Published in final edited form as:

Int J Parasitol. 2006 May 31; 36(6): 625–632. doi:10.1016/j.ijpara.2006.02.002.

Voltage-gated calcium channel subunits from platyhelminths: Potential role in praziquantel action☆

Michael C. Jeziorski^a and Robert M. Greenberg^{b,*}

^aInstituto de Neurobiología, Universidad Nacional Autónoma de México, Campus UNAM-UAQ Juriquilla, Querétaro, Qro. 76001, México

^bMarine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA

Abstract

Voltage-gated calcium (Ca^{2+}) channels provide the pathway for Ca^{2+} influxes that underlie Ca^{2+} -dependent responses in muscles, nerves and other excitable cells. They are also targets of a wide variety of drugs and toxins. Ca^{2+} channels are multisubunit protein complexes consisting of a pore-forming α_1 subunit and other modulatory subunits, including the β subunit. Here, we review the structure and function of schistosome Ca^{2+} channel subunits, with particular emphasis on variant Ca^{2+} channel subunits (Ca_v var) found in these parasites. In particular, we examine the role these subunits may play in the action of praziquantel, the current drug of choice against schistosomiasis. We also present evidence that Ca_v var homologs are found in other praziquantel-sensitive platyhelminths such as the pork tapeworm, *Taenia solium*, and that these variant subunits may thus represent a platyhelminth-specific gene family.

Keywords

Calcium channels; Praziquantel; *Schistosoma mansoni*; *Taenia solium*; Platyhelminthes

1. Introduction

Diseases caused by platyhelminthic parasites represent an international health crisis. Schistosomiasis, caused by trematode flatworms of the genus *Schistosoma*, affects approximately 200 million people, killing as many as 280,000 people per year (van der Werf et al., 2003), with a large percentage of infected individuals residing in Africa (Engels et al., 2002; Chitsulo et al., 2004). Cestodes of the genus *Taenia* and other platyhelminths infect millions more. The drug of choice against platyhelminth parasites is praziquantel, a compound that is generally ineffective against parasites from other phyla. The platyhelminth-specific efficacy of praziquantel remains unexplained, as its mode of action at the cellular and molecular level is not yet fully understood. Recent evidence suggests that voltage-gated Ca^{2+} channels (VGCCs) in schistosomes possess a subunit with an unusual structure that may underlie their sensitivity to praziquantel. Here, we will focus on the structure and function of VGCCs in platyhelminths and present new data demonstrating that the variant VGCC form found in schistosomes is shared by other classes of platyhelminths.

☆Note: GenBank accession numbers for sequences reported here are AY624029 for TsCa_v and AY624030 for TsCa_v var.

© 2006 Australian Society for Parasitology Inc Published by Elsevier Ltd. All rights reserved.

*Corresponding author. Tel.: +1 508 289 7910; fax: +1 508 540 6902. rgreenberg@mbi.edu (R.M. Greenberg).

2. Voltage-gated Ca^{2+} channels

VGCCs belong to the superfamily of voltage-gated ion channels that respond to changes in membrane potential by selectively allowing ions to flow down their electrochemical gradient. VGCCs play a unique role within this family by coupling changes in membrane potential to the influx of Ca^{2+} necessary to elicit intracellular signaling (reviewed by Catterall, 2000; Hofmann et al., 1999). In addition to propagating impulses, VGCCs gate the Ca^{2+} influx that underlies excitation–contraction coupling, excitation–secretion coupling and other Ca^{2+} -dependent processes. The importance of VGCCs to cellular function is underscored by their role as targets for both therapeutic drugs and components of venoms used by a wide variety of predators.

VGCCs comprise a pore-forming α_1 subunit and smaller auxiliary subunits known as α_2 , α_3 and α_4 (Fig. 1). Although the α_1 subunit largely determines both the pharmacology of the channel and the nature of the current gated by it, the auxiliary subunits play important roles in shaping the response of the channel. The α_1 subunit contains four homologous domains, connected by intracellular loops, that form the sides of the membrane pore (Doyle et al., 1998). Each domain includes six transmembrane segments (labeled S1–S6), with the S4 segment containing positively charged residues that act as a voltage sensor and the extracellular P loop between S5 and S6 determining the ion selectivity of the channel.

The currents gated by VGCCs are divided into high voltage-activated (HVA) and low voltage-activated (LVA) currents, defined by the membrane potential at which the channels open. HVA currents have been further divided on the basis of pharmacological response. L-type HVA currents are sensitive to classical organic Ca^{2+} channel blockers such as nifedipine, verapamil and diltiazem. Non-L-type HVA currents include N-type, P/Q-type and R-type currents, each insensitive to the L-type channel blockers but distinguished by selective sensitivity to particular toxins.

Physiological and pharmacological distinctions between VGCCs are mirrored in the molecular variation among α_1 subunits. Phylogenetic analysis divides α_1 subunits into three clusters known as Ca_v1 , Ca_v2 and Ca_v3 , which correspond to L-type, non-L-type and LVA channels, respectively (Catterall et al., 2005). Although mammals and other vertebrates possess three or four subtypes of each channel type differing in tissue and subcellular distribution, only single genes for each type are found in the invertebrate species that have been studied. For example, thorough analysis of the fully sequenced genomes of *Caenorhabditis elegans* and *Drosophila melanogaster* has revealed one homolog each of Ca_v1 , Ca_v2 and Ca_v3 α_1 subunits, each of which presumably fulfills the roles taken by multiple gene products in mammalian tissues.

A further source of variation in VGCC currents is the influence of auxiliary subunits, of which the α_2 subunit has been most extensively studied (reviewed by Walker and DeWaard, 1998; Birnbaumer et al., 1998; Hanlon and Wallace, 2002; Dolphin, 2003; Richards et al., 2004). The α_2 subunit is a cytoplasmic protein that has four subtypes in vertebrates but only single homologs in most invertebrate species. The α_2 subunit increases current density and ligand binding to the α_1 subunit, in part by masking an endoplasmic reticulum retention signal on the α_1 subunit, enhancing its trafficking to the plasma membrane. α_2 subunits also modulate various kinetic properties of the channel, such as the voltage-dependence of activation and inactivation, the rate of inactivation and recovery from inactivation.

