Pharmacological effects of nematode FMRFamide-related peptides (FaRPs) on muscle contractility of the trematode, *Fasciola hepatica*

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SUMMARY

The physiological effects of synthetic replicates of the nematode FaRPs, AF1 (KNEFIRFamide), AF2 (KHEYLRFamide), PF1 (SDPNFLRFamide), PF2 (SADPNFLRFamide), AF8/PF3 (KSAYMRFamide) and PF4 (KPNFIRFamide) were examined on muscle preparations of the liver fluke, *Fasciola hepatica*. Changes in contractility following the addition of the test compound were recorded using a photo-optic transducer system. Unlike the varied effects these peptides have on nematode somatic musculature, all were found to induce excitatory responses in the muscle activity of *F. hepatica*. While qualitative effects of the nematode peptides were similar in that they induced increases in both the amplitude and frequency of *F. hepatica* muscle contractions, they varied considerably in the potency of their excitatory effects. The threshold activity for each peptide was as follows: 10 μM, PF1 and PF2; 3 μM, AF1 and PF3; 1 μM, AF2; and 30 nM, PF4. The results demonstrate, for the first time, the cross-phyla activity of nematode neuropeptides on the neuromuscular activity of a trematode.

Key words: *Fasciola hepatica*, neuromuscular activity, physiology, FMRFamide-related peptides, FaRPs, nematode peptides.

INTRODUCTION

Following the isolation of the cardioexcitatory peptide, Phe-Met-Arg-Phe-amide (FMRFamide) from molluscan extracts (Price & Greenberg, 1977), the number of FMRFamide-related peptides (FaRPs) identified in invertebrates has increased substantially. To date, 4 FaRPs have been isolated from platyhelminth species, namely: GNFFRFamide (*Moniezia expansa*) (Maule *et al*. 1993), RYIRFamide (*Artioposthia triangulata*) (Maule *et al*. 1994*a*), GYIRFamide (*Dugesia tigrina*, *Bdelloura candida*) (Johnston *et al*. 1995, 1996) and YIRFamide (*B. candida*) (Johnston *et al*. 1996). However, the majority of known helminth FaRP primary structures have been gleaned from extracts or deduced from gene sequences of members of the phylum Nematoda. Thus far, more than 20 FaRPs have been identified from nematodes and there is evidence of structural homologies in the neuropeptides of parasitic and free-living forms. Of these, 12 FaRPs have been isolated and sequenced from extracts of *Ascaris suum* and designated AF1-AF12 (Cowden, Stretton & Davis, 1989; Cowden & Stretton, 1993, 1995). The remaining 9 FaRPs have been isolated from *Haemonchus contortus* (AF2) (Keating *et al*. 1995) and from the free-living nematodes *Caenorhabditis elegans* (CF1-CF8 and AF2) (Rosoff *et al*. 1992; Marks *et al*. 1995*b*) and *Panagrellus redivivus* (PF1, PF2, PF3 and PF4) (Geary *et al*. 1992*b*; Maule *et al*. 1994*b*, 1995*b*). Although a number of physiological experiments have been carried out to examine the effects of neuropeptides in nematodes, similar studies using trematodes or cestodes are few in number (Day *et al*. 1994; Marks *et al*. 1996). This may be partially explained by the lack of structural information on endogenous peptides from the latter 2 groups. To date, only 2 studies have been performed on trematodes, both of which involved examining the physiological effects of flatworm peptides on, in one instance, isolated muscle fibres of *Schistosoma mansoni* (Day *et al*. 1994) and, in the other, muscle strips of *Fasciola hepatica* (Marks *et al*. 1996). Interestingly, flatworm FaRPs all seem to have similar excitatory effects on flatworm muscle, whereas nematode FaRPs have clearly distinguishable actions on nematode tissues (Day *et al*. 1994; Maule *et al*. 1995*a*; Marks *et al*. 1996). With increasing numbers of reports of resistance to all of the broad-spectrum anthelmintics currently marketed (see review by Conder & Campbell, 1995), the search for novel therapeutic targets is becoming

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a pressing need. In this respect, helminth neuromuscular function continues to be a favoured target area for drug development. The abundance and potent actions of native neuropeptides in parasitic nematodes and platyhelminths identifies neuropeptide receptors as putative drug targets, and any homology between the neuropeptide receptors of these 2 groups may enable future effective broad spectrum drug development (for reviews see Geary *et al*. 1992*a*, 1995; Thompson *et al*. 1996).

This study aims to examine the physiological effects of the nematode FaRPs: AF1 (KNEFIRFamide), AF2 (KHEYLRFamide), PF1 (SDPNFLRFamide), PF2 (SADPNFLRFamide), PF3 (KSAYMRFamide) and PF4 (KPNFIRFamide) on somatic muscle function in *F. hepatica*. These nematode FaRPs were selected because previous studies revealed that they have distinguishable physiological effects on *Ascaris suum* somatic muscle strips (Maule *et al*. 1995*a*). Any cross-phyla activity of helminth neuropeptides is likely to indicate if nematode and platyhelminth FaRP receptors have structural similarities.

