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THE PHYSIOLOGICAL PROPERTIES OF AMINE-CONTAINING NEURONES IN THE LOBSTER NERVOUS SYSTEM

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SUMMARY

1. Our previous studies have shown that octopamine and serotonin are found associated with a system of neurones in the connective tissue sheath of the second roots of lobster thoracic ganglia. To try to understand the mechanism of activation of these neurones, we undertook an examination of their general physiological properties.

2. All of the neurones receive excitatory synaptic input that has a cholinergic pharmacology, which suggests that it may be from sensory neurones. A very limited number of cells, possibly one, provides the total synaptic input to all the cells in the roots of the second and third thoracic segments.

3. The cells within one root are electronically coupled to each other. The extent of coupling varies widely between cells; on occasion the coupling is sufficiently tight for action potentials originating in one cell to trigger action potentials in the neighbouring cell.

4. The majority of the cells show no spontaneous activity at temperatures below 14 °C, but become spontaneously active above that temperature. Cells cycle reversibly from silent to continuously active to bursting and back as the temperature is increased and decreased.

5. Octopamine and serotonin both inhibit the bursting activity. The octopamine response is blocked by phentolamine but not by propranolol, while the inhibitory action of serotonin is unaffected by either of these drugs. The amine-inhibition of the firing could be an autoregulatory mechanism for cell activity.

6. The physiological properties described in this paper suggest that the widely dispersed amine-containing neurones in lobsters behave like a neurosecretory organ in terms of their mechanism of activation.

INTRODUCTION

Our previous studies have shown that octopamine and serotonin are found associated with neurones in the connective tissue sheath of the second roots of lobster thoracic ganglia (Wallace, Talamo, Evans & Kravitz, 1974; Evans, Kravitz, Talamo & Wallace, 1976; Livingstone, unpublished). The serotonin levels are one sixth of the octopamine levels, and it is not certain whether both amines are

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found in the same or different neurones. The amine cells have the characteristic morphological features of neurosecretory neurones and can release octopamine (Evans, Kravitz & Talamo, 1976) and serotonin (Livingstone, unpublished) into haemolymph at two different sites. The first release site is close to the neurone cell bodies at a bifurcation of the second roots; the second is at the distal ends of the roots where they enlarge to form pericardial organs (special crustacean neurosecretory structures (Alexandrowicz, 1953)) (Evans, Kravitz, Talamo & Wallace, 1976; Evans, Kravitz & Talamo, 1976). From the vicinity of both of these release sites haemolymph is drawn rapidly into the heart, and amines quickly and effectively circulate throughout the body. In examining peripheral tissues for physiological actions of the amines, we found that octopamine enhances the rate of clotting of haemolymph (Battelle, B.-A. & Kravitz, E. A., unpublished) and that both serotonin and octopamine increase the frequency and strength of the heart beat (Battelle, B.-A. & Kravitz, E. A., unpublished) and induce a contracture and enhance the strength of nerve evoked contractions in exoskeletal muscles (Kravitz, Battelle, Evans, Talamo & Wallace, 1976). All of the actions we have seen can be considered 'stress-related' effects and, in at least this way, are similar to certain actions of amines in mammals.

Our understanding of the physiological function of the system of amine-containing neurones in lobsters would be greatly aided by a knowledge of the mechanism of activation of the cells. In this paper we describe the general physiological properties of the cells and we present data on their synaptic activation and the effects of temperature and amines on their spontaneous firing.

METHODS

Thoracic regions of ventral nerve cords together with their second roots and, in some experiments, their pericardial organs, were dissected from lobsters (*Homarus americanus*) weighing about 0·5 kg. The general techniques for dissecting and recording from these preparations have been described (Evans, Kravitz, Talamo & Wallace, 1976; Evans, Kravitz & Talamo, 1976). In the experiments in this paper, a second root from one of the thoracic segments (T1–T3) was removed from the nerve cord and pinned out in a dish coated with Sylgard resin (Sylgard 184, Dow Corning). Preparations were superfused continuously with lobster saline at a flow rate of 0·5–1·0 ml./min and a temperature of 10–14 °C except where noted in the Results. The root cells were viewed with darkfield illumination and a fibre optics light source was used to avoid heating the preparation. To facilitate micro-electrode penetration of cells, a small piece of Sylgard resin was inserted under the bifurcating portion of the root. Intracellular recordings were made with micro-electrodes filled with 2 M-potassium acetate and having resistances ranging from 40 to 80 MΩ. A conventional bridge circuit was used for recording and passing current pulses with a single micro-electrode. The signals were displayed on an oscilloscope through a high impedance preamplifier. In some expts. signals were also recorded with a Brush 220 pen recorder (Gould Instrument Co.). The superfusion chamber was grounded through an agar Ag–AgCl bridge. The cut ends of the second roots were drawn into suction electrodes to stimulate the root cells antidromically or to activate their synaptic inputs. In some experiments, the nerve trunks were stimulated along their course by insulated platinum wire electrodes with the wires exposed only in the narrow portions contacting the nerve trunks. For acetylcholine (ACh) ionophoresis, micro-electrodes were filled with 1 M-ACh. The resistances of ACh-containing electrodes were in the range 100–150 MΩ. In some experiments, to avoid spontaneous leakage of ACh from the electrode, a braking current of 2–5 nA was passed continuously. In certain pharmacological experiments, synaptic potentials were averaged with a Computer of Average Transients (CAT, series 400, Technical Measurement Corp.) and the effects of different blocking agents were compared.

Normal lobster saline has the following composition: NaCl, 462 mM; KCl, 16 mM; $CaCl_2$, 26 mM; $MgCl_2$, 8 mM; glucose, 11 mM; Tris, 10 mM; maleic acid, 10 mM; and the pH was adjusted to 7·4 with NaOH. In some experiments, low Ca (2·6 mM)–high Mg (32 mM) saline was used to block synaptic transmission.

