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Calcium channels of schistosomes: unresolved questions and unexpected answers

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Abstract

Parasitic flatworms of the genus *Schistosoma* are the causative agents of schistosomiasis, a highly prevalent, neglected tropical disease that causes significant morbidity in hundreds of millions of people worldwide. The current treatment of choice against schistosomiasis is praziquantel (PZQ), which is known to affect Ca^{2+} homeostasis in schistosomes, but which has an undefined molecular target and mode of action. PZQ is the only available antischistosomal drug in most parts of the world, making reports of PZQ resistance particularly troubling. Voltage-gated Ca^{2+} (Ca_v) channels have been proposed as possible targets for PZQ, and, given their central role in the neuromuscular system, may also serve as targets for new anthelmintic therapeutics. Indeed, ion channels constitute the majority of targets for current anthelmintics. Ca_v channel subunits from schistosomes and other platyhelminths have several unique properties that make them attractive as potential drug targets, and that could also provide insights into structure-function relationships in, and evolution of, Ca_v channels.

Schistosomes are trematode flatworms that parasitize humans and other mammals (as well as birds), and cause schistosomiasis, a prevalent tropical disease. Schistosomes have a complex life cycle that requires freshwater snails as intermediate hosts, and they infect the mammalian host via water-borne contact with the free-swimming larvae shed from those snails (Figure 1)[1-3]. According to the World Health Organization in its publication “Preventive chemotherapy in human helminthiasis” (http://whqlibdoc.who.int/publications/2006/9241547103_eng.pdf), there are an estimated 200 million people whose quality of life is severely impaired by schistosomiasis. More recent estimates suggest the number may be closer to 450 million, with a burden on human

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Further Reading/Resources Excellent summaries of the basics of schistosomiasis can be found at the CDC website, http://www.cdc.gov/parasites/schistosomiasis/, and at the World Health Organization website, http://www.who.int/topics/schistosomiasis/en/. Additional review articles on the parasites and the disease are cited in the references.

Related Articles

Subtopic	Article title
CALCIUM ION CHANNELS	Calcium channels as therapeutic targets
CALCIUM ION CHANNELS	Calcium channels: Novel roles
CALCIUM ION CHANNELS	Voltage-gated calcium ion channels

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health similar to that of tuberculosis or malaria (http://iom.edu/~/media/Files/Activity%20Files/PublicHealth/MicrobialThreats/2010-SEP-21/King%20CH.pdf). These facts alone provide an important impetus for research into the basic neuromuscular physiology of these parasites. Indeed, the majority of anthelmintic drugs in current use target ion channels of the worm's neuromuscular system, where they typically act as agonists or as positive allosteric modulators[4] (eg, ivermectin, levamisole). Our lab focuses on the structure, function, and modulation of schistosome voltage-gated calcium (Cav) channels, as Ca_v channels are widely recognized as targets in pharmacotherapy[5-7]. However, our interests go beyond identification of possible therapeutic targets, and focus also on using a comparative approach to understand how these channels fit into the biology and life cycle of parasitic platyhelminths, and perhaps add insights into mammalian channel function.

Ca_v channels initiate the contraction of the schistosome musculature[8]. Interestingly, neuropeptide-based signalling, which is well established as of major importance in the neuromuscular system of flatworms [9, 10], appears to be functionally coupled with Ca_v channel activity in schistosome muscle[11], emphasizing their physiological significance in this system. In addition, schistosome Ca_v channels are also almost certainly key players in other important Ca^{2+}-dependent events, such as synaptic transmission, enzyme activity, and gene expression.

