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MODULATION OF CONTRACTILITY AND BASIC TONUS IN TWO SERIALLY HOMOLOGOUS MUSCLES IN THE CRICKET TELEOGRYLLUS OCEANICUS

Iowa State University PH.D. 1986

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Modulation of contractility and basic tonus in two serially homologous muscles in the cricket *Teleogryllus oceanicus*

by

Bruce A. O'Gara

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GENERAL INTRODUCTION

Segmentation is a prominent feature of annelids, arthropods and chordates. Presumably the ancestors of these groups were initially composed of similar segments, but through the course of evolution segments became regionally specialized for different activities and behaviors. It is presumed that along with segmental specializations appropriate modifications of the segmentally arranged nervous system also occurred.

In the mid-body segments of annelids there is little obvious specialization of the segments. Correspondingly, the nervous system within each of these segments contains repeated sets of identifiable and segmentally homologous neurons (Gunther, 1972; Blackshaw, 1981). Nevertheless, there are serial gradations and regional specializations in the anatomy and physiology of the central nervous system of many annelids (Thompson and Stent, 1976a,b; Schafer and Calabrese, 1981; Leake, 1986; Smith and Mittenthal, 1980; Drewes and McFall, 1980; Drewes et al., 1980; Pallas and Drewes, 1981). These differences are established during embryonic development prior to hatching (Prosser, 1933; O'Gara et al., 1982; Weisblat, 1981).

Arthropod segmental ganglia also contain serially homologous, identifiable neurons. These neurons can be strikingly similar in anatomy (Tyrer and Altman, 1974;

Robertson et al., 1982), and neurotransmitter content (Bishop and O'Shea, 1982; Tyrer et al., 1984). However, similar neuronal anatomy does not necessarily imply similar function. For example, while the anatomy of spiracular motoneurons of the hissing segment of the hissing cockroach *Gromphadorina portentosa* is the same as that of other abdominal segments, the tracheal system is modified to produce hissing. Spiracular motoneuron activity of the hissing segment differs from other segments during normal respiration as well as during hissing (Nelson, 1979).

The anatomy of the insect thoracic segments is highly differentiated compared to the abdominal segments. The most obvious modifications of the thorax are the presence of legs and wings. In jumping orthopterans the metathoracic legs are much larger than either the mesothoracic or prothoracic legs. Corresponding to this specialization, dramatic alterations of neuronal function occur in some leg motoneurons of the locust. For example, the fast and slow motoneurons of the extensor-tibiae in the metathoracic ganglion have switched function compared to the other thoracic segments (Wilson and Hoyle, 1978; Wilson, 1979a,b).

The process of segmental differentiation in arthropods is not neccessarily restricted to embryonic development. For example, in the holometabolous insect, *Manduca sexta*, differentiation of both the nervous system and muscles occurs during metamorphosis. The motoneurons of the dorsal

longitudinal muscle (DLM) are conserved through metamorphosis (Casaday and Camhi, 1976) although their anatomy can be greatly modified (Truman and Reiss, 1976). The thoracic DLM has very different functions in the larva and the adult. In the caterpillar this muscle is used for crawling and other relatively slow movements. The ultrastruture and mechanical responses are typical of arthropod slow muscle (Rheuben and Kammer, 1980). During metamorphosis to the adult moth, the muscle remains innervated by the same motoneurons, but undergoes the transformation into flight muscle. The ultrastructure and mechanical responses of the DLM are then typical of arthropod fast muscle (Rheuben and Kammer, 1980). Interestingly, during this transformation from slow to fast muscle little change in synaptic morphology occurs (Rheuben and Kammer, 1980; Schaner and Rheuben, 1985). Although the cause of this transformation is generally attributed to hormonal influences, the specific mechanisms involved in transformation of the muscles are unknown.

Some clues to how the differentiation of muscle types occurs have been obtained in studies of the dimorphic claws of certain crustaceans. During the juvenile stages of lobsters, one claw differentiates into a crusher and the other into a cutter. The fibers of the claw closer muscle also become differentiated, with a greater percentage of fast muscle fibers in the cutter than the crusher (Costello et al., 1981). Two alternative hypotheses have been proposed to

explain such differentiation. The myogenic hypothesis states that the muscle plays an active role in development, dictating the type of synapses which form (Frank, 1973). The neurogenic hypothesis states that the motoneurons, through some trophic factor, determine fiber type (Atwood, 1973). These two hypotheses are not mutually exclusive and the actual situation appears to be more complex than either model by itself suggests. Neural factors have been shown to be important in determining fiber type, but, other factors play a major role in determining fiber type (Trinkaus-Randall, 1982).

The purpose of the present studies was to examine the modulation of twitches and basic tonus by octopamine and proctolin in two serially homologous muscles of the cricket *Teleogryllus oceanicus*. One of these muscles, the metathorcic DLM, is specialized for rapid contraction to power wing movements. The other muscle, the abdominal DLM, is specialized for relatively slow movements and postural control of the abdomen.

Explanation of Dissertation Format

This dissertation is composed in the alternate format. The sections (Parts I - II) of this dissertation are complete manuscripts modified to conform to the specifications of the Iowa State University Thesis Office. Each part consists of

an introduction, methods and materials, results, discussion, summary and references. A general introduction precedes Part I and a general summary follows Part II.

SECTION I. DIFFERENTIAL PHARMACOLOGICAL SENSITIVITY OF SERIALLY HOMOLOGOUS MUSCLES IN THE CRICKET *TELEOGRYLLUS OCEANICUS*

I. THE METATHORACIC DORSAL LONGITUDINAL MUSCLE

INTRODUCTION

Tension production of invertebrate muscle can be modulated through means other than classical excitatory and inhibitory neurotransmission (reviewed by Hoyle, 1983a,b). In arthropods, two important modulators of tension production are octopamine (reviewed by David and Coulon, 1985; Orchard, 1982; Evans, 1980) and proctolin. Effects of octopamine on arthropod muscle include: increases of twitch amplitude in locust extensor-tibiae (O'Shea and Evans, 1979), crayfish skeletal muscle (Fischer and Florey, 1983), *Limulus* skeletal muscle (Rane et al., 1984), and lobster skeletal muscle (Harris-Warrick and Kravitz, 1984). Octopamine also produces increases in relaxation rate of locust extensor-tibiae (O'Shea and Evans, 1979; Evans and Siegler, 1982), as well as potentiation of synaptic transmission in locust extensor-tibiae (O'Shea and Evans, 1979), crayfish opener muscle (Fischer and Florey, 1983), *Limulus* skeletal muscle (Rane et al., 1984), lobster skeletal muscle (Harris-Warrick and Kravitz, 1984), and *Manduca* dorsal longitudinal muscle (Klaassen and Kammer, 1985).

Evans (1981) presented evidence for three octopamine receptors based on differences in the mechanical responses of the locust extensor-tibiae to several octopamine agonists and antagonists. The octopamine$_1$ receptor modulates a myogenic rhythm, while the octopamine$_{2A}$ receptor produces increases in

twitch amplitude and the octopamine$_{2B}$ receptor mediates an increase in relaxation rate. It is not known if this classification scheme is generally applicable to other octopamine-responsive systems.

The pentapeptide proctolin has at least two distinct types of actions on arthropod muscle; these include increases in basic tonus of a muscle (Adams and O'Shea, 1983; Schwarz et al., 1980; Wordon et al., 1985) and increases in the amplitude of muscle contraction (Bishop et al., 1984a; Mercier and Wilkens, 1985). These effects are probably mediated by proctolin-containing motoneurons (O'Shea and Bishop, 1982; Agricola et al., 1982; Bishop et al., 1984a,b; Keshishian and O'Shea, 1985; Keshishian, 1985; Siwicki and Bishop, 1986).

The metathoracic dorsal longitudinal muscle (DLM) of the cricket *Teleogryllus oceanicus* is specialized for rapid contraction associated with wing movements (Ready and Josephson, 1982). The metathoracic DLM is innervated by 5 motoneurons, each innervating a different muscle band (Bentley, 1973; Clark, 1976a). Each muscle cell is innervated by a single fast motoneuron (Clark, 1976b; Neville, 1963). The metathoracic DLM is also innervated by a dorsal unpaired median neuron (DUMDL)(Bentley, 1973; Clark, 1976a; Davis and Alanis, 1979; Hoyle et al., 1980). All known DUM neurons 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 and proctolin on the production of tension by the metathoracic DLM of *T. oceanicus*. Electrophysiological correlates of octopamine action on tension production as well as the roles of cAMP and DUMDL in mediating these responses were examined. In addition, the octopamine receptor properties were examined to determine if they fit into the classification scheme of Evans (1981).

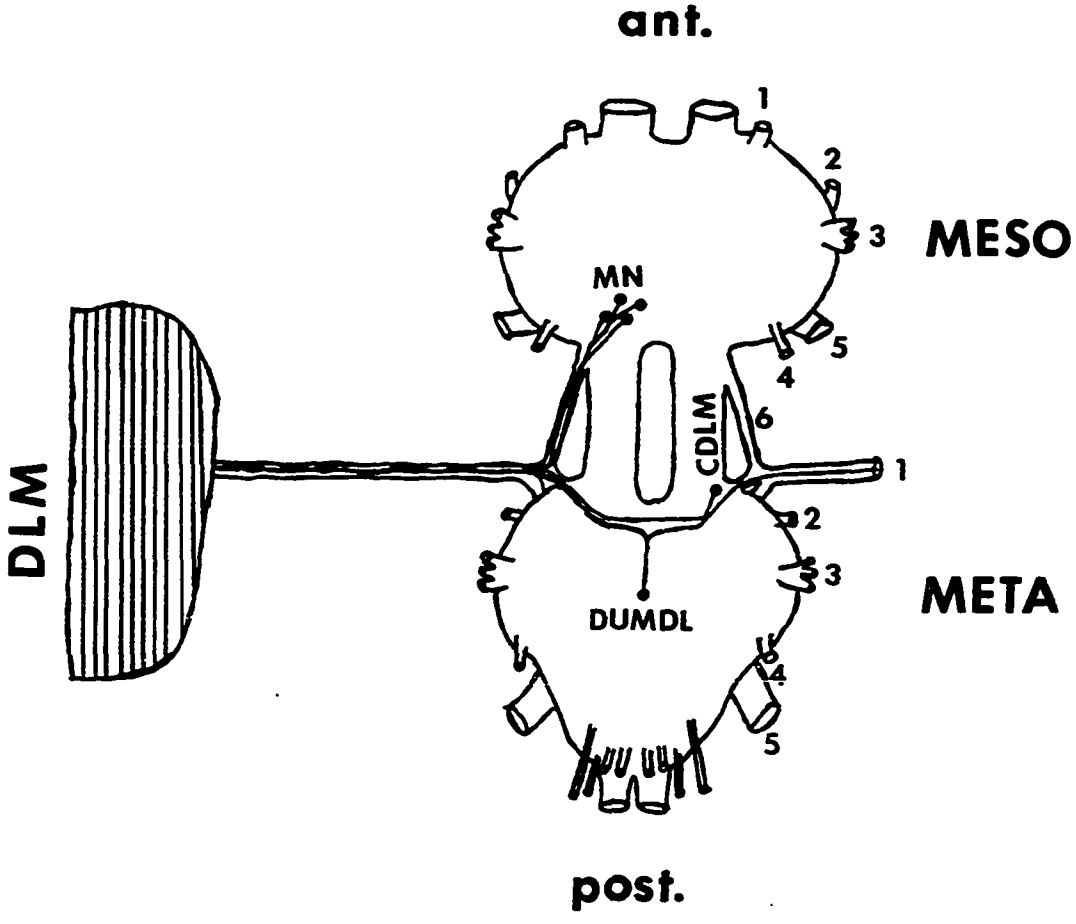
MATERIALS AND METHODS

Male crickets, *Teleogryllus oceanicus*, were raised from eggs in 12 l clear plastic boxes containing approximately 60 crickets. Crumpled paper toweling was placed in the boxes to increase surface area. The crickets were fed romaine lettuce daily, and kept on a 15:9 LD cycle at approximately 30°C.

The mechanical responses of the metathoracic DLM to various drugs were studied using young adult male crickets. 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 as well as the posterior half of the abdomen were removed. The prothoracic ganglion was then 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) which measured isometric tension. The muscle was stretched to approximately its normal length *in vivo*. Muscle responses

Figure 1. Innervation of the metathoracic DLM by nerve 1 of the metathoracic ganglion

The numbering of nerves follows Campbell (1961). Abbreviations: meso. - mesothoracic ganglion; meta. - metathoracic ganglion; ant. - anterior; post. - posterior; DLM. - metathoracic dorsal longitudinal muscle; MN. - mesothoracic motoneurons of the metathoracic DLM; CDLM. - contralateral dorsal longitudinal motoneuron; DUMDL - dorsal unpaired median neuron of the DLM.


ant.
1
2
3
MESO
MN
5
4
6
CDLM
1
DLM
2
3
META
DUMDL
4
5
post.

were evoked by supramaximal electrical stimulation of mesothoracic nerve 6 (Fig. 1) with a suction electrode (stimulus frequency = 1 Hz, 0.5 ms duration, 15 V). The force transducer was positioned for optimally recording twitch amplitude. All calculations involving twitch tension were based on relative rather than absolute values. Therefore, absolute tension was uncalibrated. After dissection, twitch amplitude decreased steadily for 1 - 2 hr until a stable amplitude was reached; preparations continued to be viable for several more hours. This decrease of twitch amplitude over time may indicate that the DLM is modulated by endogenous octopamine, which was released due to the stress of capture and dissection (c.f., 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 ml/min, 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} M octopamine and noting the response. The preparation was then exposed to 10^{-5} M antagonist for about 8 min, followed by 10^{-6} M octopamine plus 10^{-5} M

antagonist.

Muscle intracellular electrical activity was recorded with borosilicate glass microelectrodes filled with 1 M potassium acetate (20 - 50 M-ohm resistance). Input resistance was measured with two microelectrodes placed within the same muscle cell less than 60 micrometers 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 5 different muscle cells of each preparation (n = 10). Octopamine (10^{-5} M) was then applied to the preparation for 5 min and EJPs in 5 more cells were examined. Resting membrane potential, EJP amplitude, and EJP duration at one-half amplitude ($T_{1/2}$) were measured with a Tektronix 5D10 waveform digitizer. When miniature endplate 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 pipette. The octopamine in the bolus was diluted by the saline already present in the body cavity in a 1:1 ratio; therefore concentrations are expressed as the concentration after dilution in the body cavity.

Intracellular records from neurons in the metathoracic ganglion were obtained with aluminosilicate glass microelectrodes filled with 2.5% Lucifer Yellow (Sigma) and 1

M LiCl (50 - 70 M-ohm resistance). The metathoracic ganglion was supported with a small metal platform. Extracellular nerve recordings were made with suction electrodes. The preparations were superfused with saline at 0.5 ml/min.

The saline contained: 152 mM NaCl, 8 mM KCl, 1 mM $MgCl_2$, 11 mM $CaCl_2$, 4 mM $NaHCO_3$, 5 mM TES, 5 mM trehalose, 105 mM sucrose, pH 6.7. DL-octopamine, naphazoline, tolazoline, clonidine, metoclopramide, chlorpromazine, 5-hydroxytryptamine creatine sulfate, theophylline, 3-isobutyl-1-methyl-xanthine (IBMX) and proctolin were purchased from Sigma (St Louis, MO). Forskolin was purchased from Calbiochem-Behring (La Jolla, CA). All drugs were dissolved in saline, except forskolin which was dissolved in ethanol prior to dilution with saline. All experiments were conducted at room temperature (21 - 25°C).

RESULTS

Amine-mediated modulation of tension production in the metathoracic DLM

Octopamine applied to the metathoracic DLM during neuronal stimulation caused dose-dependent increases (i.e., facilitation) of twitch amplitude, (Figs. 2, 3 and 4) contraction rate and relaxation rate (Figs. 2, 3). Slight decreases in basic tonus were often seen, especially at high concentrations of octopamine (Fig. 3). The threshold concentration for twitch amplitude facilitation was about 10^{-8} M with maximal facilitation (88%) seen at 10^{-5} M. The EC_{50} (c.f., Fig. 4) for twitch amplitude facilitation was 4.0 x 10^{-7} M (Table 2). Threshold for increasing relaxation rate was about 10^{-8} M with maximal facilitation (74%) at 10^{-4} M and the EC_{50} (c.f., Fig. 14) at 3.0 x 10^{-7} M. Increases in relaxation rate developed more slowly than increases in contraction rate or twitch amplitude, as shown in the differentiated recordings of tension (Fig. 3). Such differences are 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 than the effects on twitch amplitude or contraction rate facilitation.

The octopamine-mediated increases in all three parameters persisted for a dose-dependent time after washing.

Figure 2. 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} M) response. Twitch amplitude was increased by 94%, contraction rate by 33%, and relaxation rate by 83%.

