THE MODULATORY EFFECTS OF SEROTONIN, NEUROPEPTIDE F1 AND PROCTOLIN ON THE RECEPTOR MUSCLES OF THE LOBSTER ABDOMINAL STRETCH RECEPTOR AND THEIR EXOSKELETAL MUSCLE HOMOLOGUES

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Summary

The muscle receptor organ (MRO) of the lobster is a complex proprioceptive system lying in parallel with the axial extensor musculature. Two peripherally located sensory neurones extend stretch-sensitive dendrites into individual receptor muscle strands one tonic (RM_1) and one phasic (RM_2). Previous studies have shown that the sensitivity of the sensory neurones to passive stretch could be enhanced by serotonin and proctolin. Here we show that the receptor muscles and their exoskeletal muscle homologues are also responsive to serotonin, proctolin and, in addition, to neuropeptide F1 (TNRNFLRF-NH_2).

Two measures of motor performance were enhanced by all three neurohormones: EJP amplitude and nerve-evoked tension development. Serotonin was the most effective modulator of both tonic and phasic muscles. F1 had powerful effects on the phasic extensor muscle. A low incidence of tonic muscle fibres with synapses responding to the neurohormones suggests that there are distinct populations of synapses: those sensitive to specific modulators and others that are insensitive. These findings, taken together with the enhancing effects of modulation on the primary sensory afferents, suggest that circulating neurohormones may act at multiple loci in the MRO system in a concerted and hormone-specific manner to alter the flow of proprioceptive feedback.

Introduction

Our understanding of the cellular mechanisms by which neurohormones influence motor activity has progressed significantly since Livingstone *et al.* (1980) first reported that injections of octopamine and serotonin could cause freely moving lobsters to assume stereotyped, natural postures. In a recent review, Kravitz (1988) concludes that amines and peptides, released by neurones both within the central nervous system and into the peripheral circulation, act at many loci in a concerted manner to enhance the effectiveness

of particular motor patterns. They include central pattern generating circuits, motor neurones, neuromuscular junctions and muscle fibres. We have been interested in the concept that proprioceptors are targets for neurohormones and that the modulation of proprioceptive feedback might make a significant contribution in biasing motor behaviour.

It has been shown that the sensitivity of several arthropod mechanoreceptors can be influenced by the presence of serotonin, octopamine and the pentapeptide proctolin (Pasztor and Bush, 1987, 1989; Pasztor and Macmillan, 1990; Cooper and Hartman, 1992; El Manira *et al.* 1991). Octopamine is a potent modulator of insect wing stretch receptors (Ramirez and Orchard, 1990) and of *Limulus* claw closer receptors (G. R. Wyse and V. M. Pasztor, in preparation). Most of these mechanoreceptors are simple sense organs where the sensory endings are associated with either cuticular structures or connective tissue strands. They lack efferent neural mechanisms for gain control and peripheral modulation involves the sensory units themselves. In the lobster oval organ, a stretch receptor of the second maxilla, for example, it was clearly demonstrated that both the receptor potential and spike generation were enhanced by proctolin and diminished by octopamine and serotonin (Pasztor and Bush, 1987, 1989).

By contrast, the well-known abdominal stretch receptor of decapod crustaceans, the muscle receptor organ (MRO), is a much more complex system (Kuffler, 1954). Each MRO consists of two sensory neurones, one which fires only tonically (SN_1) and another (SN_2) which is fast-adapting or phasic. The sensory endings of each neurone are embedded in separate receptor muscle strands, RM_1 and RM_2 respectively (Alexandrowicz, 1967). These neurones not only fire in response to passive changes in strand length as the body segments move, but are stimulated by active contractions of the receptor muscles evoked by motor efferents (for a review, see Bush and Laverack, 1982). In addition, firing control is exerted by accessory efferents which encrust the sensory endings with inhibitory synapses. This much-studied reflex system, which is involved in several distinct postures and behaviours involving the abdominal musculature (Kennedy *et al.* 1966), offers interesting possibilities for the study of interactive effects of neuromodulators working concurrently at several loci.