The primary site on the α_1 subunit for interaction with the α_2 subunit is a region of the intracellular loop between domains I and II labeled the alpha interaction domain (AID). Early studies described a similarly conserved site in the α_2 subunit, the 30-residue interaction domain (BID; DeWaard et al., 1994). Subsequent work has resolved the crystal

structure of the conserved core of the α_1 subunit, both alone and complexed with the AID of the α_1 subunit (Chen et al., 2004; Opatowski et al., 2004; Petegem et al., 2004; commentary by Yue, 2004). These studies have verified the prediction (Hanlon et al., 1999) that Ca_v s are members of the membrane-associated guanylate kinase (MAGUK) family of proteins. MAGUKs are often concentrated at synapses and are thought to play important roles in clustering ion channels and neurotransmitter receptors (reviewed by Dimitratos et al., 1999). MAGUK proteins typically comprise one or more PDZ domains (Ponting et al., 1997) located N-terminally to a Src-homology 3 (SH3) domain, a bridging region (the HOOK domain) and a guanylate kinase (GK)-like domain. Ca_v s differ from other members of the MAGUK family in important respects, lacking the N-terminal PDZ domains and a nucleotide binding site within the GK domain.

Although the newly revealed crystal structure of α_1 subunits indicates that the AID of the α_1 subunit binds not to the BID but within the modified GK domain, the BID plays an essential structural role in α_1 subunits (Chen et al., 2004). It spans the SH3 and GK domains and the connecting HOOK region, contains two β -strands that are integral parts of the SH3 and GK domains and may be necessary for proper folding of the α_1 subunit.

Most of the heterologous characterisation of α_1 and α_2 subunits has been conducted with genes cloned from mammalian species. Mammalian subunits were the first cloned and many invertebrate homologs that were subsequently identified were not as amenable to expression in heterologous expression systems. Until molecular biological approaches began to be applied in recent years to studying helminth Ca^{2+} currents, their direct characterisation depended upon electro-physiological recordings, which are technically difficult in flatworms. For example, although Ca^{2+} influx has been implicated in contractility, no native Ca^{2+} currents from schistosome cells have yet been published, though a preliminary report has indicated that these currents can be found in muscle fibers (Cobbett and Day, 2002), along with previously described K^{+} currents (Day et al., 1993). Some information on native Ca^{2+} currents has been gained from experiments in other platyhelminths, but pharmacological characterisation has proved challenging, which is one reason why the molecular target of praziquantel (see below) remains undefined (for review see Greenberg, 2005).

3. Voltage-gated Ca^{2+} channel subunits in schistosomes

Molecular cloning approaches have now begun to clarify the structure and functional properties of schistosome VGCC subunits (Kohn et al., 2001a,b; reviewed by Greenberg, 2005). Three full-length HVA α_1 subunits have been cloned from adult *Schistosoma mansoni*. Although, none has yet been heterologously expressed, two can be classified as non-L-type α_1 subunits and one as L-type based on sequence similarity to mammalian proteins (Kohn et al., 2001b). The presence of two non-L-type α_1 subunits in schistosomes is unusual. In other invertebrates that have been examined, including several complete invertebrate genomes, only single L-type and non-L-type α_1 subunits are found (Littleton and Ganetzky, 2000; Jeziorski et al., 2000).

In addition to the α_1 subunits, two subtypes of Ca_v bs have been cloned from schistosomes (Kohn et al., 2001a, 2003a). Again, this finding in itself is noteworthy in that only one Ca_v subtype has been identified in the genomes of other invertebrates. One of the schistosome Ca_v s resembles other invertebrate Ca_v s in its size and pattern of conserved regions. The other, a variant subtype which we have named Ca_v var, displays clear similarity to the family of Ca_v subunits, but differs in several fundamental ways. The predicted protein is significantly larger than conventional Ca_v s and similarity at the amino acid level in conserved regions is markedly reduced, suggesting a separate gene family. Although

Ca_v var contains a domain similar to the BID of conventional α_1 subunits, it lacks two highly conserved serine residues (see Table 1). These conserved serines each constitute consensus protein kinase C (PKC) phosphorylation sites. The Ca_v var subunit was simultaneously cloned from two species of schistosomes, *S. mansoni* (SmCa_v var, formerly called SmCa_v A) and *Schistosoma japonicum* (SjCa_v var, formerly SjCa_v) but no homologs of the variant subtype could be identified by similarity searches in any vertebrate or other invertebrate genome.

Studies using heterologous expression revealed that schistosome Ca_v var possesses functional properties that are not shared with other Ca_v s (Kohn et al., 2001a). The Ca_v var subunit associates with α_1 subunits when expressed in *Xenopus* oocytes, as it causes the current/voltage relationship of α_1 subunits to shift in a hyperpolarising direction much as conventional α_1 subunits do. However, all conventional α_1 subunits studied to date also increase the amplitude of the current gated by α_1 subunits. In contrast, co-expression of schistosome Ca_v var subunits with either a jellyfish (CyCa_v1) or human (Ca_v2.3) α_1 subunit markedly reduces current through the channel.

Another intriguing aspect of the schistosome Ca_v var subunit is its ability to confer praziquantel sensitivity to α_1 subunits. Praziquantel is the drug of choice to treat schistosomiasis, due to its efficacy against all schistosome species and relatively benign side effect profile (Andrews et al., 1983; Fenwick et al., 2003; Hagan et al., 2004). Although praziquantel acts against a wide variety of platyhelminths, it is generally ineffective against other organisms such as nematodes (Andrews et al., 1983). The molecular target of praziquantel has not been defined but it is known to induce rapid influx of Ca²⁺, leading to a rapid, sustained Ca²⁺-dependent muscular contraction (for reviews see Andrews, 1985; Day et al., 1992; Redman et al., 1996; Cioli and Pica-Mattoccia, 2003; Greenberg, 2005).

