J. exp. Biol. ***190***, *253–264 (1994)*
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THE EFFECTS OF MYOMODULIN AND STRUCTURALLY RELATED NEUROPEPTIDES ON SKELETAL NEUROMUSCULAR TRANSMISSION IN THE LOCUST

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Accepted 2 February 1994

Summary

1. The modulatory actions of myomodulin A on tension generated in the extensor-tibiae muscle of the locust hindleg by stimulation of the slow excitatory motoneurone (SETi) depend upon the frequency of stimulation. Myomodulin A has no consistent effect on the tension induced by the fast extensor motoneurone (FETi) or upon the myogenic rhythm present in the extensor. The effects of a range of structurally related neuropeptides have also been assessed.

2. At low frequencies of SETi stimulation (1 Hz and below), the predominant modulatory effects are increases in the amplitude, contraction rate and relaxation rate of twitch tension. At higher frequencies, where twitches summate but tetanus is incomplete (up to 20 Hz), these effects are superimposed upon an increase of maintained tension.

3. The modulatory actions of myomodulin-like peptides show some similarities to and some differences from the modulatory actions of octopamine, proctolin and FMRFamide-like neuropeptides in this preparation, but are likely to be mediated *via* a distinct set of receptors.

4. The results of the present study, taken together with the localization of myomodulin-like immunoreactivity in specific sets of neurones in the locust nervous system, suggest the presence of a novel modulatory system in insects that uses myomodulin-like neuropeptides. It also indicates that myomodulins, which were first identified in molluscs, may represent another interphyletic family of neuropeptides.

Introduction

The molluscan neuropeptide myomodulin (PMSMLRL-NH_2) was first isolated as a neuropeptide present in an identified cholinergic buccal motoneurone (B16) from *Aplysia californica*, where it serves to modulate neuromuscular transmission to the accessory radula closer muscle (Cropper *et al.* 1987; Whim and Lloyd, 1990). Six other structurally related myomodulin-like neuropeptides have since been isolated from *Aplysia* (Cropper *et al.* 1991; Miller *et al.* 1991), and the gene encoding this family of myomodulin-related neuropeptides has also been characterized in *Aplysia* (Miller *et al.* 1993). In addition, a myomodulin-like peptide has been isolated from *Fusinus* (Kobayashi and Muneoka,

Key words: neuropeptides, myomodulin, neuromuscular transmission, locust, *Schistocerca gregaria*.

1990), and *Mytilus* catch-relaxing peptide (CARP) has been shown to be a homologous peptide that differs from myomodulin A at only two residues (Hirata *et al.* 1987). Myomodulin-like neuropeptides have only been isolated from molluscan species.

Recently, however, immunocytochemical evidence has been presented for the existence of myomodulin-like immunoreactivity in the central nervous system of the locust *Schistocerca gregaria* (Swales and Evans, 1994). In the suboesophageal ganglion of the locust, myomodulin-like immunoreactivity occurs in five groups of cells. The processes from the two anterior ventral midline groups of cells, where myomodulin-like immunoreactivity is co-localised with that for bovine pancreatic polypeptide (BPP), for FMRFamide (Myers and Evans, 1985*a*,*b*) and for locustamyotropin (Schoofs *et al.* 1992*b*), project to the corpora allata *via* nervi corpora allata II. Myomodulin-like neuropeptides may therefore be involved in the control of the release of juvenile hormone in the locust. The thoracic ganglia of the locust contain three groups of myomodulin-immunoreactive cells, including a bilaterally symmetrical group of 12–15 posterior lateral cells that are also immunoreactive to BPP and FMRFamide (Myers and Evans, 1985*a*,*b*). The latter cells project *via* the thoracic median nerves to the median neurohaemal organs, suggesting a neurohormonal role for myomodulin-like peptides in the locust. Since the latter cells have also been shown to project *via* the median nerves to innervate a group of small skeletal muscles, which disappear during the first few days of adult life (Myers and Evans, 1985*a*), it is also possible that myomodulin-like peptides in the locust could function as locally released neuromodulators of neuromuscular transmission.

