available at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Effect of allopurinol on brain adenosine levels during hypoxia in newborn piglets

Peter J. Marro[a,*], *Om P. Mishra*[b], *Maria Delivoria-Papadopoulos*[b]

[a]*Departments of Pediatrics, Division of Neonatology, Barbara Bush Children's Hospital at Maine Medical Center, 22 Bramhall Street, Portland, ME 04102-3134, USA*

[b]*Department of Pediatrics, Drexel University College of Medicine, St. Christopher's Hospital for Children, Philadelphia, PA 19129, USA*

ARTICLE INFO

Article history:
Accepted 12 November 2005
Available online 26 January 2006

Keywords:
Hypoxia
Xanthine oxidase
Adenosine
Purines
Allopurinol

ABSTRACT

Adenosine, a purine nucleoside, is a potent inhibitory neuromodulator in the brain which may provide an important endogenous neuroprotective role during hypoxia–ischemia. Allopurinol, a xanthine oxidase inhibitor, blocks purine degradation and may result in the accumulation of purine metabolites, including adenosine, during hypoxia. The present study determines the effect of allopurinol administration prior to hypoxia on brain levels of adenosine and purine metabolites in the newborn piglet. Twenty-two newborn piglets (age 3–7 days) were studied: 5 untreated normoxic and 6 allopurinol-treated normoxic controls were compared to 5 untreated hypoxic and 6 allopurinol-treated hypoxic animals. Brain tissue energy metabolism was continuously monitored during hypoxia by ^{31}P NMR spectroscopy. Brain tissue levels of purines increased in both hypoxic groups during hypoxia, however, there were significantly higher increases in brain tissue levels of adenosine (66.5 ± 30.5 vs. 19.4 ± 10.7 nmol/gm), $P < 0.01$ and inosine (265 ± 97.6 vs. 162.8 ± 38.3 nmol/gm), $P = 0.05$ in the allopurinol-treated hypoxic group. Allopurinol inhibits purine degradation under severe hypoxic conditions and results in a significant increase in brain tissue levels of adenosine and inosine. The increased accumulation of CNS adenosine during hypoxia which is seen in the allopurinol-treated animals may potentiate adenosine's intrinsic neuroprotective mechanisms.

1. Introduction

Adenosine is an endogenous neuromodulator which potentially plays a significant protective role in cerebral hypoxic–ischemic injury (Daval et al., 1989; Fern et al., 1994; Gidday et al., 1995; Zhou et al., 1994). Adenosine promotes vasodilation of cerebral vessels during ischemia (Dunwiddie, 1985; Laudignon et al., 1991; Sciotti and Van Wylen, 1993) but can also inhibit the release of numerous neurotransmitters including aspartate and glutamate (Poli et al., 1991; Sciotti et al., 1992; Simpson et al., 1992). A high density of presynaptic adenosine A1 receptors is found in the hippocampus, notably, the CA1 region, which is enriched with glutamate receptors of the NMDA type. This area is known to be especially vulnerable to ischemia. Adenosine may induce a hyperpolarizing outward potassium current inhibiting the release of glutamate via presynaptic A1 receptors which may lead to a voltage-dependent block of NMDA receptors (Lee and Lowenkopf, 1993; MacDonald et al., 1986; Trussell and Jackson, 1985), thus reducing NMDA-receptor-mediated calcium influx and

* *Corresponding author*. Fax: +1 207 871 6063.
E-mail address: marrop@mmc.org (P.J. Marro).

0006-8993/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.11.061

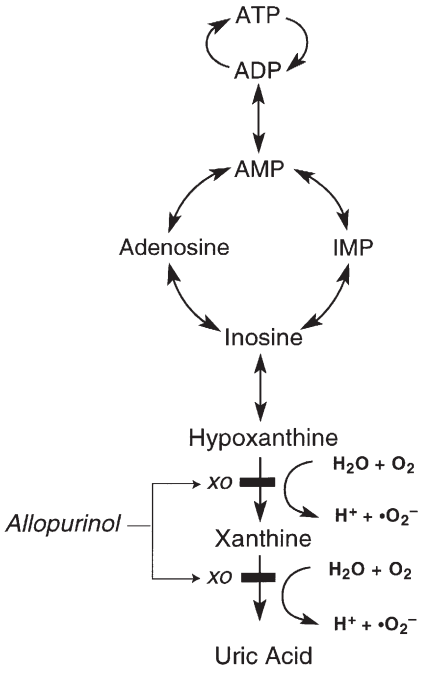

Fig. 1 – Purine metabolism and the generation of oxygen-free radicals via the xanthine oxidase pathway, with inhibition by allopurinol. XO, xanthine oxidase; $\cdot O_2^-$, superoxide radical.

excitotoxicity (Craig and White, 1992; de Mendonca and Ribeiro, 1993; Finn et al., 1991).