Co-expression of the schistosome Ca_v var subunit with a praziquantel-insensitive mammalian α_1 subunit (Ca_v2.3) yields a current that is responsive to praziquantel. Peak Ca²⁺ currents are increased in the presence of 100 nM praziquantel in *Xenopus* oocytes coexpressing the two subunits but not with either subunit alone (Kohn et al., 2001a). The praziquantel-dependent increase in Ca²⁺ current amplitude is consistent with the reported ability of the drug to induce Ca²⁺ influx. Other Ca_v s, including the conventional schistosome α_1 subunit, do not confer praziquantel sensitivity to the mammalian α_1 subunit (Kohn et al., 2003b).

Site-directed mutagenesis has revealed that the absence of the two consensus PKC sites in the BID region of Ca_v var subunits is responsible in part for both the current reduction and the ability to confer praziquantel sensitivity (Kohn et al., 2003a,b). Restoring the consensus PKC sites in Ca_v var by substituting serine residues at either or both of these sites restores the ability of the subunit to enhance currents through α_1 subunits like other Ca_v s, while sensitivity to praziquantel is eliminated. When one such mutant containing a single serine is further altered to eliminate a basic residue and disrupt the consensus PKC site, the phenotype reverts to that of the wildtype schistosome Ca_v var. Thus, the novel effects of the variant subunit result from a lack of PKC sites in the BID rather than the absence of serines. The importance of PKC sites within the BID of Ca_v var is consistent with the finding that phosphorylation of VGCC subunits is a critical regulator of channel properties (reviewed by Rossie, 1999; Kamp and Hell, 2000; Keef et al., 2001). Although recombinant mammalian subunits can be phosphorylated by PKC in vitro (Puri et al., 1997), the specific sites of phosphorylation have not yet been determined.

In recent years, several reports of schistosome isolates with reduced susceptibility to praziquantel have appeared (reviewed by Cioli et al., 1993; Day and Botros, 2006; Brindley,

1994; Geerts and Gryseels, 2001). The finding that a single-base mutation at a critical site can eliminate the ability of Ca_v var to confer praziquantel sensitivity might suggest such a mechanism for acquiring praziquantel resistance. *Schistosoma mansoni* strains reported to have reduced praziquantel sensitivity show no changes in primary structure or expression levels of schistosome subunits (Valle et al., 2003). However, Egyptian isolates with reduced praziquantel susceptibility show differential stability and reproductive fitness, which may indicate that there are several pathways for acquiring reduced sensitivity to praziquantel (William et al., 2001) and schistosomes may make use of more than one mechanism.

If the variant subunit found in schistosomes is in fact an essential target of praziquantel, the Ca_v var gene should be found in other organisms that exhibit sensitivity to the drug. An important target of praziquantel is *Taenia solium*, the pork tapeworm, which represents a significant health problem in developing countries (Carpio, 2002; Flisser et al., 2003; Garcia et al., 2003). Praziquantel is effective in treating both the intermediate porcine host and humans infected with either cysticerci or adult *T. solium* (Andrews et al., 1983; Cioli and Pica-Mattoccia, 2003; Garcia et al., 2003). As in schistosomes, the precise manner in which praziquantel acts upon *Taenia* has not been extensively studied.

4. Variant $\text{Ca}_v\beta$ s in *T. solium* and other platyhelminths

We used reverse transcription of RNA plus PCR (RT-PCR) to search for subunit homologs in *T. solium* cysticerci obtained from muscle tissue of an infected pig (Veterinary School, Universidad Nacional Autónoma de México). Using degenerate primers designed against conserved regions of subunit genes, we amplified fragments of two distinct subunit cDNAs from *T. solium* and the full cDNAs were then isolated via standard cloning techniques and sequenced.

One cDNA, named Ts Ca_v , is 2249 bp long and encodes a protein of 640 amino acids with a predicted molecular weight of 72.5 kDa that resembles the conventional subunits found in *S. mansoni* and in many other invertebrate and vertebrate organisms (Fig. 2A). The second cDNA, Ts Ca_v var, more closely resembles Sm Ca_v var and Sj Ca_v var, the variant subunits identified only in schistosomes to date. The cloned Ts Ca_v var cDNA is 2772 bp in length and contains an open reading frame encoding a protein of 809 amino acids with a predicted molecular weight of 86.6 kDa (Fig. 2B). Two spliced forms of the Ts Ca_v var cDNA were isolated during the process of cloning. One lacks a cassette of 18 amino acids between residues 684–701 within an unconserved region near the carboxyl terminus. A second cDNA lacks the 133 amino acids between residues 391–523, including a segment that is moderately conserved with the schistosome subunits. Although, neither of the two alternate cDNAs is a product of mispriming during PCR, it is still unclear whether they represent splicing artifacts or functionally relevant mRNAs.

Phylogenetic analysis reveals that the conventional and variant subunits form two subfamilies that are related but separate (Fig. 3). As described above, an important structural distinction between the two types of subunits resides within the BID, in which variant subunits lack two conserved PKC sites. Ts Ca_v differs from other conventional subunits by containing threonine in place of the first serine but threonine can also be phosphorylated by PKC (Woodgett et al., 1986). In both Sm Ca_v var and Sj Ca_v var, the two serines are replaced by cysteine and alanine. Within the Ts Ca_v var sequence the serines are replaced by asparagine and alanine (Table 1), which is consistent with the BIDs of the variant schistosome subunits. As in Sm Ca_v var and Sj Ca_v var, the remainder of the BID is conserved with respect to conventional subunits.

We investigated whether variant α_1 subunits are found in other classes of platyhelminths by screening two turbellarians for α_1 subunit BID regions. Adapter-ligated, amplified cDNA pools (Matz, 2002) were generated from *Dugesia dorotoce-phala* (Carolina Biological Supply) and *Bdelloura candida* (collected from the legs and gills of *Limulus polyphemus* maintained at the Whitney Laboratory, University of Florida). cDNA fragments amplified as described in Fig. 2 from each species encoded a BID lacking the two serine residues. As in the variant schistosome subunits, the turbellarian sequences incorporate cystine and alanine residues in the corresponding positions (Table 1). A second fragment resembling the BIDs of conventional α_1 subunits was also amplified from *B. candida*, indicating that both forms of the α_1 subunit are expressed in three different classes of platyhelminths.

