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POTENTIATION OF NEUROMUSCULAR TRANSMISSION BY AN OCTOPAMINERGIC NEURONE IN THE LOCUST

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SUMMARY

1. Spikes in the octopaminergic dorsal unpaired median (DUM) neurone which innervates the extensor tibiae muscle of the locust metathoracic leg (DUMETi) produce direct and indirect effects on muscle tension.

2. Direct effects include a slowing of an intrinsic rhythm of contraction and relaxation, a relaxation of muscle tone and a small hyperpolarization of the muscle membrane potential. The latter two effects are weak and variable. All three effects are mimicked by superfusion of octopamine and are mediated by octopamine receptors on the muscle fibres.

3. Indirect effects are found when the DUMETi neurone is stimulated at the same time as the motoneurones innervating the extensor muscle. They include (*a*) potentiation of tension generated in the extensor muscle by spikes in the slow excitatory motoneurone (SETi), (*b*) reduction in duration of each twitch contraction generated by SETi due to an increase in the rate at which the muscle relaxes, (*c*) increase in the amplitude of the synaptic potential generated by SETi. These various effects have a time course of several minutes and far outlast the duration of DUMETi stimulation. They can be mimicked by superfusion of octopamine.

4. The effect of DUMETi on neuromuscular transmission is mediated by receptors with a high affinity for octopamine located both on the muscle and on the terminals of the slow motoneurone. The presence of the presynaptic receptors is revealed by the increase in the frequency of spontaneous miniature end plate potentials recorded in the muscle in the presence of octopamine.

5. DUMETi is a member of a group of similar aminergic neurones and it is suggested that they may share a role in modulating transmission at peripheral neuromuscular synapses, and possibly central synapses.

INTRODUCTION

Biogenic amines are capable of modulating transmission at vertebrate neuromuscular junctions (Orbeli, 1923; Bowman & Zamis, 1958; Kuba, 1970). However, the natural release sites of the modulating amines and the physiological significance

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of the experimental effects have yet to be established (Bowman & Nott, 1969). In contrast, in invertebrates skeletal muscle has recently been shown to receive a direct aminergic input in addition to its motor innervation, for instance in the marine mollusc, *Aplysia* (Weiss, Cohen & Kupfermann, 1975, 1978) and in locusts (Evans & O'Shea, 1977, 1978; O'Shea & Evans, 1977).

In the locust the aminergic input to the extensor muscle of the tibia of the hindleg is provided by one of the neurones of the dorsal unpaired median (DUM) group.* The particular neurone that innervates this muscle is unpaired in the metathoracic ganglion and has been called DUMETi (Dorsal Unpaired Median cell to Extensor Tibiae muscle) (Hoyle *et al.* 1974). Both left and right extensor tibiae muscles receive an axon from the single unpaired DUMETi neurone. Each extensor muscle is also innervated by three paired motoneurones (Pearson & Bergman, 1969; Cochrane, Elder & Usherwood, 1972; Hoyle & Burrows, 1973), whose cell bodies lie in the metathoracic ganglion.

In previous publications (Evans & O'Shea, 1977, 1978) we have shown that DUMETi is an octopaminergic neurone. One physiological effect of stimulating DUMETi is to lower the frequency of a myogenic rhythm of contraction found in some of the fibres of the extensor muscle. This effect can be mimicked by superfusing the muscle with low concentrations of octopamine (Evans & O'Shea, 1978). This slowing is mediated by receptors on the myogenic muscle fibres with a high affinity for octopamine. These fibres comprise only a very small fraction of the total, and the above finding is not therefore evidence for the widespread presence of octopamine receptors on the extensor muscle.

The aim of the present study is to determine the action of the octopamine released from the terminals of DUMETi in the non-myogenic parts of the extensor muscle. Here we show the absence of a dramatic direct effect of DUMETi stimulation on the muscle. This finding led us to suspect that the function of DUMETi might only be revealed by its interactions with the effects produced by the motoneurones. Here we show that DUMETi is able to potentiate the strength of contractions produced by the slow excitatory motoneurone (SETi) and that this effect can be mimicked by the application of low concentrations of octopamine. The presynaptic terminals of the SETi motoneurone possess octopamine receptors capable of mediating at least a part of the observed potentiation of neuromuscular transmission. Other octopamine receptors, present on the muscle fibres themselves, increase the rate at which the muscle relaxes after being excited by either the slow or the fast excitatory motoneurone. Octopamine can also reduce the effectiveness of the common inhibitory motoneurone to the extensor muscle (Evans & O'Shea, 1977). Here we show that this effect is not responsible for the potentiating effect of DUMETi and octopamine on SETi neuromuscular transmission.

* Not all of the neurones with spiking cell bodies in the dorsal median group conform to the same pattern as DUMETi (unpublished observations and C. S. Goodman, personal communication). Some are confined to the ganglion and do not have peripheral axons, while others are bilaterally asymmetrical. There is a question, therefore, as to whether the DUM neurones are all really 'unpaired' in the adult. The term unpaired is, however, appropriate if one considers the unique origin of the neurones from the single, unpaired median neuroblast (C. S. Goodman, personal communication). At this time, therefore, a change of generic name is not necessary but may become necessary later as more is discovered about individuals of this group of neurones.

The results suggest that the DUMETi neurone functions as a modulator of the effectiveness of a synaptic junction, between a slow motoneurone and a muscle. This conclusion, and the implications of the involvement of a biogenic amine in neural modulation are discussed. Brief accounts of some of this work have already been published (Evans & O'Shea, 1977; O'Shea & Evans, 1977).

MATERIALS AND METHODS

Experiments were performed at room temperature (21 °C) on adult *Schistocerca americana gregaria* (Dirsch, 1974) (formerly *S. gregaria*) of either sex. The animals were obtained from our own crowded laboratory cultures fed on wheat seedlings.

Dorsal dissections to expose the ventral nerve cord were performed on animals which were restrained on a dish. Full details of the physiological techniques used are given in Evans & O'Shea (1978). Intracellular recordings were made from the somata of DUM neurones in the metathoracic ganglion using glass microelectrodes filled with 2 M-potassium acetate and having resistances of 20–50 MΩ. DUMETi was identified by showing that its soma spikes always corresponded with its axon spikes recorded extracellularly in both nerves innervating the left and right metathoracic extensor tibiae muscles (nerve $5b_1$; nomenclature of Pringle, 1939). Spikes in DUMETi were elicited either by passing depolarizing current into the soma through the recording microelectrode via a bridge circuit, or by antidromic stimulation from one pair of silver hook electrodes placed on the extensor tibiae nerve ($5b_1$) of one side. The latter technique allowed us to elicit a higher frequency of spikes in the DUMETi axons.

Motoneurones of the extensor tibiae muscle were excited by stimulating the peripheral nerve roots which contain their axons. The motor axon of SETi was stimulated by a pair of silver hook electrodes placed around nerve 3b. This nerve also contains a branch of the CI motoneurone but this was not excited at threshold for extracellular stimulation of SETi. This was confirmed by intracellular recording from muscle fibres innervated by both motoneurones. The axon of the fast extensor motoneurone of the tibiae (FETi) was stimulated by paired hook electrodes placed around nerve 5. This nerve contains the axons of many neurones, including that of DUMETi, but that of FETi is the largest in diameter and has the lowest threshold to extracellular stimulation.

The effect of stimulating DUMETi on the muscle tension developed by the motoneurones was investigated. Known concentrations of drugs were superfused directly on to the extensor tibiae muscle. The muscle was exposed by opening the metathoracic femur along the dorsal ridge. Solutions were superfused at a flow rate of 1 ml/min using a peristaltic pump. Tension was measured almost isometrically with a force transducer attached to the distal tendon of the muscle. Intracellular recordings were made from the muscle fibres innervated by the SETi motoneurone. All results were recorded on magnetic tape and filmed after the experiments.

The amines and blocking agents were dissolved in physiological isotonic saline (pH 6·8) containing 140 mM-NaCl, 10 mM-KCl, 4 mM-$CaCl_2$, 4 mM-$NaHCO_3$, 6 mM-NaH_2PO_4 (Usherwood & Grundfest, 1965) plus 90 mM-sucrose.

We would like to acknowledge the gift of N,N,dimethyl-octopamine (Sterling Winthrop Res. Inst.). Other drugs were obtained from the Sigma Chemical Co. except for phentolamine mesylate (Ciba) and phenylethanolamine HCl (Regis Chemical Co.).

RESULTS

(1) *Direct effects of stimulating DUMETi and of octopamine application on membrane potential and resting tension in the extensor muscle*

Stimulation of DUMETi produces a small hyperpolarization (~ 3–5 mV) in the resting membrane potential of certain muscle fibres of the extensor tibiae muscle. This effect is quite variable and is often not present. While monitoring the direct effects of DUMETi stimulation (and also of direct octopamine application) on the muscle, we have often attempted to measure associated changes in conductance of intracellularly recorded muscle fibres. We have consistently been unable to measure such changes and assume therefore that the muscle's receptors for octopamine do not mediate large conductance changes.

DUMETi stimulation produces a reduction in the overall muscle tonus. This relaxation can be marked although it is again highly variable. It can be mimicked by superfusion of octopamine on to the muscle. Low concentrations of DL-octopamine (10^{-8} M) produce small and variable effects whereas high concentrations (10^{-5} M) produce consistently large effects. These actions of octopamine on the extensor muscle persist in the presence of picrotoxin at a concentration (5×10^{-4} M) which blocks the effect of the inhibitory motoneurone on the muscle (Usherwood & Grundfest, 1965). The direct superfusion of GABA (5×10^{-5} M) on to the extensor muscle produces no reduction in basal tonus. The above findings suggest that the relaxation in basal tonus is not caused by a release of GABA from the terminals of the common inhibitory (CI) motoneurone. The relaxing effect on muscle tonus is therefore a direct post-synaptic effect of octopamine on the extensor muscle.

(2) *Effects of combined DUMETi and excitatory motoneurone stimulation*

When the axon of SETi is stimulated at 6 Hz, each spike evokes a small twitch in the muscle fibres it innervates. At 6 Hz these twitches are discrete, constant in amplitude and do not summate. Stimulating DUMETi at 12 Hz while stimulating SETi at 6 Hz produces a fall in basal tension and potentiates the amount of tension produced by the slow motoneurone. In Fig. 1 A the basal tension of the muscle is reduced during the 12 s burst of DUMETi spikes and returns to its original value after about 30 s. SETi induced twitches are increased in amplitude by about 30% at maximum and potentiation is detectable for at least 2 min. This suggests that the spikes in DUMETi induced a long-term change somewhere in the process from the mobilization of the motoneurone transmitter substance to the activation of the contractile mechanism.

