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MODULATION OF TENSION PRODUCTION BY OCTOPAMINE IN THE METATHORACIC DORSAL LONGITUDINAL MUSCLE OF THE CRICKET *TELEOGRYLLUS OCEANICUS*

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Summary

1. Application of octopamine to the metathoracic dorsal longitudinal muscle (DLM) of the cricket *Teleogryllus oceanicus* produced dose-dependent increases of twitch amplitude, contraction rate and relaxation rate. The threshold for octopamine effects was between 10^{-8} and 10^{-7} mol l^{-1}, while maximal effects were seen at approximately 10^{-5} mol l^{-1}.

2. The octopamine receptors were classified as octopamine$_2$ receptors on the basis of the differential responsiveness of the muscle to the octopamine agonists naphazoline, tolazoline, clonidine and the octopamine antagonists metoclopramide and chlorpromazine. It was not possible to distinguish between octopamine$_{2A}$ or octopamine$_{2B}$ receptors in this preparation.

3. Octopamine had both presynaptic and postsynaptic effects, since it increased both miniature end-plate potential (mEPP) frequency and muscle relaxation rate.

4. At a calcium concentration of 11 mmol l^{-1}, octopamine did not affect muscle membrane potential, input resistance or EJP amplitude, but the EJP duration at half amplitude ($T_{1/2}$) was slightly increased. In low-calcium saline (1.8 mmol l^{-1}), octopamine did not affect membrane potential or $T_{1/2}$, but EJP amplitude was increased.

5. Stimulation of the octopaminergic dorsal unpaired median neuron (DUMDL), which innervates the metathoracic DLM, increased twitch amplitude in about 25% of the preparations. Failure in the other preparations was apparently due to spike conduction failure within the metathoracic ganglion.

6. These results show that octopamine can be an important modulator of metathoracic DLM tension production.

Introduction

Octopamine is now known to be an important neuromodulator in invertebrates (reviewed by David and Coulon, 1985). One action of octopamine is to modify tension production by muscles: octopamine produces increases in twitch ampli-

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tude, contraction rate and relaxation rate and decreases of basal tonus (O'Shea and Evans, 1979; Klaassen and Kammer, 1985; Whim and Evans, 1988, 1989). In the locust extensor tibiae, some of these effects have been attributed to specific octopamine receptor subtypes (Evans, 1981). However, only a few studies have been performed to determine if this receptor classification is valid in other preparations (Morton, 1984; Lafon-Cazal and Bockaert, 1985; Nathanson, 1985; Orchard and Lange, 1986; Pannabecker and Orchard, 1986; Reale *et al.* 1986).

The metathoracic dorsal longitudinal muscle (DLM) of the cricket *Teleogryllus oceanicus* is specialized for the rapid contractions associated with wing movements during both flight and stridulation (Ready and Josephson, 1982). The metathoracic DLM is innervated by five motoneurons, each innervating a different muscle band (Bentley, 1973; Clark, 1976*a*). Each muscle cell is innervated by a single fast motoneuron (Clark, 1976*b*; Neville, 1963). In addition, the metathoracic DLM is innervated by a dorsal unpaired median neuron (DUMDL) (Bentley, 1973; Clark, 1976*a*; Davis and Alanis, 1979; Hoyle *et al.* 1980). Although the neurotransmitter content of DUMDL is unknown, all DUM neurons which have been examined contain octopamine (Evans and O'Shea, 1978; Dymond and Evans, 1979; Christensen *et al.* 1983; Morton and Evans, 1984; Orchard and Lange, 1985).

The purpose of this study was to examine the effects of octopamine on tension production by the metathoracic DLM of *T. oceanicus* and to compare these responses with those of the locust extensor tibiae (O'Shea and Evans, 1979; Evans, 1981; Evans and Siegler, 1982) and the DLMs of the locust (Whim and Evans, 1988) and the hawkmoth *Manduca sexta* (Klaassen and Kammer, 1985; Fitch and Kammer, 1986; Klaassen *et al.* 1986). We examined the electrophysiological correlates of octopamine's effects on tension production, as well as the role of DUMDL in mediating these responses. In addition, the properties of the octopamine receptor(s) were examined to determine if they fit into the classification scheme of Evans (1981). Octopamine was found to increase twitch amplitude, contraction rate and relaxation rate. The octopamine receptors were classified as octopamine$_2$ receptors, but were not identical to those in the locust extensor tibiae (Evans, 1981). A brief report of some of these results has been previously published (O'Gara, 1986).

Materials and methods

Male crickets, *Teleogryllus oceanicus*, were raised from eggs in 12-l clear plastic boxes containing crumpled paper towelling. About 60 crickets were raised in each box. The crickets were fed romaine lettuce daily, and kept on a 15 h: 9 h L: D cycle at approximately 30 °C.

The mechanical responses of the metathoracic DLM to various drugs were studied using young adult male crickets aged from 3 days to 3 weeks after their imaginal ecdysis. At this age the metathoracic DLM is large and pink, whereas approximately 1 month after the imaginal ecdysis the muscle turns white and becomes greatly reduced in volume. Increases in twitch duration are correlated