The following drugs were used: atropine sulphate (Calbiochem), α-bungarotoxin (kindly supplied by Dr L. Hall), edrophonium chloride (Hoffmann LaRoche), hexamethonium chloride and neostigmine methylsulphate (Pfaltz and Bauer), nicotine (Eastman Organic Chemicals), D-tubocurarine chloride (Mann Research Lab), Phentolamine (Ciba Pharmaceuticals) and ACh chloride, eserine sulphate, 5-hydroxytryptamine creatinine sulphate, DL-octopamine hydrochloride, DL-propranolol (Sigma).

RESULTS

Electrophysiological properties of root neurones. When the root neurones are impaled with micro-electrodes there is a brief period (a few min) of repetitive firing at frequencies up to 20 pulses per sec, and then, at temperatures below 14 °C, most of the cells become silent. Above that temperature, however, many cells generate recurrent bursts of spikes in a highly regular fashion, or fire continuously (see later section). Resting potentials are in the range of -50 to -70 mV ($-59 \pm 0{\cdot}8$ mV, mean $\pm$ S.E. of mean, $n = 77$) and depolarizing current pulses delivered through the recording electrode trigger trains of action potentials (see Fig. 7). The action potentials overshoot the resting potential by about 10 mV.

Stimulation of the lateral branch of a second root usually elicits an action potential in a root cell body followed by a slow depolarizing change. By changing the stimulus intensity one can activate these two components separately. Low intensities of stimulation produce action potentials alone (Fig. 1*A*) while higher stimulus intensities produce the slow component as well (Fig. 1*B*). That the action potential is due to the antidromic activation of the axon of the cell, while the slow potential results from synaptic activation, is suggested in an experiment in which the preparation is soaked in a low Ca–high Mg solution. In this solution only the slow potential is abolished, the action potential is not (Fig. 1*C*). Stimulation of the central end of a second root also produces only a slow potential (Fig. 1*D*, but see below) and this also is blocked in the low Ca–high Mg solution (Fig. 1*E*). Whether the synaptic activation from the central end and the lateral branch of the root is from the same or different fibres is considered below.

We explored next whether root cells are homogeneous in their general physiological properties. There are approximately twelve cells per root in the roots used in the present studies (T1, T2 and T3) (Evans, Kravitz, Talamo & Wallace, 1976). With two different T1 roots, in one preparation we recorded from eleven cells and in a second from nine cells. In both cases the responses evoked by stimulating the lateral branch and central end of the roots were observed. In grouping the cells from the two roots together, three patterns of responses were seen. First, seventeen cells (85%) showed the responses described above, i.e. stimulation of the central end evoked only a synaptic response while lateral-branch stimulation produced both a synaptic response and an antidromic action potential. Secondly, in two cells, stimulation at either end of the root yielded both an antidromic action potential and a synaptic potential. We were able to demonstrate by injection of procion yellow or horseradish peroxidase (not shown) that some cells have an axonal process directed toward the central end of a root in addition to the normal axonal

process heading towards the lateral end of a root. We expect that centrally-directed axonal processes are the origin of action potentials evoked by central stimulation of cells in this category, but we do not know whether these processes extend into the central ganglia or terminate in roots before entering ganglia. Finally, in one cell, stimulating either end of a root produced only synaptic activation. Occasionally, we have seen cells similar to this one in other experiments even though neighbouring cells in these experiments showed the more usual response. We cannot be certain whether the rare cells that show only synaptic activation belong to a true sub-population of the root neurones, or whether they respond in this manner owing to partial damage of the lateral branch of a root during an experiment.

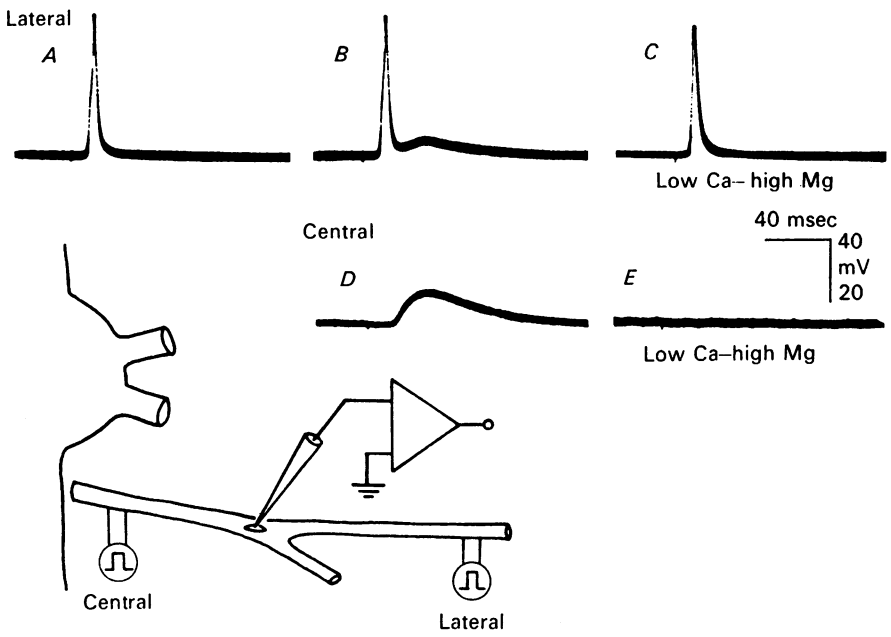

Fig. 1. Antidromic action potentials and synaptic potentials recorded from a root neurone. The inset diagram shows the recording and stimulating arrangement. The proximal portion of the second root (C.N.S. end) is cut away from the thoracic ganglion in this and all subsequent experiments. Stimuli are applied to the *central* end and *lateral* branch of the root. *A*, an antidromic action potential evoked by a weak stimulus to the lateral branch; *B*, an action potential followed by a synaptic potential when the stimulus intensity is increased; *C*, the same stimulus as in *B* in a low Ca–high Mg solution; *D* and *E*, responses evoked by stimulating the central end of the root in normal (*D*) and low Ca–high Mg solution (*E*).

