Neurochemistry International 98 (2016) 19–28

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

GLT-1: The elusive presynaptic glutamate transporter

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ARTICLE INFO

Article history:
Received 21 January 2016
Received in revised form
21 April 2016
Accepted 22 April 2016
Available online 26 April 2016

Keywords:
Glutamate homeostasis
Synaptic plasticity
Synaptogenesis
Aging
Neurodegeneration
Glutamate-glutamine cycle
Schizophrenia
Addiction
Metabolism
Mitochondria

ABSTRACT

Historically, glutamate uptake in the CNS was mainly attributed to glial cells for three reasons: 1) none of the glutamate transporters were found to be located in presynaptic terminals of excitatory synapses; 2) the putative glial transporters, GLT-1 and GLAST are expressed at high levels in astrocytes; 3) studies of the constitutive GLT-1 knockout as well as pharmacological studies demonstrated that >90% of glutamate uptake into forebrain synaptosomes is mediated by the operation of GLT-1. Here we summarize the history leading up to the recognition of GLT-1a as a presynaptic glutamate transporter. A major issue now is understanding the physiological and pathophysiological significance of the expression of GLT-1 in presynaptic terminals. To elucidate the cell-type specific functions of GLT-1, a conditional knockout was generated with which to inactivate the GLT-1 gene in different cell types using Cre/lox technology. Astrocytic knockout led to an 80% reduction of GLT-1 expression, resulting in intractable seizures and early mortality as seen also in the constitutive knockout. Neuronal knockout was associated with no obvious phenotype. Surprisingly, synaptosomal uptake capacity (V_{max}) was found to be significantly reduced, by 40%, in the neuronal knockout, indicating that the contribution of neuronal GLT-1 to synaptosomal uptake is disproportionate to its protein expression (5–10%). Conversely, the contribution of astrocytic GLT-1 to synaptosomal uptake was much lower than expected. In contrast, the loss of uptake into liposomes prepared from brain protein from astrocyte and neuronal knockouts was proportionate with the loss of GLT-1 protein, suggesting that a large portion of GLT-1 in astrocytic membranes in synaptosomal preparations is not functional, possibly because of a failure to reseal. These results suggest the need to reinterpret many previous studies using synaptosomal uptake to investigate glutamate transport itself as well as changes in glutamate homeostasis associated with normal functions, neurodegeneration, and response to drugs.

1. Introduction

In the mammalian brain, small molecules called neurotransmitters function to transmit signals across chemical synapses. There are 4 lines of evidence that need to be assembled to establish a substance as a neurotransmitter—synthesis in the presynaptic terminal, release from the presynaptic terminal by action potentials, the presence of receptors on the postsynaptic membrane that produce a response that can be measured postsynaptically, and the presence of a clearance mechanism for the transmitter in the extracellular space. Transmitter clearance is required for several reasons: 1) to prevent the accumulation of the transmitter in the extracellular space; 2) to prevent the metabolic waste of leaving the transmitter in the extracellular space unrecovered for cellular utilization; 3) to restore transmitter to presynaptic terminals that might become depleted of transmitter.

Glutamate is now widely accepted as the excitatory neurotransmitter in the mammalian brain, but its role as a neurotransmitter was slow to become established because of its central role in intermediary metabolism, its ubiquitous distribution, and presence in high concentration in the brain. One approach to testing the hypothesis that glutamate is a neurotransmitter that was taken in the 1970's was to focus on the requirement that a transmitter must

Abbreviations: DHK, dihydrokainate; EAAT1 (GLAST), excitatory amino acid transporter 1; EAAT2 (GLT-1, slc1a2), excitatory amino acid transporter 2; EAAT3 (EAAC1), excitatory amino acid transporter 3; EAAT4, excitatory amino acid transporter 4; EAAT5, excitatory amino acid transporter 5; EM-ICC, electron microscopy immunocytochemistry; H^+, proton; K^+, potassium; LM-ICC, light microscopy immunocytochemistry; LTD, long-term depression; LTP, long-term-potentiation; mGluR, metabotropic glutamate receptor; nGLT-1−/− (synGLT-1 KO), conditional neuronal GLT-1 knockout; Na^+, sodium.

