The effect of the anthelmintic emodepside at the neuromuscular junction of the parasitic nematode *Ascaris suum*

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SUMMARY

Here we report on the action of the novel cyclo-depsipeptide anthelmintic, emodepside, on the body wall muscle of the parasitic nematode, *Ascaris suum*. Emodepside caused (i) muscle relaxation, (ii) inhibition of muscle contraction elicited by either acetylcholine (ACh), or the neuropeptide, AF2 (KHEYLRFamide) and (iii) a rapid relaxation of muscle tonically contracted by ACh. The inhibitory action of emodepside on the response to ACh was not observed in a denervated muscle strip, indicating that it may exert this action through the nerve cord, and not directly on the muscle. Electrophysiological recordings showed emodepside elicited a Ca^{++}-dependent hyperpolarization of muscle cells. Furthermore, the response to emodepside was dependent on extracellular K^+, similar to the action of the inhibitory neuropeptides PF1 and PF2 (SDPNFLRFamide and SADPNFLRFamide). Thus emodepside may act at the neuromuscular junction to stimulate release of an inhibitory neurotransmitter or neuromodulator, with a similar action to the PF1/PF2 neuropeptides.

Key words: anthelmintic, *Ascaris suum*, neuropeptide.

INTRODUCTION

Cyclo-octadepsipeptides are a new class of anthelmintic (Sasaki *et al*. 1992) which inhibit nematode motility (Samson-Himmelstjerna *et al*. 2000; Terada, 1992). The mechanism for this is poorly understood (Chen, Terada & Cheng, 1996). A previous study has shown that PF1022A, the parent compound for this class of anthelmintics, may have an action on the membrane properties of *Ascaris suum* body wall muscle (Martin *et al*. 1996). To provide further insight into this we have used *in vitro* preparations of body wall muscle of the parasitic nematode *A. suum*.

The body wall muscle of *Ascaris* receives projections from excitatory and inhibitory motorneurones. The excitatory motorneurones use acetylcholine (ACh) as their transmitter. ACh acts on nicotinic receptors on the muscle to elicit depolarization and muscle contraction (Stretton *et al*. 1985; Pennington & Martin, 1990; Colquhoun, Holden-Dye & Walker, 1991). The inhibitory motorneurones release gamma-amino butyric acid (GABA) which activates ligand-gated chloride channels on the muscle to hyperpolarize and relax muscle (Martin, 1982; Holden-Dye *et al*. 1989). In addition, both classes of motorneurone have neuropeptide-like immunostaining (Sithigorngul, Stretton & Cowden, 1990). Although the identity of the neuropeptides that co-localize with ACh and GABA remains to be determined, there are a number of peptides that have potent activity on the muscle, strongly suggesting that they, or closely related sequences, have a physiological role in muscle regulation (Brownlee, Holden-Dye & Walker, 2000).

In the context of this study, the action of the inhibitory peptides, SDPNFLRF-amide (PF1) and SADPNFLRFamide (PF2), is of particular interest. Both these peptides have been biochemically isolated from 2 species of nematode (Geary *et al*. 1992; Rosoff *et al*. 1993), and have a potent inhibitory action on the somatic muscle of *A. suum* (Franks *et al*. 1994). At least part of this action is due to a direct effect on the muscle (Holden-Dye *et al*. 1995), suggesting that there is an inhibitory receptor for PF1-like peptides on the somatic muscle.

Here we investigate the mechanism of action of the cyclodepsipeptide, emodepside, using *in vitro* muscle preparations of *A. suum* muscle to measure effects on muscle tension and membrane potential. In particular, we address the question of whether the effect of emodepside is pre- or post-synaptic to the neuromuscular junction, and how the action of emodepside compares to the inhibitory neurotransmitter GABA and the inhibitory neuropeptide, PF2.

MATERIALS AND METHODS

The methods used were similar to those described by Trim *et al*. (1997). *A. suum* were obtained from a local abattoir and maintained for up to 5 days in artificial

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perienteric fluid (APF composition in mM, NaCl 67, CH_3COONa 67, $CaCl_2$ 3, $MgCl_2$ 15·7, KCl 3, Trizma® base 5, pH 7·6, with glacial acetic acid, 3 mM glucose at 37 °C).

Muscle tension measurements for the dorsal muscle strip

Ascaris muscle strips were prepared by dissecting a 1 cm strip of the body wall muscle immediately anterior to the genital pore. Dorsal muscle strips (DMS) were excised from this section by cutting along both lateral lines. Dorsal muscle is devoid of motorneurone somata, which are only present in the ventral nerve cord, however, it does contain the dorsal inhibitory and dorsal excitatory motorneurone terminals, which have reciprocal synapses with each other. For denervated muscle strips, the dorsal cord was removed from the muscle strip. This was obtained by cutting along a lateral line and opening up the muscle exposing both nerve cords. The muscle strip was then cut on the inner side of the nerve cords so that only body wall muscle (consisting of half of the ventral muscle field and half of the dorsal muscle field) between the nerve cords remained.