with this developmental change (Ready and Josephson, 1982). Prior to dissection, the head, wings and legs were removed. A mid-dorsal incision was made and the gut and the posterior half of the abdomen were removed. To reduce movement artifacts, the prothoracic ganglion was removed and the connectives severed posterior to the metathoracic ganglion. The postscutum, the exoskeletal plate to which the metathoracic DLM is attached posteriorly, was isolated from the rest of the exoskeleton. A minuten pin, which had loops bent into each end, was attached by one end to the postscutum with cyanoacrylate glue. The other end of the pin was attached to a transducer (Narco Biosystems F50) to measure isometric tension. The muscle was stretched to approximately its normal length *in vivo*. Muscle twitches were evoked by supramaximal electrical stimulation of the four DLM motoneurons in mesothoracic nerve 6 (Campbell, 1961) with a suction electrode (stimulus parameters: 1 Hz, 0.5 ms duration, 15 V). DUMDL and the contralateral dorsal longitudinal motoneuron (CDLM), whose somata are located in the metathoracic ganglion (Neville, 1963; Clark, 1976*a*), were not activated by stimulation of mesothoracic nerve 6. After dissection, twitch amplitude decreased steadily for 1–2 h until a stable amplitude was reached; it was at this point that experiments were begun. Preparations continued to be viable for several more hours. This decrease of twitch amplitude over time may be the unavoidable result of the DLM being modulated by endogenous octopamine released during the stress of capture and dissection (Evans, 1981). Tension recordings were displayed on an oscilloscope and chart recorder (Brush RD 1684-00 or Gould 2200S). Tension recordings were also differentiated to determine the rates of contraction and relaxation of the muscle twitch. The preparation was superfused with saline or drugs at $0.75\,\mathrm{ml\,min^{-1}}$, a rate which exchanged the fluid volume covering the preparation every few seconds. A bubble was introduced into the perfusion system to mark the beginning and end of a drug application.

The action of octopamine antagonists was examined by first applying $10^{-6}\,\mathrm{mol\,l^{-1}}$ octopamine and noting the response. The preparation was then exposed to $10^{-5}\,\mathrm{mol\,l^{-1}}$ antagonist for about 8 min, followed by $10^{-6}\,\mathrm{mol\,l^{-1}}$ octopamine plus $10^{-5}\,\mathrm{mol\,l^{-1}}$ antagonist. The antagonist decreased the amount of facilitation produced by octopamine, and these effects were expressed as the percentage reduction of twitch amplitude, contraction rate or relaxation rate.

Muscle intracellular electrical activity was recorded with borosilicate glass microelectrodes filled with $1\,\mathrm{mol\,l^{-1}}$ potassium acetate (20–50 MΩ resistance). Input resistance was measured with two microelectrodes placed within the same muscle cell less than 60 μm apart. Such measurements were made using hyperpolarizing current pulses (50 ms duration) and 10–15 mV displacements of membrane potential. Excitatory junctional potentials (EJPs) were examined prior to pharmacological treatment in five different muscle cells of each preparation ($N=10$). Octopamine ($10^{-5}\,\mathrm{mol\,l^{-1}}$) was then applied to the preparation for 5 min and EJPs in five more cells were examined. Resting membrane potential, EJP amplitude and EJP duration at half amplitude ($T_{1/2}$) were measured with a Tektronix 5D10 waveform digitizer. Intracellular records from neurons in the

metathoracic ganglion were obtained with aluminosilicate glass microelectrodes filled with 2.5 % Lucifer Yellow (Sigma) and 1 mol l^{-1} LiCl (50–70 MΩ resistance). The metathoracic ganglion was supported upon a small metal platform. Extracellular nerve recordings were made with suction electrodes. The preparations were superfused with saline at 0.5 ml min^{-1}.

When miniature end-plate potentials (mEPPs) were examined the perfusion system was not used since it introduced electrical noise sufficient to obscure small mEPPs. Therefore, octopamine was applied to the preparation in a bolus using a pipet. The octopamine in the bolus was diluted by the saline already present in the body cavity; concentrations are expressed as the approximate concentration after dilution. mEPP amplitude ranged from noise levels (0.5 mV) to 3 mV. When mEPP frequency or amplitude was measured only those mEPPs with amplitudes at least 0.3 mV larger than background noise were counted. The reported mEPP amplitude (Fig. 5B) was the size of the deflection above the noise level.

The saline contained (mmol l^{-1}): NaCl, 152; KCl, 8; $MgCl_2$, 1; $CaCl_2$, 11; $NaHCO_3$, 4; TES, 5; trehalose, 5; sucrose, 105; pH 6.7. DL-Octopamine–HCl, DL-synephrine–HCl, naphazoline–HCl, tolazoline–HCl, clonidine–HCl, metoclopramide–HCl, chlorpromazine–HCl and 5-hydroxytryptamine (creatinine sulfate complex) were purchased from Sigma (St Louis, MO). All drugs were dissolved in saline. Experiments were conducted at room temperature (21–25 °C).

Results

Amine-mediated modulation of tension production

Octopamine applied to the metathoracic DLM during 1 Hz stimulation of mesothoracic nerve 6 caused dose-dependent increases (i.e. potentiation) of twitch amplitude (Figs 1, 2 and 3A,C), contraction rate (Figs 1, 2, 3B) and relaxation rate (Figs 1, 2, 3D). Very slight decreases of basal tonus were sometimes seen, especially at high octopamine concentrations. However, this effect was weak and variable. The threshold concentrations for potentiation of twitch amplitude, and contraction and relaxation rates were all between 10^{-8} and 10^{-7} mol l^{-1}. The maximal potentiation of twitch amplitude, contraction rate and relaxation rate were seen between 10^{-6} and 10^{-4} mol l^{-1} (Fig. 3A,B,D). The EC_{50} values (the concentration which produces 50 % of the maximal effect of the drug) for potentiation of twitch amplitude, and contraction and relaxation rates were determined from the graphs in Fig. 3 and are shown in Table 1. Twitch duration was unaffected or slightly increased by octopamine. It was never reduced by octopamine or its agonists, unlike in the locust DLM (Whim and Evans, 1988).

Increases in relaxation rate developed more slowly than increases in contraction rate or twitch amplitude, as shown in the differentiated recordings of tension (Fig. 2). Such differences were most easily seen at high concentrations of octopamine. These results suggest that the effect on relaxation rate was mediated through a different mechanism(s) or site(s) of action from the effects on twitch amplitude or contraction rate.