Currently, the treatment of choice against schistosomiasis is praziquantel, which is highly effective against all schistosome species, has minimal side effects, and has been demonstrated repeatedly to control schistosomiasis in large-scale treatment efforts[12-17]. Due to these advantages, as well as steadily reduced costs, PZQ has become the only commercially available antischistosomal treatment in most parts of the world[15, 18]. However, this success comes at a potential cost. Reliance on a single drug to treat such a hugely prevalent disease represents an ultimately untenable situation[8], as there is no readily available alternative should drug resistance develop. In that light, reports of PZQ resistance in the field[9-11] and in the laboratory after drug selection[12, 13] are particularly troubling. Furthermore, treatment failures can arise because juvenile schistosomes are refractory to PZQ, and do not become sensitive until egg deposition begins at approximately 6 weeks following infection[23-26].

In this review we will focus on what we know about Ca_v channel expression and function in native schistosome cells and on the function of schistosome Ca_v channel subunits, including their apparent role in PZQ action, as surmised by expression in heterologous systems.

Ca^{2+} CURRENTS IN NATIVE SCHISTOSOME CELLS

Ca_v channel activity in schistosomes was first indicated by experiments showing that rapid contraction of worm muscle was dependent upon the presence of Ca^{2+} in the bathing medium[8]. In that study, the reported speed at which schistosomes contracted following introduction of external Ca^{2+} was consistent with a Ca^{2+} gate in the plasma membrane that is opened by a characteristically rapid change in membrane voltage. However, the first electrophysiological study geared towards recording voltage-gated ion currents from schistosome muscle cells, which used the whole-cell patch-clamp technique, detected the prominent outward K^+ currents in these cells but no Ca^{2+} or other inward currents[14]. In contrast, Ca^{2+} currents of dissociated cell preparations from ectoparasitic and free-living platyhelminths were more readily obtainable. Thus, neurons from the polyclad flatworm *Notoplana acticola* express a typical complement of ionic currents, including cadmium-sensitive Ca^{2+} currents[15]. Similarly, both nerve and muscle cells of the triclad ectoparasitic flatworm *Bdelloura candida* express Ca^{2+} currents that activate at −30 mV, reach peak

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amplitude in approximately 5 ms, and inactivate slowly[16, 17]. The neuronal Ca^{2+} current is relatively insensitive to Ca_v channel blockers such as nifedipine and verapamil, is not blocked by ω-conotoxin GVIA, and exhibits no sensitivity to 10 μM praziquantel[18]. The muscle Ca^{2+} current was too unstable for determination of pharmacological sensitivities. Muscle cells from the free-living freshwater flatworm *Girardia tigrina* also express Ca^{2+} currents that tend to run-down under whole-cell patch clamp[19]. Interestingly, although 10 μM PZQ had no apparent effect on intact *B. candida*, it did produce a rapid Ca^{2+}-dependent contracture in approximately 20% of the isolated *B. candida* muscle fibers when applied within one hour of dispersion of the cells[16]. Our preliminary data indicate that *Dugesia spp*. respond to PZQ, though with less sensitivity and rapidity than schistosomes (unpublished observations), suggesting free-living planarians may have potential for use as models to study PZQ targets.

More than a decade after the first patch-clamp studies on schistosomes, the schistosome muscle preparation was revisited in two studies[11, 20]. In the first[33], voltage-gated Ca^{2+} currents were detected in muscle fibers, but only after extensive block of prominent outward K^+ currents in these cells. These Ca^{2+} currents peaked at approximately +20 mV and were relatively small (less than 100 pA), activated within 30 ms after depolarization, and did not inactivate for at least 250 ms if the depolarization was maintained. This time-course of the Ca^{2+} current in schistosome muscle was not unlike that of the L-type Ca^{2+} currents of mammalian muscle. Similar to the Ca^{2+} currents recorded from other flatworms[16, 19], the Ca^{2+} currents from schistosome muscle fibers ran down rapidly, within a few minutes of establishing the whole-cell configuration, thus complicating pharmacological analysis. However, depolarization-initiated contractions of the muscle fibers, which are presumably dependent on Ca_v channel activity, were blocked by nicardipine with an IC_{50} of 4.1 μM. Surprisingly, other dihydropyridines such as nifedipine and nitrendipine were largely ineffective at blocking these depolarization-induced contractions. Diltiazem was also relatively ineffective, as were conotoxins that inhibit non-L type mammalian Ca_v channels. The more recent study[11] investigated neuropeptide enhancement of Ca^{2+} currents. As with depolarization-induced muscle fiber contraction, peptide (YIRFamide)-induced contractions were sensitive to nicardipine, and also to high concentrations of the phenylalkylamines verapamil and methoxyverapamil. Recording of robust Ca_v currents was aided by using a combination of Ca^{2+} and Ba^{2+} as charge carrier, and rundown was managed by recording within a brief window of time during which currents were relatively stable. The currents recorded under these conditions were enhanced by the peptide YIRFamide. Due to technical issues, the effect of dihydropyridines such as nicardipine and nifedipine were not tested, but these currents were partially inhibited (~50%) by 10 μM verapamil[11]. Thus, what we know so far about the pharmacological profile of schistosome and other platyhelminth Ca_v channels sets them apart from their mammalian homologues.