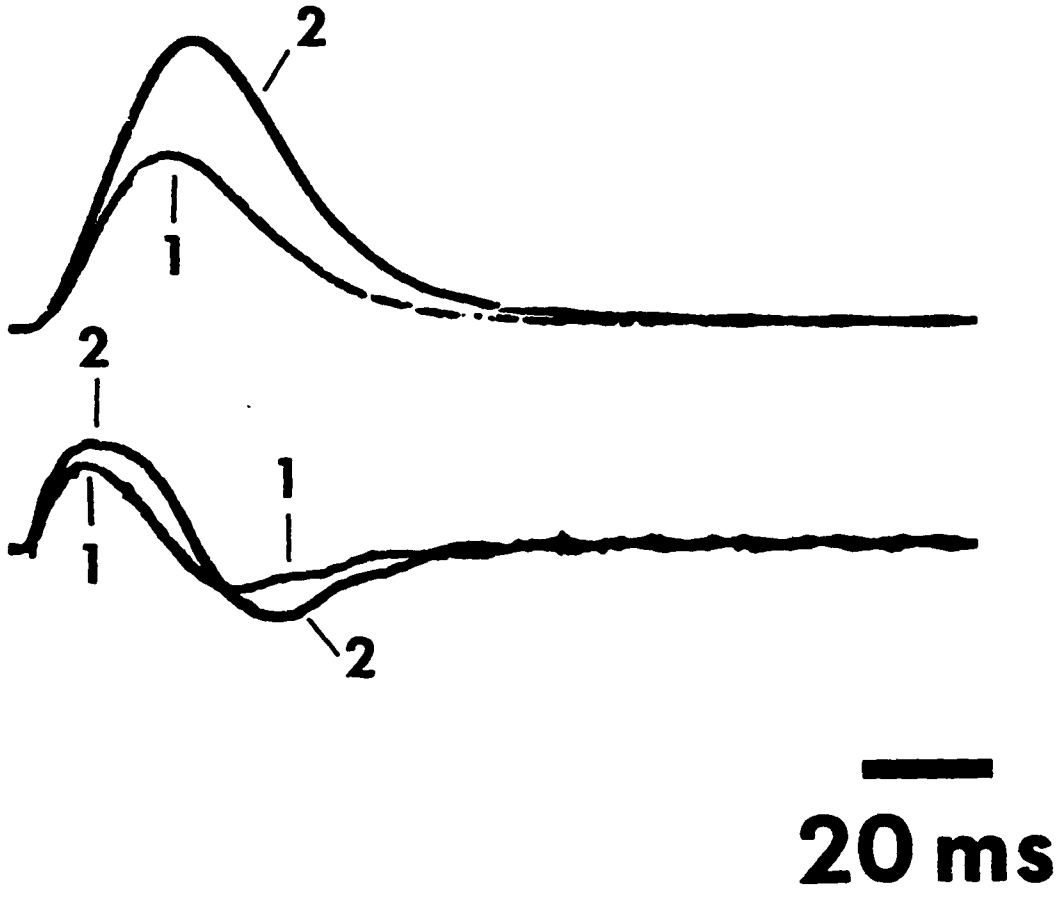
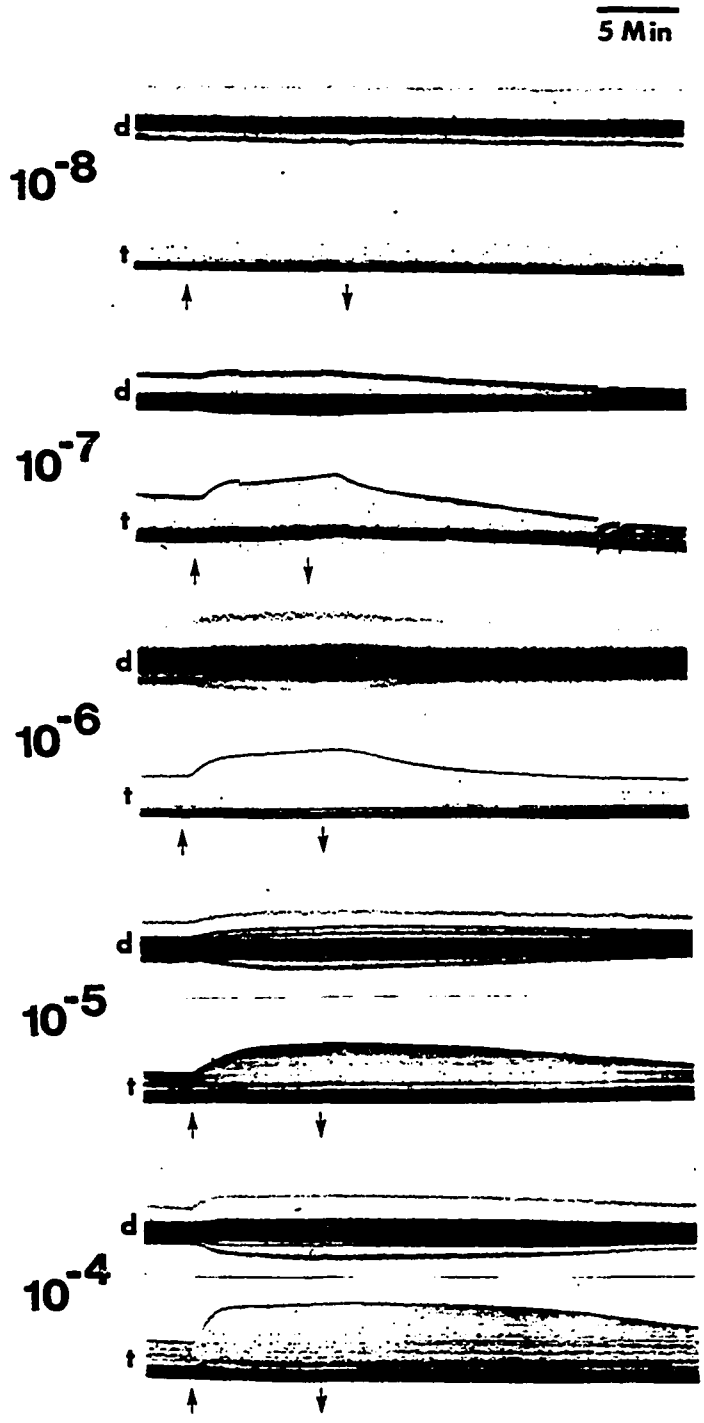

2
1
2
1
1
2
20 ms

Figure 3. Effect of several concentrations of octopamine on the metathoracic DLM

Top traces (d) are differentiated twitches (c.f., Fig. 2); deflections above the dark center line indicate the contraction rate and deflections below the dark center line indicate the relaxation rate. Bottom traces (t) are mechanical records of twitch tension. The preparation was stimulated at 1 Hz. Due 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; octopamine was washed off at the downward pointing arrow.


5 Min
d
10^{-8}
t
d
10^{-7}
t
d
10^{-6}
t
d
10^{-5}
t
d
10^{-4}
t

At concentrations below 10^{-6} M the octopamine effects began to decrease within 1 min 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 hr after washing.

Synephrine was more potent in increasing twitch amplitude than octopamine. The threshold for twitch amplitude facilitation was about 10^{-7} M and maximal facilitation occurred at 10^{-5} M (Fig. 4). The EC_{50} of synephrine was 5.0×10^{-6} M. In contrast, dopamine was approximately two orders of magnitude less potent than octopamine (threshold about 10^{-4} M)(Fig. 4). In 5 preparations serotonin was inactive at concentrations between 10^{-7} M and 10^{-4} M.

One of the physiological consequences of octopamine-mediated increases in twitch amplitude and relaxation rate is shown in Figure 5. When the muscle was stimulated to contract at a frequency similar to that used during flight or stridulation (25 Hz)(Bentley and Hoy, 1970), increases in the amplitude of tension transients and peak tension were evident during octopamine application. In 6 preparations the mean increase of tension transients was 146.6% $\pm$ 7.5 SE.

Figure 4. Facilitation of twitch amplitude by octopamine, synephrine, and dopamine

Each point represents the mean of between 5 and 27 preparations. Vertical bars represent the SE. SEs for 10^{-8} M octopamine and 10^{-6} M dopamine are smaller than the symbols.

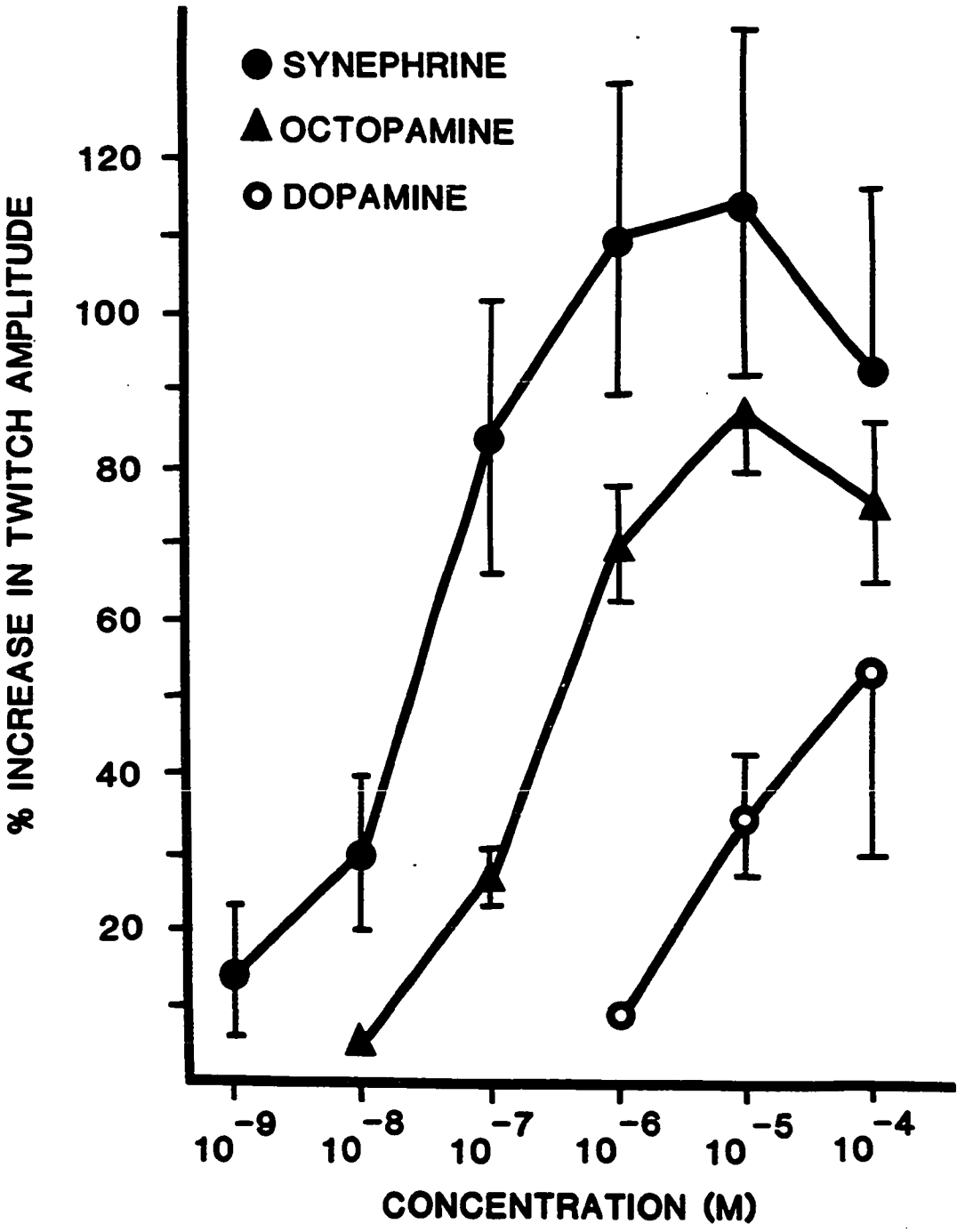
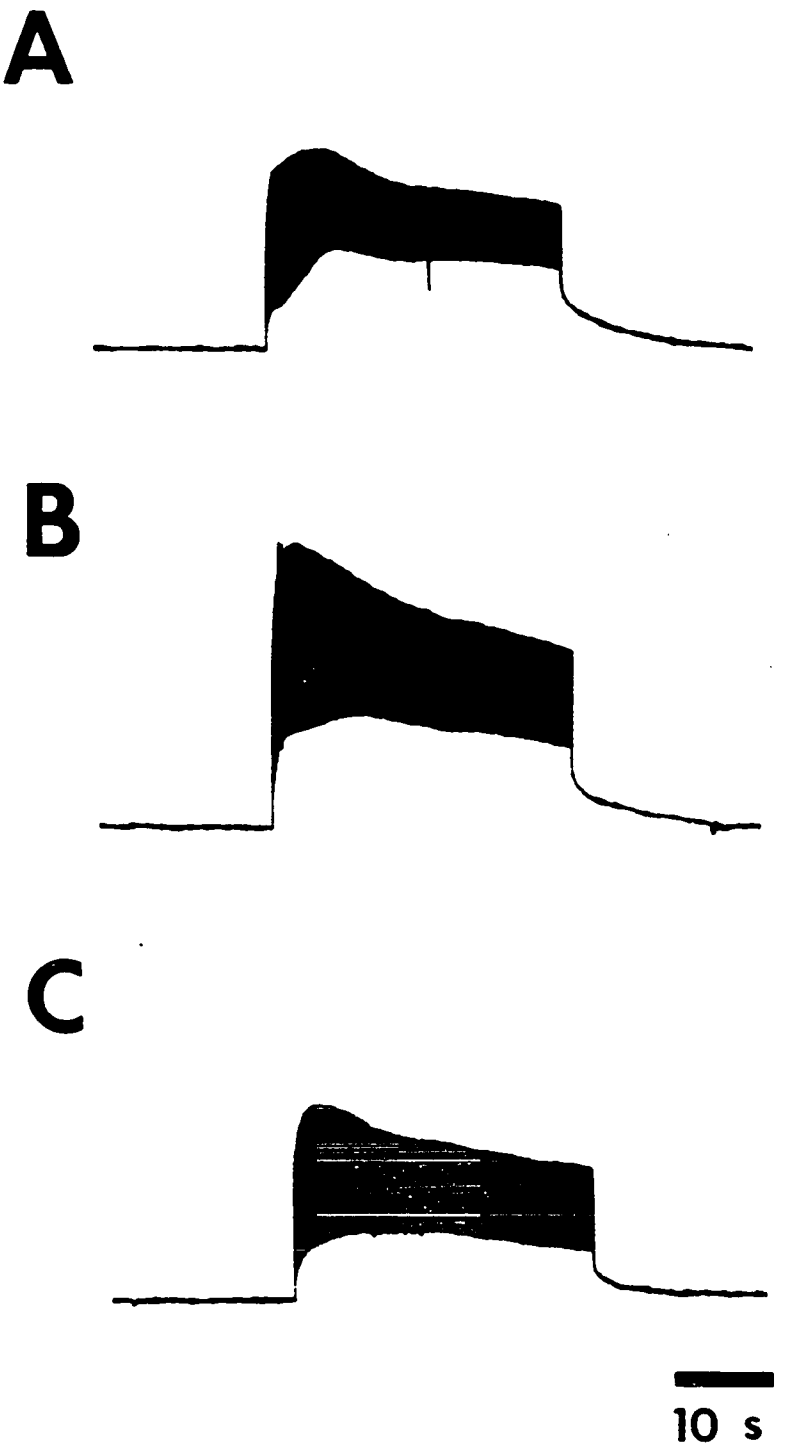

SYNEPHRINE
OCTOPAMINE
DOPAMINE
% INCREASE IN TWITCH AMPLITUDE
120
100
80
60
40
20
10^-9
10^-8
10^-7
10^-6
10^-5
10^-4
CONCENTRATION (M)

Figure 5. Octopamine 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} M octopamine, and C) after washing 1 hr. Note the increased amplitude of tension transients in (B).


A
B
C
10 s

Electrophysiological effects of octopamine on the metathoracic DLM

The effects of octopamine on EJPs were examined by evoking unitary EJPs through neural stimulation. Results from these experiments confirm that each metathoracic DLM cell is innervated by a single fast motoneuron (Clark, 1976b; Fig. 6).