These findings demonstrate that a homolog of the variant α_1 subunits identified in the trematodes *S. mansoni* and *S. japonicum* is also expressed in the cestode *T. solium*, as well as in two species of turbellarians. Although it remains to be determined whether a homologous protein is found in all platyhelminths, our results establish a stronger correlation between the presence of the α_1 var subtype and sensitivity to praziquantel. As noted above, the α_1 var isoform may be unique to platyhelminths, as no sequence resembling that of variant Ca_v s has yet been found outside of the phylum. For example, only a single, conventional α_1 subtype has been found in the mollusc *Lymnaea stagnalis* (Spafford et al., 2003). Furthermore, a BLAST search for α_1 subunit homologues within the fully sequenced genomes of *C. elegans*, *D. melanogaster* and *Ciona intestinalis* detects only a single conventional α_1 subunit in each. A distant homolog of known α_1 subunits is present in some genomes (e.g. the w10c8.1 gene in *C. elegans*), but the translated sequence deviates significantly from both α_1 subunits described here, and the *C. elegans* protein appears not to modulate α_1 subunits when expressed in *Xenopus* oocytes (Jeziorski, unpublished observations).

5. Conclusions

The results presented here provide further evidence for the hypothesis that a variant subunit of VGCCs is the clinically relevant target of praziquantel in schistosomes and other platyhelminths. Heterologous expression has demonstrated that the Ca_v α_1 var subunit can associate with an α_1 subunit, induce alterations in channel kinetics and current amplitude and confer sensitivity to praziquantel in a normally insensitive subunit. The increase in Ca^{2+} current in such channels in the presence of praziquantel is consistent with previously described physiological effects of the drug. Conserved PKC sites that exist in all known conventional Ca_v subunits, but in no Ca_v α_1 var subunits, account for the differential responses of the two families of subunits to praziquantel. We now show that the Ca_v α_1 var subunit is not limited to schistosomes, but is also found in *T. solium*, an important clinical target of praziquantel, as well as two species from a third class of platyhelminths. To date, the presence or absence of the Ca_v α_1 var subunit in a given organism is consistently correlated with the presence or absence of sensitivity to praziquantel.

Nevertheless, several key questions remain before the Ca_v α_1 var subunit can be definitively established as the essential target of praziquantel. Due to technical obstacles in expressing cloned schistosome α_1 subunits, the effects of the Ca_v α_1 var subunit have been studied only upon non-helminth channels. We do not yet know whether Ca_v α_1 var associates with one or all of the α_1 subunits present in schistosomes or whether the association is limited to L-type, non-L-type or as yet undescribed T-type channels. Furthermore, it is not clear whether Ca_v α_1 var competes with the conventional Ca_v subunit or whether the two have complementary or exclusive roles in modulating trafficking, kinetic response or pharmacology of the α_1 subunit. Ca_v α_1 var may associate with multiple α_1 subunits, but induce praziquantel sensitivity in only a subset. In addition, the precise mechanism of

praziquantel interaction with Ca^{2+} channel subunits remains unresolved. Does it interact directly with the α_1 subunit, interfere with α_1/α_2 interaction, or act indirectly on Ca^{2+} channels containing these variant Ca_v s?

Beyond the questions related to praziquantel sensitivity are the biological roles these variant Ca_v s play in platyhelminths. The growing evidence that the Ca_v var subunit is a platyhelminth-specific family of α subunits raises intriguing issues about their importance to helminth physiology. Unlike other α subunits, the variant α subunits reduce Ca^{2+} currents in oocytes instead of increasing them. If this response is true of native schistosome currents, what is the importance of this unusual type of modulation? Are these α subunits interacting with other receptors or channels? Genomic, post-genomic and physiological studies may allow some of these questions to be addressed directly.

Acknowledgments

We wish to thank Dr Anaid Antamarian Salas and Jessica Roberts-Misterly for technical assistance, the laboratory of Dr Edda Sciotto, UNAM, for assistance in obtaining *T. solium* specimens and Dr Kathy Hancock at the Centers for Disease Control and Prevention for providing *T. solium* cDNA libraries. Lynn Milstead helped with manuscript preparation. This work was supported by PAPIIT grant IN-221702 to MCJ. RMG is supported by NIH grant AI 40522 and by the Neal Cornell Research Fund at the Marine Biological Laboratory.

References

- Andrews P. Praziquantel: mechanisms of anti-schistosomal activity. *Pharmacol. Ther.* 1985; 29:129–156. [PubMed: 3914644]
- Andrews P, Thomas H, Pohlke R, Seubert J. Praziquantel. *Med. Res. Rev.* 1983; 3:147–200. [PubMed: 6408323]
- Birnbaumer L, Qin N, Olcese R, Tareilus E, Platano D, Costantin J, Stefani E. Structures and functions of calcium channel α subunits. *J. Bioenerg. Biomembr.* 1998; 30:357–375. [PubMed: 9758332]
- Brindley PJ. Drug resistance to schistosomicides and other anthelmintics of medical significance. *Acta Trop.* 1994; 56:213–231. [PubMed: 8203304]
- Carpio A. Neurocysticercosis: an update. *Lancet Infect. Dis.* 2002; 2:751–762. [PubMed: 12467692]
- Catterall WA. Structure and regulation of voltage-gated Ca^{2+} channels. *Annu. Rev. Cell Dev. Biol.* 2000; 16:521–555. [PubMed: 11031246]
- Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J. International Union of Pharmacology XLVIII. Nomenclature and structure–function relationships of voltage-gated calcium channels. *Pharmacol. Rev.* 2005; 57:411–425.
- Chen Y-H, Li M-H, Zhang Y, He L-L, Yamada Y, Fitzmaurice A, Shen Y, Zhang H, Tong L, Yang J. Structural basis of the α_1 – α_2 subunit interaction of voltage-gated Ca^{2+} channels. *Nature.* 2004; 429:675–680. [PubMed: 15170217]
- Chitsulo L, Loverde P, Engels D. Schistosomiasis. *Nat. Rev. Microbiol.* 2004; 2:12–13. [PubMed: 15035004]
- Cioli D, Pica-Mattoccia L. Praziquantel. *Parasitol. Res.* 2003; 90:S3–S9. [PubMed: 12811543]
- Cioli D, Pica-Mattoccia L, Moroni R. Drug resistance in schistosomes. *Parasitol. Today.* 1993; 9:162–166. [PubMed: 15463744]
- Cobbett P, Day TA. Functional Voltage-gated Ca channels in muscle fibers of platyhelminths. *Int. Congress Parasitol.* 2002; 10:105.
- Day, TA.; Botros, S. Drug resistance in schistosomes. In: Maule, AG.; Marks, NJ., editors. *Parasitic Flatworms: Molecular Biology, Biochemistry, Immunology and Physiology*. Wallingford: CAB International; 2006. p. 256-268.
- Day TA, Bennett JL, Pax RA. Praziquantel: the enigmatic antiparasitic. *Parasitol. Today.* 1992; 8:342–344. [PubMed: 15463532]
- Day TA, Orr N, Bennett JL, Pax RA. Voltage-gated currents in muscle cells of *Schistosoma mansoni*. *Parasitology.* 1993; 106:471–477. [PubMed: 8341583]

