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AF2, an *Ascaris* Neuropeptide: Isolation, Sequence, and Bioactivity

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COWDEN, C. AND A. O. W. STRETTON. *AF2, an Ascaris neuropeptide: Isolation, sequence, and bioactivity.* PEPTIDES **14**(3) 423–430, 1993.—A FMRFamide-like neuropeptide, KHEYLRFamide (Lys-His-Glu-Tyr-Leu-Arg-Phe-amide; AF2), was isolated from a head extract of the nematode *Ascaris suum* by using a three-step HPLC separation. In a dorsal muscle strip preparation, synthetic AF2 produced multiple effects on muscle tension: a slow relaxation was followed by contraction and rhythmic activity. Sulfated AF2 was no more potent than AF2. The effects on muscle tension were correlated with electrical activity recorded intracellularly from muscle cells. AF2 markedly increased the tension change associated with changes in muscle membrane potential.

Nematode Neuropeptide FMRFamide Muscle tension *Ascaris suum*

CONSIDERABLE progress has been made in demonstrating modulation at neuromuscular junctions in invertebrate preparations [for review, see (5)]. The pattern emerging from these studies is that several signalling molecules released by motorneurons and by modulatory neurons alter the effects of neurotransmitters on the amplitude, speed, and duration of muscle contraction. These effects may involve changes in neuromuscular transmission (either in pre- or postsynaptic elements, or both) and/or in excitation–contraction coupling. Many of the molecules that mediate these effects are peptides.

Prominent among the families of peptides that affect neuromuscular systems are the FMRFamide-like peptides (FLPs). The sequences of a wide variety of FLPs from many different organisms, both vertebrate and invertebrate, have been obtained either directly by peptide isolation and sequencing, or indirectly by cloning and sequencing DNA that encodes these peptides (2,10,19,22,25,27,28,31,33,35,42,44,47,50,52). In many cases, it has been shown that the FLPs modulate muscle contraction by their actions on either the presynaptic or the postsynaptic cell, or both (6,20,25–27,30,32,36,38,40,46,48,49).

We are investigating the role of neuropeptides in the control of locomotion in *Ascaris suum* (9,58). Immunocytochemical studies have shown that there are many different families of putative neuropeptides in *Ascaris* (54,56). However, in order to study the chemical structure and bioactivity of peptides that affect the motornervous system, we have initially concentrated on the FLPs for two reasons. Firstly, FMRFamide-like immunoreactivity (FLI) is found in more than 60% of the 298 neurons in the *Ascaris* nervous system [including sensory neurons, interneurons, putative neuroendocrine cells, and motorneurons (7)], so the FLPs are likely to be present in relatively large amounts compared to peptides expressed in only a few neurons. Indeed, radioimmunoassay (RIA) showed that 100 fmol of FLI per head could be detected in high performance liquid chromatography (HPLC) fractions of head extracts, so chemical isolation appeared feasible. Secondly, the fact that FLI is present in several identified motorneurons in *Ascaris* makes it likely that at least some of the FLPs have direct effects on muscle, facilitating the analysis of their mechanisms of action.

Many peaks of FLI are evident after HPLC separation of extracts of *Ascaris* heads, suggesting that a large family of FLPs is present in the *Ascaris* nervous system (7,9). In order to understand the physiological significance of this chemical diversity, it is necessary to isolate the peptides. The quantities of natural peptides that can be isolated from *Ascaris* are too small to allow extensive physiological experiments to be performed; the natural peptides are therefore isolated in sufficient amounts for microsequence determination, and then large amounts of synthetic peptides are prepared. AF1, a peptide with the sequence KNEFIRFamide, was isolated from an acid methanol extract of 10,000 *Ascaris* heads in five steps of HPLC (9). It is a potent modulator of *Ascaris* inhibitory motorneurons; it reduces the input resistance and blocks slow oscillatory potentials in these cells. We report in this paper the chemical and physiological characterization of a second *Ascaris* FLP, termed AF2.

METHOD

Ascaris lumbricoides var. *suum* were obtained from the small intestine of pigs at a local slaughterhouse. They were transported and maintained at 37°C in phosphate-buffered saline (PBS; 8.5 m*M* sodium phosphate, 150 m*M* sodium chloride, pH 7.4).

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Purification of FLP From Ascaris

Freeze-powdered *Ascaris* heads were extracted with acid methanol and fractionated on C18 cartridges as previously described (9). HPLC separations were performed on a Beckman HPLC system and monitored at 214 nm with a Gilson Model 116 UV detector. All HPLC steps were performed on a Whatman ODS 3 column (4.6 mm × 25 cm) at a flow rate of 1 ml/min. Fractions (0.5 ml) containing FLI were identified by RIA. This assay was performed as previously described (9) except that the primary antibody and the tracer were added to the samples at the same time. The assays were incubated for 24 h before separating the bound and unbound tracer.

