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THE ROLE OF CYCLIC NUCLEOTIDES AND CALCIUM IN THE MEDIATION OF THE MODULATORY EFFECTS OF OCTOPAMINE ON LOCUST SKELETAL MUSCLE

BY PETER D. EVANS

From the A.R.C. Unit of Insect Neurophysiology and Pharmacology, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ

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SUMMARY

1. The role of cyclic AMP and calcium in the mediation of the effects of octopamine has been investigated in the extensor tibiae muscle of the hind leg of the locust.

2. Elevation of cyclic AMP levels in the preparation by means of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX), by means of the diterpene adenylate cyclase activator, forskolin, and by means of cyclic nucleotide analogues mimics the post-synaptic effects of octopamine application at different frequencies of neuronal stimulation. These conditions also mimic the presynaptic effects of octopamine on spontaneous release of transmitter from the slow motoneurone.

3. The effects of octopamine on the preparation are calcium sensitive, with the maximal sensitivity occurring between 0·5 and 4·0 mM-external calcium.

4. The results are discussed in terms of the role of cyclic AMP and calcium in the mediation of the effects of octopamine.

INTRODUCTION

An identified octopamine-containing modulatory neurone functions to increase the level of cyclic AMP in the extensor tibiae muscle of the locust hind leg (Evans, 1984). Unlike the motoneurones to this muscle the modulatory neurone does not form discrete synaptic junctions with the muscle fibres, but rather ends as blind neurosecretory terminals in the outer layers of the sarcolemmal complex of the muscle fibres (Hoyle, Colquhoun & Williams, 1980). The octopamine release from these modulatory terminals acts as a local neurohormone which activates multiple classes of octopamine receptors, some of which are located presynaptically on motoneurone terminals and others of which are located post-synaptically at extrajunctional locations on the muscle surface (Evans, 1981). The different receptor classes have been characterized pharmacologically (Evans, 1980, 1981).

The presynaptic octopamine receptors (class 2A) function to increase the spontaneous and neurally evoked release of transmitter from the slow motoneurone to this muscle (O'Shea & Evans, 1979; Evans, 1981). This contributes to the potentiation of twitch tension by octopamine (Evans & O'Shea, 1977; O'Shea & Evans, 1979). The post-synaptic octopamine receptors (class 2B) serve to increase the rate of relaxation

of twitch and tetanic tension (O'Shea & Evans, 1979; Evans & Siegler, 1982) but may also contribute to the potentiation of twitch tension. It is likely that changes in the relaxation rate induced by octopamine in this muscle are of much more physiological significance than the effects on twitch amplitude (Evans & Siegler, 1982). A similar conclusion has also been drawn for the modulatory actions of catecholamines on vertebrate skeletal muscle (Bowman, 1982).

Thus changes in cyclic AMP level induced by octopamine in the extensor muscle could result from the activation of both pre- and post-synaptic receptors. Undoubtedly the major changes in cyclic AMP levels occur post-synaptically in the muscle, since this represents a far larger proportion of the tissue than the presynaptic terminals of the motoneurone (Evans, 1984). However, it is not possible to determine from biochemical measurements alone whether any changes are due to the activation of the presynaptic octopamine receptors. Furthermore, additional evidence, other than the fact that a neuromodulator mediates changes in cyclic AMP levels, is required before it can be concluded that cyclic AMP directly mediates the effects of the neuromodulator.

The involvement of cyclic AMP in the mediation of both the pre- and post-synaptic actions of octopamine can, however, be investigated in physiological studies where the receptor-activation stage of the process is bypassed by artificially elevating the cyclic AMP levels. Such experiments should mimic the actions of the activation of adenylate cyclase activity by octopamine receptors, if cyclic AMP is directly involved in mediating the responses of these receptors. This criterion is one of those generally included in lists of experimental evidence needed for the acceptance of cyclic nucleotide changes as the direct mediators of a neurotransmitter action (Robison, Butcher & Sutherland, 1971; Beam & Greengard, 1976). However, recent evidence suggests that care must be taken to ensure that cyclic nucleotide levels are not being changed indirectly by the actions of the drugs used, such as methylxanthines and cyclic nucleotide derivatives, on adenosine receptors (Wolff, Londos & Cooper, 1981).

In the present study the actions of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX), the diterpene adenylate cyclase activator, forskolin, and a series of cyclic AMP analogues have been assessed for their ability to mimic the pre- and post-synaptic actions of octopamine on the extensor tibiae muscle of the locust. In addition, the calcium sensitivity of octopamine responses has been examined. The results are discussed in terms of the role of cyclic AMP and calcium in the mediation of the octopamine responses of the extensor muscle.

METHODS

Adult locusts (*Schistocerca americanca 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 h before use to reduce any persisting effects of endogenous activators of octopamine receptors (see Evans, 1981).

Tension in the extensor tibiae muscle of a hind leg was measured almost isometrically with a tension transducer attached to the distal apodeme. The slow extensor tibiae motoneurone (SETi) was excited through a pair of silver hook electrodes on nerve 3b (nomenclature of Pringle, 1939) (see O'Shea & Evans, 1979; Evans & Siegler, 1982). An operational amplifier signal differentiator was used to measure continuously the rates of contraction and relaxation of neurally evoked tension (Buchan & Evans, 1980). Miniature end-plate potentials were recorded intracellularly from extensor

tibiae muscle fibres using micro-electrodes filled with 2 M-potassium acetate which had d.c. resistances in saline of 15–30 MΩ. The majority of the recordings were made from the muscle fibres of the distally located accessory extensor bundle which are innervated only by SETi and the common inhibitor (CI) (Hoyle, 1978; Evans & O'Shea, 1978). Recordings were also made from slow fibres innervated only by SETi and CI in other parts of the leg (Evans, 1981).

