Neurochemistry International xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Neurochemistry International

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

Glutamate transporter control of ambient glutamate levels

Weinan Sun [a], Denis Shchepakin [b], Leonid V. Kalachev [a,b], Michael P. Kavanaugh [a,*]

[a] Center for Structural and Functional Neuroscience, The University of Montana, Missoula, MT 59812, United States
[b] Department of Mathematics, The University of Montana, Missoula, MT 59812, United States

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Glutamate transport
Ambient neurotransmitter
Tonic signaling
Microdialysis

ABSTRACT

Accurate knowledge of the ambient extracellular glutamate concentration in brain is required for understanding its potential impacts on tonic and phasic receptor signaling. Estimates of ambient glutamate based on microdialysis measurements are generally in the range of ~2–10 μM, approximately 100-fold higher than estimates based on electrophysiological measurements of tonic NMDA receptor activity (~25–90 nM). The latter estimates are closer to the low nanomolar estimated thermodynamic limit of glutamate transporters. The reasons for this discrepancy are not known, but it has been suggested that microdialysis measurements could overestimate ambient extracellular glutamate because of reduced glutamate transporter activity in a region of metabolically impaired neuropil adjacent to the dialysis probe. We explored this issue by measuring diffusion gradients created by varying membrane densities of glutamate transporters expressed in *Xenopus* oocytes. With free diffusion from a pseudo-infinite 10 μM glutamate source, the surface concentration of glutamate depended on transporter density and was reduced over 2 orders of magnitude by transporters expressed at membrane densities similar to those previously reported in hippocampus. We created a diffusion model to simulate the effect of transport impairment on microdialysis measurements with boundary conditions corresponding to a 100 μm radius probe. A gradient of metabolic disruption in a thin (~100 μm) region of neuropil adjacent to the probe increased predicted [Glu] in the dialysate over 100-fold. The results provide support for electrophysiological estimates of submicromolar ambient extracellular [Glu] in brain and provide a possible explanation for the higher values reported using microdialysis approaches.

1. Introduction

During synaptic transmission, glutamate transporters restrict the spatiotemporal pattern of ionotropic and metabotropic glutamate receptor signaling (for review see Tzingounis and Wadiche, 2007). In addition to their roles in shaping the dynamics of synaptically released glutamate, glutamate transporters also help maintain low steady-state glutamate levels. Given the stoichiometry of ion coupling to glutamate uptake, the theoretical lower limit of extracellular glutamate in brain is approximately 2 nM (Zerangue and Kavanaugh, 1996; Levy et al., 1998). Many studies using intracerebral microdialysis have reported levels of ambient glutamate ⩾ 2 μM, three orders of magnitude higher than the theoretical lower limit (Benveniste et al., 1984; Lerma et al., 1986; for reviews see Cavelier et al., 2005; Nyitrai et al., 2006). By contrast, reports of ambient glutamate concentration estimated from electrophysiological measurement of tonic NMDA receptor activity in hippocampal slice range from 87 to 89 nM (Cavelier and Attwell, 2005; Le Meur et al., 2007) to as low as 25 nM (Herman and Jahr, 2007).

Accurate knowledge of the ambient glutamate concentration in different brain regions is important for evaluating its effects on synaptic transmission. Several ionotropic and metabotropic glutamate receptor subtypes are activated by low micromolar concentrations of glutamate, and tonic exposure in this range profoundly inhibits synaptic circuitry *in vitro* (Zorumski et al., 1996). Glutamate transporters play a dominant role in limiting ambient glutamate, as pharmacological inhibition of transport has been shown to lead to a rapid increase in ambient glutamate causing increased tonic NMDA receptor signaling (Jabaudon et al., 1999; Cavelier and Attwell, 2005; Le Meur et al., 2007; Herman and Jahr, 2007).

In this work we attempt to integrate data in the literature with new *in vitro* measurements and *in vivo* modeling of diffusion gradients formed by glutamate transporters. Proceeding from the assumption that in steady-state conditions, the volume-averaged rates of release and uptake of glutamate are equal, we show the

* Corresponding author. Tel.: +1 4062434398; fax: +1 4062434888.
E-mail address: michael.kavanaugh@umontana.edu (M.P. Kavanaugh).

http://dx.doi.org/10.1016/j.neuint.2014.04.007
0197-0186/

influence of glutamate transporter membrane density on steady-state diffusion gradients in a density range relevant to *in vivo* brain expression. We suggest that metabolic impairment of glutamate transport in a shallow boundary region of a microdialysis probe can account for the discrepancies between estimates of ambient glutamate from dialysis and electrophysiological approaches.

