International Journal for Parasitology 39 (2009) 315–326

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Effects of SDPNFLRF-amide (PF1) on voltage-activated currents in *Ascaris suum* muscle

S. Verma, A.P. Robertson, R.J. Martin *

Department of Biomedical Science, Iowa State University, Ames, IA 50011, USA

ARTICLE INFO

Article history:
Received 12 May 2008
Received in revised form 20 June 2008
Accepted 13 July 2008

Keywords:
PF1
AF3
Ascaris suum
Voltage-activated currents
Calcium currents
Potassium currents

ABSTRACT

Helminth infections are of significant concern in veterinary and human medicine. The drugs available for chemotherapy are limited in number and the extensive use of these drugs has led to the development of resistance in parasites of animals and humans (Geerts and Gryseels, 2000; Kaplan, 2004; Osei-Atweneboana et al., 2007). The cyclooctadepsipeptide, emodepside, belongs to a new class of anthelmintic that has been released for animal use in recent years. Emodepside has been proposed to mimic the effects of the neuropeptide PF1 on membrane hyperpolarization and membrane conductance (Willson et al., 2003). We investigated the effects of PF1 on voltage-activated currents in *Ascaris suum* muscle cells. The whole cell voltage-clamp technique was employed to study these currents. Here we report two types of voltage-activated inward calcium currents: transient peak (I_{peak}) and a steady-state (I_{ss}). We found that 1 μM PF1 inhibited the two calcium currents. The I_{peak} decreased from −146 nA to −99 nA ($P = 0.0007$) and the I_{ss} decreased from −45 nA to −12 nA ($P = 0.002$). We also found that PF1 in the presence of calcium increased the voltage-activated outward potassium current (from 521 nA to 628 nA ($P = 0.004$)). The effect on the potassium current was abolished when calcium was removed and replaced with cobalt; it was also reduced at a higher concentration of PF1 (10 μM). These studies demonstrate a mechanism by which PF1 decreases the excitability of the neuromuscular system by modulating calcium currents in nematodes. PF1 inhibits voltage-activated calcium currents and potentiates the voltage-activated calcium-dependent potassium current. The effect on a calcium-activated-potassium channel appears to be common to both PF1 and emodepside (Guest et al., 2007). It will be of interest to investigate the actions of emodepside on calcium currents to further elucidate the mechanism of action.

© 2008 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

There is a group of 13 parasitic and bacterial infectious diseases listed as neglected tropical diseases in the Millennium Declaration of the United Nations (Hotez et al., 2007). Ascariasis is the most common parasitic infection in the list, with an estimated 807 million people infected and 4.2 billion people at risk (de Silva et al., 2003; Bethony et al., 2006). Helminth infections are also a welfare and economic concern in animals (Coles, 2001; Wolstenholme et al., 2004).

Chemotherapy is widely used to control these parasitic infections. The drugs available for chemotherapy are limited in number and the extensive use of these drugs has led to the development of resistance in parasites of animals and humans (Geerts and Gryseels, 2000; Kaplan, 2004; Osei-Atweneboana et al., 2007). The cyclooctadepsipeptide, emodepside, belongs to a new class of anthelmintic that has been released for animal use in recent years (Harder et al., 2003). Emodepside has been proposed to mimic the effects of PF1 (Willson et al., 2003), an inhibitory FMRFamide like neuropeptide (FLP) in nematodes (McVeigh et al., 2006).

FLPs have been isolated from both free living and parasitic nematodes (Geary et al., 1992, 1999; Husson et al., 2005; Li, 2005; McVeigh et al., 2006). There are more than 31 nematode *flp* genes that have been identified and found responsible for the synthesis of more than 90 FLPs (McVeigh et al., 2005). FLPs are associated with all the major neuronal systems in nematodes (Stretton et al., 1991; Brownlee et al., 1996; Brownlee and Walker, 1999; Geary and Kubiak, 2005). PF1 (SDPNFLRFamide) is a peptide that was originally isolated from an acetone extract of *Panagrellus redvivius* (Geary et al., 1992). PF1 has marked paralytic and hyperpolarizing effects on *Ascaris suum* muscles (Franks et al., 1994; Bowman et al., 2002). Although PF1 has not been recovered from *A. suum*, Yew et al. (2005) have isolated related peptides with the C-terminal PNFLRFamide from *A. suum*. PF1 has been reported to antagonize the effects of acetylcholine and levamisole induced contractions (Franks et al., 1994; Geary et al., 1999). The effects of PF1 appear to be mediated by nitric oxide in *A. suum* (Bowman et al., 1995). The hyperpolarizing effect of PF1 is abolished by a combination of potassium channel antagonists and nitric oxide synthase (NOS)

* Corresponding author. Tel.: +1 515 294 2470; fax: +1 515 294 2515.
E-mail address: rjmartin@iastate.edu (R.J. Martin).

0020-7519/$34.00 - see front matter © 2008 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijpara.2008.07.007

inhibitors. It has been shown that nematode NOS is partially dependent on calmodulin and completely dependent on calcium (Bowman et al., 1995, 2002). These effects suggest a role for calcium for the mode of action of PF1.

Voltage-gated calcium channels play a major part in regulation of calcium entry from extracellular sources in nematodes (Jeziorski et al., 2000). Entry of calcium through ion channels plays an important role in the physiological processes of contraction, secretion, synaptic transmission and signal transduction pathways (Catterall et al., 2005). Voltage-gated calcium channels are modulated positively and negatively by G-protein coupled receptors in many species (Tedford and Zamponi, 2006) including neuropeptide receptors in *A. suum* (Verma et al., 2007).