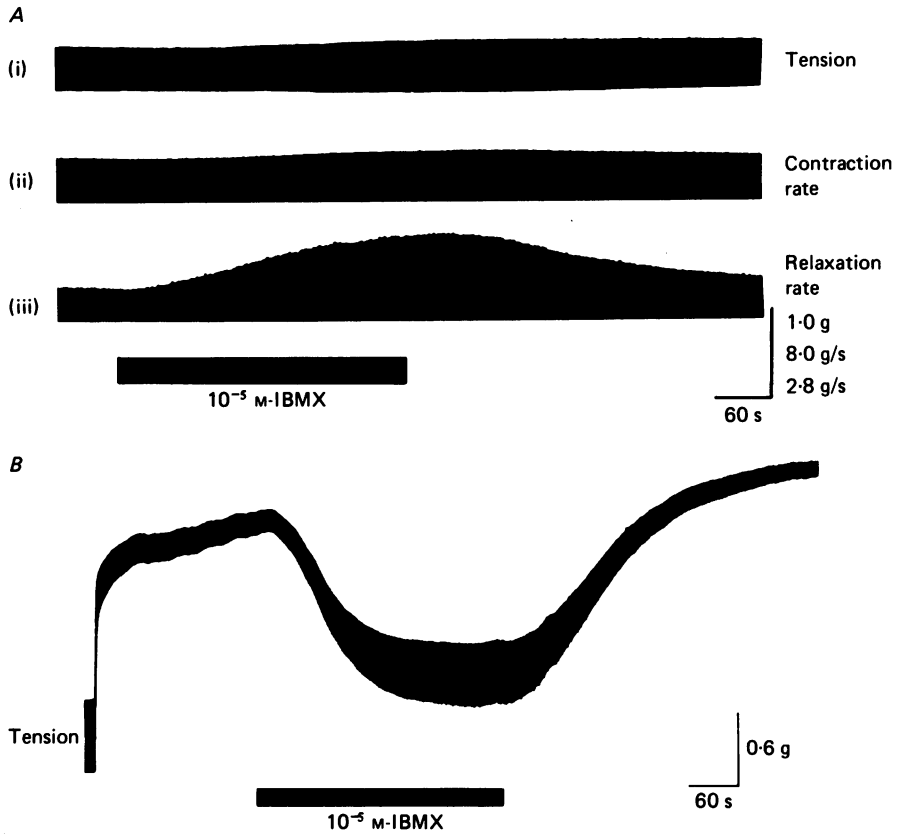


Fig. 1. The effect of a 5 min pulse of 10^{-5} M-IBMX (black bars) on tension induced in extensor muscle by firing SETi at different frequencies. *A*, the effect on twitch amplitude (i), contraction rate (ii) and relaxation rate (iii) when SETi is fired at 1 Hz. *B*, the effect on maintained tension and twitch fusion when SETi is fired at 7 Hz. The initial tension maintained by firing SETi at 1 Hz is shown at the beginning of the trace.

Drugs, which were superfused directly onto the surface of the extensor tibiae muscle, were dissolved in a physiologically 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. In some experiments the calcium content of the saline was varied between 0·5 and 10·0 mM. Stock solutions of forskolin were made by dissolving 1 mg in 100 μl ethanol. Appropriate ethanol controls were run for each experiment.

Drugs were obtained from the following sources: 3 isobutyl-1-methylxanthine (IBMX) (Aldrich); 8-(4-chlorophenylthio)adenosine 3′:5′monophosphate, cyclic (Boeringer Mannheim); and forskolin (Calbiochem). All other drugs were obtained from the Sigma Chemical Co.

RESULTS

Effects of drugs that elevate cyclic AMP levels

Phosphodiesterase inhibitors. The levels of cyclic nucleotides in a tissue are controlled by the balance of synthetic and degradative processes. Thus, inhibition of the enzyme phosphodiesterase, which breaks down cyclic nucleotides, can elevate the levels of cyclic nucleotides in a tissue. The methylxanthine IBMX is a potent inhibitor of phosphodiesterase activity in the locust extensor tibiae muscle. It elevates the levels of both cyclic AMP and cyclic GMP (Evans, 1984). Fig. 1 shows

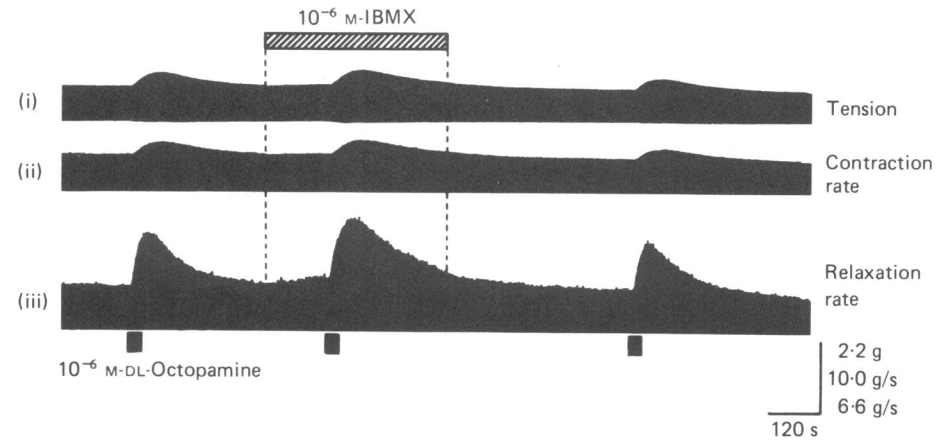


Fig. 2. Potentiating effecting of 10^{-6} M-IBMX (hatched bar) on responses to a 30 s pulse of 10^{-6} M-DL-octopamine (black bars) introduced into the superfusate. SETi was fired at 1 Hz and the Figure shows twitch amplitude (i), contraction rate (ii) and relaxation rate (iii) of tension in the extensor muscle.

the effect of applying a 5 min pulse of 10^{-5} M-IBMX on tension induced in the extensor muscle by firing the slow motoneurone (SETi). When SETi is stimulated at 1 Hz (Fig. 1*A*) IBMX increases the amplitude of twitch tension by 21·7 %, increases its rate of contraction by 12·5 % and increases its rate of relaxation by 155·6 %. IBMX superfusion also induces a relaxation of the maintained tension induced by firing SETi at 7 Hz (Fig. 1*B*). At this frequency IBMX also reduces the degree of fusion of the individual tension transients, which (at the slow time course of Fig. 1*B*) results in an increase in the width of the tension trace. The effects of IBMX at both 1 and 7 Hz are qualitatively the same as those induced in this muscle by the application of octopamine, but are of a much slower time course. The effects on twitch amplitude and contraction rate are small at 10^{-5} M-IBMX and exhibit thresholds between 10^{-6} and 10^{-5} M-IBMX. In contrast the increases in relaxation at both 1 and 7 Hz are much more sensitive and exhibit a threshold between 10^{-8} and 10^{-7} M-IBMX.

