Biochemical Pharmacology 135 (2017) 1–11

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Commentary

Potentiating SLC transporter activity: Emerging drug discovery opportunities

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ARTICLE INFO

Article history:
Received 3 January 2017
Accepted 13 February 2017
Available online 16 February 2017

Keywords:
SLC
Glutamate
Monoamine
Transporter
Potentiator

ABSTRACT

Maintaining the integrity of cellular membranes is critical to protecting metabolic activities and genetic information from the environment. Regulation of transport across membranes of essential chemicals, including water, nutrients, hormones and many drugs, is therefore key to cellular homeostasis and physiological processes. The two main transporter superfamilies are ATP-binding cassette (ABC) transporters that primarily function as efflux transporters, and the solute carrier (SLC) transporters. SLC transporters encompass 52 gene families with almost 400 different human transporter genes. Although long under-explored, SLC transporters are an emerging drug target class and the molecular target of several approved inhibitor drugs, such as selective serotonin reuptake inhibitors (SSRIs) for depression and sodium/glucose co-transporter (SGLT2) inhibitors for diabetes. Interestingly though, although loss-of-function mutations in numerous human SLC transporters are linked to Mendelian diseases, few reports of SLC transporter activators have appeared, and only inhibitors have been advanced to clinical studies. In this commentary, we discuss several strategies for potentiating SLC transporter function, from direct acting potentiators to modulators of transcription, translation or trafficking. We review the progress made in recent years toward the understanding of the structural and molecular basis of SLC transporter function and the pathways and mechanisms that regulate SLC expression, and describe the opportunities these new insights present for discovery of SLC transporter potentiators. Finally, we highlight the challenges associated with the various approaches and provide some thoughts on future directions that might facilitate the search for SLC potentiators with therapeutic potential.

1. Introduction

Solute carrier (SLC) transporters constitute the second largest family of membrane proteins in the human genome. The SLC transporter superfamily encompasses 52 diverse gene families. Little to no sequence homology exists between SLC sub-families and multiple structural folds are represented across the SLC superfamily. Consequently, the classification of the almost 400 human SLC transporter genes is based solely on the function of the corresponding protein as a solute transporter, rather than any sequence or structural similarity to other family members. SLC transporters regulate the intake and/or efflux across cellular membranes of a wide variety of essential molecules, such as sugars, amino acids, inorganic ions, neurotransmitters, hormones, vitamins and drugs. The importance of SLC transporters in disease is illustrated by human genetic data which suggest that >50% of SLC family members are associated with a human disease compared to only ~20% for the broader human genome [1,2]. Since the SLC gene nomenclature was originally established in the 1990s, SLC transporters, long under-explored, have increasingly become relevant drug targets for the treatment of numerous diseases [3]. SLC drug targets span all therapeutic areas, with important targets identified

Abbreviations: SLC, solute carrier; PAM, positive allosteric modulator; SLC1A1, solute carrier family 1 member 1; EAAT1, excitatory amino acid transporter member 1; Glt_{ph}, glutamate transporter homolog from *Pyrococcus horikoshii*; TM, transmembrane; HP, hairpin; smFRET, single molecule fluorescence energy transfer; LeuT, leucine transporter from *Aquifex aeolicus*; SLC6A1, solute carrier family 6 member 1; SERT, serotonin transporter; DAT, dopamine transporter; NET, norepinephrine transporter.

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http://dx.doi.org/10.1016/j.bcp.2017.02.010
0006-2952/

in neuroscience, metabolism, inflammatory diseases and oncology [1]. SLC transporters have been successfully targeted with small molecule inhibitor drugs, i.e., selective serotonin reuptake inhibitors (SSRIs) for depression, and sodium/glucose cotransporter (SGLT2) inhibitors for diabetes. In addition to inhibitors, strong therapeutic rationales also exist for drugs that enhance the function of transporters. The rationale for drugs that increase transporter function is based on: 1) SLC transporter-related Mendelian diseases are almost exclusively caused by loss of function rather than gain of function mutations [1,4–6], and 2) SLC transporter expression is frequently down-regulated in animal disease models and in diseased human tissues in post-mortem studies [7,8]. However, despite the strong rationale, very few reports describing drugs that increase transporter function have appeared in the scientific literature [9–11]. This lack of progress is striking given that the pharmacology of several SLC families has been intensely studied over the last several decades (e.g. neurotransmitter transporters; for review [12]) and that the discovery of potentiators was the stated aim of several published studies [10,13–15].