To understand fully the functional role(s) of myomodulin-like neuropeptides in the locust it will be necessary to sequence the endogenous myomodulin-like peptides present in the locust nervous system. However, since a quantitative assay has not yet been established for myomodulin-like peptides, all isolation and purification studies on peptides of this family in molluscs have relied on bioassays (Cropper *et al*. 1987, 1991). I have therefore initiated a range of studies to identify possible physiological target sites for myomodulin-like peptides in the locust, which could provide useful bioassays for the isolation and characterization of the endogenous neuropeptides from this family in the locust nervous system.

The present study has examined the ability of myomodulin A, and of a range of structurally related neuropeptides, to modulate neuromuscular transmission and muscle contraction in the extensor-tibiae neuromuscular preparation of the hindleg of the locust. The insect skeletal neuromuscular junction provides a very simple system in which to study the effects of a range of neuromodulatory compounds, such as biogenic amines and neuropeptides. In particular, many studies have focused attention on the modulation of neurotransmission in the extensor-tibiae neuromuscular preparation from the hindleg of the locust *Schistocerca gregaria* (see Evans and Myers, 1986*a*) because of the large size of this muscle and the simplicity of its neuronal innervation (Hoyle, 1955*a*,*b*; Pearson and Bergman, 1969; Hoyle and Burrows, 1973). Neuromuscular transmission mediated by the slow excitatory motoneurone to the extensor-tibiae muscle (SETi) was first shown to be modulated by the biogenic amine octopamine. This amine increases both the amplitude of SETi-induced twitch tension and its rate of relaxation, and acts at both pre- and

postsynaptic sites on the muscle (Evans and O'Shea, 1977, 1978; O'Shea and Evans, 1979; Evans, 1981, 1984*a*,*b*,*c*, 1985). In addition, octopamine alters the 'catch'-like properties of the muscle and its ability to produce maintained tension (Evans and Siegler, 1982). These properties of this neuromuscular system have been shown to be further modulated by a range of neuropeptides, including proctolin (May *et al.* 1979; Evans, 1982; O'Shea, 1985) and members of the Phe-Met-Arg-Phe-NH_2 (FMRFamide)-like family of neuropeptides (Evans and Myers, 1986*b*; Robb *et al.* 1989; Cuthbert and Evans, 1989).

The results of the present study, taken together with the localization of myomodulin-like immunoreactivity in specific sets of neurones in the locust ventral nerve cord (Swales and Evans, 1994), brain and retrocerebral complex (L. S. Swales and P. D. Evans, in preparation), suggest the presence in insects of a modulatory system that uses myomodulin-like neuropeptides. They also indicate that myomodulins, which were first identified in molluscs, may represent another interphyletic family of neuropeptides.

Materials and methods

Adult locusts (*Schistocerca gregaria* Forskål) of either sex were obtained from crowded laboratory colonies fed on wheat seedlings. Small batches of animals were left for 1–2 h before use, after removal from the main culture, to minimize any initial potentiation effects due to elevated levels of octopamine in the haemolymph (see Evans, 1981; Davenport and Evans, 1984*a*,*b*).

Tension in the extensor-tibiae muscle of the locust hindleg was measured almost isometrically with a tension transducer attached distally to the apodeme. The slow extensor-tibiae (SETi) and the fast extensor-tibiae (FETi) motoneurones were excited by stimulating nerve 3b or 5, respectively, with a pair of silver hook electrodes. Patterned pulse trains were computer-generated and used to trigger the output of a stimulator (Evans and Siegler, 1982). An operational amplifier signal-differentiator was used to measure continuously the rates of contraction and relaxation of neurally evoked tension in the different muscle regions (Buchan and Evans, 1980).

Myomodulin A and related peptides were superfused directly onto the surface of the muscle dissolved in a physiologically isotonic saline (pH 6.8) containing (in $mmol\,l^{-1}$): NaCl, 140; KCl, 10; $CaCl_2$, 4; $NaHCO_3$, 4; NaH_2PO_4, 6; and sucrose, 90. Dose–response curves were measured for concentrations between 10^{-9} and $10^{-5}\,mol\,l^{-1}$ on at least four separate preparations for each peptide tested.