The excitatory junctional potentials (EJPs) elicited in the muscle fibres by the SETi motoneurone are also potentiated by the stimulation of the DUMETi neurone at 20 Hz (Fig. 1 B). The increase outlasts the stimulation of DUMETi by about 2 min and has a time-course similar to the potentiating effect on the amplitude of the tension. The effect on EJP amplitude is generally small and variable, whereas the potentiating effect on tension is larger and highly reproducible.

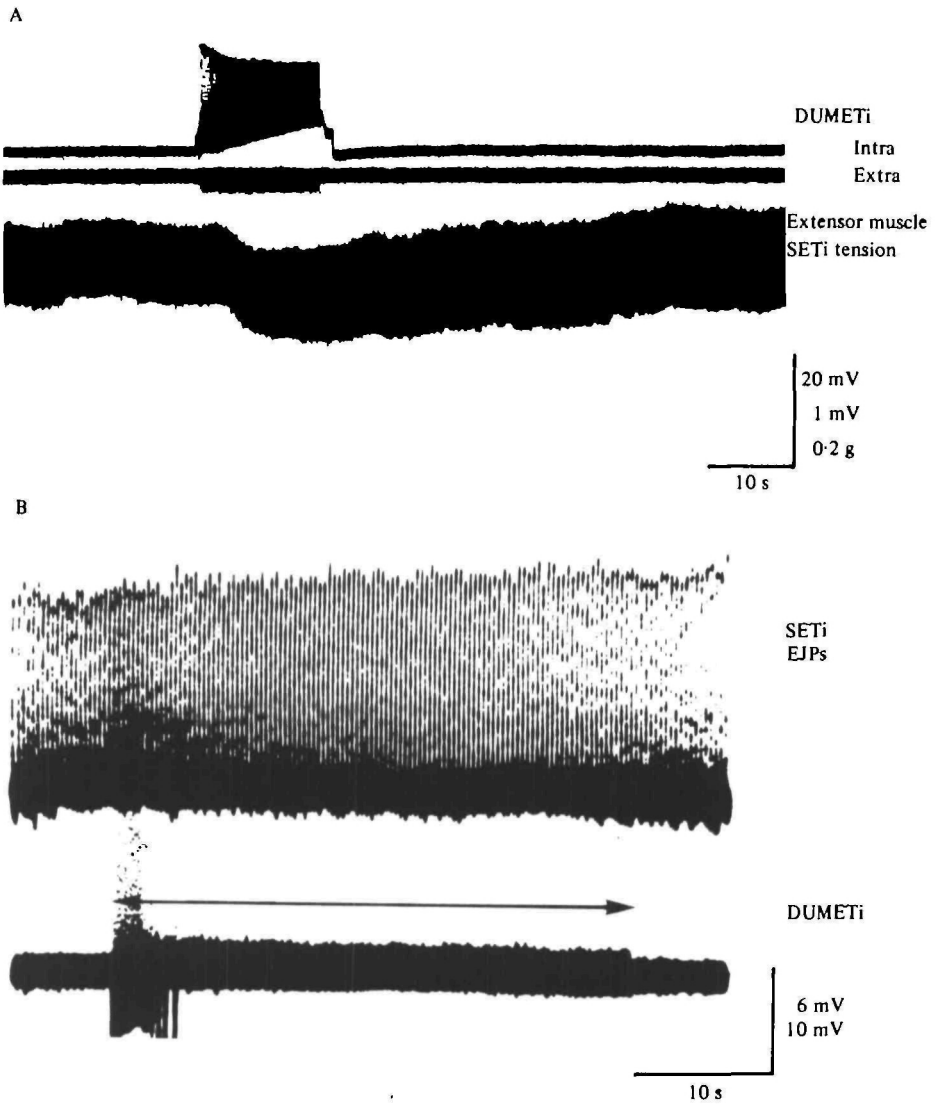

Fig. 1. (A) The effect of spikes in DUMETi on the basal tonus of the extensor tibiae muscle and on the amplitude of twitch tension generated by stimulating the slow extensor tibiae motoneurone (SETi). The upper trace is a recording intracellularly from the soma of DUMETi and the middle trace is an extracellular recording from the animal's right extensor tibiae nerve. The lower trace records tension in the animal's left extensor tibiae muscle while the left SETi motoneurone is stimulated at 6 Hz.

A depolarizing pulse of current, about 15 seconds in duration, is passed into the soma of DUMETi and causes the neurone to fire at about 12 Hz. Axon spikes of DUMETi project peripherally as confirmed by the middle trace. Note the marked fall in basal tonus and the slow and long-lasting potentiation in the amplitude of twitches generated by SETi.

(B) Effect of spikes in DUMETi (lower trace) on the amplitude of synaptic potentials (EJPs) generated by the SETi motoneurone (stimulated at 3 Hz) and recorded intracellularly in a muscle fibre of the left extensor tibiae muscle.

The arrowed line marks the duration of the spike train in DUMETi. Spikes in DUMETi were initiated by antidromic stimulation at 20 Hz of the animal's right extensor tibiae nerve. These spikes invade the soma of DUMETi, but after about five seconds of stimulation fail to initiate soma spikes and thereafter only the smaller axon spike is recorded in the soma. Spikes in SETi were initiated by stimulating the animal's left nerve 3b. Note the slow potentiation of EJPs.

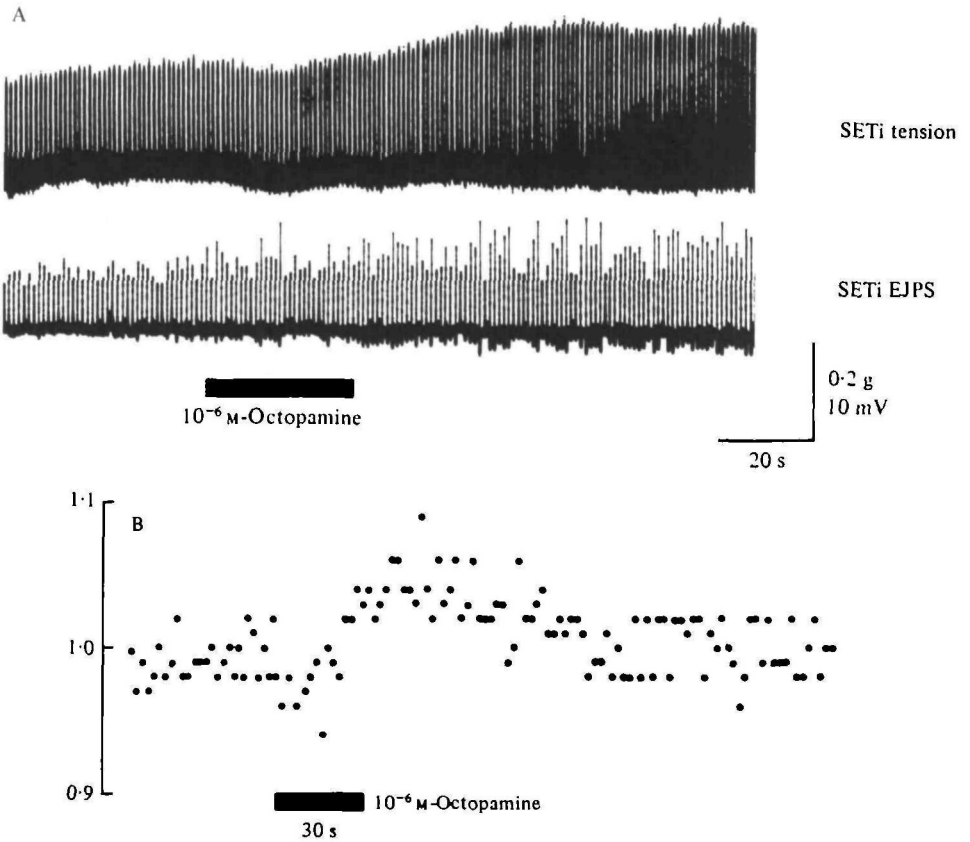

Fig. 2. (A) The potentiation by a pulse of 10^{-6} M-DL-octopamine of the amplitude of tension and EJPs induced by the SETi motoneurone. The SETi motoneurone is stimulated at 1 Hz by extracellular stimulation of nerve 3b. Note that the slow rise in SETi-induced tension (upper trace) is accompanied by a smaller and more variable rise in the amplitude of SETi-induced EJPs (lower trace). (B) Effect of a 30-second pulse of 10^{-6} M-DL-octopamine on the amplitude of EJPs induced by stimulating the SETi motoneurone at 1 Hz. The EJP amplitude is potentiated and rises to a maximum of about 1·1 of the initial value about one minute following application of octopamine. Note that the effect outlasts the presence of octopamine as does the effect of octopamine on SETi-induced tension but that the magnitude is smaller.

(3) *Effects of octopamine*

The effects of stimulating DUMETi can be mimicked by the application of low concentrations of octopamine to the extensor muscle (Fig. 2). A 30 s pulse of 10^{-6} M-DL-octopamine potentiates the amplitude of SETi-induced EJPs, increases the amplitude of the individual twitches by approximately 30% and causes a small reduction in basal tonus (Fig. 2A). The potentiating effects on contractions and EJPs are prolonged, and outlast the octopamine pulse by several minutes. The effect on EJP size of any muscle fibre was much more variable and generally smaller than the effect of octopamine on SETi-induced tension recorded from the whole muscle. Fig. 2B shows another example of the potentiating effect of octopamine on EJP size, in which a 30 s pulse of DL-octopamine (10^{-6} M) produces about a 10% potentiation. This concentration of octopamine typically produces a 30% increase in the amplitude of the contraction induced by SETi.

