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Research Report

Intrastriatal injection of hypoxanthine alters striatal ectonucleotidase activities: A time-dependent effect

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ABSTRACT

The aim of this study was to investigate the effects of intrastriatal injection of hypoxanthine on ectonucleotidase (E-NTPDases and ecto-5′-nucleotidase) activities and expressions in the striatum of rats. The effect of pre-treatment with vitamins E and C on the effects elicited by this oxypurine on enzymatic activities and on thiobarbituric reactive substances (TBARS) was also investigated. The effect of pre-incubation with hypoxanthine on nucleotide hydrolysis in striatum homogenate was also determined. Adult Wistar rats were divided into (1) control and (2) hypoxanthine-injected groups. For ectonucleotidase activity determination, the animals were sacrificed at 30 min, 24 h and 7 days after drug infusion. For the evaluation of the expression of NTPDase 1–3 and also ecto-5′-nucleotidase, TBARS assay and the influence of the pre-treatment with vitamins on ectonucleotidase activities, the animals were sacrificed 24 h after hypoxanthine infusion. Results show that hypoxanthine infusion significantly inhibited ectonucleotidase activities and increased TBARS only 24 h after administration. Pre-treatment with vitamins was able to prevent these effects. Moreover, ecto-5′-nucleotidase expression was increased (80%) at 24 h after hypoxanthine infusion. We suggest that these hypoxanthine-induced biochemical modifications could, at least in part, participate in the pathophysiology of Lesch Nyhan disease.

1. Introduction

Lesch Nyhan disease is a hereditary X-linked disorder caused by a deficiency of hypoxanthine–guanine phosphoribosyltransferase (HPRT) activity (Nyhan et al., 1965; Henderson, 1968; Rijksen et al., 1981; Jinnah and Friedmann, 2001), in which tissue accumulation of hypoxanthine occurs. Affected patients present cognitive deficits, hyperuricemia, spasticity, dystonia and self-mutilation behavior, characterized by biting of the lips, tongue and fingers with apparent tissue loss (Mizuno, 1986; Jinnah and Gage, 1990; Matthews et al., 1999; Jinnah and Friedmann, 2001). In addition, patients present a prominent loss of striatal dopamine (Jinnah and Friedmann, 2001).

Although the connection between neurological and behavioral dysfunctions present in Lesch Nyhan disease and the

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alteration in the purine metabolism is not elucidated, tissue accumulation of oxypurines such as hypoxanthine has been demonstrated to contribute to the neurological dysfunction present in this disease (Dasheiff, 1980; Kisch et al., 1985; Visser et al., 2000; Ma et al., 2001). In this scenario, a recent study demonstrated that intrastriatal administration of hypoxanthine significantly inhibited Na^+, K^+-ATPase, acetylcholinesterase and catalase activities in a time-dependent manner (Bavaresco et al., 2007). Moreover, it has been demonstrated that hypoxanthine/xanthine oxidase acts as a source of oxidative stress in the vascular system (Oliveira et al., 2001) and might contribute to the destruction of blood-brain barrier observed in ischemic brain tissue (Beckman et al., 1987). A previous study demonstrated that *in vitro* hypoxanthine increased thiobarbituric reactive substances (TBARS) and reduced tissue antioxidant capacity (TRAP) in rat striatum, suggesting an induction of oxidative stress (Bavaresco et al., 2005).

It is well known that the biological role of ATP is not only as metabolic fuel, since ATP and also ATP hydrolysis products (ADP, AMP and adenosine) can act as signaling molecules (Komoszynski and Wojtczak, 1996). In this context, studies indicate that ATP and its metabolites, as well as some purines can act as neuromodulators in different brain regions (Schiffmann and Vanderhaeghen, 1993; Komoszynski and Wojtczak, 1996). It has also been shown that adenosine exerts a regulatory mechanism on the monoamines system (Schiffmann and Vanderhaeghen, 1993; Zhu et al., 2007) and seems to be involved in motor and behavioral changes through its action on specific receptors, named A_1, A_2, A_{2B} and A_3 (Ribeiro et al., 2003; Martinelli and Tuccinardi, 2008). On the other hand, evidence suggests the involvement of the adenosine receptor in aggressive behavior after clonidine administration in mice (Ushijima et al., 1984). Extracellular concentrations of adenosine depend on a regulatory mechanism between the release of this compound from the intracellular medium and the catabolism of ATP, ADP and AMP by ectonucleotidases, such as NTPDases (nucleoside triphosphate diphosphohydrolase) and ecto-5′-nucleotidase (CD73) (Zimmermann, 2006).

