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Review

Neurotransmitter transporters in schistosomes: Structure, function and prospects for drug discovery

Paula Ribeiro *, Nicholas Patocka

Institute of Parasitology, McGill University, Macdonald Campus, 21, 111 Lakeshore Road, Ste. Anne de Bellevue, Quebec H9X 3V9, Canada

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ABSTRACT

Neurotransmitter transporters (NTTs) play a fundamental role in the control of neurotransmitter signaling and homeostasis. Sodium symporters of the plasma membrane mediate the cellular uptake of neurotransmitter from the synaptic cleft, whereas proton-driven vesicular transporters sequester the neurotransmitter into synaptic vesicles for subsequent release. Together these transporters control how much transmitter is released and how long it remains in the synaptic cleft, thereby regulating the intensity and duration of signaling. NTTs have been the subject of much research in mammals and there is growing interest in their activities among invertebrates as well. In this review we will focus our attention on NTTs of the parasitic flatworm *Schistosoma mansoni*. Bloodflukes of the genus *Schistosoma* are the causative agents of human schistosomiasis, a devastating disease that afflicts over 200 million people worldwide. Schistosomes have a well-developed nervous system and a rich diversity of neurotransmitters, including many of the small-molecule ("classical") neurotransmitters that normally employ NTTs in their mechanism of signaling. Recent advances in schistosome genomics have unveiled numerous NTTs in this parasite, some of which have now been cloned and characterized *in vitro*. Moreover new genetic and pharmacological evidence suggests that NTTs are required for proper control of neuromuscular signaling and movement of the worm. Among these carriers are proteins that have been successfully targeted for drug discovery in other organisms, in particular sodium symporters for biogenic amine neurotransmitters such as serotonin and dopamine. Our goal in this chapter is to review the current status of research on schistosome NTTs, with emphasis on biogenic amine sodium symporters, and to evaluate their potential for anti-schistosomal drug targeting. Through this discussion we hope to draw attention to this important superfamily of parasite proteins and to identify new directions for future research.

Contents

Abbreviations: CNS, central nervous system; DAT, dopamine transporter; GABA, gamma-aminobutyric acid; GAT, gamma-aminobutyric acid (GABA) transporter; GLT, glutamate transporter; iDAT, invertebrate dopamine transporter; NAT, noradrenaline transporter; NTT, neurotransmitter transporter; OAT, octopamine/tyramine transporter; PZQ, praziquantel; RNAi, RNA interference; SERT, serotonin transporter; siRNA, short interfering RNA; SLC, solute carrier; SSRI, selective serotonin reuptake inhibitor; VMAT, vesicular monoamine transporter.

* Corresponding author. Tel.: +1 514 398 7607; fax: +1 514 398 7857.
E-mail address: paula.ribeiro@mcgill.ca (P. Ribeiro).

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1. Introduction

Neglected tropical diseases cause millions of deaths each year and pose health risks to more than a billion people worldwide. The amount of resources that is devoted to their control and management runs in billions of dollars [1]. Schistosomiasis in particular afflicts over 200 million people, the majority residing in sub-Saharan Africa [2,3]. The disease is caused by a parasitic flatworm (Platyhelminth), a trematode of the genus *Schistosoma*. Several species of schistosomes are known to cause disease in humans, primarily *Schistosoma mansoni*, *Schistosoma haematobium*, *Schistosoma japonicum* and, to a lesser extent, *Schistosoma mekongi* and *Schistosoma intercalatum*. It has been estimated that schistosomiasis results in 70 million DALYs (disability adjusted life years) lost each year [4], a disease burden comparable to that of malaria and tuberculosis. There is no vaccine for schistosomiasis and treatment relies heavily on a single drug, praziquantel (PZQ). PZQ is a relatively effective and inexpensive drug but it is not active against larval (schistosomulae) stages of the parasite [5,6]. Moreover, with only one drug available, the prospect of drug resistance is a real concern [7–9], particularly as PZQ usage increases due to growing mass treatment programs. There is a strong urgency to develop other chemotherapeutics for either treatment alone or co-administration with PZQ.