MATERIALS AND METHODS

Parasite collection

Specimens of the liver fluke, *Fasciola hepatica* were collected from the bile duct and liver of experimentally-infected gerbils (*Meriones unguiculatus*) 4 weeks post-infection. The worms were placed in RPMI-1640 (Gibco, 22511–018) at 37 °C and upon examination some specimens had regurgitated their gut contents. To reduce experimental variability, all worms were allowed to regurgitate their gut contents before use. This also avoided the release of fluke gut contents into the experimental chamber during experimentation. Following regurgitation of gut contents, the worms were then maintained in fresh RPMI-1640 (37 °C) until use. Immediately prior to their use in tension experiments, the lateral edges of the flukes (0·5 mm) were trimmed to facilitate peptide penetration. Note that untrimmed flukes were found to be non-responsive to peptide addition.

Neuromuscular function

The physiological effects of the peptides on *F. hepatica* muscle preparations were examined using a balance-beam optic-isolator transducer system (Fetterer, Pax & Bennett, 1977; Atchison *et al*. 1992; Marks *et al*. 1996). The fluke was securely attached to an inflexible suction pipette attached to the ventral surface of the parasite; the procedure was repeated with a second, flexible pipette, attached at the ventral surface exactly 1 mm posterior to the inflexible pipette. The load against which the parasite muscle segment contracted was adjusted electronically by way of a field coil positioned near the fulcrum of the balance-beam. The movement generated by the muscle between the 2 pipettes was measured using a photo-optic system, and the signal thus generated was amplified and stored on a chart recorder (for details of the methodology, see Marks *et al*. 1996).

Data collection

Once attached to both pipettes, the muscle preparation was allowed to equilibrate for approximately 10 min, thus allowing a steady baseline to be recorded. A test compound was then added to the experimental chamber and contractility of the muscle preparation monitored for a 20-min period. To test for reversibility and within-preparation repeatability of the peptide effects, the medium in the chamber was replaced with fresh medium. Following reversal of the response after wash-out (20 min), the same test compound was added a second time. In all cases, this protocol was used to test each compound on a naive (previously untreated) worm. All experiments were repeated a minimum of 10 times.

Data analysis

The physiological effects of the peptides on *F. hepatica* muscle preparations were analysed using 3 parameters: (a) baseline tension, (b) contraction frequency and (c) contraction amplitude at the time points: 0 (i.e. immediately prior to peptide addition), 1, 5, 20, 25 and 30 min post-addition (for details, see Marks *et al*. 1996). For the purposes of statistical analysis, the amplitude and frequency of spontaneous contractions were examined during the 1-min period prior to each of these time-points. For each preparation, values for each of the 3 parameters were normalized to the value recorded at the 0-min time-point. Contractions were defined as fast transient tension changes, superimposed on baseline tension, which were $\geqslant$ 0·5 mg/mm in amplitude (exact amplitudes recorded as mm pen deflections above the minimum tension immediately prior to contraction). The minimum average tension recorded during the 1-min period prior to peptide addition was taken as baseline tension, i.e. an average of the minimum tension recorded during each relaxation. Responses were noted on spread sheets and entered into a PRIME computer program. Data were analysed electronically using a SAS programme which computes the mean, standard error and number of observations used to determine each response variable (for details, see Atchison *et al*. 1992). Student's *t*-test, non-paired, was used to determine if differences in the 3 contractility parameters between treated and vehicle (60 μl distilled H_20) control groups, time-matched, were significant

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($P \leqslant 0{\cdot}05$). All of the motility parameters presented were significantly different from those recorded during control periods ($P \leqslant 0{\cdot}05$).

Peptides

AF1, AF2, PF3 and PF4 were purchased from Sigma Chemical Company (St Louis, MO, USA). PF1 and PF2 were synthesized by sequential solid-phase synthesis, using BOC chemistry and a *p*-methylbenzhydryl amino resin on an Applied Biosystems 430A peptide synthesizer. Peptides were dissolved and diluted in distilled water immediately prior to use to 1000-fold the desired test concentration. Final test concentrations were achieved by adding the peptide solutions to the RPMI-1640 in the experimental chamber.

