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# OCTOPAMINERGIC MODULATION OF FLIGHT MUSCLE IN THE LOCUST

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## SUMMARY

1. The modulatory actions of octopamine on neurally induced twitch tension in the dorsal longitudinal flight muscles of the locust are described. Octopamine increases the amplitude of twitch tension, the rate of twitch contraction and the rate of twitch relaxation in this fast twitch muscle. The specificity of the receptors mediating these octopamine responses is also described.

2. Evidence is presented to suggest that the dorsal unpaired median neurone to the locust dorsal longitudinal flight muscles (DUMDL) is an octopaminergic neurone whose activation mimics the application of exogenous octopamine to the muscle.

3. The effects of both DUMDL and octopamine on the dorsal longitudinal muscle depend upon the frequency of stimulation of motor neurone input to the muscle.

4. The results are discussed in terms of the behavioural significance of the release of octopamine during the first few minutes of locust flight. It is suggested that such a release is likely to be an important modulatory factor influencing the kinetics of contraction of the dorsal longitudinal muscles, resulting in an increase in the force generated by each muscle contraction together with an energy-saving adaptation due to a reduced overlap in the duration of twitches in antagonistic muscles.

## INTRODUCTION

Octopamine is a biogenic amine with a widespread occurrence in insect nervous systems, that can function as a neurotransmitter, a neuromodulator and a neurohormone (Evans, 1980, 1985*a*). A role for octopamine in the control of various aspects of insect flight has been suggested. Since octopamine levels in haemolymph rise rapidly during the first few minutes of flight and then decline to lower plateau levels (Goosey & Candy, 1980; Bailey, Martin & Downer, 1983), it has been suggested that this amine functions as an important hormonal regulator of carbohydrate and lipid metabolism in contracting flight muscle (Candy, 1978). In addition, octopamine may also function as a modulator of neuromuscular transmission in flight muscle. In the tobacco hawkmoth, *Manduca sexta*, octopamine

Key words: octopamine, flight muscle, locust, insect.

increases the amplitude of excitatory junctional potentials (EJPs) in the dorsal longitudinal muscles of immature and adult moths but, paradoxically, increases the amplitude and frequency of miniature EJPs in immature animals whilst having the opposite effect on adults and pharate adults ready to eclose (Klaassen & Kammer, 1985; Fitch & Kammer, 1986; Klaassen, Kammer & Fitch, 1986). These results suggest that octopamine produces both presynaptic and postsynaptic effects in this preparation that interact in a complex way that varies with the age of the animal. The strength of locust flight muscle contractions has also been shown to be increased by octopamine in a half-locust preparation (Candy, 1978). More recently it has also been suggested that octopamine affects the centrally generated flight motor patterns of insects (Sombati & Hoyle, 1984; Claassen & Kammer, 1986), but it is not clear from these studies if it has specific direct actions on the central pattern generator neurones or a more general effect due to an enhanced efficacy of sensory transmission producing an increase in arousal.

To date, however, the source of any endogenous octopamine mediating these effects has not been definitely determined. For instance, it is not clear if the actions on flight muscle described above are brought about by the increased levels of octopamine in the haemolymph, which might be produced by the release of octopamine from octopamine-containing neurohaemal organs such as the corpora cardiaca and median neurohaemal organs (see Evans, 1985*a*). Alternatively, the raised haemolymph levels of octopamine could be a secondary consequence of the spill over into the haemolymph of octopamine released from the terminals of modulatory octopaminergic neurones innervating specific skeletal muscles. One such group of octopaminergic neurones, the dorsal unpaired median (or DUM) cells (Hoyle, Dagan, Moberly & Colquhoun, 1974), has been shown to be directly responsible for many of the physiological actions of octopamine in insects (see Evans, 1985*a*). Thus the DUM cell to the extensor tibiae muscle (DUMETi) has been shown to be octopaminergic (Evans & O'Shea, 1977, 1978), to modulate neuromuscular transmission and muscle contraction in this muscle (O'Shea & Evans, 1979; Evans, 1981; Evans & Siegler, 1982) and to release octopamine specifically in a frequency-dependent fashion (Morton & Evans, 1984). The DUM cells innervating the locust ovarioles have also been shown to be octopaminergic and to modulate the contractile properties of the visceral muscle in this preparation (Lange & Orchard, 1984; Orchard & Lange, 1985, 1986). In addition, the photomotor neurones to the firefly light organ have been shown to be octopaminergic DUM cells (Christensen & Carlson, 1981, 1982; Christensen, Sherman, McCaman & Carlson, 1983). The above evidence raises the possibility that the physiological actions of octopamine on locust flight muscle might be mediated by another octopaminergic DUM cell. Anatomical evidence for the innervation of locust dorsal longitudinal flight muscle by a DUM cell, called DUMDL, has been presented (Hoyle, Colquhoun & Williams, 1980). There remains a certain amount of confusion as to how many DUM cells actually innervate this muscle since Altman & Tyrer (1977) suggested there were two whereas other workers have only found one (Hoyle *et al.* 1980; Watson, 1984). Homologous neurones may be present in other species including *Manduca* (Wasserman, 1985)

and the cricket *Acheta domestica* (Davis & Alanis, 1979). However, evidence for the octopaminergic nature of DUMDL is lacking.

In the present study we have compared the physiological and pharmacological effects of stimulating DUMDL and of applying exogenous octopamine to the dorsal longitudinal flight muscles of the locust, and provide evidence for its octopaminergic nature. Further, we compare the physiological actions of octopamine on this flight muscle which is a purely fast muscle, with the previous findings on the octopaminergic modulation of fast and slow skeletal muscle fibres in the locust hindleg extensor tibiae muscle. Any modulation of the contractile properties of locust flight muscle, particularly the twitch duration, are likely to have important consequences in terms of energy expenditure since during the first few minutes of locust flight the overlapping of antagonistic twitches results in wastage of energy as far as aerodynamic output is concerned (Neville & Weis-Fogh, 1963; Wilson, 1968). This is just the time when octopamine levels increase in locust haemolymph. Furthermore, since the dorsal longitudinal muscle is composed of five motor units (Neville, 1963), we compared the actions of octopamine and DUMDL on each of the units to see if any regional differentation in responsiveness could be detected, as has been described for the extensor tibiae muscle (Evans, 1985*b*).

## MATERIALS AND METHODS

Male locusts (*Schistocerca gregaria*) were taken from a crowded laboratory culture fed on wheat seedlings and bran. All experiments were performed at room temperature (20°C), and used adult animals from 1 day old. No qualitative differences in the response of preparations to octopamine were observed with adult animals up to 3 weeks old. However, there were quantitative differences in the responsiveness of animals at different ages which will be the subject of a separate communication (M. D. Whim & P. D. Evans, in preparation). Animals were removed from the colony at least 1 h before use to minimize the possible potentiating effects of any released octopamine (Evans, 1981). They were immobilized on a block of Plasticine, and the legs and wings removed. A dorsal incision along the length of the thorax exposed the thoracic cavity. The gut and tissues overlying the metathoracic ganglion were then removed. Any fat body adhering to the metathoracic dorsal longitudinal flight muscles was also removed. Care was taken not to damage the tracheal supply to the muscle. The proximal end of the muscle was dissected clear of its attachment to its mesothoracic homologue and attached to a force transducer which measured tension in the muscle almost isometrically. The distal end of the muscle was pinned securely to the Plasticine using minuten pins to secure its position. An operational amplifier signal differentiator was used to measure continuously the rates of contraction and relaxation of neurally evoked tension in the muscle (Buchan & Evans, 1980).

