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PARASITOLOGY (A VAIDYA, SECTION EDITOR)

Ion Channels and Drug Transporters as Targets for Anthelmintics

Robert M. Greenberg

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Abstract Infections with parasitic helminthes, such as schistosomes and soil-transmitted nematodes, are hugely prevalent and are responsible for a major portion of the global health and economic burdens associated with neglected tropical diseases. In addition, many of these parasites infect livestock and plants used in agriculture, resulting in further impoverishment. Treatment and control of these pathogens relies on anthelmintic drugs, which are few in number, and against which drug resistance can rapidly develop. The neuromuscular system of the parasite, and in particular, the ion channels and associated receptors underlying excitation and signaling, have proven to be outstanding targets for anthelmintics. This review surveys the different ion channels found in helminths, focusing on their unique characteristics and pharmacological sensitivities. It also briefly reviews the literature on helminth multidrug efflux that may modulate parasite susceptibility to anthelmintics and may prove useful targets for new or repurposed agents that can enhance parasite drug susceptibility and perhaps overcome drug resistance.

Keywords Ion channels · Drug transporters · Helminths · Anthelmintics · Neglected tropical diseases · Schistosomiasis

Introduction

Parasitic helminths, or worms, are mainly represented by members of two phyla, the nematodes, or roundworms, and the platyhelminths, or flatworms. Parasitic nematodes include the soil-transmitted worms (e.g., ascarids, hookworms) and the filarial worms that cause diseases such as elephantiasis (lymphatic filariasis) and river blindness (onchocerciasis). Parasitic platyhelminths include the flukes (trematodes), such as schistosomes, and the tapeworms (cestodes). Helminths infect a huge proportion of the world's human population, with over 1 billion people in the developing world estimated to be infected with one or more species of worms [1–3]. Helminth infections represent a major portion of the neglected tropical diseases, and have devastating - although often underestimated - effects on the health and development of individuals and societies [4]. The causative agents and the prevalence of the most significant human infections are listed in Table 1. Parasitic helminths also infect livestock and plants (as well as companion animals), resulting in significant economic losses and production shortfalls [5, 6].

Three primary approaches for limiting and controlling these pathogens are: improved sanitation and hygiene; vaccination; and treatment with anthelmintic compounds. Educational interventions and infrastructural improvements have been shown to be highly effective at preventing helminth infection and reducing transmission [7], as well as having long-lasting benefits for general public health. However, implementation requires a substantial investment of resources and levels of management and coordination that are often not feasible in the developing world. Effective preventative and therapeutic vaccines are obviously highly desirable, but despite strong research efforts and the identification of promising candidate antigens [8, 9], there are currently no vaccines approved for use against helminth infections. Thus, both treatment and control of helminth infections depend almost entirely on a relatively small set of anthelmintic drugs. Although effective, chemotherapy as the sole line of defense is ultimately unsustainable, as reinfection, the development of drug resistance, and inconsistencies in maintenance of ongoing treatment and monitoring can hinder long-term efficacy [10].

R. M. Greenberg (✉)
Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia, PA 19104, USA
e-mail: rgree@vet.upenn.edu

Table 1 Major Helminth Infections of Humans (adapted from [1])

Agents	Disease	Prevalence
Nematodes: soil-transmitted		
Ascaris lumbricoides (roundworm)	Ascariasis	800 million
Trichuris trichuria (whipworm)	Trichuriasis	600 million
Necator americanus, *Ancylostoma duodenale*	Hookworm	575 million
Strongyloides stercoralis (threadworm)	Strongyloidiasis	30–100 million
Nematodes: filarial		
Wucheria bancrofti, *Brugia malayi*	Lymphatic filariasis	120 million
Onchocerca volvulus	River blindness (onchocerciasis)	37 million
Loa loa	Loiasis	13 million
Dracunculus medinensis	Dracunculiasis (guinea worm)	10,000
Platyhelminths: flukes (trematodes)		
Schistosomes (blood flukes)	Schistosomiasis	200–300 million
Schistosoma mansoni, *S. haematobium*		
S. japonicum, *S. mekongi*, *S. intercalatum*		
Food-borne trematodes	Food-borne trematodiases	>40 million
Clonorchis sinensis (liver fluke)		
Opsthorchis viverinni (liver fluke)		
Fasciola hepatica (liver fluke)		
Paragonimus spp. (lung flukes)		
Fasciolopsis buski (intestinal fluke)		
Platyhelminths: tapeworms (cestodes)		
Taenia solium (pork tapeworm)	Cysticercosis	>400,000