Extensor-tibiae muscles were prepared for cyclic AMP assays as described previously (Evans, 1984*a*). Experimental and control muscles were pre-incubated for 10 min in $10^{-4}\,mol\,l^{-1}$ isobutylmethylxanthine (IBMX) before exposure of the experimental muscle to concentrations of myomodulin A up to $10^{-5}\,mol\,l^{-1}$ plus IBMX for 10 min; the control muscle was exposed to a 10 min incubation in IBMX. At the end of the incubation period, the muscles were frozen and rapidly dissected away from their surrounding cuticle. The muscles were homogenized and cyclic AMP levels assayed as described previously (Evans, 1984*a*).

Myomodulin A, catch-relaxing peptide, pheromone biosynthesis activating neuropeptide (PBAN-1), locustatachykinins I and II and crustacean neuropeptide F1 were

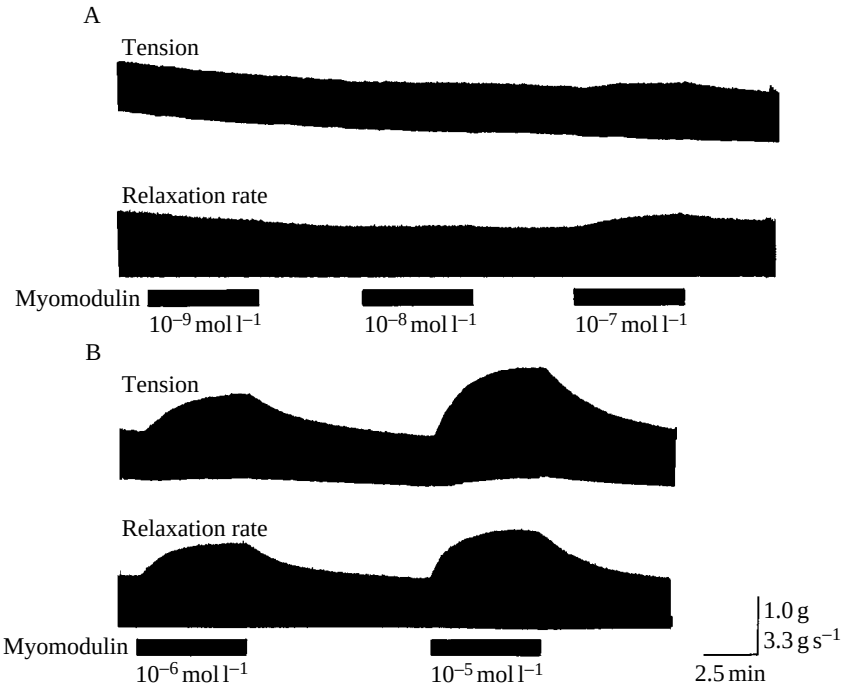

Fig. 1. The effect of 5 min pulses of myomodulin A on SETi-induced twitch tension in the extensor-tibiae muscle. SETi was stimulated at a frequency of 1 Hz. (A) Effects of pulses of 10^{-9}, 10^{-8} and 10^{-7} mol l^{-1}; (B) effects of pulses of 10^{-6} and 10^{-5} mol l^{-1}.

all obtained from Peninsula Laboratories Inc. L-Pro-L-Met and buccalin were obtained from Bachem (UK) Ltd. Leucopyrokinin and leucopyrokinin (4-8) were obtained from Sigma. Locustamyotropin I and II were the kind gift of Dr G. M. Holman.

Results

Myomodulin produces similar increases in both the amplitude and relaxation rate of twitch tension generated in the extensor-tibiae muscle by stimulation of the slow motoneurone at 1 Hz (Fig. 1). These effects outlast the presence of the peptide in the superfusate by several minutes. During a prolonged pulse of 10^{-5} mol l^{-1} myomodulin, maximal effects on each of these variables are reached after 3–4 min. Myomodulin, at concentrations up to 10^{-5} mol l^{-1}, produces no observable effects on twitch tension generated by stimulating the fast motoneurone to the extensor-tibiae muscle (FETi) at 1 Hz. In addition, it has no consistent observable effect on the amplitude or frequency of the myogenic rhythm of contraction and relaxation observed in this muscle.

