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PEPTIDERGIC AND AMINERGIC MODULATION OF INSECT SKELETAL MUSCLE

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SUMMARY

Insect skeletal muscles are frequently innervated by small numbers of motor neurones, all of which can be uniquely identified physiologically. They therefore present excellent model systems in which to study the basic principles of neuromuscular transmission and the modulation of these effects by biogenic amines and peptides. The extensor-tibiae muscle of the hind leg of the locust is a much studied, large muscle that is innervated by three identified motor neurones and one identified modulatory neurone. Much attention has recently been focused on the modulation of neuromuscular transmission and muscular contraction in this muscle by biogenic amines and peptides.

One proximal bundle of muscle fibres in the extensor-tibiae muscle exhibits a myogenic rhythm of contraction and relaxation. The rhythm is stimulated by a variety of peptides including proctolin, the AKH-related peptides M_1 and M_2, and by small cardioactive peptide (SCP_B). In addition, it is activated by 5-hydroxytryptamine and by one class of adenosine analogues. The rhythm is inhibited by octopamine and by a second class of adenosine analogues. The actions of these various modulatory compounds will be discussed in terms of the likely numbers of pharmacologically distinct receptors in this preparation and their modes of action.

Neuromuscular transmission and muscular contraction in the extensor-tibiae muscle is modulated by the biogenic amine octopamine and by the peptides, proctolin and FMRFamide. The actions of these modulators are discussed in relation to differences in the responsiveness of various regions of the muscle, to the frequency dependence of their effects on motor neurone activity and to their modes of action.

The cellular locations and mode of transmission to the muscle of some of these modulators will be considered. Octopamine and proctolin are contained within neurones which innervate the muscle, whilst FMRFamide- and SCP_B-like peptides appear to be released into the locust haemolymph as neurohormones.

INTRODUCTION

An understanding of the physiological significance of the synaptic interactions between two neurones besides requiring a detailed knowledge of the direct actions of the neurotransmitter released by the presynaptic neurone, also needs to take into account the effects of variations in the levels of modulatory substances present in the local hormonal environment of the synapses. In recent years the actual definition of a modulatory action, as opposed to a true synaptic interaction, has been the subject of

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much debate (see Kupfermann, 1979). Indeed, rather than being distinct, rigidly definable entities, it needs to be emphasized that the terms really only designate different regions in a continuous spectrum of chemical communication between cells. The exact point where a neurotransmitter becomes a neuromodulator, and a neuromodulator becomes a neurohormone is impossible to establish. Further, some anatomically defined synaptic contacts release a specific neurotransmitter, together with a cotransmitter that has modulatory functions (Iversen, 1984). Equally, it has become apparent that many of the chemical effectors used in the nervous system, such as particular peptides or biogenic amines, may function as either neurotransmitters, neuromodulators or neurohormones in different contexts. Thus for any specific chemical effector, its functional role must be studied individually in relation to the particular synaptic system under consideration and, whenever possible, in relation to the effects of uniquely identifiable neurones.

Invertebrate nervous systems have many advantages for the study of basic synaptic mechanisms because of the large size of many of their neurones and the ease with which individual neurones can be identified from one preparation to the next. Insect skeletal muscles, for instance, are frequently innervated by small numbers of motor neurones, all of which can be uniquely identified physiologically (Hoyle, 1983). They, therefore, present excellent model systems in which to study the basic principles of neuromuscular transmission and the modulation of these effects by biogenic amines (see Evans, 1980) and neuropeptides (O'Shea, 1985; O'Shea & Schaffer, 1985). These modulatory effects can be studied in detail all the way from the pharmacology of the receptors mediating the effects to the molecular details of their modes of action.

A considerable amount of information is now available on the modulatory actions of a variety of different biogenic amines and peptides on several identified cell systems in invertebrates (e.g. Kupfermann, 1979; Kravitz *et al.* 1985; Mayeri & Rothman, 1985). Nevertheless, we have chosen to review the actions of these modulators on a single insect muscle, namely the extensor-tibiae muscle of the hindleg of the locust. The neuronal innervation to this muscle from the third thoracic (metathoracic) ganglion of the locust has been well studied (Hoyle, 1955*a*,*b*; Pearson & Bergman, 1969; Hoyle & Burrows, 1973) (see Fig. 1). It is innervated by one fast excitatory motor neurone (FETi), one slow excitatory motor neurone (SETi) and by a branch from a common inhibitory motor neurone (CI). In addition, it is innervated by a specific modulatory neurone, designated DUMETi (Dorsal Unpaired Median cell to Extensor-Tibiae muscle) (Hoyle, Dagan, Moberly & Colquhoun, 1974). Both the left and the right extensor-tibiae muscles receive an axon from the single unpaired DUMETi neurone in the metathoracic ganglion. All the neurones innervating the muscle can be activated selectively either by stimulation from an intracellular microelectrode or by stimulation from extracellular electrodes at the points indicated in Fig. 1.

The muscle fibres of the extensor-tibiae muscle have receptor molecules for the neurotransmitters released from the neurones innervating the muscle and also for a variety of substances which probably act as circulating hormones. Thus they have

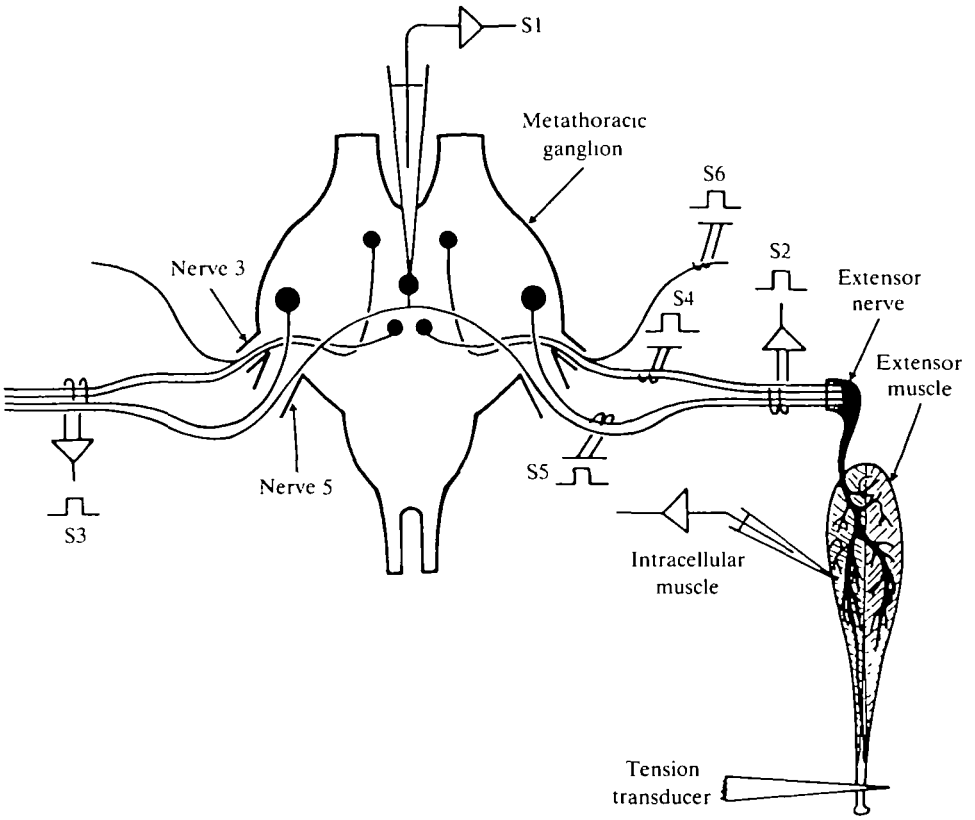


Fig. 1. Experimental arrangement used in the identification of the soma of DUMETi and in stimulating the axons of the extensor-tibiae motor neurones. Cell bodies of neurones which innervate the extensor-tibiae muscle are shown in their appropriate positions (black circles) in the metathoracic ganglion. Of the four axons contained in each extensor nerve, two (DUMETi and the fast extensor motor neurone, FETi) leave the ganglion *via* nerve 5 and two (SETi and the common inhibitor, CI) *via* nerve 3. The axon of SETi is stimulated (S4) with hook electrodes placed around a branch of nerve 3 (3b). At threshold stimulus intensity for SETi, the axon of CI, also contained in this nerve, is not excited because it has a much smaller diameter and therefore a higher threshold to extracellular stimulation. DUMETi is stimulated either intracellularly (S1) or extracellularly (S3). Stimulation at S2 and S3 is used during the identification of the DUMETi cell body. The axon of FETi is stimulated (S5) with hook electrodes placed around nerve 5. At threshold stimulus intensity for FETi, the axon of DUMETi is not excited. CI is stimulated (S6) by hook electrodes on nerve 3a which contains its axon but lacks other axons which project to the extensor muscle (from Evans & O'Shea, 1978).

receptors for glutamate, which is thought to be the rapidly acting excitatory transmitter released by both FETi and SETi, and also for γ-amino butyric acid, the transmitter released from CI (Usherwood, 1978). In addition the muscle has receptors for the biogenic amine, octopamine, which is the modulatory effector released by DUMETi (see Evans, 1985*a*) and also for the pentapeptide, proctolin, which has recently been shown to be released as a slow acting cotransmitter from the terminals of SETi (O'Shea, 1985). Furthermore, the muscle responds to a range of presumed circulatory peptidergic neurohormones.

This review will consider our current knowledge of the pharmacology and mode of action of some of the receptors mediating the actions of modulatory compounds on the extensor-tibiae muscle. First, the differential modulation of a myogenic rhythm of contraction and relaxation found in a specific small bundle of proximal muscle fibres will be considered. Second, the effects of various modulatory compounds on neuromuscular transmission and muscle contraction will be reviewed. Third, recent information on the cellular location of the modulatory compounds will be presented. Finally, the physiological significance of such a multimodulated muscle will be considered in relation to the behaviour of the locust.

MODULATION OF A MYOGENIC RHYTHM

The myogenic rhythm within the extensor-tibiae muscle of the locust hindleg was first described by Voskresenskaya (1959). It probably functions to pump haemolymph along the long narrow hindleg (Usherwood, 1974) and may also aid in ventilation (Evans & O'Shea, 1978) in similar ways to myogenic pumping structures present at the bases of many other insect appendages (see Wigglesworth, 1965; Jones, 1977). The rhythm is confined to a bundle of muscle fibres at the proximal end of the leg (Evans & O'Shea, 1978; Hoyle, 1978*a*) (see Fig. 2). Within the myogenic bundle the rhythm is thought to be initiated by a small number of pacemaker fibres and then to spread electrotonically to the other follower muscle fibres in the bundle (Burns & Usherwood, 1978). The frequency of the rhythm can be modulated by a variety of pharmacologically active substances including glutamate and γ-aminobutyric acid, the presumed rapidly acting neurotransmitters of the

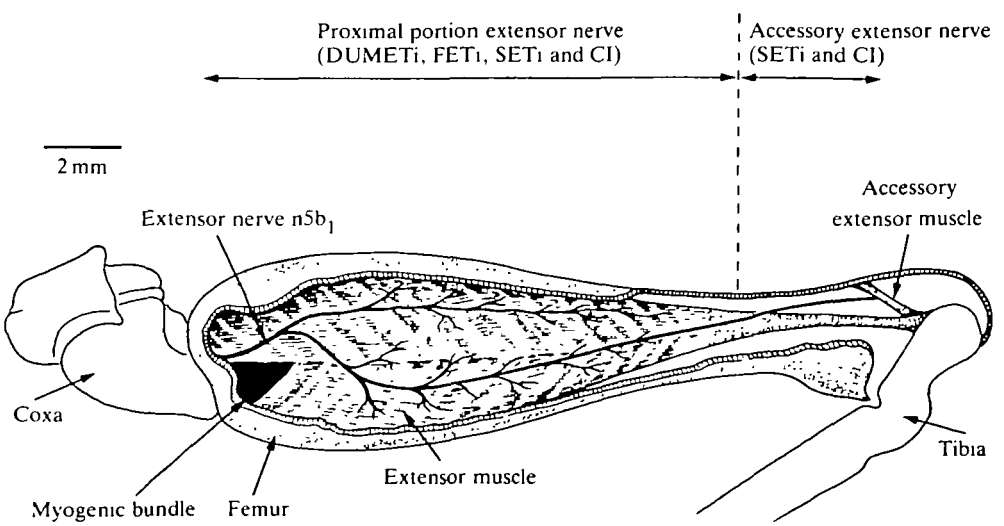


Fig. 2. A diagram of the metathoracic femur, to show innervation of extensor-tibiae muscle by extensor nerve (nerve $5b_1$). The dashed line represents division between 'proximal portion of extensor nerve' and 'accessory extensor nerve'. The neurones known to be present in the various sections of the extensor nerve are also shown. The position of the myogenic bundle is shown in black (modified from Evans & O'Shea, 1978).

