Functional expression of a glutamate-gated chloride channel (GLC-3) from adult *Brugia malayi.*

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Abstract

Parasitic worms are causative agents for six highly prevalent neglected tropical diseases of humans which include ascariasis, lymphatic filariasis, schistosomiasis, trichuriasis, hookworm infection, and onchocerciasis that affect a high percentage of the world's population. Resistance to available anthelminthic drugs especially for the benzimidazole anthelmintic agents (e.g., albendazole and mebendazole) and ivermectin is a serious concern (this is a real problem for veterinary medicine and a growing concern in human medicine). Previous studies involving ivermectin have shown that it has a limited action against adult filarial worms. Here, we describe the functional expression of a glutamate-gated chloride channel (GLC-3) from adult *Brugia malayi*, a filarial parasite. We expressed GLC-3 in *Xenopus laevis* oocytes and used two-electrode voltage-clamp electrophysiology to study the resulting ion channel. Application of various receptor agonists (1 mM): L-aspartate, glycine, γ-aminobutyric acid (GABA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) failed to activate the GLC-3 receptor. Application of 1mM L-glutamate and ibotenate produced robust inward currents. Further experiments revealed that GLC-3 is a glutamate-gated channel with an EC_{50} of 64.8 ± 4.01 µM and a Hill coefficient of 2.56 ± 0.46 µM. We recorded the responses to 300 µM L-glutamate over a range of holding potentials (-80 to +20 mV) and constructed a current-voltage plot. The current-voltage relationship revealed a reversal potential (E_{rev}) of -35.3 ± 3.2 mV indicating the GLC-3 channel is selective for chloride ions. The GluCl channel antagonists picrotoxin and fipronil had negligible inhibitory effects on the L-glutamate responses. We investigated the ivermectin effects on the GLC-3 receptor which reveals ivermectin to be an agonist with an EC_{50} of 3.6 nM (pEC_{50} = -2.4 ± 0.4). Interestingly, we also observed that Ivermectin has an inhibitory effect on the GLC-3 glutamate response at very low concentrations, IC_{50} = 73 pM (pIC_{50} = -4.1 ± 11.4). This is in stark contrast to the previously reported positive allosteric (PAM) effects of ivermectin on many invertebrate GluCls.

Keywords: Glutamate-gated chloride channel; *Brugia malayi;* Ivermectin, Picrotoxin; Fipronil; Two-electrode voltage-clamp electrophysiology.

Abbreviations: GluCls, glutamate-gated chloride channels; ML, macrocyclic lactone; LGICs, ligand-gated ion channels; *Bma*, Brugia malayi; I-V, current-voltage; E_{rev}, reversal potential; PCR, polymerase chain reaction; NCBI, National Center for Biotechnology Information; NEB, New England BioLabs; $GABA_A$, γ-aminobutyric acid type A; AMPA, α-amino-3-hydroxy-5-methyl-4-

isoxazolepropionic acid; NMDA, N-methyl-D-aspartate; IVM, ivermectin; PTX, picrotoxin; FIP, fipronil; DMSO, dimethyl sulfoxide.

Introduction

Parasitic nematodes are responsible for many diseases in humans and animals as well as infecting crops affecting global economic growth and production. These nematodes are the causative agents for highly prevalent neglected tropical diseases in tropical and subtropical regions of the world (Garcia, 2007). Lymphatic filariasis causes a high burden of disease, with more than 120 million individuals globally infected (NIAID, 2007). Filarial nematodes (e.g., *Brugia malayi, Brugia timori, and Wuchereria bancrofti*) are predominantly responsible for lymphatic filariasis (Rick et al., 2011). The filarial worms are transmitted through the bite of insect vectors Such as members of *Anopheles*, *Culex*, *Aedes*, *Mansonia*, and *Ochlerotatus* genera of mosquitoes (Bobbi, 2015).

In sub Saharan countries such as Burkina Faso, Niger, Nigeria, Ghana, Togo, and Uganda, efforts to control neglected tropical diseases are in progress. However, there are challenges to eradicating these diseases which include proper assessment of safety, compatibility, and amenability (Hotez et. al., 2007). The World Health Organization (WHO) launched the Global Program to Eliminate Lymphatic Filariasis (GPELF) in the year 2000, with the aim to eliminate lymphatic filariasis as a public health problem by 2020. While, the GPELF also ensures morbidity management and disability prevention (MMDP), mass drug administration (MDA) has been effective for control of infection such as the use of Diethylcarbamazine and albendazole for Lymphatic filariasis.