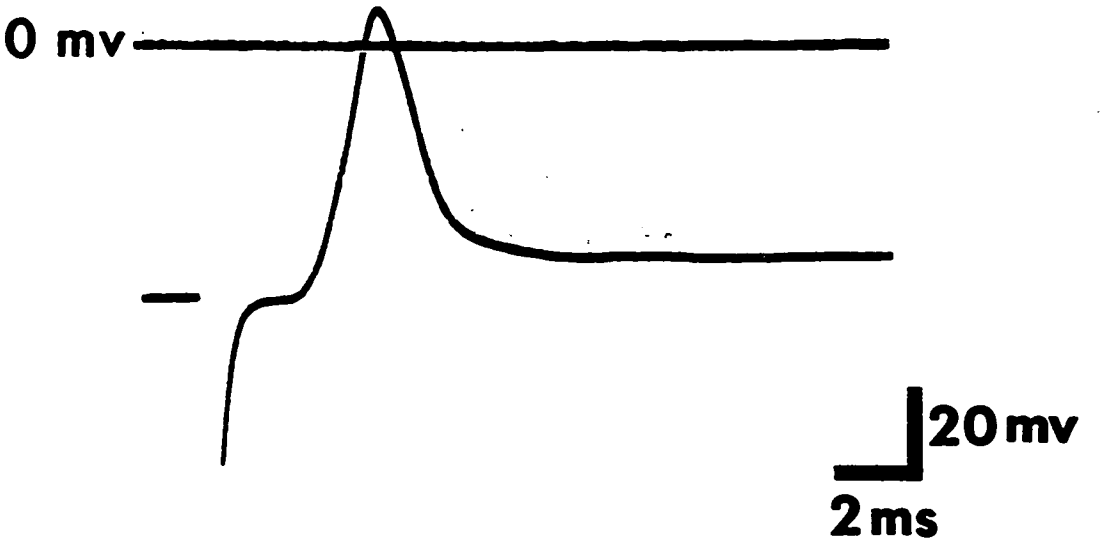
The effects of 10^{-6} M octopamine on resting membrane potential (Em), EJP amplitude and duration at one-half amplitude ($T_{1/2}$) are presented in Table 1. Resting membrane potential and EJP amplitude were unaffected by octopamine. However, there was a slight but statistically significant increase in $T_{1/2}$ ($p < .01$, $t = -4.27$, $df = 9$; paired difference t-test).

These parameters were also examined in low calcium saline which tends to reduce active membrane responses (Klaassen and Kammer, 1985). Low calcium saline alone resulted in significantly reduced EJP amplitude ($p < .001$, $t = 12.07$, $df = 18$; independent means t-test), and significantly increased $T_{1/2}$ ($p < .001$, $t = -5.11$, $df = 18$; independent means t-test). However, membrane potential was unaffected ($p > .05$, $t = -0.99$, $df = 18$; independent t-test)(Table 1).

Following octopamine application in low calcium saline (1.8 mM) resting membrane potential and $T_{1/2}$ were unaffected (Table 1). However EJP amplitude was significantly increased

Figure 6. Intracellular recording of an action potential from the metathoracic DLM

This response was elicited by a single suprathreshold stimulus to mesothoracic nerve 6.


0 mv
20 mv
2 ms

($p < .01$, $t = -3.78$, $df = 9$; paired difference t-test)(Table 1).

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 (Fig. 7). Input resistance was unaffected by 10^{-5} M octopamine. There was a tendency for a slight hyperpolarization (up to 3 mV), although similar shifts (both depolarizing and hyperpolarizing) occurred during control periods.

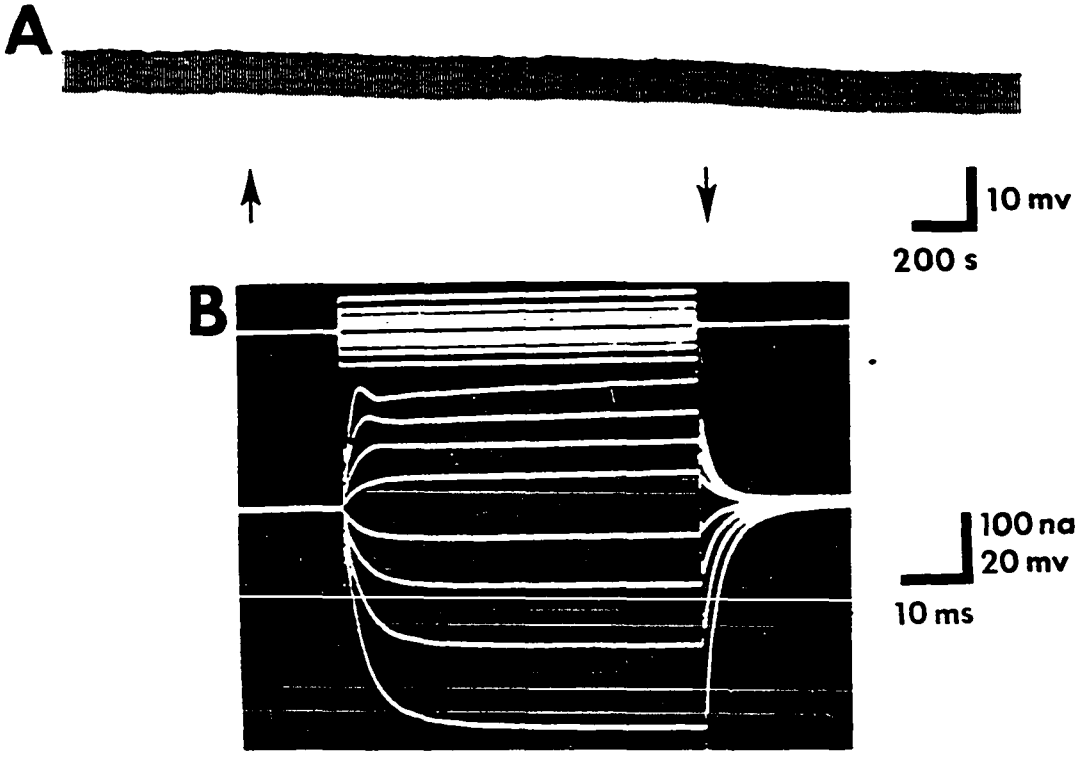
Table 1. Octopamine effects on membrane potential, EJP amplitude and EJP duration

	Parameter	Control (±SE)	Octopamine (±SE)
11 mM Ca^{++}			
	Em	65.0(±1.9)mV	64.9(±2.5)mV
(n=10)	EJP	70.4(±3.4)mv	68.1(±4.2)mV
	$T_{1/2}$	1.20(±0.15)ms	1.31(±0.16)ms*
1.8 mM Ca^{++}			
	Em	66.6(±4.6)mV	65.5(±4.6)mV
(n=10)	EJP	43.3(±6.7)mV	50.2(±6.3)mV*
	$T_{1/2}$	1.82(±0.38)ms	1.74(±0.27)ms

*$p<.01$.

Figure 7. Effect of octopamine on membrane potential and input resistance

A) Octopamine (10^{-5} M) was applied (upward pointing arrow) and then washed off (downward pointing arrow). A slight hyperpolarizing drift was evident but there was no change in input resistance (600 kilo-ohms) during hyperpolarizing current pulses (20 na; 50 ms duration). B) Current-voltage relationship of the metathoracic DLM using 50 ms pulse durations. The small transients at the onset of depolarization may indicate an active membrane response or delayed rectification.


A
10 mv
200 s
B
100 na
20 mv
10 ms

Possible presynaptic effects of octopamine were examined by comparing the frequency of mepps before and after octopamine application. In untreated preparations mepp amplitude varied from noise level (0.5 mV) up to 3 mV. The amplitude of most mepps was about 1 mV, duration about 5 ms (Fig. 8[A]), and frequency about 0.3 Hz. As shown in Figures 8 and 9, octopamine (10^{-5} M) caused a statistically significant increase in mepp frequency ($p < .05$, $t = -3.32$, $df = 5$; paired difference t-test; $n = 6$). These effects occurred within 30 s and persisted for the duration of octopamine application.

Role of cyclic nucleotides in octopamine responses

The possible role of cyclic nucleotides in octopamine-mediated responses of the metathoracic DLM was examined. Previous studies by Evans (1984a) showed that inhibition of phosphodiesterase (the enzyme which degrades cyclic nucleotides) by IBMX or theophylline resulted in elevated levels of cAMP and cGMP in locust extensor-tibiae. In the metathoracic DLM of *T. oceanicus*, IBMX or theophylline alone increased twitch amplitude and relaxation rate by approximately 20%. When octopamine was applied in the presence of theophylline (concentrations from 10^{-6} M to 10^{-4} M) no potentiation in twitch amplitude or relaxation rate was observed (Fig. 10[A]). IBMX also failed to potentiate octopamine responses.

Since IBMX or theophylline alone caused small increases

Figure 8. The effect of octopamine on mepp frequency

A) Two typical mepps are shown at a fast sweep speed. B) Control period (1) showing spontaneous mepp frequency. No change of mepp frequency occurred just after a 1 ml bolus of saline was applied (2). Mepp frequency approximately doubled just after a 1 ml bolus of 10^{-4} M octopamine was applied to the preparation (3).

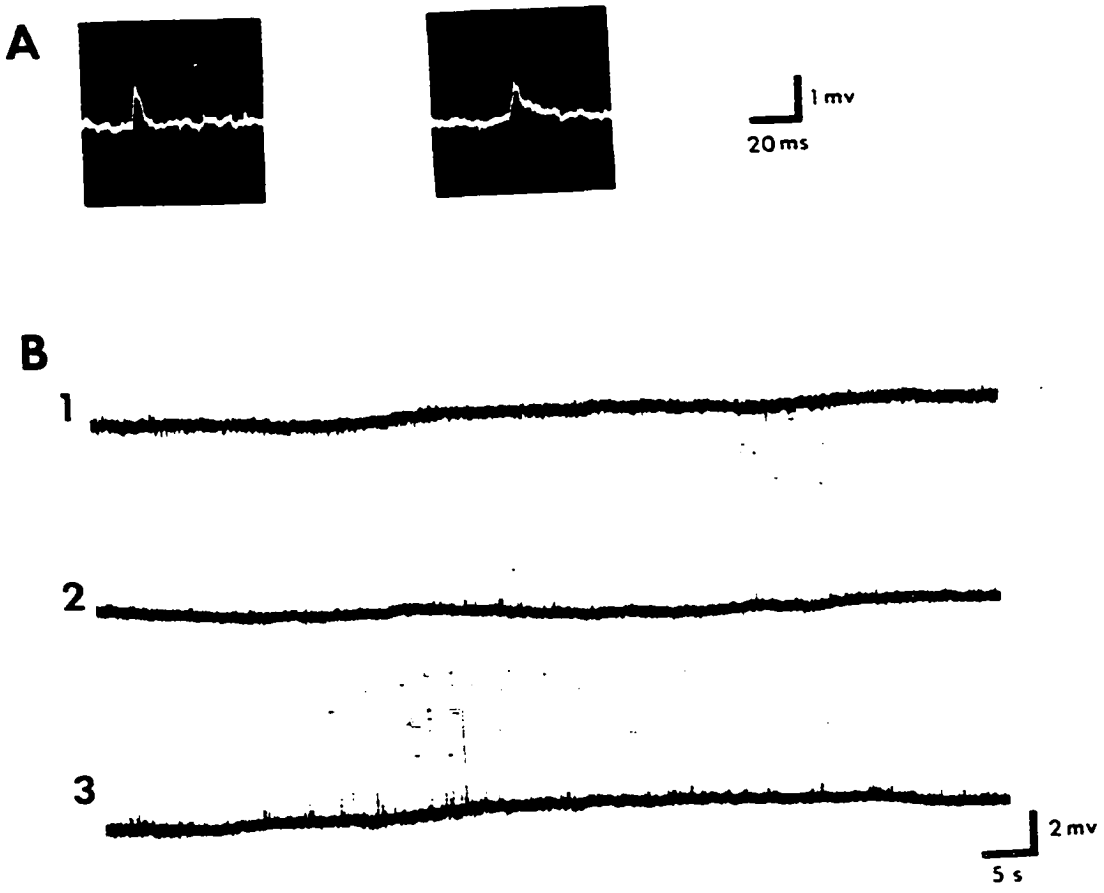
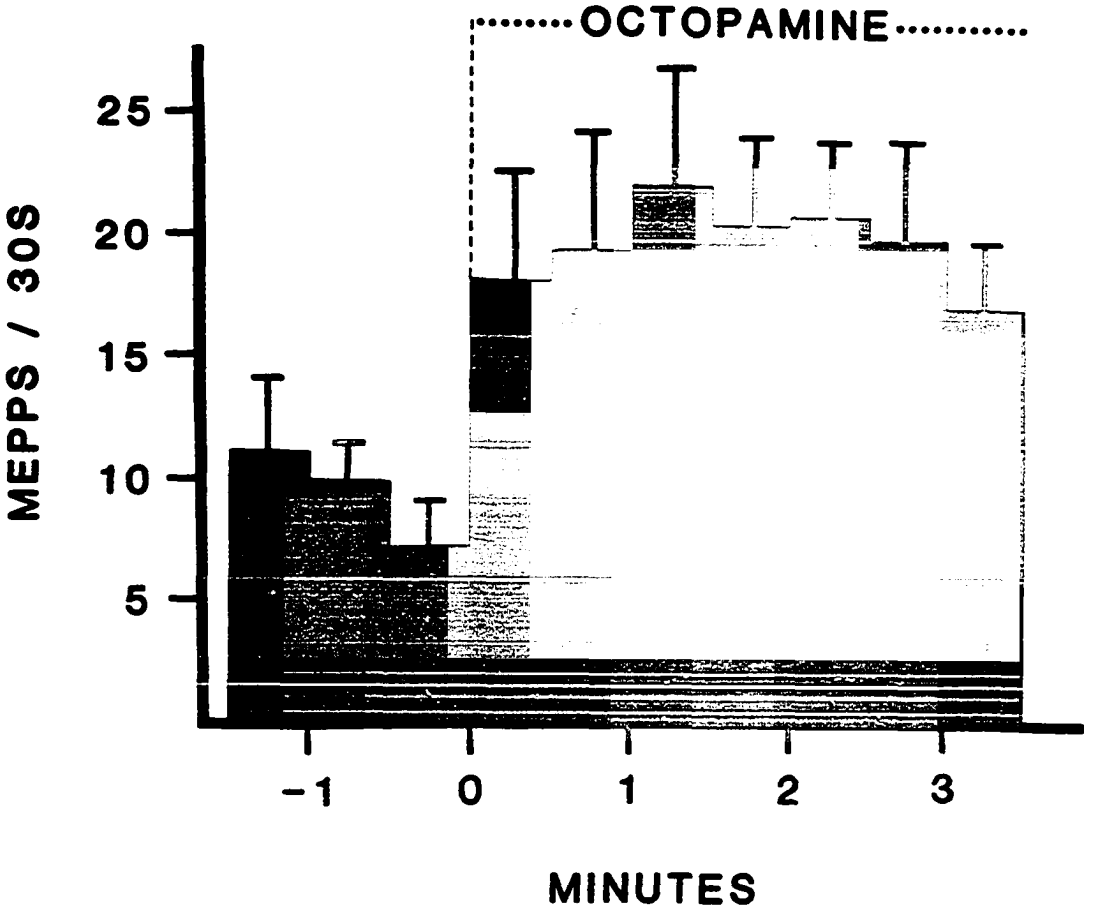

A
1 mv
20 ms
B
1
2
3
2 mv
5 s

Figure 9. Histogram showing the effect of octopamine on average mepp frequency

Octopamine (10^{-5} M), applied at time 0, produced a significant increase in mepp frequency. Bars indicate the mean number of mepps/30 s. Vertical lines indicate SE.


OCTOPAMINE
MEPPS / 30S
25
20
15
10
5
-1
0
1
2
3
MINUTES

in twitch amplitude and relaxation rate, the effect of the non-specific adenylate cyclase stimulator forskolin was examined. Relatively high concentrations of forskolin (10^{-4} M) caused a 20% increase in twitch amplitude, slight increases in relaxation and contraction rates, and a reduction of basic tonus (Fig. 10[B]). These results suggest that alterations in cyclic nucleotide levels in the metathoracic DLM could not fully account for the effects of octopamine.

Role of DUMDL in modulating metathoracic DLM function

An obvious candidate neuron for octopamine modulation of the metathoracic DLM is DUMDL. 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 *T. oceanicus* DUMDL was similar to that of other orthopterans (Bentley, 1973; Clark, 1976a; 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 other DUM neurons (Heitler and Goodman, 1978; Hoyle and Dagan, 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

Figure 10. The role of cyclic nucleotides in tension production

A) Theophylline failed to potentiate octopamine-mediated increases of twitch amplitude. (1) A control application of 10^{-6} M octopamine produced a 69.2% increase of twitch amplitude. (2) Theophylline (10^{-4} M) was applied at the first arrow; then 10^{-4} M theophylline plus 10^{-6} M octopamine was applied, at the second arrow, producing a 64.3% increase of twitch amplitude. B) Forskolin (10^{-5} M) was applied at the first arrow and washed off at the second arrow. A 23.5% increase of twitch amplitude and a reduction of basic tonus were evident (t). The differentiated twitches (d) show a 13.3% increase in contraction rate but no change in relaxation rate.