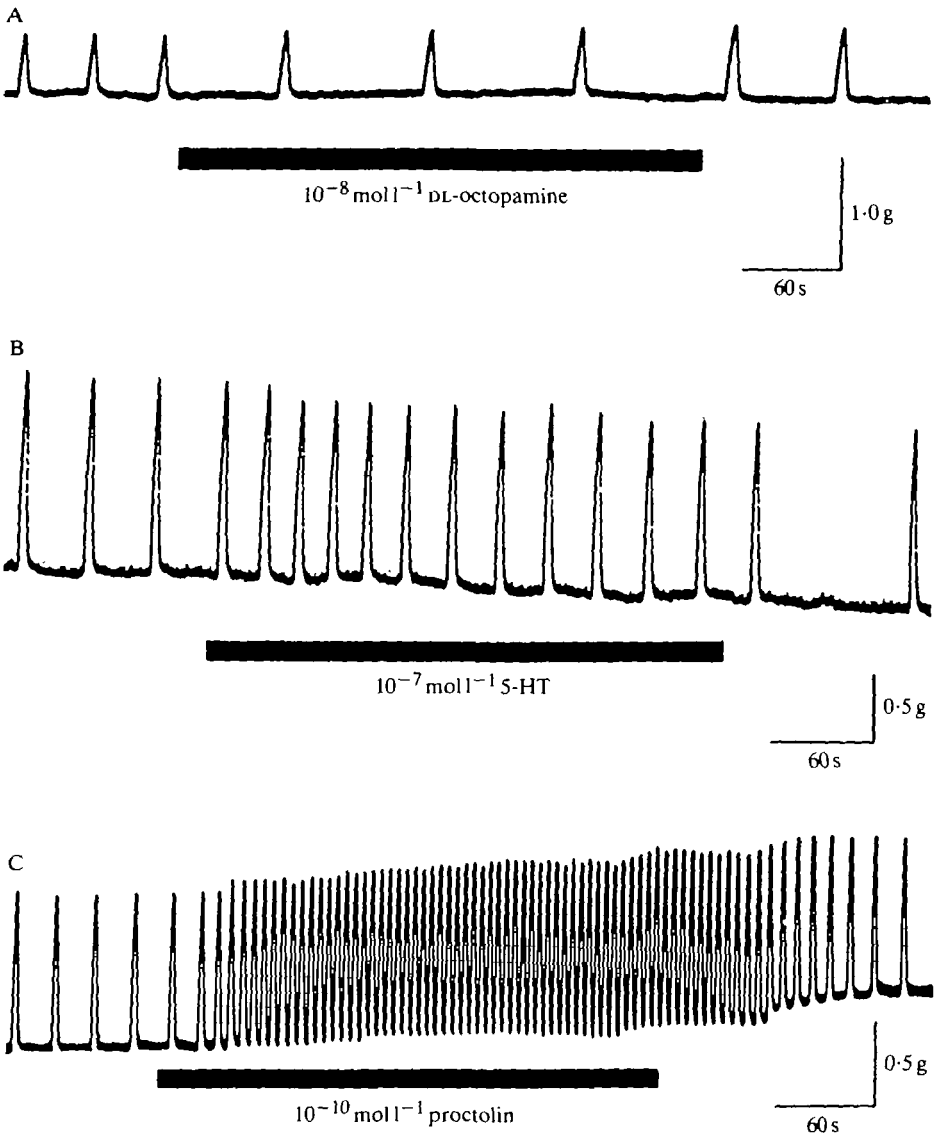


Fig. 3. The effect of modulators on the myogenic rhythm. (A) A 5-min pulse of 10^{-8} mol l^{-1} DL-octopamine reduces the frequency of the rhythm and it recovers in saline. (B) A 5-min pulse of 10^{-7} mol l^{-1} 5-hydroxytryptamine (5-HT) speeds up the rhythm and it recovers in saline. (C) A 5-min pulse of 10^{-10} mol l^{-1} proctolin again speeds up the rhythm and also generates an increase in basal tension. The frequency recovers in saline (from Evans, 1984*c*).

excitatory and inhibitory motor neurones to the muscle (Burns & Usherwood, 1978; Evans & O'Shea, 1978). In addition the myogenic rhythm can be bidirectionally modulated by a range of biogenic amines and peptides, some modulators increasing its frequency and others decreasing it (see Fig. 3; Table 1).

The octopamine-induced reduction in myogenic rhythm frequency is the most well studied receptor system of this myogenic preparation (Hoyle, 1975; Evans &

O'Shea, 1978; Evans, 1981). It has a threshold of between 10^{-9} and 10^{-10} mol l^{-1} for an observable slowing of the rhythm and the effect can be mimicked by the stimulation of DUMETi, the octopamine-containing neurone. The pharmacology of the octopamine receptors mediating these effects has been described (Evans, 1981). The receptors are stereospecific for the naturally-occurring D(−) isomer of octopamine and are maximally sensitive to monophenolic biogenic amines with an OH group on the β-carbon of the side chain (i.e. to octopamine and synephrine). The receptors mediating the actions of octopamine on the myogenic rhythm have been designated OCTOPAMINE$_1$ class receptors, and can be pharmacologically distinguished from the OCTOPAMINE$_2$ class receptors that mediate the effects of octopamine on neuromuscular transmission and muscle contraction in the rest of the muscle (Evans, 1981). Drugs such as chlorpromazine and yohimbine are much better antagonists of OCTOPAMINE$_1$ receptors than metoclopramide, whilst the converse is true for OCTOPAMINE$_2$ receptors. In addition agonists such as clonidine are more effective than naphazoline on OCTOPAMINE$_1$ receptors and the converse is again true for OCTOPAMINE$_2$ receptors. This distinction between the different subclasses of octopamine receptors also extends to their modes of action. The OCTOPAMINE$_2$ receptors mediate their actions *via* increases in the levels of the intracellular second messenger cyclic AMP (Evans, 1984*a*,*b*), whilst OCTOPAMINE$_1$ receptors mediate their actions *via* a different second messenger system that does not involve changes in cyclic AMP levels (Evans, 1984*c*). The latter conclusion is based on the fact that drugs such as forskolin, that increase intracellular cyclic AMP levels by a direct activation of adenylate cyclase, produce an increase in the frequency of the rhythm, not a decrease. In addition, the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX) does not potentiate the effects of octopamine on the myogenic rhythm. However, the calcium ionophore, A23187 can also reduce the frequency of the myogenic rhythm, suggesting that octopamine may act by increasing the intracellular calcium levels in the pacemaker fibres. Changes in the extracellular calcium concentration did not cause significant differences in

Table 1. *Modulators of the myogenic rhythm*

Modulators that decrease frequency of rhythm	
Octopamine	Hoyle, 1975; Evans & O'Shea, 1978
Adenosine + range of analogues	Evans, 1984*c*
Modulators that increase frequency of rhythm	
2′,5′-Dideoxyadenosine	Evans, 1984*c*
5-Hydroxytryptamine	Evans & O'Shea, 1978
Proctolin (Arg-Tyr-Leu-Pro-Thr)	Piek & Mantel, 1977
AKH (*p*-Glu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2)	O'Shea, 1985
MI (*p*-Glu-Val-Asn-Phe-Ser-Pro-Asn-Trp-NH_2)	O'Shea, Witten & Schaffer, 1984; Witten *et al.* 1984
MII (*p*-Glu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-NH_2)	
BPP_{5a} (*p*-Glu-Lys-Trp-Ala-Pro)	Piek, Visser & Mantel, 1979
SCP_B (Met-Asn-Tyr-Leu-Ala-Phe-Pro-Arg-Met-NH_2)	P. D. Evans & C. M. Myers, in preparation

the response of the myogenic bundle to octopamine application. At present an octopamine-mediated increase in internal calcium levels, due to a release from an internal calcium store, is not ruled out by the evidence available. Indeed it has recently been suggested that in a variety of tissues various receptor agonists, including biogenic amines, can use inositol 1,4,5-trisphosphate as a second messenger to induce the release of calcium from non-mitochondrial intracellular calcium stores (see Berridge & Irvine, 1984).

The only other pharmacological agent that has been shown to reduce the frequency of the myogenic rhythm is adenosine and certain of its analogues (Evans, 1984*c*). This effect is not blocked by phentolamine, indicating it is not mediated by the octopamine receptors described above. Studies with a range of adenosine analogues have revealed that there are in fact two sites of action for adenosine derivatives on the myogenic bundle, since 2′,5′-dideoxyadenosine accelerates the rhythm (Evans, 1984*c*). The physiological significance of these adenosine regulatory sites is not known, although it is interesting to speculate that adenosine may be released as a cotransmitter along with octopamine.

The myogenic rhythm can also be accelerated by a variety of peptides and by 5-hydroxytryptamine (Table 1). The pentapeptide proctolin accelerates the myogenic rhythm and can also induce a rhythm in a quiescent preparation (Piek & Mantel, 1977; May, Brown & Clements, 1979). Dose–response curves reveal a threshold of between 10^{-11} and 10^{-12} mol l^{-1} for proctolin (Evans, 1984*c*). The actions of proctolin on the myogenic bundle are potentiated in the presence of IBMX and are mimicked by forskolin, suggesting that proctolin mediates its actions *via* an increase in cyclic AMP levels within the pacemaker fibres (Evans, 1984*c*). Recent evidence suggests that proctolin is released as a cotransmitter by SETi (O'Shea, 1985). This evidence can be summarized as follows. First, a neurone copositional with the SETi soma is immunoreactive with an antiserum raised against proctolin (Keshishian & O'Shea, 1985), whilst none of the other motor neurones to the muscle, or DUMETi, stains with the antiserum. Second, there are proctolin immunoreactive nerve terminals on the surface of the extensor-tibiae muscle, including the myogenic fibres. Third, proctolin can be released from the SETi terminals by high potassium saline in a calcium-dependent fashion. Fourth, when SETi is induced to fire a short burst of action potentials, the myogenic rhythm can be seen to be accelerated after a short delay. The duration and decay of this increased frequency parallel the actions of proctolin. It is likely that SETi uses glutamate for transient contractions and proctolin to produce much longer-lasting effects (O'Shea, 1985).

5-Hydroxytryptamine (5-HT) can also accelerate the myogenic rhythm and has a threshold in the range of 10^{-9} mol l^{-1} (Evans & O'Shea, 1978; Evans, 1984*c*). Proctolin and 5-HT act on separate receptors since gramine (10^{-5} mol l^{-1}) does not alter the response of the myogenic rhythm to proctolin (10^{-10} mol l^{-1}), but completely inhibits the actions of 10^{-7} mol l^{-1} 5-HT (Evans, 1984*c*). In addition, bromoLSD inhibits the actions of 5-HT but not of proctolin (May *et al.* 1979). Preliminary pharmacological data on the 5-HT-activated receptors (P. D. Evans, unpublished) indicates that they have a very narrow specificity range. In this respect

their responses appear to be more similar to those produced by the activation of the receptors for the peptide diuretic hormone on *Rhodnius prolixus* Malpighian tubules, which are also activated by 5-HT (Maddrell, Pilcher & Gardiner, 1971), rather than those produced by the activation of specific 5-HT receptors, such as those on the blowfly salivary gland (Berridge, 1972). This raises the possibility that the endogenous activator of these receptors on the locust myogenic bundle may be a second peptide hormone, rather than 5-HT itself.

Indeed, the myogenic rhythm can also be accelerated by a variety of other peptides. Several members of the adipokinetic hormone (AKH) family can accelerate the myogenic rhythm, including AKH itself (O'Shea, 1985) and the recently isolated myoactive factors MI and MII from the cockroach corpus cardiacum (O'Shea, Witten & Schaffer, 1984; Witten *et al.* 1984). The significance of the effects of MI and MII on the locust myogenic rhythm is unclear since they are probably not present in the locust (O'Shea, 1985). It is more likely that their structural similarity to AKH allows them to act on a receptor whose endogenous ligand is either the AKH peptide itself (Stone, Mordue, Batley & Morris, 1976) or its related analogue AKHII (Carlsen, Herman, Christensen & Josefsson, 1979), since both the latter peptides have been isolated from the locust corpora cardiaca.

Another peptide which increases the frequency of the myogenic rhythm is bradykinin-potentiating peptide (BPP_{5a}) (Stewart, Ferreira & Greene, 1971). Piek, Visser & Mantel (1979) report that this peptide was active between 10^{-8} and 10^{-9} mol l^{-1} making it several orders of magnitude less potent than proctolin. It is not clear if this peptide is mimicking the effects of AKH, since it also has an *N*-terminal pyro-Glu residue, or whether it is mimicking the actions of proctolin, since Piek (1985) suggests that BPP_{5a} and proctolin have some similarities in the nature of the amino acids in part of their sequences.