Synaptic input to the root neurones. Stimulation of either the lateral branch or the central end of a root produces a synaptic potential in all cells tested. Varying the intensity of stimulation yields no evidence of multiple synaptic inputs to single cells. In all cases, at a certain critical intensity of stimulation a response occurs. Stimuli of greater intensity give no further increase in the size of the response. When recordings are made from pairs of neighbouring cells in the same roots, synaptic potentials are evoked simultaneously in both cells at the same intensity of stimulation (not shown, but see Fig. 4). If one of the electrodes is moved to a third cell, the

threshold of the synaptic response in that cell is identical to that seen in the first two. Similar experiments have been carried out with twelve pairs of cells with similar results.

To test whether the synaptic response produced by stimulating the lateral branch or central end of a root is from the activation of the same or different nerve fibres, we carried out collision experiments. Two successive stimuli are applied to a root, one to the lateral branch and one to the central end, and the interval between the stimuli is changed gradually. With intervals beyond a certain critical time (in this experiment, greater than 40 msec), the second stimulus always produces a synaptic

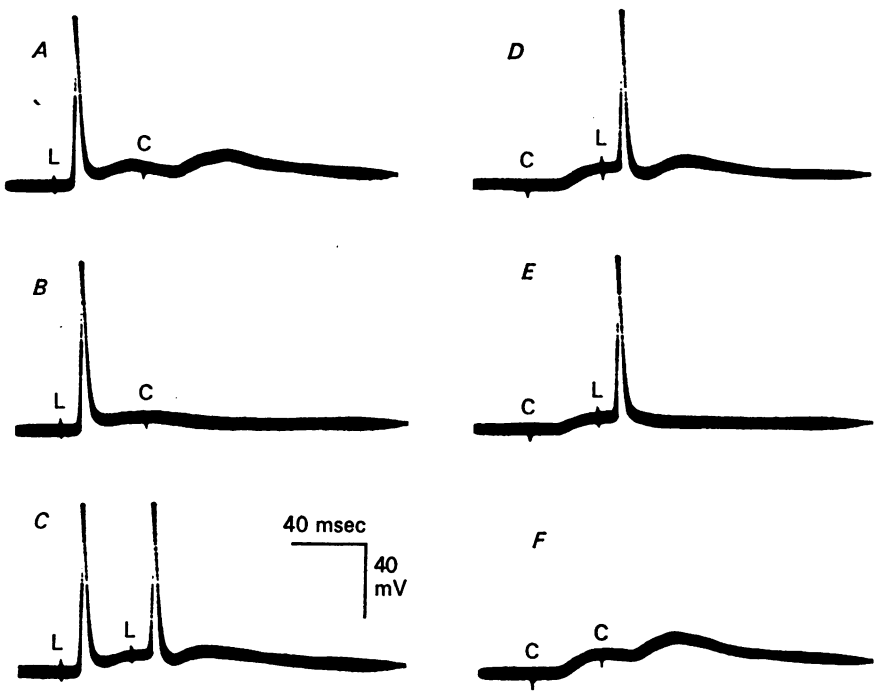

Fig. 2. A 'collision' experiment. In *A* and *B* stimuli are applied first to the lateral branch (L) and then to the central end (C) of a second root. From *A* to *B* the interval between the two stimuli is shortened. *C*, two stimuli are delivered to the lateral branch alone at a shorter inter-stimulus interval than in *B*; *D–F*, the same experiment as in *A–C* except that the first stimulus is applied to the central end of the root and in *F* both stimuli are delivered to the central end.

potential following the first synaptic response (Fig. 2*A*, *D*). When the interval between stimuli is shortened to 40 msec or less, the second response disappears (Fig. 2*B*, *E*). To exclude the possibility that the failure of the second response is due to a conduction block in the axon generating the synaptic response or to the desensitization of post-synaptic receptors or to an inhibitory synaptic response following the excitatory response, two successive stimuli separated by 36 msec are applied only to the lateral branch (Fig. 2*C*) or central end (Fig. 2*F*) of the root. In these cases, the second stimulus still produces a synaptic response.

The results of this group of experiments suggest that a single through fibre or a

small group of fibres in which individual fibres cannot be stimulated separately under our experimental conditions, produces the synaptic potential seen in all cells when we stimulate either end of a root.

Shared synaptic input between roots. The pericardial organs at the distal ends of the second roots are linked together by nerve trunks that run in a rostro-caudal direction in the pericardial cavity (Alexandrowicz, 1953). In three experiments two thoracic roots (T2 and T3) together with the nerve trunk linking their pericardial