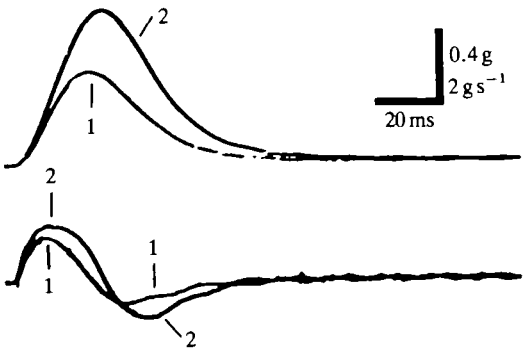


Fig. 1. Effect of octopamine on twitch amplitude, contraction rate and relaxation rate. Superimposed traces at top show mechanical records of metathoracic DLM twitch tension. Superimposed traces at bottom show differentiated twitches, upward deflections indicating contraction rates and downward deflections indicating relaxation rates. In both sets of records trace 1 was recorded prior to octopamine application and trace 2 was recorded during the maximal octopamine (10^{-6} mol l^{-1}) response. In this record, twitch amplitude was increased by 68 %, contraction rate by 26 % and relaxation rate by 46 %. Twitch duration (from the initiation of contraction to 90 % of relaxation) was 76 ms both before and during octopamine application.

The receptor(s) mediating these responses was specific for octopamine-like phenolamines. Synephrine was more potent in increasing twitch amplitude than was octopamine. The threshold for twitch amplitude potentiation was between 10^{-9} and 10^{-8} mol l^{-1} and maximal potentiation occurred between 10^{-7} and 10^{-4} mol l^{-1} (Fig. 3A). The EC_{50} of synephrine was 5.0×10^{-8} mol l^{-1}. Dopamine (a catecholamine) was approximately two orders of magnitude less potent than octopamine (threshold between 10^{-6} and 10^{-5} mol l^{-1}) (Fig. 3A). In contrast to the effects on the locust extensor tibiae (O'Shea and Evans, 1979), serotonin was inactive on the *T. oceanicus* metathoracic DLM at concentrations between 10^{-7} and 10^{-4} mol l^{-1} (N=5).

The octopamine-mediated increases of twitch amplitude, contraction rate and relaxation rate persisted for a dose-dependent time after washing. At concentrations below 10^{-6} mol l^{-1} the octopamine effects began to decrease within 1 min

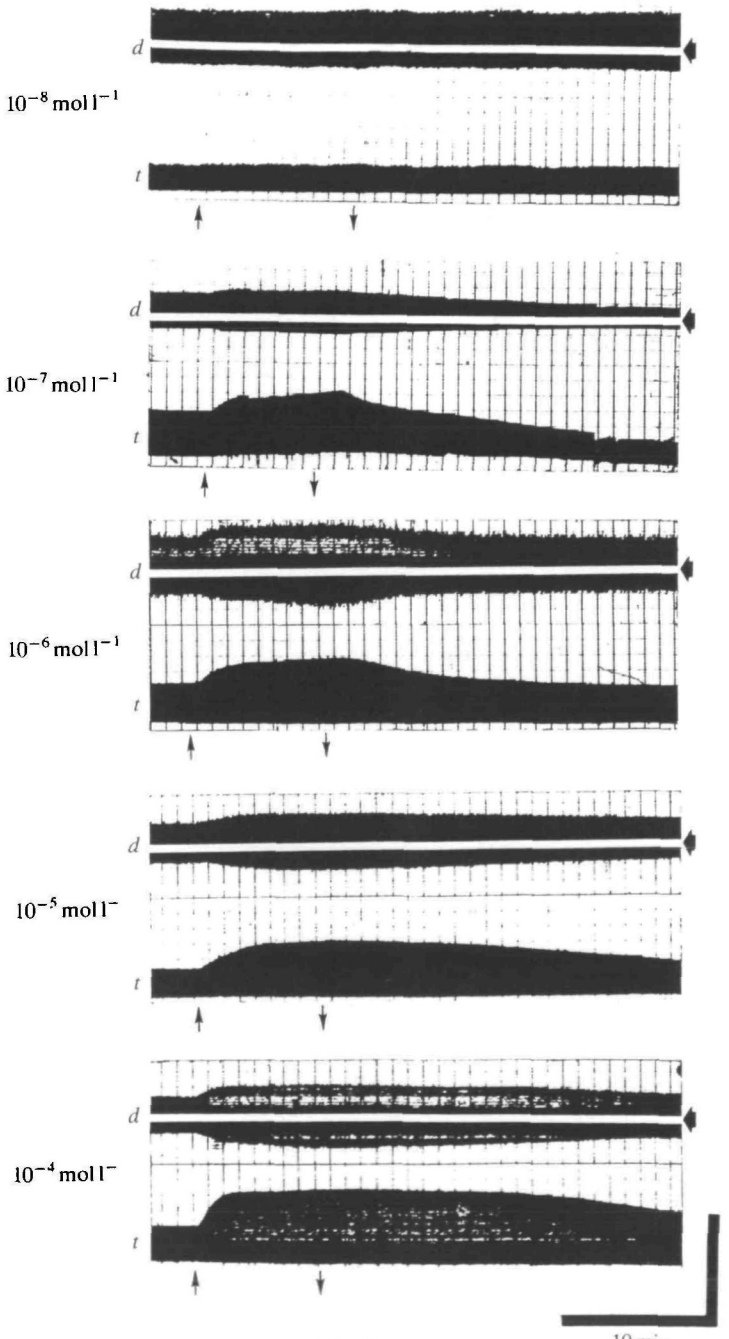
Table 1. *EC_{50} values for twitch amplitude, contraction rate and relaxation rate*

	EC_{50}			$\frac{EC_{50}\text{ amplitude}}{EC_{50}\text{ relaxation rate}}$	
	Twitch amplitude	Contraction rate	Relaxation rate	Cricket	Locust*
Octopamine (mol l^{-1})	4.0×10^{-7}	4.5×10^{-7}	3.0×10^{-7}	1.34	1.65
Naphazoline (mol l^{-1})	3.7×10^{-6}	1.0×10^{-7}	1.0×10^{-7}	36.85	0.06
Tolazoline (mol l^{-1})	7.7×10^{-6}	5.0×10^{-7}	6.7×10^{-6}	1.15	5.33
Clonidine (mol l^{-1})	2.7×10^{-4}	2.5×10^{-4}	5.4×10^{-4}	0.53	0.32

* Values from Evans (1981).

after washing. However, with higher concentrations of octopamine (or other agonists) all three parameters continued to increase for up to 20 min and persisted for over 2 h after washing.