Very recently, whilst looking for possible antagonists of the responses of the extensor-tibiae muscle to the molluscan peptide, FMRFamide (see below), we observed that another peptide first isolated from the molluscan nervous system, namely small cardioactive peptide (SCP_B) (Morris *et al.* 1982), was also capable of initiating and accelerating a myogenic rhythm in the extensor-tibiae muscle of the locust (P. D. Evans & C. M. Myers, in preparation) (see Fig. 4A). SCP_B antagonizes the actions of FMRFamide on various molluscan preparations (Murphy, Lukowiak & Stell, 1985; P. E. Lloyd, personal communication), but does not appear to do so on the locust muscle. The effects of SCP_B on the myogenic rhythm were dose-dependent, with a threshold for an observable effect occurring between 10^{-8} and 10^{-9} mol l^{-1} (Fig. 4B). The actions of SCP_B were not blocked by gramine, suggesting that this peptide is not acting on the same acceleratory receptor as 5-HT.

At present very little is known about the pharmacology of the receptors mediating the accelerating effects of the various peptides on the myogenic rhythm. In the absence of specific inhibitors for each of the active peptides, it is even impossible definitively to determine how many distinct peptidergic receptors are present. On the basis of the structures of the various peptides it seems likely, however, that there will be at least three distinct peptidergic receptors present; one for proctolin, one for the

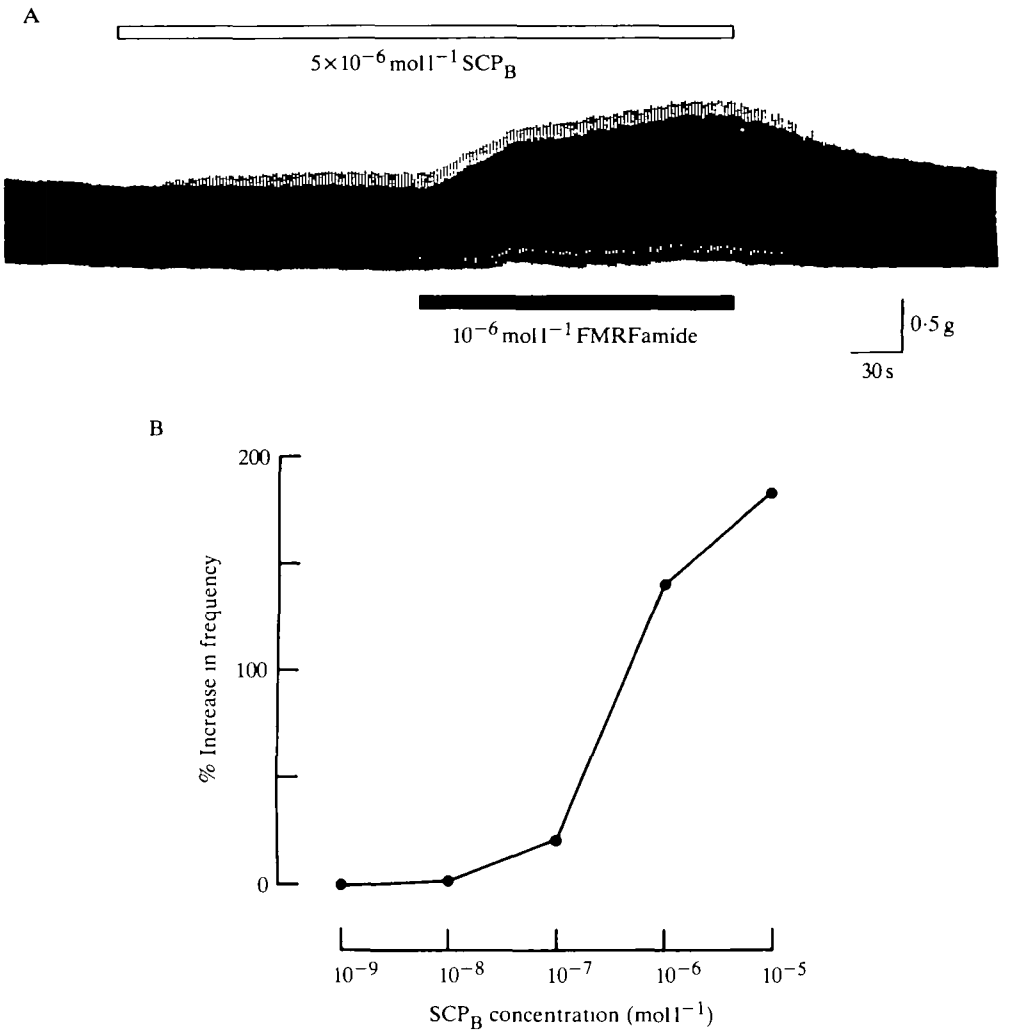


Fig. 4. The effect of small cardioactive peptide (SCP_B) on the locust extensor-tibiae muscle. (A) shows that when 5×10^{-6} mol l^{-1} SCP_B is introduced into the muscle superfusate it initiates a myogenic rhythm which can be seen superimposed upon the twitch tension transients caused by stimulation of SETi at 1 Hz. It can also be seen that this concentration of SCP_B does not prevent the potentiating action of a pulse of 10^{-6} mol l^{-1} FMRFamide upon the amplitude of the SETi-induced contractions. (B) A dose–response curve for the accelerating action of SCP_B on the frequency of the myogenic rhythm (P. D. Evans & C. M. Myers, in preparation).

AKH-like peptides and one for a peptide related to SCP_B which has not yet been isolated from the locust. The putative peptidergic site upon which 5-HT acts could represent a fourth site, since it is distinct from those acted upon by proctolin and SCP_B. Furthermore, the physiological significance of multiple modulatory receptors in the myogenic bundle is not clear at the present time. In many respects the diversity of the pharmacological profile of the receptors present on this myogenic bundle suggests it has more in common with insect visceral muscles, such as the heart

or gut, rather than with the rest of the skeletal muscle fibres in the other regions of the extensor-tibiae muscle.

The myogenic bundle of the locust extensor-tibiae muscle is an excellent preparation in which to expand our knowledge of the pharmacology of a wide variety of insect peptidergic receptors. However, it is a difficult preparation in which to combine biochemical studies on the modes of action of these receptors with the physiological observations. The physiological responses to peptide application measure the responses originating from the very small number of pacemaker fibres in the myogenic bundle. On the other hand, any biochemical responses would represent the sum of changes in both the pacemaker and larger number of follower fibres (approx. 20) present in the bundle, due to the difficulties involved in identifying and separating these two classes of muscle fibre.

MODULATION OF NEUROMUSCULAR TRANSMISSION AND MUSCLE CONTRACTION

The modulatory effects of biogenic amines and neuropeptides are not confined to the proximal bundle of myogenic muscle fibres in the extensor-tibiae muscle of the locust hindleg. In addition, the other regions of this highly specialized muscle respond in different ways to a range of modulators. The different regions of the muscle are specialized with respect to the degree of innervation they receive from each of the four neurones projecting to the muscle and also with respect to the proportions of fast, slow and intermediate muscle fibre types they contain (Hoyle, 1978*b*).

Octopamine

The modulatory effects of stimulation of the DUMETi neurone, and of the application of exogenous octopamine, upon neurally generated tension in this muscle have been described extensively (Evans & O'Shea, 1977, 1978; O'Shea & Evans, 1979; Evans, 1981, 1985*a*; Evans & Siegler, 1982). In brief, the most potent action of octopamine is to increase the rate of relaxation of both fast and slow motor neurone generated tension (Fig. 5A). In addition, it increases peak twitch tension, and the rate of contraction, generated by SETi but not by FETi. These effects are dose-dependent with thresholds occurring in the range of 10^{-8}–10^{-9} mol l^{-1} (Evans, 1981).

The effects of octopamine on SETi-induced tension depend on the frequency and pattern of motor neurone stimulation (Evans & Siegler, 1982). When SETi is stimulated at a frequency of 1 Hz, each stimulus produces a muscle twitch. By contrast, when SETi is stimulated at frequencies higher than 15–20 Hz, a smooth tetanic contraction results. Between these extremes stimulation produces an incomplete fusion of twitches. As the frequency of stimulation increases, the individual tension transients decrease in size but the base line of maintained tension increases (Fig. 5B). Pulses of octopamine introduced into the superfusate produce a pronounced decrease in maintained tension whilst increasing the height of individual

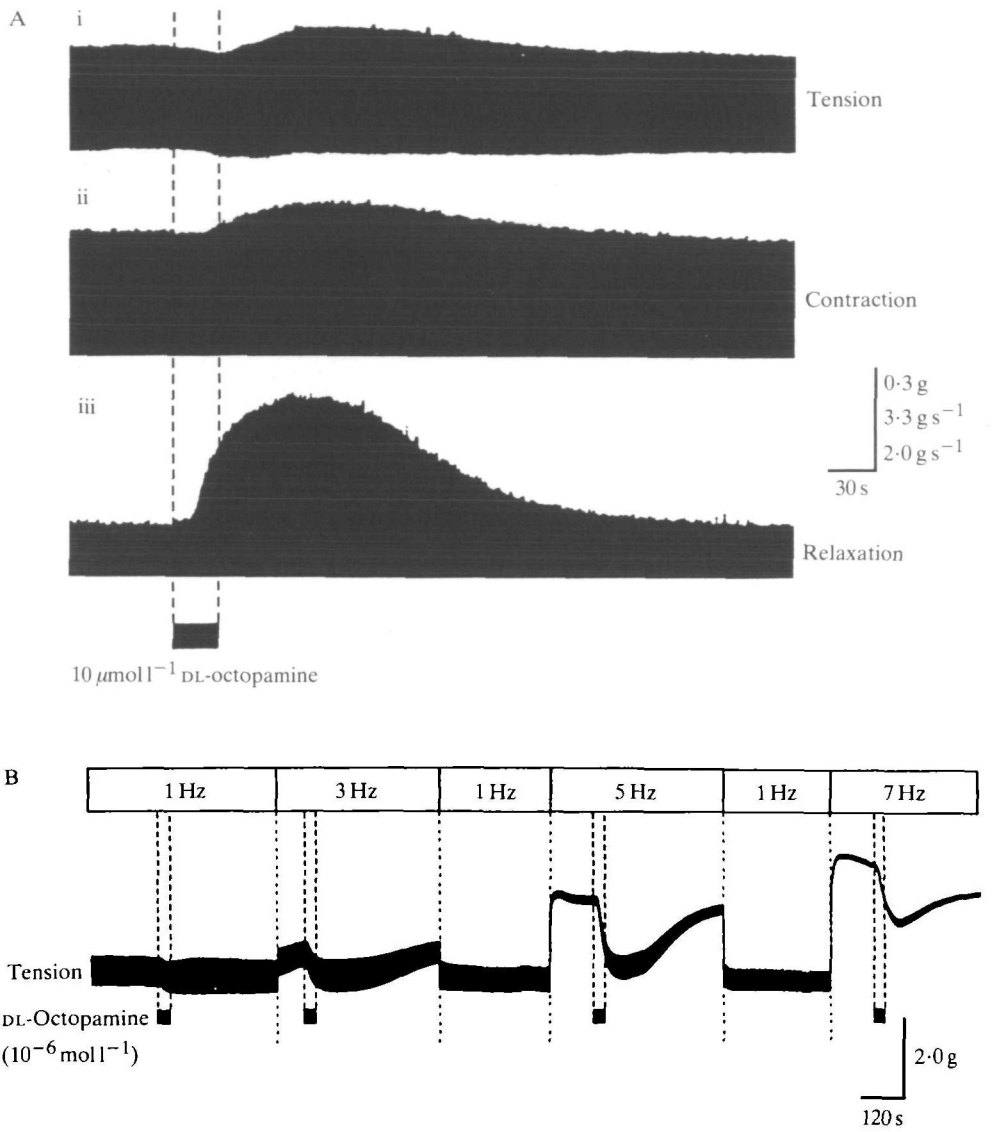


Fig. 5. The effect of octopamine on SETi-induced tension in the extensor-tibiae muscle. (A) The effect of a 30-s pulse of 10 μmol l^{-1} DL-octopamine (black bar) introduced into the muscle superfusate on twitch tension response elicited by stimulating SETi at a frequency of 1 Hz. Trace i shows the response of twitch tension and traces ii and iii show the effects on contraction and relaxation rates respectively. (B) The frequency dependence of octopamine effects on maintained tension. A continuous recording of the tension profile from a metathoracic extensor-tibiae muscle produced by firing SETi at different frequencies is shown. 30-s pulses of 10^{-6} mol l^{-1} DL-octopamine (bars) were introduced into the superfusate. The preparation was returned to 1 Hz stimulation for 5 min between the 3- and 5-Hz stimulation and also between the 5- and 7-Hz stimulation. The maximum reduction of maintained tension induced by octopamine application occurred at 5 Hz (from Evans, 1982; Evans & Siegler, 1982).

tension transients and the effects are maximal between SETi stimulation frequencies of 5 and 7 Hz. The threshold for a noticeable change in maintained tension when SETi was stimulated at 7 Hz occurred between 10^{-9} and 10^{-10} mol l^{-1} for a 30-s pulse of DL-octopamine. Octopamine also reduces a variety of other forms of maintained tension in this muscle, including tetanic tension and catch-like tension (Evans & Siegler, 1982). Thus the major effect of octopamine on this muscle is to increase the frequency at which individual twitches fuse. In addition, the hysteresis effects observed in maintained tension due to the catch-like property of the muscle are reduced, so that the tension responses of the muscle can follow more closely rapidly changing patterns of motor neurone input. Overall, octopamine changes the response of the muscle from one that favours the maintenance of posture, to one that favours rapid changes in joint position or force, such as might occur during locomotion.