The first HPLC step used a gradient of *n*-butanol (2–8%) in 0.1% trifluoroacetic acid (TFA). To avoid overloading the column, this step was performed 11 times and the column was cleaned with 70% acetonitrile (ACN) in TFA after each run. Fractions corresponding to the peak of activity from which AF2 had been purified and partially sequenced in a preliminary experiment were pooled, concentrated (SpeedVac), and diluted to 10% ACN in TFA. This sample was further purified (step 2) by using a gradient of ACN (25–40%) in 0.1% heptafluorobutyric acid (HFBA). This step was performed twice and the fractions corresponding to the major peak of activity were prepared for the next step as before. In the final step of HPLC, a gradient of ACN (5–35%) in TFA was used.

Characterization of FLP

The sequence of the purified peptide was determined by automated Edman degradation as previously described (9). Fast atom bombardment mass spectroscopic (FAB-MS) analysis of the natural peptide was performed by S. Kelly (M-Scan Inc., West Chester, PA) on a VG Analytical ZAB-2SE high field mass spectrometer operating at Vacc = 8 kV. A cesium ion gun was used to generate ions for the mass spectra, which were recorded with a PDP 11-250J data system. Mass calibration was performed with cesium iodide or cesium iodide/glycerol.

Synthetic Peptide Preparation, Characterization, and Identity With Natural Peptide

Synthetic KHEYLRFamide (AF2) was prepared on an automatic solid-phase peptide synthesizer as previously described (9). The FAB-MS analysis of the synthetic peptide was carried out by R. Randall at the University of Wisconsin Biochemistry Department Mass Spectroscopy Laboratory. Crude synthetic peptide was purified on a Beckman Ultrasphere ODS column (10 mm × 25 cm) with a gradient of ACN (20–30%) in TFA at a flow rate of 5 ml/min.

Elution times of the natural and synthetic peptides were compared in four HPLC systems: step A: isocratic RP-HPLC (solvent, 20% ACN in TFA); step B: isocratic RP-HPLC (solvent, 25% ACN in HFBA); step C: gradient RP-HPLC (all conditions the same as the final step of purification); and step D: gel filtration HPLC with two Spherogel TSK 2500PWHR columns (Beckman, 7.8 mm × 30 cm) in series (solvent, 45% ACN in TFA; flow rate, 0.3 ml/min). In all cases, the synthetic peptide was run after the natural peptide, so that there was no risk of contaminating the natural peptide with synthetic peptide.

Sulfated AF2 was prepared using a modification of the method developed previously (45). Crude synthetic AF2 (5 mg) was added to 250 μl conc. sulfuric acid at −8°C with vigorous shaking for 4 min as the peptide went into solution, followed by occasional shaking for a further 30 min at −8°C. The reaction was stopped with 2 ml ice-cold water and 10 ml ice-cold TFA, and peptides were reisolated by absorption onto an activated C18 cartridge, washing with TFA and eluting with 2 ml 50% ACN in TFA. The peptides were then fractionated by isocratic HPLC (solvent, 32.5% ACN in HFBA). In addition to a peak with the same mobility as AF2, there were two new, more hydrophilic peaks. After hydrolysis in 1 *M* HCl at 100°C for 5 min (28), one of them could be converted from a peptide more negatively charged than AF2 to a peptide with the same electrophoretic mobility as AF2 at pH 6.4 (high-voltage paper electrophoresis); it was therefore identified as sulfated AF2. The other new component was resistant to this mild acid hydrolysis (as was AF2 itself), and we surmise that it is sulfonated AF2.

Physiology

In preliminary muscle tension experiments, the most consistent results were obtained with muscle strips taken from the region of the gonopore, rather than from anterior portions of the worm. A 2–2.5 cm piece of worm, including the gonopore, was taken [from segment IV (57)] and the ventral half removed by cutting along the lateral lines. The dorsal half was pinned down along the anterior end and along the most anterior 1.0 cm of both lateral lines in a Sylgard chamber (volume 1 ml). The posterior end was attached to an isometric force transducer (Gould model FT03) with a suture thread that was drawn through the free end of the dorsal muscle strip with a surgical needle [modified from (53)]. Baseline tension was set at about 5 g.

