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INACTIVATION OF OCTOPAMINE IN LARVAL FIREFLY LIGHT ORGANS BY A HIGH-AFFINITY UPTAKE MECHANISM

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SUMMARY

1. Uptake studies using radioactive octopamine have revealed that the larval lantern of the *Photuris* firefly possesses a concentrative, high-affinity uptake system for octopamine. This compound has been shown previously to be the neurotransmitter of the photomotor neurones.

2. Imipramine is a potent inhibitor of the uptake of octopamine in the lantern. At 10^{-5} mol l^{-1}, it reduces specific uptake of radioactive octopamine with a K_i of $5{\cdot}8\times10^{-6}$ mol l^{-1}. Incubation of lanterns in $2{\cdot}5\times10^{-4}$ mol l^{-1} imipramine induces an increase in sensitivity of the luminescent response to endogenously released and exogenously applied octopamine. Release of endogenous octopamine is potentiated in imipramine-treated lanterns, which also show a significant reduction of octopamine content with this treatment.

3. Uptake of octopamine does not appear to be affected by denervation of the lanterns, suggesting that nerve terminals are not the principal sites of octopamine uptake.

INTRODUCTION

Considerable evidence suggests that octopamine is the neurotransmitter responsible for the induction of luminescence in the firefly lantern. It has been detected in adult lanterns (Robertson & Carlson, 1976; Copeland & Robertson, 1982) and in larval lanterns, lantern ganglia and photomotor neurone somata (Christensen, Sherman, McCaman & Carlson, 1983). It also mimics the pharmacological actions of the endogenous transmitter (Carlson, 1968*b*, 1969). The lantern contains an octopamine-specific adenylate cyclase which stimulates the production of cyclic

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AMP, believed to play an intermediate role in light production (Nathanson, 1979; Nathanson & Hunnicutt, 1979). The photomotor neurones closely resemble DUM neurones in other insect species (Christensen & Carlson, 1982) and, in the locust, one of the thoracic DUM neurones (DUMETi) has been shown to be octopaminergic (Evans & O'Shea, 1978). In addition, the larval photomotor neurones have been found to be the major, if not the exclusive source, of octopamine in the lantern (Carlson & Jalenak, 1986). Further, larval lanterns incubated in saline containing high potassium and normal calcium concentrations release octopamine, but they release significantly less when the calcium is replaced by magnesium (Carlson & Jalenak, 1986).

Neurotransmitters must be rapidly inactivated. In insects such inactivation has been found to involve either active uptake processes or enzymatic degradation (see Evans, 1980). In this paper, we have investigated the active uptake of octopamine in the lantern of the larval firefly, using the tricyclic antidepressant imipramine which is known to inhibit the uptake of amines in the nervous system (Iversen, 1967; Evans, 1978). We have studied the effects of imipramine upon [^{3}H]octopamine uptake and upon luminescence responses of the lantern. The larva, rather than the adult, was chosen because the nerves terminate directly upon the lantern (Peterson, 1970), making the analysis of synaptic transmission much easier. Furthermore, when the larval lanterns are immersed in aerated saline, electrical stimulation of the nerve has been shown to produce a luminescence that is directly proportional to the stimulation (Christensen & Carlson, 1982).

MATERIALS AND METHODS

Experimental animals

Larval *Photuris* fireflies were collected on Long Island, New York, in Autumn 1984, and in Princeton, New Jersey, in Spring 1985. They were maintained at 10°C on moistened towelling, in which state they required no feeding.

Chemicals

Imipramine HCl was a gift of Ciba Geigy Ltd. All other drugs were supplied by Sigma Chemical Co.

Preparation of lanterns

The paired larval lanterns occupy the lateral sternite of the 8th abdominal segment and are innervated by lantern nerves from the last (8th) abdominal ganglion within the 6th abdominal segment (Christensen *et al.* 1983). For physiological studies, the lanterns and their associated nerves were dissected free, still attached to the ventral cuticle which overlies the photogenic tissue. Each lantern was positioned ventral side down on a Sylgard-filled dish, surrounded by a Plexiglas ring of 1 cm diameter and immersed in firefly saline (Carlson, 1968*a*). Air was bubbled into the saline through a tube in the wall of the ring. Solutions, contained in syringes, were introduced to the

lantern through a manifold that fed a single, short tube to the bottom of the chamber. Excess solution was removed by continuous aspiration. The lantern nerve was stimulated *via* a suction electrode connected to a Grass SD9 stimulator triggered in turn by a Grass S44 stimulator. Light was monitored by a photomultiplier facing the ventral side of the lantern (the photogenic layer) and its output, along with the stimulator output, was recorded on a Grass four-channel polygraph. Output from the photomultiplier showed a linear relationship with light intensity.

To observe a relatively rapid physiological effect of imipramine on the neurally-induced luminescence of intact larval lanterns still attached to the ventral cuticle, a concentration of $2{\cdot}5\times10^{-4}$ mol l^{-1} imipramine was used. At this concentration of imipramine profound increases in luminescence intensity and extinction times could be seen within 3 h. The luminescence induced by octopamine in lanterns dissected free of their cuticle could be enhanced by application of imipramine at concentrations as low as 10^{-5} mol l^{-1}, presumably because access of imipramine to the photogenic tissue was greatly increased.