The simplest explanation for the above results would be for the increases in SETi-induced twitch contraction and contraction rate to be mediated *via* presynaptic receptors on the SETi terminals and for the increases in relaxation rate of twitch tension to be mediated *via* postsynaptic, or rather extrajunctional, receptors since DUMETi forms no discrete synapses with the muscle fibres (Evans, 1981). Independent evidence for the existence of presynaptic octopamine receptors comes from studies on the spontaneous release of transmitter from the SETi terminals. The frequency, but not the amplitude, of slow, spontaneous miniature end-plate potentials is increased by octopamine application, which implies that the increases in neurally induced excitatory junctional potentials must, by definition, be due to an increase in quantal content. The pre- and postsynaptic octopamine receptors have been designated OCTOPAMINE$_{2A}$ and OCTOPAMINE$_{2B}$ receptors respectively. They can be distinguished pharmacologically on the basis of the relative potencies of a range of agonists and antagonists (Evans, 1981). They can also be distinguished pharmacologically from the OCTOPAMINE$_1$ receptors on the myogenic bundle, as outlined above.

A considerable amount of evidence suggests that both the OCTOPAMINE$_{2A}$ and OCTOPAMINE$_{2B}$ receptor subtypes mediate their actions on neuromuscular transmission and muscle contraction *via* a mechanism that involves an increase in intracellular levels of the second messenger cyclic AMP (Evans, 1984*a*,*b*). Octopamine application and DUMETi stimulation both produce increases in cyclic AMP levels, but not cyclic GMP levels, that are potentiated by the phosphodiesterase inhibitor IBMX (Fig. 6A). The octopamine effects are dose-dependent and the DUMETi effects depend on the firing frequency. The changes in cyclic AMP levels have a similar time course, amine specificity and pharmacological sensitivity to agonists and antagonists as the corresponding physiological responses. In addition, artificially raising intracellular cyclic AMP levels by the application of IBMX, the diterpene adenylate cyclase activator, forskolin, or cyclic AMP analogues, such as 8-chlorophenylthiocyclic AMP, mimic all the physiological actions of octopamine. Since this includes the action of octopamine on increasing the frequency of miniature end-plate potentials, it suggests that even the presynaptic actions of octopamine are

mediated by changes in the levels of cyclic AMP within the terminals of SETi on the muscle, an effect for which it is difficult to obtain direct biochemical measurements.

An unusual feature of the dose–response curve for the octopamine-mediated increases in cyclic AMP levels in the locust extensor-tibiae muscle (Fig. 6A) is that the rising phase of the sigmoid curve extends over more than 2 log units of concentration before entering the linear portion. This suggests that there may be more than one component to the response. This can be seen more clearly by replotting the data from Fig. 6A on a log–log plot (Fig. 6B). This reveals clearly that there are two proportionally related components to the response. Each component has an initial linear functional slope of unity where a ten-fold increase in octopamine concentration produces a ten-fold change in cyclic AMP accumulation. Thus in these regions of the curve there is no cooperativity between agonist molecules. The existence of these two linear components to the curve, joined by a non-linear section, could mean that the preparation has a single receptor type that increases cyclic AMP levels. In addition, at higher concentrations it may undergo some form of agonist-induced conformation change that alters its affinity for octopamine. An alternative explanation is that there are, in fact, two distinct independent receptor sites involved. A further point about this curve is that a comparison of the dose–response curves for the physiological effects of octopamine on neuromuscular transmission and muscle tension in the extensor muscle (Evans, 1981) with the above curves for cyclic AMP accumulation, indicates that the physiological responses are all maximal at concentrations of octopamine (10^{-5}–10^{-4} mol l^{-1}) where the cyclic AMP accumulation is still increasing. This could perhaps be explained by the existence of 'spare receptors' for octopamine (c.f. Levitzki, 1976; Fain & Berridge, 1979). The occupancy of such receptors would result in the redundant production of second messenger which is not accompanied by any further increase in the physiological response. It is assumed that spare receptors enable a preparation to respond to lower doses of hormone, since the probability of a hormone activating a specific receptor is proportional to the number of receptors on the cell surface (Berridge, 1980). At physiological threshold levels of octopamine (between 10^{-8} and 10^{-9} mol l^{-1}, see Evans, 1981), only very low levels of cyclic AMP accumulation were observed in the whole muscle. However, the relationship between cyclic AMP levels and physiological responses is complex since the increases in cyclic AMP levels could be confined to a functionally distinct subcompartment of muscle fibres within the muscle.

A direct test of this hypothesis reveals that octopamine-induced changes in cyclic AMP levels are different in different regions of the muscle (Evans, 1985*b*) (Fig. 7). The maximal effects occur in regions *e,f* and 135*c, d* of the muscle, which are also the regions where octopamine has the maximal effects on the relaxation rates of SETi twitch tension (Table 2). These regions contain the largest proportions of slow and intermediate type muscle fibres (Hoyle, 1978*b*) and contribute most of the tension developed by the muscle during a SETi-induced twitch. An examination of the dose-responsiveness of cyclic AMP levels to octopamine concentration in the different regions of the muscle again provides evidence for the existence of more than one component in the response. In all regions of the muscle, the main component of the

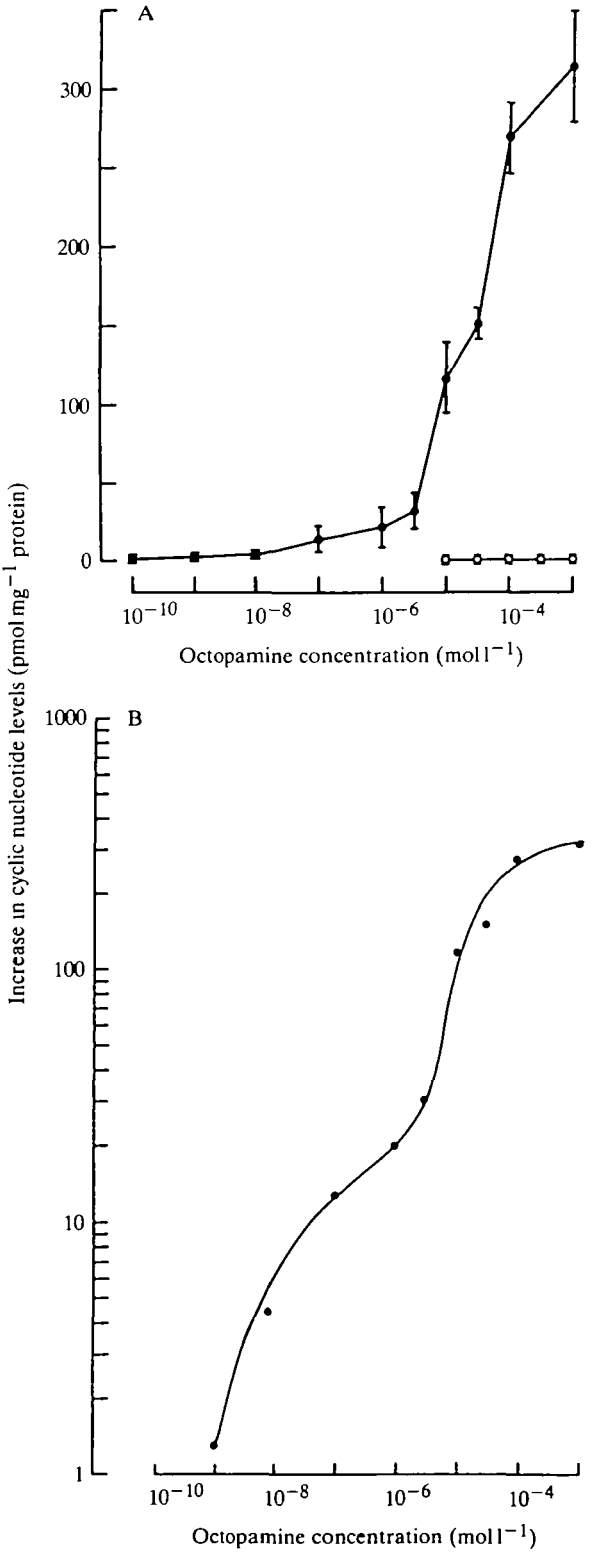

A
300
200
100
0
10^{-10}
10^{-8}
10^{-6}
10^{-4}
Octopamine concentration (mol l^{-1})
Increase in cyclic nucleotide levels (pmol mg^{-1} protein)
B
1000
100
10
1
10^{-10}
10^{-8}
10^{-6}
10^{-4}
Octopamine concentration (mol l^{-1})

Fig. 6

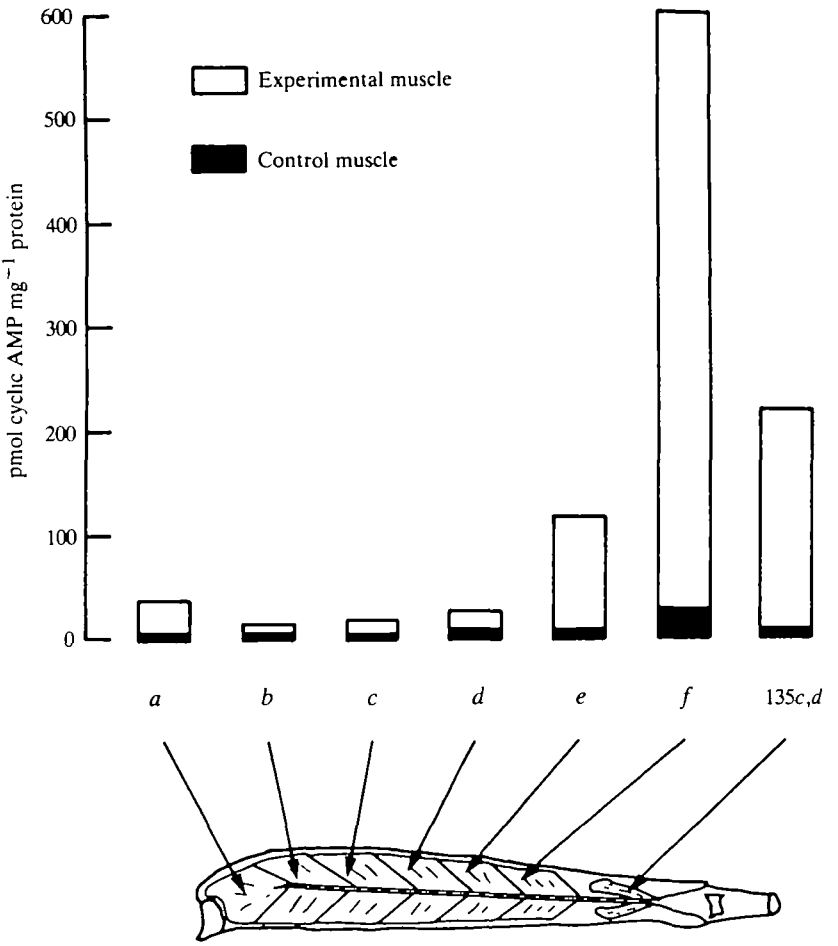


Fig. 7. The effect of a 10-min exposure to 10^{-5} mol l^{-1} DL-octopamine on cyclic AMP levels in different regions of the extensor-tibiae muscle of the locust hind leg. The results are expressed as pmol cyclic AMP mg^{-1} protein for each of the blocks of muscle fibres from a single muscle. Open bars show the levels in the experimental muscle and filled bars the levels in the contralateral control muscle. Both experimental and control muscles were pre-incubated for 10 min in 10^{-4} mol l^{-1} isobutylmethylxanthine (IBMX) for 10 min, and the control for a further 10 min incubation in IBMX. The diagram indicates the location of the blocks of muscle assayed (from Evans, 1985*b*).