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http://dx.doi.org/10.1016/j.neuint.2016.04.010
0197-0186/

have a specific clearance mechanism in the terminals from which it is released. This approach was pioneered by Julie Axelrod and colleagues, who demonstrated monoamine uptake systems in synaptosome preparations, and also that the uptake that was observed was specifically into presynaptic terminals. With this as a background, Wofsey, Kuhar, and Snyder reasoned: "Transport systems into nerve terminals have been described for several putative neurotransmitters, including norepinephrine, serotonin, GABA. If certain amino acids are neurotransmitters, then perhaps analogous uptake systems might transport them into nerve terminals. If such is the case, the exogenous amino acid which would label selectively a "neurotransmitter pool" should be more highly localized in synaptosomal fractions than would be the endogenous amino acids, which would include metabolic as well as transmitter pools" (Wofsey et al., 1971).

These authors showed that ^{3}H-glutamate and aspartate accumulate in a unique low density synaptosomal fraction containing pinched off nerve terminals, a similar result to one obtained using brain slices (Kuhar and Snyder, 1970). It was subsequently shown that the uptake system for glutamate into brain synaptosomes is a high affinity sodium dependent carrier (Bennett et al., 1972; Logan and Snyder, 1971). The conclusions of these studies were reinforced by EM autoradiographic studies demonstrating uptake of ^{3}H-glutamate into axon terminals of pigeon and rat (Beart, 1976a, b; Divac et al., 1977; Iversen and Storm-Mathisen, 1976; Storm-Mathisen, 1977; Storm-Mathisen and Iversen, 1979). These studies, in aggregate, provided biochemical evidence for a neurotransmitter pool of glutamate that was at least in part sustained by a specific high affinity transport system, and were important in providing part of the foundation of evidence to support the identification of glutamate as the excitatory neurotransmitter (Fonnum et al., 1981).

In 1992, the 3 glutamate transporters of the forebrain were cloned, and, none of them proved to be localized in presynaptic terminals of excitatory synapses. However, astrocyte membranes adjacent to synapses expressed high levels of the transporters GLT-1 and GLAST, and eventually the notion that there is a glutamate transporter in presynaptic terminals was abandoned as being unsupported by molecular evidence and biologically unnecessary. A detailed history of these developments has been provided in a comprehensive review by Danbolt (2001) concerning glutamate transport that appeared in 2001 in which the problem was alluded to as the "elusive presynaptic glutamate transporter". The present review represents a brief overview of the project started by Snyder and colleagues over 40 years ago to characterize a presynaptic glutamate transport system in the brain and a more detailed update to cover the time period after 2001. For a more detailed coverage of the period before 2001, the reader is referred to the review by Niels Danbolt.

1.1. Glutamate transporter family

Excitatory neuronal activity is accompanied by release of glutamate into the synaptic cleft (Seifert et al., 2006) (Fig. 1). The amino acid L-glutamate is not only the major excitatory neurotransmitter in the central nervous system (CNS) but also a potent neurotoxin (Billups et al., 1998; Choi and Rothman, 1990; Lipton and Rosenberg, 1994; Meldrum and Garthwaite, 1990; Olney, 1990). To maintain low, non-toxic extracellular glutamate concentrations and ensure the fidelity of synaptic transmission, in addition to diffusion, rapid glutamate removal from the synaptic cleft is essential. In the case of excitatory synapses in the mammalian central nervous system clearance is provided by glutamate transporters. By controlling the concentration profile of glutamate in and around the synaptic cleft after release (Huang and Bergles, 2004),