We have already reported that both the tonic and phasic sensory neurones of the MRO display increased sensitivity to passive stretch stimulation in the presence of serotonin and proctolin (Pasztor and Macmillan, 1990). The present paper is the first in a series investigating other putative targets for neuromodulators in the MRO system. Here we investigate the effects of serotonin, proctolin and a member of the FMRF-amide family, neuropeptide F1 ($TNRNFLRF-NH_2$), on the receptor muscles RM_1 and RM_2 and their excitatory motor innervation. Since the receptor muscles are closely coupled to, and share innervation with, their exoskeletal abdominal extensor muscle homologues, the responses to these neuromodulators of the tonic superficial extensor muscles (SEM) and phasic deep abdominal extensors (DAE) are included for comparison.

Previous studies of hormonal actions of amines and peptides on crustacean exoskeletal musculature include reports of the effects of octopamine and serotonin on lobster tonic abdominal flexors and extensors (Harris-Warrick and Kravitz, 1984), of the effects of proctolin on crayfish tonic abdominal flexors (Bishop *et al.* 1987, 1991*a*,*b*) and of the

FMRF-amide family on crayfish phasic abdominal extensors (Mercier *et al.* 1990). This paper is the first investigation of neuromodulation involving the muscular component of a proprioceptive system.

We address the following questions: (1) are receptor muscles sensitive to neuromodulators; (2) are their responses to neuromodulators similar to those of their exoskeletal muscle homologues; (3) do the same amines and peptides which modulate sensory firing during passive stretch have potent effects upon nerve-evoked receptor muscle contractions?

Materials and methods

Lobster, *Homarus americanus* (Milne-Edwards), of carapace length 7.5–9.0cm were purchased locally from commercial sources and maintained in artificial sea water at 12–16°C. Most of the experiments (with the exception of some of the SEM trials) were performed during the summer months.

The nerve–muscle preparation

The dorsal half of the abdomen was pinned flat in a Sylgard-lined dish, cut surface uppermost and thoroughly washed in chilled, aerated saline to remove residual neurohormones. The complete superficial extensor musculature of one hemisegment (usually the third) was excised, together with the MRO and the superficial branch of the dorsal nerve. Fragments of exoskeleton were left attached to the ends of the muscle bundles to preserve the integrity of the muscle fibres. The skeletal and receptor muscles were mounted together in the preparation bath to maximize the probability that their shared innervation would remain intact. The most lateral bundle of the phasic dorsal abdominal extensor was prepared in a similar manner.

Recordings of junctional potentials

Muscle fibres selected for study were supported from below with a small Sylgard platform and transilluminated. Microelectrodes filled with $3 \, \text{mol} \, l^{-1}$ KCl (10–15MΩ) were used to record intracellular excitatory junctional potentials (EJPs). Usually two fibres from different muscles were tested concurrently. Increasing voltage pulses (2ms; 1–10Hz) were delivered *via* suction electrodes to the dorsal nerve until first simple and then compound EJPs occurred at the muscle membrane. At these low stimulation rates there was little movement of the tonic muscles. However, the twitching of the phasic fibres precluded the successful long-term recording needed for trials of DAE. A modified high-magnesium, low-calcium saline as described by Mercier *et al.* (1990) was used to eliminate active membrane responses and to reduce contraction amplitudes. The normal saline contained (in $\text{mmol} \, l^{-1}$): 462 NaCl, 16 KCl, 26 $CaCl_2$, 8 $MgCl_2$, 11 glucose, 10 Tris, 5 maleic acid, pH7.4. In the modified saline $MgCl_2$ was increased to $15 \, \text{mmol} \, l^{-1}$, $CaCl_2$ decreased to $8.5 \, \text{mmol} \, l^{-1}$ and additional NaCl was added to restore normal osmolality.

Muscle tension measurements

Tension was recorded from whole muscles using a purpose-built transducer (Bishop *et*

al. 1987). Each muscle was pre-loaded with 18–50mg, as the size of the muscle dictated, in order to take up any slack. All increases in contraction have been expressed as percentages of pre-trial transducer output amplitudes. Nerve-evoked contractions were elicited from tonic muscles with trains of short, high-frequency bursts of stimuli (50–80Hz for 0.2s) delivered at 5s intervals and from phasic muscles with single pulses delivered to the dorsal nerve at repetition rates of 0.2–0.5Hz. Unless otherwise indicated, the voltages used were supra-threshold for all the excitors that could be recruited without stimulating an inhibitory motor neurone.