Clearly, studying native Ca^{2+} currents in schistosome cells is technically challenging. Furthermore, as in any native system, but particularly in invertebrates where channel pharmacology is not as well established, Ca^{2+} currents are often contaminated by other ionic currents that are not always possible to eliminate. An alternative and complementary approach is to use heterologous expression of cloned channel genes in *Xenopus* oocytes or mammalian cells. Prior to the availability of a genome database, cDNAs encoding three Ca_v channel α_1 ($SmCa_v1$, $SmCa_v2A$ $SmCa_v2B$) and two β ($SmCa_v\beta$, $SmCa_v\beta_{var}$) subunits were cloned by Greenberg and collaborators[21, 225]. Publication of the *S. mansoni* genome[23] confirmed these results and also uncovered genes for additional subunits. A summary of Ca_v channel subunits found in the *S. mansoni* genome is shown in Sidebar 1.

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STRUCTURE OF SCHISTOSOME Ca_v CHANNEL α_1 SUBUNITS

Original cloning of cDNAs using RT-PCR with degenerate primers revealed three high voltage-activated (HVA) Ca_v channel α_1 subunits in *S. mansoni*[35]. One of these cDNAs ($SmCa_v1$) has highest similarity to L-type (Ca_v1) channels, and the other two ($SmCa_v2A$, $SmCa_v2B$) appear to be non L-type (Ca_v2) channels. Analysis of the genome of *S. mansoni* confirmed those three sequences, as well as a second L-type-like α_1 subunit (Smp_159990), making four $Ca_v\alpha_1$ genes in total. Other invertebrates that have been examined typically contain three α_1 subunit genes[24]. Despite the larger number of α_1 subunit genes in *S. mansoni*, there may in fact be less functional diversity, as all four of the *S. mansoni* α_1 subunits appear to be HVA channels; in other invertebrates, one of their three α_1 subunits is typically a low voltage-activated (LVA; T-type; Ca_v3) channel sequence. Surprisingly, neither the *S. mansoni* nor the *S. japonicum* genomes appear to contain LVA channel-like sequences. Thus, schistosome excitable cells may uniquely lack a requirement for the functions normally carried out by LVA channels. Alternatively, schistosomes may recruit HVA subunits to perform the roles typically fulfilled by LVA subunits, and such a change in channel properties could likely be dependent upon specific interaction with auxiliary subunits. In either case, schistosomes, and perhaps other platyhelminths, clearly differ from other metazoans in their repertoire of Ca_v channel α_1 subunits. The *Schmidtea mediterranea* genome contains a sequence fragment that appears to have highest similarity to LVA subunits, as well as other α1 (and β) subunit representatives that are found in schistosomes. Whether these sequence fragments are within genes that in fact code for LVA channels remains to be determined.