Muscle strips were placed in a 15 ml organ bath and connected by thread to a 2 g isometric transducer. The preparation was subject to a 1 g load and maintained at 37 °C with a heated water jacket. Drugs were added in volumes of no greater than 1% of the bath volume, and were rapidly mixed within the organ bath by gassing the bath with room air. Drugs were then washed out by at least 3 times the bath volume of APF. A hard copy of the data was obtained on a flat bed chart recorder (BBC, Goerz, Metrawatt, Austria).

Electrophysiological studies from dorsal muscle

The DMS was pinned cuticle side down on a Sylgard® elastomer 184 (Dow Corning Wiesbaden, Germany) lined Perspex chamber and continuously perfused with APF at 32–34 °C, directed at the *Ascaris* muscle cell via a fine bore tube. Individual muscle bags were impaled with 2 microelectrodes (10–30 MΩ; 10 mM KCl in 3 M CH_3COOK) connected to an Axoclamp 2A amplifier. One electrode was used to record membrane potential, and the second to pass current pulses (20 nA, 0·2 Hz, 500 ms). The chamber was grounded using a 3 M KCl agar bridge/AgCl electrode. Drugs were added in the perfusate for 2 min. Bath temperature was continuously monitored with a temperature probe placed adjacent to the muscle strip. Hard copy of data was recorded on a Gould model 35, 2-channel chart recorder (Gould Instruments, Ohio, USA).

Drugs and materials

Both ACh (Sigma Chemical Co., Poole, UK) and emodepside solutions were prepared on the day of the experiment. Emodepside (supplied by Bayer AG, Leverkusen, Germany) was diluted in 100% ethanol at 10^{-1} M and then diluted down in APF so that the final concentration of ethanol in the organ bath or in electrophysiology experiments was no greater than 0·1%. Neuropeptides, AF2 and PF2, were supplied by Alta Bioscience, Birmingham UK (>90% purity) and were stored at −20 °C at 10^{-3} M in 50 μl aliquots. All other drugs were obtained from Sigma Chemical Co., Poole, UK.

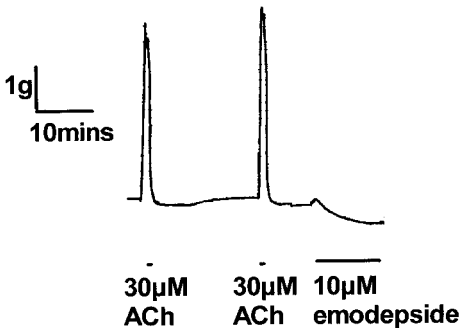

Fig. 1. Emodepside causes a relaxation of the basal tone of *Ascaris* DMS. A representative response showing relaxation of the basal tone of *Ascaris* DMS following 10 μM application of emodepside. The bars indicate duration of drug application.

Data analysis

For each individual muscle strip tension experiment a consistent response to 30 μM ACh was obtained at the beginning of the experiment. The peak contractions of the responses were measured and deemed the 'control' response. Subsequent responses were normalized with respect to the 'control' response.

To test for statistical significance, the untransformed data were used and analysed using the Student's *t*-test (two-tailed, and either paired or unpaired, as appropriate). Significance was assumed at $P<0·05$.

RESULTS

Muscle tension recordings

Emodepside, at concentrations up to 10 μM, produced a small relaxation of basal tension of the DMS ($-0·19\pm0·04$ g; $n=22$; Fig. 1). This effect was irreversible within the time-course of the experiment (i.e. no recovery after 30 min wash out). As the effect on basal tension was very small, and probably dependent on the resting tension, we further investigated the inhibitory action of emodepside against the contraction elicited by ACh. We measured the time-dependent and concentration-dependent effect of emodepside on the contraction elicited by ACh. ACh (30 μM) produced a rapid contraction of the DMS which readily reversed on washing. Application of