Fig. 6. Dose–response curves for the action of DL-octopamine on cyclic nucleotide levels in the extensor-tibiae muscle. (A) A semi-log plot of the effects of cyclic AMP levels (●) and cyclic GMP levels (○). The results are expressed as the increase in cyclic nucleotide levels in pmol mg^{-1} protein in the experimental muscle above that found in the contralateral control muscle. Both experimental and control muscles were pre-incubated for 10 min in 10^{-4} mol l^{-1} isobutylmethylxanthine (IBMX) before the exposure of the experimental muscle to octopamine plus IBMX for 10 min, and the control to a further 10 min incubation in IBMX. Each value is the mean of four determinations and the bars represent standard errors of the mean. (B) A log–log plot of the data shown in A for the effects on cyclic AMP levels.

Table 2. *Octopamine potentiation of twitch tension in different regions of the extensor muscle*

Muscle regions	SETi		FETi
	% Increase in twitch amplitude	% Increase in relaxation rate	% Increase in relaxation rate
a	32·7 ± 5·6	65·3 ± 15·0	59·9 ± 10·4
b	—	—	58·0 ± 8·2
c	—	—	50·0 ± 9·7
d	—	—	64·7 ± 20·7
e	32·2 ± 7·8	115·8 ± 8·9	45·3 ± 6·1
f	29·1 ± 1·2	146·1 ± 13·9	53·9 ± 8·8
135*c,d*	—	—	—

The values indicate the maximal responses ± standard error ($N = 4$) to a 30-s pulse of 10^{-6} mol l^{-1} DL-octopamine introduced into the muscle superfusate.

The slow motoneurone (SETi) and the fast motoneurone (FETi) were stimulated at 1 Hz.

Regions *b* and *c* are not innervated by SETi. The twitches induced in regions *d* and 135*c,d* by SETi are very small and the effects of octopamine were not quantifiable. Region 135*c,d* is not innervated by FETi. For muscle regions, see Fig. 7.

response is one that has a half-maximal effect between 10^{-4} and 10^{-5} mol l^{-1} and a maximum in the region of 10^{-3} mol l^{-1} octopamine. However, a second smaller effect is evident in some regions of the muscle, particularly in regions *d,e,f* and 135*c,d*, which produces a shoulder on the dose–response curve at 10^{-6} mol l^{-1} (Evans, 1985*b*). These data support the hypothesis that the two components in the dose–response curve for the whole muscle represent two distinct receptor sites. At present it is not possible to decide how the two biochemically identified receptor sites relate to the $OCTOPAMINE_{2A}$ and $OCTOPAMINE_{2B}$ receptors identified pharmacologically or if an additional receptor type is present that increases cyclic AMP levels at high concentrations but for which we do not yet know the physiological response.

Another interesting aspect of the mechanism of cyclic AMP production in the extensor-tibiae muscle is its interaction with forskolin, the diterpene adenylate cyclase activator. As mentioned above, forskolin can specifically increase cyclic AMP levels in this preparation and mimics all the physiological effects of applying octopamine. However, in other preparations forskolin has been shown to have a second effect in that it can potentiate the actions of agonist stimulation at concentrations below that required for a direct activation of adenylate cyclase (Daly, 1984; Seamon & Wetzel, 1984). These effects are believed to be due to its binding to a site on the guanine nucleotide regulatory protein, N_S, which couples receptor occupancy to activation of adenylate cyclase. Thus we performed experiments to see if low doses of forskolin could cause any potentiation of the effects of octopamine. Fig. 8A shows that the effects of forskolin on cyclic AMP accumulation were additive to those of a fixed dose of DL-octopamine (10^{-6} mol l^{-1}) and that, within the concentration range tested, forskolin did not appear to produce any potentiation. Fig. 8B shows the converse experiment, where the actions of a fixed dose of forskolin were examined at

various concentrations of octopamine. Again no evidence was found for potentiation. Similar physiological experiments using low doses of forskolin (10^{-7} and 10^{-6} mol l^{-1}), which by themselves produced no, or extremely small, effects on the relaxation rates of SETi-induced twitch tension, again failed to show any potentiating effects on the actions of submaximal doses of octopamine. At present the reason for this discrepancy with the literature on forskolin effects in vertebrate tissues is not clear. Suggestions have been made that some of the potentiating actions of forskolin may be related to its ability to overcome agonist-induced desensitization (Harper, 1984; Guild & Drummond, 1984). Thus the fact that the physiological responses of the locust extensor-tibiae muscle to prolonged exposure to octopamine do not appear to exhibit desensitization might account for a lack of potentiation by low doses of forskolin. Further, it suggests that N_S, which couples the occupied receptor to the catalytic subunit of adenylate cyclase, may not, in locust muscle, have a regulatory binding site for forskolin.

Proctolin

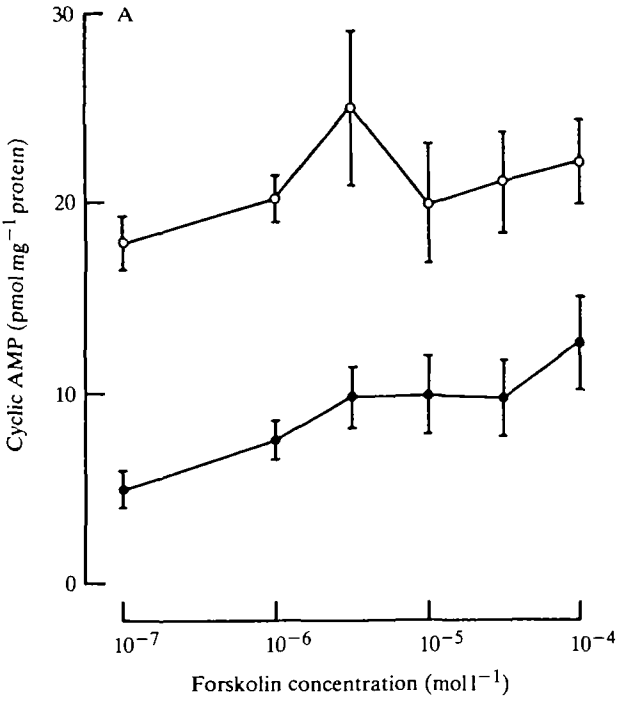
The insect pentapeptide, proctolin (Brown, 1967, 1975, 1977; Starratt & Brown, 1975; Brown & Starratt, 1975) can also modulate neurally evoked tension and basal tension in the extensor-tibiae muscle, in addition to its effects on the frequency of myogenic contractions described above. May *et al.* (1979) report that low doses of proctolin (approx. 5×10^{-7} mol l^{-1}) enhance the force of neurally evoked tetanic contraction in the slow tonic bundle of muscle fibres at the proximal end of the muscle when SETi is stimulated at 10 Hz for 3 s every 30 s. This is the opposite effect to octopamine, since octopamine reduces the height of SETi-induced tetanic contractions (Evans & Siegler, 1982). In contrast, the amplitude of slow twitch tension (SETi fired at 1 Hz) is increased by both low doses of proctolin (in the range 10^{-9}–10^{-10} mol l^{-1}) (P. D. Evans, unpublished) and by octopamine (Evans & O'Shea, 1977; O'Shea & Evans, 1979). At higher concentrations (i.e. above 10^{-9} mol l^{-1}), proctolin induces a slow contraction which is concentration-dependent (May *et al.* 1979). The magnitude of this slow contraction is also dependent on the amount of basal tension already being maintained by the muscle (Fig. 9) since it varies with the frequency of stimulation of SETi, being maximal between 5 and 7 Hz (Evans, 1982). Thus the action of proctolin is again opposite to that of octopamine, since both octopamine and DUMETi stimulation decrease maintained tension with the effect also being maximal in the region of 7 Hz (Evans & Siegler, 1982). The relationship between the frequency of SETi stimulation and the effect of proctolin is probably due to changes in the basal level of intracellular calcium maintained in the muscle fibres at the different stimulation frequencies which can alter the effects produced by proctolin on the contractile apparatus. Both the effects of proctolin on basal maintained tension (i.e. its inductions of slow contractions) and on the amplitude of twitch tension vary with changes in the external calcium concentration of the medium. If the external calcium concentration is varied between 0·5 and 10·0 mmol l^{-1} both effects of proctolin show a maximum in the range 1–2 mmol l^{-1} (P. D. Evans, unpublished). This contrasts with the effects of octopamine on twitch

tension, which again varied with extracellular calcium concentration but showed a maximum at 4 mmol l^{-1} (Evans, 1984*b*). The effects of proctolin on the extensor-tibiae muscle are not confined to those of the tonic proximal bundle used by May *et al.* (1979) since experiments similar to those described above for octopamine (Evans, 1985*b*) reveal effects of proctolin on slow fibres in regions *e* and *f* of the extensor muscle (P. D. Evans, unpublished).

Very little is known about the pharmacology of proctolin receptors on the extensor-tibiae muscle. The most extensive studies on proctolin pharmacology to date have been carried out on the cockroach hindgut (Starratt & Brown, 1979; Sullivan & Newcomb, 1982). These studies replaced each of the amino acids of proctolin by either alanine or a D-amino acid analogue, and found that all five amino acids in the chain were necessary for activity. In addition they found that the presence of a free carboxy terminal was required.

Similarly, very little consistent evidence has been provided for the mode of action of proctolin in any preparation. In the extensor-tibiae muscle, May *et al.* (1979) suggest that in the proximal bundle of tonic muscle fibres proctolin mediates its effects *via* a depolarizing action on the muscle fibres. However, evidence discussed above also implicates the involvement of cyclic nucleotides in the proctolin-mediated increase in myogenic rhythm frequency (Evans, 1984*c*). In the whole extensor muscle, and also in the isolated myogenic muscle bundle, however, 10-min incubations in the presence of proctolin at concentrations from 10^{-10} to 10^{-4} mol l^{-1} did not produce any consistent changes in either the levels of cyclic AMP or cyclic GMP (P. D. Evans, unpublished). One possible explanation for the above observations would be that proctolin mediates its effects by different mechanisms in different regions of the muscle, in parallel with the recent findings for octopamine (Evans, 1984*a*,*b*,*c*, 1985*b*). Thus in the pacemaker fibres for the myogenic rhythm proctolin increases cyclic AMP levels, whilst octopamine mediates its actions *via* a mechanism that may involve the release of calcium from an internal store (Evans, 1984*c*). However, since the pacemaker fibres only represent a small proportion of the fibres of the myogenic bundle, the biochemistry of the bundle is dominated by that of the follower fibres which, like the rest of the muscle, do not demonstrate proctolin-mediated increases in cyclic AMP levels. In the rest of the extensor muscle the proctolin responses of the slow muscle fibres are largely opposite to those of octopamine. They seem likely to be mediated by a mechanism involving increases in

Fig. 8. The interactions of the effects of octopamine and forskolin on the levels of cyclic AMP in the extensor-tibiae muscle. (A) The effect of a fixed concentration of DL-octopamine (10^{-6} mol l^{-1}) on the dose-dependent effects of forskolin. The cyclic AMP levels in the presence of octopamine and various concentrations of forskolin (○) and in the presence of forskolin alone (●) are shown. The results are plotted as pmol cyclic AMP mg^{-1} protein for each of the superfused muscles examined. (B) The effect of a fixed concentration of forskolin (10^{-6} mol l^{-1}) on the dose-dependent effects of DL-octopamine. The cyclic AMP levels in the presence of forskolin and various concentrations of octopamine (●) and in the presence of octopamine alone (○) are shown. The results are plotted as the increase in cyclic nucleotide levels in pmol mg^{-1} protein in the experimental muscle above that found in the contralateral control muscle. Each value is the mean of four determinations and the bars represent standard errors of the mean.


A
30
20
10
0
Cyclic AMP (pmol mg^{-1} protein)
10^{-7}
10^{-6}
10^{-5}
10^{-4}
Forskolin concentration (mol l^{-1})

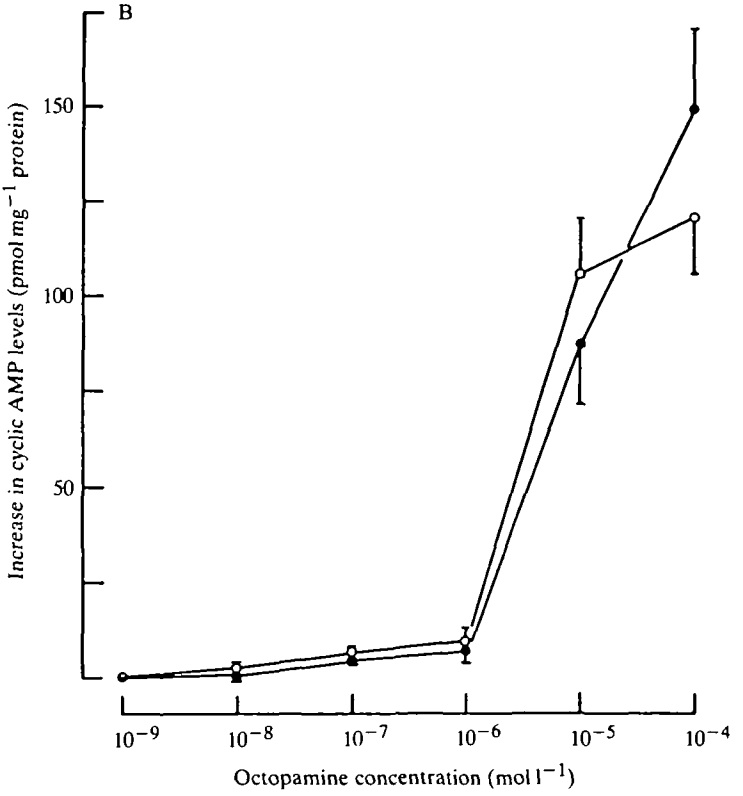

B
150
100
50
Increase in cyclic AMP levels (pmol mg^{-1} protein)
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
Octopamine concentration (mol l^{-1})

Fig. 8

intracellular calcium levels (see Berridge & Irvine, 1984) since octopamine has been proposed ultimately to act by reducing intracellular calcium levels in the muscle fibres (see Evans, 1984*a*,*b*, 1985*a*).

