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Whole-cell patch-clamp recording of nicotinic acetylcholine receptors in adult *Brugia malayi* muscle

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Abstract

Lymphatic filariasis is a debilitating disease caused by clade III parasites like *Brugia malayi* and *Wuchereria bancrofti*. Current recommended treatment regimen for this disease relies on albendazole, ivermectin and diethylcarbamazine, none of which targets the nicotinic acetylcholine receptors in these parasitic nematodes. Our aim therefore has been to develop adult *B. malayi* for electrophysiological recordings to aid in characterizing the ion channels in this parasite as anthelmintic target sites. In that regard, we recently demonstrated the amenability of adult *B. malayi* to patch-clamp recordings and presented results on the single-channel properties of nAChR in this nematode. We have built on this by recording whole-cell nAChR currents from adult *B. malayi* muscle. Acetylcholine, levamisole, pyrantel, buphenium and tribendimidine activated the receptors on *B. malayi* muscle, producing robust currents ranging from > 200 pA to ~1.5 nA. Levamisole completely inhibited motility of the adult *B. malayi* within 10 min and after 60 min, motility had recovered back to control values.

Keywords

Brugia malayi; acetylcholine receptor; anthelmintic

The Neglected Tropical Diseases (NTDs) are a group of parasitic, bacterial, fungal and viral infections that include lymphatic filariasis and soil-transmitted helminth infections (Hotez *et al.*, 2007). Lymphatic filariasis, also known as elephantiasis, is a debilitating disease caused by adult *Brugia malayi*, *Brugia timori* or *Wuchereria bancrofti* lodging in the lymphatic system of the hosts. Although the disease is largely asymptomatic, acute and chronic infections results in blockage of the lymphatic vessels and fluid accumulation (WHO, January 2012). About 1.3 billion people are at risk of suffering from lymphatic filariasis. Anthelmintics are the main chemotherapeutic agents used for treating parasitic nematode infections because there are currently no effective vaccines on the market. The treatment regimen recommended by the World Health Organization for lymphatic filariasis is albendazole with either diethylcarbamazine or ivermectin administered as a single dose through mass drug administration (MDA) (Ottesen *et al.*, 2008). These drugs help in eliminating circulating microfilariae but do not kill adult parasites. Although MDA is preferable for prevention and treatment of parasitic nematode infections, there are concerns about the development of resistance. Anthelmintic resistance has been reported to the

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mainstay anthelmintics, namely the cholinergic agonists/antagonists, benzimidazoles and macrocyclic lactones in several species of veterinary parasite (Wolstenholme *et al.*, 2004). There is therefore an urgent need to improve the use of existing anthelmintics, anthelmintic combinations and to develop new ones.

Recent introduction of novel nicotinic anthelmintics to the market has both increased the significance and emphasized the importance of nicotinic acetylcholine receptors as anthelmintic target sites. Tribendimidine has been approved for human use in China (Xiao *et al.*, 2005) and has the potential for single-dose MDA (Zhang *et al.*, 2008). Derquantel, a nAChR antagonist (Robertson *et al.*, 2002) has been formulated with abamectin and marketed as Startect®, and last but not least, monepantel (Kaminsky *et al.*, 2008). Nicotinic AChRs on nematode muscles are also the target for ‘old’ anthelmintics like levamisole and pyrantel. The recent publication of *B. malayi* draft genome has permitted identification of nAChR subunit genes. According to Williamson *et al.* (2007), there are apparently a reduced number of nAChR subunit genes in the *B. malayi* genome, with orthologues of the nAChR subunit genes *lev-1* and *lev-8* absent. Interestingly, *lev-8* has not been found in the trichostrongylid parasites *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* and the role of *lev-1* in forming a receptor has been questioned as *lev-1* seems to lack a signal peptide in these parasites (Neveu *et al.*, 2010). There are molecular similarities between *B. malayi* and *Ascaris suum*, both belonging to phylogenetic clade III (Blaxter *et al.*, 1998). Single-channel recordings have resulted in the identification of N-, L- and B-subtypes of the nAChR present on *A. suum* somatic muscles (Qian *et al.*, 2006).

Our aim recently has been to demonstrate that adult *B. malayi* are amenable to electrophysiological recordings and to engender interest in characterizing the nAChRs in this parasite. In that regard, we have demonstrated that adult *B. malayi* can be dissected to expose muscle cells for single-channel patch-clamp recordings (Robertson *et al.*, 2011). We recorded single-channel currents from this parasite and observed that the percentage of *B. malayi* membrane patches containing nAChR events were comparable to what was obtained in *A. suum* but unlike in the latter parasite, a narrow range of single-channel conductance values were observed for *B. malayi*. In order to obtain a pharmacological profile of the nicotinic agonists/anthelmintics that can open these channels on *B. malayi* muscles, we have used the whole-cell patch-clamp technique. Live adult female *B. malayi* were shipped overnight from the National Institutes of Health/National Institute of Allergy and Infectious Disease (NIH/NIHAI) Filariasis Research Reagent Resource Centre (FR3; College of Veterinary Medicine, University of Georgia, USA). Adult females were maintained in RPMI 1640 medium supplemented with 1% penicillin–streptomycin changed daily. The parasites were held in an incubator at a temperature of 37 °C. Adult worms were used for experiments up to 7 days post delivery. The dissection of *B. malayi* to expose muscle cells for recordings, collagenase treatment and the pipettes used for the whole-cell recordings are unchanged from our previous report (Robertson *et al.*, 2011). As figure 1 shows, the dissection exposed clear, strap-like muscle cells. The bath solution used was, in mM: NaCl 23, NaAc 110, KCl 5, CaCl₂ 6, MgCl₂ 4, HEPES 5, D-glucose 11, and sucrose 10, pH adjusted to 7.2 with NaOH. The pipettes were filled with a pipette solution of composition KCl 120 mM, KOH 20 mM, MgCl₂ 4 mM, Tris 5 mM, CaCl₂ 0.25 mM, NaATP 4 mM, sucrose 36 mM, EGTA 5 mM, pH adjusted to 7.2 with KOH. The preparation was continuously perfused with bath solution at 1 ml/min. We applied 30 µM of the drugs for 5 sec by perfusion. Stock concentrations of the drugs were made in bath solution, except bephenium and tribendimidine which were made in DMSO. The final concentration of DMSO in the bephenium and tribendimidine containing perfusate was 0.1%. An Axopatch 200B amplifier (Axon Instruments Inc., Union City, CA, USA) was used to amplify the current signals which were filtered at 2 kHz (3-pole Bessel filter) and sampled at 25 kHz. The signals were