Glutamate-gated chloride channels (GluCls) are members of the Cys-loop ligand-gated ion channel family present in neurons and myocytes. Glutamate-gated chloride channels have been described to be conserved in invertebrate species. They play major roles in invertebrate nervous systems, by controlling locomotion, regulating feeding and mediating sensory inputs. Although GluCls are only found in invertebrates (Cleland, 1996), they are closely related to vertebrate γ-aminobutyric acid-A $GABA_A$ and glycine receptors (Vassilatis et. al., 1997). GluCls are targets for the avermectin/milbemycin (A/M) family of anthelmintics such as ivermectin that cause paralysis of pharyngeal muscles in gastrointestinal nematodes. Ivermectin is also useful for filarial control programs because of its activity against microfilariae that often cause ocular disease and severe dermatitis (Ben et. al., 2014). The invertebrate GABA-gated chloride channels are also targeted by insecticides such as the phenylpyrazole, fipronil, providing further evidence of the importance of the ligand-gated anion channel superfamily in pest control (Lucy et. al., 2001). GluCls are also targets of the avermectin/milbemycin (A/M) family as endectocides and insecticides (Cully et al., 1994; Dent et al., 2000).

Adult *B. malayi* are mainly found in the lymphatic vessels of humans, causing lymphatic filariasis, sometimes characterized with swelling of the limbs (elephantiasis). *B. malayi* can be maintained in the laboratory, unlike the more common cause of lymphatic filariasis, *Wuchereria bancrofti,* which makes the former a suitable model parasite (Verma et. al., 2017).

Most studies involving Ivermectin have shown that it has a limited action against adult filarial worms and is not an effective adulticide *in vivo*. The objective of this study was to examine the functional expression of a GluCl (Bma-GLC-3) from adult *B. malayi*. We directly measured the effects of IVM on application of L-glutamate to oocytes expressing the GLC-3 receptor.

Materials and Methods

Cloning of B. malayi glc-3

Total RNA from 3 adult female *B. malayi* was extracted using TRIzol Reagent™ (Invitrogen™, Carlsbad, CA, USA) and cDNA was synthesized from the total RNA using oligo(dT)$_{12-18}$ as primers. Reagents used were from the SuperScript™ IV VILO™ Master Mix (Invitrogen™, Carlsbad, CA, USA). cDNA was used as template for PCR full length amplification of the *B. malayi glc-3* sequence (WormBase ID: 8050) using Phusion® High Fidelity DNA Polymerase (Thermo Scientific, Carlsbad, CA, USA) at an annealing temperature of 55°C and for 40 PCR cycles. Using the online NEB Tm calculator, specific primers were designed and used for PCR amplification of *Bm-glc-3* (forward primer: 5'-AAA*gaattc***CCACC**ATGCTTCTTCCGC ACTACG -3'; reverse primer:5'-AAA*gggccc*TTATGTTGGTCTTGATA CCGG -3'). Restriction enzyme*EcoRI* (*gaattc*) and *ApaI* (*gggccc*) sites were respectively added to the 5' end of the forward and reverse primers to enable sub-cloning into the pTB 207 expression vector. For optimal translation of *Bma-glc-3,* the Kozac motif (**CCACC**) was also added to the start of the forward primer. The resulting PCR products were digested with restriction enzymes *EcoRI* and *ApaI* and gel-purified using the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel Inc., Bethlehem, PA, USA). The purified PCR products were then ligated into the pTB207 expression vector which was linearized by *EcoRI* and *ApaI* restriction enzymes. The final constructs were sequenced with pTB207 vector primers (forward, T7) and (reverse, SP6) and only positive clones were used for cRNA synthesis.

cRNA Preparation for oocyte injection

cDNA/pTB207 plasmids were linearized using *PstI* at the 3' end of the untranslated *Xenopus* β-globulin region. The linearized plasmids were purified using the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel Inc., Bethlehem, PA, USA) and served as template for cRNA transcription using the T7 RNA polymerase and a mMessage mMachine *in vitro* transcription kit (Invitrogen TM, Carlsbad, CA, USA). The quality of cRNA was checked on a 1% agarose gel and quantified by absorption microscopy. cRNA was stored at – 80^{o}C until needed for microinjection into *Xenopus* oocytes.