The recent finding that SETi uses proctolin as a slow-acting cotransmitter along with glutamate as its presumed rapidly acting transmitter (O'Shea, 1985), has

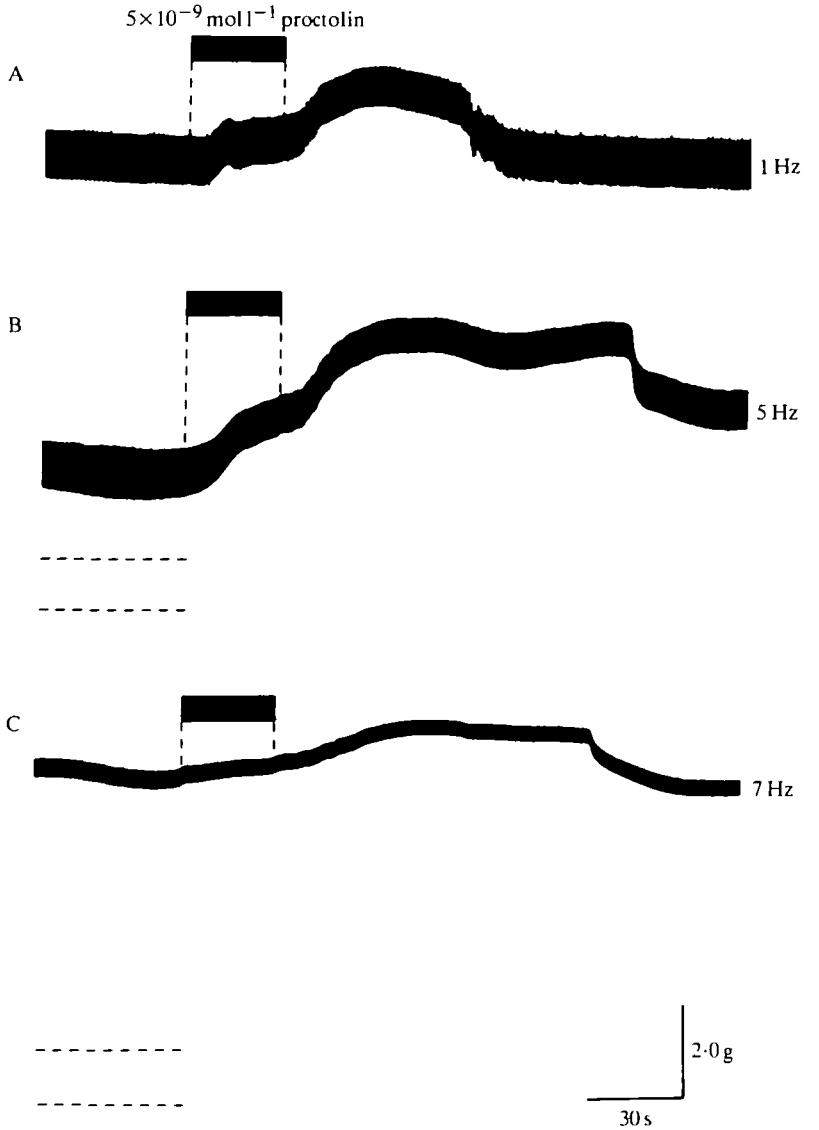


Fig. 9. The frequency-dependent effect of a 30-s pulse of 5×10^{-9} mol l^{-1} proctolin (black bars) introduced into the muscle superfusate on SETi-induced tension at various stimulation frequencies in the extensor-tibiae muscle. Horizontal dashed lines indicate level of tension responses at 1 Hz for traces B and C. Proctolin increases the level of maintained tension in the muscle and the effect is maximal at 5 Hz. Note the abrupt relaxation of muscle tension during the recovery phase (from Evans, 1982).

opened up new exciting possibilities for the study of proctolin in a system where all the other neuronal inputs are known. The information now available about the slow time course of action of the proctolin released from SETi should enable some detailed studies to be conducted on its modes of action in the different regions of the extensor-tibiae muscle. It will also be of much interest to see if the apparently different modes of action for proctolin in the different regions of the muscle are mediated by different pharmacological classes of proctolin receptor, as has been found for octopamine (Evans, 1981, 1984*a*,*b*,*c*).

FMRFamide

Immunocytochemical evidence has recently been presented for the presence of peptides related to the neuropeptide FMRFamide (Phe-Met-Arg-Phe-NH_2) in specific subsets of neurones within the locust nervous system (Myers & Evans, 1985*a*,*b*). FMRFamide was originally isolated and identified from the nervous system of the clam *Macrocallista nimbosa* (Price & Greenberg, 1977). Since that time FMRFamide, and a family of structurally related peptides, have been shown to be present in nervous tissue from a wide range of invertebrate species, including the snail *Helix* (Price *et al.* 1985) and the horseshoe crab *Limulus* (Watson *et al.* 1984). Structurally related peptides have also been reported to be present in vertebrate nervous tissue (Dockray & Williams, 1983; Williams & Dockray, 1983; Dockray *et al.* 1983; O'Donohue *et al.* 1984).

In molluscs, FMRFamide is cardioexcitatory and also stimulates a variety of non-cardiac muscles (Price & Greenberg, 1980; Painter, 1982; Cottrell, Schot & Dockray, 1983*b*; Cottrell, Greenberg & Price, 1983*a*). In the locust, the fact that immunoreactive neurones could be traced to identified skeletal muscles, and also that it appeared concentrated in neurohaemal areas (Myers & Evans, 1985*a*,*b*), suggested that locust skeletal muscle would be an interesting target site in which to investigate the effects of FMRFamide-related peptides (P. D. Evans & C. M. Myers, in preparation). Also, whilst this study was in progress, a short communication appeared (Walther, Schiebe & Voigt, 1984) reporting the effects of FMRFamide-like peptides on locust skeletal muscle. In the rest of this section we will describe how our own investigation on the effects of FMRFamide-like peptides on the locust extensor-tibiae muscle has confirmed and extended those presented in the above short communications.

In the locust extensor-tibiae muscle preparation, FMRFamide-like peptides can modulate SETi-induced twitch tension (Walther *et al.* 1984; P. D. Evans & C. M. Myers, in preparation) and also alter basal tension in the muscle, but they do not alter fast motor neurone generated twitch tension or affect the myogenic rhythm (P. D. Evans & C. M. Myers, in preparation). This means that again the extensor muscle displays differential effects in different regions of the muscle, with the largest effects occurring in the slow fibres located in regions *e* and *f* of the muscle. Fig. 10A shows that a 5-min pulse of either FMRFamide, or the structurally related peptide YGGFMRFamide at a concentration of 10^{-6} mol l^{-1} increases the amplitude, contraction rate and relaxation rate of SETi-generated twitch tension. The responses

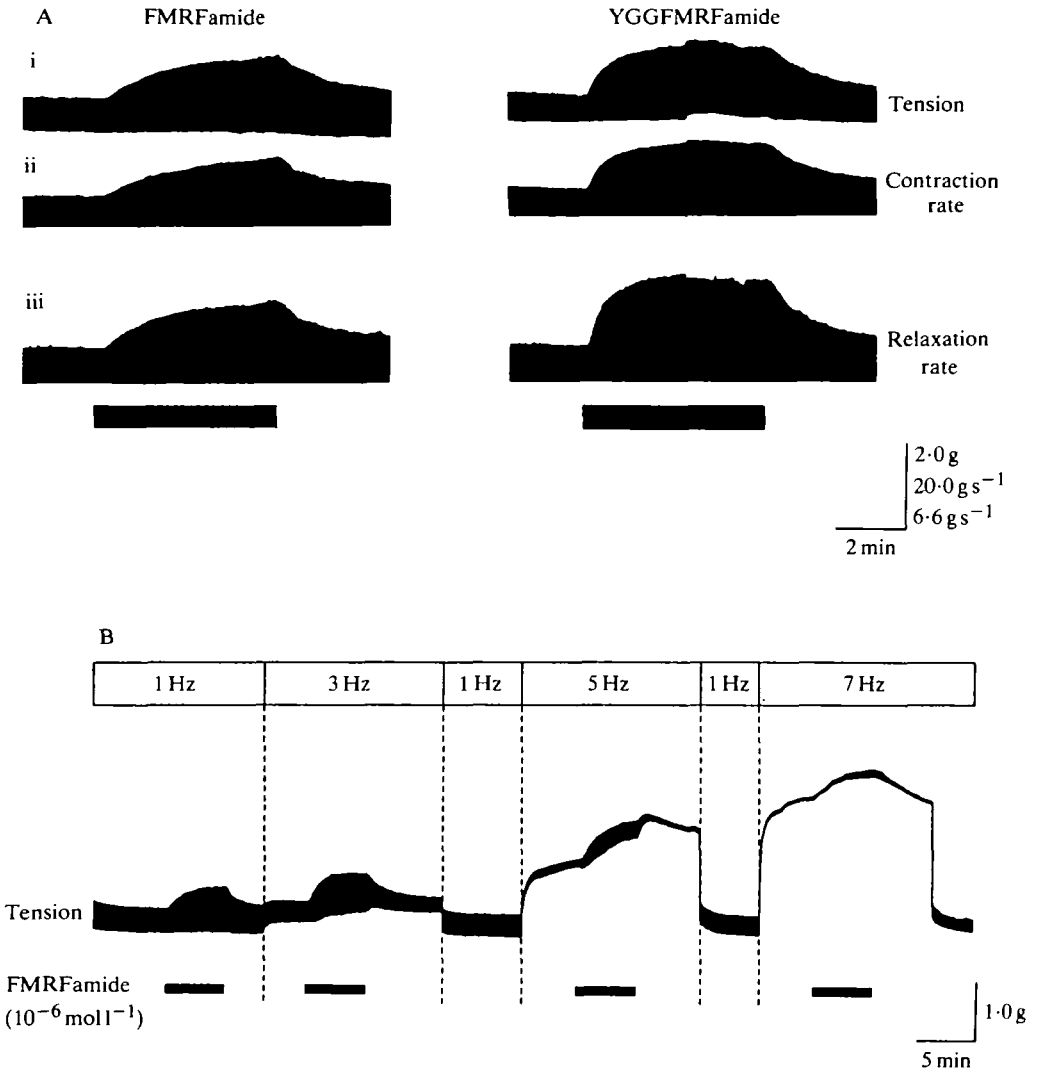


Fig. 10. The effect of FMRFamide-like peptides on SETi-induced tension in the extensor-tibiae muscle. (A) The effect of 5-min pulses of 10^{-6} mol l^{-1} FMRFamide (left-hand side) and YGGFMRFamide (right-hand side) (black bars) introduced into the muscle superfusate on twitch tension response elicited by stimulating SETi at a frequency of 1 Hz. Trace i shows the response of twitch tension, and traces ii and iii show the effects of contraction and relaxation rates respectively. (B) The frequency dependence of FMRFamide effects on maintained tension. A continuous recording of the tension profile from a metathoracic extensor-tibiae muscle produced by firing SETi at different frequencies is shown. 5-min pulses of 10^{-6} mol l^{-1} FMRFamide (bars) were introduced into the superfusate. The preparation was returned to 1 Hz stimulation between the 3- and 5-Hz stimulation and also between the 5- and 7-Hz stimulation (P. D. Evans & C. M. Myers, in preparation). Details of peptides are given in Table 3.

to YGGFMRFamide develop more rapidly than those due to FMRFamide itself and saturate after a 2-min exposure. Shorter pulses in the region of 30 s produce effects that persist for several minutes after the removal of the peptide from the superfusate.