One practical consequence of octopamine-mediated increases in twitch ampli-



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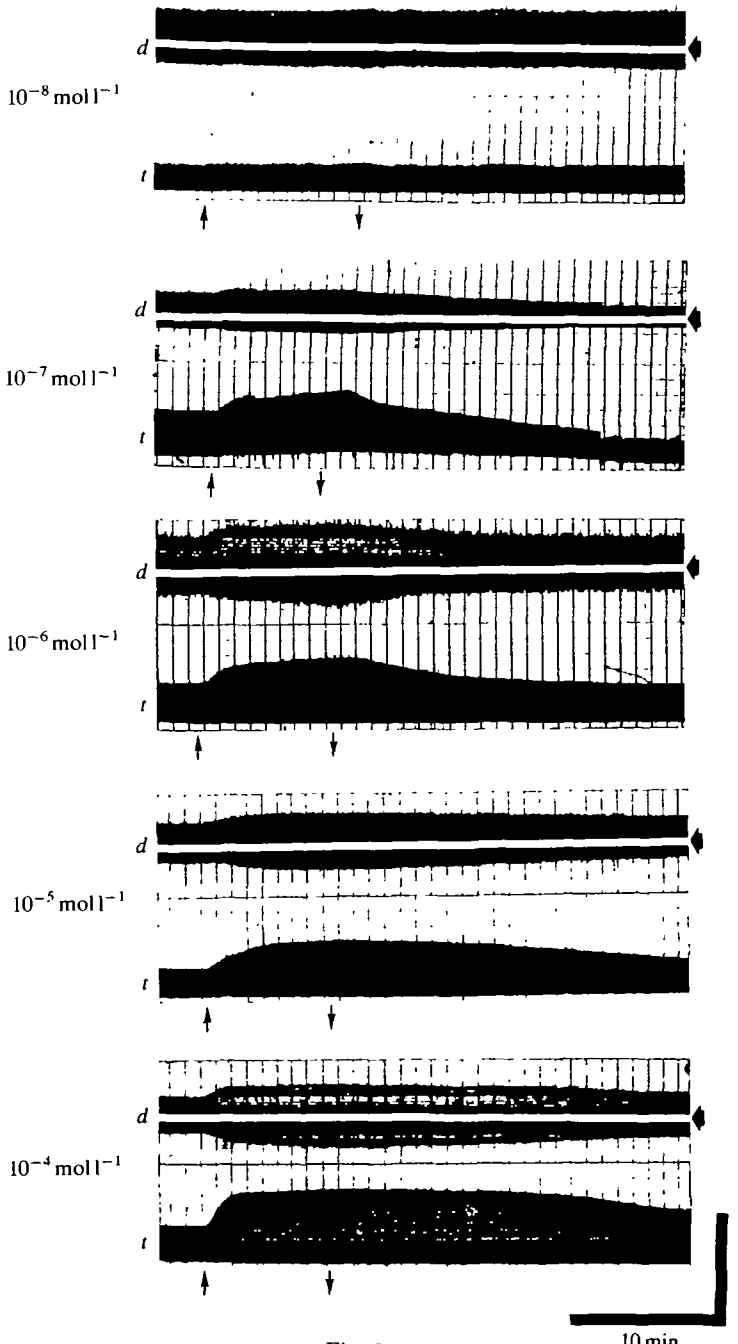