Oocyte preparation and microinjection

De-folliculated *Xenopus laevis* oocytes obtained from Ecocyte Bioscience (Austin, TX, USA) were injected with 15-60 ng of

Bma–glc-3 cRNA in 50 nl in nuclease-free water as previously described (Abongwa et al., 2016). Briefly, each oocyte was injected at the animal pole cytoplasmicaly with cRNA using a nanoject II microinjector (Drummond Scientific, PA, USA). Injected oocytes were incubated in sterile oocyte incubation solution (88 mM NaCl, 1 mM KCl, 0.4 mM $CaCl_2 \cdot 2H_2O$, 0.33 mM $Ca(NO_3)_2$, 0.8 mM $MgSO_4$, 5 mM Tris-HCl, 2.4 mM $NaHCO_3$, 2.5 mM Na pyruvate, 100 U/mL penicillin, 100 µg/mL streptomycin, pH 7.4), one oocyte per well of 96-well culture plates. The oocytes were then incubated at 19^{o}C for 2-7 days to allow proper receptor expression with replacement of incubation solution at 24 hours intervals.

Chemicals

L - glutamate, L - aspartate, glycine, γ - aminobutyric acid (GABA), α - amino - 3 - hydroxy - 5 - methyl - 4 - isoxazolepropionic acid (AMPA), N - methyl - D - aspartate (NMDA), ivermectin, picrotoxin and fipronil were purchased from Sigma - Aldrich (St Louis, MO, USA). Ibotenic acid (Ibotenate), kainic acid (kainate) and quisqualic acid (quisqualate) were obtained from Tocris Bioscience (Bio - Techne Corporation, Minneapolis, MN, USA). All chemicals were dissolved in recording solution (100 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2.2H_2O$ and 5 mM HEPES, pH 7.3), except ivermectin, picrotoxin and fipronil which were dissolved in DMSO.

Electrophysiology recordings

Membrane current measurements of oocytes expressing *Bma*–glc-3 were made using the two-electrode voltage-clamp electrophysiology technique as described by Abongwa et al. (2016). Microelectrodes were pulled using a Flaming/Brown horizontal electrode puller (Model P - 97; Sutter Instruments, Novato, CA, USA). The pulled microelectrodes were filled with 3 M KCl, and their tips were cautiously broken with tissue paper to attain a resistance of 2-5 MΩ in recording solution. Each oocyte was positioned in an oocyte recording chamber and impaled with two microelectrodes. An Axoclamp 2B amplifier (Warner Instruments, Hamdem, CT, USA) was used for recording oocytes voltage-clamped at –80 mV except when otherwise stated. Non-injected oocytes served as control for these experiments. Data were stored on a computer using Clampex 9.2 (Molecular Devices, Sunnyvale, CA, USA).

Drug applications

L - glutamate, L - aspartate, glycine, GABA, AMPA, NMDA, ibotenate, kainate and quisqualate were tested as agonists at an initial concentration of 1 mM. Each chemical was applied for 5 s, after that, a wash period of 2 mins with recording solution in between the drug applications was allowed, to reduce the effects of desensitization. A dose-response relationship for L-glutamate was generated by first challenging the oocytes with a control application of 1 mM L-glutamate for 5 s, followed by increasing concentrations of L - glutamate (3 µM to 1 mM), and 2 mins wash with recording solution in between applications.

GLC-3 responses to 5 s applications of 300 µM L-glutamate were recorded over a range

of holding potentials (–80 to +20 mV). The oocyte was held at a membrane potential of –80 mV and 300 μM glutamate was applied for 5 s. This was followed by a 2-3 mins wash with recording solution after which the glutamate application was repeated at –60, –40, –20, 0 and +20 mV. Again, a 3 mins wash with recording solution was allowed between drug applications.

For studies on the antagonists' fipronil and picrotoxin, oocytes were analyzed in different antagonist test concentrations. Briefly, 300 μM L-glutamate was applied followed by a 2-minute wash, antagonist (0.3 μM-100 μM) was applied for 30 seconds before 300 μM L-glutamate was applied again (5 s). After a 2-minute wash period the process was repeated with a higher antagonist concentration. The amplitude of the response to co-application of antagonist and L-glutamate was normalized to the values obtained in the presence of 300μM L-glutamate in physiological saline.