The presence of a phosphodiesterase inhibitor should also potentiate the effects of any neurotransmitter or neuromodulator that mediates its effects by an increase in cyclic nucleotide levels. Fig. 2 shows the effect of the presence of 10^{-6} M-IBMX on the responses of the extensor tibiae muscle to a 30 s pulse of 10^{-6} M-DL-octopamine. Addition of IBMX alone at this concentration produces effects that vary in

magnitude from one preparation to another. In the preparation illustrated in Fig. 2 it caused a small increase in relaxation rate but had little effect on amplitude of tension or on the contraction rate. However, the responses of all three parameters to the pulse of octopamine are potentiated in magnitude in the presence of IBMX compared with the control pulses before and after exposure to IBMX. In the presence of IBMX the octopamine-mediated increase in twitch amplitude and contraction rate both rise from 36 to 46 %, whilst the increase in relaxation rate rises from 103 to 120 %. The time courses of the octopamine responses are also more prolonged in the presence of IBMX.

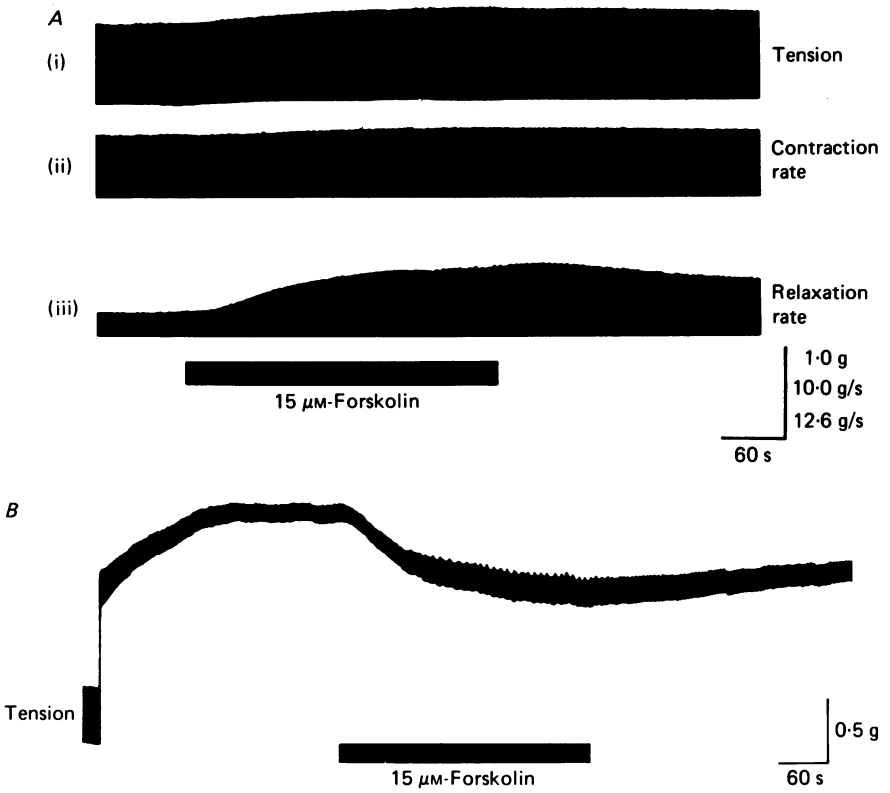


Fig. 3. The effect of a 5 min pulse of 15 μM-forskolin (black bars) on tension induced in extensor muscle by firing SETi at different frequencies. *A*, effect on twitch amplitude (i), contraction rate (ii) and relaxation rate (iii) when SETi is fired at 1 Hz. An initial 5 min control pulse of 0·1 % ethanol in locust saline produced no change in the tension parameters. *B*, the effect on maintained tension and twitch fusion when SETi is fired at 7 Hz. The initial tension maintained by firing SETi at 1 Hz is shown at the beginning of the trace.

Forskolin. The experiments above with IBMX indicate that changes in cyclic nucleotide levels in the extensor muscle can mimic the effects of octopamine application. Nonetheless, they do not distinguish between the effects of cyclic AMP and cyclic GMP, the levels of both nucleotides being increased by IBMX in this muscle (Evans, 1984). Recently, it has been shown that the diterpene, forskolin, selectively

increases cyclic AMP levels in both membrane fragments and intact cells by a direct activation of adenylate cyclase activity (Seamon & Daly, 1981). Forskolin also selectively increases cyclic AMP levels in the extensor muscle (Evans, 1984).

Forskolin mimics the effects of octopamine exposure to the extensor muscle. Fig. 3*A* indicates that a 5 min pulse of 15 μM-forskolin can potentiate the amplitude of

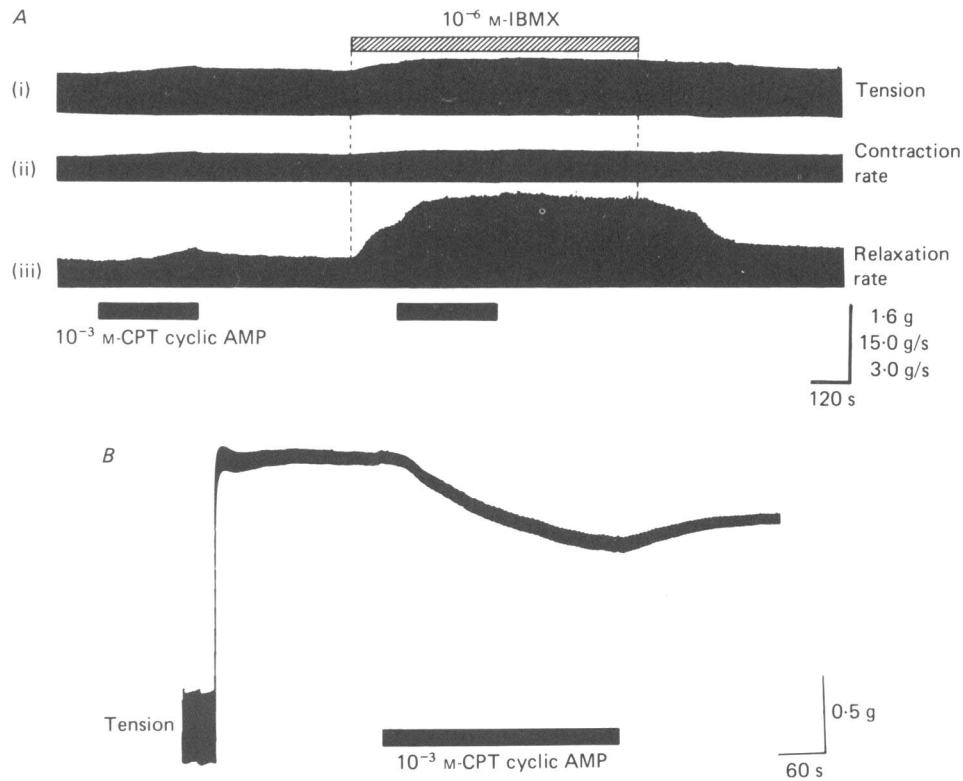


Fig. 4. The effects of a 5 min pulse of 10^{-3} M-CPT cyclic AMP (black bars) on tension induced in extensor muscle by firing SETi at different frequencies. *A*, the effect on twitch amplitude (i), contraction rate (ii) and relaxation rate (iii) when SETi is fired at 1 Hz. The second pulse of CPT cyclic AMP is given whilst the muscle is exposed to 10^{-6} M-IBMX, which in this preparation itself produced some potentiation of the response, but in addition increased and much prolonged the responses to CPT cyclic AMP. *B*, the effect of maintained tension and twitch fusion when SETi is fired at 7 Hz. The initial tension maintained by firing SETi at 1 Hz is shown at the beginning of the trace.