Platyhelminths and nematodes are multicellular animals with relatively well-developed neuromuscular systems that constitute a large proportion of the organisms' cells and gene activity, and are essential for parasite survival, development, and reproduction [11–13]. Indeed, within their definitive hosts (and also at other stages) worms often exhibit (and indeed require) exquisite control of motility, navigation, feeding, and reproductive behaviors, all dependent upon a highly-regulated, fully-functional neuromuscular system. Interference with any of these activities has the potential to disrupt a parasite's life cycle, either by killing the worm (directly, or by compromising its ability to feed or fend off host defenses), or by rendering it incapable of reproducing. It is not surprising therefore that the neuromuscular system of these organisms has been a prime target of some of the most effective anthelmintic agents available.

Underlying neuromuscular function are networks of ion channels, ligand receptors (many of which are also ion channels), neurotransmitters, peptides, transporters, and intracellular signaling pathways, all of which must be coordinated and regulated, and all of which must be integrated with the normal metabolic functioning required to maintain cell viability. Ion channels, proteins which underlie electrical excitability in cells, have been particularly useful as targets for anthelmintics. Indeed, most of the antinematodal drugs in use today target ion channels [14•]; similarly, praziquantel, the drug of choice against parasitic platyhelminths such as schistosomes and many cestodes, also appears to act on parasite ion channels (see below) [15, 16•].

Interestingly, ion channels are highly-conserved, ancient proteins [17–19], with representatives in both parasites and their hosts. However, many of these channel families are highly diverse, often with invertebrate-specific members. Furthermore, even extremely well-conserved proteins such as tubulin can be selectively targeted; the widely-used benzimidazole anthelmintics (e.g., albendazole, fenbendazole) inhibit microtubule polymerization by targeting tubulin [20]. Subtle variations in subtypes or structural changes (sometimes a single amino acid change) are sufficient to provide selectivity against the parasite protein.

In 2011, Wolstenholme comprehensively surveyed the literature on nematode ion channels and other neuromuscular receptors as targets of anthelmintics [14•]. Since publication of that review, several exciting new findings have appeared, including publication of new helminth genomes. Here, I revisit the drugs and their targets covered in that 2011 review, incorporating platyhelminths as well as nematodes, and include updates on some exciting recent findings. I also briefly discuss the possible role of parasite drug transporters in modulating susceptibility to anthelmintics, including evidence implicating them in drug resistance and their potential as targets for new or repurposed drugs that either potentiate

current anthelmintics, or that act on their own to disrupt the normal physiological functions of these proteins.

Ion Channels

Ion channels are pore-forming membrane proteins and protein complexes that underlie electrical excitability and fast neurotransmission, as well as other rapidly-occurring biological functions in cells. They form a gated pathway for charged ions to flow passively across the normally impermeable lipophilic cell membrane, down the electrochemical gradient that is maintained through active (ATP-dependent) transport. As Hille has pointed out [21], the relationship of ion channels to electrical signaling is comparable with that of enzymes to metabolism. Ion channels are involved in a huge array of cellular functions, and channelopathies caused by channel mutations constitute a substantial disease burden [22]. Different ion channels have a range of selectivity, from relatively non-selective (e.g., non-selective cation channels) to highly selective for a particular ion. Activation, or gating, of ion channels can be by changes in voltage, ligand binding (extracellular or intracellular), or mechanical force. These categories are not rigid, however, as some channels can be gated by multiple types of stimuli, and channel activity can also be modulated by intracellular signals. Importantly, ion channels exhibit a rich pharmacology, and are targets of a wide variety of both naturally-occurring toxins and synthetic compounds [23], including many anthelmintic drugs (Table 2).