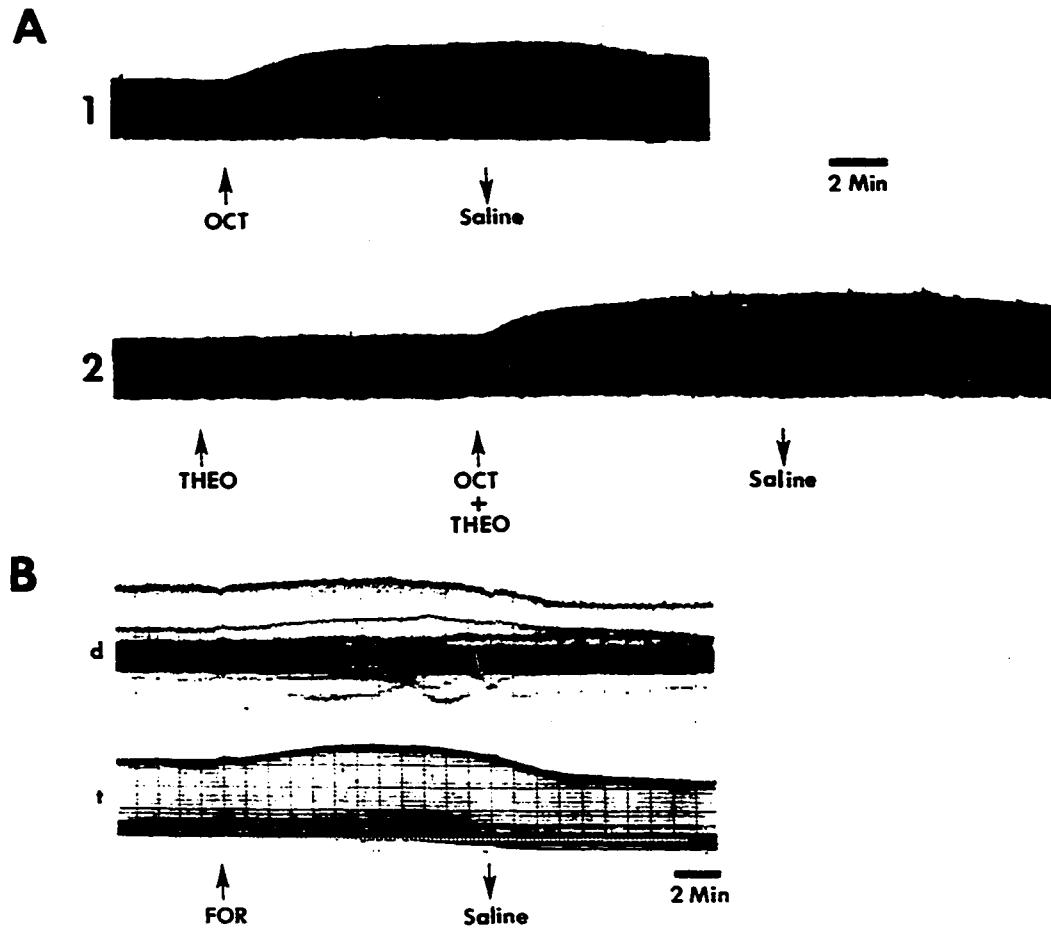
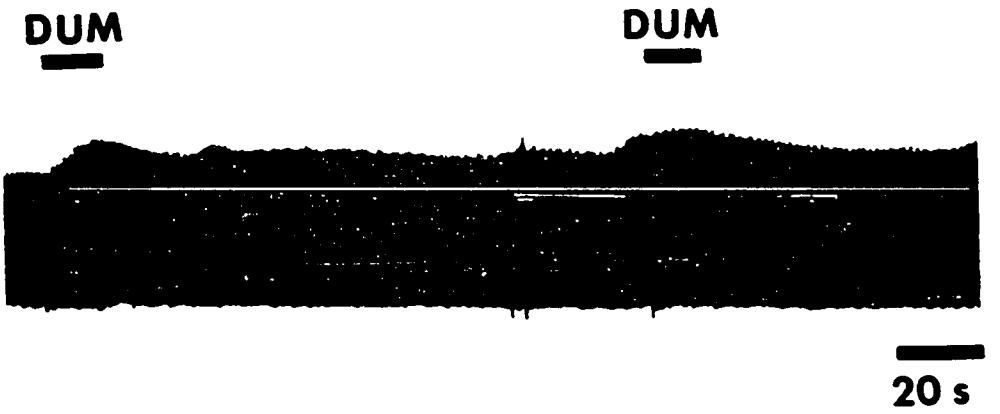

A
1
OCT
Saline
2 Min
2
THEO
OCT
+
THEO
Saline
B
d
t
FOR
Saline
2 Min

Figure 11. Facilitation 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 bars) through the contralateral metathoracic nerve 1. These increases of twitch amplitude were the largest produced by DUMDL stimulation.


DUM
DUM
20 s

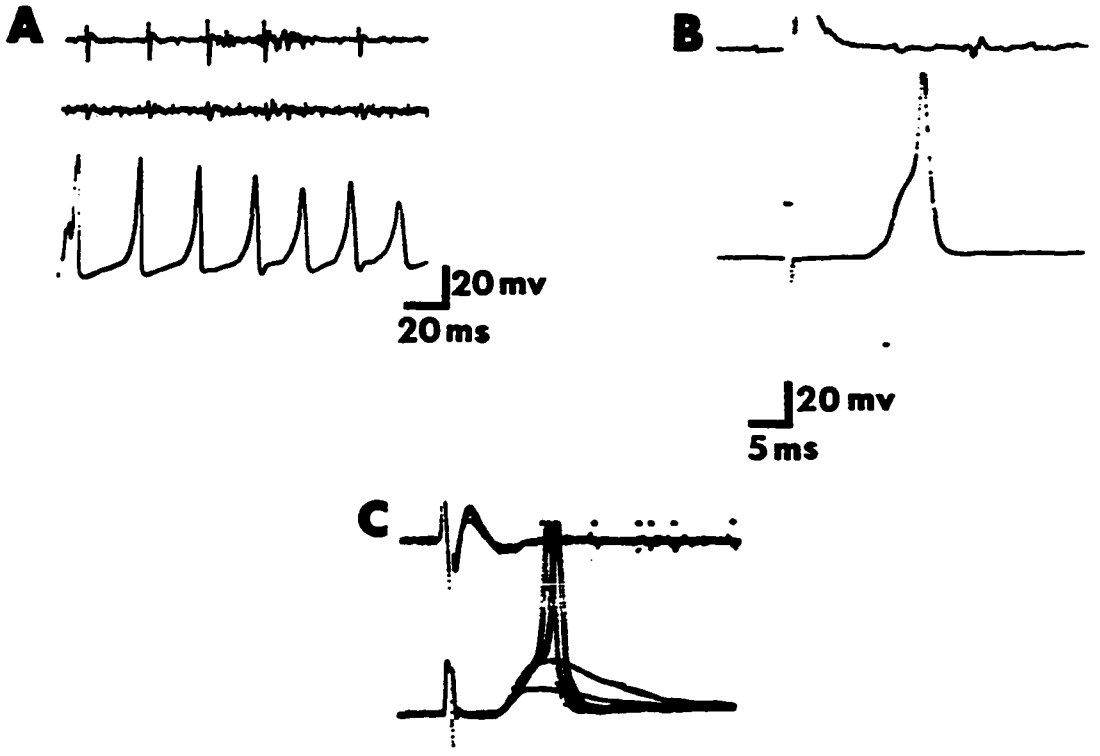
spikes superimposed upon a compound postsynaptic potential. Injection of depolarizing current caused repeated spiking, although a rapid decrease of spike amplitude was seen (Fig. 12[A]). When spike amplitude was reduced below a critical level there was failure of spike propagation into the periphery (Fig 12[A]).

To examine the action of DUMDL on evoked contractions of the metathoracic DLM, DUMDL was activated by stimulating the metathoracic nerve 1 contralateral to the muscle while muscle contractions were evoked by stimulation of the ipsilateral mesothoracic nerve 6. In 7 of 9 preparations no increase in twitch amplitude occurred following DUMDL stimulation, even though many different stimulation frequencies and durations were used. However in 2 of 9 preparations small increases (up to 20%) in twitch amplitude were seen (Fig. 11).

The possibility that conduction failure in the DUMDL pathway accounted for the frequent failure of DUMDL stimulation to increase twitch amplitude was then examined. Recordings from metathoracic nerve 1, in response to stimulation of the contralateral nerve 1, showed that DUMDL spikes were initially propagated across the ganglion and into the contralateral nerve (Fig. 12[B,C]). However, with repeated stimulation, the intraganglionic delay increased and spike propagation across the ganglion and into the contralateral nerve eventually failed (Fig. 12[C]). Reliable spike conduction across the metathoracic ganglion was never

Figure 12. 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 eventually 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 repeated stimulation the onset of the spike at first increased, but then spike amplitude decreased in two distinct increments. In the extracellular record of the contralateral nerve 1 the latency of DUMDL spikes (dots) increased and the spike eventually failed. Time scale: (A) 20 ms; (B),(C) 5 ms.


A
20 mv
20 ms
B
20 mv
5 ms
C

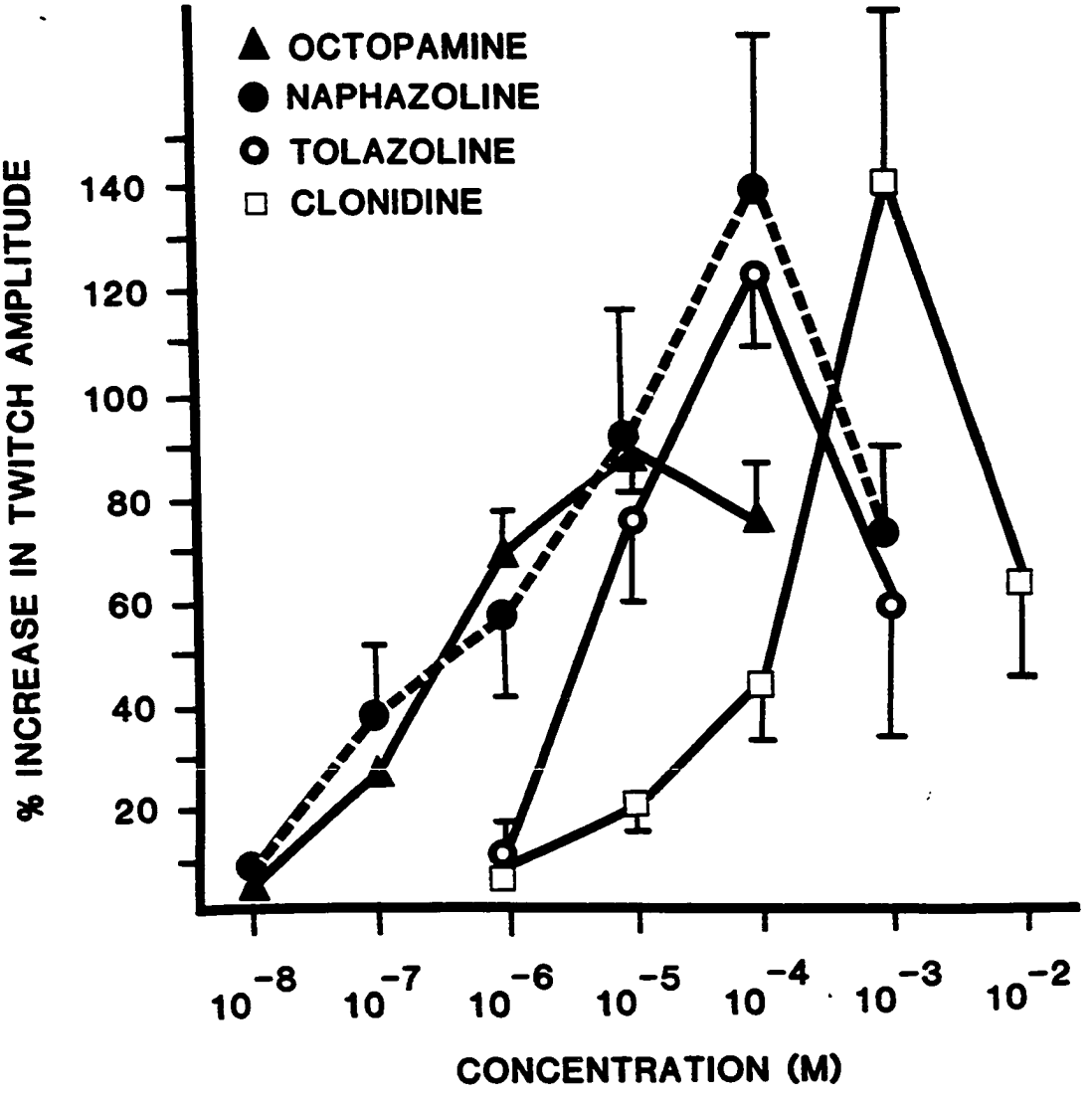
observed at stimulation frequencies above 0.1 Hz. Intracellular recordings from the DUMDL soma confirmed that progressive spike failure occurred during repetitive stimulation. Conduction failure was indicated by two incremental decreases in spike amplitude (Fig. 12[C]). Similar variations in spike amplitude have been previously 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

Evans' (1981) classification scheme included three octopamine receptor types based on the differential mechanical responses of the locust extensor-tibiae muscle to various octopamine agonists and antagonists. The agonists and antagonists which most effectively permitted differentiation of these three classes of octopamine receptors were utilized to determine if comparable receptor types existed in the metathoracic DLM of *T. oceanicus*. Figure 13 shows the effects of three different octopamine agonists on twitch amplitude. Although all agonists increased twitch amplitude, clonidine (an octopamine$_1$ agonist) was markedly less potent than naphazoline and tolazoline which are octopamine$_2$ agonists (Fig. 13; Table 2). The same basic patterns were seen when comparing increases in relaxation rate produced by these agonists (Fig. 14; Table

Figure 13. Dose-response curves for effects of octopamine and three octopamine agonists on twitch amplitude

Vertical bars represent SE. SE for 10^{-4} M clonidine is smaller than the symbol.


OCTOPAMINE
NAPHAZOLINE
TOLAZOLINE
CLONIDINE
% INCREASE IN TWITCH AMPLITUDE
140
120
100
80
60
40
20
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
CONCENTRATION (M)

3). Because the octopamine$_1$ receptor agonist (clonidine) was much less potent than either of the octopamine$_2$ agonists (naphazoline and tolazoline), the receptors mediating increases in twitch amplitude and relaxation rate are most likely octopamine$_2$ receptors.

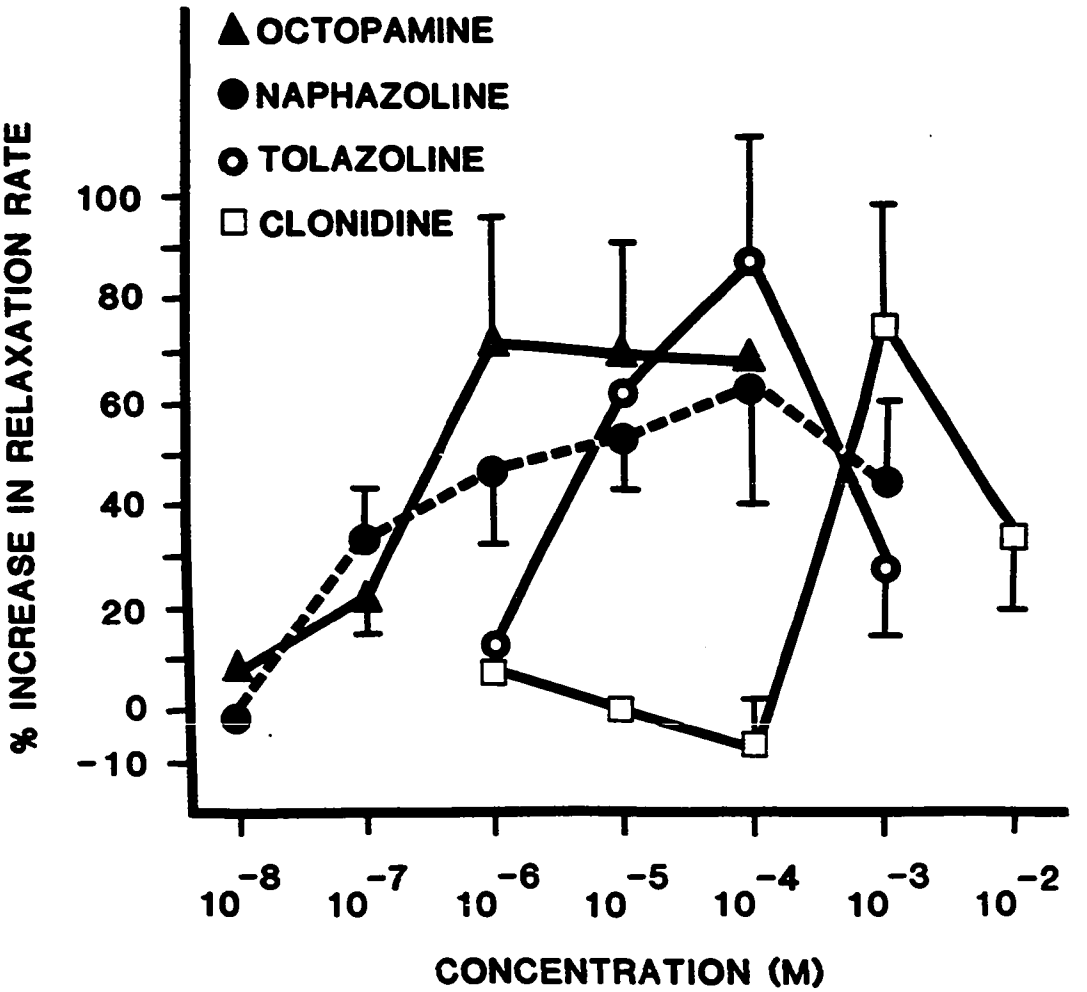
Table 2. Action of agonists on twitch amplitude

	Percent Increase(±SE) at 10^{-5} M	EC_{50}
Octopamine	88.0(±7.2)	4.0×10^{-7}
Naphazoline	93.4(±22.1)	3.7×10^{-6}
Tolazoline	75.9(±15.5)	7.7×10^{-6}
Clonidine	19.7(±3.0)	2.7×10^{-4}

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 rate (Table 4). 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

Figure 14. Dose-response curves for effects of octopamine and three octopamine agonists on relaxation rate

Vertical bars represent SE. SEs for 10^{-8} M octopamine and naphazoline, 10^{-6} M tolazoline and clonidine, and 10^{-5} M clonidine are smaller than the symbols. SE for 10^{-5} M tolazoline and 10^{-4} M naphazoline were omitted for clarity but were 12.7 and 25.7 respectively.