The effects of myomodulin on SETi-induced twitch tension are dose-dependent. A 5 min pulse of the peptide shows a threshold for both effects between 10^{-8} and 10^{-7} mol l^{-1} (Figs 1 and 2). Fig. 2 shows that a closely related molluscan peptide, catch-relaxing peptide, also produces similar dose-dependent effects to those of myomodulin, but is slightly less effective at lower concentrations.

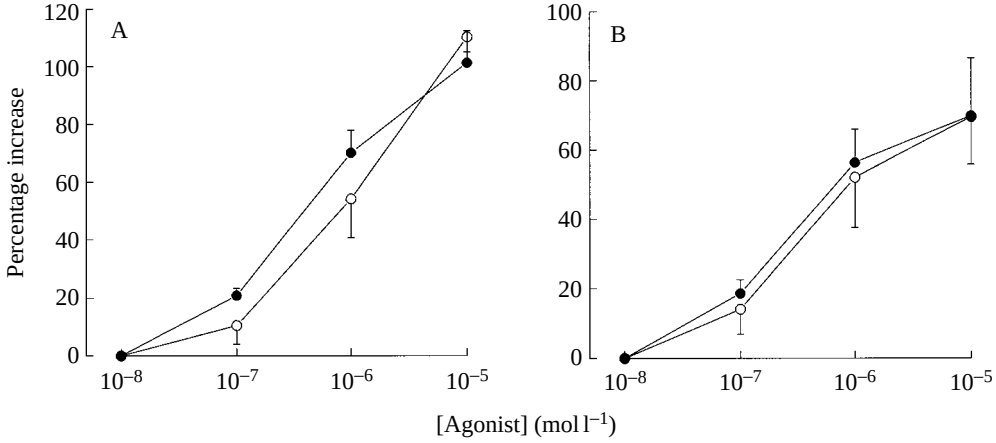

Fig. 2. Dose–response curves for the actions of myomodulin A (●) and catch-relaxing peptide (○) on SETi-induced twitch tension. (A) Maximal effects on twitch amplitude; (B) maximal effects on the rate of relaxation of twitch tension. SETi was fired at a frequency of 1 Hz, and each of the peptides was introduced into the superfusate for a period of 5 min. Each point represents the mean of at least four determinations, each on a separate animal, and the bars represent standard errors.

Table 1. *The specificity of myomodulin-related peptides on the locust extensor-tibiae muscle*

Peptide	Structure	Increase in twitch amplitude (%)	Increase in relaxation rate of twitch tension	Initiation of myogenic rhythm	*N*
Myomodulin	PMSMLRL-NH_2	70.1±7.8	56.5±9.5	–	7
Catch-relaxing peptide	AMPMLRL-NH_2	54.1±13.5	52.1±14.5	10^{-7}–10^{-6}	3
L-Pro-L-Met	PM	0	0	–	3
Leucopyrokinin	QTSFTPRL-NH_2	8.8±0.9	12.6±0.9	10^{-8}–10^{-7}	3
Leucopyrokinin (4–8)	FTPRL-NH_2	10.7±0.9	20.0±1.2	10^{-7}–10^{-6}	3
PBAN1	26_{aa}+RYFSPRL-NH_2	0	0	10^{-7}–10^{-6}	3
Locustamyotropin I	GAVPAAQFSPRL-NH_2	30.2±3.2	10.4±1.2	10^{-8}–10^{-7}	3
Locustamyotropin II	EGDFTPRL-NH_2	6.7±1.6	10.2±0.9	10^{-8}–10^{-7}	3
Buccalin	GMDSLAFSGGL-NH_2	0	0	–	3
Locustatachykinin I	GPSGFYGVR-NH_2	30.2±2.6	41.7±4.6	–	3
Locustatachykinin II	APLSGFYGVR-NH_2	18.2±1.9	38.4±6.2	–	3

5 min pulses of peptides were applied to the preparation at various concentrations. The effects of the peptides on tension elicited by stimulating the slow motoneurone at 1 Hz are compared at a concentration of 10^{-6} mol l^{-1}.

Values are mean ± 1 S.E.M.

