In conclusion, a dispositional mechanism, possibly associated with changes in the permeability of the blood-brain barrier, may be responsible for the expression of morphine tolerance as represented by an alteration in the ED_{50} for subcutaneously given morphine. In addition, the observation of cross-tolerance to the highly lipophilic narcotic heroin after removal of a tolerance-inducing morphine pellet may be associated with the phenomenon of withdrawal tolerance. This tolerance, as characterized by an increase in the ED_{50} of systemically administered narcotics, should be distinguished from the central or neuronal tolerance that is demonstrated by normal sensitivity to a painful stimulus and normal ED_{50} for heroin and morphine administered intracerebroventricularly in the presence of high levels of morphine in the CNS.

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Formamidine Pesticides: Octopamine-Like Actions in a Firefly

Abstract. *The formamidine pesticide chlordimeform and its* N*-demethylated metabolites cause the light organ of the firefly* Photinus pyralis *L. to glow brightly. Monodemethyl chlordimeform is active at doses as low as 5 nanograms per insect when applied topically. This action is postsynaptic and probably involves membrane-bound receptors since cyproheptadine blocks the glows induced by both monodemethyl chlordimeform and octopamine, the putative neurotransmitter in the light organ. The pesticidal and pestistatic properties of the formamidines may result from actions on octopaminergic systems.*

The formamidines are a relatively new class of pest control agents effective against lepidoptera, certain other insects, and mites and ticks (*1*). Their effectiveness in plant and animal protection results, at least in part, from the induction of abnormal behavior in the pest rather than by direct lethality. Reduced feeding, dispersal from plants, erratic mating behavior, and detachment of ticks from their host are typical of these behavioral effects (*1-3*). Such actions have been termed pestistatic (*2*) rather than pesticidal. Several biochemical actions, including mitochondrial uncoupling, inhibition of monoamine oxidase, blockage of cholinergic neuromuscular transmission, and local anesthetic effects have been proposed to explain various facets of the toxicology of these agents (*2-5*). However, none of these can plausibly explain their striking effects on invertebrate behavior (*2-4*). We have found that the formamidine chlordimeform and some of its metabolites are potent effectors of the lighting response of the photocytes of the firefly *Photinus pyralis* L. This response is believed to be controlled by octopaminergic neurons (*6, 7*). In combination with other evidence, this result raises the possibility that interactions with octopaminergic

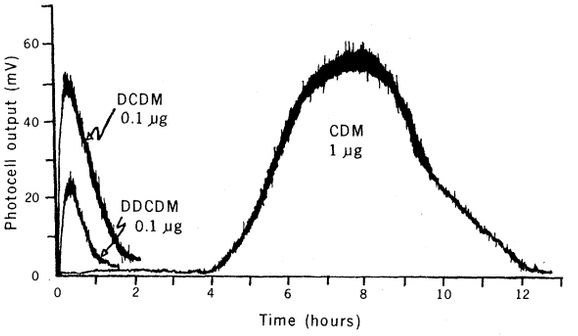

Fig. 1. Light output from adult fireflies treated topically with chlordimeform (*CDM*) or with two sequential *N*-demethylation products (*DCDM* and *DDCDM*). Acetone-treated controls gave no response.

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systems may be responsible for some or all of the pestistatic and pesticidal actions of the formamidines. Such a conclusion would be significant since no pesticide has yet been shown to have a major biological action as a result of interference with octopaminergic or other monaminergic systems.

Adult male fireflies were collected locally and kept in glass jars containing grass clippings and a cotton wick soaked in sucrose solution. Chemicals were administered either in 1 μl of acetone, topically to the ventral abdomen, or in 1 or 2 μl of insect saline by injection into the abdominal hemocoel (*8*). Lighting responses were assessed either visually or with a selenium photocell connected directly to a potentiometric recorder. The visual rating scale was: 0, lanterns uniformly dark; 1, dim uniform glow, or small, circumscribed areas with glows of intermediate brightness; 2, moderate uniform brightness, or numerous areas with moderate to bright glows; 3, uniform bright glowing of both light organs. The photocell was used with both in vivo and in vitro firefly preparations. The in vivo method consisted of taping a firefly to the inside of a small plastic lid that was then positioned so that the lantern almost touched the photocell. Measurements in vitro were made similarly with isolated light organs (*9*) in a small petri dish containing the test solution and placed on top of the photocell. Chlordimeform [*N'*-(4-chloro-*o*-tolyl)-*N*,*N*-dimethylformamidine; CDM], its *N*-monodemethyl and *N*,*N*-didemethyl analogs (DCDM and DDCDM, respectively), and the non-formamidine metabolites *N*-formyl-4-chloro-*o*-toluidine (NFT) and *N*-(4-chloro-*o*-tolyl) urea (CTU) were prepared and characterized by standard methods. In every case the chemical purity was at least 95 percent.