After the muscle strip was prepared as described above, intracellular recordings of electrical activity were obtained from muscle cell bellies directly over the nerve cord and near the anterior end of the preparation using standard electrophysiological techniques developed for *Ascaris* (59).

Synthetic peptides were dissolved in normal *Ascaris* saline (sodium acetate, 125.1 m*M*; NaCl, 3.9 m*M*; KCl, 24.5 m*M*; $MgCl_2$, 4.9 m*M*; and $CaCl_2$, 5.9 m*M*; MOPS buffer, 5 m*M*; adjusted to pH 6.8 with NaOH) and superfused over the preparation at a flow rate of 1.5 ml/min at 37 ± 1°C. HPLC-purified synthetic AF2 was quantified by its optical density at 280 nm and the extinction coefficient of tyrosine. HPLC-purified synthetic AF1 and sulfated AF2 were quantified by comparing their optical densities at 214 nm with that of AF2.

RESULTS

Isolation and Confirmation of AF2

AF2 was first isolated and sequenced concurrently with the final purification of AF1; however, the identity of the PTH amino acid in the second cycle was unclear. Since there was insufficient purified peptide to resolve this problem by coelution with synthetic peptides or by FAB-MS, another purification was performed. The extraction and fractionation procedure was virtually identical to the purification of AF1 (9), as was the first step of HPLC (Fig. 1A).

In order to increase the yield of purified peptide, the second step was changed. Gel filtration HPLC had been used in the first purification to separate AF2 from a larger peptide that coeluted with it in RP-HPLC; however, the recovery was low compared with that obtained with RP-HPLC. Switching from TFA to HFBA as counter-ion in RP-HPLC separates peptides on the basis of the number of positive charges (3). As is shown in Fig. 1B, this procedure led to the successful separation of AF2 from most of the other material detected by optical density at 214 nm, including the larger peptide that appears as a broad peak eluting after AF2.

A third step of RP-HPLC using a gradient of ACN in TFA gave only a single peak (Fig. 1C). Microsequencing showed that