RESULTS

General

Intact parasites, i.e. those with the lateral margins untrimmed, exhibited a similar pattern of spontaneous muscular activity to that of trimmed-worm preparations. The contractility of intact parasites was not affected by application of peptides to the bath (not shown). The spontaneous, transient contractions of trimmed specimens was somewhat variable, but usually consisted of low amplitude ($< 0{\cdot}5$ mg/mm), high frequency (4–16/min) contractions which were superimposed on a background of irregular slow-wave changes in baseline tension. Results which stipulate contraction frequency refer only to fast-wave contractions ($\geqslant 4$/min), $\geqslant 0{\cdot}5$ mg/mm in amplitude, unless otherwise stated. All of the nematode peptides tested were found to be excitatory on trimmed *F. hepatica* preparations, increasing both the amplitude and frequency of fast muscle contractions. In all cases, there was an almost immediate increase in fast contraction frequency and amplitude following the addition of peptide; these effects usually persisted until wash-out. The increase in fast muscle contractions was often accompanied by a decrease in slow-wave activity. Despite the increase in amplitude and frequency of fast contractions, none of the test peptides (with the exception of PF4, see next sentence) was found to elicit a significant change in baseline tension. PF4 (10 μM) was found to cause a significant decrease in baseline tension 5 min post-peptide addition ($P < 0{\cdot}5$) as the contractions increased in both amplitude and frequency. However, tension changes were not significantly altered at any other time-point during the 20-min period of the PF4 treatment (Fig. 1). The effects of the test peptides on the rate of contraction of *F. hepatica* somatic muscle are summarized in Table 1. The effects of all of the peptides tested were reversible and could be repeated following wash-out.

Positive controls included the addition of arecoline (10 μM) and high-K^+ (90 mM) to the muscle preparation. Arecoline caused a flaccid paralysis, whereas high K^+ induced a marked spastic (i.e. sustained) contraction (Fig. 2) and both were found to significantly alter baseline tension ($P < 0{\cdot}5$). The addition of warm RPMI-1640 (37 °C) or distilled water to the experimental chamber ($\leqslant 600$ μl; $n = 12$) had no discernible effects on the contractility of the muscle preparation.

AF1 and AF2

The addition of AF1 and AF2 had similar physiological effects on trimmed *F. hepatica* preparations, causing an increase in the frequency of the fast muscle contractions. The effects of AF2 were 3-fold more potent than those of AF1, exhibiting a threshold concentration for excitation of 1 μM.

AF1 ($\geqslant 3$ μM; $n = 48$) consistently increased fast-contraction frequency and amplitude in *F. hepatica* muscle preparations (Fig. 3). These effects were accompanied by a reduction in the amplitude of slow-wave activity (Fig. 3). The threshold concentration for activity of AF1 was 3 μM (Fig. 4). Irrespective of the peptide concentration, contraction frequency returned rapidly to pre-treatment levels following replacement of the medium with fresh RPMI-1640. AF1 increased muscle activity in a concentration-dependent manner, such that AF1 (100 μM; $n = 12$) stimulated contraction frequency by 62% within 1 min of adding the peptide; contraction frequency increased to 225% after 20-min of exposure to the peptide. The addition of AF1 (10 μM; $n = 12$) to the muscle strip resulted in an increase in fast-contraction frequency of 75% by 1 min post-addition; as with the higher peptide concentrations, fast contraction frequency continued to increase during the 20 min exposure period (Fig. 4). In comparison, addition of the threshold concentration for activity, i.e. 3 μM AF1, resulted in a maximal increase of 62% within 5 min, thereafter the excitatory effects slowly declined over the remainder of the test period. Nevertheless, the effects of 3 μM AF1 were still evident immediately prior to wash-out. AF1 treatment also resulted in a concentration-dependent increase in fast contraction amplitude (Table 1). At 10 μM ($n = 12$), the peptide induced a 22% increase in the amplitude of fast muscle twitches within 1 min; the amplitude of the fast contractions further increased to 44% by 20 min post-addition of the peptide.

The addition of AF2 to the muscle preparation ($\geqslant 1$ μM; $n = 60$) consistently increased both the amplitude and frequency of fast contractions (Figs 5 and 6; Table 1). AF2 had a threshold concentration for activity of 1 μM ($n = 12$), compared to 3 μM for AF1. AF2 (100 μM; $n = 12$) induced a rapid increase in fast-contraction frequency, with 59% and 176%

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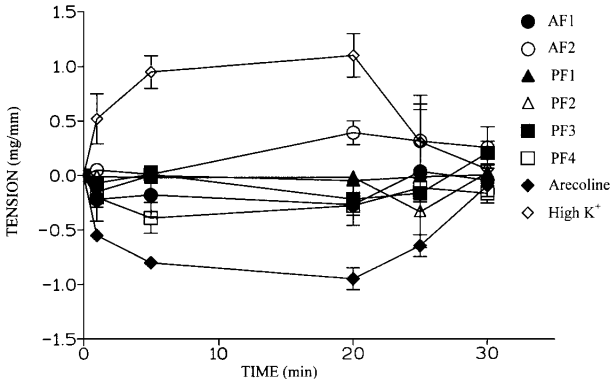

Fig. 1. Graph showing the effects of test compounds on baseline tension of *Fasciola hepatica* muscle preparation. Addition of test compounds was at 0 min and wash-out at 20 min. Data points represent mean ± S.E. ($n \geqslant 10$). Note that nematode FaRPs did not significantly alter baseline tension.