Fig. 2

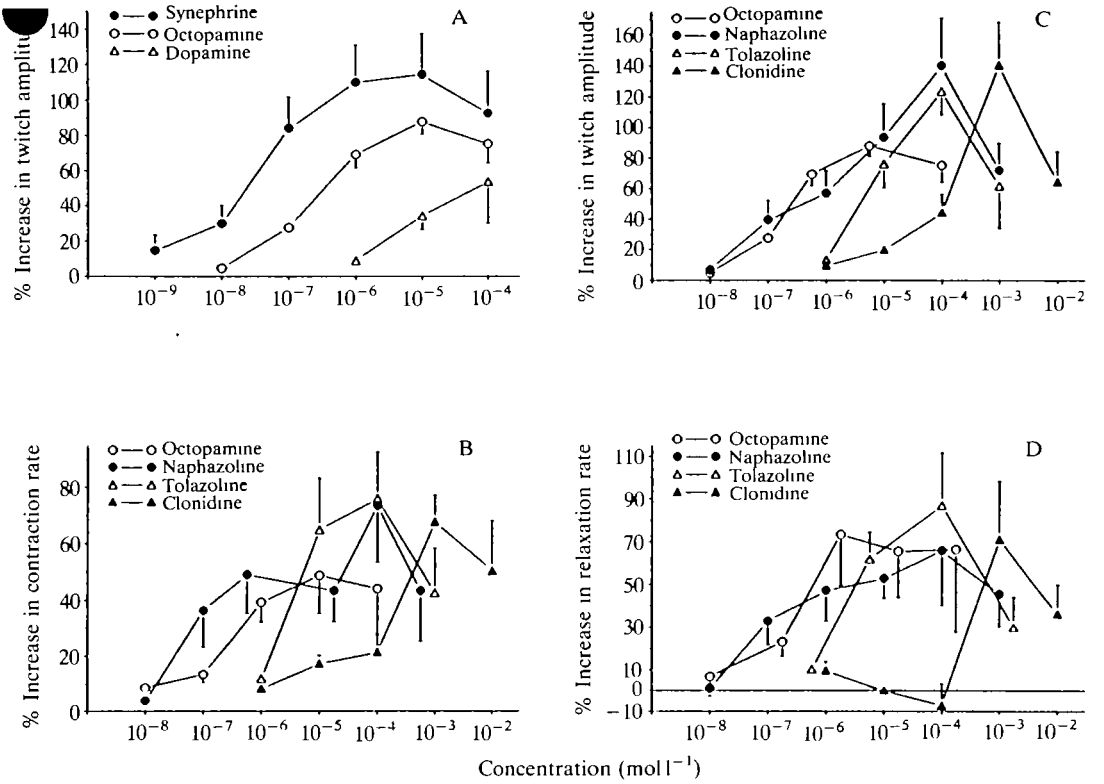

Fig. 3. Facilitation of twitch amplitude, relaxation rate and contraction rate by octopamine and its agonists. Each point represents the mean of 5–27 preparations. Vertical bars represent the S.E. When error bars are not present the S.E. is smaller than the symbol. Some points have been offset slightly for clarity. (A) Potentiation of twitch amplitude by octopamine, synephrine and dopamine. (B,C,D) Dose–response curves for the effects of octopamine and three agonists on contraction rate (B), twitch amplitude (C) and relaxation rate (D).

tude and relaxation rate is shown in Fig. 4. When the muscle was stimulated to contract at 25 Hz, a frequency similar to that used during flight or stridulation (Bentley and Hoy, 1970), octopamine increased the range of tension production (between maximum and minimum) and increased peak tension. In six preparations the mean increase in the range of tension production in response to

Fig. 2. Effect of several concentrations of octopamine on the metathoracic DLM. Top traces (*d*) are differentiated twitches (cf. Fig. 1); deflections above the center line (arrow) indicate the contraction rate and deflections below the center line indicate the relaxation rate. Bottom traces (*t*) are mechanical records of twitch tension. The preparation was stimulated at 1 Hz. Owing to the slow paper speed, individual twitches are not evident in the records. Octopamine was applied in the indicated concentrations at the upward-pointing arrow; and washed off at the downward-pointing arrow. Vertical scale: 10^{-8}, 10^{-7}, 10^{-6} mol l^{-1}; 2 g and 2 g s^{-1}: 10^{-5}, 10^{-4} mol l^{-1}; 4 g and 4 g s^{-1}.

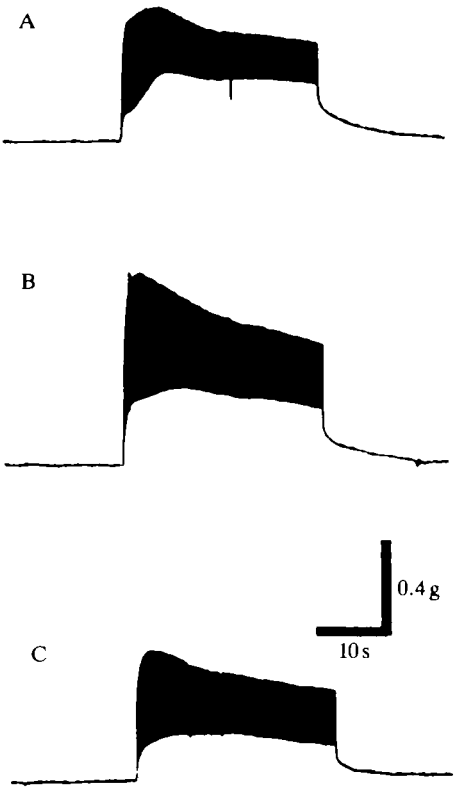

Fig. 4. Octopamine's effect on twitches during 25 Hz stimulation of the metathoracic DLM. The metathoracic DLM was stimulated (A) before octopamine application, (B) 10 min after 10^{-5} mol l^{-1} octopamine, and (C) after washing for 1 h. Note the increased maximum tension and range of tension production, as indicated by the increase in the width of the trace in B.

10^{-5} mol l^{-1} octopamine when stimulated at 25 Hz was 46.6±7.5 % (±S.E.). Although we did not see complete relaxation between twitches, Ready and Josephson (1982) found that when they stimulated the *T. oceanicus* metathoracic DLM at a similar frequency, complete relaxation occurred between twitches. The reason for this difference between their study and ours is not clear.

Electrophysiological effects of octopamine

The effects of octopamine on EJPs were examined by evoking unitary EJPs through neural stimulation. Results from these experiments (not shown) confirm that each metathoracic DLM cell is innervated by a single fast motoneuron (Clark, 1976*b*).

The effects of 10^{-5} mol l^{-1} octopamine on resting membrane potential (E_M), EJP amplitude and EJP duration at half amplitude ($T_{1/2}$) are presented in Table 2. Resting membrane potential and EJP amplitude were unaffected by octopamine

Table 2. *Effects of octopamine on membrane potential, EJP amplitude and EJP duration*

Parameter	Control	10^{-5} mol l^{-1} octopamine
11 mmol l^{-1} Ca^{2+}		
E_M (mV)	65.0±1.9	64.9±2.5
EJP (mV)	70.4±3.4	68.1±4.2
$T_{1/2}$ (ms)	1.20±0.15	1.31±0.16*
1.8 mmol l^{-1} Ca^{2+}		
E_M (mV)	66.6±4.6	65.5±4.6
EJP (mV)	43.3±6.7†	50.2±6.3*
$T_{1/2}$ (ms)	1.82±0.38†	1.74±0.27

* Significantly different from control, $P<0.01$.
† Significantly different from value in 11 mmol l^{-1} Ca^{2+} saline.
Values are mean±S.E. ($N=10$).