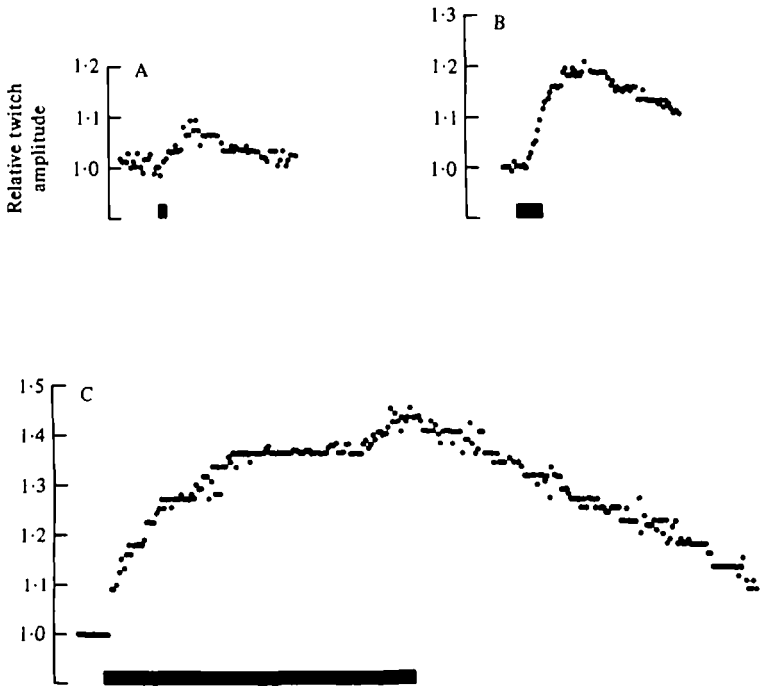

Fig. 3. The effect of three different durations of 10^{-6} M-DL-octopamine pulses on the amplitude of SETi-induced tension responses of the extensor tibiae muscle. In each case (A, B, C) the SETi motoneurone is stimulated at 1 Hz and the effects on the tension response of the muscle are normalized to the initial twitch amplitude (= 1·0) for comparison. The solid bars represent the octopamine pulse durations which are 10 s, 30 s and 6 min 15 s for A, B and C, respectively.

One possible explanation for the potentiating effect of octopamine is that the effect is secondary to a suppression of spontaneous release of transmitter (GABA) from the inhibitory motoneurone. This explanation seemed plausible since 10^{-6} M-DL-octopamine can reduce the amplitude of IJPs induced by the common inhibitory motoneurone (Evans & O'Shea, 1977). The potentiating effect of 10^{-6} M-DL-octopamine, however, is not blocked in the presence of the GABA blocking agent, picrotoxin, at a concentration (5×10^{-4} M) which is known to block the effect of exogenously applied GABA (10^{-6} M). The potentiating effect of octopamine on SETi-induced contractions appears, therefore, to be a direct effect on SETi neuromuscular transmission and not a secondary consequence of reducing tonic, spontaneous release of GABA.

To examine the characteristics of the potentiating effect of octopamine on the tension produced by the extensor muscle we varied both the length of the octopamine pulse and its concentration. A 30 s pulse induces a larger potentiation than a 10 s pulse (cf. Fig. 3 A, B). The half-time of the maximal response to continuous application of octopamine was 1·2 min (Fig. 3 C) where a 6·25 min pulse of 10^{-6} M-DL-octopamine was applied to the muscle. The potentiating effect of superfused octopamine on the extensor muscle is thus proportional to the length of the octopamine pulse. Increasing the concentration of the octopamine in the superfusate from 10^{-6} M to 10^{-5} M increased the magnitude of the potentiating response when the length of the pulse is kept constant at 30 s (cf. Fig. 4A with Fig. 3B). At 10^{-5} M-octopamine, prolonging the exposure

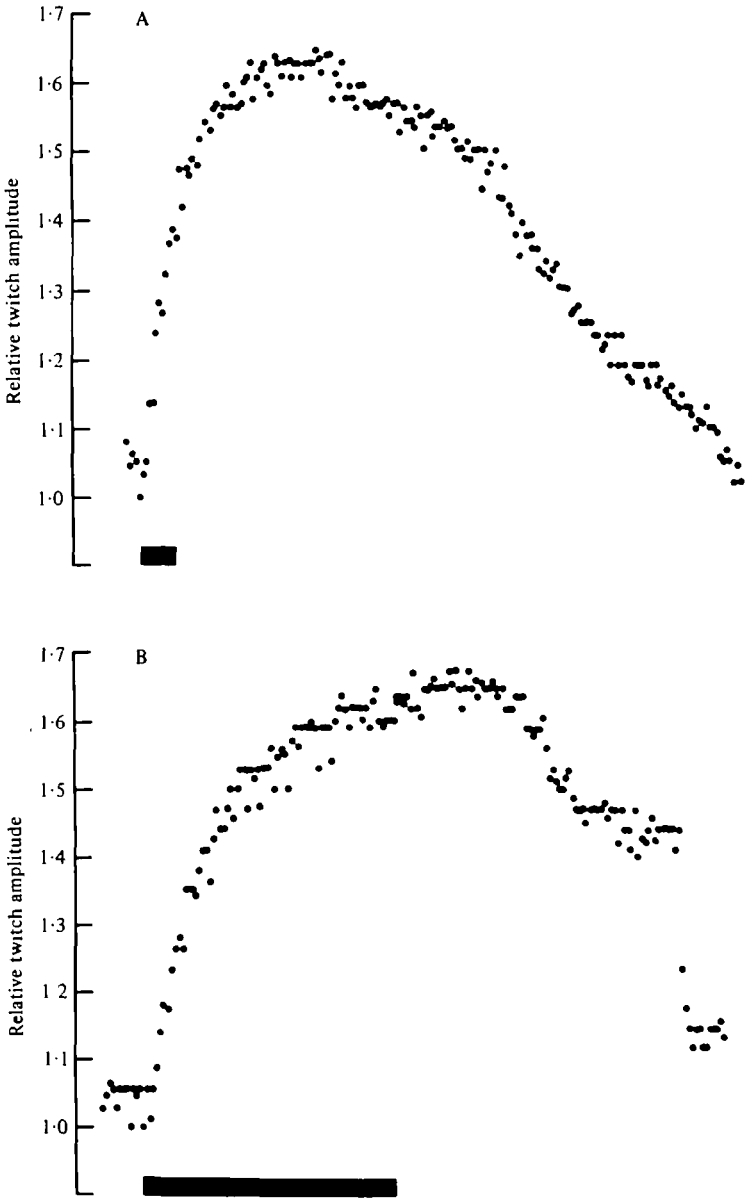

Fig. 4. The effect of two different durations (30 s in A and 220 s in B) of 10^{-4} M-DL-octopamine pulses on the amplitude of SETi-induced tension responses of the extensor tibiae muscle. The tension response of the muscle is normalized to the initial twitch amplitude (= 1·0) for comparison.

time to 220 s produced only a slight increase in the extent of the potentiation (Fig. 4B). A comparison of the effect of short pulses of octopamine (Figs. 3A, 3B, 4A) with those of longer pulses (Figs. 3C, 4B) indicates that the maximal tension response takes longer to develop in the extended exposures suggesting that under these conditions a desensitization of the octopamine receptors may be occurring.

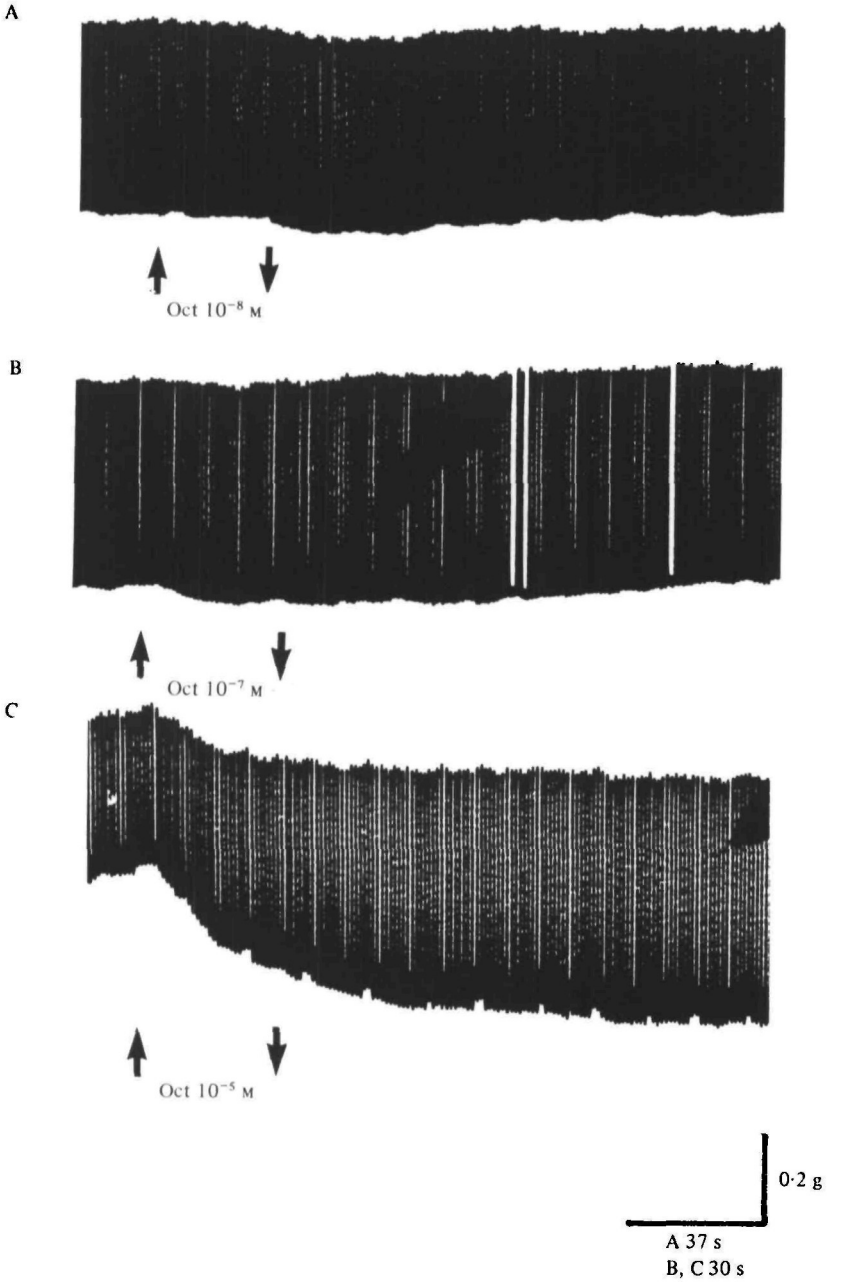

Fig. 5. Effect of DL-octopamine at three different concentrations (10^{-8} M, 10^{-7} M and 10^{-5} M for A, B and C respectively) on the amplitude of twitches generated by the SETi motoneurone. In each case the upwardly directed arrow (↑) indicates the introduction of the octopamine into the saline superfusate and the down arrow (↓) indicates the end of the octopamine pulse. Note slight differences in the pulse durations. In A there is a small fall in basal tonus but no potentiation of twitch tension. In B at 10^{-7} M there is a significant potentiation and in C at 10^{-5} M there is a marked effect on basal tonus and amplitude of twitch tension.