Neuromodulator trials

The 5-hydroxytryptamine creatinine sulphate (serotonin; Sigma Chemical Co.), proctolin (Peninsular Laboratories) and neuropeptide F1 (Cambridge Research Biochemicals) were applied as long pulses in the perfusate. A peristaltic pump provided continuous flow of 2.5mlmin^{-1} through the 10ml preparation bath. Reservoirs of control and test solutions were maintained at identical temperatures in a Lauda refrigerated waterbath. A Peltier plate proximal to the preparation bath kept the perfusate at a constant temperature (15 ± 2°C) throughout all trials.

Each trial consisted of 5min of recording of either EJP or nerve-evoked contractions in normal saline and then 5 or 10min of modulator perfusion followed by a minimum of 20min washout in normal saline flow. Stimulation parameters were selected to minimize facilitation, summation or fatigue and sufficient pre-trial stimulation was delivered to ensure a stable control baseline. At least 90min elapsed before any neuromodulator was presented for a second trial. The sequence of drugs was changed from one experiment to another.

The data presented here were taken from 217 trials using 10^{-6} mol l^{-1} modulator. This concentration is commonly used in comparable muscle studies appearing in the literature and was selected here to give direct comparisons with them and with data on the sensory neurones of the MRO (Pasztor and Macmillan, 1990).

Results

Tonic muscles

In lobsters, there are three distinct bundles of superficial extensor muscles (SEM) in each abdominal hemisegment: one medial to the MRO and two lateral. Together they receive five excitor and one inhibitor motor neurones (Parnas and Atwood, 1966). The various axons supplying SEM could be recruited in turn by increasing the stimulation voltage to the nerve, revealing that most muscle fibres received only one excitor (mean EJP size 6.7 ± 0.8mV, s.e.; N=22), many received two excitors (mean compound EJP amplitude 8.8 ± 1.0mV; N=18) and a few received three (giving a total EJP size of 14.4 ± 2.0mV; N=8). Muscle fibres were sampled from all three bundles and all appeared to share similar tonic properties. Most trials involved those SEM fibres adjacent to RM$_1$.

The tonic receptor muscle RM$_1$ is a thin strand of muscle fibres possessing considerable tensile strength due to a tough connective tissue sheath. Authors have
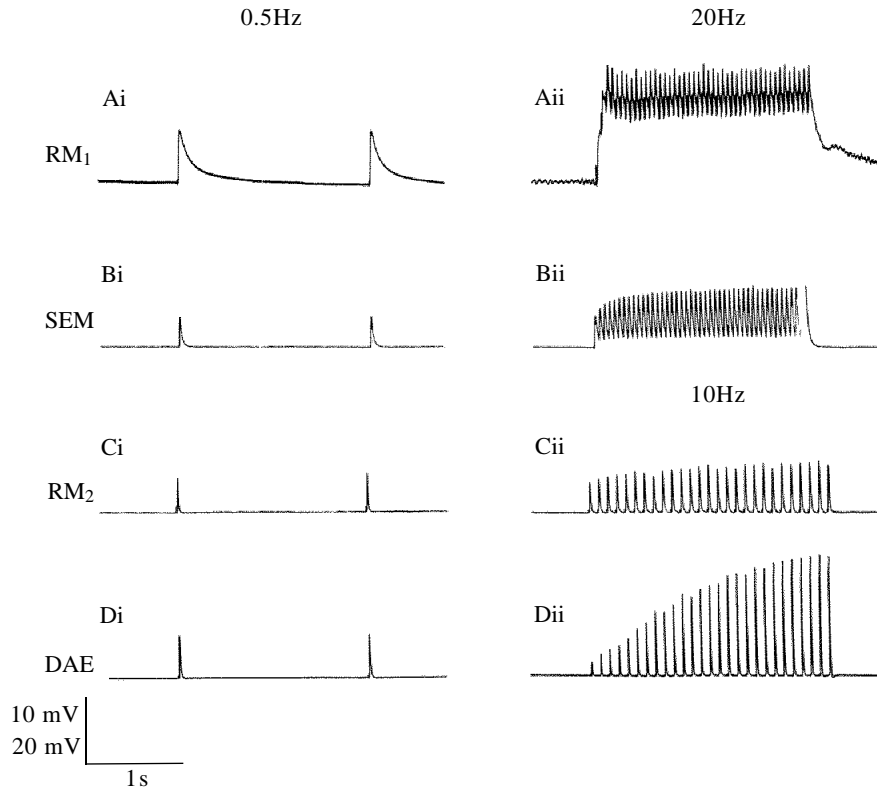
Fig. 1. Simple EJPs showing the characteristic differences in duration and facilitation between the four muscles. Pairs of intracellular recordings were made concurrently from the tonic (RM_1 and SEM) and phasic (RM_2 and DAE) muscles. Fibres in Ai and Bi were innervated by the shared excitor. All other pairs received individual excitors. Ci, Cii, Di and Dii (same preparation) were taken from fibres in modified high-Mg^{2+}, low-Ca^{2+} saline. Calibration: A and B, 10mV; C and D, 20mV; A–D, 1s.