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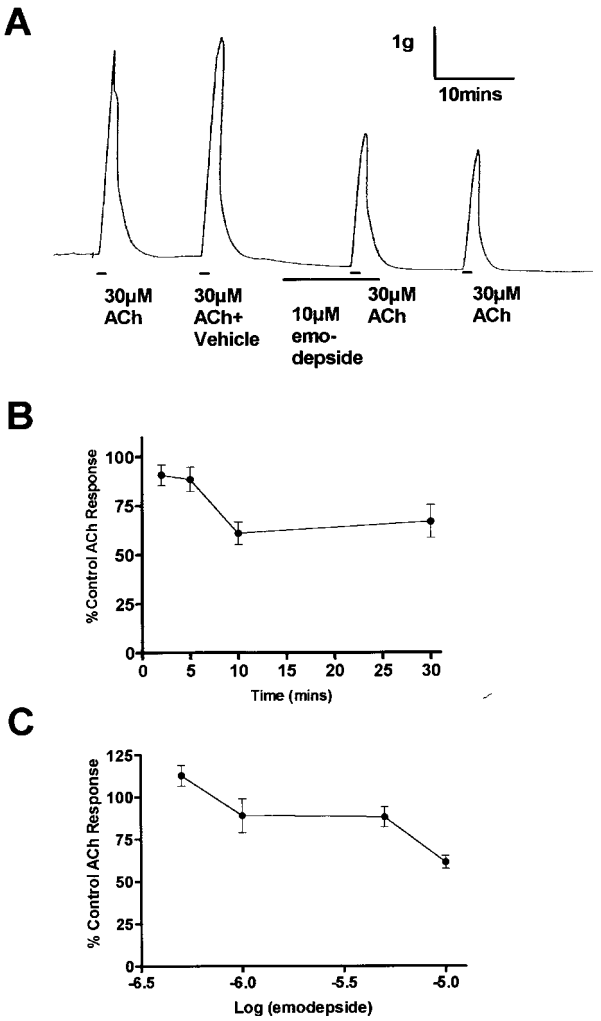

Fig. 2. The time-dependent effect of emodepside (10 μM) on the response to ACh. (A) Example trace for the response to ACh after 10 min pre-incubation with emodepside. The bars indicate duration of drug application. (B) Summary of the data from 6 experiments. The 'control' contraction is the response to 30 μM ACh in the absence of emodepside. Emodepside was then applied to the DMS for 2, 5, 10 and 30 min before the response to 30 μM ACh was measured. Data points are mean $\pm$ S.E. mean. (C) The effect of varying concentrations of emodepside on the 30 μM ACh induced contraction of *Ascaris* dorsal muscle. Contractions were obtained for ACh. Emodepside was then applied to the DMS at concentrations of 500 nM, 1 μM, 5 μM and 10 μM for 10 min before application of ACh. Data points are mean $\pm$ S.E. mean, $n \geqslant 6$.

emodepside (10 μM) to the muscle prior to addition of ACh (30 μM) caused a time-dependent reduction in the amplitude of the ACh contraction (Fig. 2A,B). This effect was not reversed following wash-out of emodepside (Fig. 2A). The maximal effect was observed at 10 min (39 $\pm$ 6% inhibition; $n=6$), as there was no further reduction in the contraction when emodepside was applied for 30 min (Fig. 2B). Therefore 10 min was selected as the time-point to investigate the concentration-dependence of the inhibitory action of emodepside. The threshold concentration for inhibition of the ACh contraction was around 1 μM, but the inhibition did not achieve significance until 10 μM ($P<0{\cdot}05$; $n=5$; Fig. 2C).

To investigate relaxation of the muscle by emodepside more directly, the muscle was pre-contracted with ACh (30 μM). Emodepside was applied to the muscle at the peak of the contraction, and the time-course of the subsequent relaxation followed for 10 min, at which point the preparation was washed. The relaxation rate per minute was calculated as follows

$$\%\ \text{relaxation rate}\,\text{min}^{-1} = \frac{\text{P}-\text{R}}{10} \times \frac{1}{100},$$

where 'P' is the muscle tension during the peak contraction and 'R' is muscle tension immediately prior to the wash (Fig. 3A). At concentrations greater than 0·5 μM, emodepside significantly increased relaxation rate ($n=5$; $P<0{\cdot}05$; Fig. 3B).

In order to provide some insight into the mechanism for the emodepside induced relaxation, we compared the effect of emodepside with the action of the inhibitory neurotransmitter, GABA, and an inhibitory neuropeptide, PF2. GABA relaxes nematode muscle via a well-characterized GABA-gated chloride channel (Holden-Dye *et al.* 1989). Therefore we compared the response of muscle to GABA, and emodepside. In contrast to the slow, partial relaxation of the muscle observed with emodepside, the response to GABA was rapid and the relaxation was back to base-line tension ($n=3$; data not shown). To further compare the response to GABA and emodepside we investigated the effect of reduced chloride APF. For these experiments chloride salts were replaced with the impermeant anions, either isethionate or acetate. The response to GABA changed from a relaxation to a contraction in low chloride. In contrast, emodepside caused a slow relaxation (data not shown). Therefore the response to emodepside did not resemble the response to GABA. (This is further supported by the results of electrophysiological recordings, see later.) However, the inhibitory neuropeptide PF2 relaxed muscle tonically contracted with ACh, causing a slow and incomplete relaxation at a similar rate to that observed for emodepside (Fig. 3C). A similar reduction in the response to 30 μM ACh was observed in the presence of PF2 as for 10 μM emodepside (1 μM PF2 for 2 min 36 $\pm$ 6%; $n=10$; Fig. 4). Previous studies have indicated that the response to PF1-like peptides is long-lasting and requires more than a 30 min wash-out for recovery (Franks *et al.* 1994). In muscle that was pre-treated with 1 μM PF2, there was no additional reduction in the response to ACh on addition of emodepside (10 μM; Fig. 4; $n=4$).