The dorsal longitudinal muscles of the locust are innervated by five excitatory motor neurones (Neville, 1963; Bentley, 1970) (see Fig. 1). In the metathoracic segment one motor neurone lies contralateral to the muscle and its axon crosses the

midline and leaves the metathoracic ganglion *via* the ipsilateral nerve 1. The remaining four motor neurones to the metathoracic longitudinal muscles lie ipsilaterally in the mesothoracic ganglion. Their axons leave *via* nerve 6 which then fuses with nerve 1 from the metathoracic ganglion. Thus all five motor neurones, which innervate the separate motor units of the muscle, can be stimulated sequentially by increasing the stimulus strength to a pair of silver hook electrodes placed under the ipsilateral branch of nerve 1 after its fusion with nerve 6 from the mesothoracic ganglion. Increasing the stimulus strength gives five separate sizes of twitch tension amplitude. The dorsal unpaired median cell to the dorsal longitudinal muscles (DUMDL) has projections in both the left and right branches of nerve 1 in the metathoracic ganglion. It can be selectively stimulated by a pair of hook electrodes on the contralateral branch of nerve 1 since, owing to the small size of the DUMDL axon, it is not activated by ipsilateral stimulation of nerve 1 at the threshold of stimulation needed to fire the motor neurones to the dorsal longitudinal muscle. Stimulating nerve 1 produces only a single action potential recorded

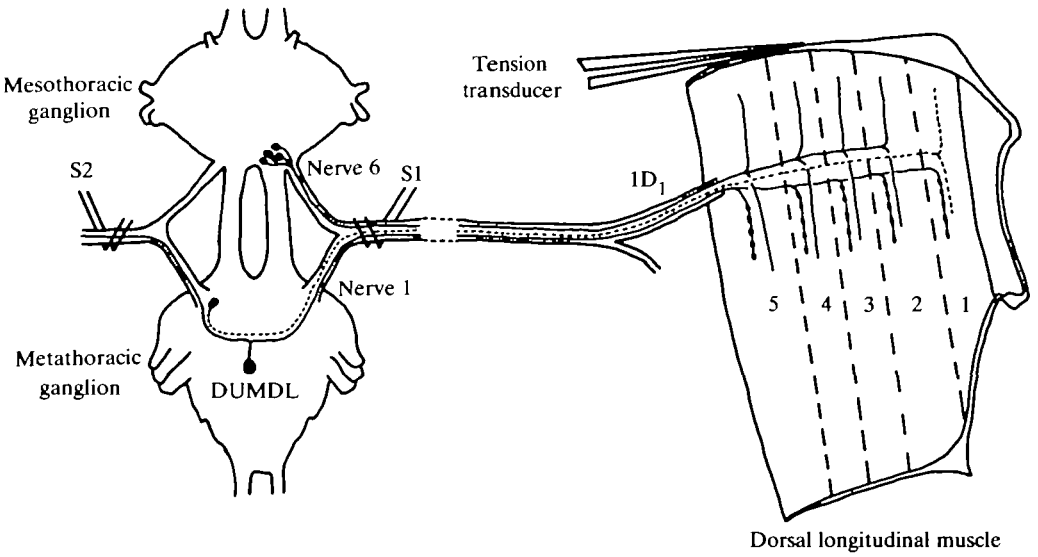



Fig. 1. Experimental arrangement used in stimulating the motor neurones to the dorsal longitudinal muscle (DLM) and in stimulating DUMDL. The cell bodies of the motor neurones innervating the DLM are shown in their appropriate positions (black dots) in the mesothoracic (four cells) and metathoracic (one cell) ganglia (after Bentley, 1970). They can be stimulated simultaneously by a pair of hook electrodes (S1) placed on the metathoracic nerve 1 after its fusion with metathoracic nerve 6. At the threshold stimulus intensity for the five motor neurones the axon for DUMDL, also contained in this nerve, is not excited because it has a much smaller diameter and therefore a higher threshold to extracellular stimulation. DUMDL can be selectively fired by a pair of hook electrodes (S2) on the contralateral metathoracic nerve 1. The numbers on the DLM correspond to the individual motor units (see Neville, 1963). The distribution of the motor neurone and DUMDL processes on the muscle is shown diagrammatically. $1D_1$, branch of nerve 1 innervating dorsal longitudinal muscle.

extracellularly in the contralateral nerve 1 which we presume to be that in the contralateral axon of DUMDL.

The stimulation paradigm employed in the tetanic contraction experiments was to fire the motor neurones for 10 s periods at the experimental frequency and then to return to continuous 1 Hz stimulation until the twitch amplitude and relaxation and contraction rates had returned to baseline values before stimulating at the next frequency in the series. Initially the range of frequencies was tested in saline and then repeated in the presence of $10^{-5}$ mol $l^{-1}$ DL-octopamine in the muscle superfusate after the responses to 1 Hz continuous stimulation had reached a maximum.

In some experiments the tension responses from single motor units each innervated by a single motor neurone were recorded. To do this motor neurones were stimulated as outlined above but tension responses were recorded from only single motor units, the other four muscle blocks being dissected clear at their points of attachment to the cuticle. Confirmation that only one motor unit was active was obtained by the observation that in these experiments tension twitches had a single intensity threshold and a constant height and did not increase in a stepwise fashion as the stimulus intensity was increased.

Drugs were superfused at 1 ml $min^{-1}$ directly onto the surface of the muscle. They were dissolved in a physiologically isotonic saline (pH 6·8) containing (in mmol $l^{-1}$): NaCl, 150; $CaCl_2$, 1·3; $KHCO_3$, 4; $KH_2PO_4$, 6; sucrose, 90. Preparations remained viable in this medium for 4–5 h, although in the present study individual preparations were rarely maintained beyond 3 h. Initial experiments were performed at a $Ca^{2+}$ concentration of 4 mmol $l^{-1}$, but these resulted in octopamine responses that were somewhat variable from one preparation to the next, possibly because of the induction of active membrane responses in some preparations (see Klaassen & Kammer, 1985). Thus we tested a range of $Ca^{2+}$ concentrations (0·5–8·0 mmol $l^{-1}$) and found that maximal octopamine-dependent effects were observed at 1·3 mmol $l^{-1}$ $Ca^{2+}$ which was then used in all subsequent experiments. This is close to the $Ca^{2+}$ concentration of 2 mmol $l^{-1}$ used by Weis-Fogh (1956) in early studies on the physiological properties of the locust dorsal longitudinal muscle.

All drugs were obtained from Sigma except for phentolamine mesylate which was a gift from Ciba and, D(−)- and L(+)-octopamine which were kindly donated by Dr M. D. Armstrong.

