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Research Report

Acute intrastriatal injection of quinolinic acid provokes long-lasting misregulation of the cytoskeleton in the striatum, cerebral cortex and hippocampus of young rats

*Paula Pierozan, Carolina Gonçalves Fernandes, Fernanda Ferreira, Regina Pessoa-Pureur**

Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, UFRGS, Porto Alegre, RS, Brasil

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ABSTRACT

Quinolinic acid (QUIN) is a neuroactive metabolite of the kinurenine pathway, considered to be involved in aging and some neurodegenerative disorders, including Huntington's disease. In the present work we have studied the long-lasting effect of acute intrastriatal injection of QUIN (150 nmol/0.5 μL) in 30 day-old rats on the phosphorylating system associated with the astrocytic and neuronal intermediate filament (IF) proteins: glial fibrillary acidic protein (GFAP), and neurofilament (NF) subunits (NFL, NFM and NFH) respectively, until 21 days after injection. The acute administration of QUIN altered the homeostasis of IF phosphorylation in a selective manner, progressing from striatum to cerebral cortex and hippocampus. Twenty four hours after QUIN injection, the IFs were hyperphosphorylated in the striatum. This effect progressed to cerebral cortex causing hypophosphorylation at day 14 and appeared in the hippocampus as hyperphosphorylation at day 21 after QUIN infusion. PKA and PKCaMII have been activated in striatum and hippocampus, since Ser55 and Ser57 in NFL head domain were hyperphosphorylated. However, MAPKs (Erk1/2, JNK and p38MAPK) were hyperphosphorylated/activated only in the hippocampus, suggesting different signaling mechanisms in these two brain structures during the first weeks after QUIN infusion. Also, protein phosphatase 1 (PP1) and 2B (PP2B)-mediated hypophosphorylation of the IF proteins in the cerebral cortex 14 after QUIN injection reinforce the selective signaling mechanisms in different brain structures. Increased GFAP immunocontent in the striatum and cerebral cortex 24 h and 14 days after QUIN injection respectively, suggests reactive astrocytes in these brain regions. We propose that disruption of cytoskeletal homeostasis in neural cells takes part of the long-lasting molecular mechanisms of QUIN toxicity in adolescent rats, showing selective and progressive misregulation of the signaling mechanisms targeting the IF proteins in the striatum, cerebral cortex and hippocampus with important implications for brain function.

*Correspondence to: Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Departamento de Bioquimica, Rua Ramiro Barcelos 2600 anexo, CEP 90035-003 Porto Alegre - RS, BRASIL. Fax: +5551 3308 5535.
E-mail address: rpureur@ufrgs.br (R. Pessoa-Pureur).

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1. Introduction

Intrastriatal injection of quinolinic acid (QUIN), an endogenous metabolite of tryptophan pathway and N-methyl-D-aspartate (NMDA) receptor agonist, has been reported to cause an increase in Ca^{2+} influx, a decrease in ATP production, and excitotoxic cell death producing a pattern of striatal cell loss that mimics the depletion of projections of striatal neurons observed in Huntington Disease (HD) (Beal et al., 1986). It was also shown that QUIN induces lipid peroxidation and mitochondrial dysfunction in the brain in a concentration-dependent manner (Perez-De La Cruz et al., 2005).

Huntington Disease is a hereditary neurodegenerative disorder characterized by an extensive neurodegeneration in striatum and cerebral cortex, which results in motor, cognitive and neuropsychiatric disorders (Martin and Gusella, 1986). The juvenile-onset or Juvenil Huntington's disease (JHD) and adult-onset forms of HD appear to differ in their pattern of striatal projection neuron loss and in the onset of symptoms (Albin et al., 1990). The mechanisms underlying the selective neurodegeneration in HD are still unknown, but is has been proposed that NMDA receptor-mediated excitotoxic processes may be involved.

The cytoskeleton, consisting of microtubules, intermediate filaments (IFs) and actin filaments is indispensable for any eukaryotic cell. The IF proteins constitute an important network of cytoskeletal proteins of vertebrate cells expressed in a tissue-and development-specific manner.

Neurons express specific IF named neurofilaments (NFs) which consist of three subunits divided according to their molecular mass: NF heavy-chain (NFH), NF middle chain (NFM) and NF light chain (NFL). Neurofilaments are important elements of the axonal cytoskeleton where they are longitudinally oriented and crosslinked to other structures constituting the most important supporting network for adult axons. Although a considerable body of evidence supports that the most essential function of NFs is maintaining the axonal diameter and thereby the conduction velocity (Friede and Samorajski, 1970; Zhu et al., 1997), recent evidence points the axonal cytoskeleton as a highly dynamic and responsive structure, able to alter their homeostasis in response to a variety of physiological and pathological signals. (Zamoner and Pessoa-Pureur, 2011a; Zanatta et al., 2012).