2. Materials and methods

2.1. Xenopus oocyte recording

Approximately 50 ng of human EAAT3 cRNA was microinjected into stage V–VI Xenopus oocytes and recordings were made 1–6 d later. Recording solution contained 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 5 mM Hepes (pH 7.5). Microelectrodes were pulled to resistances between 1 and 3 MΩ and filled with 3 M KCl. Data were recorded with Molecular Devices amplifiers and analog–digital converters interfaced to Macintosh computers. Data were analyzed offline with Axograph X (v.1.0.8) and KaleidaGraph (v 3.6; Synergy) software. For stopped flow measurements, oocytes were voltage clamped at −60 mV in a perspex recording chamber in which glutamate depletion in the absence of perfusion was <1% of the total in the recording chamber. Transporter surface density was estimated from current measurements assuming a coupled current of 2 charges/cycle at E_{Cl} (−20 mV), turnover rate of 15/s, oocyte surface area 2.85×10^7 μm^2, and transport voltage-dependance of e-fold/76 mV (Wadiche et al., 1995; Zerangue and Kavanaugh, 1996). Current amplitudes were fitted to the Michaelis–Menten relationship:

$$I_{[Glu]} = I_{max}[Glu]/\{K_M + [Glu]\}$$

2.2. Mathematical modeling of [Glu] profile near the microdialysis probe

Our microdialysis probe model can be described by the following diffusion equation in polar coordinates with sink and source in the right hand side:

$$\partial u/\partial t = D \cdot \ (1/r) \cdot \ \partial/\partial r \ [r \cdot \partial u/\partial r] - J \cdot \ u/(K_m + u) + K_L$$

where u corresponds to L-glutamate concentration. The first term in the right hand side is a Laplace operator in polar coordinates multiplied by a diffusion coefficient D. The second term represents the Michaelis–Menten transport sink in the tissue, and the third term K_L represents the leak, which is treated as a constant. The parameter J is a function of distance r from the probe center, and describes the spatial dependence of transporter impairment between the healthy and damaged tissue. The spatial metabolic damage near the probe is approximated as a Gaussian curve, and we define the function J as:

$$J(r) = 0 \text{ when } 0 \le r \le L$$

$$J(r) = J_{max} \cdot 1 - e\text{^}[-(r-L)2/2 \cdot \ sigma^2] \text{ when } r > L$$

where L is the radial boundary for the microdialysis probe and sigma represents the distance from the probe boundary characterizing the Gaussian damage function. The boundary conditions for the model are:

$$\partial u/\partial r|_{r=0} = 0$$

$$u(t, \infty) = u_s$$

The initial condition is

$$u(t, r) = u^* \text{ when } 0 \le r \le L$$

$$u(t, r) = u_s \text{ when } r > L$$

This model cannot be solved analytically because of the nonlinear term in the right hand side of the equation, so it was solved numerically by space discretization, which transforms it into system of ordinary differential equations. The leak rate constant (K_L) is related to ambient [Glu], volumetric glutamate transporter concentration [GluT] (140 μM, Lehre and Danbolt, 1998), transporter K_M value, and maximal turnover rate J_{max} by the equation:

$$K_L = [Glu]_{ambient}/(K_m + [Glu]_{ambient}) \cdot [GluT] \cdot J_{max}$$

3. Results

3.1. Diffusive concentration gradients formed by glutamate transporters

Co-expression studies of NMDA receptors with transporters for its co-agonists glycine and glutamate have shown that transporters can limit receptor activity by establishing diffusion-limited transmitter concentration gradients (Supplisson and Bergman, 1997; Zuo and Fang, 2005). We studied the concentration gradients formed by passive diffusion from a pseudo-infinite glutamate source in a perspex chamber to the glutamate sink established by transporters on the cell surface. Oocytes expressing the human neuronal glutamate transporter EAAT3 were voltage-clamped at −60 mV and superfused with varying concentrations of glutamate at a linear flow rate of 20 mm/s flow followed by a stopped-flow interval (Fig. 1). Steady-state currents elicited by glutamate perfusion relaxed to a lower steady-state level when flow was stopped, and following resumption of flow, currents rapidly recovered to initial values. The reduction in current amplitude during zero flow conditions was likely due to the formation of a diffusion-limited concentration gradient resulting in reduced surface [Glu], because the ratio of the current amplitudes with and without flow were dependent on the concentration of glutamate in the perfusate, and in all cases the amount of glutamate transported was <1% of the total glutamate in the chamber (i.e. a pseudo-infinite glutamate source; Fig. 1B–D). This gradient was also reflected in a significant shift in the concentration-dependance of steady-state currents in flow and stopped-flow conditions (K_M value for L-glutamate of 32 ± 2 and 216 ± 37 μM, respectively, $n = 4$; $p < 0.002$), while the I_{max} values were not significantly different.

3.2. Transporter density influence on kinetic parameters

Glutamate transporters are expressed at different densities among structures in the CNS, and transporter density and/or kinetics can be altered in different pathological circumstances such as trauma and ischemia. Because steady-state ambient [Glu] reflects a homeostatic balance of uptake and leak sources, changes in transport may result in significantly different steady state glutamate levels. We tested the influence of the surface density of glutamate transporters on the concentration gradient formed by passive glutamate diffusion during stopped-flow experiments by monitoring currents induced by 10 μM glutamate. With increasing transporter expression levels, the steepness of the concentration gradient formed during stopped-flow conditions was increased, as reflected in the changing ratio of the steady-state currents in flow and stopped-flow conditions (Fig. 2A and B).

Even with continuous flow, evidence for formation of a concentration gradient between the cell surface and bulk solution was observed. Oocyte membranes have a microvillar structure that can act as tortuous diffusion barrier (see Supplisson and Bergman, 1997). In a group of 29 oocytes with varying expression levels, steady-state K_M values measured with chamber flow

Please cite this article in press as: Sun, W., et al. Glutamate transporter control of ambient glutamate levels. Neurochem. Int. (2014), http://dx.doi.org/10.1016/j.neuint.2014.04.007