When CDM was applied topically (1 μg per insect, ~ 20 mg/kg), a weak general glowing was seen after a few minutes; after a lag of 3 to 5 hours, the glow increased to a peak intensity and then declined (Fig. 1). DCDM also caused continuous intense glowing but without the lag phase and with greater potency than CDM (Fig. 1). As little as 5 ng per insect caused a faint but detectable glow. DDCDM also acted rapidly but with a lesser potency than DCDM. By contrast, neither of the related non-formamidines, NFT or CTU, caused glowing at 10 μg per insect. These compounds also lack typical formamidine-like pestistatic actions in insects and acarines.

The lantern of the adult male *Photinus* consists of paired light organs located ventrally in abdominal segments 6 and 7 (*10*). Flashing is believed to be controlled by octopaminergic neurons (*6*) that act through an octopamine-stimulated adenylate cyclase–cyclic AMP system in the photocytes (*7*). Octopamine is *p*-hydroxy-β-phenylethanolamine.

Table 1. Inhibition of the DCDM-induced glow of the firefly lantern by pretreatment with cyproheptadine and the restoration of the glow by subsequent injection of theophylline. Ratings were obtained visually according to the scale from 0 to 3 as described in the text. The data are means ± standard deviation; the number of observations are shown in parentheses.

Prior treatment	Ratings	
	After DCDM	After theophylline
None (control)	2.86 ± 0.17 (11)	2.76 ± 0.40 (11)
Cyproheptadine	1.84 ± 0.54 (12)*	2.70 ± 0.46 (12)†

*Differs from the control rating at $P = .005$. †Differs from "DCDM + cyproheptadine" rating at $P = .01$.

Table 2. Inhibition of the ability of octopamine (1 m*M*) and DCDM (0.3 m*M*) to induce a glow in isolated light organs from fireflies treated with cyproheptadine. The data are means ± standard deviations, with the number of observations in parentheses, and represent the maximum peak height in centimeters observed on the photocell recorder.

Prior treatment	Octopamine	DCDM
None (control)	7.42 ± 4.17 (6)	4.00 ± 1.66 (6)
Cyproheptadine	0.26 ± 0.21 (6)*	1.75 ± 1.49 (6)†

*Differs from appropriate control at $P = .005$. †Differs from appropriate control at $P = .05$.

Initially we were concerned whether the stimulatory action of the formamidines was pre- or postsynaptic at the light organ. DCDM was used in these studies because of its potency and speed of action. For the following reasons we believe that the action is postsynaptic. The posterior light organ can be selectively denervated by transecting the nerves passing to it from the terminal abdominal ganglion (*10*, *11*). Two days later the still-innervated anterior light organ glows after injection of *d*-amphetamine, but the posterior organ does not. *d*-Amphetamine is thought to act presynaptically by releasing endogenous transmitter; that it fails to act on the posterior organ is presumably due to the degeneration of the nerve terminals after denervation (*10*, *11*). Unlike amphetamine, but like the known agonist, norepinephrine (*9*–*11*), topically applied DCDM causes bright glowing of both organs in denervated animals. Additional experiments with *d*-amphetamine alone support the conclusion that DCDM acts postsynaptically. When normal fireflies are given a large dose of *d*-amphetamine, a bright glow ensues as the endogenous transmitter is released, and the glow subsequently declines and disappears. A second dose of amphetamine then has no effect (Fig. 2). Subsequent treatment with DCDM (Fig. 2) gives a strong lighting response in these insects depleted of endogenous octopamine. Finally, we observed that fireflies pretreated with reserpine (1 μg injected per insect) did not respond to *d*-amphetamine 48 hours later, but gave bright glows when treated with DCDM topically or norepinephrine (3 to 4 μg injected per insect). Reserpine also appears to act presynaptically by depleting octopamine from the light organs of fireflies (*9*, *11*).

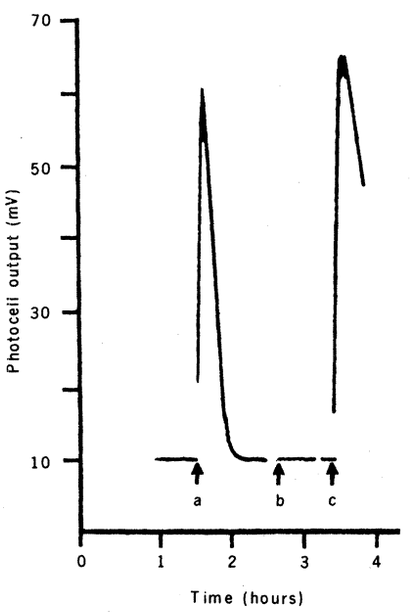

Fig. 2. Light output from a single adult firefly treated successively with two injections of 18.5 μg of *d*-amphetamine (*a* and *b*) followed by 0.1 μg of DCDM in acetone, applied topically at *c*.

Since DCDM acts postsynaptically, we sought to determine whether its stim-

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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