Emodepside also inhibited the response of the muscle to the neuropeptide, AF2. This peptide (2 min, 1 μM) elicited a biphasic effect on muscle tension, an initial relaxation followed by a prolonged

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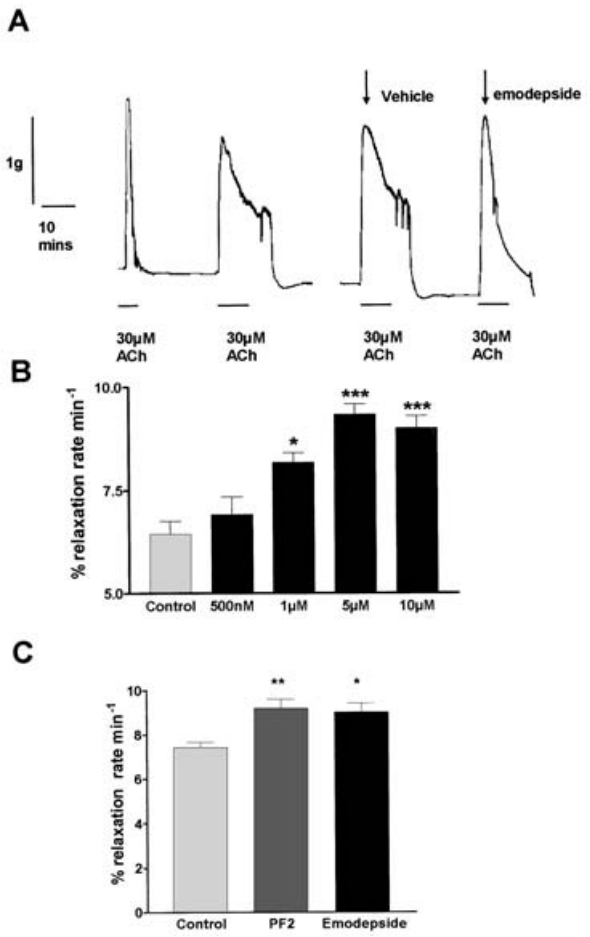

Fig. 3. Effects of emodepside on muscle relaxation. (A) Example of the effect on muscle relaxation rate. The horizontal bar indicates the duration of application of ACh. The arrows indicate the addition of either vehicle (0·1% ethanol, indicated by arrow) or 10 μM emodepside at the peak of the contraction. (B) Pooled data for different concentrations of emodepside ($n=5$). The relaxation was measured as the difference in tension at the peak of the response and prior to the wash, expressed as a percentage of the peak contraction and divided by 10, to yield the % relaxation rate min^{-1}. 'Control' is the relaxation rate with ACh alone. (C) Summary of the action of PF2 (1 μM) and emodepside (10 μM) on relaxation rate ($n=6$). * $P<0·05$; ** $P<0·01$; *** $P<0·001$.

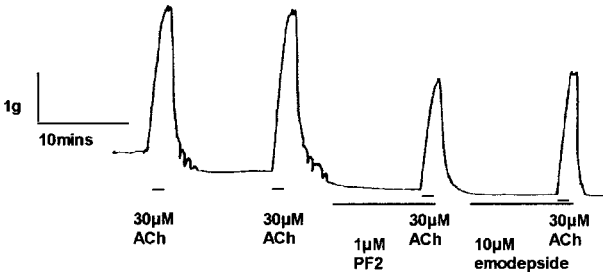

Fig. 4. PF2 (1 μM) relaxes *Ascaris* DMS in a similar manner to 10 μM emodepside. An example of the effect of pre-treatment of the muscle with PF2 and subsequent application of emodepside on the response to ACh. The horizontal bars indicate the duration of application of the drugs.