Table 1. Summary of the effects of 10 μM of the test peptides on the amplitude of *Fasciola hepatica* muscle contractions

	Contraction amplitude (% 0 min)					
Peptide	0 min	1 min	5 min	20 min	25 min	30 min
AF1	100	122	132	144	92	101
AF2	100	147	169	170	104	100
PF1	100	167	155	159	113	103
PF2	100	107	130	128	102	102
PF3	100	154	158	179	109	102
PF4	100	193	133	129	103	104

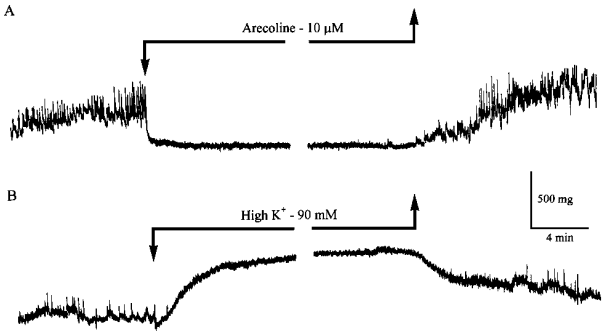

Fig. 2. Muscle tension recording showing the effects of 10 μM arecoline (A) and high (90 mM) K^+ (B) on contraction frequency and baseline tension of *Fasciola hepatica* muscle preparations.

increases in contraction frequency by 1 and 5 min post-addition, respectively. After 20 min exposure to 100 μM AF2, the contraction frequency had increased by 244 %. Replacement with fresh medium resulted in a rapid return to pre-treatment contraction frequency (Fig. 6). Similarly, addition of 30 μM AF2 ($n = 12$) resulted in an increase in contraction frequency of 169 % within 5 min; this value declined to 106 % by 20 min post-addition,

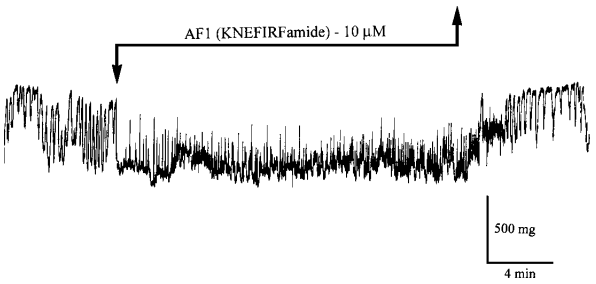

Fig. 3. Effect of the nematode peptide AF1 (10 μM) on muscle preparations of the trematode, *Fasciola hepatica*. Period of peptide addition is indicated by the arrows.

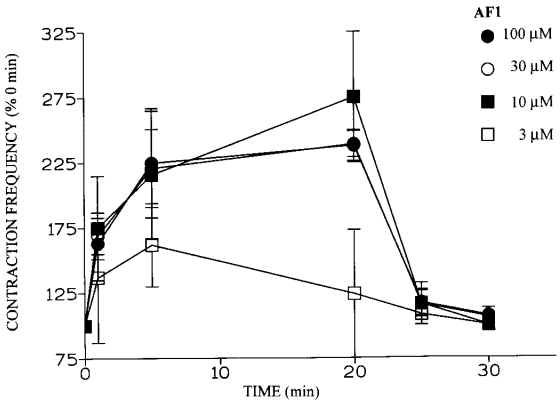

Fig. 4. Graph showing the effects of a range of concentrations of AF1 on the contraction frequency of *Fasciola hepatica* muscle preparations. Results were recorded as described in the Materials and Methods section and are presented as mean ± S.E. ($n \geqslant 12$).

and rapidly returned to pre-treatment levels following wash-out. The addition of 10 μM AF2 ($n = 12$) also resulted in a rapid increase in contraction frequency, inducing 81 % and 182 % increases by 1 and 5 min post-addition, respectively (Figs 5 and 6). The stimulation in contraction frequency slowly declined to 98 % within 20 min of peptide exposure and returned to pre-treatment levels within 5 min of wash-out (Fig. 5).

Exposure of muscle strips to AF2 ($\geqslant 1$ μM; $n = 48$) also led to an increase in the amplitude of fast contractions. Following the addition of 10 μM AF2, contraction amplitude increased by 47 % and 69 % within 1 min and 5 min, respectively; an amplitude increase of 70 % was recorded 20 min post-peptide addition. Following wash-out, the amplitude rapidly returned to pre-treatment levels (Table 1). The increase in contraction amplitude induced by 1 μM AF2 was relatively short-lived; it began to decline within 5 min of peptide addition and had returned to pre-treatment levels by 20 min post-addition (Table 1).