In the present article, we discuss several possible strategies for potentiating transporter activity. The first approach we consider is direct pharmacological potentiation, or positive allosteric modulation (PAM). Several recent structural and molecular studies are now beginning to provide insight into possible molecular mechanisms for transporter potentiation and these are reviewed. Although we focus largely on sodium-dependent glutamate transporters that have been extensively studied and for which pharmacological activators have been explored for decades [10], we also provide general hypotheses for other types of SLC transporters from other families. The second approach we discuss is modulation of SLC expression. Since maximal transport capacity of SLC transporters is directly and positively correlated with their cell surface expression, modulation of pathways and/or direct interacting proteins that regulate expression and trafficking may constitute a promising alternative strategy for the discovery of SLC transporter potentiators. The strategies we describe include 1) pharmacological chaperones 2) the identification and modulation of protein-protein interactions regulating the expression and/or activity of SLC transporters of interest and 3) phenotypic screening strategies. Such approaches may be especially relevant for the treatment of Mendelian diseases associated with loss-of-function mutations in SLC transporter genes, since, as noted above, the observed reductions in transport activity most often result from trafficking defects [1,16,17]. Finally, we discuss the challenges associated with each of the strategies, in terms of both feasibility and selectivity. In particular, we discuss how recent studies aimed at elucidating the molecular mechanism of SLC transporter activation might explain the limited progress to date and we propose future directions that might facilitate the discovery of SLC potentiators.

2. Direct acting SLC PAMs

2.1. Glutamate transporters: structure and mechanism of transport

Sodium-dependent glutamate transporters (SLC1A1-5 or excitatory amino acid transporters EAAT1-5) play essential roles in the maintenance and regulation of glutamatergic neurotransmission [18,19]. SLC1A1-5 transporters rapidly bind and buffer glutamate, removing it from the synaptic cleft, thereby contributing to the termination of synaptic activity and to the clearance of potentially cytotoxic extracellular glutamate. SLC1A1-5 transports the bound glutamate into cells where it can be recycled for use in subsequent synaptic events [19]. Glutamate transporters can also influence membrane potential and cellular excitability, either as a result of the electrogenic cotransport of $3Na^+$ and $1H^+$ and coupled counter-transport of $1K^+$ for each glutamate molecule transported or via the generation of an uncoupled chloride conductance [20]. Importantly, reduced SLC1A1-5 expression and/or function has been observed in numerous neurological and neuropsychiatric disorders characterized by dysregulation of glutamatergic neurotransmission and cytotoxicity. Potentiators of SLC1A1-5 therefore, may restore transport function, improve glutamatergic transmission and reduce excitotoxicity, and provide benefit in the treatment of diseases such as amyotrophic lateral sclerosis (ALS), Huntington's disease, addiction and depression [18,19,21–24]. However, in spite of the clear rationale for the therapeutic potential of glutamate transporter activators for neurological and psychiatric disorders [18,19], only limited progress has been made to date in the search for drugs that increase glutamate transporter function [10]. Encouragingly though, recent advances in our understanding of the structural and molecular basis of transporter function are beginning to reveal possible mechanisms by which drugs could enhance SLC1 activity.

Sodium-dependent glutamate transporters are secondary active transporters. The models proposed for most secondary active transporters involve "alternating access": a binding site for both substrate and transported ions is alternately accessible either to the external or the internal solution, but never to both solutions at the same time [25]. Transporters are thought to accomplish substrate uptake by sequentially stepping through a series of conformations in a transport cycle, including a) outward-facing open, b) outward-facing occluded c) inward-facing occluded d) inward-facing open. In glutamate transporters, the binding of cotransported sodium ions followed by binding of glutamate to the outward-open state initiates the outward- to inward-facing transition. Substrate and sodium unbinding, followed by potassium binding then triggers the return inward to outward transition.

Detailed information on the structural underpinnings of the glutamate transport cycle is now available from numerous X-ray structures of Glt_{Ph}, an EAAT homolog from the archaea *Pyrococcus horikoshii*, which shares 37% amino acid identity with human SLC1A2. The first published structures revealed the transporter in a substrate-bound, outward-facing, occluded conformation [26]. The transporter was seen as a bowl-shaped trimer with a solvent-filled extracellular basin extending halfway across the membrane bilayer. Each protomer was comprised of eight alpha-helical transmembrane segments (TMs 1–8) and two helical hairpins (HPs 1–2) with a two-fold internal structural symmetry [26]. Since the original report, numerous additional structures of Glt_{Ph} in various conformations have been published [27,28]. Overlay of the outward-open, outward-occluded, and inward-occluded states revealed that TM1, TM2, TM4, and TM5 are relatively static [29], whereas the other parts of the protein housing the substrate and ion binding sites (TM3, TM6, HP1, TM7, HP2, and TM8) undergo substantial conformational changes. Glt_{Ph} (and by inference mammalian EAATs) was therefore proposed to comprise two structural domains, a rigid 'trimerization' domain (TM1, TM2, TM4, TM5) and a dynamic 'transport' domain (TM3, TM6, HP1, TM7, HP2, and TM8) [29]. Furthermore, comparisons of the various X-ray structures show that the outward to inward transitions in glutamate transporters involve large relocations of the transport domain approximately 15 Å normal to the membrane, as a result of an "elevator" like motion. A cavity in the thinnest region of the transporter, potentially accessible to both extracellular and cytoplasmic solutions, is also seen in the structure of Glt_{ph} in an intermediate conformation between the outward- and inward-facing states [30], suggesting that movement of the transport domain along the trimerization domain may also gate the uncoupled Cl^- conductance of the transporter [31].