Invited review

Are Ca\(^{2+}\) channels targets of praziquantel action?

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Abstract

Praziquantel is the current drug of choice for the control of schistosomiasis. It is highly effective against all species of schistosomes and shows minimal adverse effects. Though introduced for the treatment of schistosomiasis more than 20 years ago, the mode of action of praziquantel remains to be elucidated. This review will focus on advances in defining the molecular target of praziquantel action, with particular emphasis on recent work indicating an important role for voltage-gated calcium channels.

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1. Introduction

Schistosomiasis is caused by trematode flatworms of the genus *Schistosoma*. The second most prevalent tropical disease, it affects approximately 200 million people worldwide, an estimated 85% of whom live in Africa (Engels et al., 2002; Chitsulo et al., 2004). As many as 280,000 deaths per year have been attributed to schistosomiasis (van der Werf et al., 2003). Potential vaccine strategies against schistosomiasis continue to be investigated (see reviews by Bergquist et al., 2002; Capron et al., 2002; Hagan and Sharaf, 2003), and public health measures can be important for reducing incidence of the disease. However, treatment of schistosomiasis remains dependent on chemotherapy intervention, particularly with praziquantel.

Praziquantel (Fig. 1) is a pyrazinoisoquinoline anthelminthic that was discovered in the 1970s, and subsequently introduced for the treatment of schistosomiasis (reviewed by Andrews et al., 1983). Commercial preparations contain equal mixtures of levo (−) and dextro (+) optical isomers. The (−) enantiomer is the active form, in vivo (Andrews et al., 1983; Liu et al., 1986; Tanaka et al., 1989; Wu et al., 1991; Xiao et al., 1999) and in vitro (Staudt et al., 1992; Xiao and Catto, 1989).

Over the past several years, praziquantel has become the drug of choice against schistosomiasis. Indeed, it has effectively become the only antischistosomal drug that is commercially available (Fenwick et al., 2003; Hagan et al., 2004). Praziquantel has activity against all schistosome species with minimal adverse effects, and it is also effective against other trematode and cestode infections (reviewed by Andrews, 1985). The value of praziquantel has been demonstrated repeatedly in large-scale schistosomiasis control efforts in a variety of countries. However, it has not seen widespread use in areas such as sub-Saharan Africa (reviewed by Fenwick et al., 2003; Hagan et al., 2004), though the Schistosomiasis Control Initiative (www.schisto.org), with major funding from the Bill and Melinda Gates Foundation, has been initiated in part to remedy this situation. In addition, schistosomes show stage- and sex-dependent differences in susceptibility to praziquantel (Xiao et al., 1985; Sabah et al., 1986; Pica-Mattoccia and Cioli, 2004).

With praziquantel becoming essentially the only antischistosomal treatment in use, prospects of emerging resistance to the drug have alarming implications, particularly since there is precedence for the development of resistance to other antischistosomals in schistosomes.
(reviewed by Cioli et al., 1993; Brindley, 1994; Geerts and Gryseels, 2001). Low cure rates for praziquantel in the field were reported from northern Senegal approximately 10 years ago (Gryseels et al., 1994; Stelma et al., 1995). However, it has also been suggested that the low cure rates in this region may result from particularly high worm burdens, high infection and reinfection rates, and the presence of large proportions of immature worms with reduced susceptibility to praziquantel during treatment (reviewed by Cioli, 2000; Doenhoff et al., 2002; Danso-Appiah and DeVlas, 2002). In the Nile delta region of Egypt, parasites with reduced susceptibility to praziquantel in vivo and in vitro have been isolated from villagers following initiation of widespread treatment programs (Ismail et al., 1996, 1999; William et al., 2001a). Interestingly, these isolates exhibit variations in the stability of their reduced susceptibility to praziquantel, and those that retain this phenotype show a reduction in reproductive fitness (William et al., 2001b). Strains of schistosomes have revealed several sequences with no clear homology to genes from other phyla (reviewed by Hu et al., 2004; LoVerde et al., 2004; McManus et al., 2004; Verjovski-Almeida et al., 2004). On the other hand, the target might instead be encoded by a gene with representatives in other phyla, but with particular structural signatures that interact with praziquantel. It is well established that changes in a few, or even single, amino acid residues in critical regions can produce dramatic alterations in the pharmacological sensitivities and functional properties of receptors and channels (see, e.g. Heinemann et al., 1992; Satin et al., 1992; Vais et al., 2000; Striessnig et al., 1998).