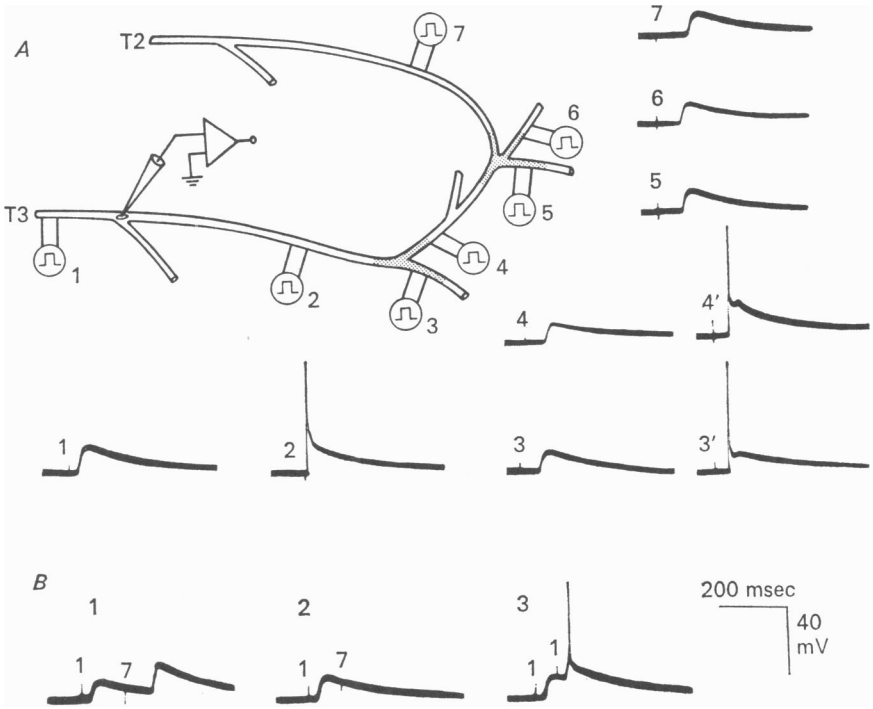

Fig. 3. Synaptic input to a root neurone. The inset diagram shows the preparation and the experimental arrangement. The second roots of two body segments (T2 and T3) are dissected together with their pericardial organs (stippled areas) and connecting nerve bundle. Intracellular recording is made from a neurone of the T3 segment. Stimuli are delivered at multiple sites along the roots and nerve bundle (1–7). *A*, the responses evoked by stimulating at points 1–7; at points 3 and 4 weak stimulation produced only synaptic potentials while at higher intensities of stimulation antidromic action potentials are seen, followed by the synaptic potentials (3′, 4′). *B*, a 'collision' experiment; 1, 2, stimuli were applied at points 1 and 7 successively. The second synaptic potential disappears when the inter-stimulus interval is shortened in 2; 3, two successive stimuli are applied only at point 1 with a shorter interval between them than in 2.

organs were dissected (see inset drawing, Fig. 3). In two of the experiments we recorded from a cell body in one root and examined the physiological responses seen by stimulating at different points along both roots and the connecting trunk. When recording from a neurone in T3, stimulation along the T3 root produces the expected results (Fig. 3, stimulus points 1–3). However, stimulating the nerve trunk linking T2 with T3 (point 4) also produces a synaptic potential as does stimulating at several locations along the T2 root (stimulus points 5–7). Furthermore, a collision

experiment suggested that the same axon or group of axons in both roots gives rise to the synaptic potential seen in the cell body in T3. For example a stimulus delivered to the central end of T3 (point 1) can eliminate the synaptic response produced by stimulating the lateral branch of T2 (point 7), when the inter-stimulus interval reaches a certain critical time period (Fig. $3B_1$, B_2). However, two stimuli delivered to the same end of a root (point 1) can be applied with a considerably shorter time interval between them, and still produce two successive synaptic potentials (Fig. $3B_3$). Comparable results can be obtained when stimuli are delivered at the central end of T3 (point 1) and each of the other points of stimulation. That the synaptic response seen in the cell in the T3 root is not due to the activation of the T3 root by the spread of current from the distant stimulating electrode is shown at the end of the experiment by removing the T2 root from the electrode or by cutting the nerve near the stimulating electrode. Antidromic action potentials are elicited by stimulating the lateral branch of T3 (point 2) and by high intensity stimulation of the pericardial organ region of the same root (3′, 4′, Fig. $3A$).

In the second experiment of this type the intracellular recording was made in a cell body in the T2 root. In this case, stimulating the T3 root generated a synaptic response in a T2 neurone and, as above, a collision experiment suggested that the synaptic input to the T2 neurone came from the same fibre (or fibres) in both roots.

In the third of this series of experiments, simultaneous recordings were made from two cell bodies, one in each root. When the central end of the T2 root is stimulated, synaptic potentials appear in both neurones at the same intensity of stimulation (Fig. 4, upper half). The delay between the stimulus and the response in the T3 neurone, however, is considerably longer than in the T2 neurone. This presumably reflects the time necessary for the conduction of the nerve impulse from the site of stimulation in the T2 root to a near (T2) and distant (T3) synaptic junction. When the stimulation is applied to the lateral branch of T3, the delays are reversed (Fig. 4, lower half).

Pharmacology

The three major types of through fibres known to be present in the second thoracic roots are efferent excitatory and inhibitory axons to exoskeletal muscles, probably using glutamate and GABA as neurotransmitters, and afferent sensory axons likely to use ACh as their transmitter substance (Barker, Herbert, Hildebrand & Kravitz, 1972). The possibility that the synaptic input to the root neurones might be from cholinergic sensory fibres in the root was examined next.

When ACh is added to the fluid bathing a root or injected onto cell bodies by iontophoresis, a dose-dependent depolarization of the neurones is observed (e.g. see Fig. $5C$). The synaptic potentials generated by stimulation of the root are reduced in size reversibly by ACh and various cholinergic blocking agents. The synaptic response is decreased by about 50% at the following concentrations of drugs: nicotine, 5×10^{-6} M; ACh, 10^{-5} M; curare, $1{\cdot}4 \times 10^{-5}$ M; atropine, 3×10^{-5} M; hexamethonium, 7×10^{-5} M. α-Bungarotoxin (from 10^{-6} to 10^{-5} M) applied to a preparation for up to 30 min has no effect on the response. In Fig. 5, the effects of hexamethonium (7×10^{-5} M) on synaptic potentials evoked by nerve stimulation

are compared with effects on potentials produced by the iontophoretic application of ACh. The synaptic responses and the ionophoretic ACh potentials are all reversibly reduced in size by hexamethonium treatment (Fig. $5A_2$, $5B_2$ and $5C_2$). The time course of the reduction in size and its subsequent recovery are approximately the same for all the responses. As expected, the action potential evoked by