## RESULTS

### *Effects of octopamine on twitch tension*

The monophenolic biogenic amine, octopamine, produced a marked modulation of twitch tension parameters in the dorsal longitudinal flight muscle of the locust. Fig. 2A shows the effect of introducing a 30 s pulse of $10^{-6}$ mol $l^{-1}$ DL-octopamine into the superfusate whilst the muscle was being stimulated to contract at a frequency of 1 Hz. The amplitude of twitch tension increased to a peak value of 18 % above the

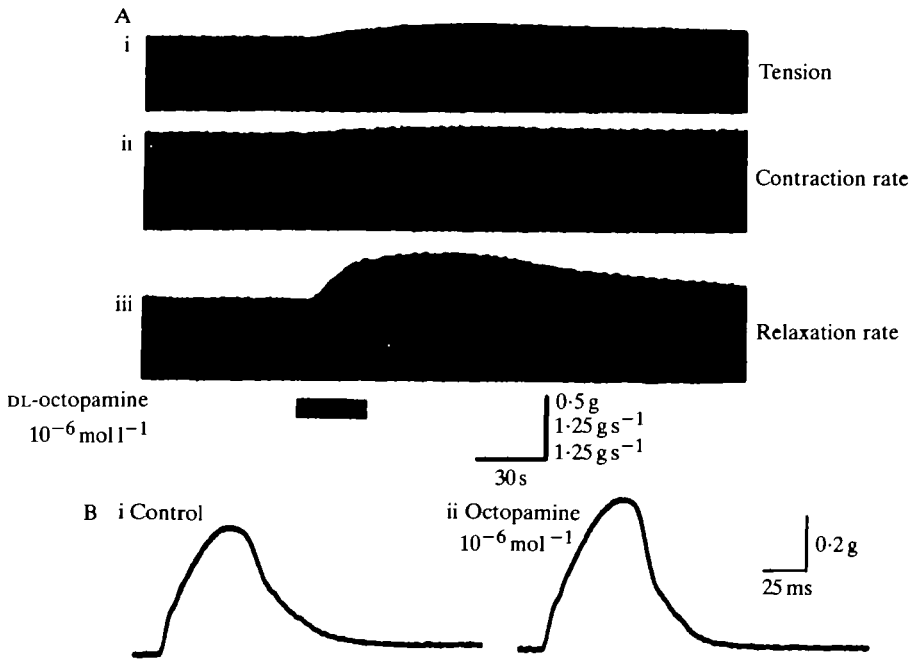



Fig. 2. (A) The effect of a 30-s pulse of $10^{-6}$ mol $l^{-1}$ DL-octopamine (black bar) on the twitch tension generated in the dorsal longitudinal muscle in response to stimulation of the motor neurones at a frequency of 1 Hz. (i) The response of twitch tension; (ii, iii) the effects on contraction and relaxation rates, respectively. The parameters returned to baseline values after 10 min of superfusion in saline. (B) Individual tension transients from Fig. 2A replayed at higher speed to show the effect of octopamine on twitch duration as measured from the initiation of contraction to 90% relaxation. (i) Control twitch before octopamine application (duration 102 ms); (ii) twitch during peak of response to $10^{-6}$ mol $l^{-1}$ octopamine (duration 84 ms). The duration of the twitch returned to 101 ms during the course of the wash-out period.

control level approximately 60 s after the end of the octopamine pulse and then gradually declined to resting values over the next 5 min. The rate of contraction of twitch tension also increased by a small amount (6% in the example shown in Fig. 2A) but this effect was found to vary from one preparation to the next. However, the most marked effect of octopamine was an increase in the rate of relaxation of twitch tension. This effect developed more rapidly than the other two and reached a peak increase of 53%.

The octopamine-induced increase in relaxation rate decreased the duration of the muscle twitch. Fig. 2B shows individual twitches before and during the octopamine pulse shown in Fig. 2A. The overall duration of the twitch decreased from 102 to 84 ms as measured from the initiation of contraction to 90% relaxation. The decreased twitch duration was almost exclusively due to a reduction in the time taken for the muscle to relax since, in the example shown, there is no significant alteration in the contraction rate.

The effects of octopamine on the above three parameters of twitch tension were dose-dependent (Fig. 3A–C). They all showed threshold increases between $10^{-8}$ and $10^{-9}$ mol $l^{-1}$ with maximal effects occurring in the range of $10^{-6}$–$10^{-5}$ mol $l^{-1}$. In addition, the octopamine response also varied with the length of the octopamine pulse (not shown). A 5-min pulse of $10^{-7}$ mol $l^{-1}$ DL-octopamine gave a half-time of

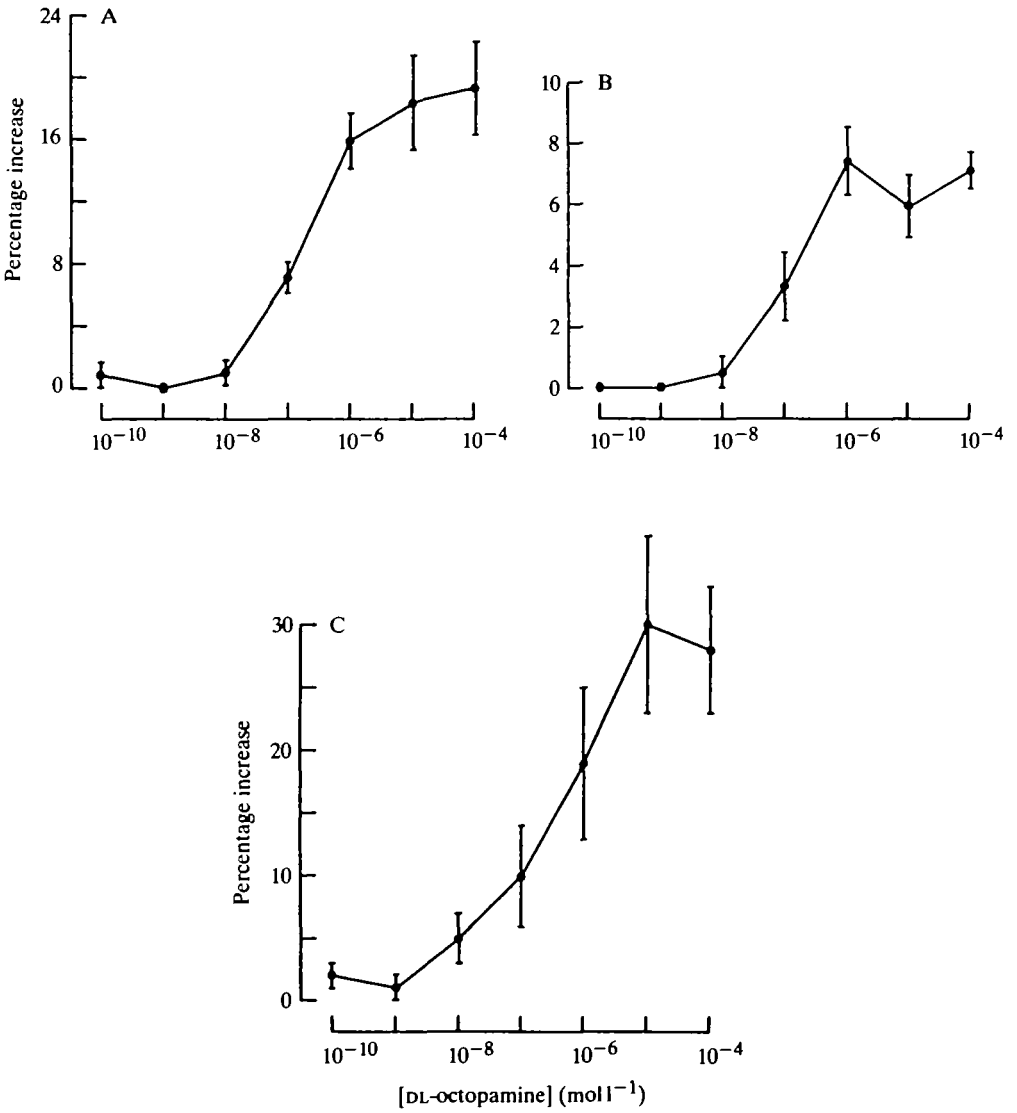



Fig. 3. Dose–response curves for the action of DL-octopamine on neurally evoked twitch tension in the dorsal longitudinal muscles. (A) Maximal effects on twitch amplitude; (B) maximal effects on contraction rate of twitch tension; (C) maximal effects on relaxation rate of twitch tension. The motor neurones were fired at a rate of 1 Hz and octopamine was introduced into the superfusate for a period of 30 s. Each point represents the mean of eight determinations and the bars represent standard errors.

