brain in a dose-dependent manner. The striatal infusion of the toxin 6-hydroxydopamine (6-OHDA) to rodents is one of the common models used to simulate Parkinson's disease (PD). Different studies have linked PD alterations with excessive glutamatergic transmission in the striatum since NMDAr antagonists exert beneficial effects in PD models. In this work we investigated the effect that a systemic administration of L-KYN + PROB exerted on the toxic model induced by 6-OHDA in rats. PROB (50 mg/kg, i.p.) + L-KYN (75 mg/kg, i.p.) were given to rats for seven consecutive days. On day two of treatment, the animals were infused with a single injection of 6-OHDA (20 μg/2 μl) into the right striatum. Fourteen days post-lesion, rotation behavior was assessed as a marker of motor impairment. The total levels of dopamine (DA) were also estimated in striatal tissue samples of 6-OHDA-treated animals as a neurochemical marker of damage. In addition, twenty eight days post-lesion, the striatal damage was assessed by hematoxylin/eosin staining and immunohistochemistry against glial fibrillary acidic protein (GFAP) in the same animals. Neurodegeneration was also assessed by Fluoro Jade staining. 6-OHDA infusion increased rotation behavior, striatal reactive gliosis and neurodegeneration, while DA levels were decreased. For all markers evaluated, we observed protective effects of L-KYN + PROB on the dopaminergic damage induced by 6-OHDA. Our results suggest that this strategy was useful to mitigate dopaminergic toxicity in the hemiparkinsonian model. The combined use of L-KYN and PROB is a valuable tool to modulate glutamatergic and cholinergic activities, presumably by means of increased levels of endogenous KYNA.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder around the World. The incidence of PD increases with age from 1 to 2% at 50 years-old to approximately 5% at 60 to 85 years-old (Shastry, 2001). This disorder is characterized by rigidity, tremor, postural abnormalities and bradykinesia. At a pathological level, PD presents progressive degeneration of dopamergic neurons in the substantia nigra pars compacta (SNpc) projecting into the striatum. Clinical parkinsonian symptoms arise when a majority (60–70%) of DAergic neurons from SNpc are damaged, resulting in reduced

Abbreviations: ANOVA, Analysis of variance; CNS, Central nervous system; DA, Dopamine; DOPAC, Dihydroxyphenylacetic acid; GABA, γ-Aminobutyric acid; GFAP, Glial fibrillary acidic protein; H&E, Hematoxylin-eosin; HVA, Homovanillic acid; 6-OHDA, 6-Hydroxydopamine; KP, Kynurenine pathway; KYNA, Kynurenic acid; L-KYN, L-kynurenine; α7-nAchr, α7-Nicotinic acetylcholine receptors; NMDAr, N-Methyl-D-aspartate receptors; PBS, Phosphate-buffered saline; PD, Parkinson's disease; PROB, Probenecid; ROI, Region of interest; SEM, Standard error; SNpc, Substantia nigra pars compacta.

* Corresponding author. Laboratorio de Aminoácidos Excitadores, Insurgentes Sur 3877, Mexico D.F., 14269, Mexico. Tel.: +52 55 5606 3822x2013.
E-mail address: absada@yahoo.com (A. Santamaría).
[1] Programa de Maestría, Posgrado en Ciencias Biológicas, UNAM.

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dopamine (DA) levels in the nigrostriatal system (Lang and Lozano, 1998a,b). One of the most widely used experimental models of PD is the one produced by the intrastriatal infusion of 6-hydroxydopamine (6-OHDA) to rats. This toxic model provides a more gradual progressive impact on the nigral–striatal system (a protracted retrograde degeneration that lasts for 1–3 weeks after lesioning) than the more immediate effects caused by injection of this agent into either the medial forebrain bundle or the SN itself (Aponso et al., 2008). 6-OHDA is a hydroxylated analogue of DA which uses the same transport system as DA to reach the intracellular domain and produce specific degeneration of catecholaminergic neurons. Following its intracerebral injection to rats, 6-OHDA causes degeneration of DAergic neurons with a dramatic loss of DA in the striatum, as well as loss of tyrosine hydroxylase immunoreactivity (Bové et al., 2005). Then, the extent of DA depletion can be assessed by evaluating rotation behavior in response to DAergic agonists (Beal, 2001).

N-methyl-D-aspartate subtype of glutamate receptors (NMDAr) are abundantly distributed throughout the brain, and are highly modulated ionotropic receptors responsible for excitatory neurotransmission in the central nervous system (CNS). Their role in physiological regulation of excitatory events is well established; however, under pathological conditions, excessive stimulation of these receptors leads to an excess of intracellular calcium through the persistent opening of the channel associated to the receptor to produce excitotoxicity, a toxic mechanism currently involved in cell death in several disorders of the brain (including Alzheimer's disease, stroke, Huntington's disease and PD) in which excess of glutamatergic transmission and mitochondrial metabolic disturbances are primary mechanisms turning neuronal cells more sensitive to excitotoxic damage (Kemp and McKernan, 2002). Noteworthy, it has been documented that antagonist of NMDAr exhibit beneficial effects in PD and related animal models (Löschmann et al., 2004; Montastruc et al., 1997; Nash and Brotchie, 2002; Nash et al., 2000, 2004), thus supporting the concept that glutamatergic transmission is a key factor triggering DAergic damage in PD. Moreover, the DAergic and glutamatergic interactions involving kynurenate-sensitive components is a topic under current investigation (Poeggeler et al., 2007).