Dose–response curves indicate that YGGFMRFamide has a threshold between 10^{-9} and 10^{-10} mol l^{-1} for a 5-min exposure for all the three parameters measured and that the dose–response curves for all three parameters are superimposible with roughly equal maximal effects of 200% increases occurring at 10^{-6} mol l^{-1}. FMRFamide is almost one order of magnitude less potent than YGGFMRFamide. Increasing the frequency of stimulation of the slow motor neurone indicates that, as for octopamine and proctolin discussed above, FMRFamide has a frequency-dependent effect on maintained tension with maximal increases in slow contractions occurring between 5 and 6 Hz (Fig. 10B). Thus FMRFamide has a very similar effect to proctolin on basal maintained contractions, both peptides increasing it rather than decreasing it, as is the case with octopamine. In addition, FMRFamide increases the amplitude, contraction rate and relaxation rate of twitch tension as does octopamine. FMRFamide, however, increases all three parameters to the same extent, whilst octopamine produces a much more marked increase in relaxation rate than in the other two parameters. In addition, the effects of FMRFamide appear to follow a much slower time course and they are not mediated *via* an action on octopamine receptors since they are not blocked by phentolamine (P. D. Evans & C. M. Myers, in preparation). It has been suggested that FMRFamide mediates its effects presynaptically on the terminals of SETi (Walther *et al.* 1984). We confirmed this directly by showing that FMRFamide could increase the frequency, but not the amplitude, of spontaneous miniature end-plate potentials from SETi (P. D. Evans & C. M. Myers, in preparation). A presynaptic site of action for FMRFamide would be consistent with its increasing EJP amplitude, twitch tension amplitude and rate of contraction of twitch tension. However, it is difficult to reconcile this with an increase in the rate of relaxation of twitch tension, since this parameter is presumably related to the rate at which Ca^{2+} is taken up by the muscle sarcoplasmic reticulum. We are, therefore, forced to postulate that FMRFamide also has direct postsynaptic or extrajunctional actions on the slow muscle fibres of the locust. Since FMRFamide did not affect the relaxation rate of fast twitch tension it seems likely that the intermediate fibres in the muscle, that are innervated by both FETi and SETi, lack FMRFamide receptors and that they are confined to the slow muscle fibres that are only innervated by SETi. At present the exact contributions of pre- and postsynaptic effects to the observed actions of FMRFamide on EJP size, twitch tension amplitude and contraction rate remain to be resolved.

The actions of FMRFamide on locust skeletal muscle have some parallels and some differences with its observed actions on other invertebrate muscles. Thus in the locust, the ability of FMRFamide to increase maintained tension or to produce a slow contracture is similar to its effects on *Helix* tentacle retractor muscle (Cottrell *et al.* 1983*a*), on the anterior byssus retractor muscle of *Mytilus edulis* (Painter, 1982), on the isolated radula protractor muscle of *Busycon contrarium* (Greenberg & Price, 1979) and on leech heart (Kuhlman, Li & Calabrese, 1985). However, unlike its actions on the *Helix* tentacle retractor muscle and leech heart, FMRFamide does not induce or modulate myogenic activity in locust skeletal muscle, although it can do in locust heart (C. M. Myers & P. D. Evans, in preparation).

Immunocytochemical blocking experiments on the locust nervous system suggest that there may be more than one endogenous FMRFamide-like peptide present (Myers & Evans, 1985*a*,*b*). However, as yet we have not isolated and sequenced these peptides. Specificity studies on various FMRFamide analogues (Table 3) indicate that NH_2-terminal extensions can increase the potency of the peptide effects on the locust extensor muscle. Thus YGGFMRFamide is more potent than FMRFamide itself. In addition substitution of leucine for the methionine does not decrease activity. The C-terminal Arg-Phe-NH_2 sequence is essential for activity in the locust and the preceding neutral amino acid (Met or Leu) may also be important since γ_1-MSH, in which the neutral amino acid is replaced by aspartate, is inactive. This is supported by the fact that the amidated dipeptide Arg-Phe-NH_2 is inactive. Since, LPLRFamide, the chicken brain peptide (Dockray *et al.* 1983), is an order of magnitude less effective than FMRFamide, it suggests that the Phe-residue at the beginning of the FMRFamide sequence may also be important for the action of these peptides on locust muscle. Some cells in the locust nervous system appear to stain with antibodies raised against bovine pancreatic polypeptide, but not to those raised against FMRFamide (Myers & Evans, 1985*a*,*b*), thus we tested the action of various pancreatic polypeptides ending in the -Arg-Tyr-NH_2 sequence. None of these were active on the extensor-tibiae muscle, confirming that the C-terminal -Arg-Phe-NH_2 sequence is essential for activity in this muscle.

Table 3. *Structure–activity relationships for effects of FMRFamide peptides on locust extensor-tibiae muscle*

Peptide	Amino acid sequence	Relative potency	Reference
YGGFMRFamide	Tyr-Gly-Gly-Phe-Met-Arg-Phe-NH_2	1	1, 2
Met^5-Enk-Arg^6-Phe^7	Tyr-Gly-Gly-Phe-Met-Arg-Phe	$0–3\times10^{-5}$	1
	Tyr-Gly-Gly-Phe-Leu-Arg-Phe-NH_2	1	1
Met-Enkephalin	Tyr-Gly-Gly-Phe-Met	0	1, 2
FMRFamide	Phe-Met-Arg-Phe-NH_2	$10^{-1}–10^{-2}$	1, 2
	Phe-Met-Arg-Phe	0	1
FLRFamide	Phe-Leu-Arg-Phe-NH_2	10^{-2}	1
LPLRFamide	Leu-Pro-Leu-Arg-Phe-NH_2	5×10^{-2}	2
RFamide	Arg-Phe-NH_2	0	2
γ_1-MSH	.....Phe-Arg-Trp-Asp-Arg-Phe-NH_2	approx. 3×10^{-5}	1
CCK-Octapeptide	.....Met-Gly-Trp-Met-Asp-Phe-NH_2	$0–3\times10^{-5}$	1
Neuropeptide Y	.....Ile-Thr-Arg-Glu-Arg-Tyr-NH_2	0	1
BPP	.....Leu-Thr-Arg-Pro-Arg-Tyr-NH_2	0	2
APP	.....Val-Thr-Arg-His-Arg-Tyr-NH_2	0	2
HPP	.....Leu-Thr-Arg-Pro-Arg-Tyr-NH_2	0	2

The potencies of the peptides are expressed relative to that of YGGFMRFamide and the table combines data from Walther, Schiebe & Voigt (1984) (1) and from P. D. Evans & C. M. Myers (in preparation) (2).

γ_1-MSH, melanocyte stimulating hormone; CCK, cholecystokinin; BPP, bovine pancreatic polypeptide; APP, avian pancreatic polypeptide; HPP, human pancreatic polypeptide.

The mode of action of FMRFamide-like peptides on locust muscle has not yet been determined. However, it appears not to involve cyclic nucleotides, since their effects are not potentiated by IBMX and changes in cyclic AMP levels in the muscle were not observed after 10-min incubations of the muscle with concentrations of FMRFamide ranging from 10^{-11} to 10^{-4} mol l^{-1} in the presence of 10^{-4} mol l^{-1} IBMX (P. D. Evans & C. M. Myers, in preparation). In other preparations, such as some molluscan neurones, FMRFamide can induce changes in potassium and calcium conductances, and some of the effects are mediated by cyclic nucleotides in some cells but not in others (Colombaioni, Paupardin-Tritsch, Vidal & Gerschenfeld, 1985).

Acetylcholine

Acetylcholine (ACh) has also been shown to extend some modulatory influences upon neuromuscular transmission from the fast motor neurone to the extensor-tibiae muscle (Fulton & Usherwood, 1977). ACh had presynaptic effects and increased both the spontaneous and evoked release of transmitter but had no postsynaptic actions. The effects were dependent on extracellular calcium and could be mimicked by carbachol, nicotine and acetyl-β-methylcholine. They were antagonized by D-tubocurarine and decamethonium. However, the effects were only observed at a concentration of 10^{-5} mol l^{-1} and above, and the effects of acetylcholinesterase inhibitors were not examined. Thus the physiological significance of these effects is not clear at present.

SOURCES OF MODULATORS FOR THE EXTENSOR-TIBIAE MUSCLE

Modulatory compounds can reach the extensor-tibiae muscle of the locust hindleg either as transmitters or cotransmitters released by neurones innervating the various regions of the muscle, as nonsynaptic modulators or local hormones released from neurosecretory terminals between the muscle fibres, or as true circulating neurohormones released into the blood from specific neurohaemal organs at some distance from the extensor muscle. The specific modulators discussed above contain examples of all three types of neuroeffector route.

Octopamine is released as a local neurohormone from the terminals of the modulatory neurone, DUMETi, which lie between the muscle fibres of the extensor-tibiae muscle (Morton & Evans, 1984). A considerable amount of evidence has been amassed for the octopaminergic nature of this cell (see Evans, 1985*a*). Anatomical evidence indicates that the terminals of the DUMETi neurone do not make discrete synapses with the muscle fibres, but rather end as neurosecretory terminals between the muscle fibres (Hoyle, Colquhoun & Williams, 1980). However, this is probably not the only source of modulatory octopamine that the muscle sees, since under stressful circumstances haemolymph levels of octopamine can rise to almost 10^{-7} mol l^{-1} (Davenport & Evans, 1984*a*,*b*), which is well above the threshold concentrations for the physiological actions of octopamine on the extensor muscle described above.

Until recently it had been assumed in the locust, that as with other arthropods, such as the lobster (see Kravitz *et al.* 1985), the major source of modulatory proctolin was as a circulating neurohormone. However, the demonstration that proctolin is released as a cotransmitter, along with glutamate, from the terminals of SETi has opened up a new perspective on the functional role of the proctolinergic modulation of this muscle (see O'Shea, 1985). Proctolinergic motor neurones have now also been demonstrated innervating skeletal muscles in crustaceans such as the lobster (Schwarz *et al.* 1984) and the crayfish (Bishop, Wine & O'Shea, 1984).

Our recent immunocytochemical studies on FMRFamide-like peptides in the locust (Myers & Evans, 1985*a*,*b*) have revealed a number of neurones in the metathoracic ganglia that stain with an antiserum raised against FMRFamide (see Fig. 11). These cells also stain with an antiserum raised against bovine pancreatic polypeptide (Furness *et al.* 1983) which additionally stains several other sets of cells in this ganglion, especially in the region of the fused abdominal neuromeres. The processes of some of the stained FMRFamide-like cells were traced in serial sections and none of them appear to send processes into the paired lateral peripheral nerves leaving the ganglion. Instead these cells could be traced into the median nerves (Myers & Evans, 1985*a*,*b*), where some of their processes end as neurosecretory terminals in the median neurohaemal organs. Others continue further along the median nerves towards the spiracle muscles giving off branches that ramify over the surface of identified skeletal muscles. Thus, although we do not know the precise structure of the immunoreactive FMRFamide-like molecules in the locust nervous system, it seems unlikely that the extensor-tibiae muscle will be modulated by a neuronal input that contains them. It is more likely that these peptides will be released into the circulating haemolymph and reach the extensor muscle as true neurohormones.

