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Hypoxic-ischemic insult decreases glutamate uptake by hippocampal slices from neonatal rats: Prevention by guanosine

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Abstract

Brain injury secondary to hypoxic-ischemic disease is the predominant form of damage encountered in the perinatal period. The impact of neonatal hypoxia-ischemia (HI) in 7-day-old pups on the high-affinity [^{3}H] glutamate uptake into hippocampal slices at different times after insult was examined. Immediately following, and 1 day after the insult there was no effect. But at 3 to 5 days after the HI insult, glutamate uptake into the hippocampus was markedly reduced; however, after 30 or 60 days the glutamate uptake into hippocampal slices returned to control levels. Also, this study demonstrated the effect of the nucleoside guanosine (Guo) on the [^{3}H] glutamate uptake in neonatal HI injury, maintaining the [^{3}H] glutamate uptake at control levels when injected before and after insult HI. We conclude that neonatal HI influences glutamate uptake a few days following insult, and that guanosine prevents this action.

Keywords: Neonatal hypoxia-ischemia; Glutamate uptake; Guanosine; Hippocampal slices

Introduction

Perinatal brain hypoxic-ischemic (HI) injury is relevant to morbidity and mortality in humans, often leading to mental retardation, seizures, and motor impairment (cerebral palsy), neuro-developmental impairment and disability (Amato and Donati, 2000; Fukamachi et al., 2001; Vannucci, 1990; Vexler and Ferriero, 2001). In neonates and children, hypoxia is a major complicating factor associated with low birth weight and other medical problems, such as those encountered in sudden infant death syndrome (Kalaria et al., 1993; Ottaviano et al., 2001; Sizonenko et al., 2003). The brain of the fetus is extremely susceptible to disorders involving oxygen supply (Valkounova et al., 2001). The vulnerability of the developing brain to HI damage is different from that seen in adult brain and is thought to be due in part to the release of excitatory amino acids (Fukamachi et al., 2001; Grow and Barks, 2002; Johnston, 2001; McDonald and Johnston, 1990; Vexler and Ferriero, 2001).

Glutamate is the main excitatory neurotransmitter in the mammalian central nervous system (CNS). It is involved in most brain functions (Meldrum, 2000; Nedergaard et al., 2002; Ozawa et al., 1998) such as memory and learning (Izquierdo and Medina, 1997), development and aging (Segovia et al., 2001), and adaptation to the environment (Danbolt, 2001; Mattson et al., 2002; Warren, 2002). Glutamate exerts its signaling role by acting on glutamate receptors located on the neural cell surface in such a way that glutamate concentration in the surrounding extracellular space usually determines the extent of receptor stimulation. The amount of glutamate in the synaptic cleft depends on the balance between its release by presynaptic neurons and its uptake that occurs mainly

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through transporters located in the astrocyte cell membrane and also in the presynaptic neuronal terminal since there is no known extracellular enzyme capable of significantly metabolizing glutamate (Attwell, 2000; Chen and Swanson, 2003; Danbolt, 2001; Rothstein et al., 1994; Ullensvang et al., 1997). The uptake of glutamate is mainly accomplished by Na^+-dependent high affinity systems mediated by a family of transporters (Danbolt, 2001). When present in high concentrations in the synaptic cleft, glutamate may lead to excitotoxicity, a process corresponding to glutamate receptor over-stimulation that subsequently leads to neuronal damage (Danbolt, 2001; Furuta et al., 1997; Maragakis and Rothstein, 2001, 2004; Mattson et al., 2002; Segovia et al., 2001). Indeed, excitotoxicity has been related to various acute and chronic neurodegenerative disorders (Brewer, 2000; Danbolt, 2001; Ingram et al., 2001; Maragakis and Rothstein, 2004; Segovia et al., 2001). Recent evidence suggests that glutamate excitotoxicity is the major mechanism for neuronal death after neonatal HI. In this situation, there is an increase in the extracellular glutamate levels, and its uptake is impaired by oxygen free radicals produced during hypoxia (Painter, 1995; Palmer, 1995; Volpe, 2001). Experimentally, it has been shown that the rat hippocampus is most susceptible at around the second postnatal week (Danbolt, 2001).

Experiments performed by our group have provided evidence that the nucleoside guanosine (Guo) enhances glutamate uptake by rat cortical astrocyte cultures and brain cortical slices from P10 rats under physiological and excitotoxic conditions (Frizzo et al., 2001, 2002, 2003; Oliveira et al., 2004). In vivo studies show that systemic administration of Guo prevented seizures elicited by quinolinic acid and alpha-dendrotoxin, which over stimulate the glutamatergic system, strongly suggesting a neuro-modulatory role of Guo in this system (Lara et al., 2001; Schmidt et al., 2000; Soares et al., 2004; Vinadé et al., 2003). As pointed out above, one of the most important effects of hypoxic ischemia is the increase in extra cellular glutamate levels; thus, mechanisms that enable maintenance of glutamate homeostasis after ischemia might protect against neuronal damage. The aim of this study was to investigate putative alteration on glutamate uptake by hippocampal slices of developing rats subject to HI, and the potential protective effect of guanosine against the HI-induced disturbance in the uptake.

Materials and methods

Chemicals

L-^{3}[H] glutamate was purchased from Amersham International. Other chemicals were of analytical reagent grade and purchased from Sigma.

Animals

Seven-day-old Wistar rats, weighting 12-16 g from the Department of Biochemistry, ICBS, UFRGS Animal House were used. They were fed ad libitum and maintained on a 12 h light/12 h dark cycle, at room temperature. All animal procedures were in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the National Institute of Health (USA). As recently reviewed by Hurn et al. (2005), long-term damage produced by unilateral HI in seven-day-old rats (as we used) is not affected by gender, since no difference in outcome between male and female pups was observed. Thus in this study we used both male and female rats.

Hypoxic-ischemic (HI) injury

In this research, we utilized the most widely used experimental model of neonatal cerebral hypoxia-ischemia—the Rice-Vannucci Model (Rice et al., 1981; Vannucci and Vannucci, 1997). It consists of the association of unilateral occlusion of the common carotid artery with exposure to a hypoxic atmosphere in order to produce unilateral damage in the rat brain. Animals were anesthetized with halothane. The left common carotid artery was identified through a longitudinal neck incision, isolated from the vagus nerve and permanently occluded with surgical silk thread. After a 2-h recovery period, groups of four pups were placed into a 1500 mL chamber and exposed to an 8% oxygen-92% nitrogen atmosphere delivered at 5 l/min for 1.5 h, with the chamber partially immersed in a 37°C water bath to maintain a constant thermal environment. Rats surviving hypoxia (the mortality rate was less than 5%) were returned to their dams. The pups were killed by decapitation immediately or 1, 3, 5, 30, or 60 days after the hypoxic insult. The brain was removed and the left hippocampus was used in all experimental assays. In this neonatal model, cerebral hemispheres of rats receiving hypoxia are differentially affected: the left hemisphere will present neuronal death since it suffers ischemia (due to carotid occlusion) in association with hypoxia, while the right hemisphere (receiving only hypoxia) suffers no overt morphological damage (Bômont et al., 1992; Rice et al., 1981). The time interval from death to assay was about 10 min.

The rat model of neonatal ischemic-hypoxia as modified by Rice et al., 1981, reproducibly caused unilateral brain injury. This model is useful for the study of neurochemical events associated with neuronal death in the affected hemisphere, as compared to the contra lateral, undamaged hemisphere (Moretto et al., 1999). However, based upon data shown in Fig. 2, in this research the controls consisted only of pups that had not suffered any insult.