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OCTOPAMINE MEDIATED RELAXATION OF MAINTAINED AND CATCH TENSION IN LOCUST SKELETAL MUSCLE

BY PETER D. EVANS* AND MELODY V. S. SIEGLER†

*From the *A.R.C. Unit of Invertebrate Chemistry and Physiology, and †Dept. of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ*

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SUMMARY

1. The modulatory actions of an identified octopaminergic neurone (DUMETi) that projects to the extensor–tibiae muscle of the locust hind leg depend upon the frequency of stimulation of the slow motoneurone (SETi) to this muscle.

2. At low frequencies of SETi stimulation (1 Hz and below) the predominant modulatory effects are increases in the amplitude and relaxation rate of twitch tension. At higher frequencies, where twitches summate but tetanus is incomplete (up to 20 Hz), the reduction of maintained tension becomes considerably more important.

3. Both octopamine application and DUMETi stimulation reduce the amount of catch tension displayed by the extensor muscle when SETi is fired in a variety of different stimulus patterns. The extensor–tibiae muscle is itself 'pattern sensitive' since is shows a 'positive spacing effect' when SETi is stimulated at an average frequency of 1 Hz.

4. It is suggested that a primary function of DUMETi is to change the response of the muscle from one that favours maintenance of posture to one that favours rapid changes in joint position or force, such as might occur during locomotion.

INTRODUCTION

Biogenic amines modulate neuromuscular transmission and muscle contraction in a wide variety of vertebrate (Bowman & Nott, 1969) and invertebrate species, including crustaceans (Kravitz, Glusman, Harris-Warrick, Livingstone, Schwarz & Goy, 1980), molluscs (Twarog & Muneoka, 1973; Weiss, Cohen & Kupfermann, 1978) and insects (Evans, 1980).

In the locust the biogenic amine octopamine is contained in a group of neurones that project to skeletal muscles (Hoyle, 1975; Hoyle & Barker, 1975; Evans & O'Shea, 1977, 1978). Those in the third thoracic ganglion, which controls the hind legs, have their cell bodies clustered dorsally at the mid line of the ganglion. One of these neurones has axons that project bilaterally to the extensor–tibiae muscles of the hind legs, and has been designated DUMETi (Dorsal Unpaired Median cell to Extensor–Tibiae muscle) (Hoyle, Dagan, Moberly & Colquhoun, 1974). In addition to DUMETi, only three other neurones are thought to innervate the extensor muscle (Pearson &

Bergman, 1969; Hoyle & Burrows, 1973) and each of the four neurones can be activated independently by stimulating the appropriate nerves.

The extensor muscle has provided an ideal preparation for studying the effects of octopamine on neuromuscular transmission. Stimulation of DUMETi, or exogenous application of octopamine to the extensor muscle, potentiates the peripheral effects of firing the slow motoneurone, SETi. The amplitude of the evoked excitatory junction potentials (e.j.p.s), and of the individual twitch contractions in the muscle are increased (Evans & O'Shea, 1977; O'Shea & Evans, 1979). The twitches also reach a higher absolute peak tension, even though maintained or basal tension may decline slightly at the same time (O'Shea & Evans, 1979). In addition, each twitch has an increased rate of contraction (Buchan & Evans, 1980) and of relaxation (O'Shea & Evans, 1979).

Recent studies have revealed the sensitivity of the latter response (Buchan & Evans, 1980; Evans, 1981*a*). Not only are the changes in the relaxation rate of twitch tension proportionally greater than changes in twitch height and rise time, they also appear more rapidly, and have a lower threshold (Evans & Gee, 1980; Evans, 1981*a*). These observations prompted us to consider the possibility that DUMETi, and octopamine, might affect yet other properties of the extensor muscle, not previously examined. It seemed likely, for example, that they would change the muscle's ability to develop or maintain tetanic tension. In particular, the catch property of the muscle might be altered (Wilson & Larimer, 1968; Wilson, Smith & Dempster, 1970). Accordingly, the slow motoneurone to the extensor was stimulated at frequencies that resulted in maintained tension rather than twitches, and in patterns that revealed the catch property of the muscle. The tension evoked by SETi after the DUMETi neurone was stimulated, or when octopamine was applied exogenously, was compared with that before treatment. A brief account of some of this work has been given elsewhere (Evans, 1981*b*).

METHODS

Adult locusts (*Schistocerca americana gregaria*, formerly *S. gregaria*) of either sex were obtained from crowded laboratory colonies fed on wheat seedlings. Animals were removed from the main colony and kept isolated for 1–2 hours before use. When animals are used directly from the main colony, many show reduced responses to the application of octopamine and the stimulation of DUMETi. This is likely to be due to the persisting effects of an endogenous activator of octopamine receptors, probably octopamine itself (see Evans, 1981*a*).

Tension in the extensor–tibiae muscle of a hind leg was measured almost isometrically with a tension transducer attached distally to the apodeme. The slow extensor–tibiae motoneurone (SETi) and the common inhibitor (CI), which has a branch to the extensor muscle, were excited by stimulating the appropriate peripheral nerves through pairs of silver hook electrodes. The axons of both neurones reach the muscle via nerve 3b, but since that of SETi is of larger diameter it can be stimulated without recruiting CI. The latter neurone also has axons in nerves 3a and 3c, and antidromic stimulation of either axon evoked orthodromic impulses of the axon in nerve 3b. DUMETi, the modulatory neurone to the extensor–tibiae muscles, has two axons, which are bilaterally symmetrical. Orthodromic impulses in one axon could be evoked by stimulating the other axon antidromically, through a pair of silver hook electrodes placed on the extensor–tibiae nerve $5b_1$ (nomenclature of Pringle, 1939). This allowed a higher frequency of impulses to be initiated in DUMETi axons than could be induced by depolarizing the DUMETi soma through an intracellular micro-electrode (O'Shea & Evans, 1979). The effects of DUMETi stimulation, and of octopamine application, were tested as described previously (Evans & O'Shea, 1978; O'Shea & Evans, 1979). In experiments where DUMETi was stimulated, 50 μl. of saline was applied to the

surface of the extensor muscle and replaced at 5 min intervals by appropriate solutions during the course of the experiment (see Evans, 1981*a*).

Pulse trains that were sinusoidally modulated in frequency were produced by driving a voltage-controlled oscillator with another function generator. Pulse trains in other patterns were computer-generated. In either case the pulse trains were used to trigger the output of a stimulator.

All drugs were dissolved in 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) and 90 mM-sucrose. DL-octopamine and picrotoxin were obtained from the Sigma Chemical Co. and phentolamine mesylate was a gift from CIBA.