However, there was a slight but statistically significant increase in $T_{1/2}$ ($P<0.01$, $t=-4.27$, df=9; paired t-test).

These parameters were also examined in low-calcium (1.8 mmol l^{-1}) saline which tends to reduce active membrane responses (Klaassen and Kammer, 1985). Low-calcium saline alone (compared with 11 mmol l^{-1} calcium saline) resulted in significantly reduced EJP amplitude ($P<0.001$, $t=12.07$, df=18; t-test) and significantly increased $T_{1/2}$ ($P<0.001$, $t=-5.11$, df=18; t-test). However, membrane potential was unaffected ($P>0.05$, $t=-0.99$, df=18; t-test) (Table 2).

Following octopamine application in low-calcium saline, resting membrane potential and $T_{1/2}$ were unaffected. However, EJP amplitude was significantly increased ($P<0.01$, $t=-3.78$, df=9; paired t-test) (Table 2).

To examine if octopamine caused detectable changes in the passive electrical properties of the muscle membrane, input resistance was monitored while the metathoracic DLM was exposed to octopamine. Input resistance (approximately 600 kΩ) was unaffected by 10^{-5} mol l^{-1} octopamine. There was a tendency for a slight hyperpolarization of membrane potential (up to 3 mV), although similar shifts (and depolarizing shifts) occurred during control periods.

The effects of octopamine on the frequency and amplitude of mEPPs before and after 10^{-5} mol l^{-1} octopamine application were examined. The amplitude of most mEPPs was about 1 mV (range 0.3–3 mV), the duration about 5 ms and the frequency about 0.3 Hz. Octopamine (10^{-5} mol l^{-1}) caused a statistically significant increase in mEPP frequency (Fig. 5A) ($P<0.05$, $t=-3.32$, df=5; paired t-test; $N=6$). This effect occurred within 30 s and persisted for the duration of octopamine application. Octopamine had no significant effect on mEPP amplitude (Fig. 5B). The mean mEPP amplitude prior to octopamine exposure was 0.70 mV and that after exposure to 10^{-5} mol l^{-1} octopamine was 0.71 mV ($t=-0.45$, not significant; paired t-test) ($N=240$ mEPPs from six preparations).

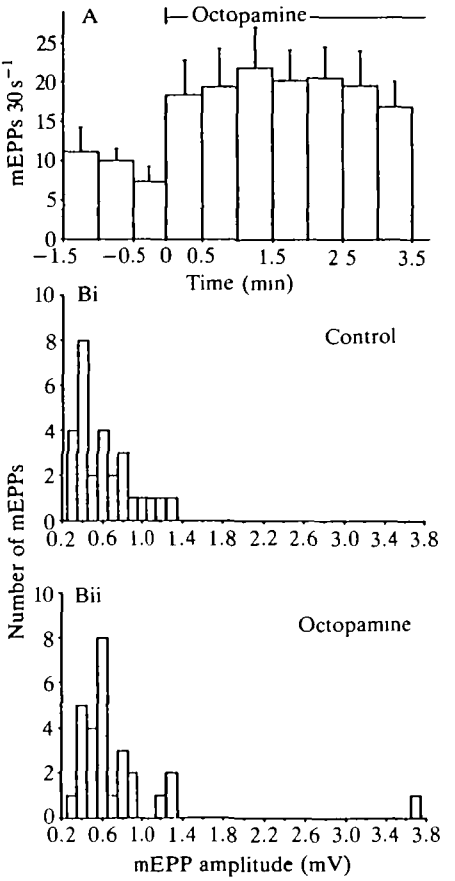

Fig. 5. The effect of octopamine on average mEPP frequency and amplitude. (A) Octopamine (10^{-4} mol l^{-1}), applied at time 0, produced a significant increase in mEPP frequency. Bars indicate the mean number of mEPPs per 30 s. Vertical lines indicate S.E. (B) The mean amplitude of mEPPs is unaltered by octopamine, although there may be a slight shift in the distribution towards increased amplitude in Bii (results from a typical preparation).

Role of DUMDL in modulating metathoracic DLM function

An obvious candidate neuron for octopaminergic modulation of the metathoracic DLM is DUMDL. Cobalt backfills indicate that the metathoracic DLM is innervated by one DUM neuron (not shown). Lucifer Yellow injections show that in *T. oceanicus* the DUMDL soma was located in the posterior half of the DUM cluster, although the exact position was variable. The anatomy of the DUMDL was similar to that in other orthopterans (Bentley, 1973; Clark, 1976*a*; Davis and Alanis, 1979; Hoyle, 1978; Hoyle *et al.* 1980; Watson, 1984; Sombati and Hoyle, 1984).

Electrophysiological properties of the *T. oceanicus* DUMDL were also similar to those of other DUM neurons (Heitler and Goodman, 1978; Hoyle and Dagan