Nicotinic Acetylcholine Receptors

Nicotinic acetylcholine receptors (nAchRs) are members of the Cys-loop ligand-gated ion channel family that underlies rapid ionotropic neurotransmission. Like other Cys-loop channels, nAchRs contain five similar subunits (at least two α subunits and no more than three non-α subunits) in a homo- or hetero-pentameric structure that surrounds a central pore. There are two extracellular ligand binding sites per receptor, and α subunits are required to form these sites, though non-α subunits can also contribute to the agonist binding site [24].

Many nAchRs localize to the neuromuscular junction, and are critical to neuromuscular signaling. Binding of extracellular acetylcholine opens the channel, allowing the flow of ions down the electrochemical gradient. nAchRs are typically cation selective and mediate excitatory responses by depolarizing the cell, although invertebrates also express anion-selective acetylcholine-gated channels that may play a role in fast inhibitory responses [25, 26•]. As with other ligand-gated channels, acetylcholine also activates a separate class of metabotropic receptors that are not ion channels, and instead act through G-protein-coupled signaling pathways.

Exposure of most organisms, including nematodes [27, 28] and some free-living flatworms [29], to cholinergic agonists causes spastic paralysis due to prolonged excitation at the neuromuscular junction. In contrast, schistosomes and other trematodes and cestodes exposed to these agents exhibit flaccid paralysis, indicative of an inhibitory response [30, 31]. This atypical reaction in schistosomes has recently been shown to be mediated by a novel family of anion-selective acetylcholine-gated channel subunits found in flatworms [26•]. Nematodes also express acetylcholine-activated channels that gate Cl^-, although they appear to be more closely related to other types of Cl^- channels than to nAChRs [32, 33].

nAchRs have proven to be especially fruitful targets for anthelmintics, particularly anti-nematodal drugs. Indeed, these receptors represent the most common targets of current anthelmintics (see Table 2). These include classic drugs such as the imidothiazoles (levamisole) and tetrahydropyrimidines (pyrantel, oxantel), which act as agonists [14•, 34], as well as more recently introduced compounds such as the amino-acetonitriles (monepantel) [35], tribendimidine [36•, 37], and the nAchR antagonist derquantel [36•]. As noted, nAchRs are pentameric structures, and the mixing and matching of the wide variety of available subunits provides the basis for a considerable diversity of receptors with distinct structural properties and pharmacological sensitivities. This potential for receptor diversity is particularly notable in nematodes, which typically contain genes encoding more nAchR subunits than do mammals [34, 38]. Striking variations in parasite nAchR pharmacology can be obtained by varying the subunits comprising the functional receptor [14•, 34, 38]. More remarkably, simply varying the stoichiometry of the same subunits can also dramatically alter receptor sensitivity to different anthelmintics [36•, 39]. Thus, parasites have the capacity to rapidly develop resistance against drugs that target these types of multi-subunit complexes simply by modifying expression patterns of existing subunits. On the other hand, this complexity in receptor composition suggests that development of resistance against one nAchR drug does not necessarily lead to cross-resistance against other drugs that act on variants of these receptors. This advantage has recently been illustrated by the development of monepantal, which targets a family of apparently nematode-specific neuronal nAchRs, and "breaks" resistance against other nAchR-targeting anthelmintics [35].