maximal increase in relaxation rate of 22 s, whereas the half-time of maximal increase in twitch amplitude was at 74 s.

*Specificity of responses*

The specificity of the dorsal longitudinal muscle responses to octopamine was examined in the presence of a range of antagonists and agonists known to be active on octopamine receptors in other insect preparations (O'Shea & Evans, 1979; Evans, 1981).

Fig. 4. shows that the effects of a 30-s pulse of $5\times10^{-8}$ mol $l^{-1}$ DL-octopamine on neurally induced twitch tension were antagonized by the $\alpha$-adrenergic antagonist,

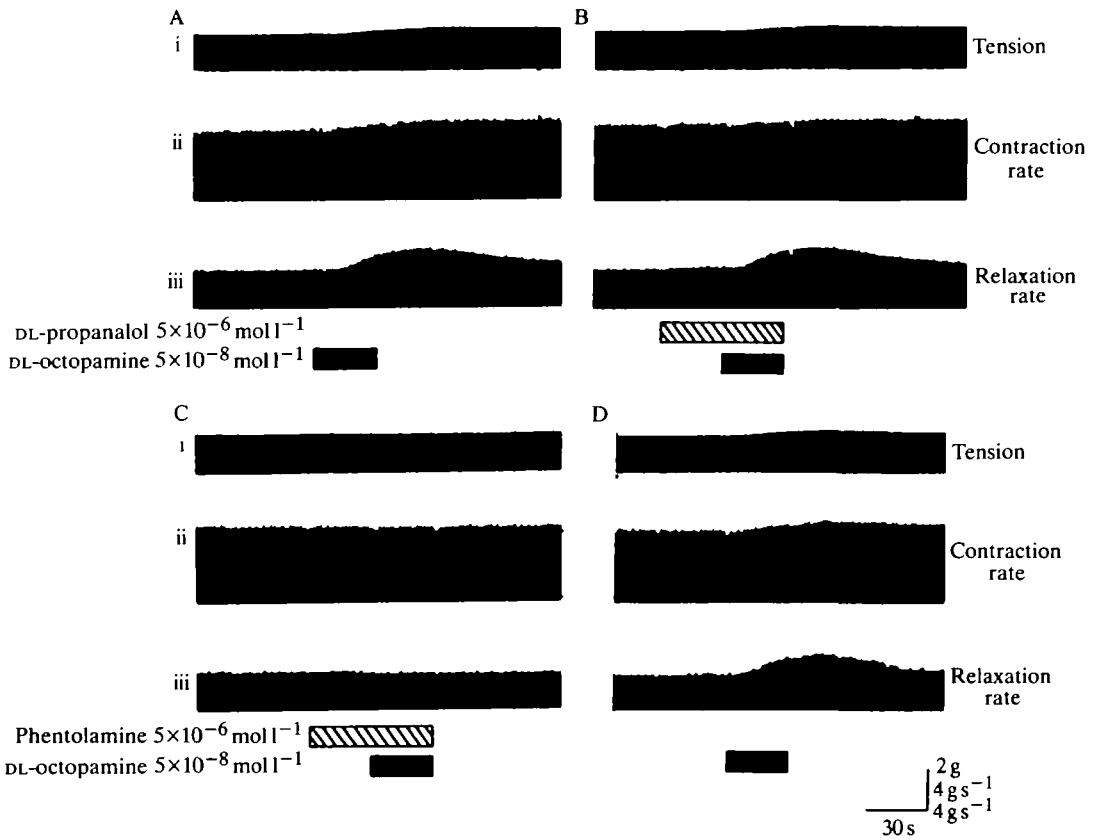



Fig. 4. The effect of blocking agents on the responses of neurally evoked twitch tension in the dorsal longitudinal muscles to 30 s pulses of $5\times10^{-8}$ mol $l^{-1}$ DL-octopamine (black bars) introduced into the muscle superfusate. (A) Control; (B) $5\times10^{-6}$ mol $l^{-1}$ DL-propranolol (hatched bar) does not block octopamine effect; (C) $5\times10^{-6}$ mol $l^{-1}$ phentolamine (hatched bar) blocks the octopamine effect; (D) octopamine response returns after wash out of phentolamine. The top trace (i) in each series shows the effects on the amplitude of twitch tension and the middle (ii) and bottom (iii) traces show the effects on contraction and relaxation rates of twitch tension, respectively. The traces shown in A–D were obtained from a single preparation with a 10 min wash period between the records.

phentolamine ($5\times10^{-6}$ mol $l^{-1}$), but not by the $\beta$-adrenergic antagonist, DL-propranolol ($5\times10^{-6}$ mol $l^{-1}$).

The effects of short pulses of compounds structurally related to octopamine upon the relaxation rate of dorsal longitudinal muscle twitch tension were compared in terms of a percentage increase in rate (Table 1). D(−)-octopamine, the naturally occurring isomer of octopamine in the locust (Goosey & Candy, 1980), was twice as potent as L(+)-octopamine indicating that the responses to octopamine are stereospecific. There was also a preference for monophenolic biogenic amines, since octopamine and synephrine were more potent than the corresponding catecholamines noradrenaline and adrenaline. The receptor showed a stronger response to biogenic amines with a hydroxyl group on the $\beta$ carbon of the side chain: octopamine was more potent than tyramine and phenethanolamine was more potent than phenylethylamine. Octopamine was thus much more active in this system compared with the other compounds tested, with the exception of synephrine, phenethanolamine and 5-hydroxytryptamine (5-HT). However, there is no evidence for the presence of synephrine and phenethanolamine in locust nervous tissue. In addition, the effects of 5-HT were not blocked by pretreatment of the muscle with $10^{-5}$ mol $l^{-1}$ gramine but were blocked by the addition of phentolamine (not shown). This suggests that 5-HT may be acting as a partial agonist of the octopamine receptors as has been shown in other locust preparations (Evans & O'Shea, 1978).