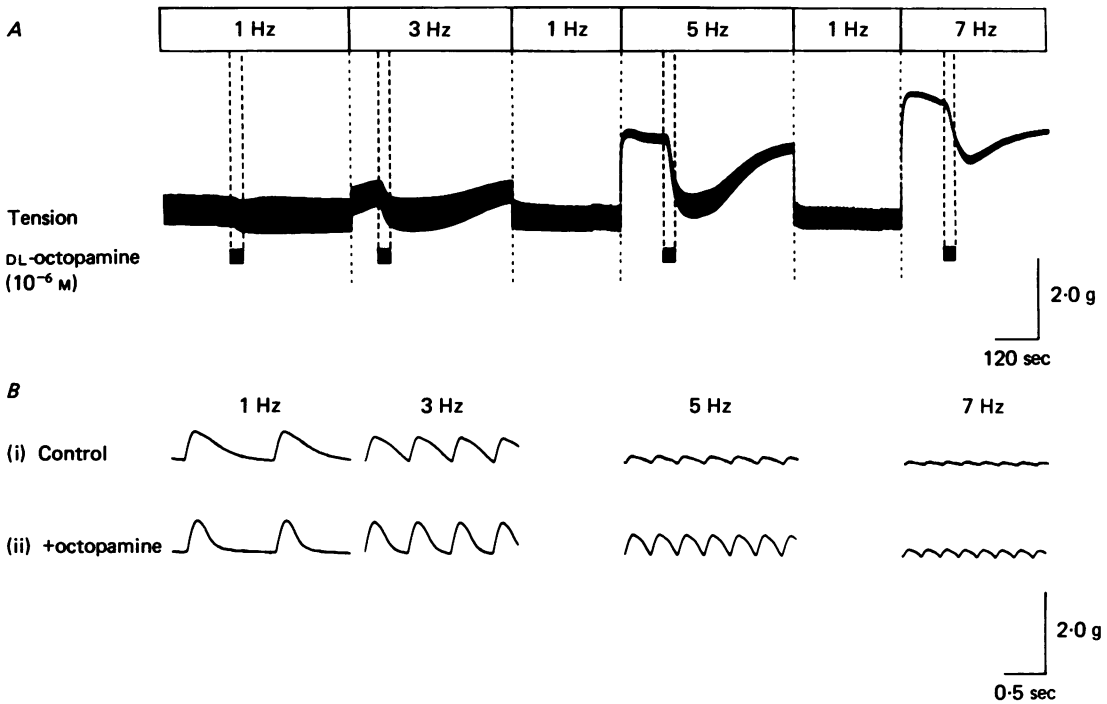

Fig. 1. Frequency dependence of octopamine effects on maintained tension. *A*, a continuous recording of the tension profile from a metathoracic extensor–tibiae muscle produced by firing SETi at different frequencies. 30 sec pulses of 10^{-6} M-DL-octopamine (bars) were introduced into the superfusate. The preparation was returned to 1 Hz stimulation for 5 min between the 3 and 5 Hz stimulation and also between the 5 and 7 Hz stimulation. The maximum reduction of maintained tension induced by octopamine application occurred at 5 Hz. *B*, selected portions of the recording in *A* on an expanded time base to show the individual tension transients (i) in normal saline and (ii) after octopamine application. Increasing the frequency of SETi stimulation reduces the size of the individual transients, whilst octopamine application increases their amplitude at each frequency, and the rates of contraction and relaxation.

RESULTS

Frequency dependence of responses

When SETi is stimulated at a frequency of 1 Hz, each stimulus produces a muscle twitch. By contrast, when SETi is stimulated at frequencies higher than 15–20 Hz (depending on the preparation), a smooth tetanic contraction results. Between these extremes stimulation produces an incomplete fusion of twitches. As the frequency

of stimulation increases, the individual tension transients decrease in size but the base line of maintained tension increases (Fig. 1).

To test the effects of octopamine upon maintained tension the SETi motoneurone was stimulated at several frequencies (Fig. 1*A*) and at each, a 30 sec pulse of DL-octopamine (10^{-6} M) was introduced into the muscle superfusate. The result depended upon the frequency at which the motoneurone was being stimulated. In

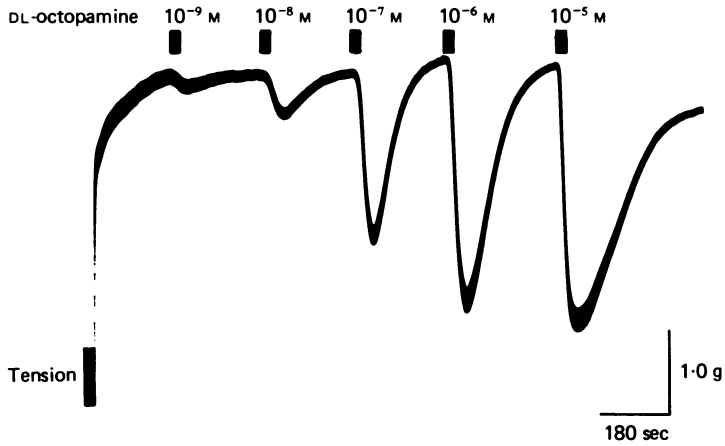

Fig. 2. Concentration dependence of octopamine effects on tension maintained in extensor muscle by firing SETi continuously at 7 Hz. 30 sec octopamine pulses of increasing concentration (bars) produce larger reductions in maintained tension. The initial tension maintained by firing SETi at 1 Hz is shown at the beginning of the trace.

the example show in Fig. 1 the effects of 10^{-6} M-DL-octopamine were maximal at 5 Hz stimulation of SETi. There was a pronounced decrease in maintained tension (Fig. 1*A*) although the individual tension transients were increased in height (Fig. 1*B*). At stimulus frequencies above 20 Hz (not shown), there was no change in muscle tension when 10^{-6} M-octopamine was applied. In all preparations the maximal effects were observed at SETi stimulation frequencies of 5–7 Hz.

The magnitude of the decline in maintained tension depended upon the concentration of DL-octopamine that was applied. In the example shown in Fig. 2, SETi was stimulated continuously at 7 Hz, and 30 sec pulses of octopamine of increasing concentrations were applied at intervals of about 4–5 mins. The threshold concentration was between 10^{-10} and 10^{-9} M, and the maximum response was obtained at 10^{-5} M. A similar, but less sensitive, concentration dependence of the maintained tension was noted previously in experiments where SETi was fired at 1 Hz (O'Shea & Evans, 1979).

The effects of DUMETi also depended upon the frequency at which the SETi motoneurone was stimulated. An experiment where DUMETi was stimulated at 10 Hz for 10 sec, at intervals, whilst SETi was stimulated in an increasing series of frequencies, is illustrated in Fig. 3. The effects of DUMETi stimulation were qualitatively similar to those of applying 10^{-6} M-octopamine, though less pronounced. In the example shown, DUMETi had maximal effects when SETi was stimulated at 7 Hz, and it had little effect at, or above, 10 Hz. In all preparations, the effects of

DUMETi were maximal between 5 and 7 Hz stimulation to SETi. The largest effects occurred at the lower frequencies in preparations where lower frequencies of SETi stimulation were needed to reach a smooth tetanic contraction.

As a further test of the effects of octopamine on muscle tension, SETi was stimulated every 60 sec with 10 sec long trains of pulses that were increased in

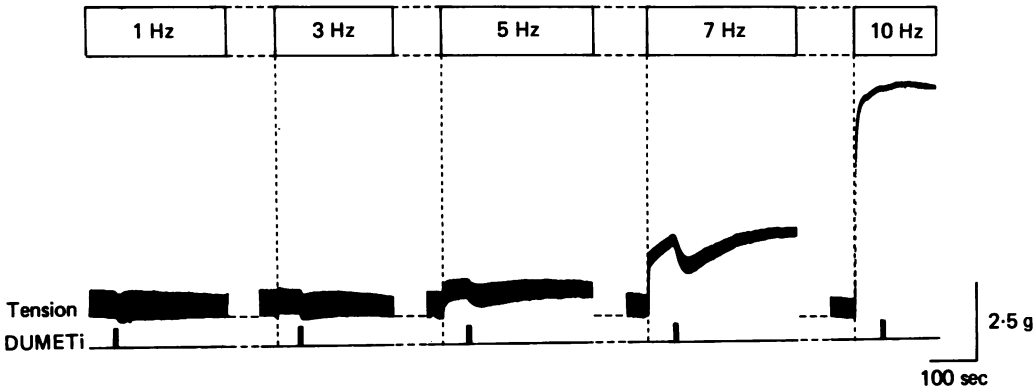

Fig. 3. Effect of firing DUMETi depends on frequency of SETi stimulation. DUMETi was fired for 10 sec bursts at 10 Hz (bars) at increasing frequencies of SETi stimulation. The preparation was allowed to recover for 10 min periods (horizontal dashed lines) between DUMETi bursts, during which time SETi was fired at 1 Hz.