- DeWaard MD, Pragnell M, Campbell KP. Ca^{2+} channel regulation by a conserved β subunit domain. *Neuron*. 1994; 13:495–503. [PubMed: 8060623]
- Dimitratos SD, Woods DF, Stathakis DG, Bryant PJ. Signaling pathways are focused at specialized regions of the plasma membrane by scaffolding proteins of the MAGUK family. *Bioessays*. 1999; 21:912–921. [PubMed: 10517864]
- Dolphin AC. β subunits of voltage-gated calcium channels. *J. Bioenerg. Biomemb.* 2003; 35:599–620.
- Doyle DA, Cabral JM, Pfuetzner RA, Kuo A, Gulbis JM, Dohen SL, Chair BT, MacKinnon R. The structure of the potassium channel: molecular basis of K^{+} conduction and selectivity. *Science*. 1998; 280:69–77. [PubMed: 9525859]
- Engels D, Chitsulo L, Montresor A, Savioli L. The global epidemiological situation of schistosomiasis and new approaches to control and research. *Acta Trop.* 2002; 82:139–146. [PubMed: 12020886]
- Fenwick A, Savioli L, Engels D, Bergquist NR, Todd MH. Drugs for the control of parasitic diseases: current status and development in schistosomiasis. *Trends Parasitol.* 2003; 19:509–515. [PubMed: 14580962]
- Flisser A, Sarti E, Lightowers M, Schantz P. Neurocystercosis: regional status, epidemiology, impact and control measures in the Americas. *Acta Trop.* 2003; 87:43–51. [PubMed: 12781377]
- Frohman MA, Dush MK, Martin GR. Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proc. Natl Acad. Sci. USA*. 1988; 85:8998–9002. [PubMed: 2461560]
- Garcia HH, Gonzalez AE, Evans CAW, Gilman RH. *Taenia solium* cysticercosis. *Lancet*. 2003; 361:547–556. [PubMed: 12932389]
- Geerts S, Gryseels B. Anthelmintic resistance in human helminths: a review. *Trop. Med. Int. Health*. 2001; 6:915–921. [PubMed: 11703846]
- Greenberg RM. Are Ca^{2+} channels targets of praziquantel action? *Int. J. Parasitol.* 2005; 35:1–9. [PubMed: 15619510]
- Hagan P, Appleton CC, Coles GC, Kusel JR, Tchuem-Tchuente LA. Schistosomiasis control: keep taking the tablets. *Trends Parasitol.* 2004; 20:92–97. [PubMed: 14747023]
- Hanlon MR, Wallace BA. Structure and function of voltage-dependent ion channel regulatory subunits. *Biochemistry*. 2002; 41:2886–2894. [PubMed: 11863426]
- Hanlon MR, Berrow NS, Dolphin AC, Wallace BA. Modelling of a voltage-dependent Ca^{2+} channel subunit as a basis for understanding its functional properties. *Fed. Eur. Biol. chem Soc.* 1999; 445:366–370.
- Hofmann F, Lacinova L, Klugbauer N. Voltage-dependent calcium channels: from structure to function. *Rev. Physiol. Biochem. Pharmacol.* 1999; 139:33–87. [PubMed: 10453692]
- Jeziorski MC, Greenberg RM, Anderson PAV. The molecular biology of invertebrate voltage-gated Ca^{2+} channels. *J. Exp. Biol.* 2000; 203:841–856. [PubMed: 10667967]
- Kamp TJ, Hell JW. Regulation of cardiac L-type calcium channels by protein kinase A and protein kinase C. *Circ. Res.* 2000; 87:1094–1102.
- Keef KD, Hume JR, Zhong J. Regulation of cardiac and smooth muscle Ca^{2+} channels ($\text{Ca}_v1.2a,b$) by protein kinases. *Am. J. Physiol. Cell Physiol.* 2001; 281:C1743–C1755. [PubMed: 11698232]
- Kohn AB, Anderson PAV, Roberts-Misterly JM, Greenberg RM. Schistosome calcium channel subunits: unusual modulatory effects and potential role in the action of the antischistosomal drug praziquantel. *J. Biol. Chem.* 2001a; 276:36873–36876. [PubMed: 11500482]
- Kohn AB, Lea JM, Roberts-Misterly JM, Anderson PAV, Greenberg RM. Structure of three high voltage-activated Ca^{2+} channel β subunits from *Schistosoma mansoni*. *Parasitology*. 2001b; 123:489–497. [PubMed: 11719960]
- Kohn AB, Roberts-Misterly JM, Anderson PAV, Greenberg RM. Creation by mutagenesis of a mammalian Ca^{2+} channel β subunit that confers praziquantel sensitivity to a mammalian Ca^{2+} channel. *Int. J. Parasitol.* 2003a; 33:1303–1308. [PubMed: 14527513]
- Kohn AB, Roberts-Misterly JM, Anderson PAV, Khan N, Greenberg RM. Specific residues in the β interaction domain of a schistosome Ca^{2+} channel β subunit are key to its role in sensitivity to the antischistosomal drug praziquantel. *Parasitology*. 2003b; 127:349–356. [PubMed: 14636021]