PF1 and PF2

The peptides PF1 and PF2 were found to have limited effects on muscle preparations from *F.*

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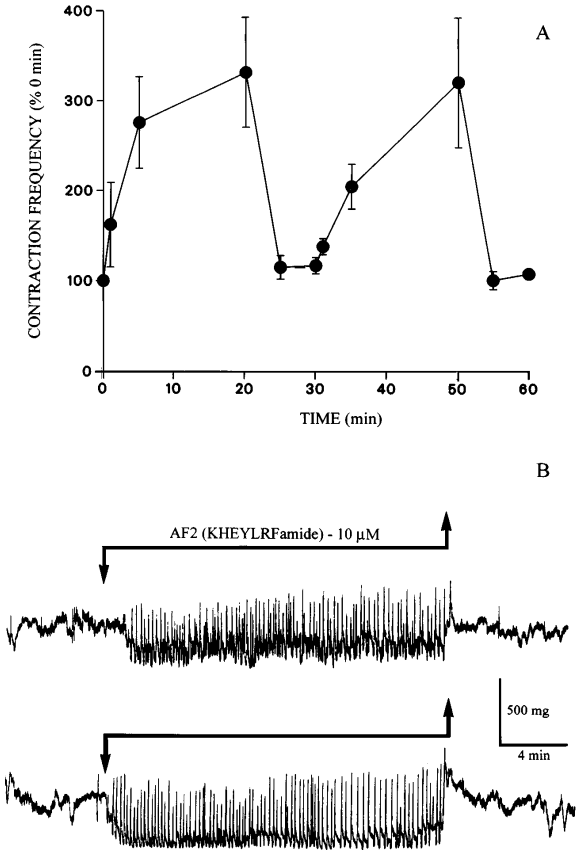

Fig. 5. Effects of first and second addition of AF2 on the motility of *Fasciola hepatica* shown in graph form (A, $n \geqslant 6$) and as an individual muscle tension recording (B). Peptide was present during the periods indicated by the arrows. Note that the lower and upper traces are continuous and that the effects were reversible.

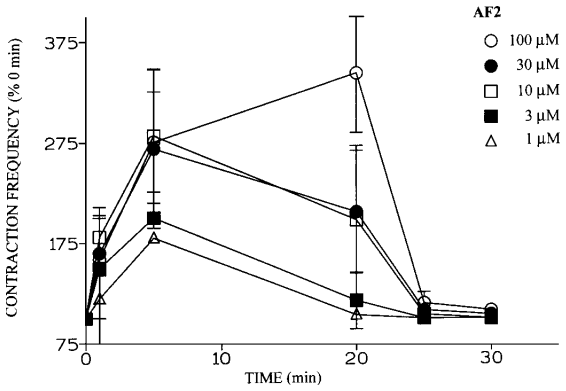

Fig. 6. Graph showing the effects of a range of concentrations of AF2 on the contraction frequency of *Fasciola hepatica* muscle preparations. Peptide was added at 0 min and wash-out at 20 min. Results were recorded as described in the text and are presented as mean ± S.E. ($n \geqslant 12$). Note that the peptide effects were rapidly and completely reversible.

hepatica, with no discernible physiological responses noted at concentrations $< 10\ \mu$M ($n = 36$).

Addition of PF1 and PF2 (10 μM; $n = 24$) to the *F. hepatica* muscle preparation induced increases in fast contraction frequency of 52 % and 92 % within 1 min

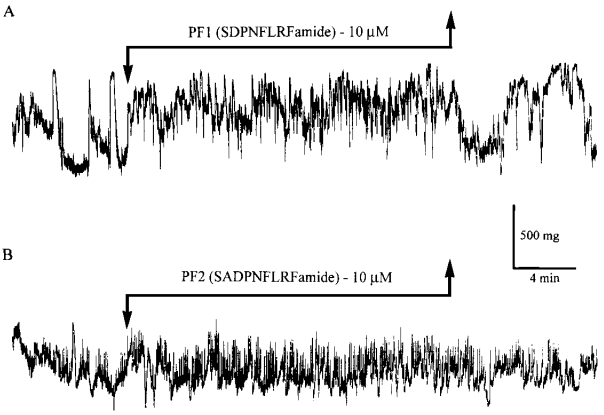

Fig. 7. Muscle tension recording showing the effects of 10 μM PF1 (A) and PF2 (B) on *Fasciola hepatica* muscle contractility. Peptide was present during the time indicated by the arrows.