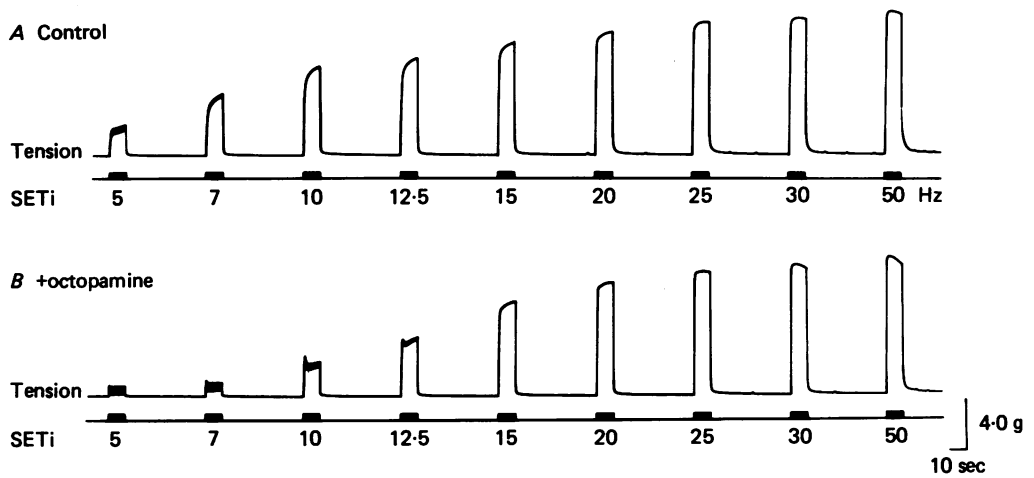

Fig. 4. Effects of 10^{-6} M-DL-octopamine on tetanic tension produced in extensor muscle by firing SETi for 10 sec at different frequencies. *A*, control series in saline before octopamine application. *B*, series repeated in presence of octopamine. Octopamine greatly reduces the height of tension developed at the lower frequencies, especially 7 Hz, but has proportionally less effect at the higher frequencies.

frequency stepwise from 5 to 50 Hz. The ability of the muscle to develop and maintain tension in normal saline was compared with that in the presence of 10^{-6} M-DL-octopamine (Fig. 4). In normal saline, tension gradually increased throughout each 10 sec stimulus, up to that of 30 Hz; at 30 and 50 Hz, tension declined after reaching a peak (Fig. 4*A*). In the presence of octopamine, significantly less tension was

developed during each 10 sec stimulation of SETi. This difference was greatest at the lowest frequency of stimulation, 5 Hz, and became less at each higher frequency, although in some preparations tension at 30 and 50 Hz was greater in octopamine. In all cases at 30 and 50 Hz, the decline from the peak tension was more abrupt than in normal saline. At the lower frequencies, 5, 7 and 10 Hz, initially there was an abrupt

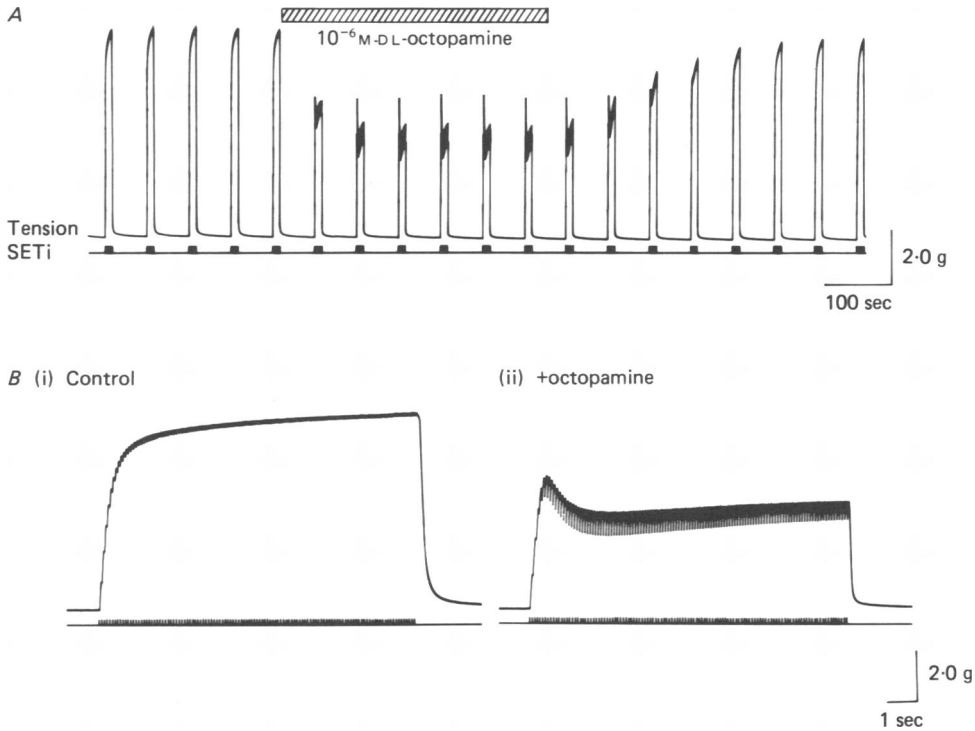

Fig. 5. Time course of octopamine effect and recovery in normal saline. *A*, the effect of a prolonged pulse of 10^{-6} M-DL-octopamine on tension in the extensor muscle produced by stimulating SETi for 10 sec bursts at 10 Hz once every minute (bars), and tension recovery in normal saline. Hatched bar indicates period of octopamine application. During the recovery phase in saline, the initial peak remains constant but the height of the plateau gradually increases. *B*, two selected pulses from *A* on an expanded time scale (i) before octopamine application and (ii) during octopamine application. Octopamine application reduces height of tetanic tension and reveals an initial tension peak. There is a more rapid development and relaxation of tension, and the tension developed after the first few impulses in the presence of octopamine is higher than that of the controls.

increase, then decrease in tension, with a more gradual increase in tension continuing throughout the remainder of the stimulus.

These effects reached a maximum within two minutes of the start of a prolonged pulse of 10^{-6} M-DL-octopamine (Fig. 5*A*). The recovery from these changes on return to normal saline was more gradual than the onset. Tetanic contractions before, and during, octopamine application are shown on an extended time course in Fig. 5*B* and *C*.

In a few preparations a small initial tension peak was present before octopamine

was applied, when the motoneurone was stimulated at frequencies of 5–10 Hz. Furthermore, the tension developed at the end of each 10 sec pulse was less than normal. These responses resemble those usually obtained after octopamine is applied and may represent the residual effects of the naturally released transmitter (see Evans, 1981*a*). There was also some variability amongst different preparations in the