Glial fibrillary acidic protein (GFAP) is the main IF protein expressed in mature astrocytes, where it is thought to help maintaining mechanical strength, as well as the shape of cells. However, recent evidence has shown that GFAP plays a role in a variety of additional astrocyte functions, such as cell motility/migration, cell proliferation, glutamate homeostasis, neurite outgrowth and injury/protection (Middeldorp and Hol, 2011). Also, after injury of CNS, astrocytes become reactive and respond in a typical manner, termed reactive gliosis (Eng et al., 2000) which is characterized by astrocyte proliferation, increased production of GFAP or by decreased GFAP turnover, causing increased protein content.

Intermediate filament proteins are known to be phosphorylated on their head and tail domains and the dynamics of their phosphorylation/dephosphorylation plays a major role in regulating the structural organization and function of IFs in a cell-and tissue-specific manner (Grant and Pant, 2000; Inagaki et al., 1990; Nixon and Sihag, 1991; Omary et al., 2006). Amino-terminal phosphorylation is mainly involved in regulating the assembly/disassembly equilibrium of GFAP, NFL and NFM subunits of NFs (Sihag et al., 2007). *In vivo* and *ex vivo* studies from our group and from others have demonstrated that the phosphate groups on the amino-terminal head domain of GFAP, vimentin and NFL are added by second messenger-dependent protein kinases, such as cAMP-dependent protein kinase (PKA), Ca^{2+}/calmodulin-dependent protein kinase II (PKCaMII) and protein kinase C (PKC) (Pierozan et al., 2010; Pierozan et al., 2012; Sihag et al., 2007). GFAP phosphorylation is possibly a key factor in astrocyte, since cell uses phosphorylation/dephosphorylation levels to regulate the dynamic of the polymerization/depolymerization of these proteins promoting cell survival and physiological roles.

Also, the assembly of NFs into a heteropolymer is dependent on the head domains of NFL and NFM and more specifically on the phosphorylation level of these domains. In this context, some of the major sites of *in vivo* and *in vitro* phosphorylation by QUIN on NFL and NFM subunits were identified to be Ser-55 (PKA) and Ser-57 (PKCaMII) (Pierozan et al., 2012).

On the other hand, phosphorylation sites in the tail domain of NFM and NFH were found to be Ser residues located in Lys-Ser-Pro (KSP) repeat regions of the tail domain of these subunits. The KSP repeats are phosphorylated by the proline-directed kinases Cdk5, the mitogen-activated protein kinases (MAPK) such as Erk1/2, JNK, p38MAPK, as well as glycogen synthase kinase 3 (GSK3) (Giasson and Mushynski, 1996; Guidato et al., 1996a, 1996b; Veeranna et al., 1998).

We have recently found that intrastriatally QUIN-injected adolescent rats showed progressive biochemical and histopathological alterations in the striatum, cerebral cortex and hippocampus, as well as behavioral deficits over a period of 21 days after drug injection, mimicking JHD (Pierozan et al., 2014). However, little is known about the role of the cytoskeleton in the cell damage induced by QUIN over the first weeks after the intrastriatal injection. In line with this, we have recently described that QUIN is able to induce hyperphosphorylation of neuronal and glial IF proteins in QUIN-exposed striatal slices of young rats and this effect was mediated by glutamate receptors and $_{i}Ca^{2+}$ levels (Pierozan et al., 2012). It is important to note that the disrupted homeostasis of the cytoskeleton of striatal neural cells was demonstrated to be an early event observed 30 min after intrastriatal QUIN injection in young rats (Pierozan et al., 2010). The evidence of a link between misregulation of cell signaling mechanisms, disruption of IF phosphorylation and cell damage in response to QUIN point to a critical role of the signaling pathways regulating the IF phosphorylation in the early events of QUIN-induced toxicity and lead us to search for the role of the cytoskeleton in the progress of brain injury.

Therefore, in the present report we investigated the long-lasting QUIN actions targeting the phosphorylating system associated with the IF-enriched cytoskeleton in the striatum, cerebral cortex and hippocampus of young rats, identifying activated kinases and phosphatases over 21 days after QUIN injection. We hypothesize that disruption of cytoskeletal