disagreed as to whether the strand is made up of several distinct muscle fibres linked by membranous bridges, or whether it is one syncitial cell deeply divided by the sarcolemma into almost separate fibrils (Komuro, 1981). Whatever may be the case in lobster, the surface membrane of RM_1 is not equipotential and dual-electrode impalements showed variations in resting potential and EJP amplitude. RM_1 receives one large unshared axon which runs parallel to the sensory MRO afferent and enters the mid region of the muscle strand. It also receives branches from at least one of the SEM excitors, which enter RM_1 at either end near its attachments. Of the 36 RM_1 fibres tested, 75% gave EJPs at one threshold only, with a mean EJP amplitude of 11.3±0.9mV and 25% showed two thresholds, with a compound EJP size of 17.9±1.1mV. As illustrated in Fig. 1, RM_1 EJPs were consistently of longer duration than those of SEM and showed almost no facilitation. However, with repetitive stimulation at physiological rates, the RM_1 membrane achieved the same level of depolarization caused by summation as did the facilitating SEM. These generalizations held true even in the rare cases when dual
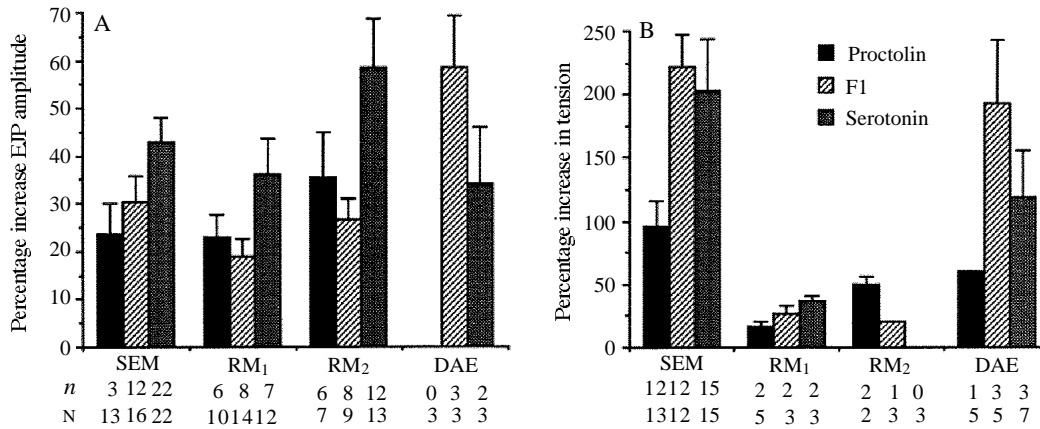
Fig. 2. Histograms summarizing the actions of proctolin, neuropeptide F1 and serotonin on the two receptor muscles (RM$_1$ and RM$_2$) and their skeletal homologues (SEM and DAE). (A) Modulation of peak EJP depolarization in response to 1Hz stimulation. (B) Modulation of total muscle tension developed during nerve-evoked contractions, elicited from tonic muscles by 0.2s bursts of 50Hz stimulation delivered at 5s intervals and from phasic muscles by trains of single 1Hz pulses. Maximal increments from control, pre-trial measurements were used in calculating mean percentage changes (+ S.E.) resulting from each modulator. The number of trials showing modulation (n) in comparison to the total number of trials performed (N) is indicated below each bar.

recordings were made simultaneously from fibres of RM$_1$ and SEM which shared an excitor (Fig. 1A,B). Neither RM$_1$ nor SEM showed any active membrane responses.