### *Stimulation of DUMDL mimics octopamine application*

When the dorsal unpaired median cell to the dorsal longitudinal muscles of the locust (DUMDL, Hoyle *et al.* 1980) was stimulated, it mimicked the effects of octopamine superfusion on twitch tension. Fig. 5 shows the effects of stimulating DUMDL at 5 Hz for 15 s *via* the contralateral branch of nerve 1 whilst stimulating

Table 1. *The action of biogenic amines on the relaxation rate of twitch tension*

| Compound | % increase in relaxation rate (±S.E.M.) | *N* |
|---|---|---|
| D(−)-octopamine | 57·6 ± 8·2 | 5 |
| DL-phenethanolamine | 53·2 ± 11·1 | 5 |
| DL-octopamine | 52·6 ± 4·9 | 15 |
| DL-synephrine | 45·8 ± 7·2 | 5 |
| 5-Hydroxytryptamine | 36·6 ± 5·8 | 5 |
| L(+)-octopamine | 26·0 ± 2·4 | 5 |
| Tyramine | 21·6 ± 3·6 | 5 |
| Dopamine | 5·6 ± 2·9 | 5 |
| L-noradrenaline | 4·8 ± 3·7 | 5 |
| L-adrenaline | 4·6 ± 2·5 | 5 |
| L-tyrosine | 3·0 ± 1·1 | 5 |
| Phenylethylamine | 2·0 ± 1·2 | 5 |

The results are expressed as the percentage increase in the rate of relaxation of twitch tension generated in response to stimulating the motor neurones at 1 Hz. All compounds were introduced into the muscle superfusate for periods of 30 s at a concentration of $10^{-5}$ mol $l^{-1}$.

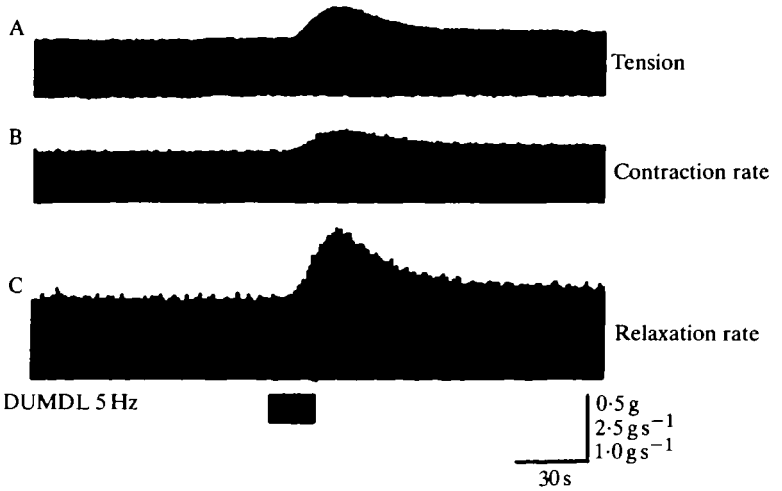



Fig. 5. The effect of DUMDL stimulation at 5 Hz for 15 s (black bar) on twitch tension induced in the dorsal longitudinal muscles by firing the motor neurones at 1 Hz. Trace A shows the effect on twitch tension and traces B and C show the effects on contraction and relaxation rates of twitch tension, respectively.

the dorsal longitudinal muscle at 1 Hz. Both the amplitude and relaxation rate of twitch tension were increased, together with a smaller increase in the rate of contraction of twitch tension, for a period which outlasted the stimulus duration. In addition, effects on twitch amplitude and contraction rate relative to the increase in relaxation rate were greater than those observed for the application of exogenous pulses of octopamine. The reason for this is not known, but it might reflect either a co-release of a second modulator along with octopamine from DUMDL or a close anatomical relationship between the DUMDL terminals and the motor neurone terminals in the muscle.

The extent of the potentiation induced by DUMDL stimulation was related to the frequency of stimulation (Fig. 6A–C). The observed effects on twitch amplitude, contraction rate and relaxation rate reached a plateau between 5 and 10 Hz, with no further significant increases up to a DUMDL stimulation frequency of 30 Hz.

The similarity between the effects of DUMDL stimulation and the application of exogenous octopamine on dorsal longitudinal muscle twitch contractions is consistent with the hypothesis that DUMDL releases octopamine as its neuroactive agent. This hypothesis receives further support from the observation that the effects of DUMDL stimulation were blocked in the presence of phentolamine. At $10^{-5}$ mol $l^{-1}$, phentolamine blocked the effects on dorsal longitudinal muscle twitch tension of firing DUMDL for 15 s at 5 Hz (Fig. 7). The blocking actions of phentolamine were reversible and the responses to DUMDL stimulation returned after the phentolamine had been washed out. The blocking action of phentolamine was selective since an equivalent concentration of DL-propranolol did not block the effects of stimulating DUMDL for 15 s at 5 Hz (not shown).

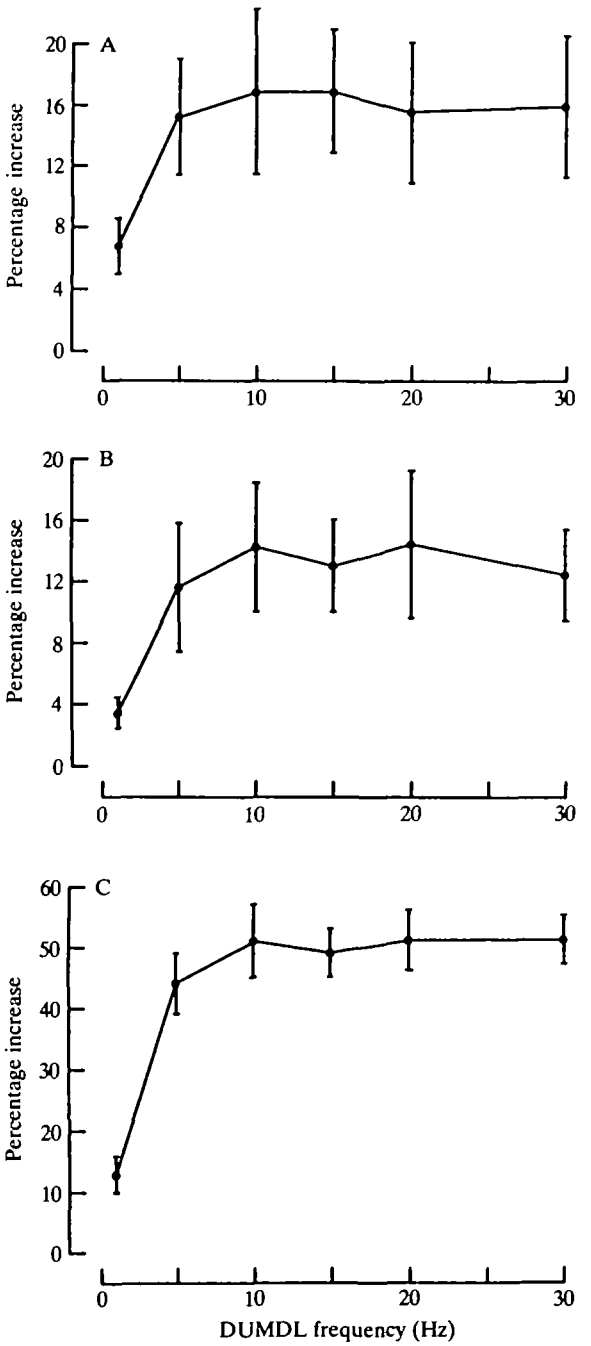



Fig. 6. Frequency-dependence of the effects of DUMDL stimulation on twitch tension induced in the dorsal longitudinal muscles by firing the motor neurones at 1 Hz. (A) The effect on amplitude of twitch tension; (B,C) the effects on contraction and relaxation rates of twitch tension, respectively. DUMDL was fired for periods of 15 s. The points represent the mean of five determinations and the bars represent standard errors.