- Kumar S, Tamura K, Jakobsen IB, Nei M. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief. Bioinform.* 2004; 5:150–163. [PubMed: 15260895]
- Littleton JT, Ganetzky B. Ion channels and synaptic organization: analysis of the *Drosophila* genome. *Neuron.* 2000; 26:35–43. [PubMed: 10798390]
- Matz, MV. Amplification of representative cDNA samples from microscopic amounts of invertebrate tissue to search for new genes. In: Hicks, UBW., editor. *GFP: Methods in Molecular Biology*. Totowa: Humana Press; 2002. p. 3-18.
- Opatowski Y, Chen C-C, Campbell KP, Hirsch JA. Structural analysis of the voltage-dependent Ca^{2+} channel subunit functional core and its complex with the α_1 interaction domain. *Neuron.* 2004; 42:387–399. [PubMed: 15134636]
- Petegem FV, Clark KA, Chatelain FC, Minor DL. Structure of a complex between a voltage-gated calcium channel subunit and an α -subunit domain. *Nature.* 2004; 429:671–675. [PubMed: 15141227]
- Ponting CP, Phillips C, Davies KE, Blake DJ. PDZ domains: targeting signalling molecules to sub-membranous sites. *Bioessays.* 1997; 19:469–479. [PubMed: 9204764]
- Puri TS, Gerhardstein BL, Zhao X-L, Ladner MB, Hosey MM. Differential effects of subunit interactions on protein kinase A- and C-mediated phosphorylation of L-type calcium channels. *Biochemistry.* 1997; 36:9605–9615. [PubMed: 9236007]
- Randall A, Benham CD. Recent advances in the molecular understanding of voltage-gated Ca^{2+} channels. *Mol. Cell. Neurosci.* 1999; 14:255–272. [PubMed: 10588383]
- Redman CA, Robertson A, Fallon PG, Modha J, Kusel JR, Doenhoff MJ, Martin RJ. Praziquantel: an urgent and exciting challenge. *Parasitol. Today.* 1996; 12:14–20. [PubMed: 15275303]
- Richards MW, Butcher AJ, Dolphin AC. Ca^{2+} channel beta-subunits: structural insights AID our understanding. *Trends Pharmacol. Sci.* 2004; 25:626–632. [PubMed: 15530640]
- Rossie S. Regulation of voltage-sensitive sodium and calcium channels by phosphorylation. *Adv. Second Messenger Phosphorylation Res.* 1999; 33:23–48.
- Spafford JD, Munro DW, Van Nierop P, Feng ZP, Jarvis SE, Gallin WJ, Smit AB, Zamponi GW, Syed NL. Calcium channel structural determinants of synaptic transmission between identified invertebrate neurons. *J. Biol. Chem.* 2003; 278:4258–4267. [PubMed: 12458203]
- Valle C, Troiani AR, Festucci A, Pica-Mattoccia L, Liberti P, Wolstenholme A, Francklow K, Doenhoff MJ, Cioli D. Sequence and level of endogenous expression of calcium channel subunits in *Schistosoma mansoni* displaying different susceptibilities to praziquantel. *Mol. Biochem. Parasitol.* 2003; 130:111–115. [PubMed: 12946847]
- van der Werf MJ, de Vlas SJ, Brooker S, Looman CW, Nagelkerke NJ, Habbema JD, Engels D. Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. *Acta Trop.* 2003; 86:125–139. [PubMed: 12745133]
- Walker D, DeWaard M. Subunit interaction sites in voltage-dependent Ca^{2+} channels: role in channel function. *Trends Neurosci.* 1998; 21:148–154. [PubMed: 9554724]
- William S, Sabra A, Ramzy F, Mousa M, Demerdash Z, Bennett JL, Day TA, Botros S. Stability and reproductive fitness of *Schistosoma mansoni* isolates with decreased sensitivity to praziquantel. *Int. J. Parasitol.* 2001; 31:1093–1100. [PubMed: 11429173]
- Woodgett JR, Gould KL, Hunter T. Substrate specificity of protein kinase C: use of synthetic peptides corresponding to physiological sites as probes for substrate recognition requirements. *Eur. J. Biochem.* 1986; 161:177–184. [PubMed: 3023081]
- Yue DT. The dawn of high-resolution structure for the queen of the ion channels. *Neuron.* 2004; 42:357–361. [PubMed: 15134631]

Fig. 1.

Simplified structure of a vertebrate voltage-gated Ca^{2+} channel. Subunits include the pore-forming α_1 subunit, which consists of four homologous domains surrounding a central pore. Each domain comprises six transmembrane regions (S1–S6). The P loop, which dips into the channel 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 α_2 and δ subunits, which modulate α_1 function. α_2 and δ subunit homologs have been identified in schistosomes. Domains of the β subunit defined by recent homology modeling and high resolution structural analysis (see text) are shown. The β subunit interacts via its guanylate kinase (GK) domain with the alpha interaction domain (AID) on the I–II loop of the α_1 subunit. Size of subunits and domains are not to scale. Adapted from elements of Randall and Benham (1999) and Yue (2004).