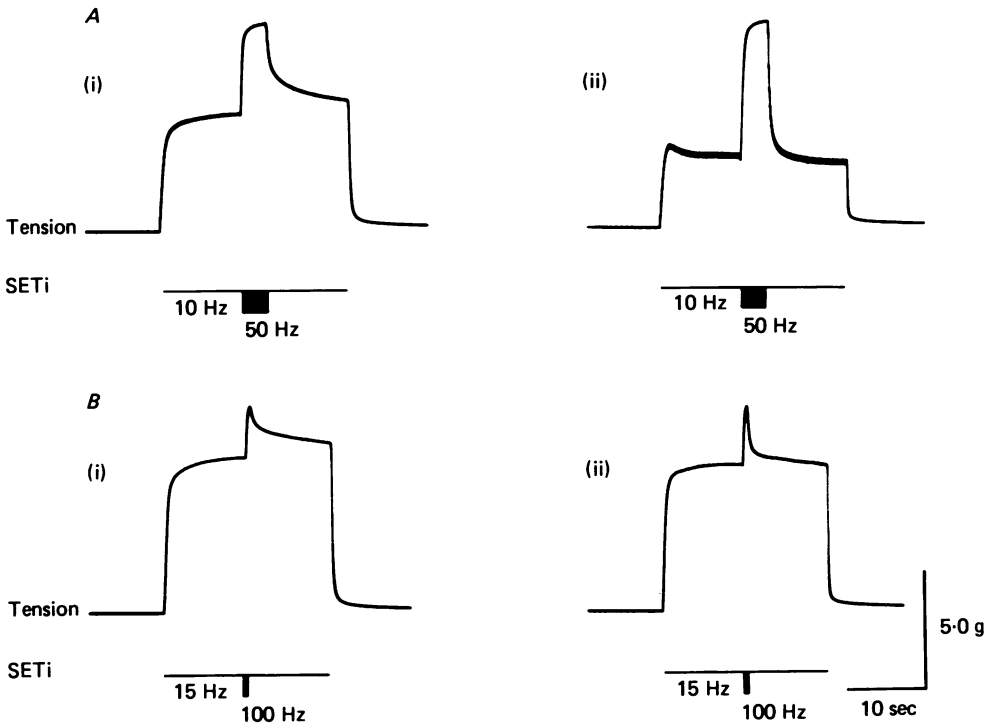

Fig. 6. Effect of octopamine on SETi-induced catch tension. *A*, Tension profiles produced by stimulating SETi continuously at 10 Hz except for an interposed period of 3 sec at 50 Hz (bar), (i) before and (ii) during the application of 10^{-6} M-DL-octopamine to the muscle. *B*, As in *A* except that SETi is stimulated continuously at 15 Hz with an interposed period of 0·5 sec at 100 Hz (bar). Octopamine reduces the catch tension generated under both stimulus regimes.

size of the initial tension peak; in exceptional cases it was much larger than the plateau of tension produced by the same frequency of stimulation before octopamine was applied (see Evans, 1981*b*).

Stimulation of DUMETi produced effects that were qualitatively similar to the effects of exogenously applied octopamine (not shown).

Effect of octopamine on catch tension

The effects of octopamine upon catch tension were tested by subjecting SETi to three different stimulus regimes. These were: (a) step increases and decreases in the frequency of the stimulus pulses, (b) sinsusoidal modulation of the frequency of the stimulus pulses, and (c) interpolation of single extra pulses in stimulus trains of different frequencies.

(a) Step changes in stimulus frequencies

If a higher frequency of stimulation to a motoneurone is briefly imposed upon a lower steady frequency, more tension is then maintained at the lower frequency. This effect, first described for crustacean muscles (Blaschko, Cattell & Kahn, 1931), is also exhibited by the extensor–tibiae muscle in the locust (Wilson & Larimer, 1968;

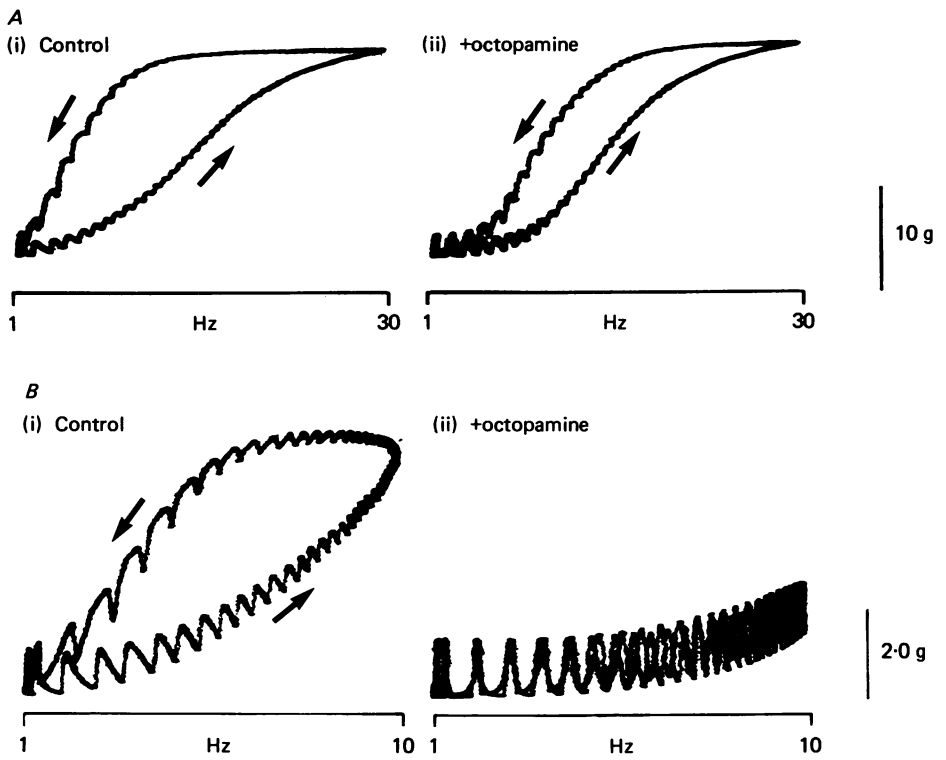

Fig. 7. Effect of octopamine on tension produced by firing SETi at sinusoidally modulated frequencies. *A*, Frequency tension loops obtained by modulating SETi stimulation frequencies between 1 and 30 Hz over a period of 10 sec (i) before octopamine and (ii) during application of 10^{-6} M-DL-octopamine. The loops are anti-clockwise and octopamine reduces the hysteresis of the loop. *B*, As in *A* except that tension is modulated between 1 and 10 Hz. Octopamine has a much larger effect in this frequency range, reducing peak tension at 10 Hz and almost completely closing tension loop.

Burns & Usherwood, 1978). Two examples of this are illustrated in Fig. 6. In the first, SETi was stimulated continuously at 10 Hz, except for a three second period when it was stimulated at 50 Hz. The tension maintained at 10 Hz after the 50 Hz pulse was greater than that before it (Fig. 6*A* (i)). When the stimulation pattern was repeated with the muscle superfusate containing 10^{-6} M-DL-octopamine the tension generated at 10 Hz was much reduced, and the catch tension previously observed after the 50 Hz step was abolished (Fig. 6*A*(ii)). In the second example SETi was stimulated continuously at 15 Hz, except for a period of 0·5 sec when it was stimulated at 100 Hz (Fig. 6*B*(i)). The catch tension induced by this firing pattern was abolished in the presence of octopamine (Fig. 6*B*(ii)).

(*b*) *Sinusoidal changes in stimulus frequencies*

When stimulation to SETi is modulated over sinusoidally varying frequencies, the change in tension in the extensor muscle is not sinusoidal. It shows considerable asymmetry or hysteresis, which is particularly clear when frequency-dependent tension loops are constructed (Wilson & Larimer, 1968). The response when SETi stimulation was modulated sinusoidally between 1 and 30 Hz is shown in Fig. 7*A*(i). There was considerably less tension in the muscle when the frequency of stimulation was increasing than when it was decreasing. When the muscle was superfused with 10^{-6} M-DL-octopamine, hysteresis was reduced (Fig. 7*A*(ii)). Tension developed more rapidly on the ascending arm of the loop, and relaxed more rapidly on the descending arm. There was, however no change in the peak tension developed. The two arms of the loop do not become symmetrical, but this would not be expected since there is necessarily a delay between the stimulation of SETi and the response of the muscle.