Concentration-response relationships for ivermectin, were performed by first challenging the oocytes with L-glutamate at concentrations of 10μM, 100μM and 1mM for 5 s, ivermectin (1 ρM – 0.1 μM) was then constantly perfused for 5 minutes before re-probing with the 3 glutamate concentrations. We measured the amplitude of the ivermectin induced currents at various concentrations to determine the dose-response relationship for ivermectin as an activator of the receptor. We also compared the glutamate responses in the presence of ivermectin with controls to determine the dose-response relationship for ivermectin as an inhibitor of glutamate responses.

Data analysis

We used Clampfit 10.2 (Molecular Devices, CA, and USA) and GraphPad Prism 5.0 (Graphpad Software Inc., CA, USA) to analyze acquired data observed and results were presented as mean ± s.e.m. For agonist rank order potency experiments, peak current responses to agonist application were normalized to a 1 mM L - glutamate response. However, for fipronil and picrotoxin antagonist experiments, peak currents evoked following drug applications were normalized to a 300 μM L - glutamate application.

Results

Sequence alignment of B. malayi Glc-3 with C. elegans Glc-3

The full length *Bma*-GLC-3 DNA sequence consists of 1461 nucleotides and encodes a protein that is 486 amino acids long. This protein has the characteristic features of a Cys loop ligand - gated ion channel (LGIC). These include: the Cys loop motif comprised of two disulphide bond forming cysteines in the N-terminal extracellular region that are separated by 13 amino acid residues and is involved in receptor assembly and channel gating; four membrane spanning regions (TM1 - TM4) which allow for passage of ions across the membrane; an intracellular domain with a role in channel conductance and receptor modulation; ligand binding loops A – F. Also present is an additional loop 'G' and a PAR motif at the very start of the TM2 region which are characteristic of ligand - gated anion channels (Green and Wanamaker, 1997; Grutter et al., 2005;

Wolstenholme, 2012). (Fig. 1). Alignment of the amino acid sequence of *Bma*-GLC-3 (WormBase ID: BM:BM8050 with the *C. elegans* GLC-3 sequence (WormBase ID: WP:CE28159) using the online EMBL - EBI Clustal Omega Multiple Sequence Alignment Tool showed a high degree of conservation, with 67.53% identity and 76% similarity.

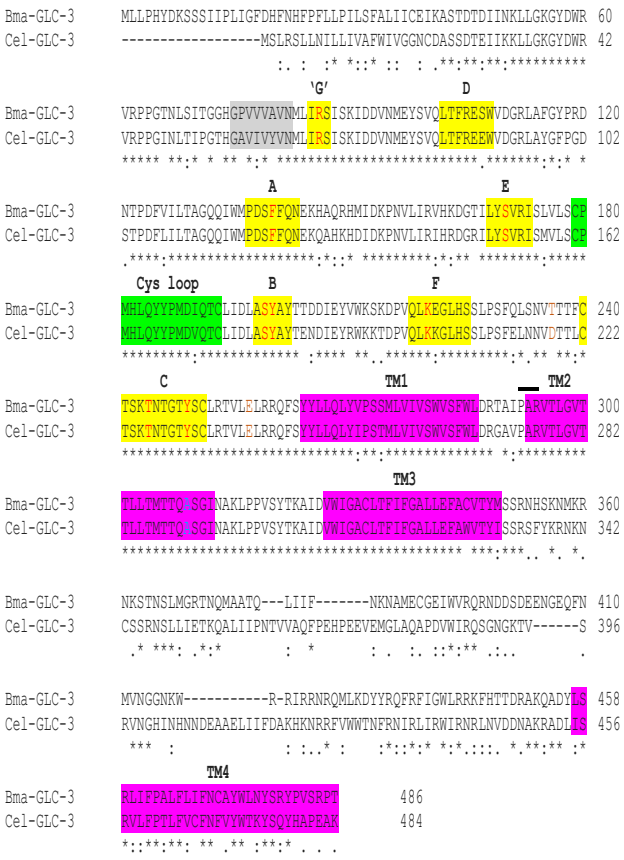


Figure 1. Amino acid sequence alignment of *B. malayi* GLC-3 with *C. elegans* GLC-3 using the EMBL-EBI Clustal O (1.2.4) Multiple Sequence Alignment online tool. Percent identity = 67.53%. Percent similarity = 76% Color code: Yellow = ligand binding loops A - 'G'; Green = Cys loop characteristic of the Cys loop ligand-gated ion channel superfamily; Purple = transmembrane regions 1 - 4 as predicted using TMHMM Server v. 2.0; Bold Line = PAR motif characteristic of ligand-gated anion channels; Residues which make contact with bound glutamate are highlighted in red; Binding loops shown following Wolstenholme et al., (2012),