NTPDases are membrane bound enzymes with catalytic sites located in the extracellular medium that are responsible for ATP and ADP breakdown. These enzymes are involved in the modulation of synaptic transmission, adult neurogenesis and thromboregulation (Zimmermann, 2006). In the central nervous system (CNS), three different NTPDases have been demonstrated, each differing with regard to substrate preference: NTPDase1 hydrolyzes ATP and ADP about equally well, NTPDase2 stands out for its high preference for ATP and NTPDase3 has a preference for ATP over ADP (Zimmermann, 2006). Moreover, ecto-5′-nucleotidase is a surface-located ecto-enzyme, anchored to the plasmatic membrane via a glycosyl phosphatidylinositol (GPI) anchor and is responsible for extracellular hydrolysis of AMP to adenosine (Zimmermann et al., 1998).

It has been suggested that a deficit in adenosine-mediated neuronal modulation could be involved in the pathological basis of Lesch Nyhan disease (Prior et al., 2006). In this context, Torres et al. (2004) demonstrated that hypoxanthine altered adenosine transport in peripheral blood lymphocytes from both control and Lesch Nyhan patients. In addition, Pesi et al. (2000) showed that cytosolic 5′-nucleotidase activity was increased in erythrocytes from individuals with Lesch Nyhan disease, suggesting the participation of oxypurine toxicity in Lesch Nyhan disease. Moreover, urinary excretion of adenosine was shown to be decreased in patients with Lesch Nyhan disease (Sweetman and Nyhan, 1970).

In the present study, we investigated the effect of intrastriatal hypoxanthine injection on ectonucleotidase activities. We also determined the relative expression of ectonucleotidases in rat striatum. The effect of pre-treatment with vitamins E and C on the hypoxanthine-mediated effects on ectonucleotidases and thiobarbituric acid reactive species (TBARS) were also investigated. Hypoxanthine was injected into the striatum because evidence suggests that many neurobehavioral features of the Lesch Nyhan disease results from the dysfunction of this cerebral structure (Visser et al., 2002).

2. Results

2.1. *Experiment 1: effect of intrastriatal hypoxanthine infusion on ATP, ADP and AMP hydrolysis in the synaptosomes of the striatum of rats*

Fig. 1 shows the effect of intrastriatal injection of hypoxanthine on nucleotide hydrolysis in the striatum of rats at different periods after oxypurine administration. As can be seen, ATP [$t(6)=3.200$; $p<0.01$] (Fig. 1A), ADP [$t(6)=2.828$; $p<0.01$] (Fig. 1B) and AMP [$t(6)=3.551$; $p<0.01$] (Fig. 1C) hydrolysis were significantly inhibited 24 h after hypoxanthine infusion, but not at 30 min {ATP [$t(6)=0.739$; $p>0.05$] (Fig. 1A); ADP [$t(6)= 1.395$; $p>0.05$] (Fig. 1B); AMP [$t(6)=0.563$; $p>0.05$] (Fig. 1C)} or 7 days {ATP [$t(6)=1.042$; $p>0.05$] (Fig. 1A); ADP [$t(6)=0.695$; $p>0.05$] (Fig. 1B); AMP [$t(6)=0.543$; $p>0.05$] (Fig. 1C)}. Nucleotide hydrolysis was not altered after hypoxanthine administration in contralateral striatum (data not shown).

2.2. *Experiment 2: relative expression of striatal NTPDases and ecto-5′-nucleotidase, analyzed by semi-quantitative RT-PCR*

Since nucleotide hydrolysis was only inhibited at 24 h after hypoxanthine administration, we also analyzed the relative expressions of NTPDases (NTPDase1, NTPDase2 and NTPDase3) and ecto-5′-nucleotidase in rat striatum after 24 h of hypoxanthine administration. As can be seen in Fig. 2, the relative expressions of NTPDase 1 (Figs. 2A–B), NTPDase 2 (Figs. 2C–D), and NTPDase 3 (Figs. 2E–F) were not altered by hypoxanthine treatment. However, the relative expression of ecto-5′-nucleotidase (Figs. 2G–H) was significantly increased (80%) after oxypurine administration.

2.3. *Experiment 3: effect of pre-treatment with vitamins E and C on the ectonucleotidase activities and on TBARS in rat striatum*

In this set of experiments, the effect of pre-treatment with vitamins E and C was determined on the effects elicited by 24 h