A

TsCav β	MIGARSDSIYSSKSTSIERQDSERSVPSSQSLFDEGDDDEGSKSLDAEAELELERLAKEOLEKARVSSVVFVAVRTNVSEF	82
SmCav β	MAGDRGYSGSDFAGNNFEEYDDEYCDRADDDDEEEDD-EDDYKEENAKQGTQEQARMLLEKAKTSKVVFVAVRTNVAFH	81
TsCav β	GSACVDCPLFGHVVSFQLKDFLHIKEKFNNEWWIGRLVKENS	163
SmCav β	GSVVDCCPVFGMAVSFQVKDFLHIKEKFNNEWWIGRLVKEGCDVGFIPSPAKLEYLRTSRPSKS-TSGNFDSSSLPRSA	160
β interaction domain		
TsCav β	SRASTPPGD-TDGEGRGDENEQTSRIKSVS-----NSKASRKTFEKKTDNIIPPYEVVPTMRPVVLIGPSLKGVEVTDMMQKA	239
SmCav β	SRPSTPPADGADTINRSYDEDSNARRETSPGKASVSARGGKPKFFKKSDNLPPYDVVPSMRPVVLIGPSLKGVEVTDMMQKA	242
TsCav β	LFDFLKRRFEGRITITRTVADISLAKRSLNNPTRAIMDKASTRNQSF	321
SmCav β	LFDFLKRRFEGRITITRTVADISLAKRSLNNPTRAIMDKSSTRNQSL	324
TsCav β	KTSLAEVNVVYVKVYSTKVLQRLIKTRGKSQRNMNVQMVAEKLQCTNDQFDVILEENQLQDACEHLAEYLEAYWRASHFA	403
SmCav β	KTSLAEITVYVKISSTKVLQRLIKTRGKSQRNMNVQMVAEKLQCTNDQFDVILEENQLQDACEHLAEYLEAYWRASHFA	405
TsCav β	IENTAKAEVVLGSGQVSTTSVPSTEKARSTPEDRVSLSPLESDVSEATEPAPIQKVPSPRWQRERGRSEYDGRWEGKRD	485
SmCav β	TKVTKAERILGIG--TGASSNQSTENEMSHG-----THRQSNAFKNDKRRGKTQDINGSRD	462
TsCav β	EMQGDDEAEVVEEGEEEEAMDMEEAEEDVDPDDEEENEVEEEDDDDEYLNSEYDEEAPQMASYYRNNYQPIAQDQRKLFL	567
SmCav β	---YNSISSPRRGSTGFENNRQRYVDEQELSDADDYRYKPHNEIRTVWDNTEKNYSFSHEPRLSSNQALQHDR---QM	538
TsCav β	VHSRQTIPTFSARLGVGSSGSRYQLQSSQNYFQCHQVQQR-RVPLDKLYWEEQEGDISYDEDERMSFRDHY	640
SmCav β	KSNRNINPTS---VG-----IHNRYNHPPQYDTEESDSPEMNRFLVDSGYPKTRSRQGSIMI----	594

B

TsCav β var	MNRASFYGNRADYSESSEDESDELLRLAELELQERALIDLDIASNAFVAFSVRTNVEFDCLLNGLDAPITPKVHSAFAK	82
SmCav β var	-----MGSRRSESTSSVDSVILEAEERLELERLVLELELAVSKPVAFSVRTNISFDGALYGLDAPSPTRVVSFGIK	73
TsCav β var	EFLQVRRREDQNWIGRVVREGAFIGELPSLKLTLRHSQNNLSQILLSEALNVAASSSPANASSSATAATS	164
SmCav β var	DFLHIKRRNQDWWIGRVVREGFPIGELPSLKLTLRHSQNNLSQILLSEALNVAASSSPANASSSATAATS	153
TsCav β var	PLNSTKASAWPAALANGNAPHSNNDKHVFFGNGKPKGAENSITPGLGVSTPDMSEKTTTQTPTLTTLTSTP--KKKPFK	244
SmCav β var	ERGGNRGLLLSSDVRHGRPTLSANRQRQSESPVHKWKDVEDDDEDDLRDTP-QTVEVPGQPTTKKQPVGEGVKRRPESK	234
β interaction domain		
TsCav β var	KGFTGPPYELVFNVRPVVVGPAKLGVEVTDMMQKALFDALRRHFEGRIVVTRVTSDISAWKRLNVLVNMDKKALTDRTGR	326
SmCav β var	KNEFVPPYELVFCMRPVVVGPAKLGVEVTDMMQKALFDALRRHFEGRIVVTRVTSDISAWKRLNVLVNMDKKALTDRTGR	316
TsCav β var	QIITALEVQRELERIEDLATSMLELVLDSEITINHPIQAKSP LAPIMVYIKTSSVRLQRLIKNRGKTQKKQISSQVAAAEK	408
SmCav β var	QLVSLTEVQQLERIFHLGSKMQLLLLDCTINHPIQAKSP LAPIMVYIKTSSVRLQRLIKNRGKTQKKQISSQVAAAEK	398
TsCav β var	LLQCAEESFDVILEQNSLESATEAMANYLESYLKAIHHYGDHGKGDROFS--PPPLMSLKTNEAKALPFMPGRPGNLIAF	488
SmCav β var	LLQCSFESFDVIDQNNLSTATEALSHFLGYYWAATHPPLIVSKAERLLGSFSAFVPETKEVDSDFPLVPMTPGHPLSVVT	480
TsCav β var	AAAGSAGFQLQELTVAGDRHACHSGTLGE-GEFEGGEGEEGDEEKEKKKGEDGAKRGEDEDDKAGHKTDTDKTKSGSV	569
SmCav β var	KSALSAGFTSQEISELTGATATGWLTEGGQVENEGLTGVHHAGSVGSEFHNTHFGGKTKTDHNN-SPDGLNTTHYSDE	561
TsCav β var	SASTDAKKKSLSLKLGKAEKREGGILKKGAGKKHQTTLTQIIPDRAHAIALAEAVVKNANAAAAAAATRLDVPGGATI	651
SmCav β var	NEGSRPNYPRNGRLHMGAAASAAAIATAAAGCTPKVHSLHPSLFQGENKRNGSVIRDINGNTTHESPLTMGLGLGIANGVAA	643
TsCav β var	GLHRSHQAAASDPSTGPKIYASRSSRQAKQREEMENARRRSEEATRAAAEAAASGAQRQKRRQRLLLAEQAAATATRH	733
SmCav β var	TISAAALFPSNSTN--HHQHS-AENYHQPTS-RIPPTIAAAAVASVSPPLKDLGIHQVSGSDGTQVYQSRSAKQAKORE	721
TsCav β var	GGGGAVKFGEAPGSEKGPVLGRGSRAMPPEELQSAFGSNLRNTSSAAEASGPLGHMPPYKNTIPLIHPSEN---	809
SmCav β var	EQLARVKQEVEAKALALQATAGRRRRRDKERRQRNPDGYWDSHDERYHAGTNDLSQHSQWNGFPVRSMARACFPDD	800

Fig. 2.