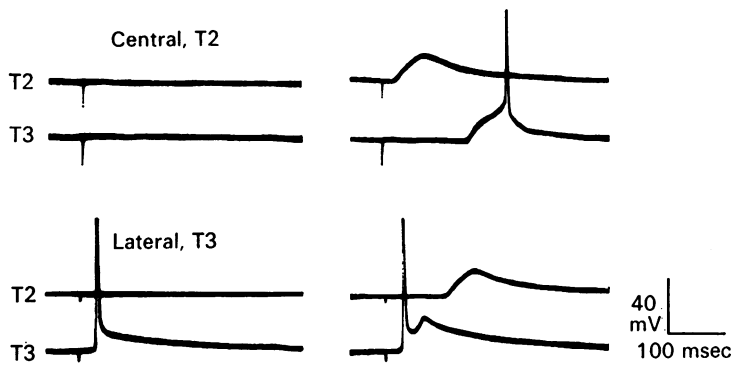

Fig. 4. Synchronous appearance of synaptic potentials in cells of two different roots. The experimental arrangement is similar to that of Fig. 3 except that two neurones are impaled, one in the T3 root, one in the T2 root. Upper traces: the responses seen in the T2 and T3 root neurones when the central end of the T2 root is stimulated. The traces on the right are at a higher intensity of stimulation than those on the left. The threshold for the appearance of the synaptic response is the same in both cells. Lower traces: the same experimental protocol except that the lateral branch of the T3 root is stimulated first. A weak stimulus (left side) produces only an antidromic action potential in the T3 neurone while a stronger stimulus yields synaptic responses in both cells.

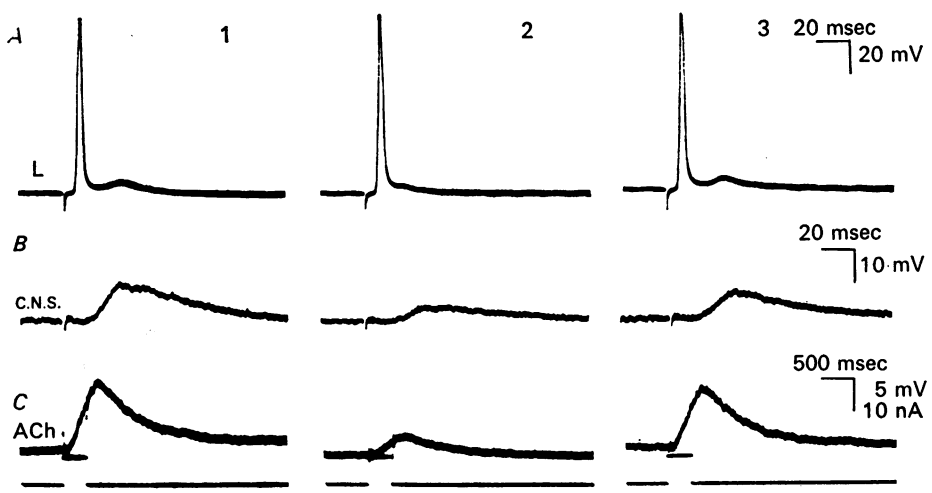

Fig. 5. The effects of hexamethonium on the synaptic and ACh induced potentials in a root neurone. *A*, antidromic action potentials followed by synaptic potentials evoked by stimulation of the lateral branch (L). *B*, synaptic potentials evoked by stimulating the central end of a root (C.N.S.). *C*, responses evoked by ACh applied ionophoretically onto the cell body of the root neurone; 1, normal responses; 2, responses after 7 min in saline containing 7×10^{-5} M-hexamethonium; 3, responses recorded 7–12 min after washing out the drug.

lateral branch stimulation is not affected by hexamethonium. The effects of anticholinesterases lend further support to the cholinergic nature of the synaptic input. Edrophonium (5×10^{-6} M) potentiates both the synaptic responses evoked by stimulation of the central end of a root (Fig. 6, upper) and ACh potentials (Fig. 6, lower). The effect of edrophonium was reversed 10 min after washout of the drug. Higher concentrations of edrophonium (5×10^{-5} M) decrease the size of the synaptic response while eserine and neostigmine at concentrations from 10^{-6} to 5×10^{-6} M have no significant effect on synaptic potentials.

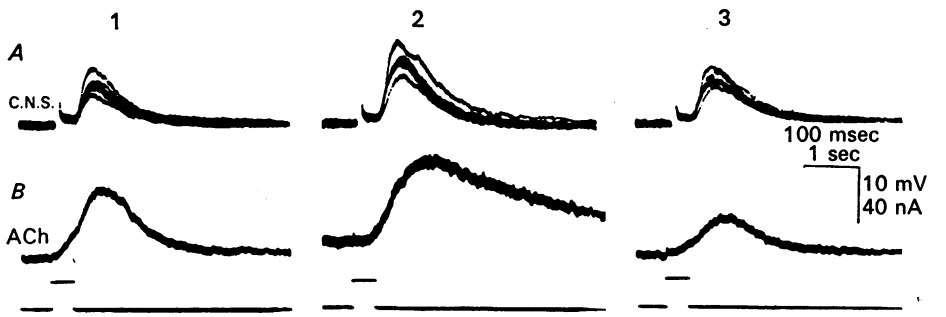

Fig. 6. Effect of the anticholinesterase, edrophonium on synaptic and ACh potentials. *A*, the synaptic potential evoked by stimulating the central end of a root; five traces are superimposed; *B*, ACh potential evoked by ionophoresis onto the cell body; 1, normal responses; 2, responses recorded 8 min after applying edrophonium, 5×10^{-6} M; 3, responses recorded 10 min after washout of the drug.