Table 1 compares the effectiveness of myomodulin with a range of structurally related neuropeptides tested as 5 min pulses at 10^{-6} mol l^{-1}. Catch-relaxing peptide differs from myomodulin by two amino acids at its N terminus and, as mentioned above, has similar

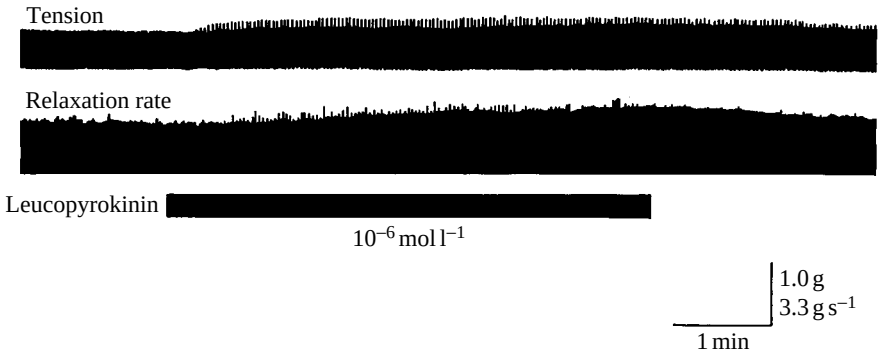

Fig. 3. The effect of a 5 min pulse of leucopyrokinin at a concentration of 10^{-6} mol l^{-1} on SETi-induced twitch tension in the extensor-tibiae muscle. SETi was stimulated at a frequency of 1 Hz. Note the induction of myogenic contractions superimposed upon those generated by SETi stimulation.

effects on SETi-induced twitch tension. However, in contrast to the actions of myomodulin, catch-relaxing peptide was consistently able to initiate a myogenic rhythm of contraction and relaxation in the extensor-tibiae muscle. A threshold for this effect occurred between 10^{-7} mol l^{-1} and 10^{-6} mol l^{-1}. The dipeptide L-Pro-L-Met, which corresponds to the first two amino acids of myomodulin, had no effect on the extensor-tibiae muscle up to a concentration of 10^{-5} mol l^{-1}. In addition, it did not block the actions of myomodulin.

We also examined the effects of a range of other insect neuropeptides ending with the sequences -Arg-Leu-NH_2 at their C termini (Table 1). Both leucopyrokinin (Holman *et al.* 1986) (Fig. 3) and leucopyrokinin (4-8) produced small increases in both the amplitude and relaxation rate of twitch tension. Like catch-relaxing peptide, they were also able to activate the myogenic rhythm, with leucopyrokinin being almost an order of magnitude more potent than leukopyrokinin (4-8). Pheromone biosynthesis activating peptide (PBAN1) (Kitamura *et al.* 1989; Raina *et al.* 1989) is a large peptide (33 amino acids) that has the same three C-terminal amino acids as leucopyrokinin, namely -Pro-Arg-Leu-NH_2. It was not able to modulate slow motoneurone neuromuscular transmission in a dose-dependent and reliable fashion. However, it consistently induced a dose-dependent increase in the frequency of the myogenic rhythm at concentrations above 10^{-7} mol l^{-1}. The last five amino acids at the C terminus of PBAN1 are the same as those of locustamyotropin I (Schoofs *et al.* 1990*a*) (Table 1). The latter peptide was able to increase both the amplitude and relaxation rate of SETi-induced twitch tension, but was less effective than myomodulin. In addition, it also initiated the myogenic rhythm when applied at concentrations above 10^{-8} mol l^{-1}. The related neuropeptide locustamyotropin II produced similar effects.

Buccalin is a neuropeptide found co-localised with myomodulin in molluscan neurones (Cropper *et al.* 1988). Both peptides have the same C-terminal ending of -Leu-NH_2. However, at concentrations up to 10^{-5} mol l^{-1}, buccalin produced no effects on SETi-induced twitch tension or the myogenic rhythm in the extensor-tibiae muscle. We also tested the effects of the recently described locustatachykinins, another class of C-