twitch tension induced by firing SETi at 1 Hz by 19·0 %, and also the corresponding rates of contraction and relaxation by 12·1 and 225·0 % respectively. Again the effects develop and decay more slowly than the corresponding octopamine effects (cf. Fig. 2). A similar exposure to forskolin also reduces the maintained tension and the degree of twitch fusion induced by firing SETi at 7 Hz (Fig. 3*B*). In this example, after forskolin was removed the maintained tension recovered very slowly and only approached the control level after a 45 min superfusion with saline. The irregularities that develop in the trace upon exposure to forskolin are due to the potentiation of a myogenic rhythm of contraction and relaxation (P. D. Evans, unpublished).

Cyclic nucleotides. Intracellular levels of cyclic nucleotides can also be increased by exposing tissues to exogenous cyclic nucleotides, though usually very high levels of cyclic nucleotides must be applied before any physiological effects can be observed. This is due to the lack of permeability of cells to many of the analogues and to their rapid metabolism by the enzyme phosphodiesterase. In the present investigation exposure of the extensor muscle to cyclic AMP, cyclic GMP, 8-bromo-cyclic AMP, 8-bromo-cyclic GMP and dibutyryl cyclic AMP at concentrations up to 10^{-2} M produced no consistent effects on SETi-induced tension. Similarly adenosine at concentrations up to 10^{-3} M produced no effects. However, the 8-(4-chlorophenylthio) adenosine 3′:5′-monophosphate cyclic derivative (CPT cyclic AMP) mimics the actions of octopamine (Fig. 4). This derivative has been reported to be a hundred times more effective than dibutyryl cyclic AMP in the activation of cyclic-AMP-dependent protein kinase in rat liver and to be more resistant to phosphodiesterase activity (Miller, Beck, Simon & Meyer, 1975). A 5 min pulse of 10^{-3} M-CPT cyclic AMP increases SETi twitch amplitude by 20 % and the rates of contraction and relaxation by 15·8 and 45·0 % respectively (Fig. 4*A*). The effects develop slowly during the 5 min exposure to CPT cyclic AMP and decay gradually over the next 8 min. The same preparation was then exposed to 10^{-6} M-IBMX for 2 min before exposure to a second 5 min pulse of 10^{-3} M-CPT cyclic AMP. In this preparation 10^{-6} M-IBMX alone produces small increases in the twitch amplitude (15·2 %) and contraction rates (10·0 %), together with a much larger increase in relaxation rate (109·1 %). However, when CPT cyclic AMP is added to the superfusate, it produces further increases in all three parameters. These are small for the twitch amplitude and contraction rate ($\sim$ 10·0 %), but the further increase in relaxation rate (55·0 %) is larger than the initial response to the control in the absence of IBMX. Furthermore, as can be best seen for the relaxation rate changes, the increases mediated by the second pulse of CPT cyclic AMP occur with a much faster time course than in the absence of IBMX. In addition, the increase is maintained for a further 8 min when muscle is superfused with saline containing 10^{-6} M-IBMX and it only gradually declines when the IBMX is removed from the saline. This suggests that the time course of the actions of a pulse of CPT cyclic AMP can be prolonged by the inhibition of phosphodiesterase activity.

A 5 min pulse of 10^{-3} M-CPT cyclic AMP can also reduce the maintained tension and degree of twitch fusion induced in the muscle by firing SETi at 7 Hz (Fig. 4*B*). As with the forskolin effect described above, the time course of the onset of the CPT cyclic AMP effect was much slower than that for octopamine, and the effect was also much longer lasting. The preparation illustrated took 30 min to recover to pre-exposure control levels.

Effects on miniature end-plate potentials. It seems very likely that most, if not all, the octopamine-mediated increase in twitch amplitude is generated by the action of the presynaptic octopamine receptors on the terminals of the slow motoneurone (O'Shea & Evans, 1979; Evans, 1981). Nonetheless, a contribution to this effect from post-synaptic actions of octopamine cannot be ruled out. Thus, although the results outlined above indicate that the elevation of cyclic AMP levels mimic the actions of octopamine on twitch amplitude, they do not prove that the cyclic AMP increases are localized presynaptically. However, a selective measure of the activation of the presynaptic octopamine receptors can be achieved by examining the increase in