Glutamate-Gated Chloride Channels

Glutamate-gated chloride channels (GluCls) are another member of the Cys-loop ligand-gated channel family. Unlike the

Table 2 Ion Channel Targets of Anthelmintics

Anthelmintic Group	Examples	Target
Imidazothiazoles	Levamisole	Nematodes; nAchR agonists [14•, 34]
Tetrahydropyrimidines	Pyrantel, oxantel, morantel	Nematodes; nAchR agonists [14•, 34]
Amino-acetonitriles	Monepantel	Nematodes; choline receptor agonists [35]
Tribendimidine		Nematodes; nAchR agonist [36•, 37]
Spiroindoles	Derquantel	Nematodes; nAchR antagonist [36•]
Macrocyclic lactones	Ivermectin, moxidectin	Nematodes; GluCl activation [28, 42]
Cyclo-octadepsipeptides	Emodepside	Nematodes; Slo-1 K^+ channels; latrophilin receptors [75, 76]
Piperazine		Nematodes; GABA receptor agonist [58]
Pyrazino-isoquinoline	Praziquantel	Schistosomes, trematodes, cestodes; voltage-gated Ca^{2+} channels? [15, 16•]
Other anthelmintics and targets:		
Benzimidazoles	Albendazole, fenbendazole	Nematodes, trematodes; β-tubulin [95]
Piperazine derivative	Diethylcarbamazine (DEC)	Filaricide; arachadonic acid pathways, anti-inflammatory, others? [96]
Oxamniquine		*Schistosoma mansoni*; DNA alkylation following activation by parasite sulfotransferase [97•]
Artemisinins	Artesunate	Schistosomes; several candidate pathways [98]
Antibiotics	Doxycycline	*Wolbachia* endosymbionts in filarial worms [99]

nAchRs, GluCls are confined to invertebrates, making them especially attractive anthelmintic (and insecticide) targets. As their name suggests, GluCls are gated by L-glutamate and permeable to Cl^-. They underlie fast inhibitory transmission, hyperpolarizing the cell. The closest relatives of GluCls are mammalian glycine channels and GABA-gated Cl^- channels. GluCls play roles in locomotion, feeding, and sensory input, among other functions. Like other Cys-loop channels, GluCls are pentameric. Although channels containing a single subunit are functional [40], the subunit stoichiometry of the native channel is unknown. Recently, the X-ray structure of a *Caenorhabditis elegans* GluCl channel has been solved [41•], the first three-dimensional structure for a Cys-loop ligand-gated channel. Exhaustive reviews on the structure and properties of these channels have been published [42–44].

GluCl channels are targets for macrocyclic lactones, which include avermectin anthelmintics such as ivermectin and moxidectin, as well as the milbemycins. The macrocyclic lactones have proven extremely successful as anthelmintics (as well as insecticides and acaricides). They are used widely in human and veterinary medicine, most notably against filarial worms that cause diseases such as onchocerciasis (*Onchocerca volvulus*) and lymphatic filariasis (*Wuchereria bancrofti*) in humans, and heartworm disease (*Dirofilaria caninum*) in canines, paralyzing and killing the microfilariae and compromising fecundity of adults. In the case of *Dirofilaria*, slow killing and stunting of adults using long-term monthly prophylactic ivermectin therapy (soft kill) can be used as an alternative or adjunct to avoid the risks of rapid killing with the arsenical melarsomine [45].

The action of ivermectin on GluCl channels is slow but effectively irreversible, thereby rendering the channel essentially non-functional. Ivermectin can activate the channel itself, and it can also potentiate glutamate-gated activation [40]. Macrocyclic lactones act as allosteric regulators; ivermectin binds to a transmembrane site distinct from the L-glutamate-binding site, and appears to stabilize an open-pore conformation [41•]. Macrocyclic lactones also have activity against mammalian neuronal receptors, most notably the $GABA_A$-gated channel [46]. Ivermectin is also a positive allosteric modulator at vertebrate neuronal nAchRs [47], while abamectin, which is used commercially in combination with derquantel, has recently been shown to non-competitively antagonize acetylcholine depolarizations and muscle contractions in *Ascaris suum* [48]. Ivermectin and other avermectins are also substrates for the multidrug transporter P-glycoprotein (Pgp) at the blood–brain barrier. Pgp mediates exclusion of these drugs from the mammalian central nervous system, thereby preventing interaction with CNS receptors; loss or disruption of host Pgp function can lead to ivermectin-induced neurological toxicity [49, 50].

