ulatory action is on the putative octopamine receptors or on later biochemical events occurring during light emission. We used cyproheptadine as an octopamine antagonist since it is a potent blocker of the octopamine-induced activation of adenylate cyclase in cockroach ganglion (*12*) and brain (*13*) homogenates. Theophylline, which increases intracellular adenosine 3′,5′-monophosphate (cyclic AMP) by inhibiting cyclic AMP phosphodiesterase and causes glowing in fireflies (*14*), was also used. In a single coded experiment fireflies were treated topically with cyproheptadine (20 μg) while controls received acetone alone. Two hours later they all received 0.1 μg of DCDM topically. After 10 to 20 minutes the glow response was rated independently by five people, averaged, and evaluated statistically (Table 1). DCDM caused significantly less intense glowing in cyproheptadine-treated fireflies than in the controls. Since cyproheptadine might have affected the ability of the light organ to produce light at some stage after the octopamine receptor–cyclic AMP synthesis stage, we subsequently injected all the fireflies with 5 μg of theophylline. After 30 minutes, the glows were again evaluated. Cyproheptadine-treated animals exhibited glows indistinguishable in intensity from the controls (Table 1), suggesting that the cyproheptadine block does occur at the receptor activation–cyclic AMP synthesis stage. Isolated light organs from cyproheptadine-treated insects responded more weakly to both octopamine and DCDM than controls (Table 2), indicating that cyproheptadine blocks the action of octopamine as well as that of DCDM.

Our results demonstrate a postsynaptic site of action for DCDM; results obtained with cyproheptadine are most simply explained if DCDM acts as an octopaminergic agonist. The *N*,*N*-dimethyl parent compound, CDM, appears to have a rather low intrinsic activity. The delay before it stimulates strong glowing (Fig. 1) may be related to its conversion to the more potent DCDM by *N*-demethylation in vivo, since injection of CDM directly into the hemocoel did not reduce the delay before strong glows were observed. Evidence has been presented that a number of the toxic actions of the formamidines may be mediated by the *N*-demethyl products generated in vivo (*15*).

The functional significance of octopamine in insects is only now being explored (*16, 17*). It has been suggested that octopamine has multiple actions as a neurohormone and neurotransmitter, some of which are analogous to those of epinephrine in vertebrates (*18*). In addition to its excitatory role in the firefly lantern, octopamine modulates the excitability and depresses inhibitory neuromuscular synaptic transmission of insect skeletal muscle (*18, 19*). It increases the rate of beating of the cockroach heart (*20*), an action also observed with CDM (*21*). No specific functions have yet been established for octopamine in the insect central nervous system (CNS), but it is likely that these exist. Octopamine is found in considerable amounts in the CNS of several insect species (*16*), and an octopamine-specific activation of adenylate cyclase has been observed in cockroach thoracic ganglion (*12, 22, 23*) and brain (*13*). We have observed that a range of formamidines induce specific motor discharges from certain cells in the isolated abdominal ganglia of tobacco hornworm larvae and this action can be mimicked by octopamine and a number of other biogenic amines (*24*).

The link between octopamine and the pest-controlling actions of the formamidines is suggestive. These agents have a potent octopaminergic action in at least one insect species and this may indicate a new biochemical site for pesticidal action.

Note added in proof: Since submitting this report we have found that DCDM stimulates firefly tail adenylate cyclase activity with a potency comparable to that of octopamine. This action is blocked by cyproheptadine and phentolamine.

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Serotonin and Octopamine Produce Opposite Postures in Lobsters

Abstract. *Serotonin and octopamine, injected into the circulation of freely moving lobsters and crayfish, produce opposite behavioral effects. Octopamine injection produces sustained extension of the limbs and abdomen; serotonin injection produces sustained flexion. Neurophysiological analyses show that these postures can be accounted for by opposing, coordinated effects of these amines on patterns of motoneuron activity recorded from the ventral nerve cord.*

The amines octopamine and serotonin are widely distributed in the lobster's nervous system, where they appear to function, at least in part, as circulating neurohormones (*1, 2*). The central ganglia of the ventral nerve cord, particularly the brain and subesophageal ganglion, have significant quantities of amines, but the highest concentrations are along the second roots of the thoracic region of the ventral nerve cord. Associated with the amines in this region are clusters of neurosecretory cells and several morphologically and biochemically distinguishable kinds of nerve endings (*2, 3*). In the same region, octopamine and

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serotonin are synthesized from precursor compounds and released into the hemolymph by depolarization (*4*). Physiological actions of amines have been observed in several potential target tissues in lobsters: hemolymph (enhanced clotting), heart (enhanced strength and rate of heartbeat), exoskeletal muscles (enhanced contractions), and the stomatogastric ganglion (change in patterns of spontaneous activity) (*5, 6*). We report here that octopamine and serotonin have opposing actions in coordinating the central neuronal activity governing flexion and extension of postural muscles in lobsters.