Actions of L-glutamate, ibotenate L-aspartate, glycine, GABA, AMPA, NMDA, kainate and quisqualate

The application of 1 mM L-glutamate and ibotenate produced slowly desensitizing inward current responses from oocytes expressing *B. malayi* GLC-3. No current responses were observed following applications of 1 mM L-aspartate, AMPA, NMDA, kainate and quisqualate, which are analogues of L-glutamate and also vertebrate glutamate receptor agonists. Application of 1 mM glycine and GABA which are agonists of the closely related glycine and GABA receptors respectively, also did not activate the *Bma*-GLC-3 receptor. The rank order potency for agonists was: L-glutamate (100 ± 0.0., n = 13) > ibotenate (73.4 ± 2.5., n = 6) >>> kainate (0.0 ± 0.0., n = 6) = L-quisqualate (0.0 ± 0.0., n = 6) = L-aspartate (0.0 ± 0.0., n = 7) = glycine (0.0 ± 0.0., n = 7) = GABA (0.0 ± 0.0., n = 7) = AMPA (0.0 ± 0.0., n = 7) = NMDA (0.0 ± 0.0., n = 7).

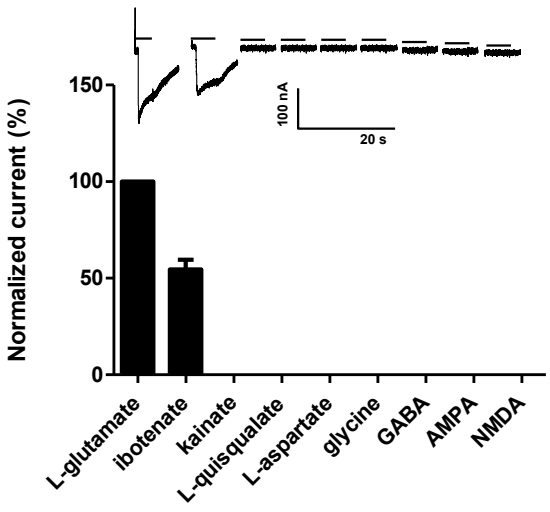


Figure 2. L - glutamate, ibotenate, kainite, L-quisqualate L - aspartate, glycine, GABA, AMPA and NMDA agonists pharmacology from oocytes expressing *B. malayi* GLC-3 at concentrations of 1 mM.

Glutamate concentration-response

The concentration-response relationship for L-glutamate was examined by applying increasing concentrations of L-glutamate from 3 µM - 1 mM to oocytes expressing *Bma*-GLC-3. We observed the slowly desensitizing inward currents following L-glutamate application to be concentration-dependent. Such effects were not observed in the non-injected control oocytes. The L-glutamate responses (normalized to 1 mM L-glutamate) were plotted using nonlinear regression and the EC_{50} was estimated to be 64.8 ± 4.01 µM and a Hill coefficient (n_H) of 2.56 ± 0.46 µM (Fig. 3).

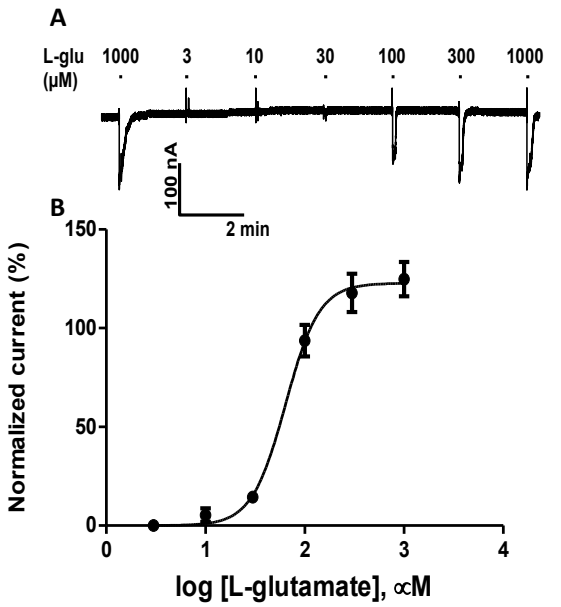
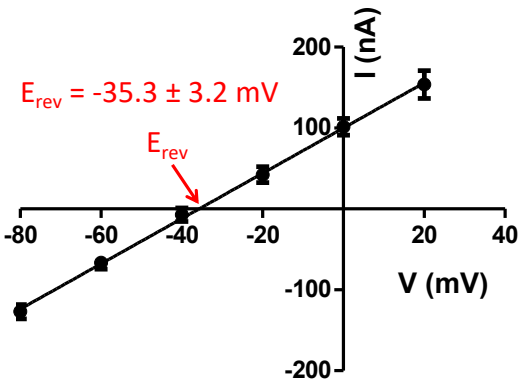