Interestingly, the recently published genome of the hookworm *Necator americanus* [51] revealed that their GluCl channel genes appear to lack key residues for ivermectin activity, a finding which may explain the relatively low ivermectin sensitivity of these worms [52]. Schistosomes and other platyhelminths are typically not sensitive to macrocyclic lactones [53, 54] (though see [55]), which could suggest an absence of GluCl channels in these organisms. However, exciting recent work [56•] has demonstrated that schistosomes in fact do express GluCl channel subunits, but that these subunits are phylogenetically distinct from those of other invertebrates, including nematodes, arthropods, and molluscs. When expressed in *Xenopus* oocytes, schistosome GluCl subunits form functional L-glutamate-gated, Cl^--permeable channels. However, these expressed channels are unresponsive to

1 μM ivermectin, thus distinguishing them from ivermectin-sensitive GluCl channels. Since GluCls are already validated as drug targets in other parasites, these pharmacologically and phylogenetically distinct receptors may prove to be outstanding candidates for new or repurposed drugs targeting GluCls in schistosomes and other parasitic flatworms.

GABA-Gated Chloride Channels

GABA-gated chloride channels are ligand (GABA)-gated inhibitory channels that mediate the relaxation phase of nematode sinusoidal muscle movement [57]. GABA channel agonists such as the anthelmintic piperazine act on this channel to produce flaccid paralysis of the worm [58]. Macrocyclic lactones also appear to interact with nematode GABA-gated channels [57], and there is some evidence that the cyclooctadepsipeptide PF1022A (see below) binds to and interacts with nematode GABA receptors [59], although electrophysiological experiments suggest that it does not act as a GABA agonist [60]. Surprisingly, schistosomes do not appear to have genes for GABA-gated channels [56•].

Other Ligand-Gated Channels

There are a host of other helminth ligand-gated ion channels with the potential to serve as attractive drug targets. These include a variety of inhibitory Cys-loop neurotransmitter (serotonin, dopamine, tyramine, AchR)-gated anion channels not found in mammals [14•, 26•, 32]. In addition to the GluCl channels, helminths also contain excitatory glutamate-gated cation channels, which play critical roles in the neuromusculature of animals. Interestingly, although schistosomes have functional (Ca^{2+}-permeable) P2X channels, nematodes apparently do not [61]. P2X channels act as receptors for extracellular ATP- and adenosine-mediated signaling, with roles in neurotransmission and intercellular signaling in a variety of tissues and organisms.

Many of these parasite ionotropic receptors still await detailed characterization, and may prove vulnerable to pharmacological disruption.

Voltage-Gated Ion Channels

Voltage-gated ion channels open in response to changes in membrane potential. They gate calcium (Ca_v channels), potassium (K_v channels), or sodium (Na_v channels), although some channels in this family are not highly ion selective, and instead permeate multiple cations; there are also voltage-gated proton channels [62], and voltage-gated chloride channels [63]. The voltage-gated ion channels underlie electrical signaling, including generation of action potentials. For example, Na_v channels initiate Na^+-based action potentials, and K_v channels regulate resting membrane potential and repolarize the cell following excitatory events (e.g., action potentials). The Ca_v channels have the additional special role of translating electrical signals into chemical signals [21], as they are a major conduit for the entry of Ca^{2+}, a critical signaling molecule, into the cell. Ca_v channels thus couple depolarization of the cell to a wide array of Ca^{2+}-dependent responses.

There are several different sub-families within the different voltage-gated channel types. The K_v channel family is hugely diverse, with almost 80 K_v channel-encoding genes in mammals [64]. K_v channels include the six-transmembrane voltage-gated K^+ channels; the six-transmembrane Ca^{2+}-activated potassium channels; the two-transmembrane, inwardly-rectifying K_{ir} channels; and the four-transmembrane, two-pore channels (e.g., TWIK). The Ca_v channel family consists of low voltage-activated (Ca_v3), and high voltage-activated (Ca_v1, Ca_v2), families of pore-forming α1 subunits, with different subtypes within each family [65].