The conclusion that octopamine and serotonin function as neurohormones in lobsters was based mainly on the morphological examination of their sites of synthesis and release from the second thoracic roots (*1, 2*). We now report that amines normally circulate in a free form (not associated with hemocytes) in lobster hemolymph. The mean concentration of octopamine was found to be $2.3 \pm 1.3 \times 10^{-9} M$ ($N = 12$; range, $1.2 \times 10^{-9} M$ to $5.5 \times 10^{-9} M$), and the mean concentration of serotonin was found to be $1.4 \pm 1.8 \times 10^{-9} M$ ($N = 20$; range, $0 \times 10^{-9} M$ to $8 \times 10^{-9} M$) (*7*). The mean concentrations of both amines are below (but within an order of magnitude of) their threshold concentrations for physiological actions on several peripheral tissues (*5, 6*). These findings (i) make it even more likely that the amines function, at least in part, through release into the hemolymph and (ii) suggest that we can introduce amines into lobsters in a manner not very different from the normal route of release—by injection directly into the circulation.

One to several minutes after DL-octopamine (1 to 10 mg) was injected into the ventral hemolymph sinus of 0.5-kg lobsters, the animals assumed rigid hyperextended postures (Fig. 1b). The legs and tail were raised off the substrate and held rigidly, with the legs and large claws fully extended and pointed anteriorly, and the tail fully extended posteriorly. The swimmerets usually beat metachronously at 1 to 2 cycles per second. If startled by a tap on the aquarium glass, the lobsters immediately relaxed and then again assumed the extended posture after several seconds. The injections frequently resulted in defecation and vomiting. Hyperextended postures lasted 30 minutes to several hours depending on the dose. Most animals recovered completely, but large doses (20 to 30 mg) were occasionally fatal.

Conversely, injection of serotonin (0.5 to 10 mg) produced a rigid flexion of the legs and abdomen (Fig. 1a). In lobsters injected with 0.5 to 2 mg of serotonin, claws were opened and held forward, walking legs were kept directly underneath the body with the distal joints slightly flexed so that the animals stood high off the substrate, and tails were tucked under, loosely flexed. Tapping on the glass did not elicit the startle response. These effects appeared within one to several minutes and lasted 10 to 30 minutes. With larger doses (5 to 10 mg), the flexion was more extreme and the effects lasted several hours.

When crayfish (*Procambarus clarkii, Astacus fluviatilis*, and *A. astacus*) were injected with proportionately smaller doses of serotonin or octopamine, the same extension or flexion postures resulted as in the lobsters (Fig. 1, c and d).

The opposing effects of octopamine and serotonin on lobster posture could result from actions at peripheral or central sites or both. To define the site of action, we first compared the actions of octopamine and serotonin on opposing muscles in the walking legs. In a study of the effects of amines on the neuromuscular preparation of the "opener" of the dactyl of the walking leg (*5*), it was found that serotonin, acting at presynaptic sites, facilitated transmitter release from excitatory nerve endings, whereas both serotonin and octopamine, acting at postsynaptic sites, caused a contracture to develop and action potentials to appear in the muscle fibers. We compared the effects of octopamine and serotonin

Table 1. Summary of the effects of octopamine and serotonin on excitatory and inhibitory innervation of slow extensor and flexor muscles. In some experiments, the muscle fibers used for recording were not innervated by all the units described. Under activity, +3 represents the greatest increase; 0, no change; and −3, the greatest decrease.

Motoneuron input	Octopamine			Serotonin		
	Activity	Times result observed	Experiments	Activity	Times result observed	Experiments
Extensor						
Excitatory units	+3	13	16	0	3	6
				−3	2	6
Inhibitory units	−2	3	6	+3	4	4
	0	2	6			
Flexor						
Tonic excitatory units	−2	15	15	−1	9	14
				0	5	14
Phasic excitatory units	0	1	8	+2*	9	11
	+1†	7	8			
Inhibitory units	+3	13	13	−3	6	7

*Firing occurred in bursts. †Firing occurred singly without bursts.