Coupling between root neurones. In the experiment shown in Fig. 7, we looked for interactions between neighbouring neurones by recording from pairs of cells in one root. When long-duration current pulses are passed into one cell, a fraction of the potential change is recorded in an adjacent neurone. Hyperpolarizing and depolarizing potential changes are transmitted almost equally well (Fig. 7*A*, upper and lower traces) and in either direction between cells. Occasionally, fairly strong coupling is observed. In Fig. 7*B* (upper) depolarizing current pulses passed into one cell body lead to a train of action potentials that generate an action potential in the neighbouring cell. In this experiment the coupling potentials persist when chemical synaptic transmission is completely blocked in a low Ca–high Mg solution or when 20 mM-$CoCl_2$ is added to the superfusion fluid. Similar experiments were performed with fifteen pairs of neurones. In twelve cases, coupling was seen, but the extent of coupling varied widely. Most often the coupling between cells is weak but it is difficult to measure accurately the coupling ratio because of the limitations of the single electrode recording technique. In three cases no coupling potential was detected.

Spontaneous firing. As mentioned earlier, penetration of a cell with a microelectrode results in an injury discharge of repetitive action potentials. At our usual low temperature range of recording (10–14 °C) most of the cells soon stop firing. At higher temperatures, however, when we examine cells after the initial discharge of action potentials we observe that many cells keep firing continuously or generate bursts of action potentials followed by silent periods (Table 1). Over 70% of the

cells are silent at the lower temperatures while over 60% show continuous firing or bursting activity at the higher temperatures. Many cells seem to cycle from silent to continuously active to bursting and back, in response to increases and decreases in temperature. An example is shown in Fig. 8. At 17 °C, the cell generates rhythmic

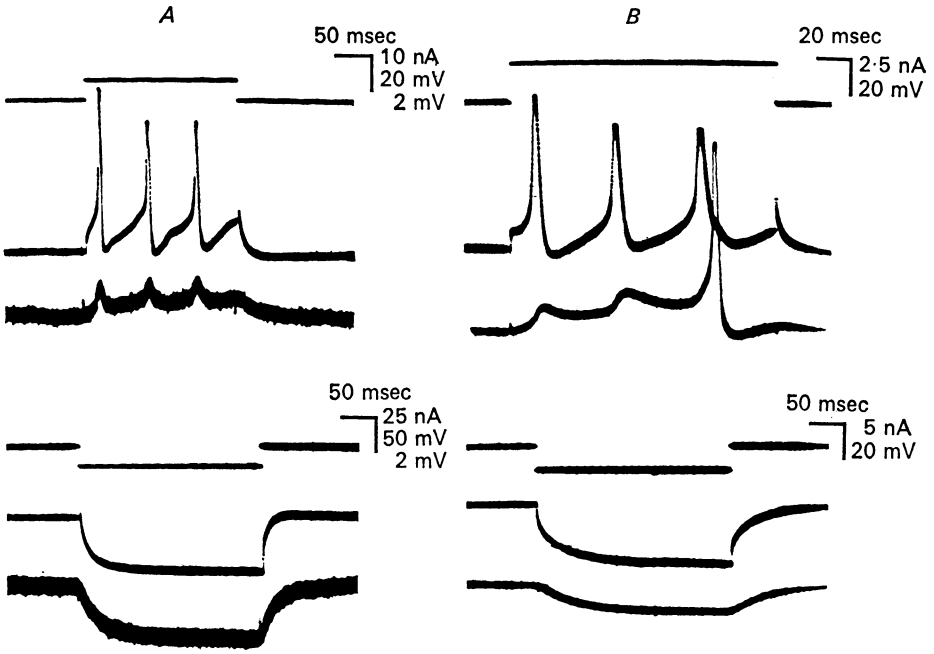

Fig. 7. Electrical coupling between root neurones. Two cells are impaled with single micro-electrodes. Current pulses are injected into one cell through the recording electrode while the potential changes are recorded in the neighbouring cell. *A*, shows the more commonly observed weak coupling between cells; *B*, an example of relatively strong coupling. In the upper sets of records depolarizing current pulses were used while in the lower sets hyperpolarizing pulses were applied. Within each set of records the uppermost trace shows the current pulse injected through the recording electrode, the middle trace shows the response in the current-injected cell and the lower trace shows the electronic coupling potential recorded from the adjacent cell.

TABLE 1. Spontaneous activity of root neurones and the effect of temperature on discharge frequency

	Numbers of cells		
	Temperature range		
Cell type	Low (10–14 °C)	High (15–18 °C)	Frequency increase*
Silent	33	16	—
Continuous discharge	6	7	3
Burst of spikes	3	21	11
Totals	42	44	14

Measurements were made with sixty-five cells and twenty-one cells were observed within both temperature ranges.

* Determined by changing the temperature from the low to the high range.

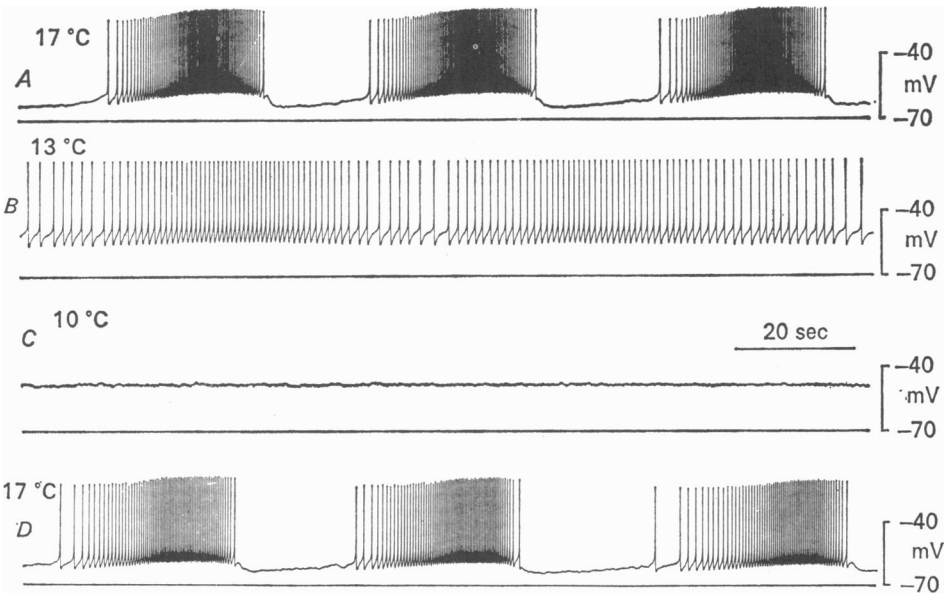

Fig. 8. The effect of temperature on the spontaneous firing of a root cell. A root cell is impaled with a micro-electrode and the spike discharge and membrane potential are displayed on a pen recorder. The spike amplitude is distorted because of the long response time of the pen recorder. The temperature is indicated at the upper left hand side of each record.