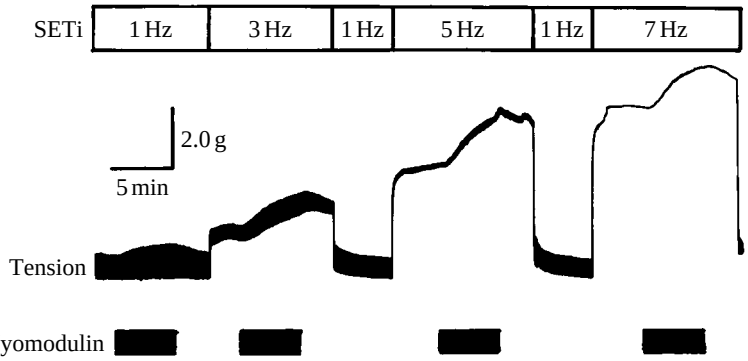

Fig. 4. The frequency-dependence of the effects of myomodulin A on maintained tension. A continuous recording of the tension profile from a metathoracic extensor-tibiae muscle produced by stimulating SETi at the different frequencies shown. 5 min pulses of 10^{-6} mol l^{-1} myomodulin A (filled bars) were introduced into the superfusate. The preparation was returned to 1 Hz stimulation between the 3 and 5 Hz stimulations and also between the 5 and 7 Hz stimulations.

terminally amidated neuropeptides isolated from the nervous system of the locust *Locusta migratoria* (Schoofs *et al.* 1990*b*). Both peptides of this class tested (Table 1) were able to increase the amplitude and relaxation rate of SETi-induced twitch tension, but had no effects on the myogenic rhythm.

The effects of myomodulin on SETi-induced tension were dependent upon the frequency of stimulation of the motoneurone. In Fig. 4 the slow motoneurone was stimulated at several frequencies and, at each frequency, a 5 min pulse of 10^{-6} mol l^{-1} myomodulin was introduced into the muscle superfusate. At 1 Hz there was a prominent increase in twitch amplitude but very little increase in basal tension. At 3 Hz there was a larger proportional increase in twitch amplitude superimposed upon an increase in basal tension. The increase in basal tension was maximal at stimulation frequencies between 5 and 7 Hz.

As a further test of the effects of myomodulin on muscle tension, the slow motoneurone was stimulated every 60 s with 10 s trains of pulses that were increased in frequency stepwise from 1 to 50 Hz. The ability of the muscle to develop and maintain tension in normal saline was compared with that in the presence of 10^{-6} mol l^{-1} myomodulin (Fig. 5). In normal saline, tension gradually increased throughout each 10 s stimulation period and, at the higher frequencies tested, produced a smooth tetanic tension plateau. In the presence of myomodulin, the height of the individual tension transients, visible up to 7 Hz, was increased and was therefore superimposed upon an increased plateau of maintained tension. At frequencies between 10 and 50 Hz, the height of the tetanic plateau was also increased. The rate of rise and fall of the tetanic tension did not appear to be altered in the presence of myomodulin.

The modulation of neuromuscular transmission in the locust extensor-tibiae muscle has parallels with some of the modulatory effects of octopamine (Evans and O'Shea, 1977; O'Shea and Evans, 1979; Evans, 1981; Evans and Siegler, 1982) and of the neuropeptide FMRFamide (Evans and Myers, 1986*b*) in this preparation. Like octopamine,

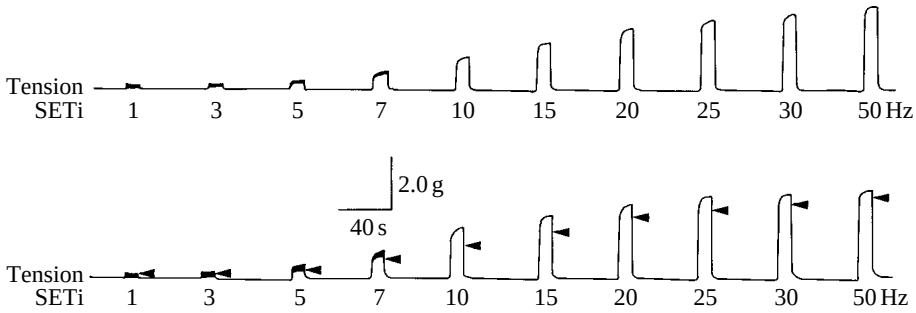

Fig. 5. The effects of 10^{-6} mol l^{-1} myomodulin A on tetanic tension produced in the extensor muscle by stimulating SETi for 10 s at different frequencies. (A) Control series in saline before myomodulin A application; (B) series in the presence of myomodulin A, which increases the height of the tetanic plateau. The arrowheads indicate the maximum tension elicited at each frequency in A under control conditions.