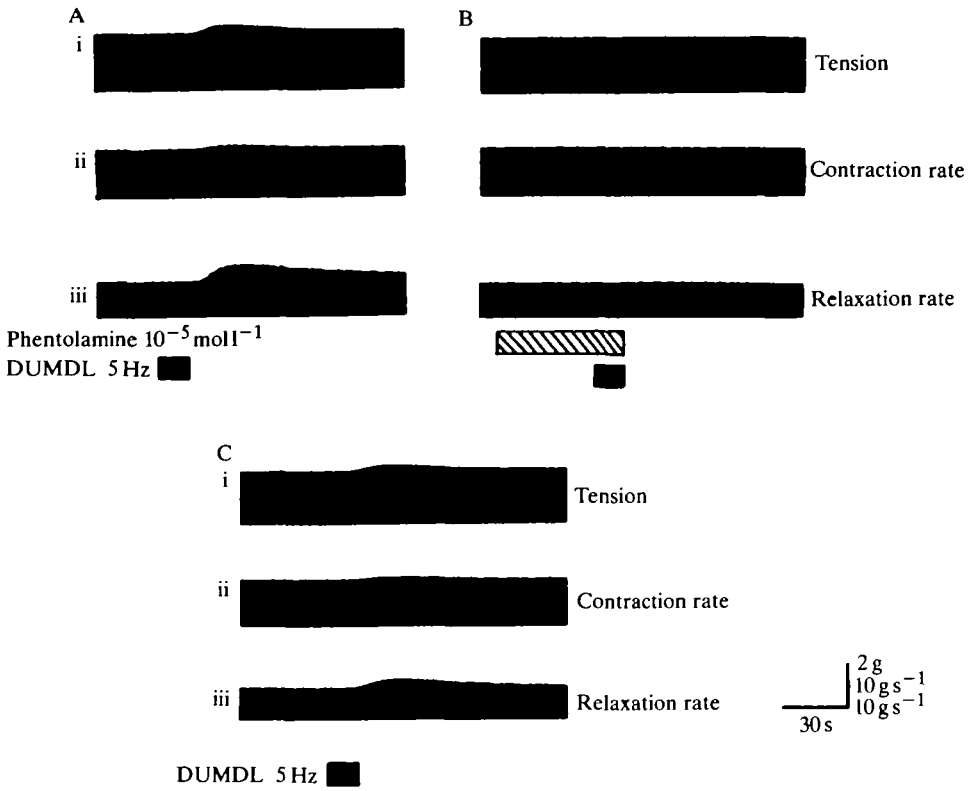



Fig. 7. Blocking actions of $10^{-5}$ mol $l^{-1}$ phentolamine (hatched bar) on the potentiating action of DUMDL stimulation at 5 Hz for 15 s (black bars). (A) Control; (B) the effect of DUMDL stimulation is blocked in the presence of phentolamine; (C) the DUMDL effect returns after washing out the phentolamine. The top trace (i) in each series shows the effects on the amplitude of twitch tension and the middle (ii) and bottom (iii) traces show the effects on contraction and relaxation rates of twitch tension, respectively. The traces shown in A–C were obtained from a single preparation with a 10 min wash period between the records.

*Dependence of effects on frequency of motor neurone stimulation*

The modulatory actions of octopamine and the peptides proctolin and FMRFamide on the tension developed in the extensor tibiae muscle of the hindleg of the locust are dependent on the frequency of stimulation of the slow motor neurone to this muscle (Evans & Siegler, 1982; Evans, 1982; Evans & Myers, 1986). The effects of exogenously applied octopamine on tension generated in the dorsal longitudinal flight muscles also depended on the frequency of stimulation of the motor neurones (Fig. 8). With stimulation periods of 10 s, at all tested frequencies above 0·1 Hz some residual tension was maintained by the muscle between the individual twitches, the amount of which increased with stimulation frequency (Fig. 8A). The muscle did not produce a smooth tetanic contraction even at frequencies up to 30 Hz (cf. Neville & Weis-Fogh, 1963), but the 10 s bursts of

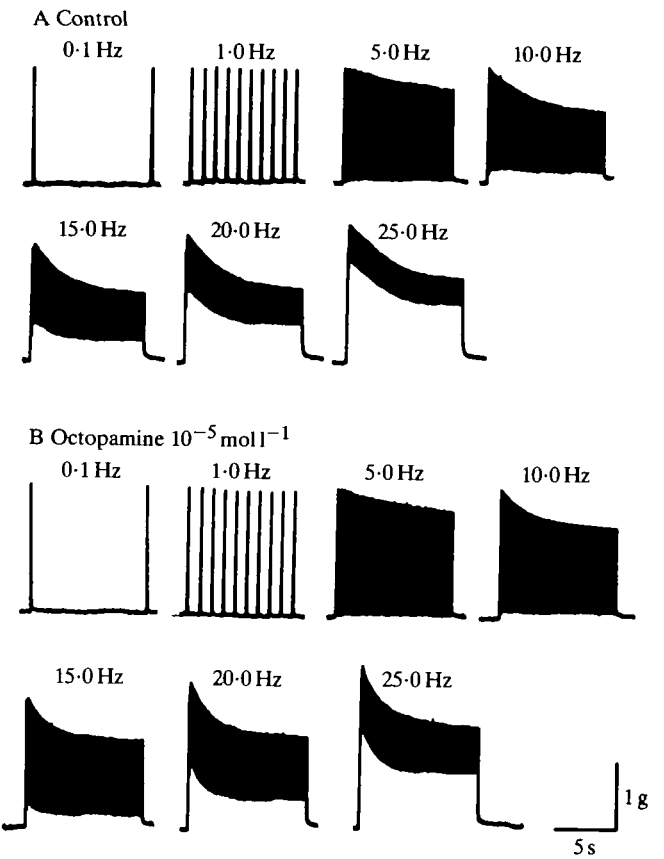



Fig. 8. The potentiating effect of octopamine upon neurally evoked tension in the dorsal longitudinal muscles depends upon the frequency of motor neurone stimulation. The effect of stimulating the motor neurones for 10 s periods at various frequencies from 0·1 to 25 Hz in the absence (A) and presence (B) of $10^{-5}$ mol $l^{-1}$ DL-octopamine.

stimulation produced incompletely fused tension transients superimposed upon an increasing baseline of maintained tension between the individual twitches. At higher frequencies the amplitude of the individual tension transients declined. Octopamine increased the amplitude of the individual tension transients at all frequencies tested (Fig. 8B). This resulted from a combination of two effects: a decrease in the amount of tension maintained between individual contractions, and an increase in the maximal levels of tension reached by each contraction. Thus octopamine serves to decrease the degree of fusion between the individual twitches and to increase the force generated by each contraction.

Qualitatively similar effects were observed when DUMDL was stimulated at 5 Hz for 10 s, 10 s prior to the stimulation of the motor neurones at different frequencies (Fig. 9A,B). In the example shown, there was also a sudden drop in the maintained tension at the beginning of each trace at frequencies above 10 Hz. This was typically

found in newly moulted adults which required time to accommodate to stimulation at higher frequencies.