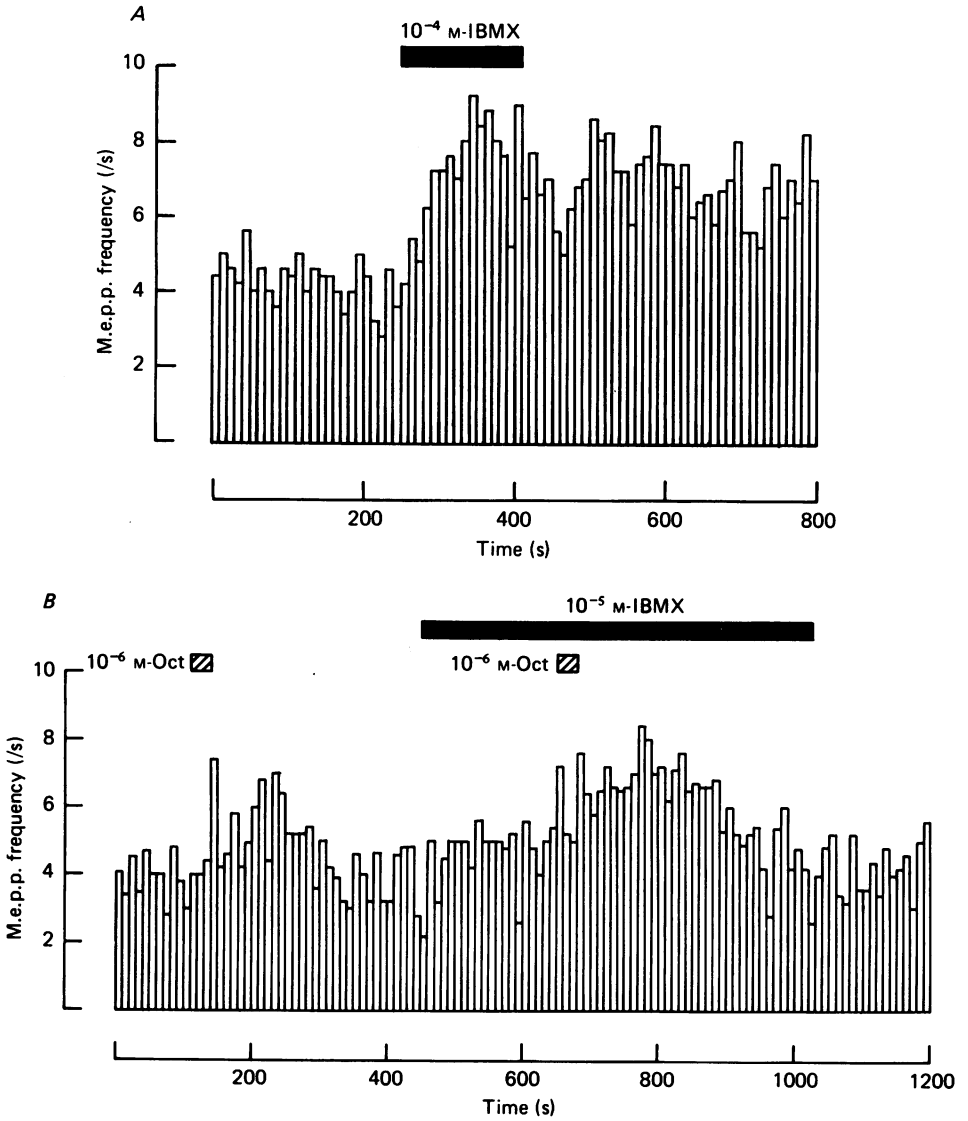


Fig. 5. Effect of elevation of cyclic nucleotide levels on frequency of spontaneous release of neurotransmitter from the terminals of the SETi motoneurone to slow distal fibres of the extensor muscle. The results are plotted as the frequency of m.e.p.p.s (mean frequency per second of ten consecutive seconds) against time. *A* (above), the effect of a 3 min pulse of 10^{-4} M-IBMX (black bar). *B* (above), the potentiating effect of 10^{-5} M-IBMX (black bar) on the response to a 30 s pulse of 10^{-6} M-DL-octopamine (hatched bar) compared with an initial control pulse of octopamine. *C* (opposite), the effect of a 5 min pulse of 10 μM-forskolin (black bar) which is preceded by a 5 min control pulse of 0·1 % ethanol in locust saline (hatched bar). *D* (opposite), the effect of a 5 min pulse of 10^{-3} M-CPT cyclic AMP.

frequency of the spontaneous release of neurotransmitter from the slow motoneurone terminals. To see whether changes in cyclic AMP levels can mimic this effect of octopamine the frequency of SETi spontaneous miniature end-plate potentials (m.e.p.p.s) has been measured in the presence of IBMX, forskolin and CTP cyclic AMP.

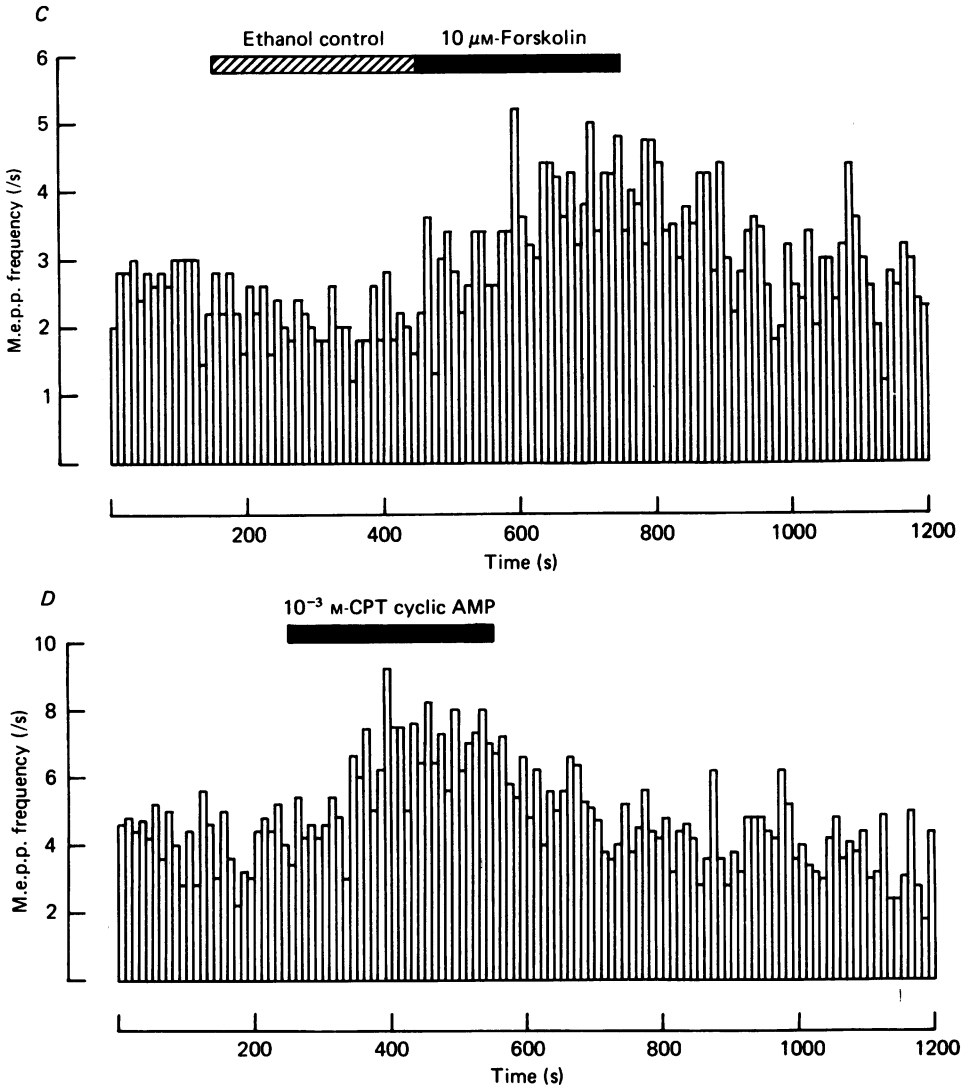
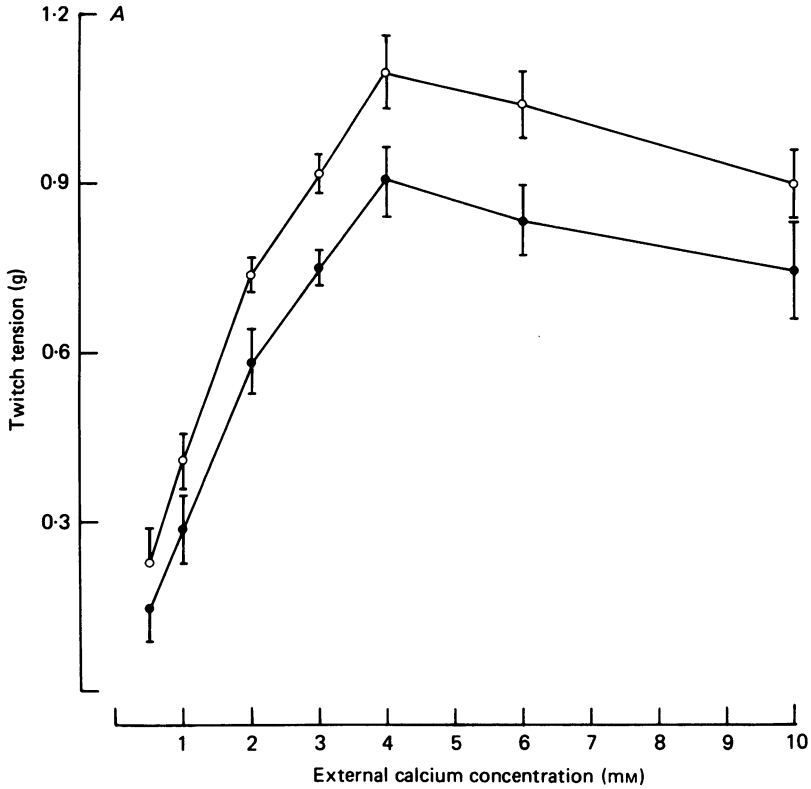