As shown in the histogram of Fig. 2A, SEM and RM$_1$ responded to all three neuromodulators with enhanced EJP amplitudes. Proctolin, neuropeptide F1 and serotonin induced increases in peak amplitude of depolarization of 24%, 31% and 43%, respectively, in the skeletal muscle SEM and 23%, 19% and 36%, respectively, in the receptor muscle RM$_1$. A detailed analysis of the sizes of the increases (in mV) classified according to the number of excitors received by the individual fibres tested is presented in Table 1. With the exception of the serotonin trials on SEM where 100% of the fibres tested showed augmentation, the incidence of responding fibres was low (proctolin trials, 21% for SEM, 50% for RM$_1$; F1 trials, 75% for SEM, 64% for RM$_1$; serotonin trials, 100% for SEM, 70% for RM$_1$). It is also noteworthy that the mean increases displayed by doubly innervated fibres (Table 1) were no greater than the increases in singly innervated fibres, although the compound, pre-trial EJPs of the former were, as expected, of greater magnitude than the latter. Taken together, these observations suggest that synapses of the various excitors supplying SEM and RM$_1$ fall into two populations: those sensitive to modulator and others that are insensitive. Four triply innervated SEM fibres responded to serotonin with very large increases (3.8–4.9 mV), indicating the presence of an excitor, not widely distributed, which responds to a greater extent than the others.

SEM fibres bearing synapses responsive to proctolin were particularly rare and all

Table 1. *Comparisons of modulation of EJP amplitude in muscle fibres receiving one, two or three excitors*

	One excitor		Two excitors		Three excitors	
A **Superficial extensor muscle (SEM)**						
Pre-trial EJP amplitudes (mV)*, responders only						
Saline	6.6±1.0;	16	8.3±1.3;	17	14.0±1.8;	4
Increments due to 10^{-6} mol l^{-1} modulator (mV)†						
Proctolin	0.9±0.2;	3(7)	–	0(4)	–	0(2)
F1	4.4±2.8;	3(5)	2.1±0.4;	9(9)	–	0(2)
Serotonin	3.1±1.0;	10(10)	3.0±1.1;	8(8)	4.6±0.3;	4(4)
B **Tonic receptor muscle (RM1)**						
Pre-trial EJP amplitudes (mV)*, responders only						
Saline	12.4±0.3;	15	18.0±0.8;	6		
Increments due to 10^{-6} mol l^{-1} modulator (mV)†						
Proctolin	2.5±0.3;	5(8)	1.5	1(2)		
F1	1.8±0.2;	6(10)	2.0±0.6;	2(4)		
Serotonin	3.6±0.6;	4(9)	3.6±0.5;	3(3)		

*Mean±S.E.; N.
†Mean±S.E.; number of fibres showing increment (total number of trials).

came from winter animals. No other seasonal variations were observed although, in general, baseline EJP amplitudes were somewhat smaller in winter animals.

There was no evidence that the modulators had any effect upon resting membrane depolarization, junctional potential waveform or facilitation properties.

The results of the nerve-evoked tension trials are summarized in Fig. 2B. The incidence of whole-muscle tension augmentation was, as expected, higher than in the EJP trials on individual fibres. All three modulators induced considerable increases in the peak tension developed during nerve-evoked contractions, with serotonin giving the greatest mean percentage increase. It was commonly observed that these amplitude effects were accompanied by changes in the relaxation times. Serotonin caused accelerated relaxation rates, whereas in the presence of either neuropeptide the onset of relaxation was delayed and relaxation rates were retarded. For example, in one representative series of trials on SEM, times were measured from peak contraction to half relaxation. Serotonin decreased the half-relaxation time by 69%, while proctolin and F1 increased half-relaxation times by 148% and 63% respectively. The time courses of both development and recovery from these effects were longer than those of amplitude changes in the same trials. This indicates that the responses of the muscles to the modulators are multifactorial. None of the modulators affected the resting tonus of the muscles.