The predicted structures for the schistosome α_1 subunits are overall very similar to their mammalian counterparts, though there are some interesting differences[35]. For example, flatworm L-type channels, including both L-type $SmCa_v$ channels, substitute a non-charged amino acid for an aspartic acid residue that is absolutely conserved in the Domain I pore region of other L-type α_1 subunits. Interestingly, L-type-like α1 subunits from molluscs (*Loligo bleekeri*), also members of the Lophotrochozoa, show a similar substitution (Fig 2). Whether this change affects ion selectivity or other channel properties, and whether it might be a potential target for highly specific antiparasitics remains to be determined. $SmCa_v1A$ also contains one less positively charged residue in the fourth transmembrane segment of Domain II than homologous L-type mammalian Ca_v1 subunits, which may suggest a slight weakening of the voltage dependence of this channel. Furthermore, the C-terminal tail of $SmCa_v1A$ contains two, instead of the normal one, IQ-like calmodulin binding domains, perhaps indicating important variation or redundancy in Ca^{2+}-dependent regulation. The non L-type $SmCa_v2A$ subunit does not have sites for interactions with syntaxin 1A and SNAP-25 in the II-III loop. These interaction sites are, however, present in $SmCa_v2B$, suggesting its involvement in synaptic transmission in neurons, whereas $SmCa_v2A$ plays other roles. Consistent with the data from schistosome muscle fiber contraction studies that indicate at most mild sensitivity to various dihydropyridines[20], the $SmCa_v1A$ subunit contains only 6 residues of the 13 thought to be involved in determining dihydropyridine sensitivity in L-type Ca_v channels[25]. In contrast, the $SmCa_v1B$ sequence is identical at 10 of these 13 residues, suggesting greater sensitivity.

To date, none of the schistosome $Ca_v\alpha_1$ subunits have been functionally expressed, making characterization problematical. In both *Xenopus* oocytes (Kohn and Greenberg, unpublished data) and in mammalian cells (Salvador-Recatalà and Greenberg, unpublished data), attempts at expression using a variety of approaches, including co-expression with schistosome or mammalian auxiliary subunits, were unsuccessful. Possible causes for this lack of expression include: unusual and/or extensive endoplasmic reticulum retention signals; the need for a specific, non-channel, schistosome chaperoning factor; the high A/T

content of the schistosome coding regions; requirement of specific lipids in the plasma membrane. The development of immortalized platyhelminth cell lines could help in this regard. These would be expected to contain helminth-specific chaperones and cell membranes with a composition more similar to that of schistosome cells, with transcription/translation machinery likely more suitable for expression of the schistosome $Ca_v\alpha_1$ subunits than the vertebrate systems. Planarian stem cells may offer a means to achieve this end[26], and recent results using cells from *Echinococcus multilocularis* have also been encouraging[27]. Current efforts to develop schistosome cell lines, including immortalization by transfection with oncogenes, have recently been reviewed[28].