One area of interest in the development of new anti-schistosomal drugs is the parasite's nervous system. The nervous system of worms holds a plethora of signaling proteins that can be targeted for anthelmintic drug discovery [10,11]. Most of the therapeutics available for treatment of nematode infections, including such mainstays as ivermectin, levamisole and the latest addition, monepantel, all function by targeting proteins of the worm's nervous system [12,13]. Of particular interest for drug targeting are those neuronal circuits that control muscle function and movement. Schistosomes rely on their neuromuscular systems to coordinate important locomotory behaviors, notably the penetration of cercariae through the skin and the ensuing migration of schistosomulae in the bloodstream. These behaviors require tight coordination, not only of the musculature that enables movement but also chemotactic and other sensory responses needed for successful navigation. Besides movement, schistosomes use their neuromuscular systems to control the muscles of the suckers, the muscle lining of the viscera, including reproductive, digestive and excretory tracts, and also the tight coupling of males and females. These activities are vital to survival of the parasite. A drug that disrupts one or more of these activities would be expected to interfere with the normal life cycle and ultimately cause elimination of the parasite from the host. It is noteworthy that PZQ, the drug of choice for treatment of schistosomiasis acts in part by disrupting normal muscle function and causing paralysis of the worm [14,15].

Neuromuscular signaling in schistosomes is mediated by many of the same neurotransmitters that have been described in other organisms, including several different types of neuropeptides and small-molecule (classical) transmitters (see [11,16–18]). Any one of these systems could be manipulated pharmacologically to disrupt muscle function and movement of the worm. To date most research on helminth neurochemistry and druggable neuronal targets has focussed on neurotransmitter receptors, either G protein-coupled receptors (GPCRs) [11,17–20] or transmitter-activated ion channels [12,21], which are known targets for anthelmintic drugs. By comparison considerably less is known about neurotransmitter transporters (NTTs), which also play a pivotal role in the control of neuronal signaling. NTTs have been the subject of much research in other organisms and they are exceptionally fruitful drug targets. Over 20 pharmaceuticals currently in use (or in clinical trials) for treatment of mood disorders and other mental illnesses have NTTs as their molecular targets (see [22,23] for an overview) and they are also being investigated among invertebrates as targets for pesticide discovery [24]. NTTs have not received much attention in any of the helminth parasites. There are, however, several of these transporters present in schistosomes and the limited research available suggests they are just as important for neurotransmitter homeostasis in worms as they are in other organisms. Moreover, as discussed later, there is growing pharmacological evidence that inhibitors of NTTs, particularly inhibitors of serotonin transporters (SERT) have potent anti-schistosomal effects *in vitro*, once again highlighting the druggability potential of these proteins.

In this chapter we will review briefly the current status of research on helminth NTTs with emphasis on schistosomes and monoamine neurotransmitter transporters, such as SERTs, which show particular promise for drug intervention. Structural and pharmacological properties of NTTs will be discussed in the context of what is known from the broader, largely mammalian field of research. Our goal is to draw attention to this important but largely neglected superfamily of parasite proteins and to evaluate their potential for anthelmintic drug targeting.

2. Neurotransmitter transporters: Classification and general properties

2.1. The different classes of transporters

NTTs are present in all animal phyla, vertebrate and invertebrate, and they play a key role in the control of neurotransmission. NTTs may be vesicular transporters, which sequester the neurotransmitter into synaptic vesicles, or plasma membrane transporters that mediate the cellular uptake of transmitter from the extracellular space (Fig. 1). Together, these transporters help to determine how much transmitter is available for release and, once released, how long it remains in the synaptic cleft, thereby controlling the intensity and duration of signaling [25]. Plasma membrane transporters may be expressed in presynaptic nerve terminals, where they mediate the reuptake of released neurotransmitter for subsequent enzymatic degradation or recycling. Alternatively, the transporter may be expressed in non-neuronal cells that serve as storage sites for the neurotransmitter. For example, in mammals, NTTs are expressed in glial cells within the central nervous system [26] and other types of cells in the periphery that accumulate neuroactive substances, such as blood platelets [27], lymphocytes [28] and others [23].

Vesicular and plasma membrane NTTs belong to different solute carrier (SLC) gene families and they differ both with respect to structure and mechanism of transport. Vesicular carriers are proton-driven antiporters [29] whereas the plasma membrane transporters are sodium-dependent symporters, which use the influx of sodium to drive neurotransmitter transport into the cell. The sodium symporters are further classified according to two major SLC gene families, SLC1 [30] and SLC6 [22,23,31]. The majority of these transporters belong to the SLC6 family and include carriers for all the biogenic monoamines (see below) and the inhibitory amino acid neurotransmitters, gamma-aminobutyric acid (GABA) and glycine. Also included in the SLC6 family are sodium-dependent amino acid carriers and several orphan transporters [23,32]. The SLC1 group is smaller and consists mainly of sodium symporters for classical excitatory neurotransmitters, glutamate and aspartate [30]. SLC6 carriers are both Na^+- and Cl^--dependent whereas SLC1 is typically Cl^--independent and uses both Na^+ and K^+ electrochemical gradients to drive the transport of neurotransmitter.

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