Praziquantel disrupts Ca$^{2+}$ homeostasis in adult schistosomes, via an unknown mechanism (reviewed by Andrews, 1985; Day et al., 1992; Redman et al., 1996). Despite several years of experiments aimed at defining praziquantel’s mode of action, the precise identity and location of the molecular targets of the drug have remained elusive (reviewed by Day et al., 1992; Redman et al., 1996; Cioli and Pica-Mattoccia, 2003). Within seconds of exposure to praziquantel, two major effects become apparent in adult worms: a rapid, sustained muscular contraction (Fetterer et al., 1980; and tegumental disruption (Becker et al., 1980; Mehlhorn et al., 1981), which subsequently leads to exposure of parasite antigens on the worm surface (Harnett and Kusel, 1986). Both responses are thought to be linked to praziquantel-dependent disruption of Ca$^{2+}$ homeostasis (reviewed by Day et al., 1992; Redman et al., 1996).

Praziquantel stimulates a rapid 45Ca$^{2+}$ uptake in male schistosomes (as well as a much slower influx of Na$^+$; Pax et al., 1978). Both contraction of the worm’s musculature and disruption of the tegument are Ca$^{2+}$-dependent processes; removal of Ca$^{2+}$ from the medium blocks both responses (Pax et al., 1978; Wolde Mussie et al., 1982; Xiao et al., 1984). Neither of these effects is immediate, however. For example, inhibition of the muscle contraction in Ca$^{2+}$-free medium requires at least 10 min to become apparent, a delay likely dependent on the depletion of sequestered intracellular Ca$^{2+}$ stores.

Experiments with detegumented worms have indicated that both the tegument and the sarcolemma indicate that emergence of praziquantel resistance could eventually become a serious problem, particularly considering that the mechanism of action of this drug remains unclear.

2. Praziquantel mode of action

Praziquantel has activity against a wide variety of platyhelminths, but it is generally ineffective against other organisms such as nematodes (Andrews et al., 1983). Based on this selectivity, the molecular target of the drug might be expected to be limited to this phylum. One possibility is that the target (or targets) is a unique gene product found only in the flatworms. Recent genome and transcriptome analyses of schistosomes have revealed several sequences with no clear homology to genes from other phyla (reviewed by Hu et al., 2004; LoVerde et al., 2004; McManus et al., 2004; Verjovski-Almeida et al., 2004). On the other hand, the target might instead be encoded by a gene with representatives in other phyla, but with particular structural signatures that interact with praziquantel. It is well established that changes in a few, or even single, amino acid residues in critical regions can produce dramatic alterations in the pharmacological sensitivities and functional properties of receptors and channels (see, e.g. Heinemann et al., 1992; Satin et al., 1992; Vais et al., 2000; Striessnig et al., 1998).

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Experiments with detegumented worms have indicated that both the tegument and the sarcolemma
contain praziquantel-sensitive sites (Blair et al., 1992). Thus, detegumented worms continue to show a response to praziquantel. However, instead of the praziquantel-dependent biphasic parasite contraction found when intact worms are bathed in high magnesium, detegumented worms show only a single, more prominent phasic contraction, indicating that a tegumental site is required for the full response. Furthermore, in the absence of Ca\(^{2+}\) in the medium, praziquantel produces a phasic contraction in intact parasites (see above), but does not stimulate contraction in detegumented worms.

Redman et al. (1996) have described several cellular factors which can regulate intracellular levels of Ca\(^{2+}\) and which might serve as targets mediating praziquantel’s effects on Ca\(^{2+}\) homeostasis. These include voltage-, ligand-, and second messenger-gated Ca\(^{2+}\) channels, intracellular Ca\(^{2+}\) release channels, and intracellular Ca\(^{2+}\) buffers. They also include pumps and transporters that regulate intracellular Ca\(^{2+}\) concentrations. Praziquantel-induced modification of membrane phospholipids and membrane fluidity have also been described (Harder et al., 1988; Lima et al., 1994), and these changes may produce alterations in membrane permeability to ions or result in indirect effects on membrane receptors and channels. Until recently, however, there has been little direct evidence connecting any of these potential targets to praziquantel action. On the other hand, Cunha and Noel (1997) have reported that concentrations of praziquantel as high as 100 μM have no effect on schistosome (Na\(^{+}\) + K\(^{+}\))-ATPase or (Ca\(^{2+}\) – Mg\(^{2+}\))-ATPase activities. Other experiments have shown that praziquantel is not acting as an ionophore (Pax et al., 1978).