myomodulin increased both the amplitude and relaxation rate of twitch tension, but the effects of myomodulin, unlike those of octopamine, are not blocked in the presence of 10^{-5} mol l^{-1} phentolamine (data not shown). This indicates that the effects of myomodulin are not initiated by the activation of the octopamine receptors in this preparation. Equally, the effects of myomodulin, unlike those of octopamine, are not potentiated in the presence of the phosphodiesterase inhibitor IBMX (data not shown). This suggests that the second messenger cyclic AMP is not involved in mediating the modulatory effects of myomodulin. This conclusion is further supported by the observation that exposure of the muscle to myomodulin A for 10 min, at concentrations up to 10^{-5} mol l^{-1}, produced no significant increase in cyclic AMP levels (control muscles, 9.8±1.1 pmol cyclic AMP mg^{-1} protein, N=6; muscles exposed to 10^{-5} mol l^{-1} myomodulin A, 11.3±1.2 pmol cyclic AMP mg^{-1} protein, N=6). The effects of myomodulin on neuromuscular transmission in the locust are all very similar to the effects demonstrated previously for the activation of one class of receptor by FMRFamide-like peptides. This raises the possibility that myomodulin produces its effects by an interaction with a receptor for FMRFamide-like peptides. This seems unlikely, however, in view of the lack of a significant structural homology between the C termini of the two peptides. In addition, low doses of the FMRFamide-like peptide F1 (TNRNFLRF-NH_2) (10^{-11}–10^{-9} mol l^{-1}) and of myomodulin (10^{-7} mol l^{-1}) produce additive modulatory effects in this preparation (data not shown).

Discussion

Myomodulin A (PMSMLRL-NH_2) is a potent modulator of neuromuscular transmission in the extensor-tibiae muscle from the hindleg of the locust. It potentiates the amplitude and relaxation rate of SETi-induced twitch tension in the muscle in a dose-dependent way. Its effects are also dependent upon the frequency of stimulation of the motoneurone. The effect was selective for SETi, with myomodulin A having no effect on FETi-induced twitch tension at concentrations up to 10^{-5} mol l^{-1}. The effects on SETi-

induced twitch tension appear to be similar to those produced by myomodulin A on tension induced by the B16 motoneurone in the accessory radula closer muscle of *Aplysia*. In the latter muscle, myomodulin A increases both the amplitude of tension and its relaxation rate in response to a burst of activity in the motoneurone (Cropper *et al.* 1987, 1991; Whim and Lloyd, 1990).

Mytilus catch-relaxing peptide (CARP), which is a structurally related homologue of myomodulin A with two amino acid substitutions in its N-terminal region (Hirata *et al.* 1987), is only slightly less potent than myomodulin A in modulating neuromuscular transmission in the locust extensor-tibiae muscle. This suggests that the activities of these molecules are specified in their amidated C-terminal regions. This idea receives further support from the finding that a range of other structurally related peptides in which only the last three amino acids at the amidated C terminus are the same (i.e. -PRL-NH_2 as in leucopyrokinin, Holman *et al.* 1986, and locustamyotropin I and II, Schoofs *et al.* 1990*a*,*c*) were also capable of modulating SETi-induced twitch tension in this muscle, albeit to a much smaller extent.

In contrast to myomodulin A, CARP also initiates, or increases, the frequency of an existing myogenic rhythm of contraction and relaxation found in a bundle of slow muscle fibres in the proximal region of the extensor muscle. The activity of this bundle of muscle fibres is also increased by proctolin, AKH-like peptides, small cardioactive peptide B (SCP_B), 5-hydroxytryptamine and 2′,5′-dideoxyadenosine and decreased by octopamine and adenosine (see Evans and Myers, 1986*a*). The contractile activity of this muscle bundle was also increased by the peptides with the conserved -PRL-NH_2 C terminus tested in the present study. One possible explanation for this difference in the effectiveness of myomodulin A and CARP could be that the proline residue in CARP is closer to the C terminus than it is in myomodulin A. The family of neuropeptides with the conserved amidated C terminus (-PRL-NH_2) has also been reported previously to increase the frequency of the endogenous contractions of locust hindgut and oviducts (see Schoofs *et al.* 1992*a*). In addition, cross-reactivity of PBAN-1 has also been reported on the same receptors in these two preparations (Fónagy *et al.* 1992).