The shapes of the tension loops depend upon several factors, including the range of frequencies over which SETi stimulation is modulated (Wilson & Larimer, 1968). Octopamine appears to be particularly effective in reducing hysteresis that occurs at lower frequencies of SETi stimulation. For example, the hysteresis that occurs when SETi is stimulated at 1–10 Hz (Fig. 7*B*(i)) is almost completely abolished when octopamine is applied to the muscle (Fig. 7*B*(ii)). There is also a reduction in the peak tension, consistent with the effects of octopamine on tetanic tension (e.g. Fig. 4).

The stimulation of DUMETi at 10 Hz for 10 sec prior to SETi stimulation has a qualitatively similar effect on sinusoidally modulated frequency changes (not shown). The effect is slight when the SETi stimulation frequency is modulated between 1 and 30 Hz but pronounced when the SETi frequency is modulated between 1 and 10 Hz.

(*c*) *Effects of extra pulses on tension*

The addition of an extra pulse to a regular train of stimuli to a motoneurone produces an increase in maintained tension in crustacean and mammalian skeletal muscle (Blaschko *et al.* 1931; Wilson & Larimer, 1968; Burke, Rudomin & Zajac, 1970). This effect does not appear to be due to the properties of the motor axon or the neuromuscular junction, and hence is considered another expression of the catch property of muscle (Wilson & Larimer, 1968; Burke *et al.* 1970).

Before examining this effect in the extensor–tibiae muscle, we tested the response of the muscle when paired pulses were delivered to SETi. The average frequency of stimulation was maintained at 1 Hz, but the interval between pairs was gradually reduced. The interval that produced the greatest extra tension in the muscle was 20 msec. At shorter intervals the effect of the second pulse declined sharply. In the presence of 10^{-6} M-DL-octopamine, the peak tensions were increased, but for all intervals the percentage increase in tension produced by the second pulse was less. Despite these changes, the stimulus interval that produced the largest effect remained the same at 20 msec.

These experiments were performed primarily to determine at what interval to introduce a single extra SETi pulse into a train to produce the maximum effect upon tension (see below). By their design they also tested whether the extensor muscle was 'pattern sensitive' or 'pattern insensitive'. According to the criteria of Wiersma &

Adams (1950) and Ripley & Wiersma (1953) the muscle was 'pattern sensitive', because the response to a given frequency of stimulation differed in magnitude according to whether the pulses were spaced uniformly, or paired. It also showed a 'positive spacing effect' at 1 Hz, in that greater tension was developed when the interval between pulses was shortened (Wiersma & Adams, 1950). For example, two pulses 20 msec apart produced a peak tension that was nearly three times that produced by each equally spaced pulse at an average frequency of 1 Hz.

The approach used to test the tension effects of additional pulses was similar to that of Burke *et al.* (1970) when studying the catch property of mammalian motor units. In the present experiments, SETi was stimulated with 2·5 sec long trains of pulses of several different frequencies. For each frequency, the resulting tension was compared with that produced when the first pulse of the train was followed 20 msec later by an extra pulse. Selected frequencies are shown in Fig. 8*A*. Traces of a pair were superimposed, and additional tension was measured as the area between the two. The extent to which an extra pulse enhanced tension depended upon the frequency at which the motoneurone was being stimulated (Fig. 8*B*). Of the frequencies tested, the peak effect occurred when SETi was being stimulated at 7·4 Hz. At frequencies above this, the extra tension produced declined gradually. The sequence of stimulus pulses was then repeated, in the presence of 10^{-6} M-DL-octopamine. The extra stimulus pulse again resulted in tension enhancement, but the peak effect now occurred at 13·4 Hz (Fig. 8*B*). Around this frequency, more extra tension was produced in the presence of octopamine than in normal saline. By contrast, at frequencies below 12 Hz, considerably more extra tension was produced in normal saline.

A comparison of the effects of DUMETi and CI on maintained tension

Activation of the common inhibitory neurone (CI) to the metathoracic extensor–tibiae muscle reduces the amount of tension maintained by SETi stimulation (Usherwood & Runion, 1970; Hoyle, 1978*a*) and has also been suggested to produce a slow reduction of catch tension in the corresponding muscle of the mesothoracic leg (Burns & Usherwood, 1978, 1979). It was thus possible that the effects described in the present paper might be mediated indirectly, through the activation of CI and the release of γ-aminobutyric acid (GABA) from its terminals.

Therefore the effects of stimulating DUMETi and CI were compared in the same preparations (Fig. 9). When SETi was fired at 7 Hz, stimulation of DUMETi at 10 Hz for 10 sec reduced maintained tension to a greater degree, and for a longer time than did stimulation of CI at 50 Hz for the same length of time. The effects of DUMETi were abolished by cutting nerve 5 but not by cutting nerve 3 close to the metathoracic ganglion, indicating that the DUMETi neurone was not mediating its effect through CI. In addition, the effects of octopamine and of DUMETi on maintained tension were antagonized specifically by 10^{-5} M-phentolamine, an α-adrenergic blocking agent, but were unaffected by 5×10^{-4} M-picrotoxin, a GABA blocking agent.

Thus the effects of DUMETi and octopamine on maintained tension are mediated by specific octopamine receptors (see Evans, 1981*a*) rather than through the direct or indirect activation of GABA receptors.