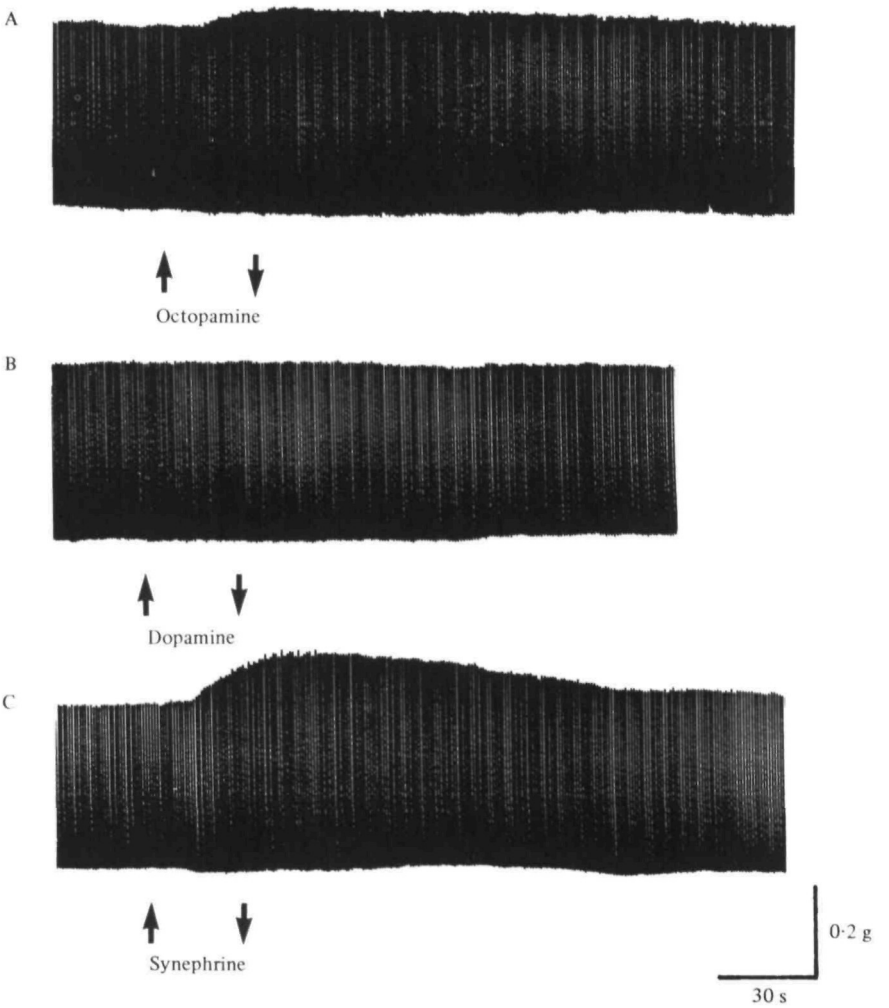

Fig. 6. Effect of 10^{-6} M-DL-octopamine (A), dopamine (B) and DL-synephrine (C) on the amplitude of SETi-induced twitches in the extensor tibiae muscle. Arrows indicate the introduction (↑) and removal (↓) of the amines from the saline superfusate. All records were obtained from the same preparation.

The threshold for the potentiating effect of octopamine on SETi-induced twitch tension occurs between 10^{-7} and 10^{-8} M-DL-octopamine (Fig. 5A–C). The exact threshold concentration varies with the individual preparation. The effect of octopamine on basal tension is also concentration dependent. It is particularly marked at 10^{-5} M-DL-octopamine (Fig. 5C) but can still be seen in some preparations at concentrations down to 10^{-8} M (Fig. 5A).

(4) *Structural specificity of tension potentiating response to biogenic amines*

In an attempt to determine the structural specificity of the potentiation of SETi-induced tension, amines related to octopamine were superfused over the extensor muscle at a concentration of 1 μM. Fig. 6 shows the results of an experiment in

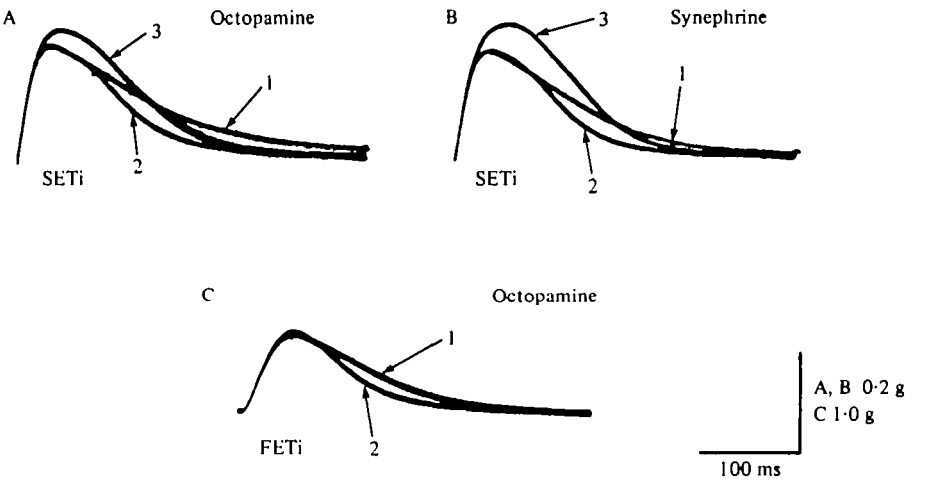

Fig. 7. Effect of 10^{-6} M-DL-octopamine and DL-synephrine on the time course and amplitude of twitches generated by stimulating SETi (A and B) and the effect of 10^{-6} M-DL-octopamine on FETi-induced tension (C). The oscilloscope time base is triggered by the rise in tension caused by stimulating either the SETi or FETi motoneurone. In A and B three sweeps are superimposed which represent twitches initiated prior to octopamine or synephrine application (1), about 20 s after application (2) and about 60 s after application (3). In C two twitches are shown, the first (1) initiated prior to octopamine application and the second (2) about 30 s after application. Note that in A and B the effect of octopamine and synephrine on the rate of relaxation occurs prior to the potentiating effect on amplitude or peak tension. Twitches induced by FETi are not increased in amplitude by octopamine but there is a marked effect on the rate of relaxation (C).

which the effects of DL-octopamine, dopamine and DL-synephrine were compared. In the same preparation 1 μM DL-synephrine (Fig. 6C) produced a more marked potentiation of SETi-induced tension than that induced by an equivalent 30 s pulse of DL-octopamine (Fig. 6A). Dopamine at this concentration had no effect (Fig. 6B). In a similar series of experiments the following amines were also shown to be without effects on SETi-induced twitch tension when applied for 30 s at 10^{-6} M; tyramine, N,N,dimethyloctopamine, L-noradrenaline, L-adrenaline, phenylethanolamine, and 5-hydroxytryptamine (5-HT). Thus the receptor mediating the potentiating effect on SETi-induced twitch tension is very specific for the β-hydroxylated monophenolic amines at concentrations below 10^{-6} M.

(5) *Effect of octopamine on rate of relaxation of tension*

Octopamine increases the rate at which the muscle relaxes following a twitch contraction induced by both SETi and FETi thereby decreasing the duration of individual contractions (Fig. 7A, C). The structural specificity of the relaxation response appears to be similar to that of the potentiating response on SETi-induced contractions. DL-Synephrine at 10^{-6} M (Fig. 7B) was again a potent agonist whilst the following amines were ineffective at this concentration: dopamine, L-noradrenaline, L-adrenaline, phenylethanolamine, N,N,dimethyloctopamine, tyramine and 5-HT.

The effect on the rate of relaxation differs in three respects from the potentiating effect of octopamine on the amplitude of the twitch. First, it has a much lower threshold being around 10^{-8} M for DL-octopamine. The same is true for DL-synephrine, the