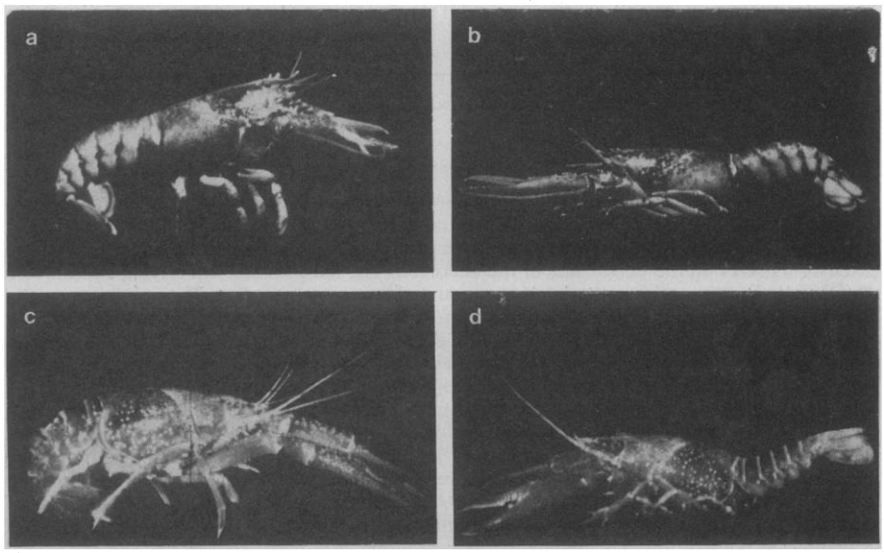

Fig. 1. Rigid postures induced by injection of serotonin or octopamine. (a) Lobster (*Homarus americanus*, 25 cm in length) injected with 5 mg of serotonin or (b) 5 mg of DL-octopamine. (c) Crayfish (*Procambarus clarkii*, 10 cm in length) injected with 1 mg of serotonin or (d) 1 mg of DL-octopamine.

on the dactyl opener muscle with their effects on the antagonistic "closer" muscle. We also compared the effects of the two amines on the main flexor and extensor muscles of the meropodite segment of the walking leg. In all the neuromuscular preparations we tested, serotonin alone showed a presynaptic facilitation of excitatory transmitter release. Both amines induced contractures in the opener and closer muscle pair, whereas neither induced contractures in the meropodite flexor and extensor pair. Thus all the muscles we tested responded to serotonin or octopamine or both, but in no case was an opposing response seen.