digitized with a Digidata 1320A (Axon Instruments Inc.) and stored on a computer hard disk.

We observed responses to all the drugs we tested, namely acetylcholine and the cholinergic anthelmintics levamisole, pyrantel, buphenium and tribendimidine. Acetylcholine (ACh), supposedly the main excitatory neurotransmitter in this parasite, generated the largest response with average currents of 1504 ± 364 pA ($n = 7$). Individual ACh responses were normalized to this value as were all other agonist responses to allow for statistical comparison. Tribendimidine was the second most potent agonist and produced average currents of 1316 ± 65 pA, 87.5 ± 4.3 % of ACh currents (Fig. 2A). Pyrantel and levamisole produced average currents of 75.2 ± 28.6 % ($p > 0.05$, paired *t*-test, $n = 4$, Fig. 2A) and 67.4 ± 8.1 % ($p > 0.05$, paired *t*-test, $n = 3$, Fig. 2A) that of ACh, respectively. Buphenium produced the smallest current response of 266 ± 125 pA, 17.7 ± 8.3 % of ACh currents ($p < 0.05$, paired *t*-test, $n = 4$, Fig. 2A). Next, we investigated the effect of levamisole on the motility of adult *B. malayi*. Adult worms with 'normal' motility were selected for this assay. Normal motility was given a score of 5 and described as worms with active, thrashing-like movement. The worms were placed in separate plates filled with $30 \mu\text{M}$ levamisole and their motility scored at 5, 10, 30 and 60 min. Because this is a subjective assay, two experienced researchers independently scored the worms. The motility of the adult *B. malayi* in the presence of levamisole dropped by 85 % in 5 min ($p < 0.001$, $n = 4$, student *t*-test, Fig. 2B) and in 10 min, the worms were completely paralyzed ($p < 0.001$, $n = 4$, student *t*-test, Fig. 2B). The motility of the worms however returned to near control values after 60 min ($p > 0.05$, $n = 4$, student *t*-test) in the drug.

In contraction assay and electrophysiological recordings from *A. suum* muscle, pyrantel > buphenium > levamisole > acetylcholine (Harrow and Gratton, 1985; Martin *et al.*, 2004; Trailovic *et al.*, 2008). In reconstituted *Haemonchus contortus* L-AChRs, levamisole > acetylcholine > pyrantel in Hco-L-AChR1 subtype and in the Hco-L-AChR2 subtype, pyrantel > acetylcholine > levamisole (Boulin *et al.*, 2011). In the *B. malayi* L-AChR, acetylcholine > tribendimidine > pyrantel $\approx$ levamisole > buphenium. Our results show that the body muscle nAChR receptor of *B. malayi* differs from those of other parasites but resembles that of *C. elegans* (Boulin *et al.*, 2008). This needs further investigation. Although levamisole has been shown to be effective in killing *B. malayi*, it's less effective on adult filariae and also less effective than DEC (Mak *et al.*, 1974; Mak and Zaman, 1980). Desensitization of the *B. malayi* body muscle nAChR may be a reason for the reduced effects of levamisole on the adult worms. Last but not least, we suggest that there are likely to be other subtypes of the nAChR in *B. malayi*. We have demonstrated through whole-cell patch-clamp recordings that nAChRs on adult *B. malayi* muscle cells are activated by the cholinergic anthelmintics levamisole, pyrantel, buphenium and tribendimidine. Levamisole reversibly paralyzed the adult worms.

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Highlights

- We report whole-cell patch-clamp recording of adult *Brugia malayi* muscle nAChR.
- Agonist potency was acetylcholine>tribendimidine>pyrantel≈levamisole>bephenium.
- Levamisole reversibly inhibited motility of adult *B. malayi*.
- Adult *B. malayi* muscle nAChR differ in pharmacology from *Ascaris suum*.
- Reduced effect of levamisole on adult *B. malayi* may be due to desensitization.

Figure 1. Whole-cell patch-clamp of *B. malayi* muscle cells. Top: Micrograph of dissected adult female showing the muscle cells (m) and arms (a) projecting to the nerve cord. Below: Diagrammatic representation of the whole-cell patch-clamp.

Figure 2.

(A) Bar chart (mean $\pm$ s.e) of normalized currents produced by the nAChR agonists/anthelmintics (30 μ M) on *B. malayi* muscles in whole-cell patch-clamp. All current response was normalized to ACh currents (* $p < 0.05$, paired *t*-test). Displayed above the bars are sample whole-cell current traces. Scale bar, horizontal 30 secs, vertical 700 pA (B) Plot of motility vs time (min) of adult female *B. malayi* in the absence and presence of 30 μ M levamisole. 4 worms/treatment were used for the assay. Comparisons of motility were made between control and treated worms at each time point, ** $p < 0.01$, *** $p < 0.001$.