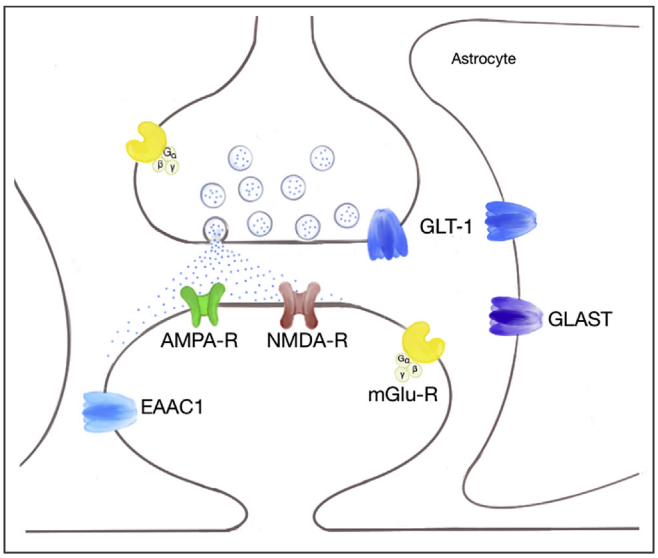

Fig. 1. Schematic diagram of the glutamatergic synapse showing locations of the glutamate transporter subtypes. GLT-1 is the major glutamate transporter and is primarily expressed in astrocytes (Lehre et al., 1995; Rothstein et al., 1994) but also in neurons, in axon terminals (Chen et al., 2004). In the hippocampus, the expression of GLT-1 protein in axon terminals is approximately 5–10% of total GLT-1 protein expression (Furness et al., 2008). GLAST is thought to be exclusively a glial transporter (Rothstein et al., 1994; Storck et al., 1992). EAAC1 is a neuronal glutamate transporter with a somatodendritic localization (Holmseth et al., 2012; Rothstein et al., 1994; Stoffel et al., 2004).

glutamate transporters play an important role in preserving the signaling functions of synapses (Katagiri et al., 2001; Stoffel et al., 2004), regulating the activation of nearby metabotropic receptors (Huang et al., 2004; Otis et al., 2004; Scanziani et al., 1997), controlling crosstalk between excitatory synapses (Arnth-Jensen et al., 2002; Asztely et al., 1997; Rusakov and Kullmann, 1998), and in some regions may shape the kinetics of excitatory postsynaptic currents (EPSCs) (Barbour et al., 1994; Takahashi et al., 1995; Tong and Jahr, 1994; Tzingounis and Wadiche, 2007).

The stoichiometry of glutamate transport is determined by the transmembrane concentration gradients for sodium (Na^+), potassium (K^+), and protons (H^+), and the membrane potential. These electrochemical gradients are maintained by the Na^+/K^+-ATPase and provide the driving force for glutamate uptake. It is generally accepted that GLT-1 co-transports one Glu^- with three Na^+ and one H^+, and countertransports one K^+ per cycle (Levy et al., 1998; Zerangue and Kavanaugh, 1996) making it an electrogenic transport process. Under physiological conditions, the inwardly directed Na^+ gradient and outwardly directed K^+ gradient, as well as the interior negative membrane potential favor the accumulation of glutamate into the cell against its concentration gradient (Kanner, 2006; Zerangue and Kavanaugh, 1996).

Two large families of high affinity, sodium dependent transporters resident in the plasma membrane have been identified. One family includes chloride dependent transporters such as those specific for catecholamines and for glycine (Amara, 1992). A separate family of potassium dependent glutamate transporters includes five members: GLT-1, isolated from rat (Danbolt et al., 1990), whose human counterpart is excitatory amino acid transporter 2 (EAAT2) (Arriza et al., 1994); GLAST, isolated from rat (Stoffel et al., 1992), whose human counterpart is EAAT1 (Arriza et al., 1994); EAAC1, first isolated from rabbit (Kanai and Hediger, 1992), whose human counterpart is EAAT3 (Arriza et al., 1994); EAAT4, expressed predominantly in the cerebellum (Fairman et al., 1995), and EAAT5, expressed predominantly in the retina (Arriza et al., 1997). These