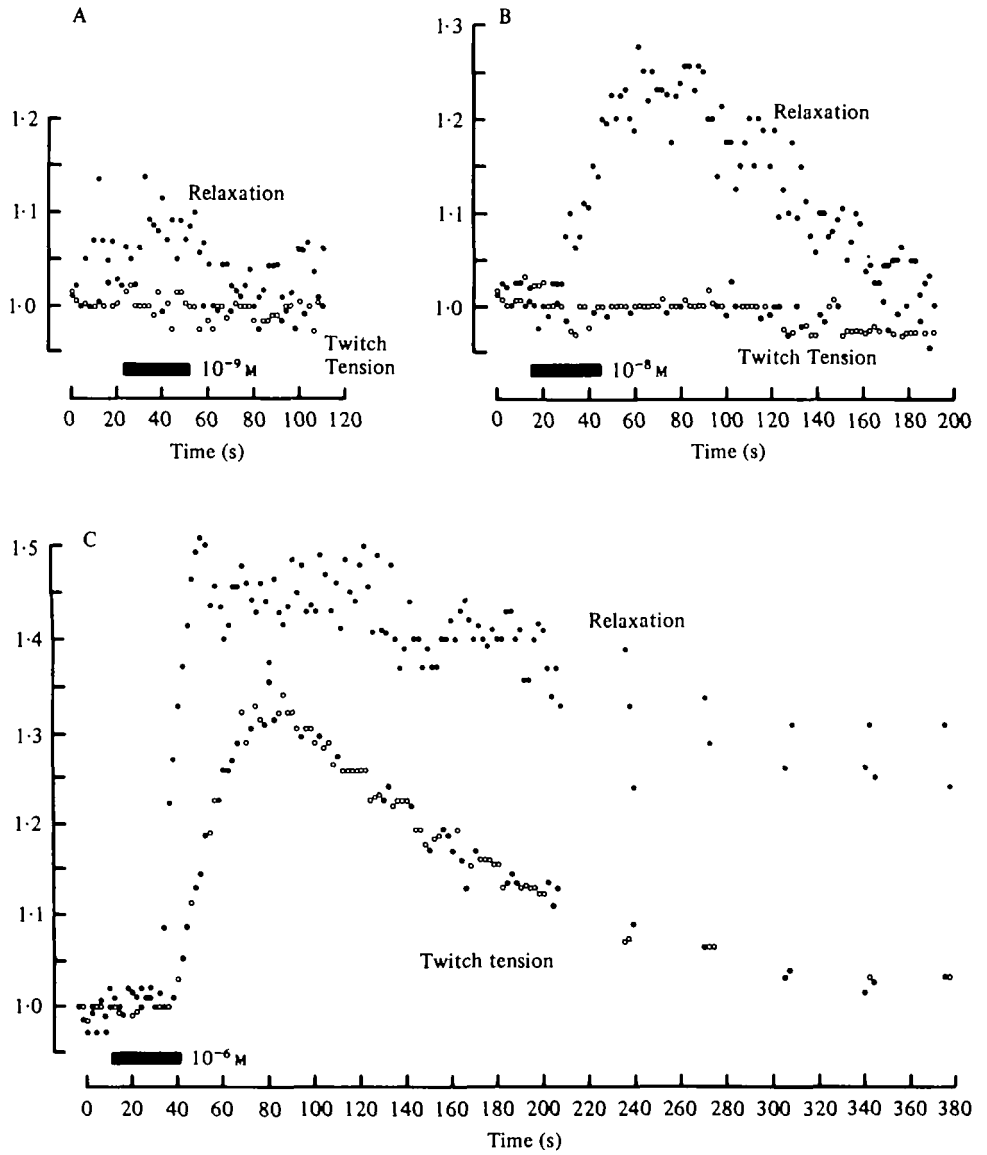

Fig. 8. Effect of three concentrations (10^{-9} M, 10^{-8} M and 10^{-6} M in A, B and C, respectively) of DL-synephrine on the amplitude of 'twitch tension' and rate of relaxation of twitches generated by the SETi motoneurone stimulated at 1 Hz. A pulse (30 s) of 10^{-9} M synephrine (black bar) produces a small but significant increase in the rate of relaxation and no effect on the twitch tension (A). At 10^{-8} M, the rate of relaxation is increased by 1·3 of the initial rate without potentiating the amplitude of twitches (B). At 10^{-6} M, both the rate of relaxation and the maximum amplitude of twitch tension attained by SETi-induced twitches are potentiated. All data points are normalized to the initial value prior to the pulse of synephrine for comparison.

most potent agonist for this response (Fig. 8A–C). The threshold for the potentiating effect of DL-synephrine on twitch amplitude is between 10^{-8} M and 10^{-7} M (Fig. 8B) whereas the threshold for the effect on relaxation is between 10^{-10} M and 10^{-9} M (Fig. 8A). Second, the effects differ in relative potency. In the normalized data shown in Fig. 9A, B, 10^{-6} M-octopamine increases the rate of relaxation by 1·5× and the

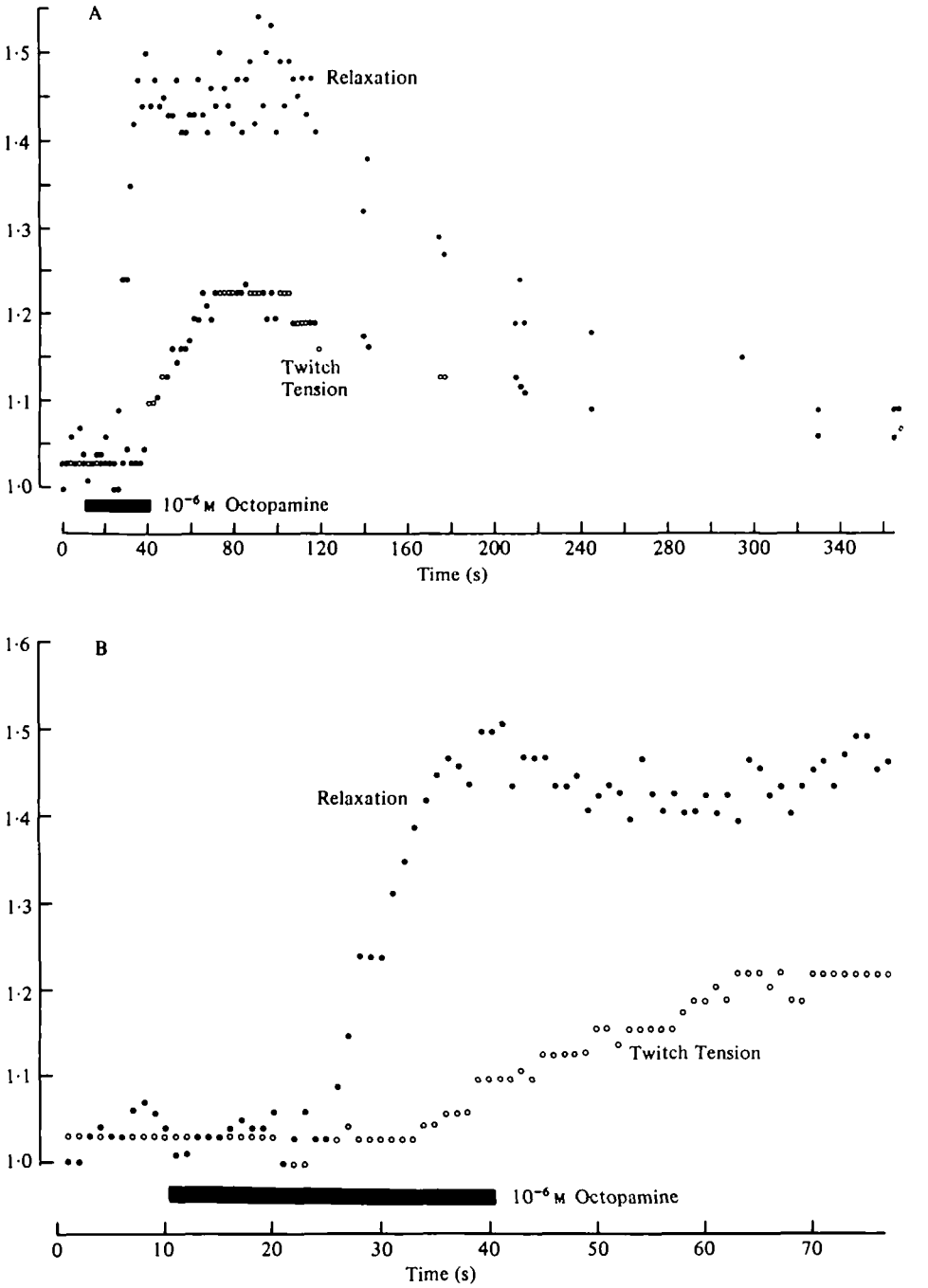

Fig. 9. Comparison of the effects of a 30 s pulse of 10^{-6} M-DL-octopamine (black bar) on the rate of relaxation and on the twitch tension induced by stimulating the SETi motoneurone at 1 Hz. The data plotted in A and B are the same, in B the time base is expanded to allow comparison of the initial effects of octopamine.

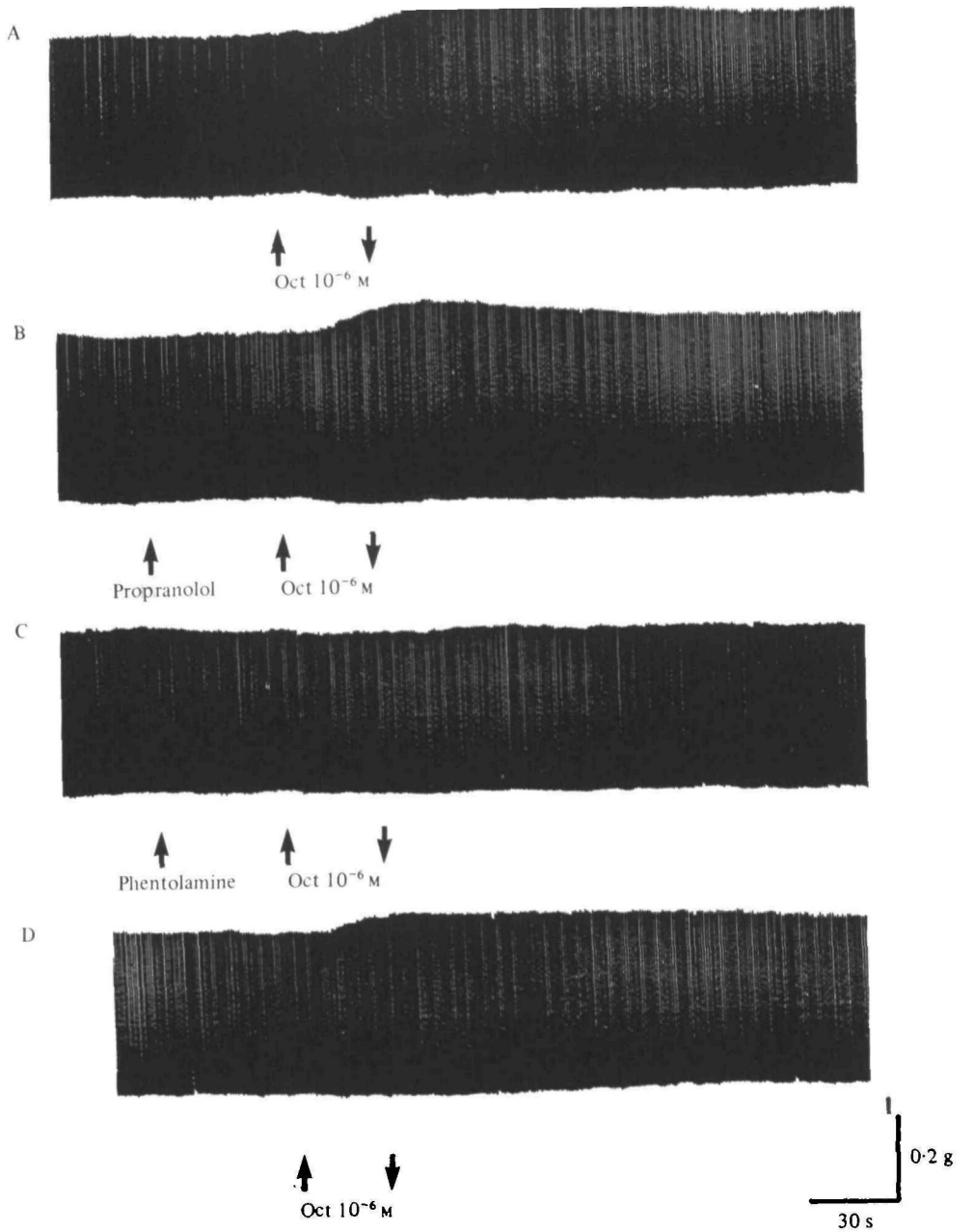

Fig. 10. Effect of 30 s pulses of 10^{-6} M-DL-octopamine on the amplitude of SETi-induced (1 Hz) twitches of the extensor tibiae muscle in the presence of DL-propranolol (β-adrenregic blocking agent) and phentolamine (α-adrenergic blocking agent). Recordings of muscle tension (A–D) are taken from a single preparation and are presented here in order. They are not, however, continuous records; after each application of octopamine the preparation was allowed to recover for a few minutes so that twitch amplitudes returned to initial levels.

