Expression of Glutamate Transporters EAAC1, GLAST, GLT-1 in Neonatal White Matter Play a Role in Brain Excitotoxicity

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Abstract

Previous studies have focussed on the expression of glutamate transporters in myelinated and mature mice optic nerves. This work investigates glutamate transporters expression in isolated immature rat optic nerves (day 0) (P0) in which myelination has not yet commenced. We have revealed the cellular distribution of glutamate transporters in the neonatal white matter using embedded Electron Microscopy. All three types of glutamate transporters found in the sub-cortical white matter in the post-natal period. Our current work examined the sub-cellular distribution of EAAC1, GLAST and GLT1 proteins in the perinatal white matter using immune histochemistry techniques.

Recent studies have revealed the rapid astrocyte swelling will liberate astrocyte glutamate into the extracellular space in a glutamate-dependent fashion usually via GLAST and GLT1 transport result in necrosis of neonatal ischaemic white matter. It is suggested that glutamate release from NF-H + axons via reverse transport which was potentially a vital factor in excitotoxic and non-excitotoxic ischemia of the perinatal white matter. We conclude that extensive expression of GLAST and GLT1 on the perinatal astrocyte processes play a role in brain excitotoxicity.

Keywords: Day 0 RONs; Glutamate transporters; Ischaemia

Introduction

Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system and has been implicated as a neurotoxic agent in several neurologic disorders including epilepsy, ischemia, and certain neurodegenerative diseases [1,2]. Unbound extracellular glutamate is deleterious primarily by sodium-dependent transport of glutamate into glia and neurons [3,4], and glutamate transport is thought to be crucial for preventing accumulation of neurotoxic levels of extracellular glutamate. Multiple subtypes of sodium-dependent glutamate transporters have been identified pharmacologically, and four rats (GLAST, GLT-1, EAAC1, and EAAT4) and five humans (EAAT1-5) transporters have been identified by molecular cloning [5]. The excitatory amino acid transporters EAAT1 (GLAST) and EAAT2 (GLT-1) are expressed primarily in astroglial cells, whereas EAAT3 (EAAC1) and EAAT4 are enriched in neurons. EAAT3/EAAC1 is the most abundant neuronal transporter and is selectively enriched in neurons of the hippocampus, cerebellum, and basal ganglia [6].

Three broad subtypes of EAA transport activity have been identified in brain preparations. One type, which is directly coupled to ATP hydrolysis, introduces glutamate into vesicles for release upon depolarization of the synaptic terminal [7]. It indirectly ensures low extracellular concentrations of EAs by reducing the driving force required to transport an EAA into the cytosol. The second activity is a chloride-dependent transport which exchanges amino acids across the plasma membrane [8]. It has a low capacity and cannot concentrate EAA intracellularly. The third type of transport activity is an active Na+-dependent system and it can be differentiated from the others by means of ion selectivity, regional distribution, and sensitivity to inhibition by analogs of EAAs. Since this transport activity is extremely high, it is generally assumed that this activity is primarily responsible for the clearance of extracellular EAAs in the brain.

Methodology

RONs of the brain from the perinatal P0 were nourished with physiological aCSF bubbled with 95%O2/ 5%CO2 to create isotonic physiological conditions surrounding the glia and white matter and transferred to 0.1 M PBS and immediately fixed with 2% paraformaldehyde for 30 min. The RONs were incubated in 20%w/v sucrose for 5 min prior to freeze sectioning. Later RONs were transferred into Tissue and frozen in dry ice/hexane. Approximately 20 µM tissue sections were obtained using a Bright cryostat microtome and the sections were mounted on Super Frost Plus slides. The sections were fixed in 2% paraformaldehyde for 20 min and then washed for 2×15 min with 0.1M PBS of approximately pH ≈ 7.45. After fixation sections were pre-incubated for 2 hrs at 4°C temperature with 0.1 M PBS with 10% blocking goat serum and 0.5% Triton-X100 (Sigma; PBGST). The sections were then washed with 0.1 M PBS and immediately fixed with 2% paraformaldehyde for 30 min. The RONs were washed for 5×15 minutes in PBGST and then mounted with coverslips of size 22 × 40 mm using mounting solution, and imaged by using IX70 scanning laser confocal microscope with Fluoview (Olympus, Japan) software.

Primary antibodies against glutamate GLAST, GLT1 and EAAC1 transporters:

1. Rabbit anti-rat Ig G GLAST antibody: 1:200
2. Rabbit anti-rat Ig G EAAC1 antibody: 1:200
3. Rabbit anti-rat Ig G GLT1 antibody: 1:200

Primary antibody omission controls were blank. The sections were washed for 5 × 15 minutes in PBGST and then mounted with coverslips of size 22 × 40 mm using mounting solution, and imaged by using IX70 scanning laser confocal microscope with Fluoview (Olympus, Japan) software.

Results

We found extensive expression of GLAST and GLT1 on the perinatal astrocyte processes. Minor levels of EAAC1 expression was observed on the perinatal astrocytes. Neurofilament (NF-H) + axons did not express GLAST but we found moderate levels of GLT1 on the perinatal astrocytes. Neurofilament (NF-H) + axons did not express GLAST but we found moderate levels of GLT1 on the perinatal astrocytes.
The expression of GLAST demonstrates the potential for transport mediated-glutamate release into the extracellular space resulting in necrosis of ischaemic white matter during the perinatal period.

Double labeling for the perinatal GFAP+ astrocytes and glutamate transporters revealed that the expression of GLAST was more when compared to the expression of the GLT1 and EAAC1. Double labeling for the perinatal NF-H (+) axons and glutamate transporters has revealed the clear co-localization of EAAC1 glutamate transporter with no GLAST and with moderate levels of GLT1.

**Conclusion**

We conclude from our practical study that the expression of glutamate transporters EAAC1, GLAST, GLT-1 in neonatal white matter play a role in brain excitotoxicity.

**Conflict of Interest**

All the authors declared that they have no conflicts to disclose.

**References**