Neuroscience 158 (2009) 1608–1615

ROLE OF GLUTAMATE TRANSPORTERS IN CORTICOSTRIATAL SYNAPTIC TRANSMISSION

C. BEURRIER,[a] G. BONVENTO,[b] L. KERKERIAN-LE GOFF[a] AND P. GUBELLINI[a]*

[a]*Institut de Biologie du Développement de Marseille-Luminy, UMR 6216 (CNRS-Université de la Méditerranée Aix-Marseille), Marseille, France*

[b]*CEA, Institute of Biomedical Imaging (I2BM), Molecular Imaging Research Center (MIRCen), F-92265 Fontenay-aux-Roses, France*

Abstract—High-affinity glutamate transporters (GTs) play a major role in controlling the extracellular level of this excitatory neurotransmitter in the CNS. Here we have characterized, by means of *in vitro* patch-clamp recordings from medium spiny neurons (MSNs), the role of GTs in regulating corticostriatal glutamatergic synaptic transmission in the adult rat. Charge transfer and decay-time, but not amplitude, of excitatory postsynaptic currents (EPSCs) were enhanced by DL-threo-β-benzyloxyaspartate (TBOA), a broad inhibitor of GTs. Moreover, TBOA also potentiated currents induced by high-frequency stimulation (HFS) protocols. Interestingly, the effect of TBOA on EPSCs was lost when MSNs were clamped at +40 mV, a condition in which neuronal GTs, that are voltage-dependent, are blocked. However, in this condition TBOA was still able to enhance HFS-induced currents, suggesting that glial GT's role is to regulate synaptic transmission when glutamate release is massive. These data suggest that neuronal GTs, rather than glial, shape EPSCs' kinetics and modulate glutamate transmission at corticostriatal synapse. Moreover, the control of glutamate concentration in the synaptic cleft by GTs may play a role in a number of degenerative disorders characterized by the hyperactivity of corticostriatal pathway, as well as in synaptic plasticity.

Key words: glutamate transporters, striatum, mGlu receptors, TBOA, electrophysiology.

Glutamate is the main excitatory neurotransmitter in the mammalian brain, exerting its action via three classes of ionotropic glutamate receptors, i.e. AMPA, kainate and *N*-methyl-D-aspartate (NMDA), and three groups of metabotropic glutamate receptors (mGluR) (Pin and Acher, 2002; Gubellini et al., 2004; Ferraguti and Shigemoto, 2006). It is involved in all aspects of brain development and function from the early stages of neurogenesis to normal ageing, through cognition, learning and motor functions (Headley and Grillner, 1990). Since glutamate is not degraded in the synaptic cleft, the most efficient way for removing this neurotransmitter from the extracellular space is via cellular uptake through glutamate transporters (GTs; for a review, see Danbolt, 2001). GTs constitute a structurally related family of Na^+- and voltage-dependent excitatory amino acid transporters (EAATs), and five have been cloned from human tissues (EAAT1 to EAAT5; Danbolt, 2001; Schousboe et al., 2004). In rodents' CNS, five GTs have been identified so far: GLAST and GLT-1 (homologous to EAAT1 and EAAT2, respectively), expressed by glial cells, EAAC1 (homologous to EAAT3) and EAAT4, expressed by neurons, and EAAT5, expressed by retinal rod photoreceptors (Pow and Barnett, 2000; Danbolt, 2001). GTs are crucial to keep the extracellular levels of glutamate low in physiological conditions, and this function can be also relevant to neuropathological situations characterized by dysfunctions of glutamatergic systems, such as amyotrophic lateral sclerosis, Alzheimer's disease, epilepsy and Parkinson's disease (PD) (Carlsson and Carlsson, 1990; Choi, 1992; Rothstein et al., 1992; Chapman, 2000; Danysz et al., 2000; Gubellini et al., 2006). In particular, regarding PD, the expression of GLT-1 mRNA is increased in the striatum and in the output structures of the basal ganglia of parkinsonian rats treated with L-DOPA inducing dyskinesia (Liévens et al., 2001; Robelet et al., 2004). Such increase could represent a compensatory mechanism to counter corticostriatal glutamatergic hyperactivity observed in this model (Gubellini et al., 2006). The striatum of the adult rat expresses GLT-1, GLAST and EAAC1 (Danbolt, 2001). In this structure, GLT-1 and GLAST are localized on astrocyte membrane processes surrounding virtually all neuronal cell bodies and synaptic complexes (Rothstein et al., 1994). It has been show in other brain structures that their localization can change with the type of structure neighboring the astrocyte (neuropil, cell bodies, pia mater or capillary endothelium; Chaudhry et al., 1995; Lehre et al., 1995), but no such information is available for the striatum. It has also been shown that striatal levels of GLT-1 are very high, while those of GLAST are low. Concerning EAAC1, it is localized in post-synaptic elements (dendritic shafts and spines) but not within presynaptic axon terminals (Rothstein et al., 1994). The striatum is the major input station of the basal ganglia, receives massive excitatory afferences from cortical fibers releasing glutamate and is involved in motor control and learning (Graybiel, 1990;