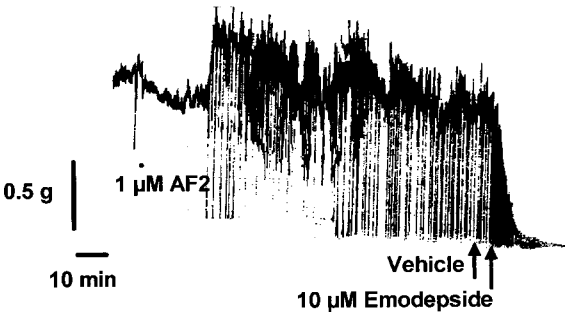

Fig. 5. The effect of emodepside on the response to the excitatory neuropeptide AF2. AF2 was added to the preparation for 2 min, as indicated. After an initial relaxation, a characteristic increase in rhythmic activity was observed. In control experiments this persisted for more than 1 h. Application of either vehicle or 10 μM emodepside is indicated by the arrows. Emodepside caused a transient increase in the frequency of the rhythmic activity, followed by a complete cessation of activity.

increase in rhythmic activity (Cowden & Stretton, 1993) that persisted for more than 1 h. Emodepside (0·5 μM) caused a relaxation (in 4 out of 6 preparations), but failed to completely inhibit the rhythmic AF2 activity. At higher concentrations, emodepside caused a relaxation and abolished the AF2 induced rhythmic activity (1 μM, 6 out of 7 preparations; 5 μM, 5 out of 5 preparations; and 10 μM, 5 out of 5 preparations). The effect of 10 μM emodepside on the response to AF2 is shown in Fig. 5.

To determine whether the inhibitory action of emodepside may involve neurotransmitter release from the nerve cord onto the muscle, we compared the action of emodepside on muscle strips with and without nerve cord. For the 'denervated' preparations the muscle was excised between the dorsal and ventral nerve cords so that neither of these were present. However, it was not possible to remove the minor lateral cords and maintain a functional preparation, therefore this neural input was still intact. All 10 preparations, with or without nerve cord, responded to ACh (30 μM) with a contraction. As with previous experiments, the response to ACh was inhibited by emodepside in the intact preparations (Fig. 6A). The response to ACh in the denervated preparations was superimposed on a falling base-line tension, and was typically lower in amplitude than the response for intact preparations, reflecting damage to the muscle during dissection. Addition of emodepside to the denervated preparations apparently caused a slight reduction in baseline tension in 9 out of 10 preparations ($-0·25 \pm 0·19$ g), but this was difficult to quantify because of the unstable base-line tension.

Furthermore, the response to ACh in the denervated preparations was not significantly inhibited by

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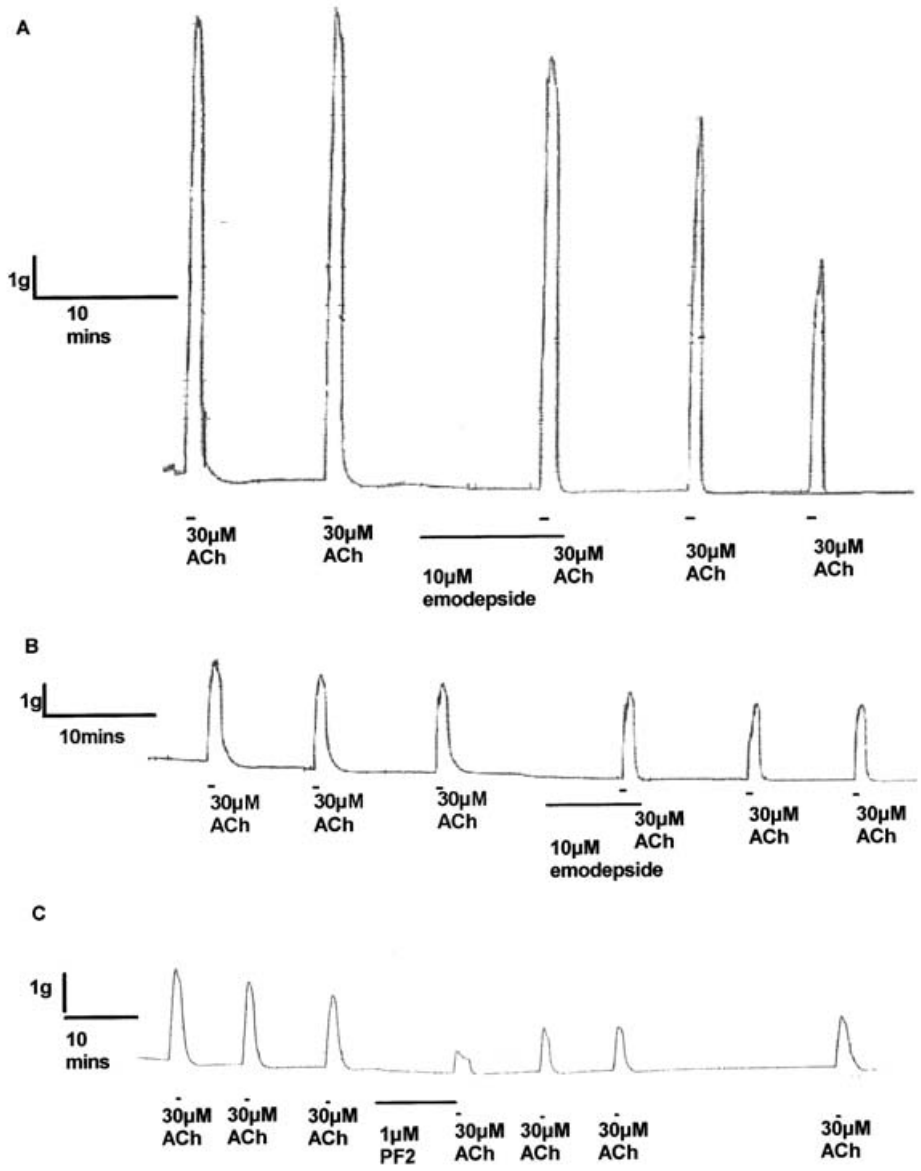