In A octopamine is added to the saline superfusate between the arrows (↑ ↓). In B the muscle is pre-treated with 10^{-5} M-DL-propranolol for 1 min (first arrow ↑) and octopamine is applied for 30 s in the presence of DL-propranolol (↑ ↓). In C phentolamine (10^{-5} M) is applied for 1 min prior to application of a mixture of phentolamine and octopamine. Finally, in D 10^{-6} M-octopamine is applied alone in the saline superfusate (↑↓). Note that in the presence of phentolamine, the potentiating effect of octopamine is blocked.

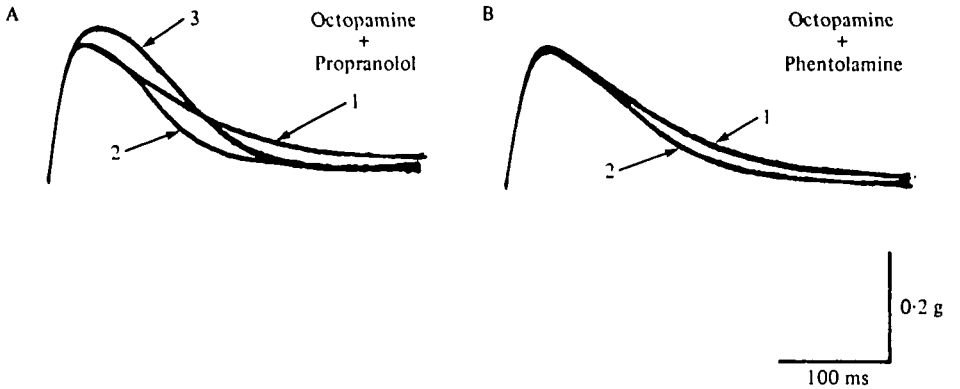

Fig. 11. Effect of DL-propranolol and phentolamine on the response of SETi-induced twitches to DL-octopamine. In A, 10^{-6} M-DL-octopamine is applied in the presence of 10^{-5} M-DL-propranolol and both the rate of relaxation and the amplitude of twitches are increased. In B, in the presence of 10^{-5} M-phentolamine, octopamine (10^{-6} M) produces a small increase in the rate of relaxation and no increase in amplitude. In A, 1 is a twitch prior to octopamine application, 2 is about 20 s and 3 about 40 s after the application of octopamine. In B, 1 is prior to octopamine and 2 is about 40 s after octopamine application.

twitch tension by 1·25×. Third, the time-course for the development of the two responses differs. The relaxation effect is initiated more rapidly (Fig. 9B), being detectable about 15 s after the application of 10^{-6} M-DL-octopamine. The effect on twitch tension is detected later, typically after about 25 s. The effect on relaxation develops more rapidly and reaches its peak sooner than the effect on twitch tension (Fig. 9B).

These differences between the threshold, potency and time course of the effect of octopamine on rate of relaxation and amplitude of twitch suggest different underlying mechanisms or different sites of action of octopamine.

(6) *Effects of adrenergic blocking agents*

We investigated the characteristics of the receptors mediating the effects of octopamine on tension induced by stimulating SETi. Pulses of octopamine were superfused over the muscle in the presence of α- and β-adrenergic blocking agents (Fig. 10). The potentiating effect of 10^{-6} M-DL-octopamine (Fig. 10A, D) was completely blocked in the presence of 10^{-5} M phentolamine (α-adrenergic blocking agent) (Fig. 10C) but was not affected in the presence of DL-propranolol (β-adrenergic blocking agent) (Fig. 10B). Similarly the effect of octopamine and synephrine on the rate of relaxation was blocked by phentolamine, but was unaffected by propranolol. In this case, however, 10^{-5} M-phentolamine produced an incomplete block (Fig. 11). The inability of 10^{-5} M-phentolamine completely to block the effect on relaxation, and its ability to eliminate the effect on twitch tension is probably related to the greatly differing thresholds for the two effects; the rate of relaxation is an order of magnitude more sensitive to octopamine than is the amplitude of the SETi-induced twitches.

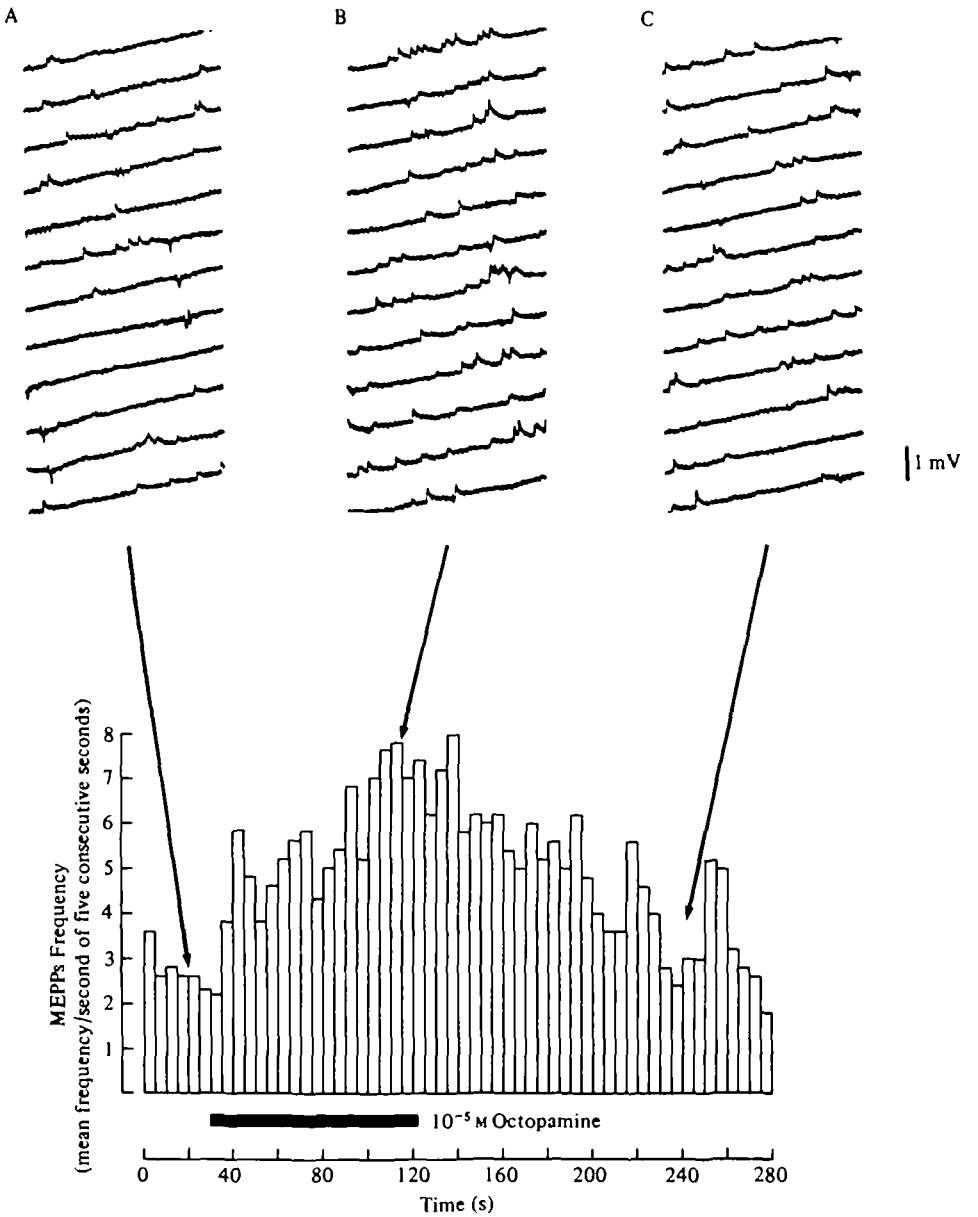

Fig. 12. Effect of 10^{-5} M-DL-octopamine (black bars) on the spontaneous release of neurotransmitter from the terminals of the SETi motoneurone on the extensor tibiae muscle. Sample intracellular records from a muscle fibre which receives SETi input are shown in the upper part of the figure (A, B, C). These recordings are representative of the condition before (A) during (B) and after (C) application of octopamine. A plot of the frequency of mepps (mean frequency per second of 5 consecutive seconds) against time is shown in the lower part of the figure and arrows indicate from where the sample recordings were taken. Note the slow rise in mepp frequency and the long-lasting effect of octopamine.