The architecture of these channels has been thoroughly studied [66]. Voltage-gated channels typically comprise four homologous transmembrane domains surrounding a central pore. Each domain typically contains six transmembrane regions, one of which acts as the voltage sensor. K_v channel subunits contain a single domain, and a functional channel is a tetramer of four of these subunits (with the exception of 2-pore, 4-transmembrane subunits, which form dimers). In contrast, Na_v and Ca_v channel subunits contain all four domains within the single protein chain; hence, these channels are monomeric. Auxiliary membrane and cytoplasmic subunits are associated with the pore-forming subunit and modulate its properties.

Both schistosomes and nematodes appear to lack Na_v channels [14•, 51, 67,]. However, this lack of Na_v channels in the platyhelminths does not appear to be universal; a Na_v channel-like cDNA has been reported in the turbellarian ectoparasitic flatworm *Bdelloura candida* [68], and a gene encoding a Na_v channel-like subunit is present in the genome of the cestode *Echinococcus granulosus* [69, 70]. Whether these subunits form functional Na_v channels remains to be determined, however.

In contrast to the situation with Na_v channels, K_v and Ca_v channels are well represented in both nematode and flatworm genomes, with multiple classes and subtypes of each. Indeed, nematode genomes (*C. elegans*, *N. americanus*) code for approximately 50–80 different K_v and Ca_v channel genes [51, 71]; the *S. mansoni* genome contains approximately 40 K_v channel genes and four genes encoding high voltage-activated Ca_v channel subunits [67]. Surprisingly, there appear to be no genes for low voltage-activated Ca_v channel subunits in either the *S. mansoni* or *S. japonicum* genomes [72], suggesting either that schistosome excitable cells uniquely lack a

requirement for the functions normally carried out by low voltage-activated Ca_v channels, or that schistosomes recruit other channels to perform those functions (e.g., pacemaker activity).

Voltage-gated ion channels have served as particularly outstanding, although underexploited, drug targets [23, 73]. For example, Ca_v channel blockers are used widely in cardiovascular medicine, and Na_v channels are targets for local anesthetics. Insecticides such as DDT and pyrethroids act on arthropod Na_v channels.

In contrast to these other systems, helminth voltage-gated (and related) ion channels are not well represented as drug targets, and further exploration is clearly warranted. Indeed, only two members of the voltage-gated ion channel superfamily have been implicated in the action of current anthelmintics: SLO-1 Ca^{2+}-activated potassium channels for the antinematodal drug emodepside; and Ca_v channels for the antischistosomal drug praziquantel.

Nematode SLO-1 Ca^{2+}-Activated Potassium Channels as Targets of Emodepside

SLO-1 potassium channels are members of the BK (big K^+ conductance) family of high-conductance K_v channels [74]. SLO-1 channels can be activated by either depolarization alone, intracellular Ca^{2+} alone, or synergistically, by both. SLO-1 channels play important roles in the neuromuscular system, as well as in secretory cells, among others. Most relevant to this discussion, SLO-1 channels are required for the action of emodepside, a relatively recent anthelmintic currently used in veterinary medicine.

Emodepside is a semi-synthetic cyclo-octadepsipeptide anthelmintic derived from the fungus *Rosellinia* spp. PF1022 [75, 76]. It has activity against a wide range of nematodes, and breaks resistance against other anthelmintics. Emodepside disrupts neuromuscular transmission, causing a flaccid paralysis; it also affects feeding and egg-laying, and slows development.

Several studies show that nematode SLO-1 channels are required for the action of emodepside. Loss-of-function mutations in the *C. elegans* SLO-1 channel-encoding *slo-1* gene render the worms resistant to the effects of emodepside on both pharyngeal and locomotor activity. The *slo-1* gain-of-function mutations phenocopy the effects of emodepside and *slo-1* null mutants are rescued by transformation with wild type *slo-1* [77]. Orthologues of *slo-1* from parasitic nematodes also restore emodepside sensitivity to the *C. elegans* loss-of-function mutants [78•]. In contrast, a human orthologue of *slo-1* does not confer emodepside sensitivity to these mutants, though it does rescue some of the behavioral deficiencies associated with the absence of the channel [79]. Electrophysiological studies on *Ascaris suum* are consistent with emodepside activation of SLO-1, but with a slow time course, possibly indicative of a non-extracellular binding site within the transmembrane domains [80].