Fig. 6. The effect of removing the major nerve cords on the inhibitory action of emodepside and PF2. All 3 recordings are from muscle obtained from the same worm. The duration of application of drugs is indicated by the horizontal bars. (A) Recording from an intact muscle strip. (B) Recording from a 'denervated' muscle strip. (C) Recording from a denervated muscle strip showing the inhibitory action of PF2.

emodepside (10 μM for 10 min), if at all (the contraction to ACh in the presence of emodepside was 99·8 $\pm$ 4·8% of the contraction before addition of emodepside; $n=10$; Fig. 6B). The inhibition of the ACh response by PF2 was still observed in the denervated preparation (Fig. 6C).

Electrophysiological recordings from muscle

Emodepside (10 μM for 2 min) caused a small, but significant, slow and irreversible hyperpolarization of muscle cells with no detectable change in input conductance (Fig. 7). After 30 min the average membrane potential change was $-5{\cdot}4 \pm 1{\cdot}0$ mV ($P<0{\cdot}001$, $n=8$; Fig. 7). The hyperpolarization elicited by 10 μM emodepside was reduced in Ca^{++} free APF. In 7 out of 8 cells there was either very little or no change in membrane potential following addition of emodepside in the absence of external Ca^{++}. Subsequent addition of Ca^{++} resulted in a rapid hyperpolarization (Fig. 8).

The hyperpolarization caused by emodepside (10 μM for 2 min) was not observed in the presence of the K^+ channel blockers 4-aminopyridine (4-AP, 250 μM) and tetraethylammonium (TEA, 5 mM). Co-application of both these blockers had a direct effect on membrane potential causing a depolarization of $2{\cdot}8 \pm 0{\cdot}5$ mV ($n=5$). Subsequent addition of emodepside (10 μM for 2 min) in the presence of TEA and 4AP caused no change in muscle cell membrane potential up to 10 min later ($-0{\cdot}1 \pm 0{\cdot}4$ mV; $n=5$). Furthermore, in the presence of the channel blockers

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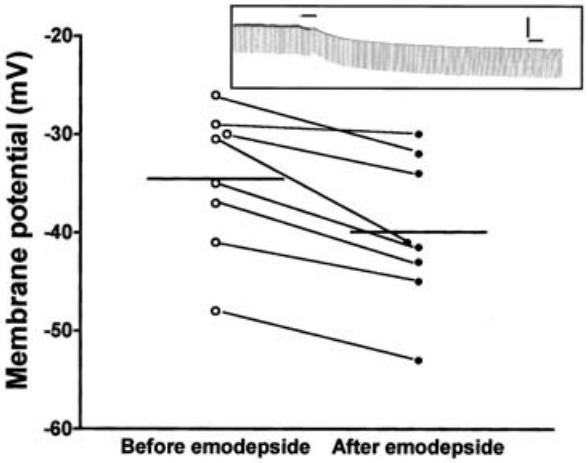

Fig. 7. The effect of emodepside on the membrane potential of *Ascaris* muscle cells. The inset shows a representative response to 10 μM emodepside. The horizontal bar indicates the duration of application. (Vehicle was without effect.) Scale bars are 10 mV and 2 min. The downward deflections are caused by injection of hyperpolarizing current pulses (0·1 Hz, 20 nA, 500 ms) and provide a measure of cell input resistance. Note the decrease in 'noise' in the presence of emodepside. This is consistent with the abolition of spontaneous activity by emodepside. The graph summarizes the data from 8 similar experiments. Resting membrane potential (○) 30 min after a 2 min perfusion of emodepside (●). Each point indicates an individual muscle cell recording. The bars indicate the average value.