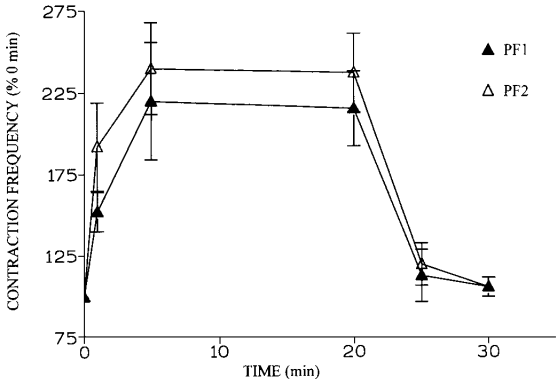

Fig. 8. Graph showing the effects of PF1 (10 μM) and PF2 (10 μM) on contraction frequency of *Fasciola hepatica* muscle preparations. Peptide was added at 0 min and wash-out at 20 min. Results were recorded as described in the text and are presented as mean ± S.E. ($n \geqslant 12$).

of peptide addition, respectively (Figs 7 and 8). The maximum increase in contraction frequency occurred within 5 min of peptide addition. Following wash-out, contraction frequency rapidly returned to pre-treatment levels (Fig. 8). An increase in the amplitude of the fast contractions was also noted following the addition of PF1 and PF2 (10 μM; $n = 24$); the maximal increase in contraction amplitude was recorded within 1 min of peptide addition (Table 1). Although these peptides had limited effects on the slow-wave contractions, the effects were variable in that both increases and decreases in frequency were recorded. However, the amplitude of slow-wave contractions diminished following peptide treatment (Fig. 7).

PF3

PF3 was found to cause a concentration-dependent excitation in the frequency and amplitude of contractions in *F. hepatica* muscle strips, beginning at

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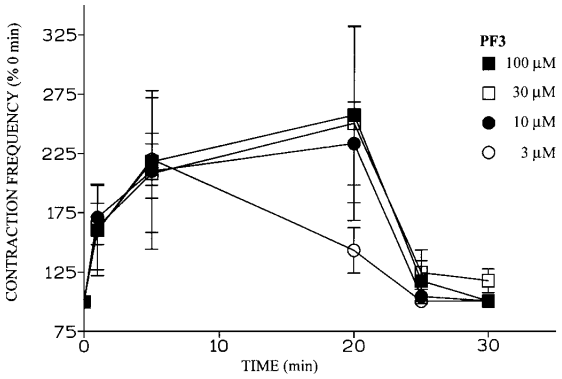

Fig. 9. Graph showing the effects of a range of concentrations of PF3 on the contraction frequency of *Fasciola hepatica*. Peptide was added at 0 min and wash-out at 20 min. Data points represent mean ± S.E. ($n \geqslant 12$).

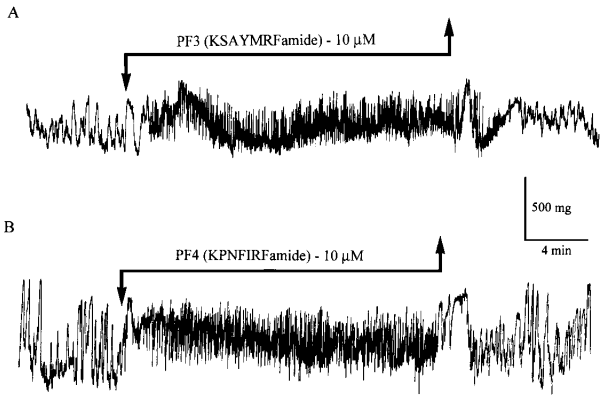

Fig. 10. Physiograph showing the effects of 10 μM PF3 (A) and 10 μM PF4 (B) on muscle contractility of *Fasciola hepatica*. Peptide was present during the period indicated by the arrows, i.e. added at time 0 and wash-out at 20 min.

3 μM (Fig. 9). PF3 (100 μM; $n = 12$) increased muscle contractility by 118% within 5 min; 20 min after addition, contraction frequency increased to 157%. Addition of 30 μM PF3 ($n = 12$) increased fast contraction frequency by 63% within 1 min and 150% by 20 min. This extended period of increased contraction frequency persisted for the 20-min duration of the experiment following the addition of $\geqslant 10$ μM PF3 (Figs. 9 and 10A). Following wash-out, contraction frequency rapidly recovered to pre-treatment levels. Addition of the threshold concentration, i.e. 3 μM PF3, resulted in a maximal increase in contraction frequency of 120% within 5 min. Following 5 min exposure to the peptide, the stimulation of contraction frequency began to decline rapidly.

Addition of PF3 at $\geqslant 3$ μM induced an increase in contraction amplitude (Table 1). At 10 μM, the amplitude of the muscle twitch increased by 54% and 58% by 1 and 5 min post-addition, respectively. Contraction amplitude returned to pre-treatment levels following wash-out (Table 1, Fig. 10A).