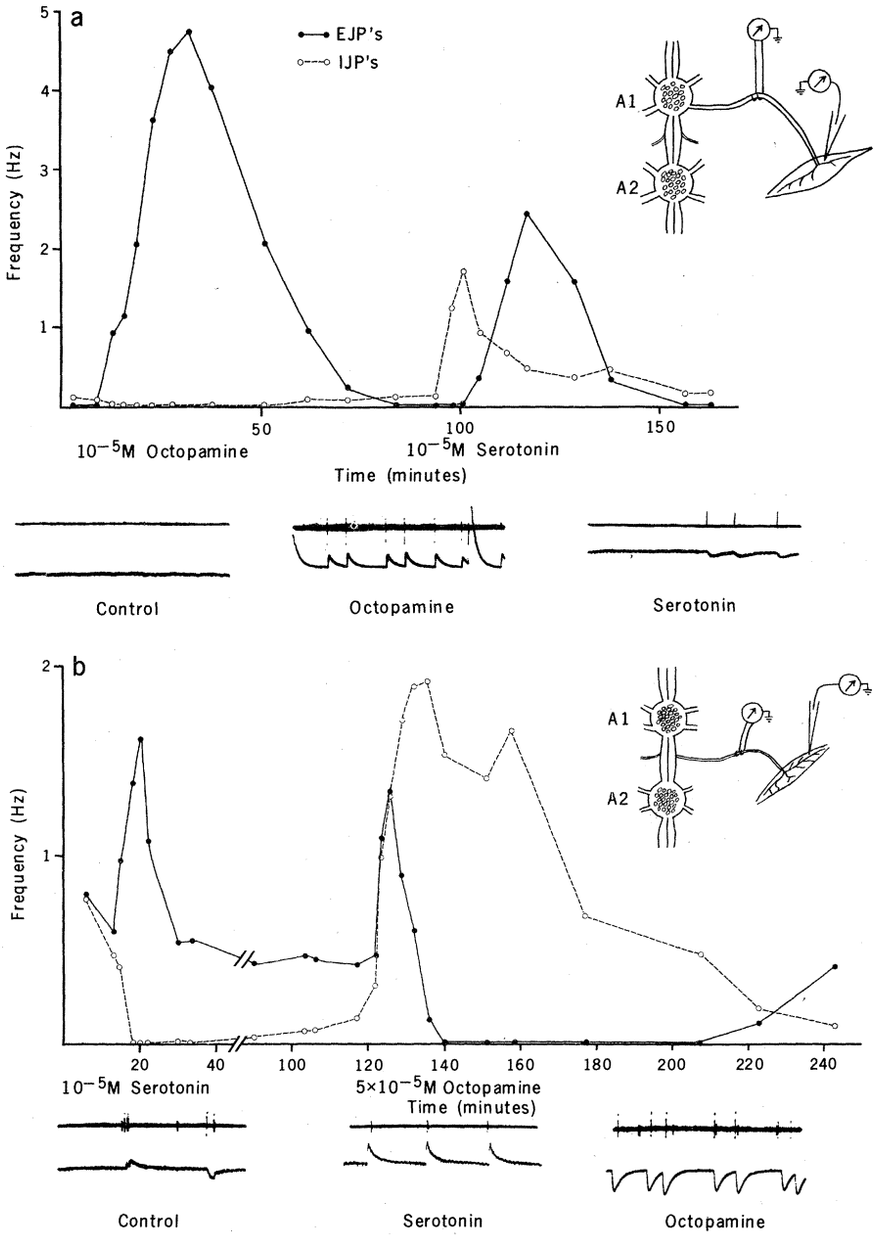

Fig. 2. (a) Effects of octopamine and serotonin on excitatory and inhibitory input to slow abdominal extensor muscles. The abdominal ventral nerve cord was exposed and the second roots from the first and second abdominal ganglia were followed peripherally to the superficial (slow) extensor muscles on the dorsal side of the abdomen. The muscles and underlying exoskeleton were cut out and the entire preparation was mounted in a small petri dish. The first three ganglia were desheathed, and the preparation was perfused with cold oxygenated lobster saline (2 ml/min) at 10°C. Unit activity in the second root of the first abdominal ganglion, distal to its division to fast and slow muscles, was recorded extracellularly with a suction electrode (upper trace of records). Intracellular recordings of muscle responses to spontaneous unit activity were made with glass microelectrodes (3 to 10 megohms) filled with 2*M* potassium acetate (lower trace of records). At the times indicated, the preparation was perfused with $10^{-5}M$ DL-octopamine or $10^{-5}M$ serotonin. The frequency of excitatory junctional potentials (from all excitatory units) and inhibitory junctional potentials was measured from photographic recordings of oscilloscope traces. *EJP*, excitatory junctional potential; *IJP*, inhibitory junctional potential. (b) Effects of serotonin and octopamine on excitatory and inhibitory input to slow abdominal flexor muscles in a different preparation. The abdominal ventral nerve cord was exposed and the superficial divisions of the third roots of the first and second abdominal ganglia were followed to the respective superficial (slow) flexor muscles that they innervate. These muscles and the ribs they attach to were excised with the nerve cord and placed in petri dishes for recording as described for extensors.

Neither serotonin nor octopamine affected neurons innervating phasic flexor or extensor muscles. Octopamine (10^{-6} to $10^{-5}M$) activated one to four different units in the nerve trunk running to the tonic extensor muscles and often excited a single unit in the root innervating the tonic flexors. In addition, it enhanced rates of firing in one to three units innervating the swimmerets. Serotonin ($10^{-5}M$) had less dramatic and less reproducible effects. The clearest result observed was that, in the nerve trunk to the tonic flexors, units showing large action potentials increased their firing rates and tended to fire in bursts rather than singly.