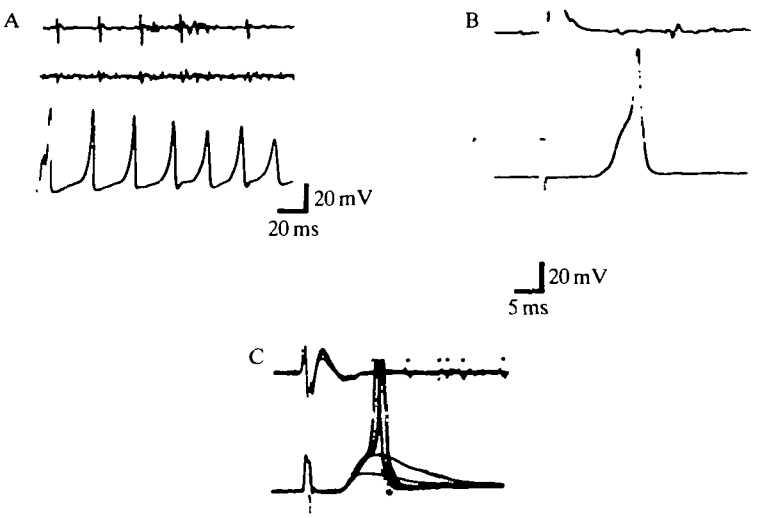

Fig. 6. Spiking activity and conduction failure in DUMDL. (A) Spiking activity was evoked by injection of depolarizing current into the DUMDL soma. Spikes were conducted peripherally to the left and right metathoracic nerves 1 (top two traces). During maintained depolarization, spike amplitude decreased and peripheral propagation failed (spikes 5 and 7) when spike amplitude dropped below a critical level. (B) Stimulation of nerve 1 produced a spike in the soma (bottom) and contralateral nerve 1 (top). (C) Conduction failure occurred during 4 Hz stimulation of metathoracic nerve 1. During repetitive stimulation, the onset of the intracellularly recorded spike was progressively delayed, and then spike amplitude decreased in two distinct increments. In the extracellular record of the contralateral nerve 1 the latency of DUMDL spikes (dots) progressively increased and the spike eventually failed.

1978; Davis and Alanis, 1979; Christensen and Carlson, 1982; Lange and Orchard, 1984). Resting potentials of DUMDL were usually near −60 mV and spike amplitudes ranged from 55 to 85 mV. DUMDL rarely exhibited spontaneous spiking or postsynaptic activity, although sensory stimuli inconsistently evoked one or two spikes superimposed upon a compound depolarizing postsynaptic potential. Injection of depolarizing current caused repeated spiking, accompanied by a rapid decrease of spike amplitude (Fig. 6A). When soma spike amplitude fell below a critical level there was failure of spike propagation into the periphery (Fig. 6A).

To examine the action of DUMDL on evoked contractions of the metathoracic DLM, DUMDL was activated by stimulating the contralateral metathoracic nerve 1, while muscle contractions were evoked by stimulation of the ipsilateral mesothoracic nerve 6. In seven of nine preparations no increase in twitch amplitude occurred following DUMDL stimulation, even though many different stimulation frequencies and durations were used (from 0.5 to 50 Hz, stimulus train durations up to several minutes). However, in two of nine preparations small increases (up to 20 %) in twitch amplitude were seen (Fig. 7).

The low success rate in producing DUMDL-mediated modulation of the

Fig. 7. Potentiation of twitch amplitude by DUMDL stimulation. The metathoracic DLM was activated by continuous 1 Hz stimulation of mesothoracic nerve 6. DUMDL was stimulated at 20 Hz (horizontal bar) through the contralateral metathoracic nerve 1. This increase of twitch amplitude was the largest produced by DUMDL stimulation.

metathoracic DLM was apparently due to conduction failure in the DUMDL pathway. Recordings from metathoracic nerve 1, during stimulation of the contralateral nerve 1, showed that DUMDL spikes were initially propagated across the ganglion and into the contralateral nerve 1 (Fig. 6B,C). However, with repeated stimulation, the intraganglionic conduction time increased and spike propagation across the ganglion and into the contralateral nerve 1 eventually failed (Fig. 6C). Reliable spike conduction across the metathoracic ganglion was never observed at stimulation frequencies above 0.1 Hz. Intracellular recordings from the DUMDL soma confirmed that progressive spike failure occurred during repetitive stimulation. Conduction failure was indicated by two incremental decreases in spike amplitude (Fig. 6C). Similar incremental variations in spike amplitude have previously been reported in the grasshopper DUMETi and have been interpreted as a soma spike, a neurite spike and an axon spike, from largest to smallest in size (Hoyle and Dagan, 1978; Heitler and Goodman, 1978).

Characterization of the metathoracic DLM octopamine receptors

The octopamine receptors of the locust extensor tibiae muscle have been classified by Evans (1981) into three receptor types, based on the differential mechanical responses to various octopamine agonists and antagonists. The agonists and antagonists which most effectively permitted differentiation of these three classes of octopamine receptors were applied to the DLM to determine if comparable receptor types existed in this muscle. Naphazoline and tolazoline (octopamine$_2$ agonists) produced markedly greater increases in twitch amplitude, contraction rate and relaxation rate than the increases produced by clonidine (an octopamine$_1$ agonist) (Fig. 3B,C,D; Table 1). These results indicate that the receptors mediating increases in twitch amplitude, contraction rate and relaxation rate are octopamine$_2$ receptors.

In Evans' (1981) classification scheme one criterion for differentiating octopamine$_{2A}$ receptors from octopamine$_{2B}$ receptors involved calculation of the ratio of the EC_{50} for twitch amplitude facilitation to the EC_{50} for increased relaxation rat

(Table 1). This ratio expresses the relative potency of an agonist for the two octopamine$_2$ receptor classes. A ratio of less than 1.0 indicates that an agonist has greater potency in increasing twitch amplitude (octopamine$_{2A}$), whereas a ratio greater than 1.0 indicates the agonist is more effective in increasing relaxation rate (octopamine$_{2B}$). A ratio of 1.0 indicates that the agonist is equally effective in potentiating the two responses. Naphazoline preferentially increased relaxation rate, with a ratio of 36.9, whereas tolazoline was equipotent in increasing relaxation rate and twitch amplitude (Table 1). These results indicate that the octopamine$_{2A}$/octopamine$_{2B}$ distinction cannot be supported in the *T. oceanicus* metathoracic DLM.