Figure 3. L-glutamate concentration-response relationship for *B. malayi*-GLC-3.
A) Representative of inward current responses from *Xenopus* oocytes expressing *Bma*- GLC-3.
B) Concentration-response plot for L-glutamate. L-glutamate EC_{50} = 64.8 ± 4.01 µM and Hill slope = 2.56 ± 0.46 µM, n = 5 - 13

Current-voltage (I-V) relationships

Recordings from 300 µM L-glutamate responses over a range of holding potentials (-80 to +20 mV) were analyzed. Thereafter, we constructed a current-voltage plot. We fitted the data using linear regression. The current-voltage relationship revealed a reversal potential (E_{rev}) of -35.3 ± 3.2 mV indicating the GLC-3 channel is selective for chloride ions. The result confirmed that GLC-3 is permeable to and selective for chloride ions. Hence, GLC-3 is a glutamate-gated chloride channel. (Fig. 4).



Ions	Equilibrium potential
Na^+	+61
K^+	-100
Cl^-	-27

Figure 4. Current-voltage relationship for the glutamate-gated currents of *B. malayi*-GLC-3 with a reversal potential (E_{rev}) of -35.3 ± 3.2 mV.

Actions of picrotoxin and fipronil

Picrotoxin (PTX) is a noncompetitive antagonist for ligand gated ion channels (LGICs), the plant-derived toxin (picrotoxinin) acts as a potent inhibitor of GABA receptors (Timothy et. al., 2013). Similarly, the phyl-pyrazole fipronil (FIP) acts by blocking chloride ion channels which are regulated by GABA receptors and also GluCls. Also, fipronil is a systemic insecticide widely used for controlling insect pests. (Simon-Delso et al., 2014; Janer et. al., 2015). We assessed the potency of PTX and FIP on expressed *Bma*- GLC-3 by testing both compounds at 0.3 to 100 µM in order to generate inhibition plots. Both PTX and FIP showed no significant inhibition at these concentrations.