*Does octopamine-dependent modulation vary between motor units?*

In the extensor tibiae muscle of the locust hindleg the modulatory effects of octopamine on slow motor neurone induced tension vary in different parts of the muscle depending on the relative percentage of slow and fast muscle fibres present (Evans, 1985*b*). The dorsal longitudinal muscle of the locust is composed of five separate motor units each innervated by a separate motor neurone (Neville, 1963). Ultrastructurally the muscle fibres in each motor unit are of the fast type (Weis-Fogh, 1961; Mizisin & Ready, 1986) and the motor neurones also induce fast-type contractions (Neville, 1963). In the present study we have examined the effects of octopamine on the twitch tension parameters from the different motor units of the dorsal longitudinal muscle. Pulses of DL-octopamine for 30 s at various concentrations produced dose-dependent increases in twitch amplitude, contraction rate and relaxation rate in both the basal and distal motor units of the dorsal longitudinal muscle (Fig. 10). However, no significant differences were observed between the

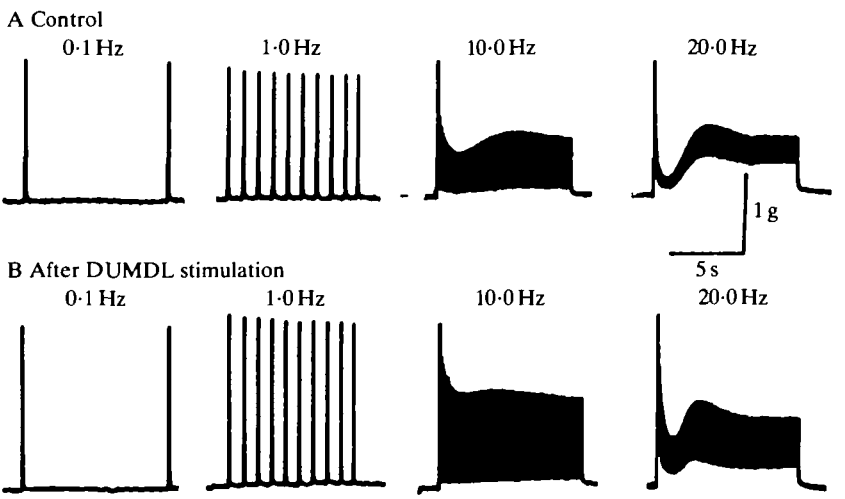
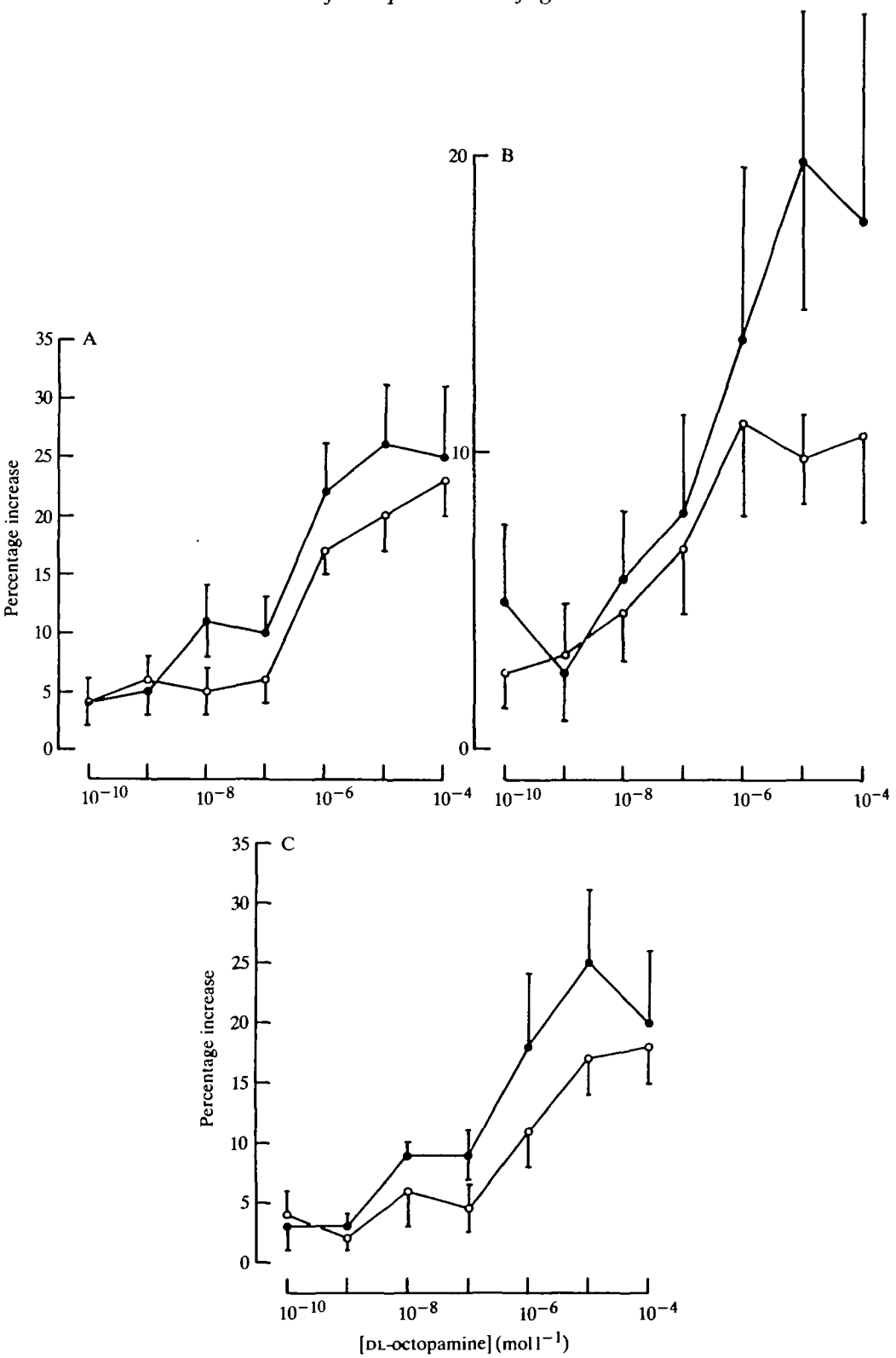



Fig. 9. The potentiating effect of stimulating DUMDL at 5 Hz for 15 s upon neurally evoked tension in the dorsal longitudinal muscles depends upon the frequency of motor neurone stimulation. The effect of stimulating the motor neurones for 10 s periods at various frequencies from 0·1 to 20 Hz before (A) and after (B) DUMDL stimulation.

Fig. 10. A comparison of the effects of octopamine potentiation of twitch tension on different motor units of the dorsal longitudinal muscle. A comparison of dose–response curves obtained from the most distal (○) and the most basal (●) motor units (Neville, 1963) in response to the introduction of 30-s pulses of DL-octopamine at various concentrations into the muscle superfusate. (A) The effects on twitch tension amplitude; (B,C) the effects on contraction rates and relaxation rates of twitch tension, respectively. The points represent the mean of five determinations and the bars represent standard errors.