This review will focus on voltage-gated Ca\(^{2+}\) channels as targets of praziquantel. These channels are critical sites for entry of extracellular Ca\(^{2+}\), and thus play an important role in the regulation of intracellular Ca\(^{2+}\) levels. Until recently, it has not been feasible to test directly whether voltage-gated Ca\(^{2+}\) channels are indeed targets of praziquantel, though there have been indications that these channels may be playing a role. Based on pharmacological characterization of praziquantel-induced contraction in intact and detegumented worms, Blair et al. (1992) suggested that Ca\(^{2+}\) channels might serve as targets for the drug. Additionally, high concentrations (50 μM) of praziquantel prolong the Ca\(^{2+}\)-dependent plateau phase of the cardiac action potential in rats (Chubb et al., 1978), a phase of the action potential carried by voltage-gated Ca\(^{2+}\) channels. On the other hand, Fetterer et al. (1980) found that methoxyverapamil (D-600), an inhibitor of one class (L-type) of mammalian Ca\(^{2+}\) channels, does not block the praziquantel-dependent Ca\(^{2+}\) influx in schistosomes, but does block the tonic contraction resulting from increased K\(^{+}\) concentrations. However, more recent evidence points to a critical role for Ca\(^{2+}\) channel proteins in praziquantel action.

3. Schistosome and flatworm Ca\(^{2+}\) currents

Aside from their possible role in praziquantel action, an important incentive for studying Ca\(^{2+}\) channels in flatworms is to better understand the physiology of excitable cells in these organisms and to obtain clues about the evolution of ion channels. Furthermore, elucidation of the structure and function of these channels might provide targets for new antischistosomal agents.

Although evidence for Ca\(^{2+}\)-dependence of contractility has been found in isolated muscle cells from Schistosoma mansoni (Day et al., 1994), native Ca\(^{2+}\) currents from schistosomes have not been published. Indeed, most likely for technical reasons, voltage-clamp experiments published to date have revealed no inward currents in muscle cells isolated from adult S. mansoni (Day et al., 1993).

Ca\(^{2+}\) currents have been recorded in cells from other flatworms, however. A normal complement of ionic currents, including cadmium-sensitive Ca\(^{2+}\) currents, are implicated in the generation of action potentials produced in neurons from the polyclad flatworm, Notoplana acticola (Keenan and Koopowitz, 1984). In Bdelloura candida, a triclad ectoparasitic flatworm that resides on the legs and gills of horseshoe crabs (Limulus polyphemus), voltage-gated Ca\(^{2+}\) currents from nerve and muscle cells have been characterized using voltage-clamp (Blair and Anderson, 1993, 1994). Both cell types had Ca\(^{2+}\) currents that activated at −30 mV, reached peak amplitude in approximately 5 ms, and slowly inactivated. The neuronal Ca\(^{2+}\) current was relatively insensitive to organic Ca\(^{2+}\) channel blockers such as nifedipine, verapamil, and ω-conotoxin GVIA, while the muscle Ca\(^{2+}\) current was too unstable for pharmacological characterization. Interestingly, the Ca\(^{2+}\) current recorded from neurons does not appear to be affected by 10 μM PZQ (Blair and Anderson, 1996). More recently, Ca\(^{2+}\) currents have been recorded from muscle cells of the triclad turbellarian Dugesia tigrina (Cobbett and Day, 2003). Again, however, the currents were not stable enough for thorough pharmacological characterization.

Considering the difficulties involved in characterizing native Ca\(^{2+}\) channels in flatworms such as schistosomes, a molecular approach offers an alternative for studying the physiological and pharmacological properties of these channels in heterologous systems. Eventually, however, characterization of native currents will be necessary to understand the properties of these channels within schistosome cells and the physiological roles they play within the organism.

4. Structure and function of voltage-gated Ca\(^{2+}\) channels

Voltage-gated ion channels underlie electrical excitability in cells. They open in response to changes in
membrane potential, selectively allowing ions to flow down the electrochemical gradient across the membrane. Voltage-gated Ca^{2+} channels are important members of this ion channel superfamily. In addition to their role in contributing to impulse propagation, Ca^{2+} channels are important regulators of Ca^{2+} homeostasis. They provide the pathway for the Ca^{2+} influxes that underlie excitation-contraction coupling, excitation-secretion coupling, and other Ca^{2+}-dependent processes in muscles, nerves, and other excitable cells (reviewed by Catterall, 2000; Hofmann et al., 1999). As such, Ca^{2+} channels are critical to the normal function of the animal. Indeed, venoms from organisms as diverse as cone snails and spiders contain toxins that target specific types of Ca^{2+} channels.

