

Excitatory Amino Acid Transporters

Key References

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Overview

It is well established that L-glutamate acts as the major fast acting excitatory neurotransmitter in the mammalian CNS. Glutamatergic neurotransmission at excitatory synapses is terminated by a family of high-affinity, sodium-dependent L-glutamate transporters which are present on both neuronal and astroglial plasma membranes. These transporters mediate the efficient clearance of extracellular L-glutamate by an uptake mechanism driven largely by the sodium electrochemical gradient across the neuronal and astroglial membranes. In addition to the transport of L-glutamate, a number of other naturally occurring excitatory amino acids, including L-aspartate, L-cysteine sulfinate and L-cysteate, are known substrates of these transporter proteins. Hence, they have been termed excitatory amino acid transporters (EAATs). Recently, a separate family of glutamate transporters localized to the synaptic vesicle membrane have been identified and termed VGLUTs 1-3 (vesicular glutamate transporters). These transporters, responsible for packaging glutamate into synaptic vesicles for neurotransmitter release, utilize a proton electrochemical gradient to drive glutamate transport into the vesicle and exhibit a very distinct pharmacology and substrate specificity from the plasma membrane localized EAATs. In addition, VGLUT affinities (K_m) for glutamate are approximately 1-3 mM, while those for the EAATs are in the range 2-50 μ M. Prior to the molecular cloning of individual EAAT subtypes, these transporter proteins were studied in classical CNS-derived preparations, including synaptosome fractions, brain slices and primary cultures of both neurons and astrocytes. These studies provided the basis for the existence of multiple EAAT subtypes based on the demonstration of pharmacologically distinct EAAT activities in preparations derived from different brain regions. The recent cloning of multiple EAAT

subtypes from both rodent and human brain has confirmed the presence of distinct EAAT subtypes.

A family of five human EAATs have been cloned and are designated EAAT1-5. The subtypes EAAT1, EAAT2 and EAAT3 are the human homologs of the transporter clones initially isolated from non-human species designated as GLAST (GLutamate/ASpartate Transporter), GLT-1 (GLutamate Transporter) and EAAC1 (Excitatory Amino Acid Carrier), respectively. EAAT1 (GLAST) is restricted to glial cells and is the most abundant of the EAATs in cerebellum and retina. EAAT2 (GLT-1) is the most abundant EAAT in the forebrain and spinal cord. Almost all the EAAT2-protein is found in astroglial cells in the mature and normal brain and spinal cord, but a few percent is expressed in nerve terminals. It should be noted that neuronal expression is observed in the adult retina and in the brain and spinal cord during development as well as in primary neuronal cultures. The expression of EAAT4 is also restricted to brain and is found predominantly in the Purkinje cells of the cerebellum. EAAT3 is found both in astroglia and neurons in addition to a number of peripheral tissues where it represents the predominantly expressed subtype. Finally, EAAT5 is expressed primarily in the retina.

As described above, the EAATs play a crucial role in glutamatergic neurotransmission by effecting the efficient removal of L-glutamate from the extracellular space. In addition, under normal physiological conditions, the activity of these transporters is responsible for the maintenance of low extracellular L-glutamate concentrations thereby preventing L-glutamate excitotoxicity. This second property has been demonstrated most convincingly for the rat GLT-1 transporter subtype by deletion of the GLT-1 gene to produce GLT-1 knockout mice. Mice deficient in GLT-1 lose 80-90% of the glutamate transport capacity measured in

forebrain preparations, and exhibit increased vulnerability to convulsants and to acute cortical injury, when compared with wild type animals. The importance of the EAAT1 subtype in the retina and cerebellum is also supported by the visual and movement abnormalities documented for EAAT1 deficient mice. Similar to the EAATs, vesicular glutamate transport was initially described in synaptic vesicle preparations isolated from rat and bovine brain. The cloned VGLUTs remained elusive for a number of years before two groups simultaneously discovered that a transporter initially described as an inorganic phosphate transporter (BNPI) exhibited robust glutamate transport capacity and termed this VGLUT1. Subsequently, two additional members of this new glutamate transporter family VGLUT2 (also DNPI) and VGLUT3 have been cloned. VGLUTs 1 and 2 exhibit widespread and overlapping distribution in glutamatergic synapses in the adult brain, while VGLUT3 has axonal and somatodendritic localization in cholinergic, GABAergic and serotonergic neurons. The cloning of individual EAAT and VGLUT subtypes represents a major breakthrough for the study of these two distinct glutamate transporter families. Their availability provides the tools for the development of subtype selective inhibitors, and modulators, which will further expand our understanding of the physiological functions of the individual transporter subtypes. Most recently, a number of studies have implicated either dysfunctional EAAT activity, or altered EAAT expression as a potential contributor to the pathogenesis of a number of CNS disorders including both neurodegenerative and psychiatric disorders. Based on these observations, drugs targeted at either the acute modulation of EAAT activity or the manipulation of EAAT expression might represent a novel therapeutic strategy for the treatment of various CNS pathologies.