*Correspondence to: P. Gubellini, Equipe IC2N-IBDML UMR6216; case 907, Parc Scientifique de Luminy, 13288 Marseille cedex 9, France. Tel: +33-0-491269248; fax: +33-0-491269244.
E-mail address: paolo.gubellini@ibdml.univ-mrs.fr (P. Gubellini).

Abbreviations: ACSF, artificial cerebrospinal fluid; AP-5, D,L-2-amino-5-phosphonopentanoic acid; cPPT, central polypurine tract; DHK, dihydrokainic acid; EAATs, excitatory amino acid transporters; EPSCs, excitatory postsynaptic currents; GFP, green fluorescent protein; GTs, glutamate transporters; HFS, high-frequency stimulation; mGluR, metabotropic glutamate receptors; MSNs, medium spiny neurons; NMDA, *N*-methyl-D-aspartate; PD, Parkinson's disease; PDC, L-trans-pyrrolyidine-2,4-dicarboxylate; PGK, phosphoglycerate kinase I promoter; PPR, paired-pulse ratio; (*RS*)-CPPG, α-cyclopropyl-4-phosphonophenylglycine; (*S*)-MCPG, (*S*)-alpha-methyl-4-carboxyphenylglycine; TBOA, DL-threo-β-benzyloxyaspartate; WPRE, woodchuck post-regulatory element.

0306-4522/09 © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2008.11.018

Levy et al., 1997). Although glutamate is the main excitatory neurotransmitter in the striatum, and although corticostriatal hyperactivity is an hallmark of PD, little is known, currently, regarding the role of GTs in modulating synaptic transmission in this structure, while this function has been already characterized in other brain regions, such as the hippocampus (Hestrin et al., 1990; Isaacson and Nicoll, 1993; Sarantis et al., 1993; Tsukada et al., 2005), the cortex (Kidd and Isaac, 2000; Campbell and Hablitz, 2004), the cerebellum (Marcaggi et al., 2003; Takatsuru et al., 2006) and the avian nucleus magnocellularis (Otis et al., 1996; Tureček and Trussel, 2000). Thus, the goal of the present work was to characterize whether and how GTs regulate corticostriatal synaptic transmission. To address this issue, we used the broad spectrum blocker DL-threo-β-benzyloxyaspartate (TBOA) and we performed *in vitro* patch-clamp recordings from striatal medium spiny neurons (MSNs) in adult rat slices. We measured the effects of TBOA on evoked glutamatergic excitatory postsynaptic currents (EPSCs) and on currents triggered by high-frequency stimulation (HFS). In addition, we tested whether mGluRs modulate the effects of GT inhibition. Here we show, for the first time in the striatum, that neuronal GTs shape the kinetic of EPSCs, while glial GTs appear to be recruited when a massive release of glutamate is triggered by HFS at corticostriatal synapses.

EXPERIMENTAL PROCEDURES

Animals and slices preparation

All animal experiments have been carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and conformed to the ethical guidelines of the French Ministry of Agriculture and Forests (Animal Health and Protection Veterinary Service). Lewis male rats weighing 250–300 g (10–12 weeks) were utilized and all efforts were made to minimize their number and suffering. To obtain rats with striatal astrocyte expressing green fluorescent protein (GFP), we used a lentiviral vector pseudotyped with Mokola with a strong tropism toward astrocytes (Colin et al., 2008; Pertusa et al., 2008) and expressing GFP, which was injected in the striatum. More precisely, we used a self-inactivated (SIN-W) lentiviral vector (Déglon et al., 2000) containing the central polypurine tract (cPPT) sequence, the mouse phosphoglycerate kinase I promoter (PGK), the woodchuck post-regulatory element (WPRE) sequence and encoding GFP (SIN-W-cPPT-PGK-GFP-WPRE). This vector was diluted in PBS-BSA to a final concentration of 200 ng p24/μl. Rats were anesthetized with a mixture of ketamine (15 mg/kg) and xylazine (1.5 mg/kg). Suspensions of lentiviral vector were injected into the striatum using a 34-gauge blunt-tip needle linked to a Hamilton (Reno, NV, USA) syringe by a polyethylene catheter. The stereotaxic coordinates were: anteroposterior (AP) +0.5 mm; lateral (L) +3.0 mm from bregma; and ventral (V) −4.5 mm from the dura. Rats received a total volume of 3 μl per striatum at a rate of 0.2 μl/min. The brains were cut in coronal slices (250 μm) in ice-cold solution (in mM: 110 choline, 2.5 KCl, 1.25 NaH_2PO_4, 7 $MgCl_2$, 0.5 $CaCl_2$, 25 $NaHCO_3$, 7 glucose, pH=7.4) bubbled with O_2/CO_2 (95/5%). Slices were stored in artificial cerebrospinal fluid (ACSF), whose composition was (in mM): 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 11 glucose, and 25 $NaHCO_3$, pH=7.4, gassed with O_2/CO_2 (95/5%), at room temperature, containing 250 μM kynurenic acid and 1 mM sodium pyruvate. Recordings were performed at 35 °C in standard ACSF solution (without kynurenic acid and sodium pyruvate).