Fig. 5*C* and *D*. For legend see opposite page.

Fig. 5*A* shows that a 3 min pulse of 10^{-4} M-IBMX introduced into the muscle superfusate can double the frequency of SETi-induced m.e.p.p.s and that the effect has a prolonged time course. The threshold for the response occurs between 10^{-6} and 10^{-5} M-IBMX. In addition, the presence of IBMX can potentiate the increase in SETi m.e.p.p. frequency induced by octopamine (Fig. 5*B*). A 30 s pulse of 10^{-6} M-DL-octopamine increases m.e.p.p. frequency, which remains elevated for 1–2 min at the end of the pulse. In the presence of 10^{-5} M-IBMX, which has little effect on its own in this preparation, the effect of an equivalent pulse of octopamine is potentiated in both magnitude and time course.

Forskolin also increases SETi m.e.p.p. frequency (Fig. 5*C*). M.e.p.p. frequency gradually increases during a 5 min pulse of 10 μM-forskolin whereas the preceding ethanol control pulse did not produce any increase. Superfusion of the muscle with



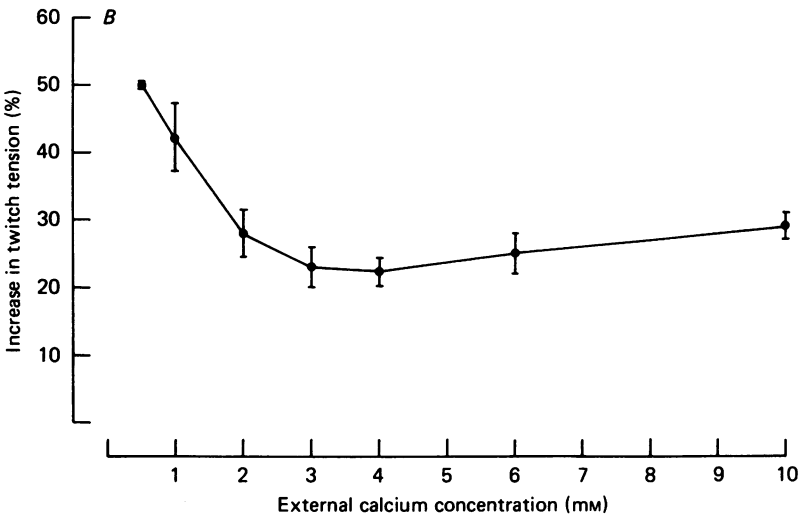


Fig. 6*A* and *B*. For legend see opposite page.