SCHISTOSOME $Ca_V\beta$ SUBUNITS

Schsitosomes and other platyhelminths, express at least two $Ca_v\beta$ subunit genes[39] instead of the single β subunit characteristic of other invertebrates. One of these subunits is structurally similar to $Ca_v\beta$ subunits from other vertebrate and invertebrate species. whereas the other, though clearly a β subunit based on sequence alignment, is different, and we have dubbed it a "variant" β subunit ($SmCa_v\beta_{var}$). For example, at ~85 kDa, $SmCa_v\beta_{var}$ is larger than other β subunits, with most of the extra sequence in the C-terminal region. Most notably, all platyhelminth β_{var} subunits lack two otherwise absolutely conserved protein kinase C (PKC) sites in the highly conserved, ~30 amino acid region of the subunit known as the BID. When co-expressed in *Xenopus* oocytes with a mammalian ($Ca_v2.3$) or jellyfish ($CyCa_v1$) α_1 subunit $Ca_v\beta_{var}$ reduces the current amplitude instead of increasing it, one of the "hallmark" effects of $Ca_v\beta$ subunits[21]. Furthermore, $SmCa_v\beta_{var}$ confers PZQ sensitivity to an otherwise PZQ-insensitive mammalian α_1 subunit. When coexpressed with $SmCa_v\beta_{var}$, voltage-gated peak currents through $Ca_v2.3$ are increased 1.5-2-fold in the presence of 100 nM PZQ compared to those in the absence of PZQ[21]. $Ca_v2.3$ expressed alone, or co-expressed with a conventional β subunit (eg, $SmCa_v\beta$), does not show this responsiveness to PZQ. Addition by mutagenesis of either one or both of the missing PKC sites in the $SmCa_v\beta_{var}$ BID region induces a more conventional modulatory phenotype, i.e. one that increases the Ca^{2+} current and that does not confer PZQ sensitivity[29]. The complementary experiment of eliminating those PKC sites in a mammalian β subunit results in a subunit that now has the ability to confer PZQ sensitivity to $Ca_v2.3$[41]. How these consensus PKC sites in the BID, a part of β subunit structure thought to be non-accessible[30-32], influence behaviour of the β subunit remains an open question, as does the mechanism by which $SmCa_v\beta_{var}$ may be mediating these effects. To help resolve these issues, we have also tried expressing $SmCa_v\beta_{var}$ with $Ca_v2.3$ in a mammalian cell line (HEK), but it shows no effect on the α_1 subunit, and indeed appears not to express at the protein level based on imaging experiments using a $Ca_v\beta_{var}$–GFP chimera (Salvador-Recatalà and Greenberg, unpublished data). Interestingly, however, in the free-living flatworm *Dugesia japonica*, PZQ disrupts normal polarity during regeneration of the worm, but this PZQ effect can be eliminated by knockdown of either $Ca_v\beta$ subunit[33]. How this fascinating, long-term effect on developmental polarity that is apparently mediated by $Ca_v\beta$ subunits relates to the shorter-term antischistosomal activity of PZQ is not clear.

Does $SmCa_v\beta_{var}$ play a role in development of PZQ resistance? To date, the only published examination of this question has found no changes in the sequence or expression levels in *S. mansoni* isolates with reduced PZQ susceptibility[34]. Furthermore, there appears to be no significant difference in β subunit expression between PZQ-sensitive adults and PZQ-refractory juveniles (our unpublished data and ref. 31). These findings, though perhaps disappointing in terms of finding a useful marker for emergence of PZQ resistance, do not eliminate the prospect that $Ca_v\beta_{var}$ and schistosome Ca_v channels are targeted by PZQ. Indeed, although juvenile worms are far less sensitive to the drug, they still respond to PZQ exposure with an influx of $^{45}Ca^{2+}$ and an initial muscular contraction[35]. However, they

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recover from exposure to concentrations of PZQ that are lethal to adult parasites. Thus, although PZQ may initially be targeting Ca_v channels (or other schistosome receptors), some downstream component of the cascade that is initiated by this interaction must differ between juvenile and adult worms, and between isolates with differential PZQ susceptibility. Screens for stage- and gender-specific differences in gene expression have revealed possible candidates[36], and we have found that higher expression of multidrug resistance transporters is one factor which correlates with reduced PZQ sensitivity[37].

The more conventional schistosome β subunit ($SmCa_v\beta$) has a predicted molecular weight of ~67.5 kDa and a predicted isoelectric point of 5.8, similar to mammalian β3 subunits. It was initially expressed in *Xenopus* oocytes with $Ca_v2.3$ as the reporter α_1 subunit, where it predictably increased $Ca_v2.3$ amplitude and shifted steady-state inactivation to more hyperpolarized potentials[29]. In contrast to $SmCa_v\beta_{var}$, $SmCa_v\beta$ does not confer PZQ sensitivity to $Ca_v2.3$. Subsequently, we showed that $SmCa_v\beta$ induces run-down of $Ca_v2.3$ channels expressed in HEK cells[38]. A systematic characterization of this phenomenon revealed that (1) it occurred even if Ca^{2+} was substituted by Ba^{2+} as the charge carrier, suggesting that it is calmodulin-independent; (2) it was dependent on chelated forms of ATP that were added to the patch pipette solution that perfuses the cell during whole-cell patch-clamp recordings, ironically with the goal of preventing run-down (more typically, rundown occurs in the absence of ATP); and (3) the structure responsible for the rapid rundown effect resides in the first 44 amino acids of $SmCa_v\beta$, a region that contains a long polyacidic motif of 15 aspartate and glutamate residues. It is tempting to suggest that the run-down that occurs in this heterologous system corresponds to the run-down of the Ca^{2+} currents recorded from platyhelminth muscle fibres[11, 16, 19, 20]. Interestingly, we have found this N-terminal polyacidic motif (NPAM) in β subunits from other parasitic platyhelminths, but not in free-living platyhelminths, nor, indeed, in any β subunits from other phyla.