Electrophysiological recordings and data analysis

In order to record striatal MSNs, whole-cell patch-clamp microelectrodes (4–5 MΩ) were filled with a CsCl solution, whose composition was (in mM): 140 CsCl, 10 NaCl, 0.1 CaCl, 10 Hepes, 1 EGTA, 2 Mg-ATP and 0.5 Na-GTP, pH=7.3, containing 0.5% biocytin. MSNs of the dorsal striatum were visualized by infrared videomicroscopy (Nikon and Olympus, Japan) before patching, and were identified a posteriori by histochemistry. For astrocyte recordings, the microelectrode solution was (in mM): KCl 130, $MgCl_2$ 2, Hepes 10, EGTA 5, $CaCl_2$ 2, Na-ATP 2, pH=7.3. Astrocytes were identified prior recordings due to their green fluorescence, and by their electrophysiological properties (Adermark and Lovinger, 2006). Electrophysiological recordings were performed by an AxoPatch 200B and a MultiClamp 700B amplifier with pClamp software (Molecular Devices, Sunnyvale, CA, USA). Picrotoxin at 50 μM was always added to the bath solution to block $GABA_A$ receptor-mediated synaptic activity. Series and input resistance was continuously monitored by sending a 5 mV pulse. Neurons showing ≥20% change in series resistance were discarded from the analysis. Synaptic stimulation for activating corticostriatal fibers and triggering EPSCs was delivered at 0.1 Hz by a bipolar electrode placed close to the recording pipette. More precisely, electrode tips were positioned in the external capsule, near the cingulum: for example, at 9.7 mm rostro-caudal level (interaural), the electrode tips were placed between coordinates 3–4 mm lateral and 6–7 mm dorsoventral (Paxinos and Watson, 1986). Data were analyzed offline by Clampfit 10.2 (Molecular Devices), Origin 7.5 (Originlab Corporation, Northampton, MA, USA) and MiniAnalysis 6.0 (Synaptosoft, Decatur, GA, USA). EPSC charge transfer was calculated starting just after the stimulus artifact to 100 ms after. Charge transfer of large currents induced by HFS trains was measured from the peak of the last EPSC in the train to baseline recovery. EPSC amplitude was measured by averaging a 0.3 or 4 ms time interval centered on the maximum amplitude value at −60 mV and +40 mV respectively. To quantify EPSC kinetics, its decay-time (from 90% to 10% of peak amplitude) was fitted with the sum of two exponentials, which allows calculating an amplitude-weighted decay-time constant (τ_w) and provides a better fit than one exponential; τ_w was calculated as:

$$\tau_w=(A_1\,\tau_1+A_2\,\tau_2)/(A_1+A_2)$$

where A_1 and A_2 are the amplitudes of the components with time constants τ_1 and τ_2 (see also Marcaggi et al., 2003). Drugs utilized were from Tocris-Cookson (Bristol, UK). Puff applications of glutamate were done by using a two-channel TooheySpritzer (Toohey Company, Fairfield, NJ, USA). Statistical analysis was performed by Wilcoxon non-parametrical test for matched pairs or by Mann-Whitney non-parametrical test for unmatched pairs (as specified in the figures' legends). Data are presented as mean± S.E.M.

Histochemistry

Following electrophysiological recordings, slices were fixed in Antigenfix (DiaPath, Rome, Italy), cryoprotected and quickly frozen for storage at −80 °C. The detection of biocytin-filled neurons was directly performed on slices with fluorescent streptavidin-Alexa 488 (Invitrogen, Paisley, UK) and a confocal microscope (Olympus Fluoview, Japan).