A
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Percentage increase
35
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0
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10
$10^{-10}$
$10^{-8}$
$10^{-6}$
$10^{-4}$
[DL-octopamine] (mol $l^{-1}$)

Fig. 10

two motor units tested. In addition, no significant differences were observed with the other motor units of this muscle.

## DISCUSSION

Octopamine is a potent modulator of neurally evoked twitch and tetanic contractions in the dorsal longitudinal flight muscles of the locust. Its effects closely parallel many of the actions of octopamine on the extensor tibiae muscle of the locust hindleg (Evans & O'Shea, 1977, 1978; O'Shea & Evans, 1979; Evans, 1981; Evans & Siegler, 1982) and on the dorsal longitudinal muscles of the moth, *Manduca sexta* (Fitch & Kammer, 1986; Klaassen *et al.* 1986). In locust fast flight muscles octopamine increases the amplitude of twitch tension and the rate of contraction but the largest increases are in the rate of relaxation, which results in a decreased twitch duration. This is similar to the actions of octopamine on slow motor neurone induced twitch tension in the extensor tibiae muscle but not to its actions on fast motor neurone induced twitch tension in this muscle where octopamine again increases the relaxation rate of twitch tension but has no effect on its amplitude and rate of contraction (O'Shea & Evans, 1979). As found for the effects of octopamine (Evans & Siegler, 1982), proctolin (Evans, 1982) and FMRFamide (Evans & Myers, 1986) on the extensor tibiae muscle, the effects of octopamine on the dorsal longitudinal muscles were frequency-dependent with maximal effects occurring at about 20 Hz which is close to the normal flight frequency of the adult locust (Kutsch, 1971). However, octopamine besides reducing the amount of maintained tension between individual tension transients in an incompletely fused tetanus, as in the extensor tibiae muscle (Evans & Siegler, 1982), in this muscle also increased the peak amplitude of the individual tension transients.

The above effects of octopamine on the dorsal longitudinal muscles of the locust are all dose-dependent with thresholds in the range of $10^{-8}$–$10^{-9}$mol$l^{-1}$. No differences were observed in the responsiveness of the different motor units in this muscle in contrast to the regional differences in responsiveness observed in the extensor tibiae muscle (Evans, 1985*b*). The receptors mediating the actions of octopamine in the dorsal longitudinal flight muscles of the locust appear to be specific for octopamine since they can be blocked selectively by antagonists that block octopamine receptors in the other preparations (O'Shea & Evans, 1979; Nathanson, 1979; Evans, 1981). The structure–specificity profile of the receptor is also broadly similar to that found for octopamine receptors in other preparations (Evans & O'Shea, 1978; Evans, 1981). The finding that synephrine was only slightly less effective than octopamine in potentiating the muscle relaxation rate suggests that the flight muscle octopamine receptor is likely to exhibit a greater similarity to the octopamine receptors found on the extensor tibiae muscle (Evans & O'Shea, 1978; Evans, 1981) and on the visceral muscle in the ovarioles (Orchard & Lange, 1985) than it does to those receptors which are responsible for the excitation-induced hypertrehalosemic response (EXIT) of the cockroach (Downer, 1979) and to those responsible for the modulation of the Schwann cell membrane potential of the squid

giant axon (Reale, Evans & Villegas, 1985) where synephrine is an ineffective agonist. As observed for all other octopamine receptors, those on the dorsal longitudinal muscles of the locust are stereospecific for the D(−)isomer of octopamine (see Evans, 1985*a*) which has been demonstrated to be the naturally occurring isomer of octopamine in the locust (Goosey & Candy, 1980).

The present study has shown that elevations in the levels of octopamine in insect haemolymph during the first few minutes of flight (Goosey & Candy, 1980; Bailey *et al.* 1983) to values of $2\times10^{-7}$ mol $l^{-1}$ would be sufficient to affect the contractile properties of the dorsal longitudinal flight muscles since the threshold for potentiation of twitch tension by octopamine lies between $10^{-9}$ and $10^{-8}$ mol $l^{-1}$. However, any modulation of the contractile properties of flight muscle by circulating octopamine would clearly be secondary to the effects produced by octopamine which is proposed to be released locally in the flight muscles resulting from the activation of DUMDL. Indeed, the former increase in haemolymph levels may well be a secondary spill over effect during the first few minutes of flight resulting from the activation of DUMDL and any other octopaminergic neurones which innervate flight muscles. In the present study we have presented physiological and pharmacological evidence for the octopaminergic nature of DUMDL. Stimulation of DUMDL mimics the effects of superfusing pulses of octopamine over the surface of the dorsal longitudinal flight muscle both on the properties of neurally evoked twitches and on tetanic contractions. The effects are dependent on the frequency of DUMDL stimulation and plateau in the range of 5–10 Hz, which is similar to the frequency-dependent responses of DUMETi described previously (Evans, 1984; Morton & Evans, 1984). In addition, the effects of DUMDL were also blocked by phentolamine, but not by propranolol, in a fashion similar to the effects of octopamine on the dorsal longitudinal muscle. Thus DUMDL is likely to be an octopaminergic neurone with modulatory properties similar to those described previously for other octopamine-containing DUM cells (see Introduction for references).

In behavioural terms the release of octopamine onto the dorsal longitudinal muscles during the first few minutes of insect flight will have several important consequences. First, it will stimulate carbohydrate metabolism which is the main source of energy for the first few minutes of locust flight (Candy, 1978). Second, it will increase the amount of force generated by the flight muscles which could be important when the insect initially takes off from rest. Third, when the locust initially takes off after a period of rest its body temperature is likely to be low (around 25 °C) and owing to the overlap in the duration of the twitches in antagonistic muscles it will waste a considerable amount of energy. This will continue until the locust reaches its normal internal body flight temperature of 30 °C when the duration of the twitches is reduced significantly and there is very little overlap of the twitches between antagonistic muscles (Neville & Weis-Fogh, 1963; Wilson, 1968). Control experiments indicate that the locust dorsal longitudinal flight muscles are still responsive to octopamine application at temperatures up to 30 °C. At this elevated temperature a given dose of octopamine produces percentage increases in twitch

amplitude, contraction rate and relaxation rate of an equivalent size to those reported in the present paper for experiments carried out at 20°C (M. D. Whim & P. D. Evans, unpublished results). However, at 30°C octopamine application does not cause any significant further decrease in twitch duration above that induced by the elevated temperature alone, since at this temperature the decrease in twitch duration due to the increased relaxation rate is exactly balanced by the increase in twitch duration due to the increase in twitch amplitude. Thus the release of octopamine during the first few minutes of locust flight is ideally suited to reduce this waste in energy until the locust warms up. By increasing the rate of relaxation of tension, octopamine will reduce the duration of the muscle twitch and so lower the degree of overlap of the contractions in antagonistic muscles whilst also increasing the force generated by each twitch. In this context Hoyle & Dagan (1978) noted that in reflex actions, DUM neurones tend to fire before the motor output occurs, so priming the muscle for activation; similarly Evans & Siegler (1982) suggested that the peripheral actions of octopamine released from DUM cells would convert skeletal muscles from a postural to a dynamic response mode. In conclusion, the results of the present study suggest that the peripheral release of octopamine from DUMDL during the first few minutes of locust flight is likely to be an important modulatory factor influencing the kinetics of the contraction of dorsal longitudinal flight muscles of the locust and may represent an energy-saving adaptation.

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