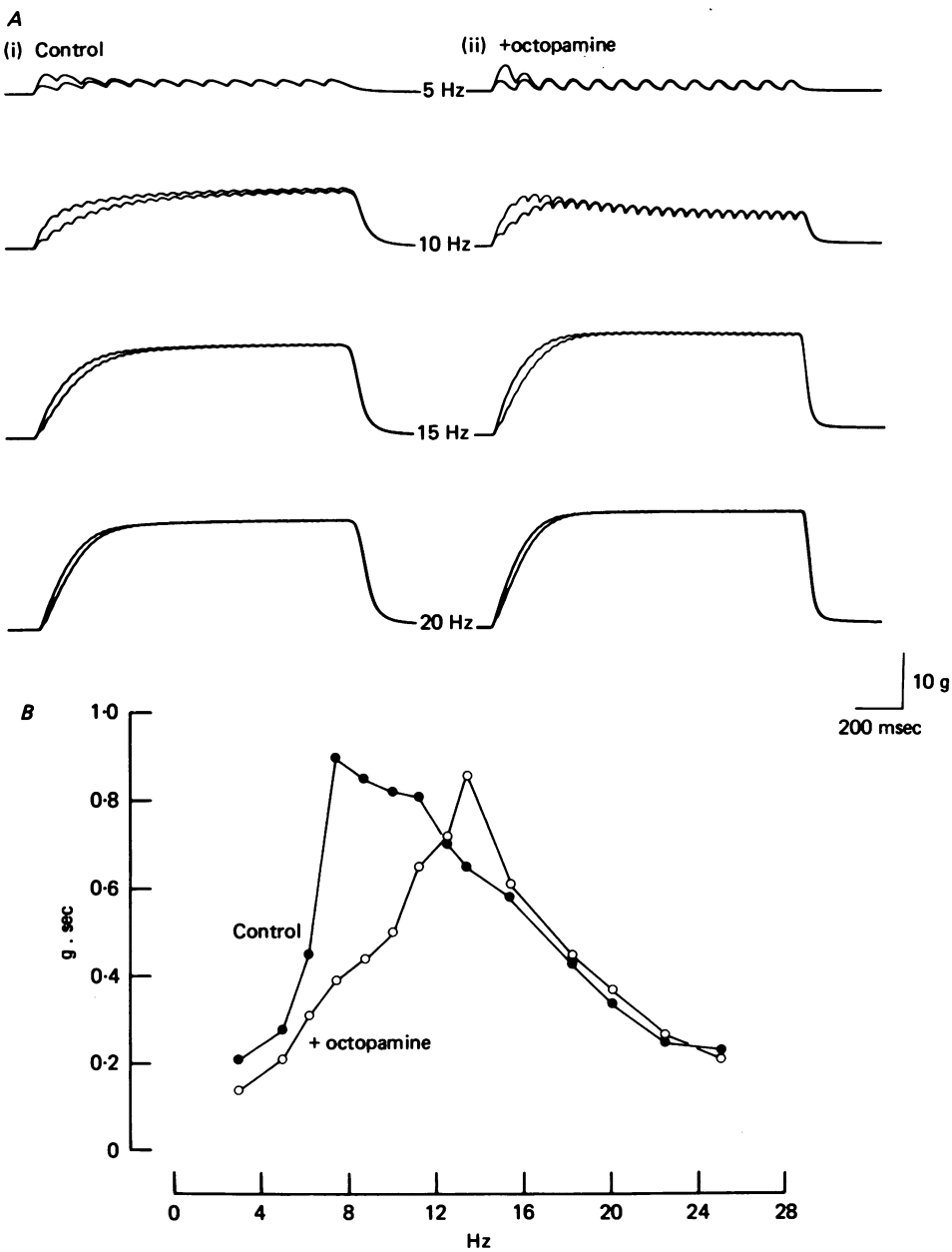

Fig. 8. Effect of extra pulse at start of train. *A*, (i) lower traces of each pair show tension profiles recorded from extensor-tibiae muscle at SETi stimulation frequencies indicated. Upper traces show the result when a single extra stimulus is introduced into the train 20 msec after the first pulse. The extent and duration of the tension enhancement varies with the frequency of SETi stimulation. (ii) as for (i) above except in presence of 10^{-6} M-DL-octopamine. There is a more rapid development of tension and initially higher tension peak (cf. Fig. 5*B* (i) and (ii)). *B*, tension enhancement expressed as area between tension curves with and without extra pulse plotted against basic frequency of SETi stimulation in presence (open circles) and absence (●) of 10^{-6} M-DL-octopamine. Octopamine depresses tension enhancement at lower frequencies but increases it at 15·4 Hz.

Effects of octopamine on tension during stimulation of SETi in stepping pattern

One way to assess the behavioural significance of the effects of octopamine would be to stimulate SETi and CI in a pattern that corresponded to one that occurred during behaviour. The pattern of SETi and CI activity in a free-walking locust has been reported by Burns & Usherwood (1979). We took the pattern of spikes during one complete step (shown in their Fig. 3) and repeated it at an appropriate interstep

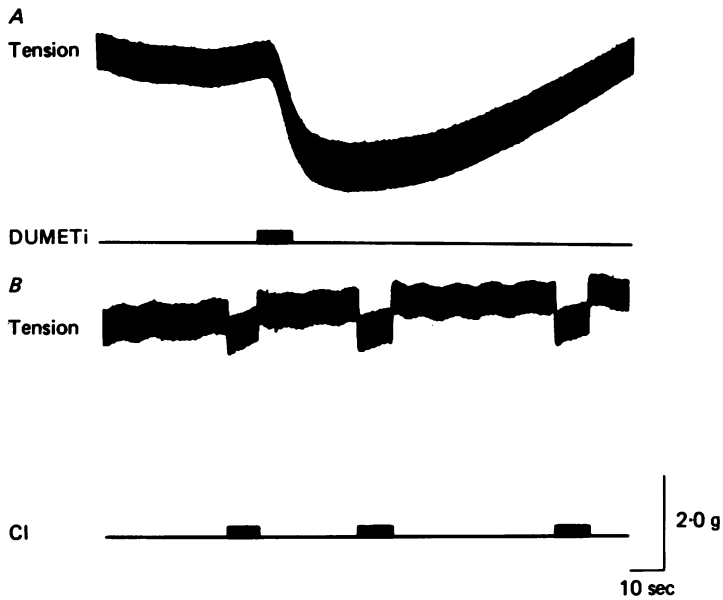

Fig. 9. Comparison of the effect of DUMETi and CI stimulation on tension maintained in the extensor muscle by firing SETi at 7 Hz. In *A*, DUMETi was stimulated for 10 sec at 10 Hz (bar). In *B*, CI was stimulated for 10 sec at 50 Hz (bars). DUMETi stimulation caused a larger and longer lasting decrease in maintained tension than that due to CI stimulation.

interval, to produce a five step sequence of stimuli to SETi and CI. The result of patterned stimuli to SETi alone is shown in Fig. 10; there was little change in tension when patterned stimuli to CI were added (not shown). The tension produced by stimulating SETi alone was compared with that produced in the presence of exogenously applied octopamine (Fig. 10*A*), or after DUMETi had been stimulated for 30 sec at 10 Hz prior to SETi activation (Fig. 10*B*). The effects of these two treatments were qualitatively similar: the amount of tension maintained between the trains of SETi activation was reduced, and there was an increase in the rate at which tension was developed, and relaxed. There was, however, no change relative to base line in the peak tension reached during each step. Thus octopamine and DUMETi stimulation both serve to increase the overall size of the individual tension profiles associated with a single step produced by the extensor–tibiae muscle due to this pattern of SETi stimulation.