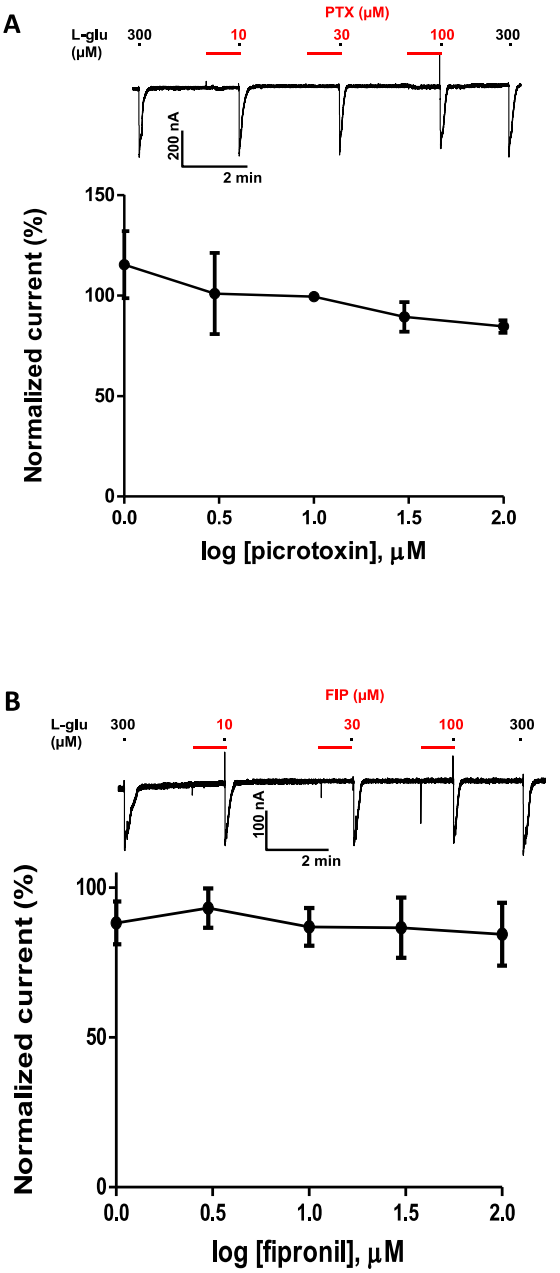


Figure 5. The effect of non-competitive antagonists, **a** picrotoxin (PTX) and **b** fipronil (Fip) on 300 µM L-glutamate responses recorded from GLC-3 homo-oligomers. For each antagonist PTX and FIP, four responses are shown 300 µM L-glutamate (control), 10 µM, 30 µM and 100 µM L-glutamate in the presence of test antagonist, 300 µM L-glutamate recovery response after a 2-5 min wash in normal saline. Concentration-response curves for the actions of each antagonist PTX and FIP, on the L-glutamate response of the GLC-3 receptor.

Actions of Ivermectin on GLC-3 receptor

We measured the effect of ivermectin as a direct activator of GLC-3 by calculating the inward current induced after 2 minutes perfusion at various concentrations. The resulting ivermectin dose-response relationship reveals an inward ivermectin sensitive current with an EC_{50} of 4.0 nM (Figure 6A). We observed that ivermectin inhibited glutamate responses. The inhibitory actions of ivermectin (IVM) on GLC-3 responses to concentrations of 10 µM, 100 µM and 1000 µM L-glutamate were dose-dependent. Figure 6B shows the dose-response relationship for ivermectin inhibition of the 1000 µM glutamate response with an IC_{50} of 73 pM (pIC_{50} = -4.1 ± 11.4).

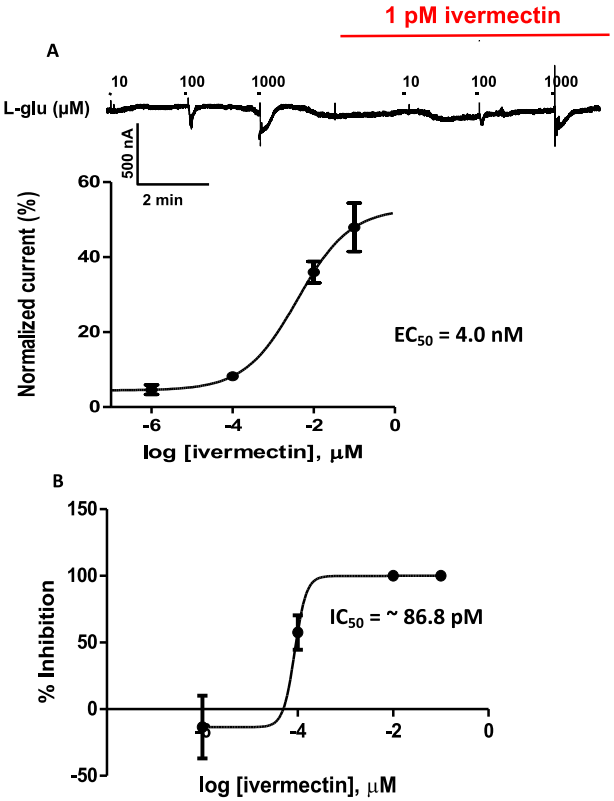


Figure 6. (A) Concentration-response curves for the actions of ivermectin (IVM) at concentration of 10 µM, 100 µM and 1000 µM L-glutamate (B) Inhibitory effect of 1pM Ivermectin on 1000 µM L-glutamate responses.

Discussion

Functional expression of Bma-GLC-3

There have been previous accounts of the glutamate gated chloride channel subunit (GLC-3) as one of the primary targets of the macrocyclic lactone anthelmintics in several parasites such as Cyathostomin species (Krystyna et al., 2013). Here we report that GLC-3 from *B. malayi* expresses as a functional homomeric channel in *Xenopus* oocytes.

The alignment of *B. malayi* GLC-3 amino-acid sequence with that of the soil nematode *C. elegans* GLC-3 present an identity of 67.53%. These comparative analyses offer a complementary approach to extensively analyze our parasite of focus (*Brugia malayi*). Our results also show that the application of L-glutamate to *Bma*-GLC-3 yielded a slowly desensitizing concentration-dependent inward currents compared to the *Bma*-AVR-14b channel where the inward currents desensitize rapidly.