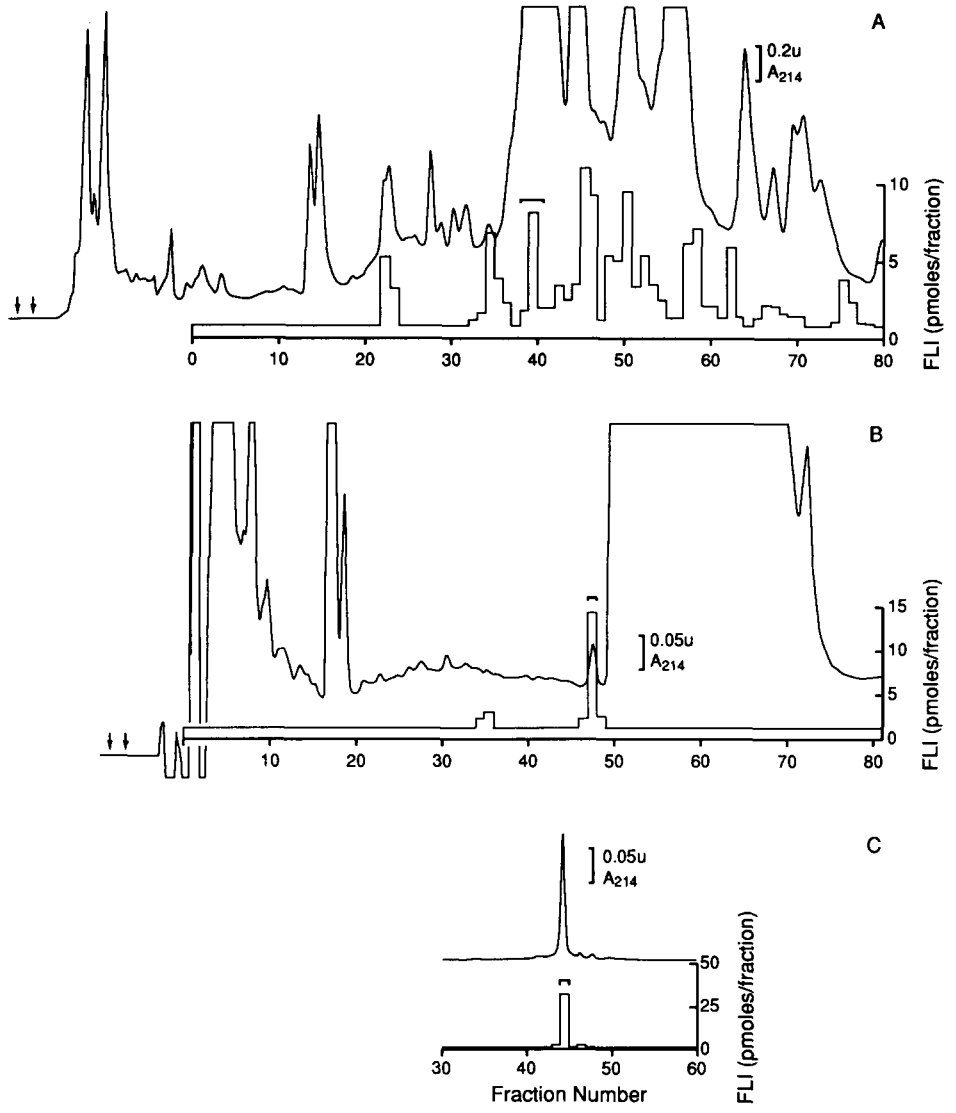

FIG. 1. Isolation of AF2 in a three-step HPLC separation. (A) First step: gradient RP-HPLC in *n*-butanol/TFA. FMRFamide-like immunoreactivity was assayed by RIA, and optical density at 214 nm (A_{214}) was monitored. The bracket shows the fractions taken to the next purification step. This step was repeated 11 times, and similar fractions were pooled with fractions 38–40 from this chromatogram. (B) Second step: gradient RP-HPLC in ACN/HFBA. This step was repeated twice and the fractions corresponding to that marked with the bracket were pooled. (C) Final step: gradient RP-HPLC in ACN/TFA. The fraction indicated by the bracket was taken for microsequencing.

it was essentially homogeneous (Fig. 2A). On the basis of the yield of PTH amino acids obtained from microsequencing, about 1 nmol of peptide was purified, more than three times the amount of peptide obtained from the earlier purification. However, the RIA detected only 30 pmol of FMRFamide-like immunoreactivity in the final peak (see Fig. 1C). Thus, the cross-reactivity of AF2 in this assay is low. Since microsequencing may underestimate the quantity of peptide present, we can estimate the cross-reactivity of AF2 to be 3% or less.

The natural peptide was analyzed in three ways. First the amino acid sequence was determined to be KHEYLRF by microsequencing (Fig. 2A). Because the RIA is specific for *C*-terminal RFamide peptides (34), a synthetic peptide was prepared in the amidated form. The mass of AF2 predicted from its chemical structure is 991.23 Da. The FAB-MS showed that the mass of the natural peptide is 991 Da (Fig. 2B) and the mass of the synthetic peptide is 991.53 (data not shown). This is independent confirmation of amidation, since the nonamidated form of this peptide would have a mass of 992.23 Da. Furthermore, the elution times of the natural and synthetic peptides were almost identical in four HPLC systems (step A: 21.5 and 21.5 min; step B: 60.7 and 60.8 min; step C: 36.9 and 36.8 min; step D: 70.2 and 70.1 min).

AF1 was reisolated in this purification using the same three steps of HPLC as for AF2; its elution time was nearly identical to that of synthetic AF1 in step 3 (data not shown). The quantity of purified AF1, estimated by comparing the optical density of the purified peptides (in step 3), was about one-fifth that of AF2.