In a follow-up study[39], we discovered that NPAM has the additional role of accelerating calmodulin-independent inactivation of a Ca_v2 subunit ($Ca_v2.3$). By constructing chimeric β subunits, we have shown that this function of the acidic motif is portable to mammalian β subunits. Perhaps by reducing Ca^{2+} entry to a necessary minimum, these atypical β subunits act as part of the unique fine-tuning of Ca^{2+} influx essential for the parasite's success. The physiological constraints that necessitate this unusually tight regulation of Ca^{2+} homeostasis remain an open question, but could provide clues to vulnerable points of attack by new antischistosomal agents.

SUMMARY AND FUTURE PERSPECTIVES

Ca_v channels of schistosomes have piqued the curiosity of researchers at least since the early 1980s. This relatively small area of ion channel research comprises similarly few investigators, but the number of questions and technical caveats is relatively large. After several decades of research, we have been able to recognize the importance of these channels in the worm musculature and nervous system, though full characterization *in situ* remains elusive. We have been able to express and characterize the function of some subunits ($Ca_v\beta$) but not others ($Ca_v\alpha_1$). Some of the findings highlight conserved features of Ca_v channels, and some highlight intriguing, non-conserved features such as in the structure of the L-type $SmCa_v1A$, which has features in the pore and C-terminus that differ from other L-type channels. Moreover, whereas the hallmark of mammalian $Ca_v\beta$ subunits is to increase the currents that pass through α_1 subunits, the β subunits of schistosomes appear to have the opposite function of reducing or limiting Ca^{2+} currents. Thus, schistosomes show us that the structure/function of $Ca_v\beta$ subunits can be profoundly modified to remodel the physiology of the neuromuscular system. Future investigations into the function of

schistosome α_1/β subunit combinations are likely to reveal more exciting features of Ca_v channels from these phylogenetically distant and clinically important organisms.

Conclusion

The majority of current anthelmintic drugs act as agonists on ion channels of the neuromuscular system of parasitic worms[4]. Additionally, Ca_v channels are validated targets of several drugs that are used to treat a variety of clinical conditions. By gaining an understanding or the structure, function, and modulation of schistosome Ca_v channels, we hope to provide information that will be useful for rational drug design against schistosomiasis. Furthermore, these types of comparative studies on the ion channels of a phylogenetically distant set of organisms are likely to provide important information about the evolution of ion channels, as well as additional insights into the structure-function relationships of mammalian Ca_v channels. The implementation of new technologies and research strategies may surprise us with further unexpected answers to lingering and unresolved questions about the Ca_v channels of these fascinating organisms. []

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APPENDIX I: ARTICLE LIST BY SUBJECT CATEGORY

VOLTAGE-GATED ION CHANNELS

CALCIUM ION CHANNELS

Voltage-gated calcium ion channels

Molecular Structure of Voltage-gated Ion Channels and comparison among different species