In this present study, we applied L-glutamate at concentrations of 3µM - 1 mM to Xenopus oocytes expressing *Bma*-GLC-3 to determine the concentration-response relationship for L-glutamate. The recordings gave rapid desensitizing inward currents following L-glutamate application. The EC_{50} and Hill coefficient were 64.8 ± 4.01 µM and 2.56 ±

0.46 µM respectively. For the agonist experiment, the rank order potency for L-glutamate was 100 ± 0.0., n = 13 and ibotenate was 73.4 ± 2.5., n = 6. While, for other agonists tested no responses were observed. This is similar to a previous report by Horoszok et. al. (2001) from *C. elegans* GLC. They reported that L-glutamate and ibotenate activated the GLC-3 receptor at 1mM, but ibotenate produced currents of a smaller amplitude and slower onset than L-glutamate.

Furthermore, we analyzed the current produced by 300 µM L-glutamate over a voltage range of –80 to +20 mV at glutamate-gated current of *B. malayi*-GLC-3. From a plot of current (I) against voltage (V), the current-voltage (I-V) relationship was observed and a reversal potential (E_{rev}) of -35.3 ± 3.2 mV was calculated by linear regression. This result shows that GLC-3 channel is selective for chloride ions.

Bma-GLC-3 is insensitive to fipronil and picrotoxin

Fipronil and PTX are non-competitive antagonists of ionotropic receptors containing chloride channels. The interactions of these non-competitive antagonists with ligand-gated chloride channels has revealed some interesting pharmacology of the Bma-GLC-3 receptor. In our experiments, we observed that the GLC-3 channels from *Brugia malayi* are insensitive to fipronil and picrotoxin at concentrations up to 100 µM. In contrast, previous experiments on Cel-GLC-3 revealed the channel to be inhibited by fipronil with an IC50 of 11.5 ± 0.11 µM but picrotoxinin-insensitive (Horoszok et. al., 2001).

Ivermectin effects on GLC-3 receptor

Ivermectin is a member of the macrocyclic lactones anthelmintic class. GluCls are targets for avermectin/milbemycin (A/M) family of anthelmintics. Ivermectin binds to glutamate-gated chloride channels (GluCls) in the membranes of invertebrate nerve and muscle cells, causing increased permeability to chloride ions, enhancing inhibitory neurotransmission, resulting in cellular hyper-polarization followed by paralysis and death.

Our results using ivermectin showed it directly active Bma-GLC-3 with an EC_{50} of 3.6 nM (pEC_{50} = -2.4 ± 0.4). Further analysis shows an inhibitory effect of Ivermectin on 1mM glutamate responses, IC_{50} of 73 pM (pIC_{50} = -4.1 ± 11.4). This is in stark contrast to the previously reported positive allosteric (PAM) effects of ivermectin on many invertebrate GluCls.

Studies relating to Ivermectin have shown that ivermectin has a limited action against adult filarial worms. Preliminary study using the worminator motility technique to show how Adult *Brugia malayi* move in the presence of Ivermectin also confirmed the poor action of Ivermectin on the adult filarial worm. However, IVM is more effective on microfilarae of *Brugia malayi*. A previous report by Yovany et. al., (2010) revealed that IVM treatment for filarial infections is characterized by an initial dramatic drop in the levels of circulating microfilariae,

followed by long-term suppression of their production.

Conclusion

The objective of this study was to examine the pharmacological properties of GLC-3 from adult *B. malayi* expressed in *Xenopus* oocytes. Our results show the complex mode of action of IVM. Ivermectin acts as a direct activator of Bma-GLC-3, similar to its effects on Cel-GLC-3 (Horoszok et. al., 2001).
Interestingly, ivermectin also acts as an inhibitor of Bma-GLC-3 glutamate responses, this is the opposite of its PAM effects on GLCs from other invertebrate species (Horoszok et. al., 2001).
It can be suggested that the GLC-3 channel from filarial adults may have a reduced sensitivity to ivermectin compared to other GluCls receptors from nematode species where the drug is effective. GLC-3 might co-assemble with one or more of the other GluCl subunits to form a component of the parasite GluCl receptor. Our results enhance our overall understanding of the glutamate-gated chloride channels in filarial nematodes.

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