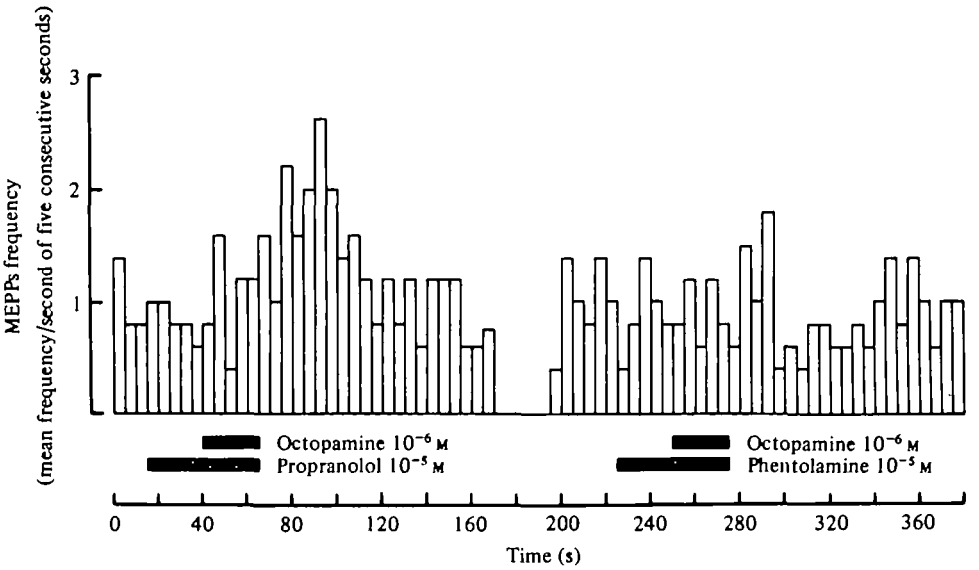

Fig. 13. The effect of octopamine on the frequency of spontaneous release of transmitter from SETi (see Fig. 15, lower) is blocked by phentolamine but not by DL-propranolol. In this experiment DL-octopamine (10^{-6} M) (black bar) is applied to the muscle with either 10^{-5} M-propranolol (left hatched bar) or 10^{-5} M-phentolamine (right hatched bar) and the muscle is pretreated with these agents alone.

(7) *Effect of octopamine on the frequency of spontaneous miniature end-plate potentials (mepps)*

Recordings from resting, unstimulated muscle fibres which receive innervation from the slow extensor and CI motoneurones revealed a low frequency of small, transient, depolarizing potentials (Fig. 12, upper). We assume these to be quantal events representing spontaneous release of transmitter from the terminals of either the SETi or CI motoneurone. Their frequency is not affected by application of picrotoxin (5×10^{-4} M) and they therefore represent mepps released from SETi. We found no spontaneous hyperpolarizing, potentials in muscle fibres known to be innervated by the CI motoneurone.

In the presence of 10^{-5} M-DL-octopamine, the frequency of mepps increases (Fig. 12 lower), but the amplitude distribution and duration of the individual events is unchanged. This effect of octopamine is blocked by the α-adrenergic blocking agent phentolamine (10^{-5} M), but not by the β-adrenergic blocking agent DL-propranolol (10^{-5} M) (Fig. 13). The effect on frequency of 10^{-5} M-DL-octopamine is also unaffected by the presence of picrotoxin at a concentration of 5×10^{-4} M. Thus octopamine appears to be acting to facilitate the amount of transmitter released from the presynaptic terminals of the SETi motoneurone by an interaction with a presynaptic receptor which has some of the characteristics of the vertebrate α-adrenergic receptor.

DISCUSSION

We have shown that an octopaminergic neurone which innervates a locust skeletal muscle modulates the force of contraction generated by an excitatory motoneurone innervating the same muscle. The finding of a direct aminergic modulatory input to a muscle has a precedent in the marine gastropod *Aplysia* in which the serotonergic metacerebral cells potentiate the force of contraction caused by motoneurones which innervate the muscles of the buccal mass (Weiss, *et al.* 1975, 1978).

The pharmacological properties, of octopamine receptors on myogenic fibres of the extensor tibiae muscle of the locust hindleg have been described (Evans & O'Shea, 1978). The myogenic fibres, however, are few in number and confined to a discrete bundle at the proximal end of the muscle. Here we have shown the presence of octopamine receptors on the remaining non-myogenic parts of the extensor muscle. This finding together with the presence of the DUMETi axon in the non-myogenic parts of the muscle (Evans & O'Shea, 1978) suggests that octopamine released from the DUMETi terminals has a more widespread functional importance in the extensor muscle than inhibition of the myogenic rhythm.

Stimulating DUMETi lead to a marked, long-lasting, potentiation of the twitch tension produced by the slow motoneurone (SETi). It also produced a slight decline in the basal tonus of the muscle. Intracellular recordings from individual muscle fibres innervated by SETi revealed that the EJPs were potentiated by DUMETi stimulation. Both potentiating effects far outlasted the period of DUMETi stimulation. The effect on EJP amplitude however was smaller than that on twitch tension and much more variable. This might suggest that the increased EJP amplitude is not responsible for the potentiated twitch tension. The effects on EJP size, however, were recorded from single muscle fibres and the effect on twitch tension was recorded from the whole muscle. Variation in the amount of EJP potentiation among single muscle fibres may be caused by variation in recording sites and proximity to neuromuscular junctions. The evidence of variation alone therefore cannot eliminate the possibility that potentiation of twitch tension is mediated by the EJP potentiation. There are post-synaptic effects of octopamine (see below) and we cannot yet say whether twitch potentiation is at least in part caused by direct postsynaptic action of octopamine on the muscle fibres.

Direct application of low concentrations of octopamine mimics the effects of DUMETi on SETi neuromuscular transmission. Concentrations of DL-octopamine as low as 10^{-7} M produced a significant potentiation of SETi-induced twitch tension. The octopamine induced potentiation of both SETi twitch tension and amplitude of SETi EJPs were both of long duration, far outlasting the stimulating pulse. We take the similarities between these two effects to indicate that the potentiation induced by stimulating DUMETi is mediated by the release of octopamine from its terminals on the muscle.

Presynaptic versus postsynaptic sites of action

The mechanism of the potentiation in the system we describe is as yet unknown. Although we have shown the presence of both pre- and post-synaptic receptors for octopamine, we do not know which causes the enhancement of contraction. Octopamine causes an increase in the size of the EJP and an enhanced contraction but we have

not shown that the enhanced contraction is caused by the increased EJP amplitude. Indeed, in *Aplysia*, Weiss *et al.* (1978) have shown that enhanced contraction response to 5-HT can occur independently of the parallel increase in EJP size.

Our only evidence on this issue is indirect. The effects which seem clearly to be mediated by post-synaptic receptors (slowing of the myogenic rhythm, increase in the rate of relaxation) have a lower threshold than the presynaptic effects of octopamine (increase in mepp frequency). The thresholds of the former are lower than the threshold for the potentiating effect on twitch tension which is close to that for the effect on mepp frequency. This evidence argues for a presynaptic mechanism for at least a part of the potentiation of twitch tension.

Pharmacology of octopamine receptors and specificity of responses

The octopamine receptors mediating the potentiating effects on SETi twitch tension and rate of relaxation have many similarities to those mediating the effects on the myogenic rhythm (Evans & O'Shea, 1978). The most potent agonist in all cases is synephrine, the N-methylated analogue of octopamine. The threshold effects for potentiation of SETi twitch tension and relaxation were respectively between 10^{-8} M and 10^{-7} M and between 10^{-10} M and 10^{-9} M for DL-synephrine. Synephrine is also a potent agonist at several other octopamine receptors such as those mediating the activation of adenylate cyclase in insect brain (Harmer & Horn, 1977) and in lobster blood cells (Battelle & Kravitz, 1978). Synephrine, however, could not be detected in significant amounts in assays of pooled DUMETi somata or in pooled lengths of extensor tibiae nerves (n $5b_1$) (Evans & O'Shea, 1978). Other biogenic amines related to octopamine were without effect on SETi twitch tension and rate of relaxation when administered at 10^{-6} M for 30 s. Thus of the naturally occurring biogenic amines in the insect nervous system, therefore octopamine is the most potent agonist of the receptors mediating the potentiating effects on SETi neuromuscular transmission.

Octopamine has been shown in the lobster to increase the tension generated in skeletal muscle by an excitatory motoneurone (Evans, Talamo & Kravitz, 1975; Kravitz *et al.* 1976). It is released into the blood from a group of peripheral octopaminergic neurones (Wallace *et al.* 1974; Evans *et al.* 1975, 1976*b*; Evans, Kravitz & Talamo, 1976*a*). Lobster skeletal muscles, however, unlike those of the locust (Evans & O'Shea, 1977, 1978) and of *Aplysia* (Weiss *et al.* 1975, 1978) are not known to receive any direct aminergic innervation but they are affected by a variety of amines which must presumably reach their targets through the blood (Dudel, 1965; Kravitz *et al.* 1976). Some of the active amines in the lobster such as 5-HT are better at potentiating the tension produced by the stimulation of the excitatory motoneurone than octopamine (Kravitz *et al.* 1976). This non-specificity of the potentiation effect is in contrast to the situation we describe here in the locust.

We examined the effects of 5-HT on neuromuscular transmission in the locust because of its known facilitatory effect on several other preparations (e.g. in lobster, Dudel, 1965; Kravitz *et al.* 1976; in *Aplysia*, Weiss *et al.* 1975, 1978) and because the muscle fibres of the myogenic component of the locust extensor tibiae muscle possess receptors with a high affinity for 5-HT which accelerate the frequency and increase the amplitude of the myogenic contractions (Evans & O'Shea, 1978). Serotonin however, had no measurable effect on the twitch tension or the EJP generated by the

SETi motoneurone. Therefore, the receptors which mediate stimulation of the myogenic rhythm do not appear also to affect neurally evoked contractions. There is no evidence therefore that these receptors, in contrast to the octopamine receptors, extend beyond the myogenic muscle fibres.

The octopamine effects described here were all blocked by the α-adrenergic blocking agent phentolamine and not by the β-blocking agent DL-propranolol. The receptors thus possess some of the characteristics of classical vertebrate α-adrenergic receptors. In this respect they are similar to octopamine receptors on the myogenic fibres of the extensor muscle (Evans & O'Shea, 1978), those that mediate the activation of adenylate cylase (Harmer & Horn, 1977; Battelle & Kravitz, 1978) and to those suggested to be involved in the autoregulation of the peripheral octopamine cells in lobsters (Konishi & Kravitz, 1978).

Pre-synaptic α-adrenergic receptors mediate the potentiating effects of noradrenaline at vertebrate skeletal neuromuscular junctions (Bowman & Nott, 1969; Kuba, 1970) and these effects have many parallels with the action of octopamine at the SETi-neuromuscular junction of the locust. Both increase twitch tension, increase EJP size and affect the frequency but not the amplitude of spontaneous mepps. Adrenaline increases the maximum twitch tension and the time to peak tension in vertebrate fast twitch muscle (Lewis & Webb, 1976) but the rate of development of tension is not significantly affected (Bowman & Nott, 1969). This again is similar to the effect of octopamine on twitch tension at the SETi neuromuscular junction in the locust.