In some experiments we made simultaneous recordings from nerve trunks with suction electrodes and from individual muscle fibers with intracellular electrodes. We identified individual units firing in the roots as excitatory or inhibitory by correlating the timing of their firing with excitatory or inhibitory junctional potentials recorded from the muscles. The results are summarized in Table 1, and some typical experiments are shown in Fig. 2. Octopamine (10^{-6} to $10^{-5}M$) had two dramatic, highly reproducible effects: it simultaneously increased the frequency of firing of the excitatory units to the extensor muscles (five- to tenfold) and of the inhibitory unit to the flexor muscles (three- to tenfold). Moreover, in three of six experiments, octopamine completely eliminated the firing of the inhibitory neuron to the extensors (Fig. 2); in other experiments, the endogenous firing frequency of this unit was very low and remained unchanged or increased slightly with oc-

topamine treatment. Octopamine had mixed effects on the excitatory input to the flexor muscles: it consistently decreased the activity of two units, characterized by small action potentials and high rates of continuous ~10-Hz activity, that produced small excitatory junctional potentials in the muscle; but it slightly increased the frequency of a unit, characterized by a large action potential, that fired infrequently and produced a large flexor excitatory junctional potential. This increase, however, was small (twofold at most) and was often transient (Fig. 2).

With superfusion of serotonin ($10^{-5}M$) onto the preparations, the most reproducible results were a decrease in inhibitory activity to the tonic flexor muscles and an increase in inhibitory activity to extensors (Table 1). In five of seven experiments, serotonin completely stopped the firing of the inhibitory units to flexors (Fig. 2). The effects of serotonin on excitatory units to flexors and extensors are less clear but support an overall activation of flexion. Usually serotonin had no effect or slightly decreased the frequency of the continuously firing small excitatory units to flexors, but it usually increased the frequency of large excitatory junctional potentials. Moreover, the pattern of activity of these units was altered so that the units fired in bursts of two to six spikes at frequencies of 50 to 100 Hz per burst. This resulted in a considerable facilitation of the excitatory junctional potentials or the production of muscle action potentials that in turn caused large contractions in the flexors. In three of six experiments, the excitatory input to extensors was unaffected by serotonin; however, in two experiments there occurred a complete short-term suppression of excitatory input accompanied by a large reciprocal increase in inhibitory activity (Fig. 2).

The opposite behavioral postures, observed after injection of octopamine and serotonin into lobsters, can therefore be explained by the actions of these amines on the central ganglia, although we have not completely ruled out the possibility that peripheral mechanisms play some role (*9*).

The postures and the selective activation and inhibition of specific sets of neurons after treatment with the two amines are strikingly similar to the postures and the patterns of neuronal firing seen upon stimulation of isolated command fibers that control abdominal position in Crustacea (*10*). For example, stimulation of a flexion-inducing command fiber increases the excitatory input to flexors and the inhibitory input to extensors while simultaneously decreasing the inhibitory input to flexors and the excitatory input to extensors (*11*). It has been suggested that command neurons function by activating "driver interneurons" that in turn activate the appropriate excitatory or inhibitory neurons to the flexor or extensor muscles (*12*). Serotonin and octopamine could selectively activate or modulate transmitter release from particular command or driver neurons and produce the observed effects. The amines could also serve directly as the neurotransmitters released by specific cells in such circuits. Some of the variability in our results may be due to the activation of several such systems.

It should be noted that the effects we observed require the use of high concentrations of amines, several orders of magnitude above the concentrations normally present throughout the hemolymph. Even if animals were to liberate their entire store of amines into the hemolymph, it is unlikely that such high levels of amines would result. Perhaps the postures we observed were only a gross manifestation of what is actually a very subtle control system. It may also be that the amines normally exert their actions on central neurons by their release from specific synaptic sites within the central ganglia, where their concentrations would be locally high, and not through the circulation. Whatever the correct explanation, it is clear that octopamine and serotonin can generate opposite postures in lobsters and thus are likely to be of general importance in lobster behavior.

Serotonergic modulation of complex movements has been seen in other invertebrate preparations (*13*). In vertebrates, parallel descending pathways of serotonergic and noradrenergic neurons from midbrain regions innervate virtually all areas of the spinal cord. In spinal rats, systemic injection of dopa produced an increased flexor reflex, whereas systemic injection of 5-hydroxytryptophan produced hyperextension (*14*). In addition, amines have been implicated physiologically and pharmacologically in the control of stereotyped motor behavior such as stepping (walking behavior in decorticated cats) (*15*). It would be intriguing if it were found that pairs of amines affect the activity of motoneurons to opposing sets of muscles in organisms from invertebrates to higher vertebrates.

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