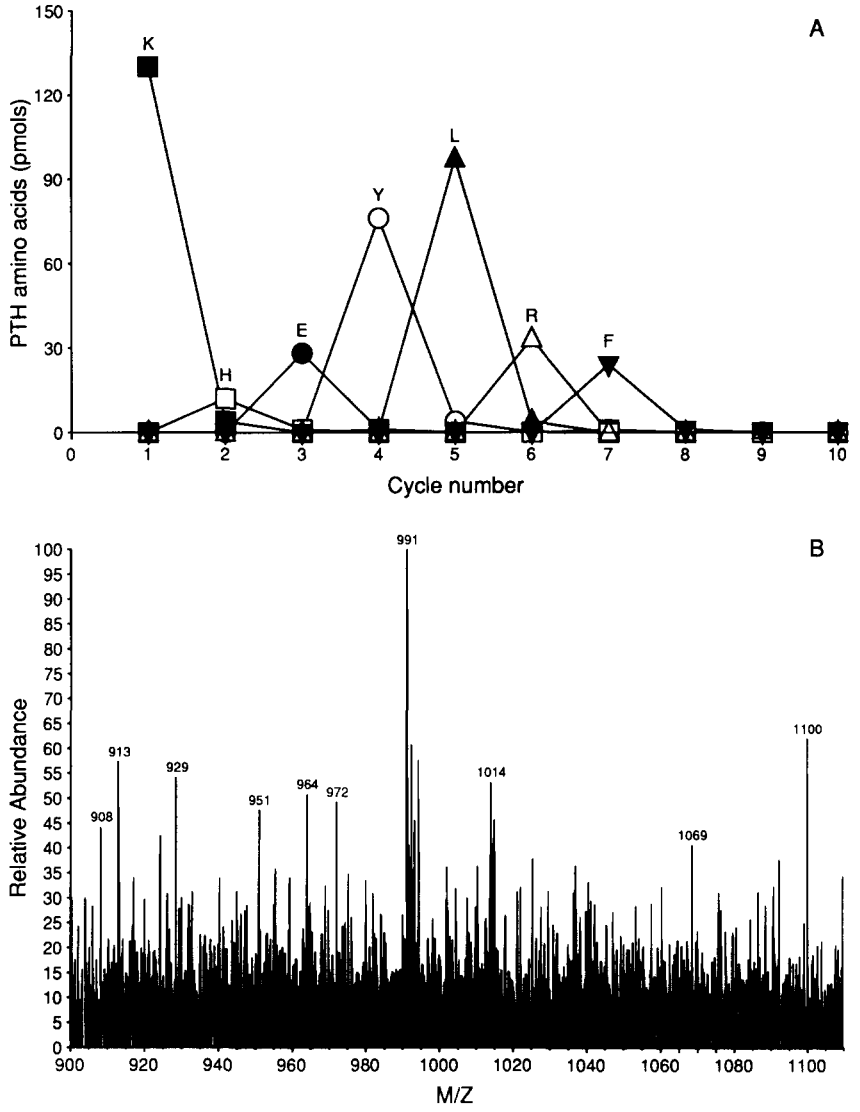

FIG. 2. Characterization of FLP. (A) Sequence determination of AF2 by Edman degradation. The quantities of PTH amino acids detected at each cycle is shown. Each amino acid is given a different symbol. The lag in each cycle is low. There is no evidence for a contaminating peptide. (B) Fast atom bombardment mass spectroscopy of peptide AF2 isolated from *Ascaris*. The M + H ion is seen at m/z 991 and the M + Na adduct ion at m/z 1014.

Biological Activity of AF2

Injection of 0.1 ml of 10^{-5} *M* AF2 into the anterior region of *Ascaris* (effective concentration ca. 10^{-6} *M*; $n = 4$) blocked locomotory movements in the region of the injection. Worms injected with control saline ($n = 4$) were not affected. This simple experiment suggested that AF2 is bioactive.

In order to explore the physiological effects of AF2 more rigorously, its effects on muscle tension (MT) were measured using a dorsal muscle strip preparation. Besides dorsal muscle, this preparation contains part of the dorsal and dorsal sublateral nerve cords. These include neuronal processes but no neuronal somata (57). The region of the gonopore was used because preliminary experiments gave more consistent results with it than with anterior portions of female worms. AF2 has multiple effects on MT; these effects depended on peptide concentration. In 34 experiments in which 10^{-7} *M* AF2 was applied for 5–30 min, relaxation was observed 31 times; a later contraction followed by rhythmic activity occurred in 30 cases (Fig. 3A). Similar but weaker effects were observed even at 10^{-11} *M* AF2 ($n = 2$; data not shown). The magnitude of these effects on MT varied between individual preparations. In addition, removing the peptide did not reverse the effects of AF2; in some cases the contraction and rhythmic activity continued for 1 to 2 h after peptide removal. Therefore, it was not possible to repeat an experiment or compare the response to different concentrations of peptide within an individual preparation. In a total of 97 experiments in which MT was measured for up to 90 min, spontaneous rhythmic activity (i.e., rhythmicity in the absence of either AF2 or other peptides) was observed in only three preparations.

The effects of AF1 on MT were also measured. At 10^{-6} and 10^{-7} *M*, AF1 has effects on MT like 10^{-7} and 10^{-8} *M* AF2; it is less potent than AF2 in this assay (data not shown).