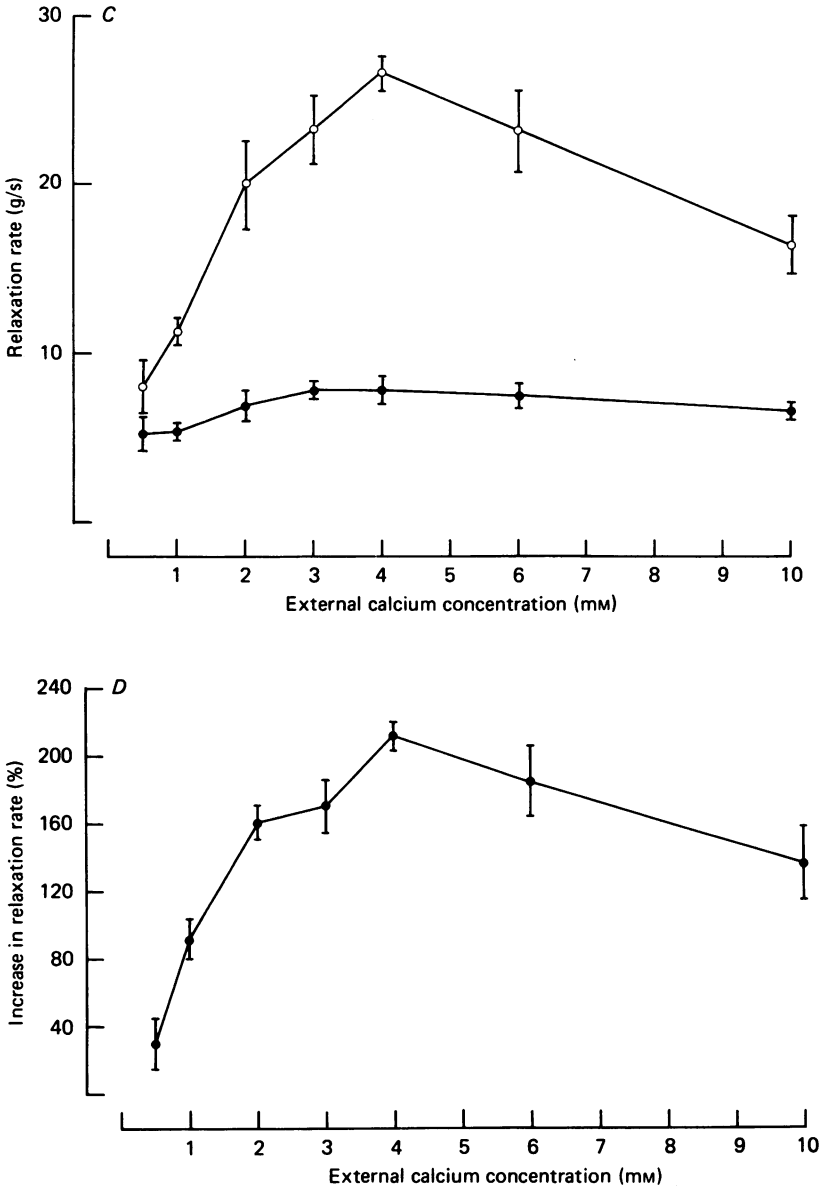


Fig. 6. Calcium sensitivity of octopamine-mediated increases in twitch tension amplitude and relaxation rate when SETi is fired at 1 Hz. The results represent the maximal responses obtained to a 30 s pulse of 10^{-6} M-DL-octopamine at various external calcium concentrations. Each value is the mean of four determinations and is expressed $\pm$ standard error of the mean. *A* (opposite), the effect of calcium on twitch amplitude in the presence (○) and absence (●) of octopamine. *B* (opposite), the effect of calcium on the octopamine-mediated increase in tension which is expressed as a percentage of the control amplitude at each calcium concentration. *C* (above), the effect of calcium on the relaxation rate in the presence (○) and the absence (●) of octopamine. *D* (above), the effect of calcium on the octopamine-mediated increase in relaxation rate which is expressed as a percentage of the control relaxation rate at each calcium concentration.

a 5 min pulse of 10^{-3} M-CPT cyclic AMP also increased SETi m.e.p.p. frequency (Fig. 5*D*), whereas equivalent pulses of 10^{-5} M-cyclic AMP, cyclic GMP, 8-bromo-cyclic AMP, 8-bromo-cyclic GMP and dibutyryl cyclic AMP had no effect on m.e.p.p. frequency.

Thus, exposure of the extensor muscle to drugs that increase cyclic AMP levels can mimic the effects of the activation of the presynaptic octopamine receptors on the terminals of the slow motoneurone.

Calcium dependence of octopamine responses

In many excitable tissues the physiological effects of changes in cyclic nucleotide levels are mediated by alterations of calcium homeostasis (see Berridge, 1975). To investigate this possibility in the locust extensor muscle, the calcium sensitivity of the octopamine responses has been examined.

The calcium sensitivity of the amplitude of twitch tension, when SETi is stimulated at 1 Hz, is shown in Fig. 6*A*. The amplitude is most sensitive to external calcium concentration between 0·5 and 4·0 mM, and reaches a peak at around 4 mM. This is in good agreement with the calcium sensitivity of potassium-induced contractions in the corresponding muscle in the mesothoracic leg of the locust (Aidley, 1965). At higher calcium concentrations (between 4 and 10 mM) there is a tendency for the amplitude to decline. The maximal increases in twitch amplitude in response to a 30 s pulse of 10^{-6} M-DL-octopamine are almost the same at all calcium concentrations examined. However, if the octopamine-mediated increase is expressed as a percentage of the control amplitude (Fig. 6*B*), the responses decline in magnitude up to 3 mM-calcium and show a slight rise from this minimum between 4 and 10 mM-calcium. This suggests that the octopamine-mediated increase in twitch tension is sensitive to the concentration of external calcium but that the relationship is complex. This is not surprising since octopamine is modulating neuromuscular transmission, a process which is itself calcium sensitive.

The calcium sensitivity of the rate of relaxation of twitch tension is shown in Fig. 6*C*. Changes in external calcium alone produce very little effect on the rate of relaxation. However, the maximum rates of relaxation of twitch tension induced by a 30 s pulse of DL-octopamine show a marked calcium dependence which peaks at 4 mM. The percentage increase in relaxation rate is also maximal at the same calcium concentration (Fig. 6*D*). It is not clear why the octopamine-mediated increase in relaxation rate should increase between 0·5 and 4·0 mM-calcium. It is possible that the octopamine-mediated increase in muscle cyclic AMP levels may exhibit a maximum at 4 mM-calcium or that its rate of breakdown by phosphodiesterase may be at a minimum at this concentration. Alternatively it is possible that this effect could be related to the calcium sensitivity of the regenerative component of the electrical response of the muscle membrane (Washio, 1972), which may itself also be octopamine sensitive. Further experimentation is required to elucidate the exact mechanism of the calcium sensitivity of the octopamine-mediated increase in relaxation rates.

DISCUSSION

Considerable evidence indicates that an increase in cyclic AMP levels mediates the activation of the octopamine receptors that modulate neuromuscular transmission and muscle contraction in the extensor tibiae muscle of the locust hind leg. Stimulation of these receptors by exogenously applied or neurally released octopamine increases cyclic AMP levels in the muscle (Evans, 1984) with a time course similar to that of the observed modulatory effects (Evans & O'Shea, 1977; O'Shea & Evans, 1979; Evans & Siegler, 1982; Evans, 1982). In this preparation, however, several pharmacological classes of octopamine receptor have been identified (Evans, 1981). Thus it is difficult to determine what proportion of the cyclic AMP increases are due to the action of each receptor type.

The results in the present paper show that drugs that artificially elevate cyclic AMP levels can mimic the physiological effects of stimulating the presynaptic $OCTOPAMINE_{2A}$ receptors on the terminals of the slow motoneurone and the post-synpatic $OCTOPAMINE_{2B}$ class receptors on the muscle fibres. Additional studies indicate that the $OCTOPAMINE_1$ class receptors, which modulate a myogenic rhythm of contraction and relaxation in a proximal bundle of muscle fibres of the extensor muscle (Hoyle, 1975; Evans & O'Shea, 1978; Evans, 1981), do not act by increasing cyclic AMP levels (P. D. Evans, unpublished).