In proctolin tension trials on summer animals, conducted at a period when EJP trials on SEM revealed no synaptic responsivity to proctolin, mean SEM tension increases of 95±21% were recorded, indicating that this modulator has major direct effects upon the muscle fibres themselves.

Table 2. *Comparisons of modulation of EJP amplitude in trials performed in normal and high-Mg^{2+}, low-Ca^{2+} salines*

	Normal saline	Modified saline
A Phasic receptor muscle (RM_2)		
Pre-trial EJP amplitudes (mV)*,†		
	16.1±2.6; 10	10.2±2.2; 16
Increments due to modulator (mV)*‡		
Proctolin	4.3±2.6; 2(2)	4.9±2.2; 4(5)
F1	5.4±1.9; 3(3)	2.1±0.7; 5(6)
Serotonin	8.3±2.6; 5(5)	4.9±2.2; 7(8)
B Deep abdominal extensor muscle (DAE)		
Pre-trial EJP amplitudes (mV)*,†		
	ND	7.6±1.0; 8
Increments due to modulator (mV)*‡		
Proctolin	ND	– ; 0(3)
F1	ND	3.6±0.2; 3(3)
Serotonin	ND	1.5±0.5; 2(3)

*Mean±s.e.; *N*.
†All fibres tested received one excitor only.
‡Mean±s.e.; numbers of fibres showing increment (total number of trials).
ND, not determined.

The phasic muscles

The deep abdominal extensor muscle, DAE, forms three distinct bundles per hemisegment in lobster, two medial and one lateral to the MRO. The lateral bundle was selected for neuromodulator trials since it shares its origin and insertion with the phasic receptor muscle RM_2. It receives one excitor from the group of six phasic extensor motor neurones (Parnas and Atwood, 1966). The synapses had high output and usually gave rise to non-propagated active membrane potentials. The resulting vigorous twitches made intracellular recording difficult and a high-Mg^{2+}, low-Ca^{2+} saline was used for EJP trials. The diminished EJPs recorded in this saline were stable when stimulated at 0.5Hz and had a mean amplitude of 7.6±1.0mV (see Table 2). They rapidly reverted to normal magnitudes upon re-introduction of normal saline.

In lobster, RM_2, like RM_1, receives an exclusive excitor plus branches from another that is shared with the exoskeletal homologue. However, in most EJP trials of RM_2, it was necessary to remove DAE to minimize movement artefacts, thus eliminating the second excitor. The RM_2 excitor has a high output (mean EJP amplitude, in normal physiological saline, 16.1±2.6mV) and active processes are common. Despite the phasic twitches, electrodes were successfully lodged in RM_2 in 10 fibres in normal saline and a further 19 where EJPs and twitching were depressed in high-Mg^{2+}, low-Ca^{2+} saline (mean amplitude 10.2±2.2mV). RM_2 synapses have similar properties to those of the parallel exoskeletal muscle, DAE, with one exception: when both were tested together in modified saline, facilitation in DAE was five times greater than in RM_2 (Fig. 1C,D).
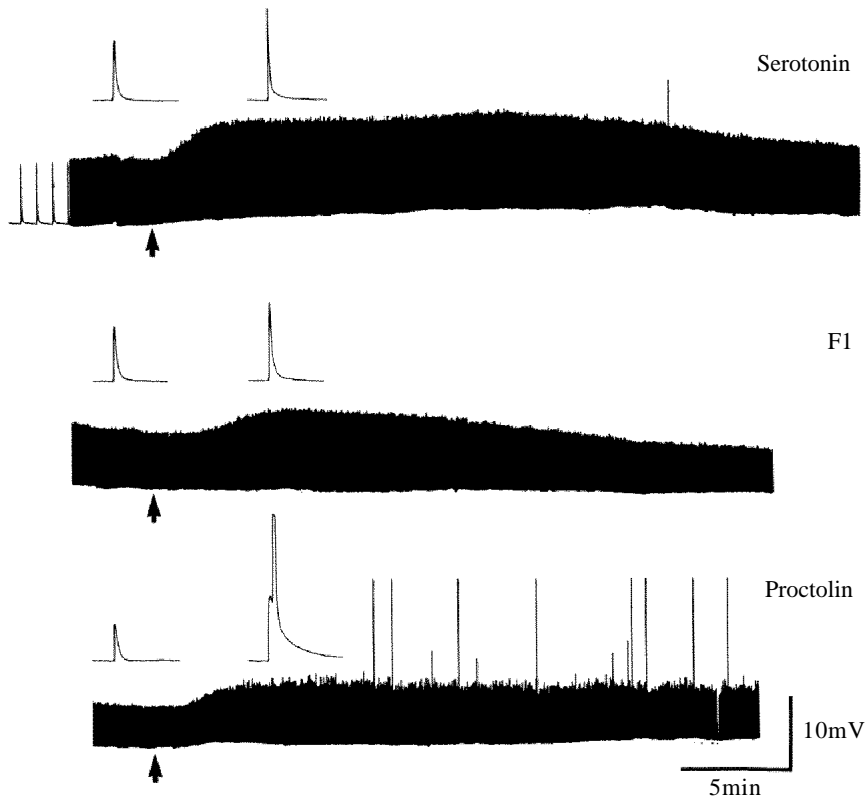
Fig. 3. Representative intracellular EJP recordings during three consecutive neuromodulator trials on the phasic receptor muscle muscle, RM$_2$. Serotonin (upper), neuropeptide F1 (middle) and proctolin (lower) were added to the superfusate at concentrations of 10^{-6} mol l^{-1} for 10min periods commencing at the arrow. Trials were performed on fibres fully facilitated to prolonged 1Hz stimulation. The last 5min of serotonin washout has been omitted from the end of the upper segment, otherwise the records are continuous. Insets: single EJPs displayed at higher speed comparing pre-trial with maximally modulated conditions. Note the high incidence of active responses arising from moderate-amplitude EJPs during the proctolin treatment but not from the larger EJPs induced by serotonin and F1. (Spike heights were cropped by the chart recorder pen.) Calibration: 10mV, 5min; insets 10mV, 1s.