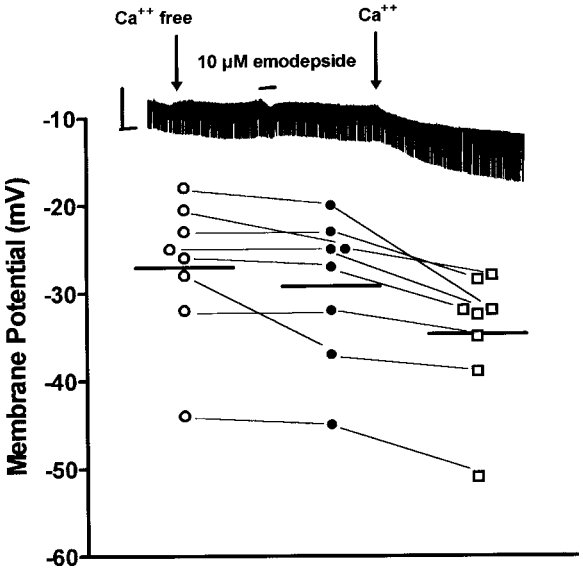

Fig. 8. The effect of removing extracellular calcium on *Ascaris* muscle cell response to emodepside. The inset shows a representative experiment. The horizontal bar indicates the duration of application. (Vehicle was without effect.) Scale bars are 10 mV and 2 min. The downward deflections are caused by injection of hyperpolarizing current pulses (0·1 Hz, 20 nA, 500 ms) and provide a measure of cell input resistance. The arrows indicate the removal and subsequent re-introduction of calcium ions. The graph shows data from 8 similar experiments; membrane potential in normal APF (○); membrane potential 30 min after 2 min emodepside perfusion in the absence of calcium (●); membrane potential 30 min after re-introduction of calcium ions (□).

there was an increase in the amplitude of spontaneous muscle potentials, and these persisted after the addition of emodepside.

Further evidence for the involvement of K^+ in the emodepside-induced hyperpolarization was provided by experiments in K^+-free APF. Perfusion of K^+-free APF resulted in muscle hyperpolarization of $-2{\cdot}5 \pm 1{\cdot}0$ mV after 10 min. Subsequent addition of emodepside (10 μM for 2 min) resulted in a further hyperpolarization of -12 ± 4 mV after 30 min ($n = 5$; compared to $-5{\cdot}4$ mV in normal APF).

DISCUSSION

Emodepside relaxes an isolated dorsal muscle strip of *Ascaris*, reflecting the ability of this anthelmintic to paralyse parasitic nematodes. Here we have investigated the cellular basis for this. As the effect of emodepside on basal muscle tension was very slight, the inhibitory action of the compound on the contraction elicited by ACh was determined. ACh has previously been shown to elicit contraction by activation of a nicotinic receptor on the somatic muscle (Colquhoun *et al*. 1991) and it would be expected that an inhibitory compound would exert a physiological antagonism of this response. Consistent with this, we found that pre-incubation of the muscle with emodepside caused a concentration and time-dependent reduction in the amplitude of the ACh contraction. This effect was significant at 10 μM emodepside and maximal at 10 min incubation. The effect was irreversible and it is likely that this reflects the highly lipophilic nature of emodepside. The ACh contraction was not decreased by more than 40% of the control value, even at the highest concentration (10 μM) of emodepside tested, and higher concentrations were not tested due to limitations of solubility of the compound. The inhibitory action of emodepside was also observed on muscle strips precontracted with ACh. In the continued presence of ACh the initial, peak contraction is followed by a slow reduction in muscle tension, or 'fade' in the response, prior to the wash. This fade may be due to desensitization of the muscle. Addition of emodepside at the peak of the contraction increased the relaxation rate of the muscle with a threshold for the effect at less than 1 μM. This was relatively slow in onset, and the tension did not relax right back to baseline before the wash. The inhibitory action of emodepside was not specific for ACh-mediated contraction as it also inhibited the action of the excitatory neuropeptide, AF2.

There are a number of possible cellular mechanisms that may underly this inhibitory action of

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emodepside and we undertook a systematic investigation of these. For example, emodepside may exert its action either pre-synaptically or directly on the muscle. To test the latter possibility, we applied emodepside to muscle from which the major nerve cords had been removed. The muscle was probably compromised by the dissection procedure as the base-line tension was not stable and the response to ACh was reduced compared to an intact preparation. In these preparations, emodepside caused a slight decrease in base-line tension. This could be due to a mechanical artefact of drug addition, an action on nerve endings of the minor nerves of the lateral cords, or a slight post-synaptic action. Most notably, however, emodepside did not inhibit the response to ACh when the nerve cord was removed. As a positive control, intact dorsal muscle strips were taken from the same worms as the denervated muscle, and emodepside inhibited the response to ACh in these preparations. Furthermore, the nerve cord was not required for the inhibitory response to PF2, consistent with the post-synaptic action of this neuropeptide (Holden-Dye *et al*. 1995).