Under conditions of tissue hypoxia, ATP is broken down to ADP, AMP, adenosine, inosine, and hypoxanthine (Fig. 1). The accumulation of adenosine and purine metabolites during hypoxia may provide an endogenous neuromodulatory and neuroprotective role. Previous studies have shown that allopurinol, a xanthine oxidase inhibitor, reduces the formation of uric acid in the plasma and in the brain tissue of newborn piglets during hypoxia (Marro et al., 1994). Allopurinol may also increase brain levels of adenosine and other purine metabolites during hypoxia by blocking the xanthine oxidase pathway and inhibiting purine degradation.

The present study measures the effect of allopurinol administration prior to hypoxia on brain levels of adenosine and purine metabolites during hypoxia in the newborn piglet.

2. Results

Physiologic measurements are presented in Table 1. All experimental animals were ventilated to achieve normal baseline arterial $PaCO_2$ measurements (35–45 mm Hg). In addition, all groups had normal baseline arterial pH and PaO_2 values. Following a decrease in FiO_2 in the hypoxic groups, arterial pH fell uniformly. The $PaCO_2$ in the allopurinol-treated animals decreased during hypoxemia but was not significantly different than the untreated group. The PaO_2 values were similar in both groups during hypoxia. Mean arterial blood pressure (MABP) decreased during hypoxia equally in the treated and untreated group (Table 1).

^{31}P NMR spectra (Fig. 2) obtained from the hypoxic study groups are summarized in Table 2. Baseline PCr/Pi values in the untreated and the treated groups were similar. No change in ATP or PCr/Pi was observed following the infusion of allopurinol. As the FiO_2 was lowered, an 80% reduction in PCr/Pi from baseline measurements was achieved in both hypoxic groups. ATP levels decreased 28% in the untreated hypoxic group, whereas ATP levels decreased by only 18% in the allopurinol-treated group. Hypoxia also resulted in a significant fall in pHi.

Plasma levels of allopurinol in the treated animals following drug infusion were 15.9 ± 3.9 mcg/ml. Brain tissue levels of purines (Table 3) increased in both hypoxic groups during hypoxia, however, there were significantly higher increases in brain tissue levels of adenosine (66.5 ± 30.5 vs. 19.4 ± 10.7 nmol/gm) and inosine (265 ± 97.6 vs. 162.8 ± 38.3 nmol/gm) in the allopurinol-treated hypoxic group.

3. Discussion

Hypoxia–ischemia produces an elevation of the excitatory amino acid glutamate in the brain secondary to enhanced presynaptic release and dysfunctional energy-dependent uptake mechanisms (Choi, 1987, 1988; McGeer and McGeer, 1989). Excessive stimulation of the NMDA receptor by glutamate can cause unregulated calcium influx, the generation of oxygen-free radicals, and activation of cytotoxic lipases, proteases, and endonucleases which may result in neuronal injury and death (Fagni et al., 1999; Johnston et al., 1991; Murphy et al., 1996; Pellegrini-Giampietro et al., 1990; Urban et al., 1990; Vannucci, 1990). The accumulation of purine metabolites during hypoxia, specifically increased levels of cerebral extracellular adenosine, may impart critical endogenous neuroprotective properties under conditions of hypoxia–ischemia in the newborn brain. Endogenous adenosine has been shown to increase cerebral blood flow, decrease metabolic rate (Blood, 2003), and inhibit synaptic transmission during hypoxia (Hentschel et al., 2003). Previous studies have shown that allopurinol attenuates the decrease in cerebral Na^+,K^+-ATPase activity (Marro et al., 1994) and prevents modification of the NMDA receptor characteristics observed

Table 1 – Physiologic measurements in newborn piglets during hypoxia

Group	pH (a)	$PaCO_2$ (mm Hg)	PaO_2 (mm Hg)	MABP (mm Hg)
Control	7.39 ± 0.08	43 ± 5	100 ± 30	72 ± 11
Allopurinol control	7.46 ± 0.05	42 ± 4	96 ± 12	72 ± 11
Hypoxia				
Baseline	7.47 ± 0.06	39 ± 5	108 ± 16	78 ± 7
Hypoxia	7.15 ± 0.12 *	39 ± 8	23 ± 3 *	54 ± 14 *
Allopurinol hypoxia				
Baseline	7.37 ± 0.06	42 ± 6	91 ± 22	79 ± 12
Hypoxia	7.04 ± 0.17 *	28 ± 10	20 ± 4 *	57 ± 10 *

* $P < 0.005$ vs. baseline mean ± SD.