Voltage-gated Ca^{2+} channels consist of a pore-forming \(\alpha_1\) subunit and associated subunits that modulate channel activity. The \(\alpha_1\) subunit of Ca^{2+} channels contains four domains, each consisting of six transmembrane regions (S1–S6) that are linked into a single molecule (see Fig. 2). The predicted structure of these \(\alpha_1\) subunits conforms to that of the ion channel superfamily (Doyle et al., 1998).

The basic building block of ion channels is a tetrameric structure comprised of the fifth and sixth transmembrane regions (S5 and S6, respectively) and the P loop, the region between S5 and S6 that forms the selectivity filter of the pore. It is here that the residues that confer a channel’s ionic selectivity and much of its pharmacology reside. The fourth transmembrane segment in each domain (S4) contains a regular pattern of positively charged amino acid residues and is thought to serve as the voltage sensor of the channel.

The two major classes of Ca^{2+} currents that have been identified in both vertebrate and invertebrate cells are Low Voltage Activated (LVA; t-type) and High Voltage-Activated (HVA). HVA currents are further divided into L-type, which, in vertebrates, are sensitive to dihydropyridine Ca^{2+} channel blockers such as nifedipine, and a variety of dihydropyridine-insensitive currents, collectively known as non-L-type. Based on data from molecular cloning combined with heterologous expression, these currents are gated by different classes of Ca^{2+} channel \(\alpha_1\) subunits. LVA channels are comprised of Ca_v3 \(\alpha_1\) subunits (Perez-Reyes, 2003). HVA L-type channels are gated by

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**Fig. 2. Simplified structure of voltage-gated Ca^{2+} channels.** Subunits include the pore-forming \(\alpha_1\) subunit, which consists of four homologous domains surrounding a central pore. Each domain is comprised of six transmembrane regions (S1–S6). The P loop which dips into the membrane between S5 and S6 forms the selectivity filter of the channel. The S4 transmembrane region contains a series of regularly spaced, positively charged residues (+) and is thought to form the voltage sensor of the channel. Also shown are auxiliary \(\beta, \alpha_2\delta,\) and \(\gamma\) subunits, which modulate \(\alpha_1\) function. Domains of the \(\beta\) subunit defined by recent homology modeling and high-resolution structural analysis (see text) are shown. The \(\beta\) subunit interacts via its guanylate kinase (GK) domain with the Alpha Interaction Domain (AID) on the I–II loop of the \(\alpha_1\) subunit. Size of subunits and domains are not to scale. Adapted from elements of Randall and Benham (1999) and Yue (2004).
Ca,1 α1 subunits, while Ca,2 α1 subunits gate non-L-type HVA currents. The pharmacological distinction between L-type and non-L-type channels does not appear to be clearly maintained in the invertebrates however (reviewed by Jeziorski et al., 2000b). Cloned invertebrate α1 subunits that are clear members of the L-type family based on structure are relatively insensitive to L-type modulators such as dihydropyridines when expressed in a heterologous system.

Auxiliary subunits associated with the α1 subunit in HVA channels include the α2δ, β, and γ subunits (Fig. 2). These proteins are important modulators of the properties of the α1 subunit (reviewed by Hofmann et al., 1999; Catterall, 2000; Arikkath and Campbell, 2003). The intracellular β subunits (Ca,βs) are critical components of Ca2+ channel complexes. When co-expressed with α1 subunits, β subunits increase current densities and ligand binding. They participate in membrane trafficking of the α1 subunit, at least in part by masking an endoplasmic reticulum retention site on the α1 subunit (Bichet et al., 2000). Ca,βs also have a major influence on several biophysical properties of the channel, including the voltage-dependence of channel activation and steady-state inactivation, rates of inactivation (reviewed by Walker and DeWard, 1998; Birnbaumer et al., 1998; Hanlon and Wallace, 2002; Dolphin, 2003), and the rate of recovery from inactivation (Jeziorski et al., 2000b).