PF4

PF4 elicited an excitatory response consisting of a concentration-dependent increase in both fast contraction frequency and amplitude when added to *F. hepatica* muscle preparations (Figs 10B and 11; Table 1). PF4 was the most potent nematode excitatory FaRP examined during the course of the present study.

Although 83% of the *F. hepatica* muscle preparations treated with 100 μM PF4 ($n = 20$) showed a pronounced increase in contractility, 17% (1 out of 6) of the preparations exhibited a gradual inhibition; the inhibitory effects were not recorded with peptide concentrations < 100 μM ($n = 60$). PF4 (100 μM; $n = 12$) increased muscle contractility by 93% and 175% within 1 and 5 min of peptide addition, respectively; but frequency levels declined 154% by 20 min post-addition. The addition of 10 μM PF4 ($n = 12$) to the muscle strip resulted in a 150% increase in contractility of the tissue by 1 min. By 5 and 20 min, this increase in fast contraction frequency

Table 2. Summary of the effects of the test peptides on nematode (*Ascaris suum*) and trematode (*Fasciola hepatica*) somatic musculature

Peptide	Physiological effect on *A. suum*	Threshold for activity on *A. suum*	Effect on *F. hepatica* muscle	Threshold for activity on *F. hepatica*
AF1	Bi-phasic, initial inhibition followed by period of increased contractility	1 mM	Excitatory	3 μM
AF2	Bi-phasic, as above	1 pM	Excitatory	1 μM
PF1	Inhibitory	1 nM	Excitatory	10 μM
PF2	Inhibitory	1 nM	Excitatory	10 μM
PF3	Inhibitory-dorsal muscle strips*	0·1 μM	Excitatory	3 μM
PF4	Inhibitory	0·1 μM	Excitatory	30 nM

* PF3 has excitatory effects on ventral muscle strips from *A. suum*.

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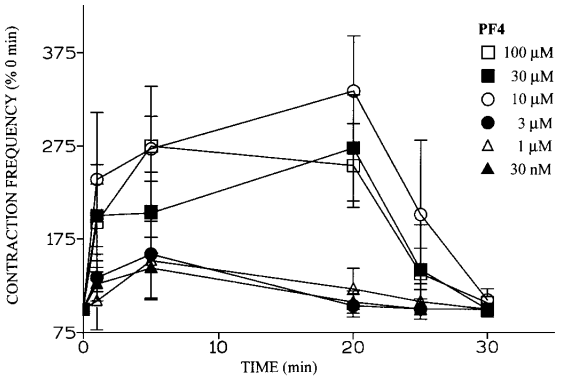

Fig. 11. Graph showing the effects of a range of concentrations of PF4 on the contraction frequency of *Fasciola hepatica*. Peptide was added at 0 min and wash-out at 20 min. Results were recorded as described in the text and are presented as mean ± S.E. ($n \geqslant 12$).

was 172 % and 234 %, respectively (Figs 10B and 11). At lower concentrations of PF4 ($< 10\ \mu$M), the excitatory responses were maximal within 5 min of peptide addition, and then slowly declined prior to wash-out. The threshold for activity of PF4 was 30 nM. At this concentration, the maximum stimulation in fast contraction frequency (44 %) occurred 5 min post-peptide addition and then declined rapidly, reaching pre-treatment levels prior to wash-out (Fig. 11). Slow wave contraction amplitude was reduced during periods of fast contraction stimulation.

Contraction amplitude also increased following treatment with PF4 ($n = 46$) in a concentration-dependent manner, beginning at 30 nM. At 10 μM, twitch height increased by 93 % and 33 % at 1 and 5 min post-addition, respectively; this increase in amplitude slowly declined over the 20-min test-period, such that the increase was only 29 % by 20 min post-addition. Following wash-out, contraction amplitude rapidly returned to pre-treatment levels (Table 1).

DISCUSSION

In the present study, all of the nematode FaRPs examined were excitatory, with the most potent being PF4 (KPNFIRFamide) which had a threshold for activity of 30 nM. PF4 elicited an increase in the frequency and amplitude of fast contractions. In comparison, the addition of PF4 to *A. suum* muscle strips causes a profound chloride-dependent relaxation and hyperpolarization (Maule *et al.* 1995*a*). Although fast contraction frequency and amplitude were increased by PF4, slow-wave contractions were inhibited. However, it is unclear whether the reduction in slow-wave activity is a direct effect of the peptide or simply a consequence of increased fast contraction activity. Following exposure to 100 μM PF4, 1 out of 6 of the *F. hepatica* muscle preparations displayed a steady decrease in baseline tension, contraction amplitude and frequency. This effect was not recorded at concentrations of PF4 $< 100\ \mu$M. Although this effect was not recorded following exposure to any of the other peptides tested, it may be due to the interaction of PF4 with receptors for structurally-related inhibitory peptides in the tissue preparation, or to rapid desensitization of the native excitatory receptor at these non-physiological concentrations.