OCTOPAMINE
NAPHAZOLINE
TOLAZOLINE
CLONIDINE
% INCREASE IN RELAXATION RATE
100
80
60
40
20
0
-10
10^-8
10^-7
10^-6
10^-5
10^-4
10^-3
10^-2
CONCENTRATION (M)

(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 on the two receptors. In the *T. oceanicus* metathoracic DLM the ratio for naphazoline was much greater than 1.0 (36.9; Table 4) indicating that naphazoline preferentially increased relaxation rate. Tolazoline was equipotent in increasing relaxation rate and twitch amplitude (Table 4).

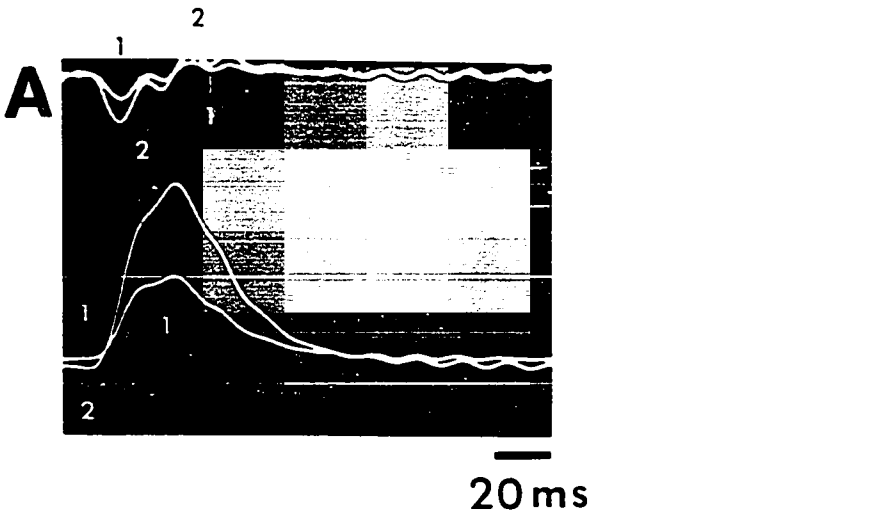
Table 3. Action of agonists on relaxation rate

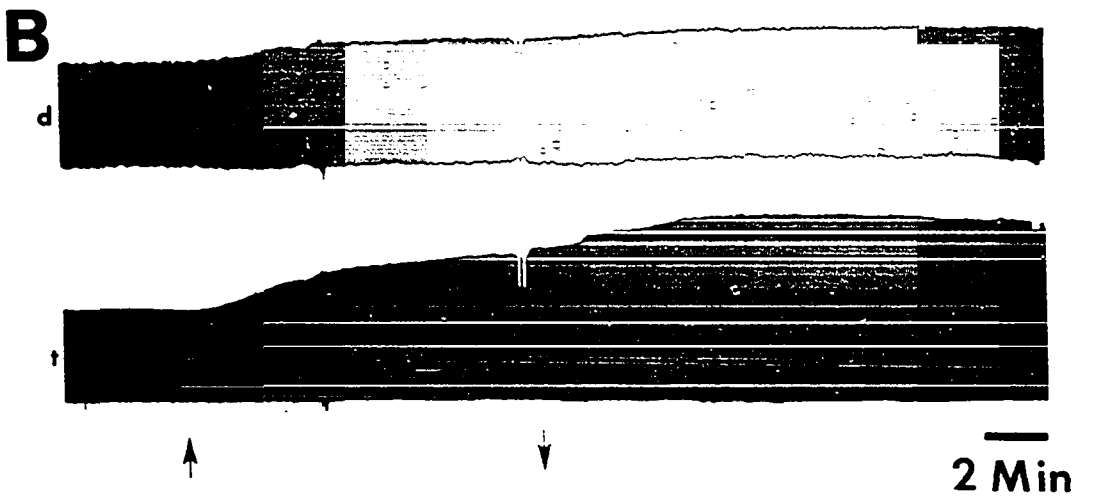
	Percent Increase(±SE) at 10^{-5} M	EC_{50}
Octopamine	65.6(±21.7)	3.0×10^{-7} M
Naphazoline	45.5(±15.3)	1.0×10^{-7} M
Tolazoline	86.9(±24.9)	6.7×10^{-6} M
Clonidine	0.0(± 0.0)	5.4×10^{-4} M

The blocking actions of the octopamine antagonists metoclopramide and chlorpromazine (10^{-5} M) on the effects of octopamine (10^{-6} M) are shown in Table 5. Both antagonists more effectively blocked increases in relaxation rate than

Figure 15. The effect of proctolin on the metathoracic DLM

A) Traces 1 show twitch tension (lower) and differentiated twitches (upper) prior to proctolin application. Traces 2 show these parameters after application of 10^{-6} M proctolin. Note that the differentiated signal is inverted compared to (B) and all other figures. B) The response of the same preparation to 10^{-6} M proctolin was recorded at a slow paper speed. Proctolin was applied at the first arrow and washed off at the second arrow. In this preparation, twitch amplitude increased by 105.9%, and contraction rate by 52.4%, but relaxation rate was not facilitated.


A
1
2
1
2
1
1
2
20 ms


B
d
t
2 Min

increases in twitch amplitude. However, chlorpromazine had very little antagonistic effect on octopamine-mediated increases of twitch amplitude.

Effect of proctolin on the metathoracic DLM

Within 1 min proctolin induced marked increases in twitch amplitude and a slight reduction of basic tonus (Fig. 15). Twitch amplitude continued to increase for as long as proctolin was applied (up to 10 min). The threshold for increases in twitch amplitude was about 10^{-7} M. Twitch amplitude was increased by 92.3% $\pm$ 18.6 (SE) at 10^{-6} M proctolin while relaxation rate increased by 31.2% $\pm$ 21.1 (SE) (n = 3). These effects persisted for at least 2 hr after exposure to 10^{-6} M proctolin.

Table 4. Comparison of octopamine receptors in the cricket DLM and locust extensor-tibiae

	$\frac{EC_{50}\ Amplitude}{EC_{50}\ Relaxation\ Rate}$	
	Cricket	Locust (Evans,1981)
Octopamine	1.34	1.65
Naphazoline	36.85	0.06
Tolazoline	1.15	5.33
Clonidine	0.53	0.32

Table 5. Action of antagonists on twitch amplitude and relaxation rate

	Reduction of Twitch Amplitude (%)	Reduction of Relaxation Rate (%)
Metoclopramide	39.8	65.4
Chlorpromazine	7.7	53.1

DISCUSSION

Effects of octopamine on mechanical events

Octopamine produced increases in twitch amplitude and relaxation rate as well as a slight reduction of basic tonus in the metathoracic DLM of *T. oceanicus* (Figs. 2, 3, 4, and 14). These actions are similar to those found in locust extensor-tibiae (O'Shea and Evans, 1979), lobster skeletal muscle (Kravitz et al., 1980; Harris-Warrick and Kravitz, 1984), crayfish skeletal muscle (Fischer and Florey, 1983), *Limulus* skeletal muscle (Rane et al., 1984) and locust flight muscle (Candy, 1978).

The thresholds for octopamine effects on twitch amplitude and relaxation rate in the *T. oceanicus* metathoracic DLM (10^{-8} M) were similar to the locust extensor-tibiae (Evans, 1981). However, the EC_{50}s for octopamine effects in *T. oceanicus* metathoracic DLM (Tables 2 and 3) were about an order of magnitude more sensitive than those for the locust extensor-tibiae (Evans, 1981).

One difference was noted between octopamine effects on the locust extensor-tibiae (the only other muscle where relaxation rate has been examined) and the metathoracic DLM. In the metathoracic DLM increases of relaxation rate developed more slowly than increases of twitch amplitude (Fig. 3). In the locust extensor-tibiae the increase in relaxation rate occurs faster than the increase in twitch

amplitude (O'Shea and Evans, 1979; Evans, 1982).

Since octopamine increased the amplitude of tension transients at stimulus frequencies similar to those occurring during flight or stridulation, it seems reasonable to suggest that such increases may be useful to the insect during these activities. Octopamine-mediated increases of relaxation rate in the locust extensor-tibiae are larger than the increases of twitch amplitude (Evans, 1981). Evans and Siegler (1982) propose that increased relaxation rate is more important in the locust extensor-tibiae than the increase of twitch amplitude. In the cricket metathoracic DLM, the twitch amplitude increase is larger than the relaxation rate increase. This suggests that the increase of twitch amplitude maybe the more important response to octopamine in the metathoracic DLM.

The idea that octopamine modulates muscle performance is supported by the observation that octopamine levels of hemolymph increase during flight or stress (Goosey and Candy, 1980; Bailey et al., 1983; Davenport and Evans, 1984a,b). In addition, octopamine levels in locust DLM and nerve 1 decrease during flight (Goosey and Candy, 1982; David et al., 1985). Results from the present study showed an apparent facilitation of twitch amplitude just after dissection. The twitch amplitude then slowly decreased for 1 to 2 hr until a stable, lower amplitude was reached. These effects may have been a physiological manifestation of octopamine release

during the stress of capture and dissection, since similar effects were subsequently produced by application of high concentrations of exogenous octopamine.

Electrophysiological effects of octopamine

Passive membrane properties of the metathoracic DLM were not changed by octopamine (Fig 7). Other preparations showing no change of input resistance include: locust extensor-tibiae (O'Shea and Evans, 1979), lobster skeletal muscle (Kravitz et al., 1980) and *Limulus* skeletal muscle (Rane et al., 1984). The membrane potential of the metathoracic DLM and other preparations tend to hyperpolarize in response to octopamine (O'Shea and Evans, 1979; Hoyle and Field, 1983; Fitch and Kammer, 1985).

Comparison of EJPs in 11 mM calcium and 1.8 mM calcium showed that membrane potential was not changed. However, EJP amplitude was significantly reduced and $T_{1/2}$ was significantly increased (Table 1). These results are consistent with the idea that calcium is a major current carrying ion in the metathoracic DLM. Octopamine, in 11 mM calcium, does not affect membrane potential or EJP amplitude (Table 1), but does produce a slight and statistically significant increase of $T_{1/2}$. It is not clear if this small increase of EJP duration is physiologically significant; however, small rises of internal calcium level are known to produce dramatic effects on tension production (Rubin et al., 1985). In 1.8 mM calcium saline, membrane potential and $T_{1/2}$

were unchanged by octopamine, but EJP amplitude was increased (Table 1). Octopamine also increased mepp frequency (Figs. 8 and 9), suggesting that transmitter release from the presynaptic terminals was increased by octopamine. However, this does not eliminate the possibility of postsynaptic modulation of EJP size since Fitch and Kammer (1985) found that octopamine increases glutamate-induced depolarization of *Manduca* DLM.

Possible role of cAMP in octopamine effects

Octopamine caused increases of cAMP levels in locust DLM (Worm, 1980) and locust extensor-tibiae (Evans, 1984a,b). However, octopamine effects on tension production in the metathoracic DLM could not be fully explained by alterations of cAMP levels. Phosphodiesterase inhibitors failed to potentiate octopamine responses (Fig. 10[A]). However, both theophylline and IBMX slightly potentiated twitch amplitude. The non-specific adenylate cyclase stimulator forskolin also caused a slight (20%) facilitation of twitch amplitude. Both forskolin and the phosphodiesterase inhibitors produced facilitations comparable to 10^{-6} M octopamine. This result contrasts with the locust extensor-tibiae where octopamine-mediated elevations in cAMP levels cause facilitation of twitch amplitude and relaxation rate (Evans, 1984a,b). However, a recent study by Evans (1985) may explain the difference. Evans found that octopamine-induced changes in cAMP levels occurred primarily in slow muscle

fibers of the locust extensor-tibiae, whereas regions of the same muscle containing primarily fast muscle fibers showed little cAMP change. Since the DLM in *T. oceanicus* is composed exclusively of fast muscle fibers, the absence of cAMP-dependent octopamine effects is not unexpected.

Role of DUMDL in producing octopamine responses

Since DUMDL innervates the metathoracic DLM, (Clark, 1976a; Hoyle et al., 1980) it is an obvious candidate for supplying octopamine to the metathoracic DLM. However, attempts to demonstrate facilitation of the metathoracic DLM by stimulation of the DUMDL axon in the contralateral metathoracic nerve 1 were usually ineffective due to impulse conduction failure across the ganglion (Fig. 12). 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). Whether such conduction failure indicates an adverse effect of dissection or the presence of an intraganglionic site for modulation of DUMDL excitability is not clear. Although release of octopamine by DUMDL may be one mechanism for octopaminergic modulation of the metathoracic DLM, octopamine levels in the hemolymph may also reach levels sufficient to cause physiological effects (Goosey and Candy, 1980; Bailey et al., 1983; Davenport and Evans, 1984a,b).

Diversity of octopamine receptor types

Pharmacological results shown in Tables 2 - 5 indicate the octopamine receptor(s) of the metathoracic DLM are probably 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) are not easily classified as octopamine$_{2A}$ or octopamine$_{2B}$ receptors. Based on Evans' data for naphazoline, an octopamine$_{2A}$ agonist, the ratio of EC_{50}s for increases in twitch amplitude and relaxation rate should be much less than 1.0 (Table 4). However, in the cricket metathoracic DLM, the ratio was 36.85. In the case of tolazoline, the ratio in the locust extensor-tibiae was 5.33 whereas in the cricket DLM the ratio was approximately 1. These results may 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 can not be made with the agonists and antagonists used in this study.

Effects of proctolin

Proctolin produced a marked increase in twitch amplitude and relaxation rate, as well as a slight reduction of basic tonus (Fig. 15). Proctolin-induced increases of twitch amplitude have been seen in the crayfish abdominal tonic flexors (Bishop et al., 1984a; Bishop et al., 1985) and

Limulus skeletal muscle (Rane et al., 1984), while increases of both twitch amplitude and relaxation rate were seen in a crab respiratory muscle (Mercier and Wilkens, 1985). The specific source of proctolin for the metathoracic DLM is unknown, but proctolin-like immunoreactivity has been localized in neurons (Bishop and O'Shea, 1982; Keshishian and O'Shea, 1985) located near those known to innervate the metathoracic DLM (Clark, 1976a).

The effects of proctolin on the metathoracic DLM contrast with the effects on the abdominal DLM of *T. oceanicus* (Part II), cockroach coxal depressor (Adams and O'Shea, 1983), locust extensor-tibiae (Wordon et al., 1985), or lobster skeletal muscle (Kravitz et al., 1980; Schwarz et al., 1980). In all these preparations proctolin increased basic tonus without greatly increasing twitch amplitude.

SUMMARY

1. Octopamine and proctolin caused dose dependent increases of twitch amplitude and relaxation rate as well as a slight decrease of basic tonus in the metathoracic DLM of the cricket *Teleogryllus oceanicus*. The threshold for octopamine effects was 10^{-8} M, while that for proctolin was 10^{-7} M.

2. The octopamine receptors were classified on the basis of the differential responsiveness of the metathoracic DLM to the octopamine agonists naphazoline, tolazoline, and clonidine; and the octopamine antagonists metoclopramide and chlorpromazine. The octopamine receptors were classified as octopamine$_2$ receptors; it was not possible to determine if octopamine$_{2A}$ or octopamine$_{2B}$ existed on the muscle.