In the intracellular trials, both DAE and RM$_2$ showed a high incidence of augmented EJP amplitudes in the presence of F1 and serotonin, but DAE did not respond to proctolin (Fig. 2A). With RM$_2$, where it was possible to compare modulation in both normal and high-Mg^{2+}, low-Ca^{2+} saline, increases in EJP amplitude expressed as percentages of pre-trial amplitude were strictly comparable in both salines (mean values in mV are given in Table 2).

Examples of EJP augmentation and recovery in RM$_2$ are presented in Fig. 3. In these three consecutive trials on the same preparation, increases of 49%, 37% and 62% were observed in serotonin, F1 and proctolin, respectively. Proctolin was notable, not only for causing an overall increase in EJP size, but also in potentiating active processes. In this series of trials, previously applied modulators had brought EJP peaks much closer to the

expected spiking threshold but, with proctolin present, relatively modest EJPs gave rise to active processes. This phenomenon was noted in other proctolin trials of RM_2 but in no other muscle.

In these two phasic muscles, F1 was a most effective modulator of tension development, especially in the exoskeletal DAE. There it invariably caused large increases (135–760%). This muscle is highly susceptible to fatigue from which, *in vitro*, it rarely fully recovers. F1 could augment nerve-evoked contractions in even the most fatigued muscle, whereas for proctolin and serotonin to have an effect, the muscle had to be twitching vigorously prior to modulator application. The absence of tension increase during RM_2 serotonin trials requires further investigation.

Discussion

We have demonstrated in this paper that both the tonic and phasic receptor muscles (RM_1 and RM_2) of the abdominal stretch receptor and their skeletal muscle homologues (SEM and DAE), responded to motor commands with augmented activity in the presence of serotonin, neuropeptide F1 and proctolin. Two variables were quantified: EJP amplitude in individual muscle fibres, to investigate whether some or all synapses were modulated and whole-muscle, nerve-evoked tension development, to assess the functional significance of neuromuscular modulation. Other investigators using the abdominal extensors have shown that quantal output is increased by these modulators, whereas the muscle membrane resistance is unchanged (Mercier *et al.* 1990). Most authors attribute observed increases in EJP amplitude to enhanced neurotransmitter output. We have extrapolated from these findings and assumed that EJP size is an indicator of presynaptic effects in the receptor muscles as well.