Complicating the situation with emodepside is evidence showing that it interacts with other receptors as well, most notably the latrophilin receptor, a conserved G-protein-coupled receptor (GPCR) that binds the black widow spider toxin latrotoxin. A range of evidence [60, 76] implicates the latrophilin receptor in emodepside action in the pharynx, but not in body wall muscle. The current view appears to be that emodepside can act either directly or indirectly on SLO-1 channels. In the pharynx, emodepside apparently acts upstream of the SLO-1 channel, likely via the latrophilin receptor, to modulate SLO-1 activity, perhaps via phosphorylation; while the latrophilin receptor is not involved in emodepside activity on body wall musculature [78•].

Flatworm Ca_v Channels as Targets of Praziquantel

Praziquantel is the current drug of choice against schistosomiasis [81, 82]. Indeed, it has become effectively the only drug available to treat a disease estimated to affect over 200 million people. Since its development in the 1970s, no new drugs (other than repurposed antimalarials such as artemisinins) have entered the market, and one drug used previously (oxamniquine) is essentially no longer available. Overall, praziquantel is quite effective, but a major shortcoming is that it is not active against immature, liver-stage schistosomes, a major concern in regions with high reinfection rates.

Furthermore, despite the availability of praziquantel for almost four decades, its molecular target is still not rigorously defined. However, substantial evidence implicates parasite Ca_v channels in praziquantel action [15, 83, 84]. Early experiments on praziquantel showed that it produces a rapid influx of Ca^{2+} into the worm, and a Ca^{2+}-dependent muscle contraction and paralysis. Several subsequent studies showed that an unusual Ca_v channel auxiliary β subunit, found only in platyhelminths, could confer praziquantel sensitivity to a mammalian Ca_v channel subunit expressed in a heterologous system, and that this capacity could be eliminated by altering a single amino acid residue in the schistosome β subunit [15].

Recent work that originated with studies on free-living planaria has substantiated the role of Ca_v channels in praziquantel action [16•, 85]. Planarians have the extraordinary ability to regenerate a head and a tail when cut at both ends. However, praziquantel disrupts this capability, causing planaria instead to regenerate two heads instead of a head and a tail. Remarkably, suppression of Ca_v channel subunit expression by RNA interference can override these effects of praziquantel, and the worms now show normal regenerative patterning in the presence of the drug. Recently, this

praziquantel-dependent activation of a neuronal Ca_v channel in planaria has been shown to regulate head structure formation by modulating dopaminergic and serotonergic pathways. Dopaminergic and serotonergic ligands that miscue regeneration also show antischistosomal activity, and compounds shown to have antischistosomal activity disrupt planarian regeneration [16•], suggesting conservation of initial signaling (e.g., activation of Ca_v channels) with divergent downstream outputs.

Related Targets

TRP channels, a highly diverse set of cation channels that mediate transduction of sensory stimuli (light, sound, chemicals, temperature, and touch) and are well represented in both nematodes and schistosomes [86], are under intensive scrutiny as candidate targets for drugs to treat pain, cancer, and a host of other conditions [87]. Dysregulation of these channels might interfere with parasite signal transduction, and could also disrupt Ca^{2+} homeostasis in worms, as these channels are important in Ca^{2+} signaling cascades. Similarly, cyclic nucleotide gated channels, which are gated by intracellular cAMP or cGMP and are critical components of sensory transduction pathways, are present in these organisms and might serve as useful targets. A homolog of the recently-discovered mechanosensitive channel Piezo is present in *C. elegans* [88], and at least one gene encoding a Piezo-like protein is represented in the *S. mansoni* genome (Smp_136560). Intracellular ion channels such as ryanodine receptors and IP3 receptors regulate cytoplasmic Ca^{2+} levels, and might also serve as attractive targets.