Excitatory Amino Acid Transporters

CURRENTLY ACCEPTED NAME	EAAT1	EAAT2	EAAT3	EAAT4	EAAT5
ALTERNATE NAME	GLAST	GLT-1	EAAC1	None	None
STRUCTURAL INFORMATION	542 aa (human)	574 aa (human)	525 aa (human)	564 aa (human)	561 aa (human)
UPTAKE INHIBITORS	<i>trans</i> -2,4-PDC (P7575) ^a	<i>trans</i> -2,4-PDC (P7575) ^a , Dihydrokainate (D1064) ^b , Kainate (K0250) ^b , T3MG, TBOA, ^c TFB-TBOA, ^c <i>trans</i> -2,3-PDC, ^d WAY-855 ^e	<i>trans</i> -2,4-PDC (P7575) ^a	<i>trans</i> -2,4-PDC (P7575) ^a	<i>trans</i> -2,4-PDC (P7575) ^a
RADIOLABELED SUBSTRATES ^f	L-[³ H]-Glutamate D-[³ H]-Aspartate	L-[³ H]-Glutamate D-[³ H]-Aspartate	L-[³ H]-Glutamate D-[³ H]-Aspartate	L-[³ H]-Glutamate D-[³ H]-Aspartate	L-[³ H]-Glutamate D-[³ H]-Aspartate
TISSUE EXPRESSION	Forebrain, astroglial	Forebrain, astroglial	Forebrain, neuronal, kidney	Cerebellum, neuronal	Retina
PHYSIOLOGICAL FUNCTION	Control [Glu] _{extracellular}	Control [Glu] _{extracellular}	Not known	Control [Glu] _{extracellular}	Control [Glu] _{extracellular}
DISEASE RELEVANCE ^g	Alzheimer's disease, amyotrophic lateral sclerosis	Amyotrophic lateral sclerosis, epilepsy, Alzheimer's disease, HIV-related dementia, stroke	Schizophrenia, bipolar disorder	Bipolar disorder, major depressive disorder, canavan disease	Retinopathies associated with Glu toxicity

Abbreviations

trans-2,3-PDC: L-*trans*-Pyrrolidine-2,3-dicarboxylic acid

trans-2,4-PDC: L-*trans*-Pyrrolidine-2,4-dicarboxylic acid

T3MG: threo-3-Methylglutamate

TBOA: threo-β-Benzyloxyaspartate

TFB-TBOA: (2S,3S)-3-(3-[4-(Trifluoromethyl)benzoylamino]benzyloxy)aspartate

WAY-855: 3-Amino-tricyclo[2.2.1.0^{2,6}]heptane-1,3-dicarboxylic acid

FOOTNOTES

a *trans*-2,4-PDC is a non-selective EAAT inhibitor. K_i values for EAATs 2, 4 and 5 are between 5-10 μM and for EAAT1 and EAAT3 are between 50-100 μM.

b Selective inhibitors of the EAAT2 subtype with K_i values between 15-60 μM. Utility limited by effects on glutamate receptors.

c TBOA is a non-substrate non selective EAAT2/EAAT3 inhibitor (K_i ~ 5 μM), 7-fold selectivity over EAAT1. TFB-TBOA reported as a more potent analog (K_i ~ 20 nM, EAAT1 and EAAT2).

d *trans*-2,3-PDC is a selective non-substrate inhibitor of EAAT2 (compared with EAAT1 and EAAT3).

e WAY-855 is a moderately selective EAAT2 inhibitor (K_i ~ 3 μM). No effects on glutamate receptors.

f L-Glutamate as the endogenous substrate and D-aspartate, a non-metabolizable substrate analog, are most frequently employed in uptake studies.

g Based on altered expression or RNA alternative splicing in disease state.

Excitatory Amino Acid Transporters

CURRENTLY ACCEPTED NAME	VGLUT1	VGLUT2	VGLUT3
ALTERNATE NAME	BNPI	DNPI	—
STRUCTURAL INFORMATION	560 aa (human)	582 aa (human)	589 aa (human)
UPTAKE INHIBITORS ^a	Evans Blue, ^b 4-methyl-glutamate, ^c <i>trans</i> -ACPD, ^c QDC, ^d Uncouplers ^e	Evans Blue, ^b 4-methyl-glutamate, ^c <i>trans</i> -ACPD, ^c QDC, ^d Uncouplers ^e	4-Methyl-glutamate, ^c <i>trans</i> -ACPD, ^c QDC, ^d Uncouplers ^e
RADIOLABELED SUBSTRATES ^f	L-[³ H]-Glutamate	L-[³ H]-Glutamate	L-[³ H]-Glutamate
TISSUE EXPRESSION	Brain, widespread glutamatergic pathways	Brain, widespread glutamatergic pathways	Brain, widespread non-glutamatergic pathways
PHYSIOLOGICAL FUNCTION	Glutamate packaging for release	Glutamate packaging for release	Not known
DISEASE RELEVANCE ^g	Schizophrenia	Not known	Not known

Abbreviations

***trans*-ACPD:** *trans*-Aminocyclopentanedicarboxylic acid

CCCP: Carbonylcyanide *m*-chlorophenylhydrazone

DIDS: 4,4'-Diisothiocyanatostilbene-2,2'-disulfonic acid

FCCP: Carbonylcyanide *p*-(trifluoromethoxy)-phenylhydrazone

QDC: Quinoline-2,4-dicarboxylic acid

FOOTNOTES

a Current tools for inhibiting VGLUTs are limited in their utility many possessing additional properties to their VGLUT inhibition

b Several dye compounds including Evans Blue, Chicago Sky Blue and Rose Bengal exhibit VGLUT inhibitory activity.

c Inhibit transport in synaptic vesicles. Not fully characterized with respect to cloned VGLUTs. Also inhibit EAATs and mGlu receptors.

d Inhibits transport in synaptic vesicles. Not fully characterized with respect to cloned VGLUTs and other glutamate targets.

e Uncouplers of the proton motive force such as FCCP, CCCP, DIDS, valinomycin, nigericin indirectly inhibit VGLUT activity by dissipating the gradient driving uptake.

f Distinct from the EAATs, L-glutamate is a VGLUT substrate, aspartate is not.

g Based on altered expression in disease state.