3. Octopamine had a presynaptic effect since miniature end plate potential (mepp) frequency was increased by octopamine.

4. In 11 mM calcium, octopamine did not affect muscle membrane potential, input resistance or EJP amplitude, but the EJP duration at one-half amplitude ($T_{1/2}$) was slightly increased. In low calcium saline, octopamine did not affect membrane potential or $T_{1/2}$, but EJP amplitude was increased.

5. Octopamine effects were not facilitated by the phosphodiesterase inhibitors theophylline or IBMX, suggesting that alterations of cyclic nucleotide levels could not fully

explain most of the octopamine effects.

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

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SECTION II. DIFFERENTIAL PHARMACOLOGICAL SENSITIVITY OF SERIALLY HOMOLOGOUS MUSCLES IN THE CRICKET *TELEOGRYLLUS OCEANICUS*

II. THE ABDOMINAL DORSAL LONGITUDINAL MUSCLE

INTRODUCTION

The abdominal dorsal longitudinal muscle (DLM) of the cricket *Teleogryllus oceanicus* is specialized for relatively slow movements and postural control of the abdomen. One example of this postural control is the tetanic contraction of the abdominal DLM just after ecdysis during wing expansion (Carlson, 1977). An unusual aspect of this tetanic contraction is that it is unaccompanied by electromyographic activity (Carlson, personal communication).

In contrast to the abdominal DLM, the metathoracic DLM is specialized for the rapid contractions which power wing movement. Results from the preceding paper (Part I) showed that the metathoracic DLM is highly responsive to the neuromodulator octopamine, which increased twitch amplitude and relaxation rate and slightly reduced basic tonus. In addition, the metathoracic DLM is responsive to the pentapeptide proctolin which also increased twitch amplitude and relaxation rate and slightly reduced basic tonus.

Proctolin-responsive muscles show two distinct types of response to proctolin. The cockroach coxal depressor (Adams and O'Shea, 1983) and locust extensor-tibiae (Wordon et al., 1985) both primarily respond by increasing their basic tonus. In response to proctolin, the crayfish abdominal tonic flexors (Bishop et al., 1984a), *Limulus* skeletal muscle (Rane et al., 1984), and crab ventilatory muscle (Mercier and

Wilkens, 1985) increase twitch amplitude, but show little change in basic tonus. These responses parallel those seen in the metathoracic DLM of *T. oceanicus* (Part I).

One likely source of proctolin for muscles in the intact animal is motoneurons. Proctolin has been localized in motoneurons which control postural movements in insects and crayfish (O'Shea and Bishop, 1982; Bishop et al., 1984b; Wordon et al., 1985). The abdominal DLM appears to be a candidate for proctolin modulation since proctolin-like immunoreactivity has been localized in neurons (Bishop and O'Shea, 1982) located in the same area as the motoneurons for the abdominal DLM (Davis, 1983). In addition, a proctolin-like substance has been localized in several abdominal muscles in two species of insects (Veenstra et al., 1985; Keshishian, 1985).

Octopamine is active on several arthropod neuromuscular systems (for a review see David and Coulon, 1985). Octopamine increased the amplitude and relaxation rate of twitches induced both by slow (O'Shea and Evans, 1979) and fast motoneurons (Part I). The sources of octopamine for these muscles are dorsal unpaired median (DUM) neurons. Although DUM neurons occur in the abdominal ganglion (Dymond and Evans, 1979), it is not known if the abdominal DLM is innervated by one of these octopaminergic neurons.

Another way in which tension production of muscles can be modulated is through inhibitory motoneurons, which utilize

GABA as their neurotransmitter. Inhibitory motoneurons have the ability to decrease basic tonus, provided the muscle is not already at its minimum tonus (Iles and Pearson, 1971; Burns and Usherwood, 1978; Chesler and Fourtner, 1981; Ballantyne and Rathmayer, 1981; Kaars, 1982; Hoyle and Field, 1983).

The purposes of this study were 1) to study the neural modulation of evoked twitches and basic tonus in the abdominal DLM; 2) to determine the effects of octopamine, proctolin, and GABA on tension production; 3) and to suggest roles for these substances in the modulation of tension production.

MATERIALS AND METHODS

Male crickets, *Teleogryllus oceanicus*, were raised from eggs in 12 l clear plastic boxes containing approximately 60 crickets. Crumpled paper toweling was placed in the boxes to increase surface area. The crickets were fed romaine lettuce daily. Animals were maintained on a 15:9 LD cycle and kept at approximately 30°C.

The cricket was decapitated and the thoracic segments discarded. The abdomen was opened with a mid-dorsal incision and the gut and nerve cord were removed. The fat and air sacs overlying the abdominal DLM were carefully removed (see Carlson [1977] for a description of the abdominal musculature of *T. oceanicus*). To monitor muscle tension, the exoskeletal attachment of the anterior end of the abdominal DLM was pinned to the bottom of the dissecting dish and a minutin pin, attached to a tension transducer (Narco Biosystems F50), was positioned to contact the posterior exoskeletal attachment of the abdominal DLM. The force transducer was positioned for optimally recording twitch amplitude. This method, involving semi-isometric monitoring of tension, was found superior to attaching the transducer to the isolated posterior attachment of the DLM because the required isolation procedure greatly reduced the viability of the preparation. Experiments were performed using all abdominal segments except the first and the highly modified, fused

posterior segments. No differences were noted in the responses of the DLM in the segments used. Calculations involving twitch tension were based on relative rather than absolute values, and absolute tension was uncalibrated in most experiments.

To study neurally evoked responses of the abdominal DLM, the lateral nerve was electrically stimulated with a suction electrode. Tension records were monitored on an oscilloscope or chart recorder (Brush RD 1684-00 or Gould 2200S). Saline or drugs were usually superfused on the preparation at 0.75 ml/min, a rate which exchanged the fluid volume covering the preparation every few seconds. During experiments utilizing GABA (Figs. 6, 8[B]) the perfusion system was not used, and drugs or saline changes were applied in boluses. This method allowed more exact control of the timing of drug application.

Intracellular electrical activity from the muscle was recorded using borosilicate glass microelectrodes filled with 1 M K acetate (15-40 M-ohms). The composition of the saline was: 152 mM NaCl, 8 mM KCl, 1 mM $MgCl_2$, 11 mM $CaCl_2$, 4 mM $NaHCO_3$, 5 mM TES, 5 mM trehalose, 105 mM sucrose, pH 6.7. Proctolin, gamma-aminobutyric acid (GABA), picrotoxin, and DL-octopamine were purchased from Sigma (St. Louis, MO). All experiments were conducted at room temperature (21 - 25°C).

RESULTS

Innervation of the abdominal DLM

Electrical stimuli applied to the lateral nerve evoked a large, overshooting EJP in DLM muscle fibers (Fig. 1[A]). The EJP was recruited as an all-or-none response and was accompanied by an observable twitch. In the absence of stimulation, several smaller spontaneous EJPs (Fig. 1[B,C]) and an inhibitory junctional potential (IJP) were also observed (Fig. 1[D]). The small EJPs often occurred during respiratory movements of the abdomen. The pattern of innervation by the neurons producing these small junctional potentials was not studied in detail. However, the size and number of junction potentials appeared similar to those in the grasshopper abdominal DLM (Tyrer, 1971).

Effects of octopamine on the abdominal DLM

Application of octopamine at concentrations up to 10^{-4} M (n = 5) caused no detectable effect on neurally evoked twitches (Fig. 2). One explanation for this result was that octopaminergic neurons (presumably in the lateral nerve) may have been inadvertently stimulated. Thus the abdominal DLM may have already been maximally facilitated before exogenous octopamine was applied. To test this idea, the octopamine antagonist metoclopramide (Evans, 1981; Part I) was applied to the preparation at 10^{-4} M for periods up to 30 min. Metoclopramide had no effect on neurally induced twitches

Figure 1. Junctional potentials in the abdominal DLM

A) Stimulation of the lateral nerve elicited a fast muscle spike. Spontaneous EJPs (B,C) and IJPs (D) were also recorded. The largest EJP in B was the same as the evoked EJP in A. Voltage scale: (A),(B),(D) 10 mV; (C) 5 mV. Time scale: (A) 5 ms; (B),(D) 100 ms; (C) 50 ms.

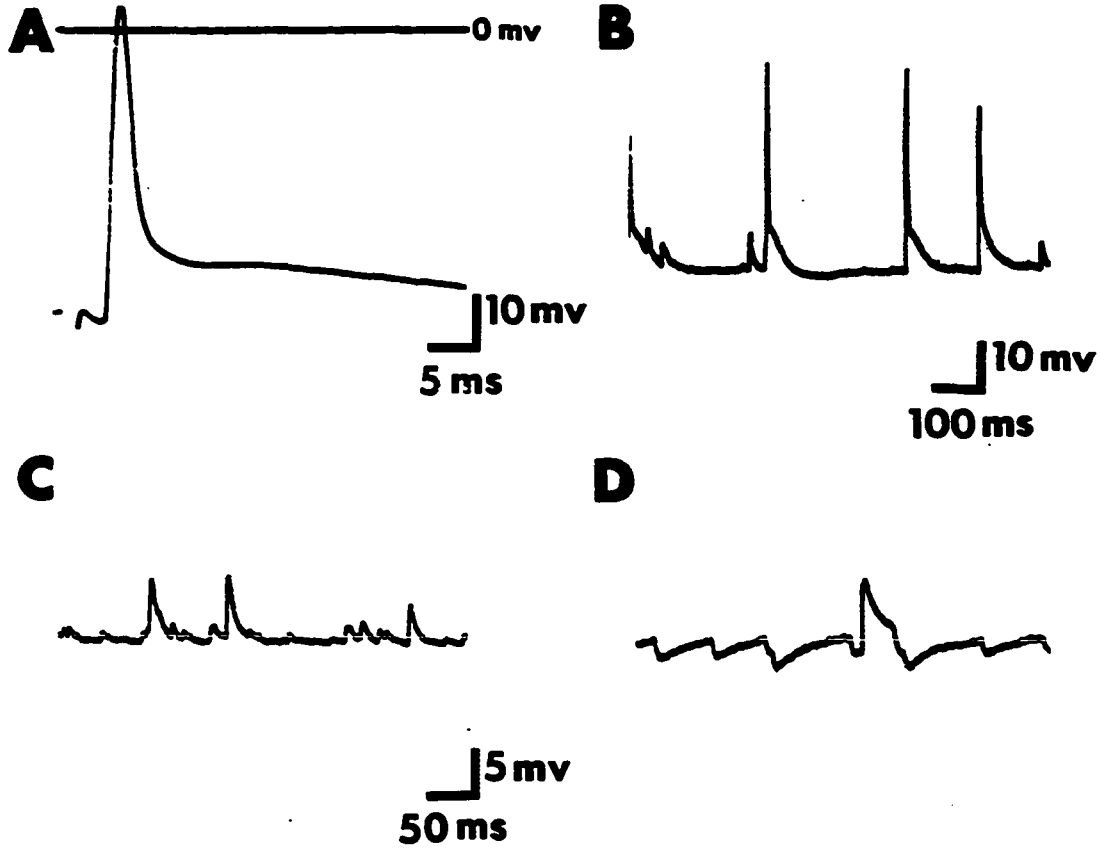
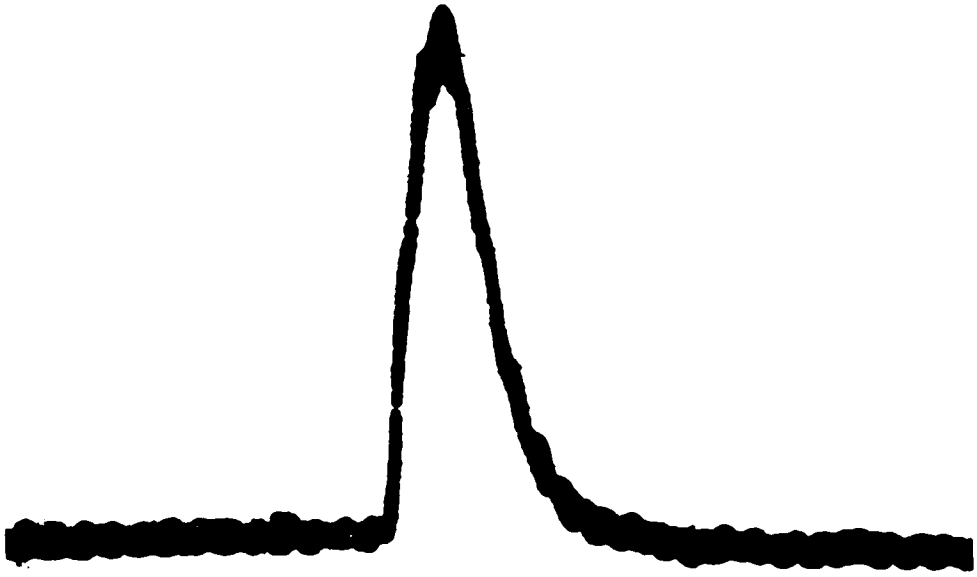

A
0 mv
10 mv
5 ms
B
10 mv
100 ms
C
D
5 mv
50 ms

Figure 2. Effect of octopamine (10^{-4} M) on abdominal DLM twitches

Three superimposed twitches were recorded, one prior to octopamine application and the other two several minutes after application.



100 ms

(n = 6), suggesting that no inadvertent octopaminergic modulation of the abdominal DLM had occurred.

Neural modulation of basic tonus

The possibility that basic tonus could be predictably modulated by neural stimulation was examined by repetitive stimulation of the lateral nerve at various frequencies. As shown in Figure 3, stimulation at 20 Hz for 3 s caused an increase in basic tonus which outlasted the neural stimulation for up to 5 min. As shown in Figure 3(B), the increase in basic tonus was dependent upon stimulus frequency. In most preparations, the lowest frequency which increased basic tonus was approximately 7 Hz, corresponding approximately to the frequency which just caused incomplete tetanus. Muscle membrane potential during neurally induced increases in basic tonus was usually unchanged, but occasionally a slight depolarization of less than 5 mV occurred (Fig. 4).

Lower stimulus frequencies (i.e., those producing no fusion of individual twitches) caused a reduction in basic tonus (Fig. 3) which did not spontaneously return to the previous levels. Such reductions occurred only if basic tonus was greater than the minimal tonus of the muscle. After dissection and isolation, the basic tonus of most preparations was above this minimal level.

In a few preparations distinct stimulus voltage thresholds were found for increasing or decreasing basic

Figure 3. Modulation of basic tonus by stimulation of the lateral nerve

A) Stimulation at 20 Hz for 3 s produced a large phasic contraction. After termination of stimulation, basic tonus was elevated. Near the end of the record, stimulation at 2 Hz evoked twitches and a decrease of basic tonus. B) At the beginning of the record the lateral nerve was stimulated at 20 Hz for 3 s. Basic tonus was then reduced by 2 Hz stimulation. Stimulation at 30 Hz for 3 s evoked a larger increase of basic tonus compared to 20 Hz stimulation. The increase of basic tonus was then released by 3 Hz stimulation of the lateral nerve.