In this manuscript we investigate the effects of PF1 on voltage-activated calcium and potassium currents in *A. suum* muscle cells. We found that PF1 reduced peak and steady-state inward calcium currents as well as increased voltage-activated potassium currents. These observations show that the inhibitory effects of PF1 also include effects on voltage-activated calcium currents. If PF1 does in fact mimic emodepside (Willson et al., 2003), our observations suggest that emodepside will also affect voltage-activated calcium and potassium currents (Guest et al., 2007).

2. Materials and methods

2.1. Collection of worms

Adult *A. suum* were obtained weekly from the Tyson's pork packing plant at Storm Lake City, Iowa, USA. Worms were maintained in Locke's solution (Composition (mM): NaCl 155, KCl 5, $CaCl_2$ 2, $NaHCO_3$ 1.5 and glucose 5) at a temperature of 32 °C. The Locke's solution was changed daily and the worms were used within 4 days of collection.

2.2. Muscle preparation

One cm muscle tissue flaps were prepared by dissecting the anterior part of the worm, 2–3 cm caudal to the head. A body muscle flap preparation was then pinned onto a Sylgard™-lined 2 ml Petri-dish. The intestine was removed to expose the muscle cells (Trailovic et al., 2005). The preparation was continuously perfused, unless otherwise stated, with APF-Ringer solution, composition (mM): NaCl 23, Na-acetate 110, KCl 24, $CaCl_2$ 6, $MgCl_2$ 5, glucose 11, and HEPES 5; NaOH was used to adjust the pH to 7.6. To study inward currents calcium-Ringer solution was prepared by adding 4-aminopyridine (4-AP) (5 mM) to APF-Ringer solution to reduce potassium currents and adjusting the pH to 7.6 by NaOH. The preparation was maintained in the experimental chamber at 34 °C using a Warner heating collar (DH 35) and heating the incoming perfusate with a Warner instruments (SH 27B) in-line heating system (Hamden, CT, USA). The perfusate was applied at 4–6 ml/min through a 19-gauge needle placed directly over the muscle bag recorded from. The calcium substitution experiments were conducted using cobalt-Ringer, composition (mM): NaCl 23, Na-acetate 110, KCl 24, $CoCl_2$ 6, $MgCl_2$ 5, glucose 11, HEPES 5 and 4-aminopyridine 5; NaOH was used to adjust the pH to 7.6. PF1 (1 μM) and AF3 (1 μM), were applied in APF-Ringer, calcium-Ringer or cobalt-Ringer as described in Section 3.

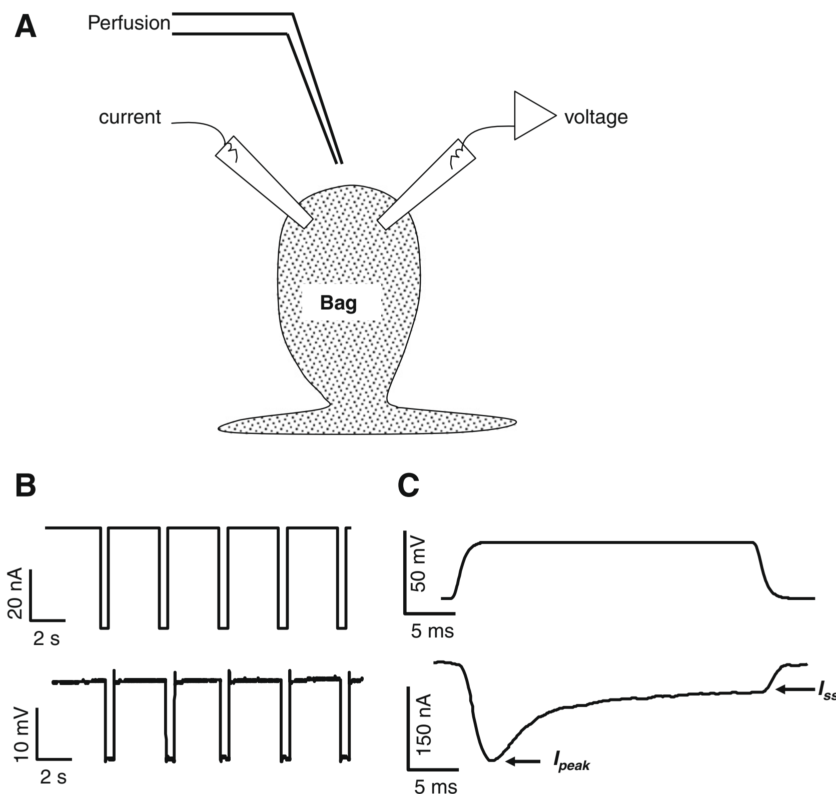

Fig. 1. *Ascaris suum* muscle bag preparation for recording current-clamp and voltage-clamp experiments. (A) Diagram of the location of two micropipettes for making current-clamp and voltage-clamp recordings from bag region of *A. suum* somatic muscle. The current-injecting pipette and the voltage-sensing pipette are shown. (B) Current-clamp recording showing 40 nA, 0.5 s, current pulses (upper trace) inducing change in membrane potential (lower trace). (C) Voltage-clamp recording showing depolarising step-voltage from a holding potential of −35 mV to 0 mV (upper trace) producing a current response (leak subtracted lower trace). Note the presence of the voltage-activated transient inward current (I_{peak}) and a sustained inward current (I_{ss}).

Download English Version:

https://daneshyari.com/en/article/2436628

Download Persian Version:

https://daneshyari.com/article/2436628

Daneshyari.com