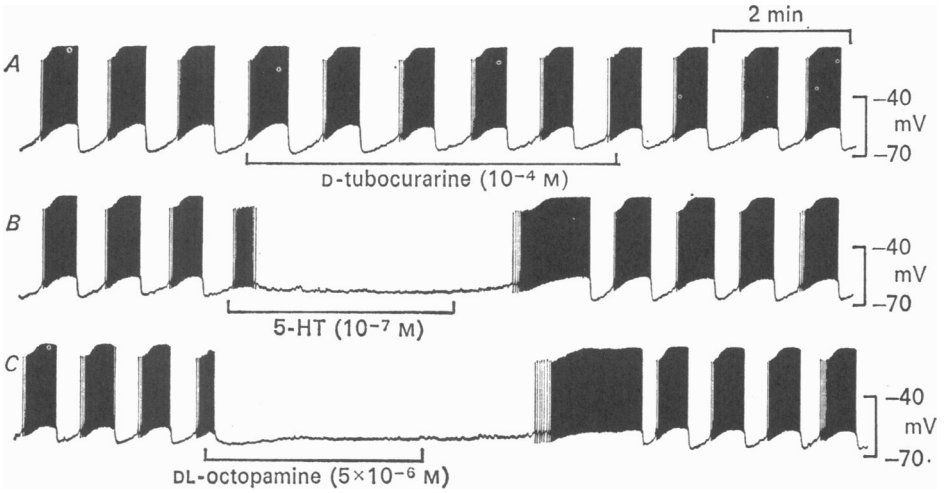

Fig. 9. The effects of D-tubocurarine, serotonin (5-HT) and DL-octopamine on the bursts of spikes in a root neurone. Spike discharges and membrane potentials are recorded from a root cell and displayed on a pen recorder. The drugs are added to the bath by superfusion during the period indicated by the line under each record. The experiment is carried out at 17 °C. The spike amplitudes are distorted as mentioned in the text of Fig. 8.

bursts of spikes (Fig. 8*A*). On cooling, the membrane potential decreases, the spike discharge becomes almost continuous at 13 °C (Fig. 8*B*), and the cell becomes silent at 10 °C (Fig. 8*C*). On returning to 17 °C the membrane potential increases and the bursting activity reappears. Bursts of action potentials at the higher temperatures are superimposed on slowly oscillating potentials ranging from 5–20 mV in size in different experiments. The bursts of spikes are likely to be an endogenous property of the root neurones as hyperpolarization of cells to the point where spikes are blocked reveals no underlying synaptic activity and D-tubocurarine, at a concentration high enough to block the cholinergic synaptic input to the cells (10^{-4} M),

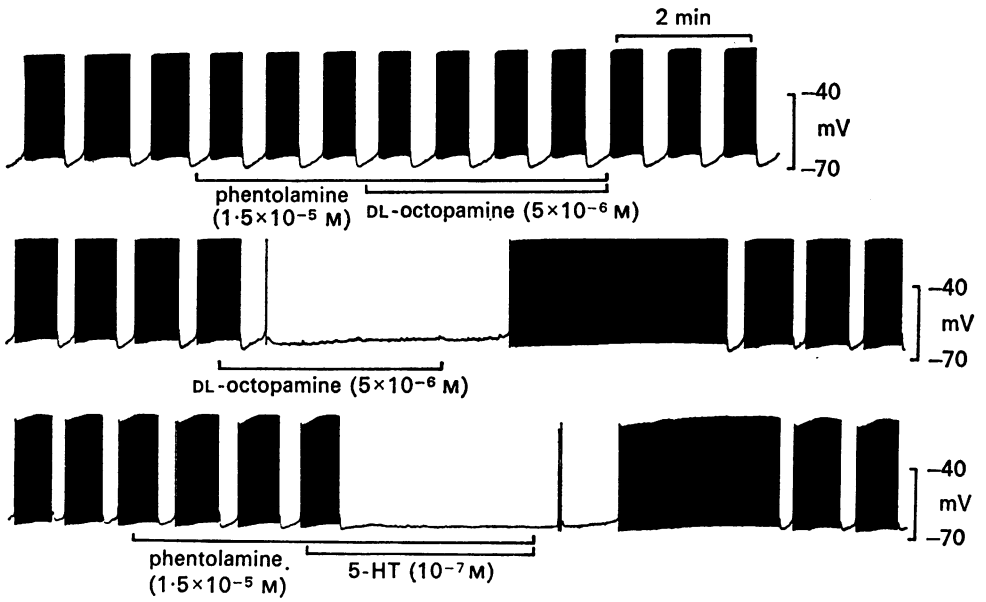

Fig. 10. The effect of phentolamine on the inhibition of the bursting activity induced by DL-octopamine and serotonin (5-HT). The experiment is performed as in the legend of Fig. 9.

causes no change in the firing rhythm (Fig. 9*A*). The bursting activity can be blocked, however, by octopamine and serotonin, the two amines thought to be released by the cells (Fig. 9*B* and 9*C*). Serotonin is about 10–20 times more effective than octopamine. Both amines cause only an inhibition of the spike discharge and a slight hyperpolarization over a wide range of concentrations (10^{-8}–10^{-5} M). At the higher concentrations the inhibitory effects last for long periods of time after washout of the amines from the bath. When the cells begin firing again after amine treatment, a prolonged rebound phase of continuous firing usually occurs (Figs. 9 and 10). Synaptic potentials evoked by stimulating the central end of a root are either not affected or sometimes slightly decreased in size by amine treatment.