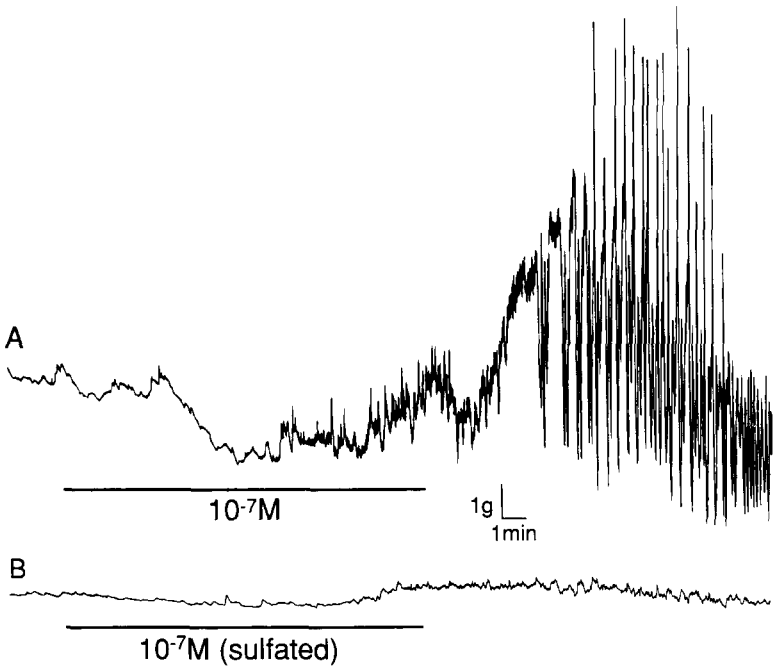

FIG. 3. Effects of AF2 or sulfated AF2 on muscle tension (MT) in two dorsal muscle strip preparations. Bar indicates exposure time to peptide: (A) and (B) = 15 min. Contraction is up. The beginning and ending of the bar shows when the changes in the superfusion system were made. Peptide enters the bath after 1.5 min. (A) A relaxation is seen following exposure to 10^{-7} *M* AF2. This is followed by contraction and the late development of rhythmic activity. (B) Action of 10^{-7} *M* sulfated AF2 produces a small relaxation followed by a small contraction and low-amplitude complex rhythmic activity.

One approach to evaluating the potential role of a sulfated form of AF2 is to determine whether it is biologically active. When 10^{-7} *M* sulfated AF2 was applied to a dorsal muscle strip, slow relaxation was observed followed by a small baseline contraction and small amplitude rhythmic activity ($n = 4$) (Fig. 3B). Due to individual variation and the long-term effects of AF2 peptides, it was not possible to make direct comparisons with the nonsulfated peptide. However, it is clear that sulfated AF2 is not more potent than nonsulfated AF2; if anything, it is less active than AF2, in that late high-amplitude rhythmic responses were not observed.

In preliminary electrophysiological experiments, the anterior end of the muscle strip was pinned sufficiently to prevent all movement. Intracellular recordings from muscle cell bellies over the dorsal nerve cord in the immobilized area showed normal resting potentials but no electrical activity, even when the free end of the muscle strip was rhythmically contracting. However, recordings from muscle bellies posterior to that area (not immobilized) consistently showed electrical activity. Thus, the electrical activity observed in this study is mechanosensitive. This activity was indistinguishable from that previously recorded from muscle cells (15) and was distinct from the movement artifacts that were occasionally observed. In all subsequent experiments, recordings of electrical activity were made from muscle bellies in a partially immobilized area pinned only along the anterior end and along the most anterior 1.0 cm of both lateral lines.

Simultaneous recordings of electrical activity in a muscle cell near the anterior end of the dorsal muscle strip (DM) and of MT in response to 10^{-7} *M* AF2 ($n = 8$) showed a small hyperpolarization (1–5 mV) during the relaxation phase (Fig. 4A, upper and middle traces), and a small depolarization (1–5 mV) and an increase in the frequency of muscle spikes (from 9.25 min^{-1} to 15.0 min^{-1}) during the beginning of the contraction phase (Fig. 4A, lower traces). After rhythmic activity had been generated and the peptide had been removed, rhythmic contractions and changes in baseline tension were correlated with slow polarizations (and associated spike events) that occurred in clusters (Fig. 4B). It is noteworthy that very large changes in MT (2–10 g) were correlated with small changes in membrane potential (0.5–5 mV) at the recording site (Fig. 4B). Changes in membrane potential of this magnitude were also observed before AF2 was applied (see individual spike events in Fig. 4A), but the correlated activity in MT was much smaller (less than 1 g, Fig. 4A).

DISCUSSION

AF2 and Other AF Peptides

AF2 is the second biologically active FLP isolated from *A. suum*. It is a new FLP, related to AF1 by three conservative amino acid substitutions, and has not been reported from other organisms.