Time course of modulatory effects – functional implications

The effects of stimulating DUMETi, and of superfusing octopamine, are long lasting and far outlast the period of stimulation. This feature was also found in the potentiating effects of the metacerebral cells in *Aplysia* (Weiss *et al.* 1975; 1978) and for the modulatory effects of octopamine and other amines at the lobster neuromuscular junction (Kravitz *et al.* 1976). The modulating receptors stimulated by amines in these examples seem, therefore, to mediate processes very different from those which are stimulated by conventional neurotransmitters. The receptors stimulated by the octopamine released from DUMETi terminals may for example have access to metabolic functions, possibly by being linked to adenylate cyclase. This possibility is suggested by the similarities between octopamine receptors on locust muscle and those known to be linked to adenylate cyclase in cockroach (Harmer & Horn, 1977) and in lobster (Battelle & Kravitz, 1978). Furthermore, in both vertebrate smooth muscle (Gomperts, 1976) and cardiac muscle (Tsien, 1977) the degree of relaxation is directly proportional to the accumulation of cAMP mediated by catecholamines. It is thus tempting to speculate that both the potentiation of twitch tension and the increased rate of relaxation caused by octopamine in locust muscle are mediated via the stimulation of adenylate cyclase.

Function of dorsal unpaired median neurones

We think that the regulation of motoneurone effects is an important function of DUMETi, which may be shared by other DUM neurones in other muscles. It seems unlikely to us that the effect of DUM neurones on the myogenic rhythm found in the extensor muscle (Hoyle, 1974; Hoyle & O'Shea, 1974) is an important role for these

cells. The octopamine receptors extend beyond the small number of myogenic fibres and the effect of octopamine on the myogenic rhythm (a slowing) is less dramatic than the accelerating effect of 5-HT (Evans & O'Shea, 1978). This fact, together with the evidence that the 5-HT receptors do not affect neurally evoked muscle activity suggesting they are confined to the myogenic fibres, is added evidence that the primary role of octopamine receptors is not the control of the myogenic rhythm; this may be the primary function of the 5-HT receptors.

The modulatory function is an important and general function which has been confirmed for biogenic amines in both vertebrates and invertebrates, in both the central and peripheral nervous systems. The various long-lasting modulatory effects of the biogenic amines have led to the idea that they may function in the long-term regulation of behavioural responsiveness or arousal (Weiss *et al.* 1975, 1978; Susswein, Kupfermann & Weiss, 1976). This idea is supported by recent evidence showing that amines can function in the vertebrate CNS to bias or modulate the effects of output produced by conventional neurotransmitters (Freedman *et al.* 1977; Hokfelt & Fuxe, 1969; Hoffer *et al.* 1973). The example we describe is probably the simplest way in which the intensity of behaviour can be increased; it is done without altering the motor output but by a bias placed directly at the neuromuscular junction. We know nothing about the possible functions of DUMETi or other DUM neurones in the central nervous system, but we speculate that the neuromuscular function provides a simple model for central effects.

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REFERENCES

BATTELLE, B. A. & KRAVITZ, E. A. (1978). Targets of octopamine action in the lobster: cyclic nucleotide changes and physiological effects in haemolymph, heart and exoskeletal muscle. *J. Pharmacol. Expt. Therap.* **205**, 438–448.

BOWMAN, W. C. & NOTT, M. W. (1969). Actions of sympathomimetic amines and their antagonists on skeletal muscle. *Pharmacol. Revs.* **21**, 27–72.

BOWMAN, W. C. & ZAMIS, E. (1958). The effects of adrenaline, noradrenaline and isoprenaline on skeletal muscle contraction in the cat. *J. Physiol., Lond.* **144**, 92–107.

COCHRANE, D. G., ELDER, H. Y. & USHERWOOD, P. N. R. (1972). Physiology and ultra-structure of phasic and tonic skeletal muscle fibres in the locust *Schistocerca gregaria*. *J. Cell Sci.* **10**, 419–441.

DIRSCH, V. M. (1974). Genus *Schistocerca* (Acridomorpha, Insects) Pubs. The Hague: Dr W. Junk, B.V.

DUDEL, J. (1965). Facilitatory effects of 5-hydroxytryptamine on the crayfish neuromuscular junction. *Naunyn-Schmied. Arch. exp. Path und Pharmacol.* **249**, 515–528.

EVANS, P. D., KRAVITZ, E. A. & TALAMO, B. R. (1976*a*). Octopamine release at two points along lobster nerve trunks. *J. Physiol., Lond.* **262**, 71–89.

EVANS, P. D., KRAVITZ, E. A., TALAMO, B. R. & WALLACE, B. G. (1976*b*). The association of octopamine with specific neurones along lobster nerve trunks. *J. Physiol., Lond.* **262**, 51–70.

EVANS, P. D. & O'SHEA, M. (1977). The identification of an octopaminergic neurone which modulates neuromuscular transmission in the locust. *Nature, Lond.* **270**, 257–259.

EVANS, P. D. & O'SHEA, M. (1978). The identification of an octopamingeric neurone and the modulation of a myogenic rhythm in the locust. *J. exp. Biol.* **73**, 235–260.

EVANS, P. D., TALAMO, B. R. & KRAVITZ, E. A. (1975). Octopamine neurons: morphology, release of octopamine, and possible physiological role. *Brain Res.* **90**, 340–347.

FREEDMAN, R., HOFFER, B. J., WOODWARD, D. J. & PURO, D. (1977). Interaction of norepinephrine with cerebellar activity evoked by mossy and climbing fibres. *Exp. Neurol.* **55**, 269–288.

GOMPERTS, B. D. (1976). Calcium and cell activation. In *Receptors and Recognition*, 2, series A (ed. P. Cuatrecasas and M. F. Greaves). London: Chapman & Hall.

HARMER, A. J. & HORN, A. S. (1977). Octopamine-sensitive adenylate cyclase in cockroach brain: effects of agonists, antagonists and guanylyl nucleotides. *Mol. Pharmacol.* **13**, 512–520.

HOFFER, B. J., SIGGINS, G. R., OLIVER, A. P. & BLOOM, F. E. (1973). Activation of the pathway from locus coeruleus to rat cerebellar Purkinje neurons. Pharmacological evidence of noradrenergic central inhibition. *J. Pharmacol. Exp. Therap.* **184**, 553–569.

HOKFELT, T. & FUXE, K. (1969). Cerebellar monoamine nerve terminals, a new type of afferent fiber to the cortex cerebelli. *Exp. Brain Res.* **9**, 63–72.

HOYLE, G. (1974). A function for neurons (DUM) neurosecretory on skeletal muscles of insects. *J. exp. Zool.* **189**, 401–406.

HOYLE, G. & BURROWS, M. (1973). Neural mechanisms underlying behaviour in the locust *Schistocerca gregaria*. I. Physiology of identified neurons in the metathoracic ganglion. *J. Neurobiol.* **4**, 3–41.

HOYLE, G., DAGAN, D., MOBERLY, B. & COLQUHOUN, W. (1974). Dorsal unpaired median insect neurons make neurosecretory endings on skeletal muscle. *J. exp. Zool.* **187**, 159–165.

HOYLE, G. & O'SHEA, M. (1974). Intrinsic rhythmic contractions in insect skeletal muscle. *J. exp. Zool.* **189**, 407–412.

KONISHI, S. & KRAVITZ, E. A. (1978). The physiological properties of amine-containing neurones in the lobster nervous system. *J. Physiol., Lond.* **279**, 215–229.

KRAVITZ, E. A., BATTELLE, B.-A., EVANS, P. D., TALAMO, B. R. & WALLACE, B. G. (1976). Octopamine neurons in lobsters. In *Society of Neuroscience Symp.*, I (ed. J. A. Ferrendelli, B. S. McEwan and S. H. Snyder), pp. 67–81. Bethesda, Maryland.

KUBA, K. (1970). Effects of catecholamines on the neuromuscular junction in the rat diaphragm. *J. Physiol., Lond.* **211**, 551–570.

LEWIS, D. M. & WEBB, S. N. (1976). Adrenaline on isotonic contractions of mammalian skeletal muscle. *Brit. J. Pharmacol.* **58**, 467P.

ORBELI, L. A. (1923). Die sympathetische innervation der skelettmuskeln. *Bull. Inst. Sci. Leshaft.* **6**, 194–197.

O'SHEA, M. & EVANS, P. D. (1977). Synaptic modulation by an identified octopaminergic neuron in the locust. *Soc. Neurosci. Abstracts* **3**, 187.

PEARSON, K. G. & BERGMAN, S. J. (1969). Common inhibitory motoneurons in insects. *J. exp. Biol.* **50**, 445–473.

PRINGLE, J. W. S. (1939). The motor mechanisms of the insect leg. *J. exp. Biol.* **16**, 220–231.

SUSSWEIN, A., KUPFERMANN, I. & WEISS, K. R. (1976). Arousal of feeding behavior of *Aplysia*. *Soc. Neurosci. Abstracts* 2.

TSIEN, R. W. (1977). Cyclic AMP and contractile activity in heart. In *Adv. Cyclic Nucleotide Res.*, **8**, (ed. P. Greengard and G. A. Robision), pp. 363–420.

USHERWOOD, P. N. R. & GRUNDFEST, H. (1965). Peripheral inhibition in skeletal muscle of insects. *J. Neurophysiol.* **28**, 497–518.

WALLACE, B. G., TALAMO, B. R., EVANS, P. D. & KRAVITZ, E. A. (1974). Octopamine: selective association with specific neurons in the lobster nervous system. *Brain Res.* **74**, 349–355.

WEISS, K. R., COHEN, J. & KUPFERMANN, I. (1975). Potentiation of muscle contraction: a possible modulatory function of an identified serotonergic cell in *Aplysia*. *Brain Res.* **99**, 381–386.

WEISS, K. R., COHEN, J. L. & KUPFERMANN, I. (1978). Modulatory control of buccal musculature by a serotonergic neuron (metacerebral cell) in *Aplysia*. *J. Neurophysiol.* **41**, 181–203.