Very little is known of the cellular location of SCP_B-like peptides in the nervous system of the locust. However, a number of facts suggest that an SCP_B-related peptide occurs in the locust and may be co-localized in some of the neurones containing the FMRFamide-like peptides. First, SCP_B has a pharmacological action on the myogenic rhythm in the locust extensor-tibiae muscle (P. D. Evans & C. M. Myers, in preparation). Second, some cells in the locust nervous system, in the same region as the cells we have described as containing FMRFamide-like immunoreactivity, have been found to be immunoreactive with an antibody raised against SCP_B (S. Kemf, W. J. Heitler & C. Hill-Venning, personal communication and in preparation). Third, co-localization of SCP_B and FMRFamide has a precedent in the finding that SCP_B occurs in a specific subset of FMRFamide-containing buccal motor neurones in the marine mollusc *Aplysia* (Lloyd, Frankfurt, Kupfermann & Weiss, 1985). Thus alternate serial sections of the locust metathoracic ganglion were stained with antisera against SCP_B and FMRFamide. This revealed that some ofthe lateral posterior FMRFamide cell group also stained positively for SCP_B-like immunoreactivity (Fig. 12), which was also traced out into the median nerves. Fig. 11 shows the relative staining patterns of FMRFamide-like and SCP_B-like immunoreactive cell bodies in the locust metathoracic ganglion. A complete

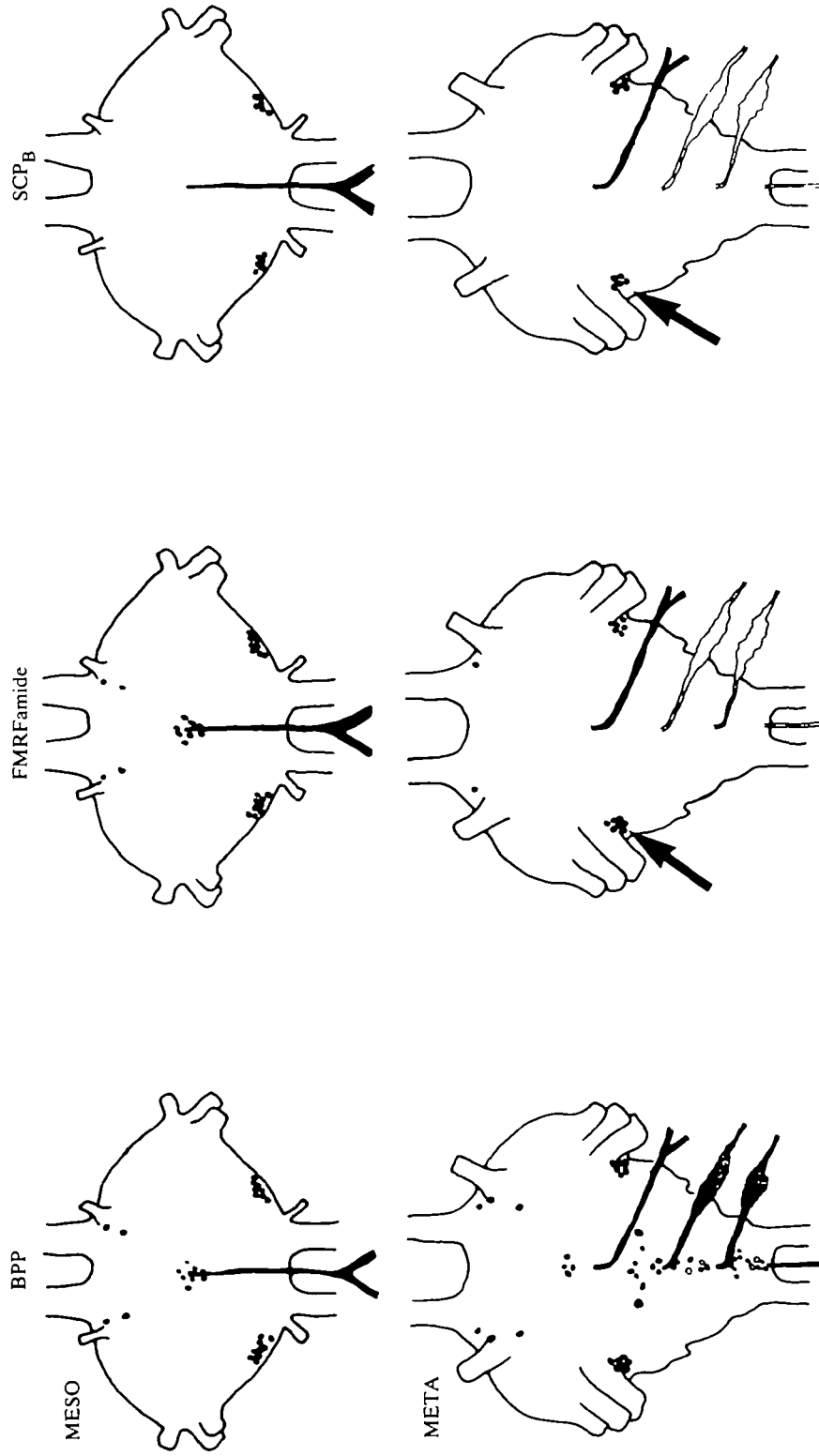


Fig. 11. Distribution of BPP-, FMRFamide- and SCP$_B$-like immunoreactive cell bodies in the meso- and metathoracic ganglia of the locust, *Schistocerca gregaria*. Filled cells occur on the ventral and open cells on the dorsal surfaces of the ganglia. The arrows indicate the positions of the posterior lateral group of stained cells shown in Fig. 12. The differential staining pattern of the median nerves is also shown. Details of peptides are given in Tables 1 and 3.

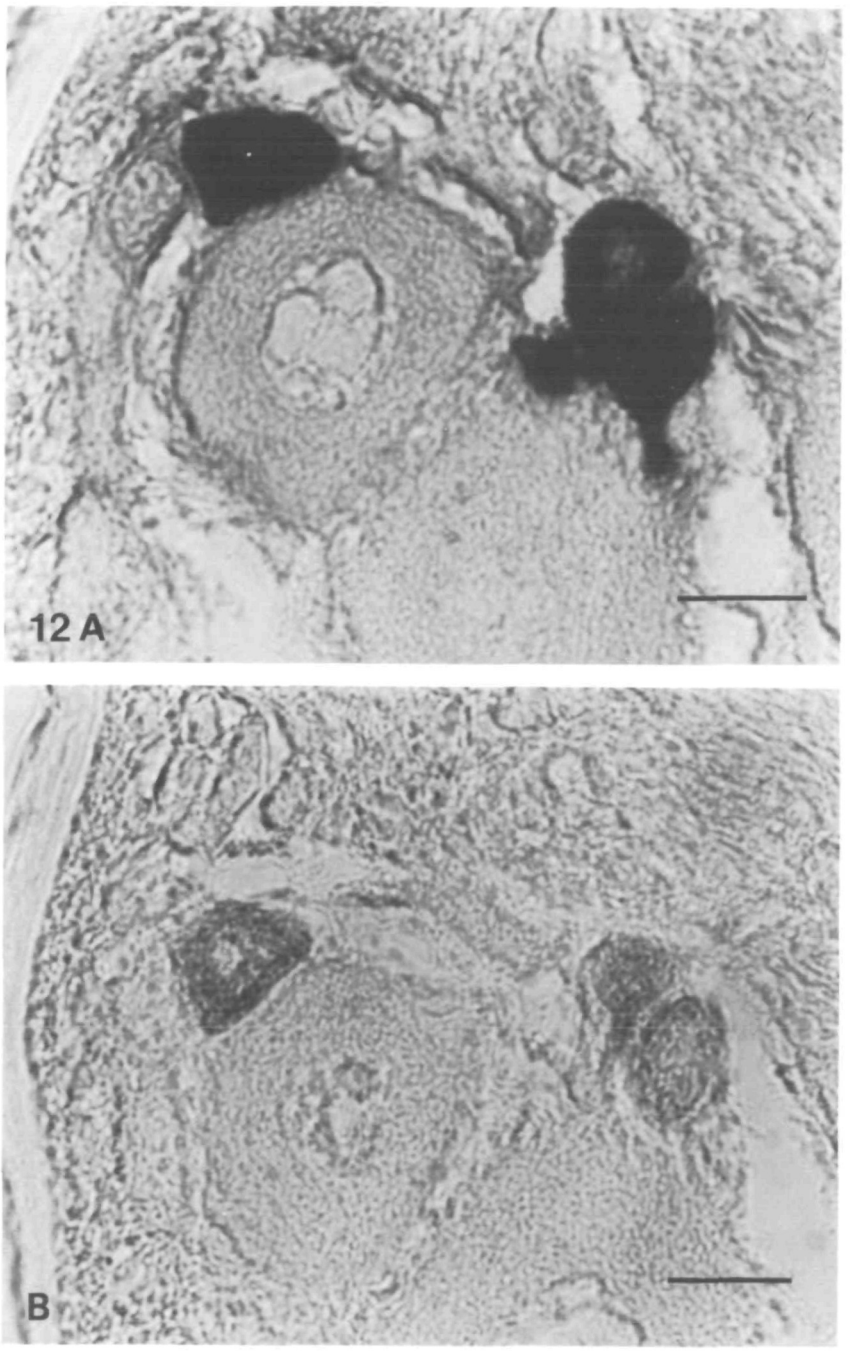


Fig. 12. Co-localization of FMRFamide- and SCP_B-like immunoreactivity in a locust thoracic ganglion. Consecutive transverse sections through the region of the metathoracic ganglion containing the posterior lateral cells show that the same cells are stained by the FMRFamide antiserum (A) and the SCP_B antiserum (B). Scale bar, 20 μm.

description of the pattern of SCP_B-like immunoreactivity in the locust nervous system is in preparation (S. Kemf, W. J. Heitler & C. Hill-Venning, in preparation). None of the neurones innervating the extensor-tibiae muscle appear to stain with the SCP_B antibody. Thus it appears likely that the SCP_B-like peptide of the locust will again reach the extensor-tibiae muscle as a neurohormone released into the haemolymph from the median neurohaemal organs.

Another group of circulatory peptide hormones in the locust that have actions on the locust extensor-tibiae muscle are the AKH-related peptides (see above). Both AKH itself (Stone *et al.* 1976) and the related peptide AKHII (Carlsen *et al.* 1979) are found in the locust corpus cardiacum, from which they are presumably released into the circulation as neurohormones (Mordue & Stone, 1981). However, recent immunocytochemical evidence, using antisera raised against AKH, has revealed neurones stained in the locust brain (Schooneveld, Tesser, Veenstra & Romberg-Privee, 1983; Schooneveld, Romberg-Privee & Veenstra, 1985), suggesting that members of this peptide family may also be released as neurotransmitter molecules from neurones. No evidence has yet been reported that any of the neurones innervating the locust extensor-tibiae muscle are immunoreactive with the antisera to AKH and so it must be assumed that these peptides again reach this muscle as circulating neurohormones.

CONCLUSION

The contractile responses of the extensor-tibiae muscle of the locust hindleg to fast-acting, neuronally-released neurotransmitters are well modulated by a variety of biogenic amines and peptides. Thus this preparation, which has already provided much basic information on the principles of neuromuscular transmission (Hoyle, 1983), can also provide a model system in which to study questions of more recent interest, such as peptidergic neurotransmission, modulation and cotransmission, as well as aminergic modulation, together with the interactions of all these different modulatory systems.

The functional significance of different modulatory systems is difficult to assess at present. We are only just starting to realize that the 'simple' model system is much more complex than originally thought. In addition there is no guarantee that we have, even on this one muscle, discovered all the receptors that exist for biogenic amines and peptides, let alone those for as yet unthought of modulatory compounds. However, if we consider the normal activity pattern of this muscle in relation to the behaviour pattern of the locust, we can get some ideas on how and when the different forms of modulation may interact. For instance, it is known that DUMETi is activated prior to any movements involving the extensor-tibiae muscle (Hoyle & Dagan, 1978), and also that octopamine levels in the haemolymph are raised by a variety of stressful stimuli (Davenport & Evans, 1984*a*,*b*). Thus the primary function of the octopaminergic modulation is likely to be to change the response of the muscle from one that favours maintenance of posture to one that favours rapid changes in joint position or force, such as might occur during locomotion. Under

such conditions the muscle's output would be more closely tuned to its neuronal input, since it would have an increased frequency-following capability and any residual maintained or catch-like tension would be removed. In addition, any interference from the myogenic rhythm would also be removed making the responses of the muscle ideally suited to deal with a stressful situation. In contrast, the muscle's responses to proctolin would seem more suited to a postural mode rather than a dynamic mode of action. The release of proctolin would become important at times of sustained activity in SETi. The induction of a long-lasting contracture of the muscle would help maintain tension independent of the neuronal input. This would be important to the locust when the extensor muscle is being used to oppose gravity in a postural state, or perhaps even during flight, when the hind legs are held in a fixed extended position close to the body (Kutsch, 1971). The actions of proctolin on the myogenic bundle would serve to speed the flow of haemolymph along the long narrow hindleg after bouts of intense activity when there would be a need to replenish energy stores in the leg muscles. Many of the actions of the AKH peptides on the extensor muscle are similar to those of proctolin, in that they can speed up the myogenic contractions and also induce slow, maintained contractions (O'Shea, 1985). These peptides are known to be released into the locust haemolymph after the initiation of flight (Mayer & Candy, 1969) and so would be ideally suited to help maintain a prolonged contraction of the extensor-tibiae muscle to keep the leg extended in its characteristic 'flight posture' (Kutsch, 1971). The physiological roles of the locust FMRFamide-like and SCP_B-like peptides are at present difficult to forecast, since nothing is known of the conditions under which they are released or of the variability of their levels in the haemolymph. However, one could envisage that there might be times when it would be of use to speed up the myogenic activity of the extensor muscle whilst not affecting its other contractile properties, a situation for which the SCP_B-like peptide would be ideally suited. The FMRFamide-like peptides appear to have some properties, such as potentiation of twitch tension and increase in twitch tension relaxation rate which are very like those of octopamine but brought about by a different mode of action. In addition, these peptides combine stimulation frequency-dependent effects on basal-maintained tension that resemble the effects of proctolin. At present the behaviour pattern that would require such a combination of responses is unclear.

It is probable that each of the modulatory amines and peptides discussed above will not be released singly, as a response to a particular behavioural stimulus, but rather that the important physiological consequence to the locust will be changes in the relative amounts of each modulator released at any one time. Thus by altering the hormonal environment of its muscles the locust is able exquisitely to tune its contractile responses to changes in the world around it.

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