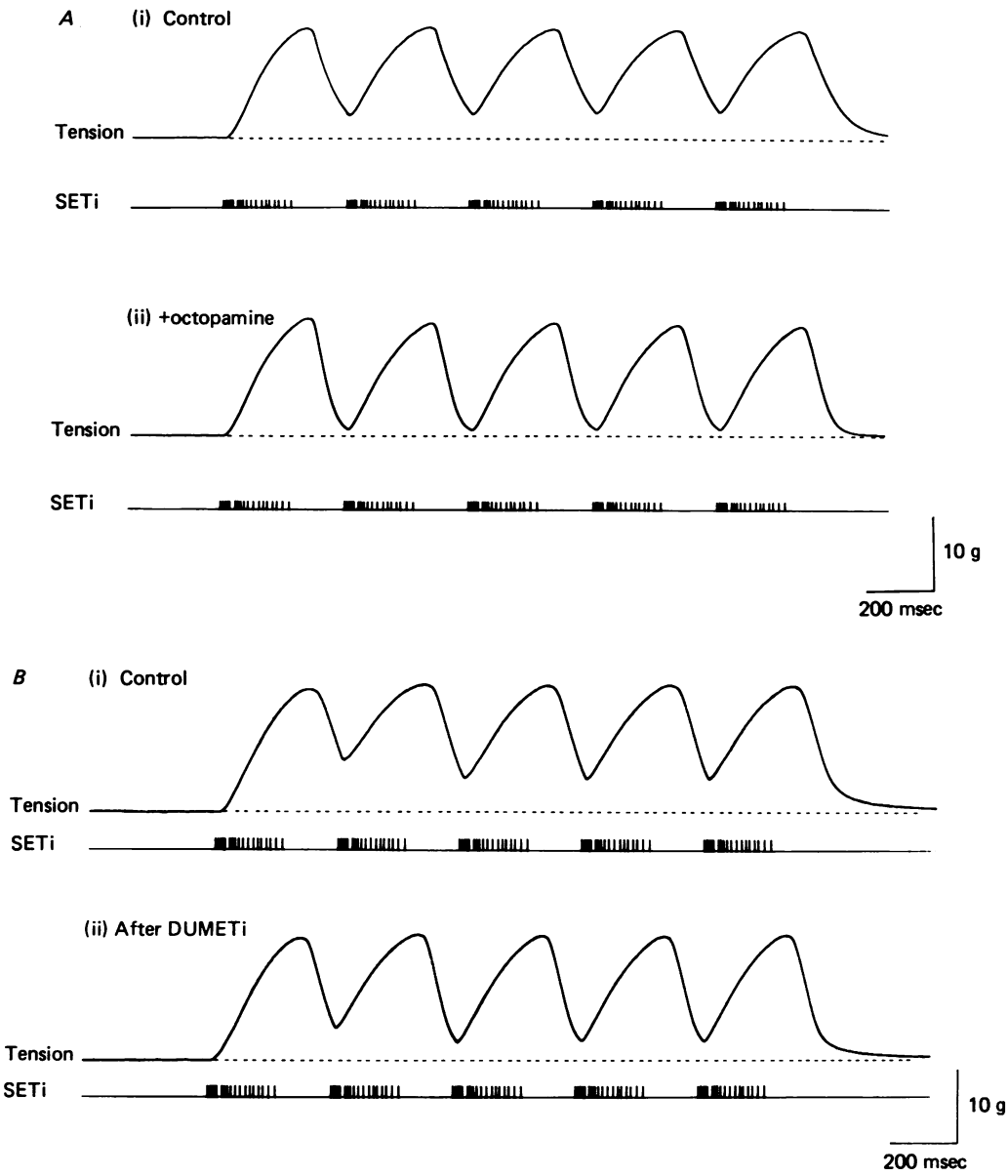

Fig. 10. Tension profiles in response to five cycles of SETi stimulation in a pattern derived from walking animal (see text for details). *A*, profile in (i) absence and (ii) presence of 10^{-6} M-DL-octopamine in muscle superfusate. There is a more rapid rise and fall of tension in presence of octopamine, and a reduction in the tension maintained between steps. *B*, profiles (i) before and (ii) after stimulation of DUMETi for 30 sec at 10 Hz. Effects are qualitatively similar to those of octopamine application.

DISCUSSION

Effects depend upon frequency and history of SETi stimulation

The effects of DUMETi, and of exogenously applied octopamine upon tension in the extensor–tibiae muscle, depend upon the frequency at which the slow motoneurone, SETi, is stimulated. At low frequencies, around 1 Hz, there is an increase in the height of the individual twitches, and a reduction of maintained or base line tension (Evans & O'Shea, 1977; O'Shea & Evans, 1979). The reduction of base line tension is relatively small and variable, and it is the increase of twitch height and relaxation of twitch tension that predominate (O'Shea & Evans, 1979; Buchan & Evans, 1980). By contrast, at higher frequencies, where twitches summate but tetanus is incomplete, the reduction in maintained tension becomes considerably more important. This effect is greatest when SETi is stimulated at around 7 Hz, either continuously (Figs. 1 and 3), or in short trains of pulses of constant frequency (Fig. 4). The effects of octopamine decline sharply at higher frequencies, and are almost negligible when tension becomes tetanic. This latter observation is consistent with those of May, Brown & Clements (1979), who failed to observe a potentiating effect of octopamine on SETi-induced tetanic tension, elicited by firing the motoneurone at 10–15 Hz for 10–15 sec.

In the present experiments, preparations differed in the degree to which tension summated at different frequencies of SETi stimulation, and in the frequency of SETi stimulation at which the largest effects of octopamine were observed. It seems likely therefore that it was the amount of tension developed, rather than the exact frequency of stimulation, that determined where the peak response occurred.

Octopamine and DUMETi stimulation not only altered the level of tension that could be maintained by a steady SETi frequency, but also the rate at which tension developed or relaxed. There were small increases in the rate of development of tetanic tension, for example when SETi was stimulated at frequencies above about 15 Hz, during sinusoidal modulation at 1–30 Hz, and when stimulated in a pattern that resembled its natural sequence of firing during stepping. The predominant effect, however, was upon the rate of relaxation. Hysteresis in the tension response to sinusoidally modulated stimuli could be abolished or reduced, depending upon the peak frequency of stimulation. This was largely due to the more rapid relaxation of tension during the decreasing half of the cycle. Similarly, relaxation of tension occurred more rapidly after step decreases in stimulus frequency, and hysteresis was again reduced. When SETi was stimulated in a stepping pattern, less tension was maintained between cycles. Thus the muscle would offer less resistance to successive flexions in the intact animal and the overall tension change during a single step would be increased.

Hysteresis in the extensor muscle is thought to arise primarily because of the 'catch' property of the muscle, rather than through effects at the neuromuscular junction (Wilson & Larimer, 1968; Wilson *et al.* 1970). The responses of the locust extensor muscle closely resemble those in other muscles of the crayfish and barnacle, and in the latter two cases it has been possible to examine directly the relationship between muscle membrane potential and muscle tension. When single muscle fibres of crayfish or barnacle were stimulated intracellularly with sinusoidally varying

current, or steps of current, there was significant tension hysteresis even though the membrane response was linear. In the crayfish claw, when the excitatory axon was stimulated sinusoidally, there was some degree of hysteresis in the membrane potential of only those muscle fibres where there was facilitation of e.j.p.s. The membrane hysteresis was not sufficient, however, to account for that of the tension response (Wilson & Larimer, 1968). It is unlikely, therefore, that changes in the degree of hysteresis seen in the present study were brought about by changing the degree of facilitation at the neuromuscular junction. Instead, the effects of octopamine upon hysteresis seem largely to be due to specific changes in the catch property of the extensor muscle. This idea is further supported by the observations that catch tension, measured in the extra pulse experiments, is greatest at a background frequency of SETi firing of about 7 Hz (Fig. 8), and likewise, the effects of octopamine on maintained tension and catch tension are greatest around this same frequency (Figs. 1, 4, 7 and 8).

It should be noted that the catch property of the muscle is less pronounced under the isometric condition studied here than under the isotonic condition (Wilson & Larimer, 1968). The latter is probably closer to that obtained in the extensor muscle during locomotion, and hence the role of DUMETi in reducing catch tension may be greater than indicated here.

All of the present results can be explained by supposing that octopamine reduces the ability of the muscle to maintain tension, but does not change, or slightly augments, the ability of the muscle to develop tension initially. This is consistent with the observation that, during trains of stimuli, the initial peak tension in octopamine may be greater than that without octopamine. The different effects upon the development and maintenance of tension described here may reflect different sites of octopamine action, for octopamine has both pre- and post-synaptic effects upon twitch tension generated by firing SETi at low frequencies. The increase in the rate of tension development (Buchan & Evans, 1980) and the increase in twitch amplitude (Evans & O'Shea, 1977; O'Shea & Evans, 1979) are at least in part mediated by receptors on SETi terminals, though an additional post-synaptic effect has not been ruled out, whereas the increased rate of relaxation is mediated by receptors on the muscle (see also Evans, 1981*a*).

The simplest explanation for the present observations is that the increase in the rate of the tension development during higher frequencies of SETi stimulation (sinusoidal or stepping pattern) and during individual twitches arises through a common presynaptic mechanism. Similarly, relaxation of maintained tension, the reduction of catch tension, and the increased rate of relaxation of individual twitches may be related results of a single action of octopamine upon the muscle. The basis of catch tension in insect or other skeletal muscle has not been investigated, however, and is still in question for molluscan 'catch' muscle. The relative importance of these different effects upon muscle tension necessarily will depend upon the frequency and the pattern of firing in the SETi motoneurone. During behaviours such as walking and jumping, the firing frequency of SETi fluctuates over wide ranges, and can be considerably in excess of the low frequencies where individual twitches are produced (Runion & Usherwood, 1968; Heitler & Burrows, 1977; Burns & Usherwood, 1978). As a consequence, the peripheral effects of the DUMETi neurone and octopamine

upon muscle catch and relaxation are likely to be of greater behavioural significance than those upon the rate of tension development and twitch height.