The action of presynaptic octopamine receptors

The activation of the presynaptic octopamine receptors on the terminals of the slow motoneurones leads to the increased activity of adenylate cyclase, resulting in the elevation of presynaptic cyclic AMP levels. The activation of the presynaptic octopamine receptors will increase the amplitude of slow motoneurone twitch tension, as well as increasing the spontaneous release of transmitter from the terminals of this neurone (see O'Shea & Evans, 1979; Evans, 1981). Since the twitch amplitude may also be altered by post-synaptic events, the spontaneous release is a better measure of the activation of presynaptic octopamine receptors. The latter effect is mimicked when cyclic AMP levels are increased by applying the phosphodiesterase inhibitor IBMX, the adenylate cyclase activator forskolin and a highly active phosphodiesterase-resistant analogue of cyclic AMP (CPT cyclic AMP).

It is unlikely that the increases in cyclic AMP levels are brought about by the activation of an adenosine receptor (cf. Wolff *et al.* 1981). In the present study superfusion of IBMX, which can block adenosine receptors as well as inhibiting phosphodiesterase, on to the preparation ensured that any endogenously released adenosine would be removed from the preparation. In addition, CPT cyclic AMP was the only analogue to have any effects, whereas it would have been expected that other less permeable compounds, such as cyclic AMP itself, would have been effective if the effects of the analogue were being mediated via external adenosine receptors. Finally, forskolin bypasses the receptors and directly activates the catalytic subunit of adenylate cyclase (Seamon & Daly, 1981).

The elevation of presynaptic cyclic AMP levels by octopamine is likely to affect transmitter release by altering the levels of free calcium in the nerve terminals, possibly by the actions of a specific protein kinase (Greengard, 1976). In the present

study the effect of octopamine on twitch amplitude has been shown to be calcium sensitive. At low calcium concentrations octopamine causes a relatively large increase in slow-motoneurone-evoked twitch tension, but at higher concentrations, where the release mechanism becomes saturated with the amount of calcium entering, the effect is proportionately smaller.

The presynaptic elevation of cyclic AMP levels has also been shown to alter transmitter release in a variety of other systems. 5-Hydroxytryptamine, for example, produces a facilitation of transmitter release through a cyclic-AMP-dependent process in the molluscan nervous system (Klein, Shapiro & Kandel, 1980) and at the crayfish neuromuscular junction (Enyeart, 1981). In addition, cyclic AMP can also facilitate the spontaneous release of transmitter at unidentified excitatory motoneurone terminals in cockroach muscle (Wareham, 1978). Also, a direct presynaptic localization of adenylate cyclase has been demonstrated in synaptosomes from rat cerebral cortex (Weller, 1977). Furthermore, in mammalian skeletal muscle catecholamines can potentiate transmitter release by the activation of presynaptic receptors (Kuba, 1970). Under normal physiological conditions the effects are mediated via α-adrenoreceptor increases in calcium availability, but in fatigued muscles a cyclic-AMP-mediated potentiation of presynaptic transmitter release may be of physiological importance (Bowman, 1982).

The action of post-synaptic octopamine receptors

The actions of the post-synaptic octopamine receptors to increase the rate of relaxation of tension in the locust extensor tibiae muscle (see O'Shea & Evans, 1979; Evans, 1981) are also mediated by increases in cyclic AMP levels. The bulk of the octopamine-mediated changes in cyclic AMP levels undoubtedly occur in the muscle fibres themselves (Evans, 1984). The changes occur in bundles of fast and slow muscle fibres (P. D. Evans, unpublished), which is consistent with the observation that octopamine increases the relaxation rate of twitch tension evoked by both the fast and the slow motoneurones (O'Shea & Evans, 1979). In addition, in the present study all three treatments used to bypass the octopamine receptors and to elevate directly the cyclic AMP levels, mimic the physiological actions of octopamine on tension generated by stimulating the slow motoneurone at different frequencies.

The increased levels of cyclic AMP generated by octopamine in the locust extensor muscle could increase the rate of relaxation of tension by altering the levels of phosphorylation of contractile proteins or of proteins controlling the availability of calcium to the contractile machinery (see Walsh & Guilleux, 1981). The latter possibility is favoured for the actions of catecholamines on vertebrate skeletal muscle, where the response of a muscle to increased cyclic AMP levels depends on its proportion of fast and slow fibres (Bowman & Zaimis, 1958; Bowman & Nott, 1974; Bowman, 1982). The locust extensor muscle contains a mixed population of slow, intermediate and fast muscle fibre types with different proportions of sarcoplasmic reticulum and mitochondria (Hoyle, 1978). It is likely that an octopamine-mediated increase in rate of relaxation could be caused by an increased rate of calcium uptake into the sarcoplasmic reticulum in all fibre types.

The elevation of cyclic AMP levels in the locust extensor muscle in the present study can also mimic the actions of octopamine at stimulation rates (5–20 Hz) where

individual SETi twitches fuse to produce an incomplete tetanus (Evans & Siegler, 1982). Under these conditions the prevalent effect is again an increase in relaxation rate that results in a decrease in the fusion of the individual twitches in a train. The initial tension peak produced by octopamine at the beginning of a period of stimulation during an incomplete tetanus may also be due to an increased accumulation of calcium in the sarcoplasmic reticulum (cf. studies on the effects of adrenaline on frog muscle by Gonzalez-Serratos, Hill & Valle-Aguilera, 1981). This action of octopamine parallels the action of adrenaline on the slow fibres of the vertebrate soleus muscle (Bowman & Zaimis, 1958). In both cases the effects of the biogenic amines can be overcome by higher frequencies of stimulation, during which it is presumed that sufficient calcium enters the muscle fibres, or is released from the sarcoplasmic reticulum, to overcome completely the biogenic-amine-mediated increase in calcium sequestration.

A general conclusion from studies of biogenic amine modulation of skeletal muscle tension is that the response of the muscle will depend on the proportion of fast and slow fibres being activated and on the relative contribution and distribution of pre- and post-synaptic mechanisms. Thus in other muscles of the locust (and in muscles of other insects) which have different proportions of fast and slow fibres from that of the extensor tibiae muscle of the hind leg, and where pre- and post-synaptic mechanisms may contribute to differing extents, it may be expected that octopamine will produce a different effect on tension in the muscle.

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