Calcium Channels as therapeutic targets

Calcium channels in Excitation secretion coupling

Calcium channels trafficking and auxiliary subunits

Calcium channels signaling to the nucleus

Calcium channels: Modulation

Calcium channels: Interfacing with other proteins

Calcium channels and disease

Calcium channels in endocrine tissue

CaV3 T-type calcium channels

Calcium channels: Novel roles

Calcium channels: Stress channels

L-type calcium channels -Role in cardiac and hearing

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Calcium channels - presynaptic

LIGAND-GATED ION CHANNELS AND RECEPTORS

PURINERGIC SIGNALING

Purinergic Signaling

Molecular structure of P2X receptors

Biophysics of P2X receptors

Pharmacology of P2X receptors

P2X-mediated synaptic transmission

P2X-mediated signaling in neuroglia

P2X receptors and inflammation

Evolution of P2X receptors

P2X receptors and pain

P2X4/7 receptors and neuropathic pain

P2X receptors in the cardiovascular system

P2X receptors in endocrine organs

P2X receptors in the bone

The pore-forming 2X7 receptor

P2X receptors in cancerous growth

P2X receptors in muscle

P2X receptors in reproductive system: the role in fertility

P2X receptors in kidneys

P2X receptors in the gut

P2X receptors in liver

METABOTROPIC GLUTAMATE (mGlu) RECEPTORS

Metabotropic glutamate receptor signaling

Molecular structure of mGlu receptors

Pharmacology of mGlu receptors

mGlu receptor-mediated synaptic plasticity

mGlu receptor-mediated signaling in neuroglia

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mGlu receptors in the retina

Evolution of mGlu receptors

mGlu receptors and pain

mGlu receptors in endocrine organs

mGlu receptors in the bone

mGlu receptors in cancerous growth

mGlu receptors in neurodegeneration

mGlu receptors in drug addiction behaviors

mGlu receptors in schizophrenia

SEROTONERGIC SIGNALING

Serotonergic signaling

SAR of 5-HT receptors

Biophysics of 5-HT receptors

Pharmacology of 5-HT receptors

5-HT signaling in eating disorders

5-HT signaling in affective disorders

5-HT7 receptors in sleep and diurnal rhythm

5-HT receptors in the gut

In vivo imaging of the 5-HT system

Internalization of 5-HT4 receptors

5-HT signaling in decision making

Localization, trafficking, signaling and ligand bias of 5-HT receptors

Differential regulation of serotonin transporter cell surface expression

Internalization of 5-HT1A receptors

AMPA/KAINATE RECEPTORS

Molecular biology of kainate receptor subunits

Pharmacology and functions of kainite receptors

Presynaptic kainate receptors in hippocampal interneurons

Metabotropic functions of kainate receptors

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Functions of kainate receptors at mossy fiber-CA3 pyramidal neuron synapses

Kainate receptors in developing presynaptic terminals

Pharmacology of kainite receptors

Kainate receptors in epilepsy

Regulation of kainite receptors by the small ubiquitin-like modifier protein (SUMO)

Modulation of GABAergic transmission by Kainate receptors

Regulation of kainate receptors by fatty acids

Gating of kainate receptors

INTRACELLULAR CHANNELS AND RECEPTORS

INOSITOL-1,4,5 TRIPHOSPHATE RECEPTORS (IP3)

Molecular biology of the InsP3Rs

Structure of InsP3R

Physiology and biophysics of InsP3R

Pathophysiology of InsP3R

Modulation of InsP3R

Mammalian InsP3R isoforms

InsP3R in Drosophila

InsP3R in C. elegans

InsP3R binding partners

InsP3R function in the brain

InsP3R function in immune cells

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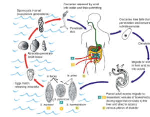