Further indirect evidence that the action of emodepside may be mediated via an action at presynaptic nerve terminals was provided from electrophysiological experiments. Intracellular recordings demonstrated that emodepside elicited a slow hyperpolarization of muscle cells. The threshold concentration for the action of emodepside on muscle membrane potential was of the same order as that observed for muscle relaxation, indicating that this hyperpolarization is likely to be directly involved in the inhibitory action of emodepside. As with the inhibitory action of emodepside on muscle tension, this hyperpolarization did not reverse on washing. Removal of calcium ions from the perfusate prevented the hyperpolarization in response to emodepside. This could be due to a requirement for calcium for activation of a current on the muscle membrane e.g. a calcium-activated potassium channel. However, it is also consistent with a role for calcium-dependent neurotransmitter release in the mechanism of action of emodepside, and taken together with the experiments of denervated muscle described above, we favour this latter explanation.

Intriguingly, the parent compound for emodepside, PF1022A, has been shown to interact with a latrophilin receptor (Saeger *et al*. 2001). This may be relevant to the action of emodepside at the neuromuscular junction as the latrophilin receptor is a G-protein coupled receptor and involved, in a not yet fully characterized manner, in neurotransmitter release. In particular, latrophilin receptors regulate the release of transmitters from dense-core vesicles e.g. amines and neuropeptides (Davletov *et al*. 1998).

If emodepside is acting to release an inhibitory transmitter or modulator on to the muscle then the question arises, what is it? Previous studies have suggested, as one possiblity, that emodepside may act by mimicking the action of the endogenous inhibitory neuromuscular junction transmitter, GABA (Martin *et al*. 1996; Chen *et al*. 1996). For example, Martin *et al*. (1996), observed a chloride-dependent increase in membrane input conductance that would be consistent with activation of a GABA-gated chloride channel (Martin, 1982; Parri, Holden-Dye & Walker, 1991; Holden-Dye *et al*. 1989). We therefore compared the inhibitory actions of GABA and emodepside on muscle tension. As would be expected of an inhibitory neurotransmitter, GABA elicited a fast relaxation of the muscle tonically contracted with ACh back to base-line tension, quite different from the slow incomplete relaxation caused by emodepside. The action of emodepside on the electrophysiological properties of the muscle are also not like the actions of GABA. In contrast to the earlier study of Martin *et al*. (1996), we found that emodepside did not cause an increase in input conductance but rather it elicited a slow, K^+-dependent hyperpolarization with no consistent change in input conductance. Taken together, our data do not support a role for GABA, or its receptor, in the action of emodepside.

A family of FMRF-amide like peptides are also known to have potent effects on nematode muscle (Brownlee *et al*. 2000). Two of these peptides, PF1 and PF2 (Geary *et al*. 1992), are structurally very similar with the sequence SDPNFLRFamide and SADPNFLRFamide, and both of these have a potent inhibitory action on *Ascaris* somatic muscle (Holden-Dye *et al*. 1995). To determine whether a PF1/2-like peptide may be involved in the response to emodepside, we compared the action of emodepside with that of PF2. As for emodepside, PF2 elicited a slow but incomplete relaxation of dorsal muscle tonically contracted with ACh. Pre-incubation of the muscle with PF2 also reduced the amplitude of subsequent contractions to ACh, in a similar manner to emodepside. The receptors for PF2 are likely to be located post-synaptically on the muscle as the inhibitory effect of PF2 on the ACh contraction was also observed in a denervated muscle strip.

Electrophysiologically the response to PF2 is also like the response to emodepside. PF2 causes a slow hyperpolarization with no consistent change in input conductance (Holden-Dye *et al*. 1995). The hyperpolarization caused by PF2 is blocked by potassium channel blockers (Walker *et al*. 2000). Similarly, the hyperpolarization caused by emodepside was blocked by potassium channel blockers, and enhanced in low external potassium. Furthermore, it has also been shown that the response to PF1/PF2 is dependent on calcium and that this reflects the involvement of a nitric oxide signalling cascade in the peptide response (Bowman *et al*. 1995). Therefore it would seem that the ionic mechanism for the response to PF2 and to emodepside is similar.

Overall, these data suggest that emodepside may mimic the action of inhibitory neuropeptides at the neuromuscular junction, possibly by triggering neuropeptide release from inhibitory nerve terminals. Whether or not this contributes to the anthelmintic action remains to be determined. Further studies on the model genetic animal *C. elegans* are being performed to refine this hypothesis.

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