Comparison with other amine mediated effects upon muscle tension

Biogenic amines have widespread and diverse effects upon both invertebrate and vertebrate muscle, and a detailed review of these effects is outside the scope of the present discussion. There are, however, a few preparations that are of particular interest, because of the marked parallels with the effects of octopamine upon the locust extensor muscle.

The anterior byssal retractor muscle of the mollusc *Mytilus* is a smooth muscle with extreme 'catch' properties, which can be reduced by the application of biogenic amines such as 5-hydroxytryptamine (5-HT) and dopamine, or by stimulating aminergic axons contained in the efferent 'relaxing nerves' (York & Twarog, 1973; Twarog & Muneoka, 1973; McLean & Robinson, 1978; Satchell & Twarog, 1979). The 5-HT-containing Retzius cells of the leech have similarly been reported to act as relaxing neurones for body wall muscles and to have prolonged effects (Mason, Sunderland & Leake, 1979). By contrast, in lobster skeletal muscle, which is not thought to receive any direct aminergic innervation, octopamine and 5-HT induce a maintained contraction in the muscle, whilst dopamine relaxes basal tension (Evans, Talamo & Kravitz, 1975; Kravitz, Battelle, Evans, Talamo & Wallace 1976; Kravtiz *et al.* 1980).

By comparison with molluscan catch muscle, the soleus muscle of the cat is more ordinary in its properties. It is a skeletal muscle containing a high proportion of slow contracting fibres, and therefore is obviously more similar in function to the locust extensor muscle than is molluscan catch muscle. In the soleus, the biogenic amine adrenaline has effects upon neurally evoked tension that are in several respects similar to those of octopamine upon the locust extensor muscle (Bowman & Zaimis, 1958). In the presence of adrenaline, individual twitches show a marked increase in the rate of relaxation. Submaximal tetanic tension developed at a particular stimulation frequency is decreased, and the individual tension transients are larger. In response to a train of pulses there is an initial peak, followed by a lower plateau. The effects of adrenaline upon tension decline as the frequency of stimulating pulses increases, with no effect apparent above a certain frequency. In contrast to the effects of octopamine in the locust, however, twitch tension decreases in amplitude and rise time. Furthermore, the effects are brought about in different ways. Mammalian skeletal muscle is thought to lack direct aminergic innervation (Bowman & Nott, 1969); by contrast, in the locust extensor muscle the octopaminergic terminals of DUMETi occur as blindly-ending neurosecretory terminals (Hoyle *et al.* 1974). Another difference is that the effects of adrenaline described above are mediated entirely post-synaptically through β-adrenergic receptors (Bowman & Nott, 1969; Kuba, 1970), although vertebrate skeletal muscle also possesses presynaptic α-adrenergic receptors which are primarily activated by noradrenaline (Kuba, 1970). In contrast, in the locust both pre- and post-synaptic octopamine receptors are involved (Evans, 1981*a*).

Like the locust extensor muscle, mammalian skeletal muscle, including the soleus, displays hysteresis and a catch property (Partridge, 1966; Burke *et al.* 1970). The

catch property, demonstrated by extra pulse experiments (see Fig. 8), is particularly prominent in slow twitch motor units (Burke *et al.* 1970). It has been stated that the effect of adrenaline upon slow contracting skeletal muscles in mammals 'probably does have some physiological significance in relation to stress, although it is not clear what it is' (Bowman & Nott, 1969). Given the similarity of effects of adrenaline and octopamine in relaxing twitch and subtetanic tension one obvious possibility is that adrenaline, like octopamine, functions to reduce hysteresis that arises from the catch property of the muscle. This would allow the tension responses of the muscle to follow more closely rapidly changing motoneurone inputs.

Comparison of CI, FETi and DUMETi in the release of catch

In the pro- and mesothoracic extensor–tibiae muscles both CI and the fast extensor–tibiae motoneurone (FETi) may release SETi-generated catch tension (Burns & Usherwood, 1978, 1979). However, the actions of CI and FETi differ in several respects from those of DUMETi. Firstly, CI and FETi exert short term effects which necessarily depend closely upon the neurones' firing patterns relative to that of SETi, during walking for example (Burns & Usherwood, 1979). By contrast, DUMETi's effects are long term (minutes), and so it would not be necessary for DUMETi to fire in a particular pattern to reduce SETi-evoked catch tension. Secondly, FETi probably reduces SETi catch tension by mechanical means, transiently removing the load upon parallel SETi-innervated fibres (Burns & Usherwood, 1978). This would be comparable to the passive resetting of tension described by Wilson *et al.* (1970) in barnacle muscle. In any case, the effects are apparent only after FETi firing has stopped. By contrast, DUMETi's effects on catch are likely to be mediated through the actions of octopamine on the metabolism of the muscle fibres. Thirdly, the effects of FETi are necessarily restricted to behaviours where FETi is active. During walking, both FETi and SETi are active in the pro- and mesothoracic legs, but only SETi is active in the metathoracic legs (Burns & Usherwood, 1979). DUMETi's effects are not restricted in this way, and in principle could release not only SETi but also FETi-evoked catch tension (Wilson *et al.* 1970). The presence of DUMETi nerve terminals in muscle fibres innervated solely by FETi (Hoyle, 1978*b*) would also be consistent with this idea. Fourthly, CI causes relaxation of maintained tension by hyperpolarizing muscle fibres, and reducing the ability of SETi to depolarize them (Usherwood & Grundfest, 1965). Although DUMETi can cause a slight hyperpolarization of resting muscle fibres, SETi-evoked e.j.p.s are enhanced (Evans & O'Shea, 1977; O'Shea & Evans, 1979).

Function of DUMETi in behaviour

Although the catch property of the extensor muscle would be advantageous in maintaining posture, allowing greater tension to be maintained at low frequencies of SETi firing, it would be a hindrance during locomotion. Activity in DUMETi would provide a way for the muscle to be changed from one state to the other. This change would be particularly useful during behaviours such as walking, when in the metathoracic leg the firing frequency of the slow motoneurone is changing rapidly, but the fast motoneurone is inactive. The timing of DUMETi activity in relation to the initiation of extensor muscle activity, and DUMETi's responses to sensory

stimuli, reported by Hoyle & Dagan (1978), are appropriate to such a function. In a quiescent animal, DUMETi fired only sporadically. When the animal was aroused, however, the frequency of DUMETi firing increased. A wide variety of external stimuli could evoke spikes in DUMETi, and the duration and intensity of the burst increased with stronger stimuli. A burst of spikes preceded every movement that included brisk tibial extension. Although the timing of the burst was variable, it generally began some 300 msec or more before the tibial movement occurred. In the present study, DUMETi produced a detectable effect on SETi maintained tension (SETi fired at its most sensitive frequency of 7 Hz) at 500–700 msec after the DUMETi axon was stimulated in the contralateral leg. Therefore, in normal behaviour, DUMETi firing is timed appropriately to reduce the catch property of the extensor muscle when locomotion begins. The effects of DUMETi are long-lasting and would continue for some time during locomotion. Additional sensory stimuli during movement could evoke further spiking in DUMETi and in so doing prolong its effects upon muscle tension.

In conclusion, it seems likely that in the periphery a primary function of DUMETi, and of the DUM neurones that innervate other skeletal muscles, is to change the response of the muscle from one that favours maintenance of posture to one that favours rapid changes in joint position or force, such as might occur during locomotion. Whatever the pattern of motoneurone activity, the effect of DUM neurones would be to match the tension output of the muscle more closely to the neuronal input, and to reduce the influence of the muscle's history of excitation. These functions are consistent with the general idea, put forward by several authors, that octopamine and other biogenic amines contribute to arousal and increased behavioural responsiveness. The present results indicate how in the periphery this increased responsiveness might be brought about.

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