Clearly, ion channels have proven to be extremely valuable as targets for anthelmintics, but other components of parasite neuronal signaling might also serve as fertile ground for new therapeutics. For example, neurotransmitter transporters, which translocate neurotransmitters across membranes, are critical for regulating neurotransmission, are validated drug targets in human medicine, and are critical to normal neuronal functioning in helminths [89]. GPCRs play essential roles in signal transduction, and are estimated to be molecular targets for more than 30 % of current drugs [90]. GPCRs also vary widely in their ligand specificity, providing the opportunity for parasite-selective activity. As noted above, the latrophilin GPCR appears to play an important role in the action of emodepside. Furthermore, neurotransmitters that activate ionotropic receptors often also act on separate classes of GPCRs (e.g., nAchRs vs. muscarinic AchRs). Downstream signaling cascades may also be targeted, as can enzymes involved in synthesis or degradation of neurotransmitters. As noted by others [14•], helminths and other invertebrates signal via neuropeptides and peptide receptors that are critical to neuromuscular function, but that are not found in vertebrates, and thus could serve as potential drug targets.

ABC Transporters

ATP binding cassette (ABC) multidrug transporters are members of the ABC protein superfamily, a large and ancient group of proteins. They are ATP-dependent efflux transporters with broad substrate specificity. P-glycoprotein (Pgp; ABCB1), multidrug resistance associated proteins (MRPs; ABCCs), and breast cancer resistance protein (BCRP; ABCG2), are implicated in multidrug resistance in mammalian cells. As mentioned above, ivermectin is a substrate for mammalian Pgp at the blood–brain barrier, and it is also a substrate for nematode Pgp. Although they are neither ion channels nor critical components of neuromuscular signaling, evidence is accruing [91–93] that by modulating effective intra-worm concentrations of compounds, parasite ABC transporters can regulate levels of anthelmintic efficacy. They also appear to play a role in the development or maintenance of anthelmintic resistance, and several studies indicate that inhibiting these transporters can overcome resistance. We and others have postulated that the roles helminth ABC transporters play in modulating drug susceptibility might be exploitable in a strategy that combines new or repurposed transporter inhibitors with current anthelmintics to enhance drug susceptibility. Notably, there are many safe, inexpensive drugs available that, in addition to targeting their primary receptors, interact with these drug transporters, and might be suitable for short-term combination therapy. Furthermore, it is likely that helminth ABC transporters play key roles in critical worm physiological functions such as excretion, reproduction, and perhaps modulation of host responses. As such, they may prove to be attractive therapeutic targets on their own.

Conclusions

There are at least two related motivations for defining targets of current anthelmintics and identifying candidate targets for new therapeutics. The most obvious is the inadequate number of currently available drugs in the face of an estimated one billion people or more infected with helminth parasites [1]. Add to these numbers the vast economic damage due to helminth infections of farm animals and agricultural crops, and the need for new anthelmintics becomes even more urgent. The second, related factor in this calculus is drug resistance. Drug resistance has emerged against essentially every class of anthelmintic used in veterinary medicine, often within as few as 3–4 years following introduction [94]. Although the problem is, as far as we know, not as dire in human medicine, and most anthelmintics appear to continue to show

effectiveness, there have been reports of insusceptibility in the field and resistance can be experimentally induced [82]. The increased implementation of mass drug administration programs may serve to accelerate the emergence of resistance, adding further urgency to the need for new therapeutics. Most helminth ion channels remain to be characterized. Doing so will provide important information about the physiology of these organisms and the evolution of ion channels and neuromuscular systems, and may ultimately provide new candidate targets for development of novel anthelmintics.

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Compliance with Ethics Guidelines

Conflict of Interest Dr. Greenberg reports grants from National Institutes of Health, and grants from Bill and Melinda Gates Foundation, during the study.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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