The presence of a glutamate residue preceding the tyrosine raised the possibility that AF2 might be sulfated in vivo (23). However, the mass of sulfated AF2 is 1071; furthermore, sulfated AF2 does not coelute with the natural peptide (data not shown). These results show that the isolated AF2 was not sulfated. A smaller peak of activity eluting in step 2 before AF2 (Fig. 2) had the same elution time as sulfated AF2; this peak was not further

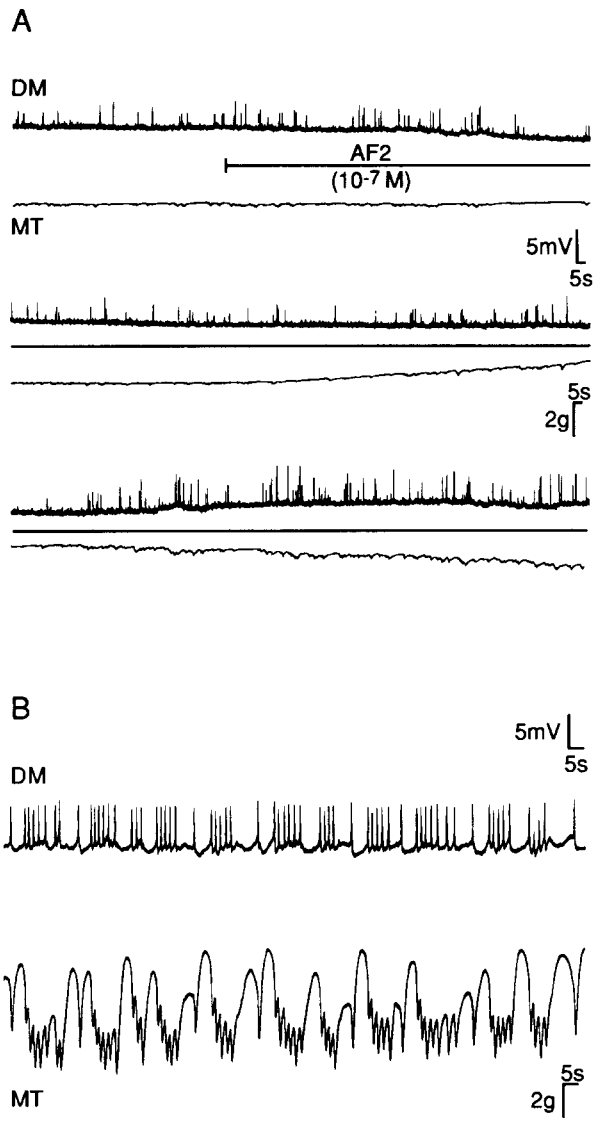

FIG. 4. Simultaneous recording of muscle tension (MT) and membrane potential of dorsal muscle (DM) in two preparations. In the MT record, contraction is down. (A) The membrane potential shows a small hyperpolarization during the relaxation phase followed by a depolarization and an increase in the frequency of muscle spikes during the beginning of the contraction phase. The bar shows when the AF2 superfusion was started. AF2 reached the chamber after 1.5 min. (B) Simultaneous recording of MT and DM after exposure to 10^{-7} *M* AF2 for 15 min. This record is taken 32 min after washout of AF2 begins. Large changes in tension are associated with relatively small changes in membrane potential.

purified or characterized. Thus, we cannot rule out the possibility that a sulfated form of AF2 is present in *Ascaris*, although it has not yet been isolated. However, the biological activity of sulfated AF2 is no higher than that of AF2 itself. This is in contrast to sulfated neuropeptides in other systems; leucosulfakinin I (LSK I), for example, is 10,000 times more potent than the nonsulfated form of the peptide (41).

AF2 has some structural features in common with cholecystokinin (CCK)/gastrin-like peptides, especially those found in insects, e.g., the locust peptide pQSDDY(SO_3H)GHMRFamide (41). These features include a tyrosine preceded by an acidic residue (DY or EY) and a *C*-terminal RFamide preceded by L (a conservative substitution for M). However, the CCK-like peptides detected in *Ascaris* head extracts have different elution times than AF2 (55), so there are other peptides that may be more similar chemically and physiologically to the CCK/gastrin family of peptides than AF2.

The relative recovered quantity of AF2 suggests that it is an important signal molecule in the *Ascaris* nervous system. More purified AF2 was recovered than the other 12 *Ascaris* FMRFamide-like (AF) peptides isolated from *Ascaris* heads so far; about five times more AF2 than AF1 was recovered in this purification. The significance of these findings to the in vivo levels of the AF peptides cannot be properly determined until the efficiency of extraction and the recovery of each of the steps used in the fractionation has been measured for each peptide. However, differential expression of AF peptides extracted from heads, tails, and pharynges and separated by HPLC has also been observed (7). At this time, it is not known whether AF1 and AF2 are encoded by the same gene in *Ascaris*. If they are, differential expression could be achieved by alternative splicing of the mRNA such that AF1 and AF2 appear on different transcripts (43) or at the posttranslational level by selective processing of the precursor protein such that AF2 is produced and/or sorted for secretion independently of AF1 (21). In addition, the gene may encode more copies of AF2 than AF1, potentially amplifying the difference in relative abundance (51).