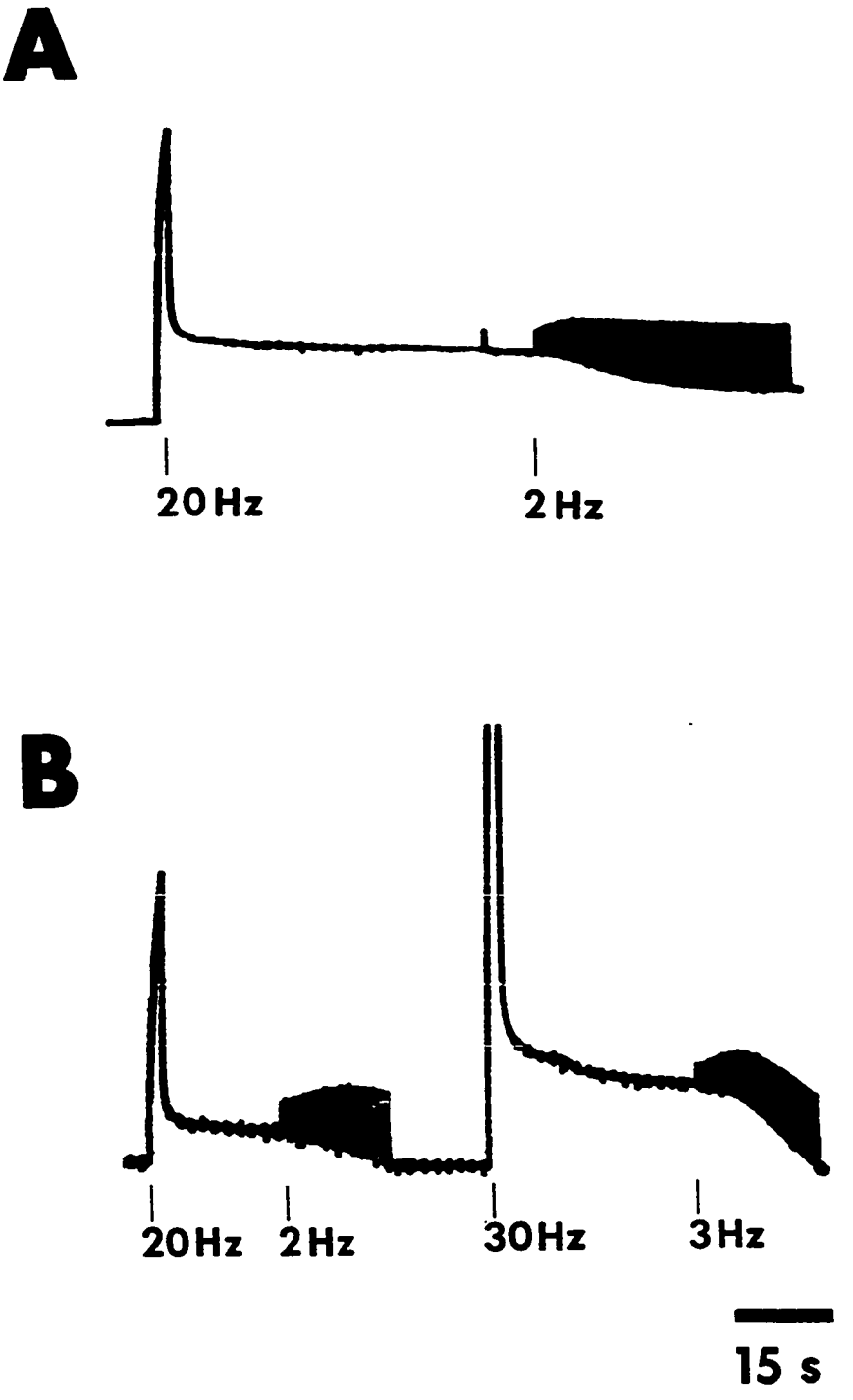
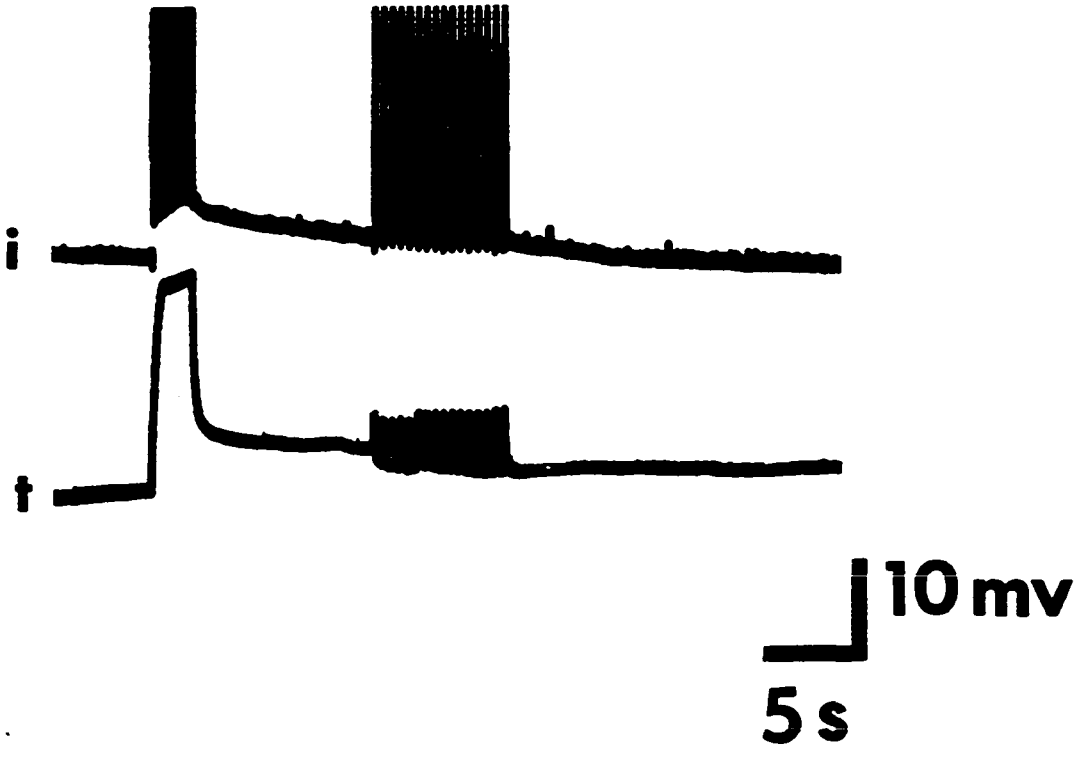

A
20Hz
2Hz
B
20Hz
2Hz
30Hz
3Hz
15 s

Figure 4. Membrane potential during neurally induced changes of basic tonus

The bottom trace (t) shows tension of the abdominal DLM. Basic tonus was increased by 20 Hz stimulation for 3 s and released by 2 Hz stimulation. The top trace (i) shows muscle membrane potential during changes of basic tonus. A series of fast muscle spikes (peaks clipped during amplification), evoked during stimulation (c.f., Fig 1[A]), was followed by an approximately 5 mV depolarization which accompanied the increase of basic tonus. This was the largest shift of membrane potential in any preparation. No large changes of membrane potential accompanied the decrease of basic tonus.


i
t
10 mv
5 s

tonus (Fig. 5). In these preparations low frequency (2 Hz) stimulation produced substantial increases or decreases in basic tonus depending on stimulation voltage (Fig. 5[B]). The intracellular electrical correlate for the decrease in basic tonus was a threshold-dependent hyperpolarizing potential that followed the fast spike (Fig. 5[C]). The amplitude and duration of this potential appeared similar to that of the spontaneously occurring IJPs (Fig. 1[D]).

The role of GABA in regulation of basic tonus

If the hyperpolarization which accompanied the decrease in basic tonus was caused by the inhibitory neurotransmitter GABA, then application of GABA should cause a similar decrease in basic tonus. In each of 8 preparations GABA application caused marked reductions of basic tonus (Fig. 6[A]).

Further evidence that these reductions in basic tonus were mediated by GABA was obtained by application of the GABA antagonist picrotoxin. In Figure 6(B) an increase in basic tonus was induced with 20 Hz stimulation of the lateral nerve for 5 s. After 45 s, 2 Hz stimulation of the lateral nerve produced a reduction of basic tonus. The preparation was then exposed to 10^{-3} M picrotoxin for 10 min and an increase in basic tonus was again induced by 20 Hz stimulation of the lateral nerve for 5 s. After 45 s the lateral nerve was again stimulated at 2 Hz but there was little reduction of basic tonus. Taken together, these results suggest that the

Figure 5. Thresholds for changing basic tonus

A) The first and third twitches were elicited by a single pulse and were followed by a reduction of basic tonus. The second twitch, elicited by a slightly lower stimulus voltage, was followed by an increase of basic tonus. B) Large increases of basic tonus were produced during 2 Hz stimulation by alternating between the stimulus voltages in A. C) The intracellular correlate (i) for the decrease of basic tonus was a hyperpolarizing potential which follows the fast muscle spike. Threshold for the hyperpolarization was identical to the threshold which produced a decrease of basic tonus in A and B. In the tension record (t) a slightly faster muscle relaxation occurred following the hyperpolarizing potential.

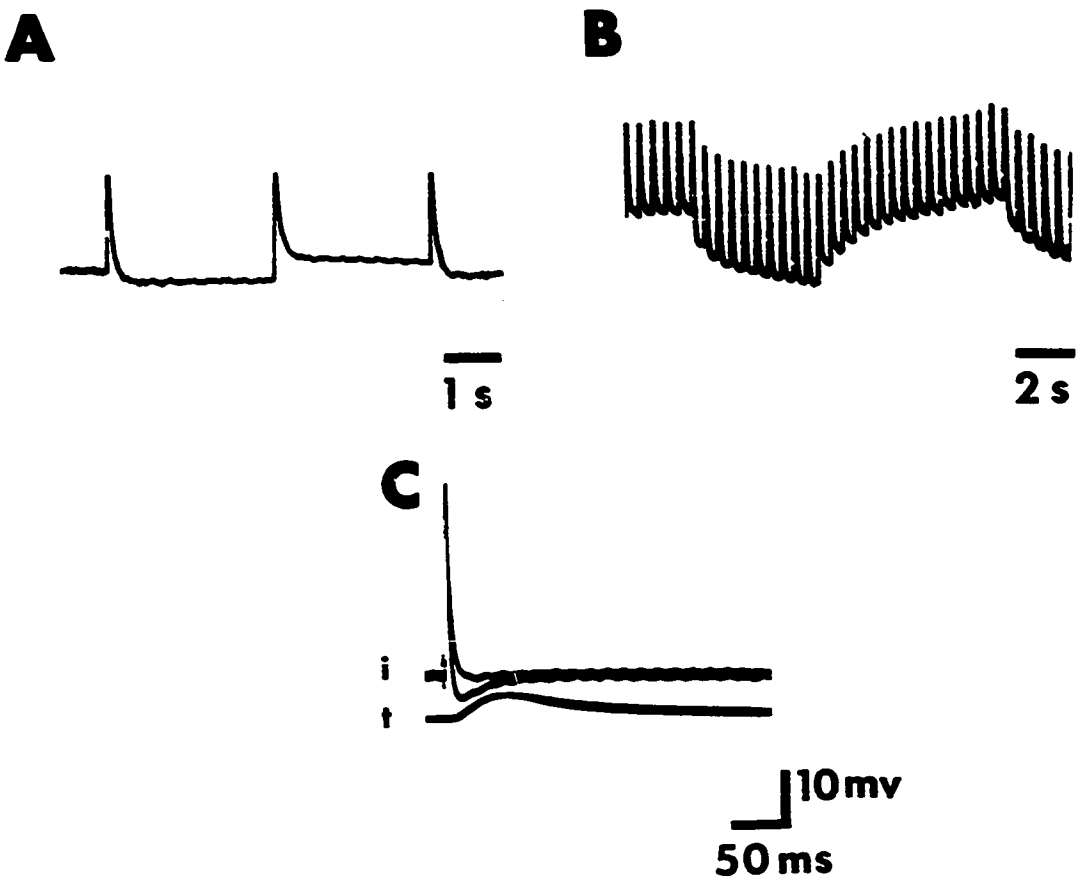
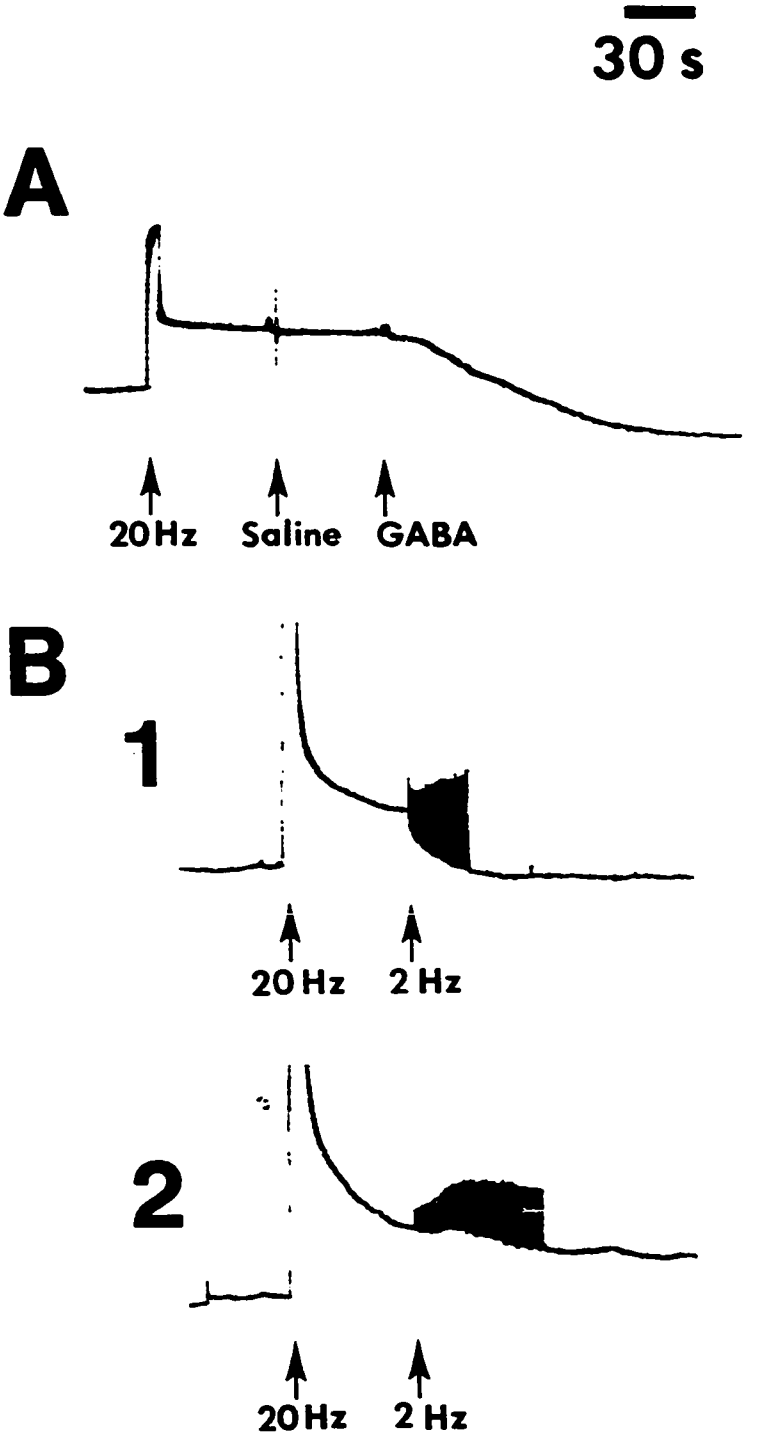

A
B
1 s
2 s
C
i
t
10mv
50 ms

Figure 6. Effects of GABA in modulating basic tonus

A) Basic tonus was increased by stimulating the lateral nerve at 20 Hz for 5 s. Addition of 0.3 ml saline had no effect, but 0.3 ml of 10^{-3} M GABA produced a reduction of basic tonus. B) Neurally-induced changes of basic tonus were blocked by picrotoxin. During a control period (1) an increase in basic tonus was produced by stimulation at 20 Hz for 5 s and a reduction of basic tonus was induced by 2 Hz stimulation. Ten minutes after picrotoxin treatment (2) an increase in basic tonus was produced by 20 Hz stimulation but the normal release of basic tonus by 2 Hz stimulation was blocked.


30 s
A
20Hz
Saline
GABA
B
1
20Hz
2Hz
2
20Hz
2Hz

reductions in basic tonus of the abdominal DLM are mediated by the release of GABA from an inhibitory motoneuron.

The role of proctolin in the regulation of basic tonus

Proctolin applied to the abdominal DLM of *T. oceanicus*, increased basic tonus, thus mimicking the increase of basic tonus produced by repetitive neural stimulation (Fig. 3). The effects of proctolin were dose-dependent at concentrations between 10^{-9} M and 10^{-6} M (Fig. 7). Concentrations higher than 10^{-6} M were not used.

Proctolin-induced increases in basic tonus were also released by lateral nerve stimulation at 2 Hz (Fig. 8[A]) or GABA application (Fig. 8[B]). The basic tonus remained reduced for at least 5 min even though proctolin was still present in the bath.

The effect of proctolin on neurally induced twitches

Because proctolin caused an increase in twitch amplitude of the metathoracic DLM (Part I), the effects of proctolin on twitches of the abdominal DLM were also studied. The lateral nerve was stimulated at 0.5 Hz, a frequency which normally caused no change of basic tonus. When proctolin was applied to the preparation basic tonus increased (c.f., Figs. 7 and 8) and twitches were superimposed on this increase in basic tonus. Comparison of twitches, before and after proctolin application, showed either no effect (2 of 5 preparations) (Fig. 9[A]) or a slight reduction in amplitude (3 of 5 preparations) (Fig. 9[B]). The maximal effect of

Figure 7. Effects of proctolin on basic tonus of the abdominal DLM

Proctolin was applied at the first arrow and was washed off at the second arrow. A dose-dependent increase of basic tonus was evident. Bars on each panel indicate equivalent amounts of tension.