The octopamine antagonists metoclopramide and chlorpromazine (10^{-5} mol l^{-1}) were able partially to block the effects of octopamine (10^{-6} mol l^{-1}). Metoclopramide caused a 39.2±3.3% (±S.E.) decrease in twitch amplitude potentiation, a 59.6±16.4% (±S.E.) reduction of relaxation rate potentiation and an 8.0±23.9% (±S.E.) reduction of contraction rate potentiation (N=6). Chlorpromazine reduced octopamine-induced twitch amplitude potentiation by 13.9±3.3% (±S.E.), relaxation rate potentiation by 50.0±31.9% (±S.E.) and contraction rate potentiation by 58.3±23.0% (±S.E.) (N=4). Both antagonists more effectively blocked increases in relaxation rate than increases in twitch amplitude. Chlorpromazine had little antagonistic effect on octopamine-mediated increases of twitch amplitude, while metoclopramide did not antagonize octopamine-mediated increases of contraction rate. The differential effectiveness of these antagonists in antagonizing octopamine-mediated increases of twitch amplitude and contraction rate suggests that separate receptors may mediate these responses even though they develop at the same rate (Fig. 2).

Discussion

Effects of octopamine on mechanical events

Octopamine produced increases in twitch amplitude, contraction rate and relaxation rate in the metathoracic DLM of *T. oceanicus* (Figs 1, 2, 3). These effects are qualitatively very similar to those found in the locust DLM (Whim and Evans, 1988). However, several differences were noted. In the cricket, octopamine caused increases of twitch amplitude, contraction rate and relaxation rate that were 2–3 times larger than those produced in the locust. Also, octopamine caused greater increases of twitch amplitude than relaxation rate, whereas the opposite effect was found in the locust. A result of the larger increase in relaxation rate in the locust was a decrease in twitch duration (Whim and Evans, 1988). However, in the cricket, twitch duration was unaffected or increased by octopamine (Fig. 1). The relative time course of development of these effects is different in the two preparations. Whim and Evans (1988) found that octopamine-induced increases in relaxation rate developed faster than twitch amplitude increases. In the cricket, increases in both twitch amplitude and contraction rate developed faster than increases in relaxation rate (Fig. 2). These results suggest

that octopamine-induced increases of relaxation rate may be more important in the locust than in the cricket. These differences between cricket and locust may reflect differences in the use of the DLM in the two insects.

The thresholds for octopamine effects on twitch amplitude, contraction rate and relaxation rate in the cricket metathoracic DLM (10^{-8} mol l^{-1}) were similar to those in the locust extensor tibiae (Evans, 1981), but were about an order of magnitude higher than in the locust metathoracic DLM (Whim and Evans, 1988). The EC_{50} values for octopamine effects in *T. oceanicus* metathoracic DLM (Table 1) were about an order of magnitude lower than those for the locust extensor tibiae (Evans, 1981). Whim and Evans (1988) did not calculate EC_{50} values for the locust metathoracic DLM, but extrapolation from their data (see Fig. 3) indicates that they would be similar to the values for the cricket.

Electrophysiological effects of octopamine

Octopamine increased mEPP frequency (Fig. 5A) and EJP amplitude (Table 2), suggesting that transmitter release from the presynaptic terminals was increased by octopamine. Similar octopamine-induced increases of mEPP frequency and EJP amplitude have been found in locust extensor tibiae (O'Shea and Evans, 1979) and *Manduca* DLM (Klaassen and Kammer, 1985; Klaassen *et al.* 1986). However, in *Manduca*, mEPP amplitude is increased by octopamine (Klaassen *et al.* 1986), but it is unchanged in the cricket (Fig. 5B) and locust extensor tibiae (O'Shea and Evans, 1979).

Role of DUMDL in modulating the metathoracic DLM

Since DUMDL innervates the metathoracic DLM (Clark, 1976*a*; Hoyle *et al.* 1980), it is an obvious candidate for providing modulatory input to the metathoracic DLM. In the locust, stimulation of DUMDL can modulate DLM function in a manner similar to octopamine (Whim and Evans, 1988). However, attempts to demonstrate modulation of the *T. oceanicus* metathoracic DLM by direct electrical stimulation of DUMDL were usually ineffective, apparently due to impulse conduction failure across the ganglion (Fig. 6). DUMDL in the adult *T. oceanicus* is more difficult to backfill with cobalt than other neurons in the same nerve, or DUMDL in juvenile crickets (Bentley, 1973; Clark, 1976*a*). In addition, Lucifer Yellow injected into the DUMDL soma spreads unusually slowly into the neurites (B. A. O'Gara, unpublished observations). It is possible that these differences indicate an anatomical alteration in DUMDL that could be correlated with increased conduction failure. For example, a constriction, which restricts the diffusion of dyes, could be a site with a low safety factor. The DUMDL in *T. oceanicus* is more prone to failure than other DUM neurons reported in the literature, including the DUMDL of another cricket species, *Acheta domesticus* (Davis and Alanis, 1979).

Diversity of octopamine receptor types

Pharmacological results shown in Table 1 indicate that the octopamine recep

tor(s) of the metathoracic DLM are octopamine$_2$ receptors (Evans, 1981), since the octopamine$_2$ receptor agonists naphazoline and tolazoline were much more potent than the octopamine$_1$ agonist clonidine. However, the metathoracic DLM octopamine receptor(s) cannot be classified as octopamine$_{2A}$ or octopamine$_{2B}$ receptors. For naphazoline, the ratio of EC_{50} values for increases in twitch amplitude and relaxation rate was 36.85, indicating a greater potency for increasing relaxation rate, in contrast to the effect in the locust (Evans, 1981). In the case of tolazoline, the ratio was approximately 1, indicating equipotency at facilitating both twitch amplitude and relaxation rate, unlike the ratio of 5.33 in the locust (Evans, 1981). These results indicate that the properties of the octopamine receptors in the cricket metathoracic DLM are sufficiently different from those of the locust extensor tibiae that pharmacological distinctions between receptor subtypes cannot be made with the agonists and antagonists used in this study.

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