A number of factors may account for the higher apparent potency of PF4 relative to the other nematode FaRPs tested in this study. Firstly, among the nematode FaRPs tested, PF4 may be the closest related structurally to an endogenous trematode FaRP. Secondly, the presence of a prolyl in position 2 from the N-terminus may confer resistance from enzymatic degradation by endogenous amino-endopeptidases (see Kubiak *et al.* 1996). That is, protection offered by the prolyl residue may enable the peptide to evade enzymatic degradation, thus allowing it to remain longer at physiologically active concentrations in the tissue-bath preparation.

The peptide AF1 (KNEFIRFamide) possesses the C-terminal motif, FIRFamide and displays considerable structural homology with PF4 (KPNFIRFamide), i.e. an asparagyl and glutamyl residue instead of a prolyl and asparagyl residue at positions 2 and 3, respectively. This peptide was less potent than PF4, with a threshold concentration for activity of 3 μM. This finding further suggests that the higher apparent potency of PF4 is due to the presence of the prolyl residue in position 2. An alternative explanation is that the N-terminal residues of PF4 are important in biological activity.

The addition of AF2 (KHEYLRFamide) to the muscle preparation resulted in an increase in the amplitude and frequency of *F. hepatica* muscle contractions. The addition of AF1 and AF2 to nematode muscle strips is known to result in a characteristic bi-phasic response, comprising an initial period of inhibition followed by a period of increased contractility (Bowman *et al.* 1995*a*). Only an excitatory effect was recorded for these two peptides in *F. hepatica*.

The inhibitory nematode peptides, PF1 (SDPNFLRFamide) and PF2 (SADPNFLRFamide) had low-potency excitatory actions on *F. hepatica* muscle preparations. This is in direct contrast to the potent inhibitory actions of these peptides on *A. suum* somatic musculature (Bowman *et al.* 1995). Bioactivity was recorded for these peptides only at concentrations $\geqslant 10\ \mu$M. The reduced potency of these peptides may be due to the fact that they are N-terminally extended, and this may limit access to the receptor they recognize.

PF3 (KSAYMRFamide) has been isolated from both the free-living nematode *Panagrellus redivivus* (Maule *et al.* 1994*b*) and from *A. suum* where it is

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referred to as AF8 (Cowden & Stretton, 1995). Muscle tension experiments on *A. suum* revealed that dorsal strips relax and ventral strips contract in the presence of PF3 (Maule *et al*. 1995*a*). PF3 caused only a marked excitatory response in the *F. hepatica* muscle preparations, with an activity threshold of 3 μM. However, the muscle preparation employed in these studies was composed of both dorsal and ventral components; also, unlike the situation in nematodes, platyhelminth muscle is not delineated into separate ventral and dorsal muscle fields. Interestingly, previous studies on *F. hepatica* muscle preparations have shown the tetrapeptide, FMRFamide, to have excitatory effects with an activity threshold of 0·1 μM (unpublished results). Therefore, PF3 and FMRFamide have a 30-fold difference in potency, even though their C-terminal tripeptides are identical. This may be due to the extended N-terminus of PF3, which may inhibit access to the receptor or receptor recognition. Conversely, the peptides may interact with different native FaRP receptors.

As found in a previous study (Marks *et al*. 1996), removal of the peptide stimulus resulted in a rapid return to pre-treatment levels of muscular activity. Also, during many of the peptide treatments, there was no evidence for desensitization of the response. Recently, the first FaRP receptor was identified as a FMRFamide-gated Na^+ channel in the mollusc, *Helix aspersa* (Lingueglia *et al*. 1996). A system of ligand-gated channels may mediate the peptide actions in *F. hepatica*. However, these effects could also be mediated by second messenger systems, as previous studies on cAMP, cGMP, IP_3 and Ca^{2+} mediated responses have shown them to be relatively short-lived in some cases, once the stimulus is removed (Chiba *et al*. 1992; Clapham & Sneyd, 1995; Falconer *et al*. 1993). Direct analysis of *F. hepatica* FaRP receptor localization, binding kinetics and affected transduction mechanisms will be required to delineate the FaRP-response.

The relatively recent realization that nematode FaRPs are extremely diverse and transcribed from a series of FaRP-encoding genes, and that many of these peptides have potent and diverse actions on nematode somatic musculature, have stimulated research in this area with a view to the exploitation of FaRP receptors as drug targets. The findings of the present study reveal that helminth peptides have inter-phyla activity. All of the nematode peptides examined were found to have excitatory effects on *F. hepatica*, indicating that at least some structural similarity between nematode and platyhelminth FaRP receptors must exist. These findings are likely to have important implications in the identification of targets for the development of novel anti-parasitic drugs, in that they suggest that compounds directed at the neuropeptide systems of helminths may have broad-spectrum activity.

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