AF2 and Spontaneous Activity in Ascaris Somatic Muscle

In this study, the effects of AF2 on muscle tension and electrical activity in a dorsal muscle strip were recorded simultaneously. Fifteen-minute superfusions of AF2 in normal *Ascaris* saline consistently generate rhythmic activity of considerable force, which continues long after the peptide is removed. This activity in muscle tension is correlated with clusters of slow polarizations and associated spike events, with clusters occurring with a mean period of 6–33 s. In female *Ascaris*, the mean period for forward moving waves is 7.1 s (range 2–12 s), or 13 s (range 7–20 s) for head-ligatured worms; for backward moving waves it is 2.6 s (range 0.6–5 s) (37). Thus, the period of the rhythmic activity generated by AF2 is comparable to that of locomotory movements. This activity is also similar to activity, previously reported by Weisblat and Russell (60), which they named modulated activity.

Several previous reports have noted that the occurrence, amplitude, and frequency of spontaneous electrical activity in *Ascaris* muscle depend on several variables, including the contents of the physiological saline, the temperature, and the details of the dissection used in making the preparation (1,4,14–18,24,60,61). In previous electrophysiological experiments, it was found that a twofold increase in calcium and magnesium concentrations (HCHM) in *Ascaris* saline suppressed spontaneous muscle activity and permitted stable intracellular recordings from muscle cells (59) or neurons (12). In preliminary experiments with HCHM saline, our preparations were quiet within a few minutes after attachment to the force transducer, and usually failed to generate modulated activity when exposed to AF2. In normal saline prior to perfusion with AF2, these preparations were not quiet but displayed irregular contractions and relaxations of low and variable amplitude (the electrical activity was also irregular); spontaneous rhythmic activity was observed only three times in 97 preparations, and it was of low amplitude.

Modulated activity has been attributed to neuronal input (60). This conclusion was drawn from the observation that dorsal muscle showed modulated electrical activity when the commis-

sures to the ventral cord [containing the neuronal somata and the interneurons that synapse upon excitatory motorneurons (57)] were intact, but not when they were cut. In our experiments with a dorsal muscle strip from which the ventral side had been removed (all commissures were cut), modulated activity was not observed in normal saline. However, we have found that AF2 consistently generates modulated activity, and this activity continues long after the peptide is removed. Thus, our working hypothesis is that AF2 has a direct effect on muscle and that modulated activity is an endogenous muscle activity. We recognize that the mechanism linking AF2 action and modulated activity may well be complex. One possible interpretation of these effects is that AF2, or an endogenous molecule with the same ability to generate modulated activity in muscle, is released either directly or indirectly by the dorsal excitatory motorneurons when they are intact but not when they are cut.

Cellular localization experiments showing that FLI is present in dorsal motorneurons (7) also support the suggestion that AF peptides act directly on muscle cells. This possibility must be treated cautiously, however, since anatomical and physiological experiments have shown that the dorsal nerve cord also contains numerous synapses from excitatory motorneurons to other motorneurons [Donmoyer and Stretton, unpublished; (11)].

While our results are compatible with the hypothesis that muscle cells are the site at which AF2 acts, they do not rule out effects on motorneurons and/or interneurons. The physiological preparation we used is complex. Processes of dorsal motorneurons are present in the dorsal nerve cord; although these processes are severed from their cell bodies, the cut ends almost certainly seal (11), so AF2 receptors, if present on these processes, might give rise to the responses in muscle reported in these experiments.

Recently, the effects of cholinergic drugs (in HCHM) on excitation–contraction coupling in a partially pinned preparation cut along the left lateral line (most of the commissures were intact) have been studied (53). In these experiments, several cholinergic agents apparently uncoupled the excitation–contraction relationship. In contrast, the apparent effect of AF2 is to amplify the magnitude of contraction in response to excitation. In this modulated relationship, excitation and contraction are still tightly coupled.

Our experiments also showed that there are mechanical effects on muscle electrical activity. Similar effects on behavior and on muscle electrical activity have previously been observed in a semi-intact preparation of *Ascaris* (37). Since stretch-activated channels appear to be ubiquitous (39), it is likely that they are present in *Ascaris* somatic muscle as well.

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