Another molluscan neuropeptide, buccalin, which modulates neuromuscular transmission in some molluscan muscles (Cropper *et al.* 1988), only shares the amidated leucine at the C terminus with myomodulin A and does not modulate neuromuscular transmission or muscle contraction in the locust hindleg extensor-tibiae neuromuscular preparation. However, the recently isolated and sequenced locustatachykinins I and II peptides (Schoofs *et al.* 1990*b*), which are C-terminally amidated peptides with no structural homologies to myomodulin, were also potent modulators of neuromuscular transmission in this preparation. This suggests that further classes of modulatory neuropeptide receptors appear to be present in this preparation and they remain to be characterized.

The modulatory effects of myomodulin-like neuropeptides on neuromuscular transmission in the locust extensor-tibiae muscle preparation show some similarities to and some differences from the previously described modulation of this preparation by the biogenic amine octopamine and the neuropeptides proctolin and members of the FMRFamide-like family (see Evans and Myers, 1986*a*). All of these substances increase

the amplitude and relaxation rate of SETi-induced twitch tension. However, the time course of the myomodulin effects is much slower than for those induced by octopamine and much more similar to those produced by the FMRFamide-like peptides. In contrast, the effects of myomodulin A on maintained basal tension are similar to those of FMRFamide and proctolin, which increase the level of basal maintained tension, rather than those of octopamine, which reduces basal maintained tension (Evans and Siegler, 1982; Evans, 1982; Evans and Myers, 1986*a*). None of the effects of myomodulin appears to be mediated *via* an interaction with octopamine receptors or by a release of endogenous octopamine, since they are not blocked by phentolamine. It also seems highly unlikely that any of the effects of myomodulin are mediated by a cross-reactivity with receptors for either proctolin or FMRFamide-like peptides because there is no significant structural homology between these peptides. In addition, the effects of myomodulin A and the crustacean FMRFamide-like peptide F1 (TNRNFLRF-NH_2), which is a potent modulator of neuromuscular transmission in the extensor-tibiae muscle, are purely additive at low concentrations. Further, in some molluscan preparations, the effects of FMRFamide-like peptides and those of myomodulin-like peptides, such as CARP, appear to be opposite, suggesting the existence of distinct receptor populations for these two families of neuropeptides (Kobayashi and Muneoka, 1990; Kuwasawa *et al.* 1992). In the locust, there is immunocytochemical evidence for the existence of distinct endogenous neuropeptides of the myomodulin- and FMRFamide-like families (Swales and Evans, 1994). It therefore appears very likely that the modulatory effects of neuropeptides from these two families, in the locust extensor-tibiae muscle, will also be mediated by distinct classes of receptor. However, definitive proof of this assertion will require the production of specific antagonists for both classes of receptor.

The results of the present study again raise the important question of why neuromuscular transmission in the extensor-tibiae muscle of the locust hindleg needs to be modulated by such an array of biogenic amines and neuropeptides. It could be that different combinations of muscles exhibit receptors for different neuropeptides, so that individual peptides could modulate the activities of specific subsets of skeletal muscles. Alternatively, it could be that this muscle needs to be modulated differentially, at different times or under different environmental conditions, and that the existence of multiple modulatory receptors increases the animal's flexibility in its responses to changes in its external or internal environment. However, it seems likely that changes in the relative amounts of the various modulators in the local hormonal environment of the muscle, rather than their presence or absence, will be the important biological signal for the animal. Myomodulin-like neuropeptides in the locust are likely to act as neurohormones or local neuromodulators, but we must await the isolation and characterization of the endogenous myomodulin-like neuropeptides from the locust nervous system, and the development of specific antagonists for their receptors, before we can draw definitive conclusions about their functional roles.

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