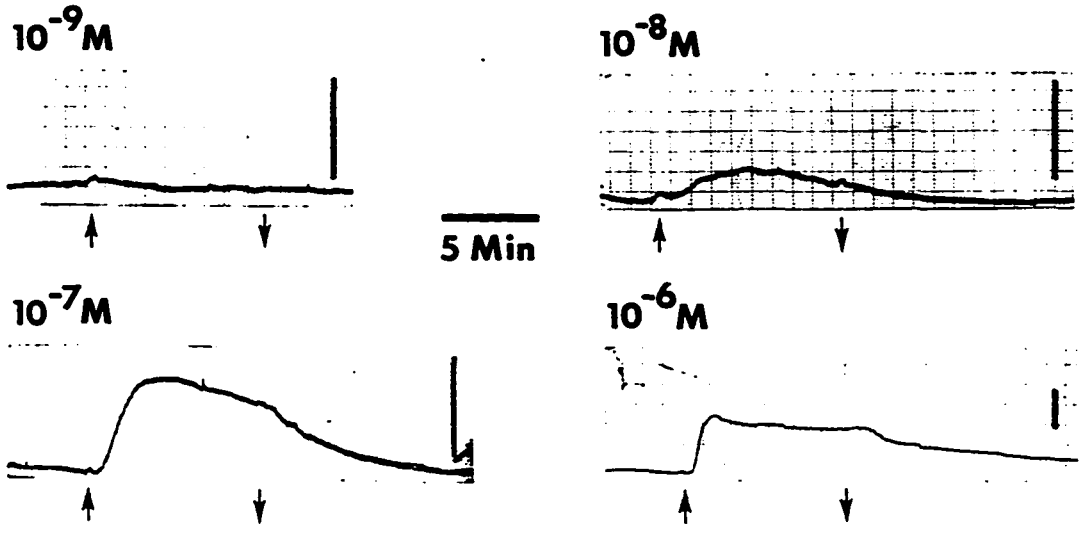
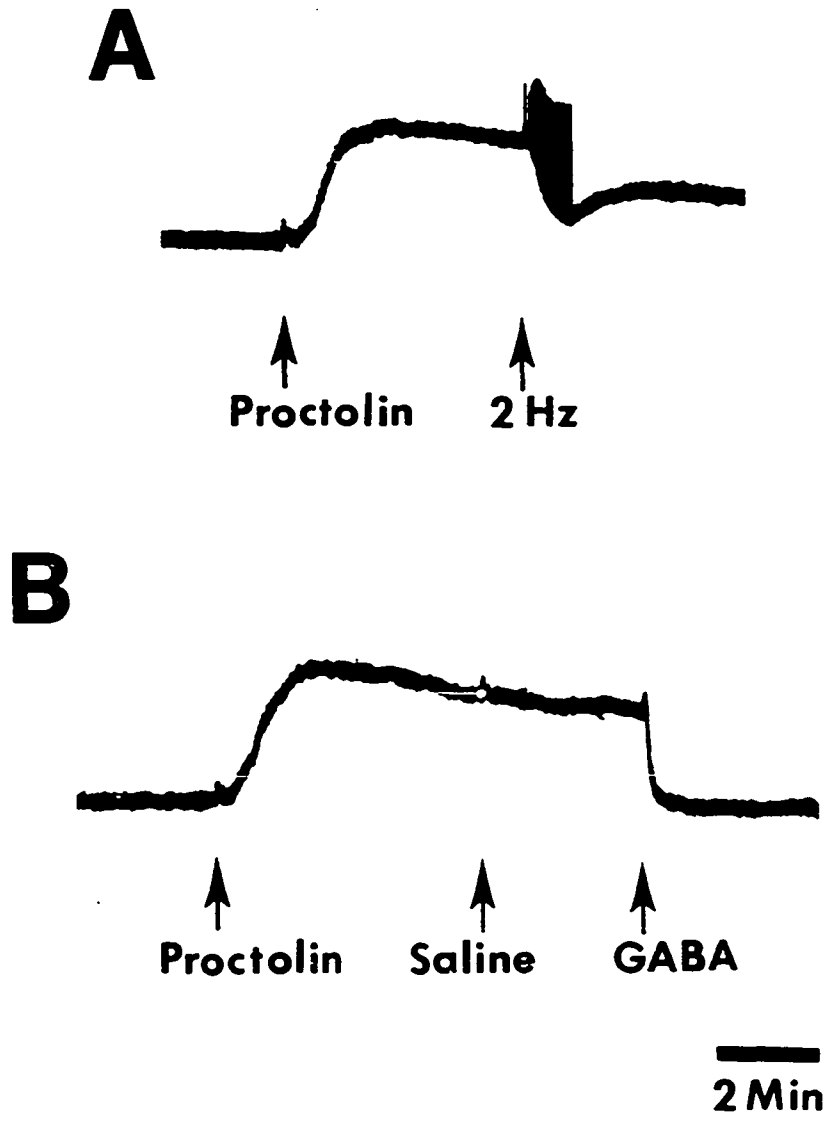

10^{-9}M
10^{-8}M
5 Min
10^{-7}M
10^{-6}M

Figure 8. Release of proctolin-induced increases of basic tonus

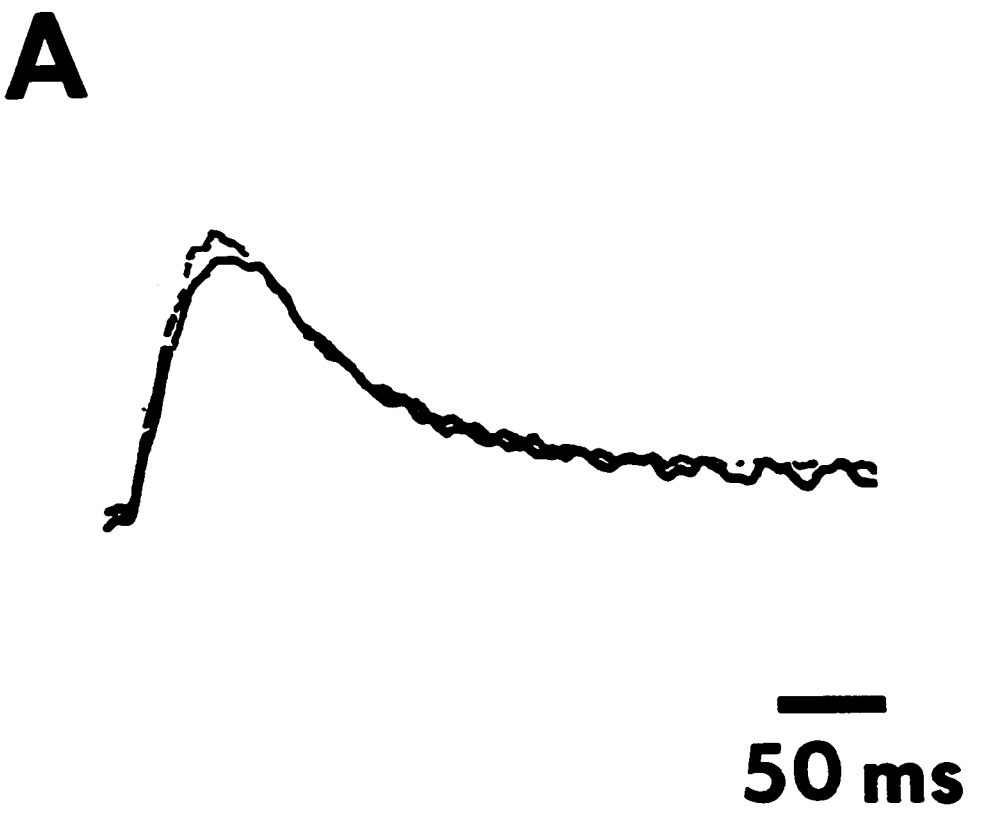
A) An increase of basic tonus was produced by addition of 0.3 ml of 10^{-6} M proctolin. Basic tonus was then released by 2 Hz stimulation of the lateral nerve. B) The proctolin-induced increase of basic tonus was released by GABA (0.3 ml of 10^{-3} M) but was unaffected by saline (0.3 ml).


A
Proctolin
2 Hz
B
Proctolin
Saline
GABA
2 Min

proctolin in any preparation was a 23% reduction in twitch amplitude (Fig. 9[B]).

Figure 9. Effect of proctolin on twitches of the abdominal DLM

Each panel shows two twitches, one prior to proctolin application and the other after basic tonus was increased maximally. Baselines were adjusted to nullify the proctolin effect on basic tonus. In some preparations proctolin either had little or no effect on twitches (A) or in other preparations produced a decrease of twitch amplitude (B).


A
50 ms
B

DISCUSSION

The abdominal DLM of the cricket exhibited the property of basic tonus; muscle tension was maintained without further neural input (Hoyle, 1983a). Changes in basic tonus were consistently modulated by stimulation of the lateral nerve. The increase of basic tonus is a "catch-like" contraction; however these contractures have not been termed catch because the criteria defining catch have not been met (Hoyle, 1983).

High frequency stimulation caused increases and low frequency stimulation decreases of basic tonus (Fig. 3). The intracellular event which correlated with reductions of abdominal DLM basic tonus was a hyperpolarizing potential (Fig. 5[C]), similar in size and duration to spontaneous IJPs. GABA mimicked the effects of low frequency stimulation and reduced basic tonus, regardless of whether the increase of basic tonus was induced by proctolin or lateral nerve stimulation (Figs. 6[A] and 8[B]). Likewise, GABA antagonizes proctolin-induced increases of basis tonus in lobster skeletal muscle (Kravitz et al., 1980). These results suggest that decreases of basic tonus in the abdominal DLM were produced by an inhibitory motoneuron. Similar actions of inhibitory motoneurons have been described in other arthropod muscles including: the cockroach coxal depressor (Chesler and Fourtner, 1981), locust extensor-tibiae (Burns and Usherwood, 1978; Wordon et al.,

1985), and weta (Orthoptera: Stenopelmatidae) extensor-tibiae (Hoyle and Field, 1983).

Increases of basic tonus in the cricket abdominal DLM were induced by high frequency stimulation but with little change of muscle membrane potential. Similar effects of high frequency stimulation have been seen in the cockroach coxal depressor muscle (Chesler and Fourtner, 1981) and locust extensor-tibiae (Burns and Usherwood, 1978). More recent evidence indicates that both of these muscles are innervated by motoneurons which utilize l-glutamate and proctolin as co-transmitters, with l-glutamate initiating twitches and proctolin causing increases of basic tonus (O'Shea and Bishop, 1982; Adams and O'Shea, 1983; Wordon et al., 1985). In the cockroach coxal depressor, a minimum frequency of motoneuron firing was required for an increase of basic tonus. Stimulation below that frequency only produced twitches (Adams and O'Shea, 1983).

In the abdominal DLM the primary action of proctolin was to increase basic tonus. Similar results have been found in the cockroach coxal depressor (Adams and O'Shea, 1983), locust extensor-tibiae, (Worden et al., 1985), weta extensor-tibiae (Hoyle and Field, 1983) and lobster skeletal muscle (Kravitz et al., 1980; Schwarz et al., 1980).

Proctolin usually had no effect or slightly reduced the amplitude of abdominal DLM twitches without affecting twitch duration (Fig. 9). This contrasts with the prolongation of

the relaxation phase of twitches in the cockroach coxal depressor treated with proctolin (Adams and O'Shea, 1983). This apparent difference between the abdominal DLM and the cockroach coxal depressor may be an artifact. Inhibitory motoneurons are known to shorten the relaxation phase of muscle contractions (Iles and Pearson, 1971; Ballantyne and Rathmayer, 1981; Kaars, 1982). In most abdominal DLM preparations, an inhibitory motoneuron was unavoidably stimulated along with the unit(s) which produced the twitches and increases of basic tonus, since both units had similar thresholds (Fig. 5). In those abdominal DLM preparations where activation of the inhibitor could be avoided (Fig. 5), twitch duration was increased compared to when the inhibitor was activated. An increase of basic tonus also persisted after a single stimulus of the lateral nerve.

Although it is unknown if the abdominal DLM motoneurons contain proctolin, several neurons in the same general location as the abdominal DLM motoneurons (Davis, 1983) exhibit proctolin-like immunoreactivity (Bishop and O'Shea, 1982). An additional indication that proctolin is involved in abdominal muscle function is the localization of a proctolin-like peptide in several abdominal muscles of the Colorado potato beetle (Veenstra et al., 1985) and *Drosophila* (Keshishian, 1985). Further work is needed on the abdominal DLM motoneurons to determine if they contain and release proctolin.

The abdominal DLM was unresponsive to octopamine at concentrations up to 10^{-4} M. This contrasts with the high sensitivity of the serially homologous metathoracic DLM (Part I). The absence of octopamine sensitivity in the abdominal DLM may be explained in several ways. First, octopaminergic innervation of the abdominal DLM may not exist. Although octopaminergic DUM neurons exist in the abdominal ganglia, the peripheral sites of innervation by these DUM neurons are unknown (Dymond and Evans, 1979). Second, octopaminergic inputs onto the abdominal DLM may exist but may have been stimulated during capture and dissection (Part I; Evans, 1981) or by electrical stimulation. Since the octopamine antagonist metoclopramide did not reduce twitch amplitude this suggests muscle facilitation had not occurred prior to octopamine application. However, since the antagonistic effect of metoclopromide is due to competition for binding sites, it would not antagonize a long lasting process induced by endogenous octopamine prior to metoclopramide application.

The pharmacological responses of the abdominal DLM contrast markedly with those of the metathoracic DLM. While proctolin produced an increase of basic tonus, it did not greatly affect twitch amplitude of the abdominal DLM. However, in the metathoracic DLM, twitch amplitude was increased and basic tonus was slightly decreased by proctolin (Part I). The two DLMs also show differences in their responses to octopamine; the metathoracic is highly

responsive (Part I), but the abdominal DLM is unresponsive.

These results show that these two serially homologous muscles have differential pharmacological sensitivities to octopamine and proctolin. Since both DLMs are innervated by serially homologous motoneurons, it is possible that the expression of neurotransmitter types by these neurons may also be different. The developmental factors which cause phenotypic differences in the quantitative or qualitative properties of these two serially homologous neuromuscular systems is unknown, but probably represents an important general mechanism which produces segmental specializations of the nervous system.

SUMMARY

1. High frequency electrical stimulation (ca. 20 Hz) of the lateral nerve in abdominal segments of the cricket *Teleogryllus oceanicus* caused an increase in basic tonus of the dorsal longitudinal muscle (DLM). This effect persisted for 1 to 5 minutes following stimulation.

2. Application of the pentapeptide proctolin (threshold between 10^{-8} M and 10^{-7} M) mimicked the increase in basic tonus produced by electrical stimulation. Individual twitches were unaffected or slightly reduced by proctolin.

3. Low frequency electrical stimulation (< 7 Hz) of the lateral nerve counteracted a previously induced increase in basic tonus, apparently by activation of an inhibitory motoneuron.

4. GABA mimicked the effect of low frequency stimulation and reduced basic tonus.

5. Octopamine, in concentrations up to 10^{-4} M, was inactive on the abdominal DLM.

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GENERAL SUMMARY

The serially homologous DLMs of the cricket metathorax and abdomen show differential pharmacological sensitivities to the neuromodulators octopamine and proctolin. The metathoracic DLM was highly responsive to octopamine while the abdominal DLM was unresponsive. The metathoracic DLM responded to octopamine by producing increased twitch amplitude and relaxation rate, as well as a decrease of basic tonus. While both muscles were responsive to proctolin the responses were qualitatively different. The metathoracic DLM responded to proctolin with increased twitch amplitude and relaxation rate as well as slightly reduced basic tonus. However, the response of the abdominal DLM to proctolin was mainly an increase of basic tonus. Twitch amplitude was unaffected or slightly reduced.

The mechanisms which cause the differential sensitivity to proctolin and octopamine are unknown, but the lack of response to octopamine by the abdominal DLM may be simply due to the lack of octopamine receptors. However, if this were true, it is unknown why octopamine receptors are present on one homologue but absent on the other. The fact that one muscle is fast while the other is a slow muscle is not an adequate explanation of the unresponsiveness of the abdominal DLM, since the slow fibers of the locust extensor tibiae are as responsive to octopamine as the fast fibers of the same

muscle (although the responses are not identical)(Evans, 1985).

An examination of the octopamine sensitivity of the metathoracic DLM at other stages may be warranted. The thoracic DLM of *Manduca sexta* develops octopamine sensitivity near the end of pupal development (Klaassen and Kammer, 1985). This occurs as the muscle changes from the slow larval form to the fast adult form. It is unknown if the larval *Manduca* DLM is responsive to octopamine. If it were not, this may indicate that the development of octopamine responsivity is related to the change of fiber type. In *Teleogryllus oceanicus* the metathoracic DLM increases dramatically in size during the last half of the last larval instar, although, this is not accompanied by changes of contraction kinetics (Ready and Josephson, 1982). It is unknown when octopamine sensitivity develops and if or in what ways it may be modified by developmental changes.

The development of the differential responsiveness of the metathoracic and abdominal DLM to proctolin poses some interesting questions. First, how are the different responses of the two muscles mediated? The proctolin receptor of each muscle may be different and thus mediate different processes in the muscle. For example, one receptor could be coupled to cAMP or other second messenger systems, while the receptors on the other muscle may use a different or no second messenger. Second, given that the mechanisms

underlying the responses differ, what are the developmental factors controlling the expression of these mechanisms? The elucidation of the developmental processes controlling the differential pharmacological sensitivity of the metathoracic and abdominal DLM will probably reveal mechanisms important for the differentiation of other tissues.

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