Several lines of evidence indicate that the primary binding site for β subunits on the α1 subunit is the Alpha Interaction Domain (AID), a specific region in the intracellular loop between Domains I and II of the α1 subunit (Pragnell et al., 1994). Until recently, the primary site on the β subunit for interaction with the α1 subunit was thought to be the Beta Interaction Domain (BID; DeWard et al., 1994). However, new insights into the structure of Ca2+ channel β subunits (Ca,βs) have altered this view.

Homology modeling has indicated that Ca,βs are members of the membrane-associated guanylyl kinase (MAGUK) family of proteins (Hanlon et al., 1999). MAGUKs are often concentrated at synapses and play important roles in clustering ion channels and neurotransmitter receptors (reviewed by Dimitratos et al., 1999). MAGUKs are comprised of one or more PDZ domains located N-terminal to a Src-homology 3 (SH3) domain, a bridging region (the HOOK domain), and a guanylate kinase (GK)-like domain. Recent resolution of the crystal structure of the conserved core of the β subunit, both alone and in complex with the AID of the α1 subunit, has shown that Ca,βs are indeed members of the MAGUK family, though with quite distinct properties (Chen et al., 2004; Opatowski et al., 2004; Petegem et al., 2004; commentary by Yue, 2004). For example, Ca,βs appear to lack the PDZ domains of MAGUKs. Furthermore, the orientation of the SH3 and GK domains has been modified, and the GK domain in Ca,βs lacks a nucleotide binding site.

The AID of the α1 subunit binds to a hydrophobic binding pocket within the modified GK domain of the β subunit. Surprisingly, the BID region is buried within the β subunit protein, and is therefore unlikely to be involved directly in protein–protein interactions such as binding to the AID. However, as discussed by Chen et al. (2004), the BID plays an essential structural role in β subunits, spanning the SH3 and GK domains and their connecting HOOK region, and containing two β-strands that are integral parts of the SH3 and GK domains.

5. Schistosome Ca2+ channel subunits

Recently, the structure and function of schistosome Ca2+ channel subunits have begun to be elucidated (Kohn et al., 2001a,b). Three HVA Ca2+ channel γ1 subunit subtypes have been described in schistosome adults. Based on their structure, two of them fall into the non-L-type class, and one into the L-type class of γ1 subunits (Kohn et al., 2001b). The presence of two types of non-L-type α1 subunits in schistosomes differs from other invertebrates that have been examined, all of which contain only one representative each of L-type and non-L-type α1 subunits (Littleton and Ganetzky, 2000; Jeziorski et al., 2000b).

Schistosomes also contain at least two subtypes of Ca,βs (Kohn et al., 2001a, 2003b). This finding in itself is unusual, in that only one Ca,β subtype has been identified in the genomes of other invertebrate species. Even more remarkable, however, are the unique structural and functional properties identified in one of these schistosome subtypes. To date, representatives of this variant subtype have not been identified in any other phyla (see Fig. 3).

The variant Ca,βs, though clearly part of the β subunit family, have very distinct structural features. For example, they are larger than other Ca,βs. However, the most unique structural signature of these Ca,βs is found in the BID. In particular, two highly conserved serine residues that constitute consensus protein kinase C (PKC) phosphorylation sites in the BID are not found in these schistosome β subunits.

The variant schistosome Ca,βs also exhibit novel functional properties (Kohn et al., 2001a). In contrast to other β subunits, which enhance currents through α1 subunits, coexpression of these variant β subunits in Xenopus oocytes with either a jellyfish (CyCa,1) or human (Ca,2.3) α1 subunit results in a dramatic decrease in current levels. In other respects, however, the variant schistosome Ca,β modulate α1 subunits in a typical manner. For example, they shift the current/voltage relationship of α1 subunits in a hyperpolarizing direction, as do other β subunits. Thus, these proteins appear to be bona fide, functional Ca,βs.

One property of these variant schistosome Ca,βs that makes them particularly interesting is that they confer praziquantel sensitivity to an otherwise...
praziquantel-insensitive mammalian α1 subunit (CaV2.3). Thus, in Xenopus oocytes coexpressing CaV2.3 with one of the variant schistosome CaVb subunits, peak Ca2+ currents are increased in the presence of 100 nM praziquantel (Kohn et al., 2001a). The praziquantel-dependent increase in Ca2+ influx through channels containing these variant b subunits is consistent with the clinical effects of the drug. Other Cavb subunits, including the conventional schistosome b subunit, do not confer praziquantel sensitivity to the mammalian α1 subunit (Kohn et al., 2003b). These results implicate these schistosome variant CaVb subunits in the mode of action of praziquantel.