(A) Alignment of the conventional *Taenia solium* subunit TsCa γ with the *Schistosoma mansoni* subunit SmCa γ . (B) Alignment of the variant *T. solium* subunit TsCa γ var with the *S. mansoni* subunit SmCa γ var. Residues that are identical between sequences are shaded. The position of the β interaction domain is indicated in each figure, and the locations of two deletions identified in certain TsCa γ var clones are indicated by dashed lines. The alignments were completed using Clustal W 1.82. To clone the two *T. solium* cDNAs, RNA was extracted from *T. solium* cysticerci with TRIzol and reverse-transcribed with SuperScript II reverse transcriptase (Invitrogen) and an oligo-dT primer. Touchdown PCR with the degenerate oligonucleotides AAYAAAYGAYGGTGGAT and

GCYTTYTGCAT-CATRTC [94° for 30 s, 49° for 40 s (−0.5°/cycle), 72° for 30 s for 22 cycles, followed by an additional 30 cycles using a 42° annealing temperature] generated two distinct cDNA fragments. The amplified products were cloned into the pTOPO II vector (Invitrogen) and sequenced on an automated sequencer. The cDNA ends were amplified using 3 RACE and 5 RACE (Frohman et al., 1988) and the full coding region of each cDNA was determined. For each of the two subunits, multiple cDNAs were cloned from separate sources and sequenced to establish a consensus sequence. The GenBank accession numbers are AY624029 for TsCa_vb, AY624030 for TsCa_v var, AAK51118 for SmCa_v B, and AAK51117 for SmCa_v var.

Fig. 3.

Phylogenetic relationships among conventional and variant Ca^{2+} channel subunits, including the distant *C. elegans* subunit homolog w10c8.1. The tree was constructed using the neighbor-joining method as implemented in MEGA 3.1 (Kumar et al., 2004). Bootstrap values from 100 replicates are shown. The GenBank accession numbers for the sequences are as follows: *Schistosoma japonicum* var, AY033597; human 1, NP_954856; rabbit 1, AAA31180; human 2, AAD33730; rabbit 2, P54288; human 3, AA 19799; rabbit 3, CAA45578; human 4, AAB53333; rat 4, A45982; *Xenopus* 3, AAA75519; *Fugu* 3, AAB96361; *C. elegans*, AAB53056; *Musca*, A54844; *Drosophila*, AAF21096; *Loligo*,

BAB88219; *Lymnaea*, AAO83844; *Cyanea*, AAB87751; and *C. elegans* w10c8.1, AAK21500.

Table 1
Alignment of a conserved region of the interaction domains of conventional and variant subunits

Conventional subunits																										
Subtype or species	Phylum	P	P	Y	D	V	V	P	S	M	R	P	I	I	L	V	G	P	S	L	K	G	Y	E	V	T
Human 1	Chordata	P	P	Y	D	V	V	P	S	M	R	P	I	I	L	V	G	P	S	L	K	G	Y	E	V	T
Human 2	Chordata	P	P	Y	D	V	V	P	S	M	R	P	V	V	L	V	G	P	S	L	K	G	Y	E	V	T
Human 3	Chordata	P	P	Y	D	V	V	P	S	M	R	P	V	V	L	V	G	P	S	L	K	G	Y	E	V	T
Human 4	Chordata	P	P	Y	D	V	V	P	S	M	R	P	V	V	L	V	G	P	S	L	K	G	Y	E	V	T
<i>Loligo bleekeri</i>	Mollusca	P	P	Y	E	V	V	P	S	M	R	P	V	V	L	I	G	P	S	L	K	G	Y	E	V	T
<i>Lymnaea stagnalis</i>	Mollusca	P	P	Y	E	V	V	P	S	M	R	P	V	V	L	I	G	P	S	L	K	G	Y	E	V	T
<i>Musca domestica</i>	Arthropoda	S	P	Y	D	V	V	P	S	M	R	P	V	V	L	V	G	P	S	L	K	G	Y	E	V	T
<i>Drosophila melanogaster</i>	Arthropoda	S	P	Y	D	V	V	P	S	M	R	P	V	V	L	V	G	P	S	L	K	G	Y	E	V	T
<i>Caenorhabditis elegans</i>	Nematoda	P	P	Y	D	V	V	P	S	M	R	P	V	V	L	V	G	P	S	L	K	G	Y	E	V	T
<i>Cyanea capillata</i>	Cnidaria	P	P	Y	D	V	V	P	S	M	R	P	V	I	F	V	G	P	S	L	K	G	Y	E	V	T
<i>Schistosoma mansoni</i>	Platyhelminthes	P	P	Y	D	V	V	P	S	M	R	P	V	V	L	I	G	P	S	L	K	G	Y	E	V	T
<i>Taenia solium</i>	Platyhelminthes	P	P	Y	E	V	V	P	T	M	R	P	V	V	L	I	G	P	S	L	K	G	Y	E	V	T
<i>Bdelloura candida</i>	Platyhelminthes	P	P	Y	E	V	V	P	S	M	R	P	V	V	L	I	G	P	S	L	K	G	Y	E	V	T
Variant subunits																										
<i>Platyhelminth species</i>	Class																									
<i>Schistosoma mansoni</i>	Trematoda	P	P	Y	E	I	V	P	C	M	R	P	V	V	F	V	G	P	A	L	K	G	Y	E	V	T
<i>Schistosoma japonicum</i>	Trematoda	P	P	Y	E	I	V	P	C	M	R	P	V	V	F	V	G	P	A	L	K	G	Y	E	V	T
<i>Taenia solium</i>	Cestoda	P	P	Y	E	L	V	P	N	V	R	P	V	V	V	V	G	P	A	L	K	G	Y	E	V	T
<i>Bdelloura candida</i>	Turbellaria	P	P	Y	E	I	V	P	C	M	R	P	V	I	F	L	G	P	A	L	K	G	F	E	V	T
<i>Dugesia dorotocephala</i>	Turbellaria	P	P	Y	D	V	V	P	C	M	R	P	I	I	L	V	G	P	A	L	K	G	Y	E	V	T

Bold indicates consensus sites for protein kinase C phosphorylation in conventional subunits.