A well-known NMDAr antagonist, kynurenic acid (KYNA), is also recognized as a neuroprotective metabolite formed at the kynurenine pathway (KP), a route that accounts for the metabolism of around 80% on non-protein tryptophan metabolism (Stone, 2001). KYNA is present in the mammalian brain at nanomolar concentrations and has been described as an inhibitory component exerting anticonvulsant and anti-excitotoxic actions (Foster et al., 1984). At physiological concentrations, KYNA blocks the glycine site of NMDAr (Kessler et al., 1989), and also inhibits α7-nicotinic acetylcholine receptors (α7nAChr) in a non-competitive manner (Hilmas et al., 2001). These receptors are abundantly localized on glutamatergic nerve endings (Carpenedo et al., 2001). KYNA is therefore conceptualized as a major endogenous modulator of physiological events associated with glutamatergic transmission (Schwarcz, 2004).

The modulation of KP through pharmacological challenges, when directed to enhance the endogenous formation of KYNA in brain tissue, has shown to constitute a successful strategy to counteract toxic events in different experimental models of brain damage (Miranda et al., 1997; Vécsei et al., 1992). When its precursor, L-kynurenine (L-KYN), is systemically administered, together or not with probenecid (PROB) - an organic acid transport inhibitor blocking the excretion of KYNA from the CNS -, the endogenous KYNA levels are increased and produce neuroprotective actions in a variety of toxic models in mammals, including those produced by quinolinic acid, MK-801, ischemia, amyloid beta peptide, etc. (Carrillo-Mora et al., 2010; Gigler et al., 2007; Hlinak and Krejci, 2006; Miranda et al., 1997; Robotka et al., 2008; Santamaría et al., 1996; Sas et al., 2008; Stone, 2000).

In consideration to the relevance that this pharmacological tool represents for therapeutic approaches at experimental and clinical levels, as well as its potential application to toxic models sharing excitotoxic components, in this work we evaluated the effect of a systemic administration of L-KYN and PROB on behavioral (locomotor asymmetry), morphological (reactive gliosis and neurodegeneration) and neurochemical alterations (changes in DA and metabolites) in rats exposed to a single intrastriatal infusion of 6-OHDA, in an attempt to provide an alternative therapeutic strategy to counteract the toxic features produced in this paradigm through the modulation of the endogenous KYNA levels. Our results demonstrate neuroprotective actions of the combined treatment with PROB + L-KYN on all toxic markers induced by 6-OHDA. The relevance of these findings for PD and related models is discussed.

2. Materials and methods

2.1. Chemicals

6-OHDA was obtained from Sigma-Aldrich (Steinheim, Germany). Sodium octyl sulphate, DA and apomorphine were obtained from Sigma Chemical Co. (St. Louis, MO). L-KYN was kindly donated by Prof. Robert Schwarcz, from the Maryland Psychiatric Research Center. All other reagents were from other known commercial sources. Deionized water from a Direct-Q3 UV system (Millipore, MA) was currently used for preparation of solutions.

2.2. Animals

Male Wistar bred-in-house rats (270–300 g) were used throughout the study. For all experimental purposes, animals were housed five per cage in acrylic box cages and provided with a standard commercial rat chow diet (Laboratory rodent diet 5001; PMI Feeds Inc., Richmond, IN, USA) and water *ad libitum*. Housing room was maintained under constant conditions of temperature (25 ± 3 °C), humidity (50 ± 10%), and lighting (12 h light/dark cycles). Forty eight rats were employed for *in vivo* experiments. All procedures with animals were carried out 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.

2.3. Experimental groups and striatal lesions with 6-OHDA

Animals (6 per group) were randomly assigned to four experimental groups: Groups I and II received seven consecutive i.p. injections of vehicle (NaOH 1 N, with pH adjusted to 10) for 7 days, while Groups III and IV received PROB (50 mg/kg, i.p.) and L-KYN (75 mg/kg, i.p.) also for seven consecutive days (both compounds were dissolved in NaOH 1 N with pH adjusted to 10). Dosing regimens were selected on the basis of a recent study demonstrating that these doses of PROB and L-KYN are neuroprotective in a toxic model induced by amyloid-β peptide in Wistar rats (Carrillo-Mora et al., 2010). Also, pilot studies were carried out to estimate the efficacy of these treatments on 6-OHDA-induced behavioral alterations. Groups I and III also received a single 2 μl-intrastriatal infusion (in caudate-putamen) of ascorbic acid [0.02% in phosphate-buffered saline (PBS) as vehicle] on the second day of systemic treatments (30 min after i.p. injections), while Groups II and IV received a similar volume of a solution containing 20 μg of 6-OHDA dissolved in 0.02% ascorbic acid, also on the second day of systemic treatments.

Stereotaxic injections were performed to animals under pentobarbital anesthesia (50 mg/kg, i.p.). Stereotaxic coordinates were as follows: AP: −0.5 mm from bregma; L: −3.5 mm from bregma; and V: −4.8 mm from dura, according to the brain atlas of Paxinos and Watson (Paxinos and Watson, 1986). Rats were unilaterally injected into the right striatum using a Hamilton microsyringe. The needle