Both the current reduction and the ability to confer praziquantel sensitivity have been localized by mutagenesis to specific amino acid residues in the β subunit, namely the two consensus PKC sites in the BID region (Kohn et al., 2003b). These results implicate these schistosome variant CaVb subunits in the mode of action of praziquantel.

Based on these results, we have hypothesized that the unusual modulatory properties and pharmacological sensitivities of the variant schistosome CaVb subunits result from the absence of the conserved consensus PKC phosphorylation sites found in the BIDs of other β subunits. Phosphorylation of voltage-gated Ca2+ channel β subunits by PKC and other protein kinases plays a critical role in regulating channel properties (reviewed by Rossie, 1999; Kamp and Hell, 2000; Keef et al., 2001). A recombinant mammalian β2a subunit can be phosphorylated in vitro by PKC, with the stoichiometry reported to be 1–2 moles of phosphate per mole of β2a subunit (Puri et al., 1997). However, of the several consensus PKC sites found in β subunits, the specific sites that are phosphorylated by PKC, either in vitro or in vivo, have not yet been defined.

Fig. 3. Phylogenetic tree of Ca2+ channel β subunits. Amino acid sequences were aligned using Clustal X (Thompson et al., 1997), and a tree constructed using the neighbor-joining method, as implemented in MEGA 2.1 (Kumar et al., 2001). The schistosome conventional and variant β subunits are boxed. Sequences and NCBI accession numbers are: Human β3, NP_000716; Rabbit β3, CAA45578; Xenopus laevis (toad) β3, AAA75519; Human β4, NP_000717; Rat β4, A45982; Human β1, NP_954856; Rabbit β1, AAA31180; Human β2, NP_000715; Rabbit β2, CAA45576; Caenorhabditis elegans (nematode) β, AAB53056; Schistosoma mansoni conventional β, AY033599; Lymnaea stagnalis (snail) β, AAO83844; Loligo bleekeri (squid) β, BAB88219; Musca domestica (housefly) β, A54844; Drosophila melanogaster (fruitfly) β, AAF21096; Cyanea capillata (jellyfish) β, AAB87751; Schistosoma japonicum variant β, AA51116; Schistosoma mansoni variant β, AAK51117; C. elegans β-like sequence (w10c8.1), AAK21500.
If the variant Ca$_{\beta}$s found in schistosomes are indeed involved in praziquantel action, and if creating a PKC site in the BID can eliminate that susceptibility, then one mechanism for acquiring praziquantel resistance might involve mutating one or both crucial sites in the BID to serine. Strains reported to have reduced praziquantel sensitivity have been tested for the presence of these (or other) mutations (Valle et al., 2003). Neither changes in primary structure of the schistosome $\beta$ subunits, nor changes in expression levels were found, indicating that these strains have not exploited altered Ca$_{\alpha}$,$\beta$s as a means of acquiring praziquantel resistance. However, evidence of differential stability of the Egyptian isolates with reduced praziquantel susceptibility indicates that there may be different pathways for acquiring praziquantel susceptibility (William et al., 2001b). It is possible that strains of parasites that acquire resistance to praziquantel can do so by multiple mechanisms.

6. Conclusions and future prospects

Several questions regarding schistosome Ca$^{2+}$ channels remain unanswered. The functional properties of the schistosome $\alpha_1$ subunits have not yet been described, either alone or in combination with the two schistosome Ca$_{\alpha}$,$\beta$ subtypes. Do only certain $\alpha_1/\beta$ combinations form functional channels? Do only certain $\alpha_1/\beta$ combinations form praziquantel-sensitive channels? Which cells express these combinations? Does praziquantel interact directly with the $\beta$ subunit, interfere with $\alpha_1/\beta$ interaction, or act somehow indirectly on Ca$^{2+}$ channels containing these variant Ca$_{\alpha}$,$\beta$s? Moreover, what precisely is the biological function of the variant schistosome Ca$_{\alpha}$,$\beta$s, which, unlike other $\beta$ subunits, reduce Ca$^{2+}$ currents instead of increasing them? Are there special characteristics of the schistosome $\alpha_1$ subunits that require this type of modulation? Of course, any results garnered from expression of sequences in heterologous systems must ultimately be compared with Ca$^{2+}$ currents found in cells of the worm itself. In the current era of genomic and post-genomic analysis, some of these questions may be able to be addressed very precisely.

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