Figure 1. Life cycle of schistosomes

Shown are the life cycles of *S. japonicum* (A), *S. mansoni* (B), and *S. haematobium* (C), the three major schistosome species that parasitize humans. The overall life cycles are quite similar, requiring both a mammalian host and an intermediate fresh-water snail host. Differences between the three species are in the species of intermediate snail host and in the predilection sites in the definitive mammalian host. Other differences include egg morphology, range of definitive mammalian hosts, and levels of egg production. Unlike most Digeneans, schistosomes have two separate sexes. Male and female adult worm pairs reside in the vasculature of the mammalian host in preferential locations depending on the species. There, they undergo sexual reproduction and deposit hundreds (*S. mansoni*) to thousands (*S. japonicum*, *S. haematobium*) of eggs per female, per day. Note that no increase in worm numbers occurs within the mammalian host. Eggs move to the lumen of the intestine or bladder, and are excreted in feces or urine. Eggs which remain within the host are the cause of the majority of pathology of chronic schistosomiasis. Excreted eggs that reach fresh water will hatch into a miracidium, a free-swimming larva that parasitizes an intermediate host snail. Within the snail host, the worms undergo developmental changes and asexual reproduction, emerging in a few weeks as free-living cercariae, the larval form that parasitizes the definitive human host. The cercariae attach to the host skin, and then penetrate it and shed their forked tail to become schistosomules. The schistosomules migrate through several tissues and mature into adults, which take up residence in their predilection sites. Adult *S. mansoni* can reside within the mammalian host for many years. Figure adapted from an image provided by the Parasitology Diagnostic Web Site (DPDx) at the Centers for Disease Control and Prevention (http://www.dpd.cdc.gov/dpdx/Default.htm).

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Figure 2. Examples of structural differences between schistosome and other L-type Ca_v channel α1 subunits

A. Charge change in the Domain I pore region. The Domain I pore region of L-type α1 subunits is shown. Note the shaded residues four spots C-terminal from the absolutely conserved glutamic acid (E) that forms part of the selectivity gate. In L-type channels from most phyla, this residue is a negatively-charged aspartic acid (D, green). However, in platyhelminths and molluscs, both of which are classed as lophotrochozoans, this residue is uncharged (red). This is interesting, because a non-charged residue at this position is characteristic of some non L-type channels (eg, Ca_v2.3). Accession numbers for sequences are: SmCa_v1A, AF361884; SmCa_v1B, Smp_159990; *S. japonicum*, Sjp_0099010; *Fasciola* (*F. hepatica*), *Schmidtea* (*S. meditteranea*), and *Bdelloura* (*B. candida*) are from DNA fragments we sequenced; *Loligo* (*L. bleekeri*), D86600; *Lymnaea* (*L. stagnalis*), AF484081; *Cyanea* (*C. capillata*), AAC63050; *C. elegans*, AAC47755; *Drosophila*, AAA81883; Ascidian (*Halocynthia roretzi*), BAA34927; Carp (*Cyprinus carpio*) Ca_v1.1, P22316; Rabbit (*Oryctolagus cuniculus*) Cav1.2, NM_001136522. B. SmCa_v1A contains one fewer positively charged residue in II-S4. Shown are residues in the fourth transmembrane region of domain II from SmCa_v1A and rat Ca_v1.2 (M67515). SmCa_v1A contains one fewer positively charged residue than Ca_v1.2. Positively charged arginines (R) and lysines (K) are highlighted in blue, and the substituted glutamine (Q) in SmCa_v1A is highlighted in red.

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Sidebar 1

Predicted Ca_v channel subunits in the *S. mansoni* genome

Accession number	Putative subtype	Predicted size (kDa)
1. Smp_020170	Non L-type α1 ($SmCa_v2A$)	236
2. Smp_004730	Non L-type α1 ($SmCa_v2B$)	156*
3. Smp_020270	L-type α1 ($SmCa_v1A$)	181
4. Smp_159990	L-type α1 ($SmCa_v1B$)	230
5. Smp_134050	$\alpha_2\delta$	129
6. Smp_124530	$\alpha_2\delta$	48*
7. Smp_135140	β ($SmCa_v\beta_{var}$)	88
8. Smp_141660	β ($SmCa_v\beta$)	56**

*partial sequence

**the gene prediction algorithm excludes the NPAM-containing N-terminus