The low incidence of neurohormone effects on EJP amplitude in the tonic muscles, SEM and RM_1, suggested that there are distinct populations of synapses on these fibres: those that expressed sensitivity to the neuromodulators and those that did not. This may favour the possibility for selective modulation of tonic muscle activity during certain behaviours.

Reports of presynaptic modulation by proctolin are rare. Bishop *et al.* (1987) were unable to detect any measurable effects on EJP size in crayfish tonic extensors, although proctolin almost doubled the amplitude of nerve-evoked contractions. By contrast, Beilin and Pasztor (1989) found that 47% of all crayfish scaphognathite depressor muscle fibres showed EJP augmentation in the presence of proctolin. It is clear from our work that proctolin EJP effects were absent or rare in both phasic and tonic skeletal extensors but were common in both receptor muscles. It may be that presynaptic proctolin sensitivity is expressed in only a few muscles and then only in situations where selective modulation of a specific population of synapses confers some advantage.

Seasonal variability in the responsiveness of neuromuscular and proprioceptive systems to neurohormones is a subject deserving careful attention. In a major study of proctolin potentiation of muscle membrane calcium channel activity, Bishop *et al.* (1991*a,b*) found that skeletal flexors of summer and spring animals were significantly

more responsive to proctolin than those of winter animals. Proctolin enhancement of EJP amplitudes in skeletal extensors of winter animals but not summer animals, found in the present study, serves to exemplify and extend the notion of diversity of neurohormone effects put forward by Pasztor and Macmillan (1990).

In Crustacea, a major source of neurohormones is the pericardial organ. This consists of a mass of neurohaemal endings associated with thoracic second roots, which store and release serotonin and neuropeptide F1 into the haemolymph (Livingstone *et al.* 1981; Trimmer *et al.* 1987). Proctolin has also been localized in pericardial tissue, although it is not detectable in the general circulation *in vivo* (Schwartz *et al.* 1984), and release sites closer to the target muscles have been sought. Some of the motor axons to the scaphognathite muscles show proctolin-like immunoreactivity (V. M. Pasztor, unpublished observations) and an additional source of proctolin is the oval organ, a stretch receptor in the scaphognathite whose sensory endings release the peptide into the haemolymph at the insertion of the proctolin-sensitive muscle (Pasztor *et al.* 1988). In the MRO, neither the receptor muscle excitors nor the endings of the sensory neurones show any proctolin-like immunoreactivity (V. M. Pasztor, unpublished observations). However, Bishop *et al.* (1987) have demonstrated that three pairs of crayfish motor neurones innervating the tonic flexors release proctolin as a co-transmitter. The presence of other abdominal ganglion neurones and axons to tonic extensors showing proctolin-like immunoreactivity, though fewer in lobster than in crayfish (Siwicki and Bishop, 1986), suggests that there are adequate sources of endogenous proctolin *in vivo* close to the receptor muscles.

The arrays of responses to modulators shown by the receptor muscles are closer to those of their exoskeletal muscle homologues than to those of their respective sensory neurones. For example, neither of the sensory neurones is responsive to neuropeptide F1 (Pasztor and Macmillan, 1990), whereas this is a potent modulator of both exoskeletal and receptor muscles. The shared motor innervation maintains comparable levels of tonus in the parallel systems of receptor and exoskeletal muscles, so it is functionally adaptive for both sets of muscles to respond to the prevailing neurohormonal concentrations with modulated nerve-evoked activity, and to do so in a coordinated manner. The apparent percentage increases in tension developed by the receptor muscles seem small in comparison to those of the skeletal muscles, but it should be remembered that the receptor muscles are not load-bearing and are not responsible for moving body segments. The augmented tension is directly transmitted to the central tendinous zone of the receptor muscle strands, where small increments can have pronounced effects upon the firing rates of the sensory units.

The potential for modulated sensitivity of the sensory neurones themselves and the increased tension generation in the receptor muscles allow the MRO to participate more vigorously in reflex activity at times of high neuromodulator release. In postural behaviour, diversity of MRO participation may be possible through the recruitment of receptor muscle synapses specifically responsive to certain neuromodulators. In repetitive locomotory behaviour, both skeletal and receptor muscles may be compensated for fatigue by the postsynaptic effects of neuropeptides or amines.

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