The inhibitory action of octopamine on the bursting activity is selectively antagonized by the α-blocking compound phentolamine ($1{\cdot}5 \times 10^{-5}$ M) (Fig. 10). Phentolamine alone at this concentration has no effect on the spontaneous firing of the cells or on the inhibitory effect of serotonin. The β-blocker propranolol (10^{-5} M) has no effect on the inhibitory actions of octopamine.

DISCUSSION

The studies presented in this paper aim to characterize the general physiological properties of the amine-containing neurones of the lobster ventral nerve cord. The work represents the first steps in an attempt to understand the physiological role of this system of neurones.

All of the amine-containing root neurones receive synaptic input. The input has a cholinergic pharmacology, suggesting that it may be sensory (Barker *et al.* 1972), and only a limited number of cells produce the synaptic response. In fact, we have no evidence that the synaptic input to an individual neurone comes from more than one fibre. We have been unable to show any gradation or series of steps in the synaptic potential by varying the duration or intensity of nerve trunk stimulation. Although a synaptic response can be produced by stimulating either end of a root, collision experiments suggest that this input is from the same through fibre in the root. It remains possible that a small group of very similar fibres gives rise to the synaptic potential. A clear-cut confirmation or denial of the latter suggestion may have to await finding a known sensory cell that forms a synaptic contact with the root cells. Not only do individual neurones receive a unitary synaptic input from a through fibre in a root, but neighbouring cells share this input, and the sharing of synaptic input extends to other roots as well. At the distal ends of the thoracic roots there are nerve trunks that link the roots together and form a ring-like structure that surrounds the heart. In the several experiments in which we dissected out two roots joined together by these nerve trunks, cells in one root could be synaptically activated by stimulating the adjoining root. Collision experiments and measurements of the threshold for the generation of synaptic responses, again suggest that the same fibre extends through both roots. We do not know how widespread the sharing of synaptic input between roots is, but the possibility exists that one or a very small number of cells generates the synaptic input that triggers the firing of the whole population of root neurones.

Some possible candidates for the 'sensory' cells that activate the root neurones might be the N-cells originally described by Alexandrowicz (1952). There are five of these cells on each side of the body in the region of the pericardial sinus. They send their axons into the central ganglia through the second thoracic roots and their function is unknown. It is, of course, of very high priority to find the cells that generate the synaptic input since then it would be possible to determine the sensory signal that turns on the entire amine system of neurones.

The observation that most of the root cells are electrotonically coupled to each other suggests further the homogeneity in the physiological responsiveness of these neurones. While the extent of coupling varies widely between neighbouring cells, the existence of this mechanism could be another way to ensure that cells fire in concert.

The present studies do not contribute to the question of whether octopamine and serotonin are found in the same or different cells along the roots. We did see some heterogeneity in the physiological properties of the cells but we cannot associate this heterogeneity in any way with the presence of one or other of the amines. On the other hand, if the cells fire in unison as we suspect, it may only be of academic

concern whether the amines are in the same or different cells, i.e. the two amines may be released together in any event. One test of this idea would depend on finding the sensory neurones that activate the root cells. Attempts could then be made to trigger the firing of the root cells with natural stimuli and to correlate this firing with the simultaneous appearance of both amines in the haemolymph. On the other hand, temperature change, with the resultant increases and decreases in spontaneous firing of the cells, may be a more immediate way to try to correlate activation of the cells with the release of amines.

In *Aplysia*, spontaneous activity has been seen in neurosecretory neurones in the abdominal ganglion (Frazier, Kandel, Kupferman, Waziri & Coggeshall, 1967), and temperature sensitive generation of spontaneous firing has been reported in neurones of the visceral ganglion (Wilson & Wachtel, 1974; Smith, Barker & Gainer, 1975; Carpenter, 1967; Carpenter & Alving, 1968). The temperature range over which the lobster cells change from silent to bursting (10–17 °C) covers the normal temperature range that lobsters encounter. Certainly in the summer in shallow water, animals will be found at water temperatures as high as 17 °C, while in the winter temperatures well below 10 °C will be common to the animal. There are many differences in behavioural traits between lobsters in summer and winter. Whether any of these differences result from the activation of the system of amine-containing neurones remains to be established.

Finally, inhibition of the spontaneous firing of the root neurones by octopamine and serotonin may be a further interesting feature of this system. This inhibition could serve as an auto-regulatory mechanism for cell activity by allowing a cell to detect increases in the local concentrations of amines that are released. The connective tissue near the cell bodies of the root neurones has a dense matrix of nerve endings of the cells (Evans, Kravitz & Talamo, 1976; Schaeffer, unpublished). It is not difficult to imagine that cells continuously monitor the amine concentration in their vicinity or in the haemolymph sinus in which the roots are located and adjust their firing rate according to the amine level. In recent studies 'auto-receptors' for amines have been reported for neurones in the mammalian C.N.S., and one of the roles suggested for these receptors is similar to the one that we suggest for the lobster neurones (Aghajanian & Bunney, 1977).

There are approximately 100 neurones associated with the amines octopamine and serotonin in the lobster's nervous system (Wallace *et al.* 1974). These neurones are on seven pairs of nerve trunks in the thoracic region of the ventral nerve cord. Thus this system of neurones consists of many individual cells that are widely distributed in the anterior region of the animal. On the other hand, the studies presented in this paper on the general physiological properties of the cells and on their synaptic activation, suggest that these neurones function more like a unit, a neurosecretory organ, than like a collection of isolated individual cells. Such a way of functioning may be reasonable for neurones containing hormonal substances that are to be released into the haemolymph for widespread actions throughout the organism.

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