Denervated lanterns were produced by transecting the lantern nerves at the level of the intersegmental membrane between the 6th and 7th abdominal segments. Following the transection, the nerves were assumed to be severed if the animal could not be induced to luminesce by manipulation. Each operated animal was maintained on moistened paper for 3 days at 20°C and tested each day to ensure that luminescence could not be induced. The lanterns were subsequently removed and tested in high K^+ saline (Carlson & Jalenak, 1986). If no luminescence was observed in this saline it was assumed that the nerves had degenerated.

Radioenzymatic assay of octopamine

Octopamine was assayed using a modification of the procedure developed by Molinoff, Landsberg & Axelrod (1969). The assay conformed to that used by Christensen *et al.* (1983) and Carlson & Jalenak (1986). The method includes the use of internal standards of octopamine added to each sample to control for any effects of the presence of imipramine in the tissue samples.

Uptake of [^{3}H]octopamine

The uptake of [^{3}H]octopamine was investigated as described by Evans (1978) and Morton & Evans (1984).

Larval lanterns were dissected free of cuticle and immersed in firefly saline. Pairs of lanterns were incubated for various lengths of time at room temperature in saline containing DL-[3,5-^{3}H]octopamine (Amersham, 22 Ci mmol^{-1}, 10 μCi ml^{-1}, approximately $0{\cdot}45\times10^{-6}$ mol l^{-1}) to examine the time course of octopamine uptake. In all subsequent experiments on the effects of different octopamine concentrations and the effect of imipramine, 10-min incubation periods were used routinely and each value presented is the mean uptake measured for three sets of two lanterns each. At the end of the appropriate incubation time, lanterns were washed for 1 min in two consecutive 10-ml volumes of ice-cold saline to remove surface radioactivity. The

lanterns were immediately homogenized in 0·5 ml of ice-cold concentrated hydrochloric acid/absolute ethanol mixed 1:60 (v/v). The homogenate was transferred to a 1·5-ml plastic tube and combined with two further 0·25-ml washings of the homogenizer. The proteins precipitated were removed by centrifugation at 12000 ***g*** for 5 min at room temperature. The precipitates were dissolved in 1 mol l^{-1} sodium hydroxide and assayed for protein according to the method of Lowry, Rosenberg, Farr & Randall (1951) using bovine serum albumin as standard. The supernatants were transferred to scintillation vials and 7·5 ml of Biofluor (New England Nuclear) was added to each. Radioactivity was estimated in a Packard Tri-Carb liquid scintillation spectrometer at a counting efficiency of 35 %.

To establish that the radioactivity extracted was truly associated with octopamine, the supernatant of the centrifuged homogenate was co-chromatographed with authentic octopamine using thin layer chromatography (TLC) on silica gel plates in butanol:acetic acid:water (12:3:5 v/v). Over 90 % of the radioactivity in the lantern extracts co-migrated with authentic octopamine.

The results are expressed as picomoles of octopamine taken up per minute per lantern. The uptake relationships for octopamine were qualitatively similar when expressed as picomoles of octopamine taken up per minute per microgram protein but showed an increased variability. The latter may be due to errors introduced in the measurement of the very small quantities of protein in each lantern (approx. 2–6 μg per lantern) and/or the relatively small amount of protein in the lantern actually involved in octopamine uptake.

RESULTS

Luminescence induced by electrical stimulation and exogenous octopamine

Luminescence in the larval lantern can be induced by direct stimulation of the lantern nerve (Fig. 1A) (Carlson, 1968*a*), application of exogenous octopamine (Fig. 1B) or its analogues (Carlson, 1969) or immersion of the lantern in high K^+ saline (Carlson, 1968*a*). A 0·5-s stimulation of the lantern nerve at 10 Hz causes a rapid rise and fall of luminescence (Fig. 1A). One possible explanation for this may be that the transmitter released by electrical stimulation is rapidly inactivated, resulting in extinction of luminescence.

In comparison, the luminescence response to exogenously applied $2{\cdot}5\times10^{-5}$ mol l^{-1} octopamine follows a much longer time course (Fig. 1B). Not only is the rising phase of luminescence slower, but extinction of luminescence is a much lengthier process. Actually, the luminescence time courses in these two experimental situations are not true reflections of the inactivation of octopamine since the time course of light extinction may reflect processes subsequent to octopamine action, such as production of cyclic AMP and active complex (luciferin-luciferase-AMP). Still, assuming that octopamine is the endogenous transmitter, the results do illustrate differences in the time course of the effectiveness of octopamine under the two different conditions of application to the lantern.

Uptake of [^{3}H]octopamine by the larval lantern

The time course of uptake of a fixed concentration ($4{\cdot}5\times10^{-7}$ mol l^{-1}) of DL-octopamine into the larval lantern is shown in Fig. 2. The uptake of octopamine was linear for 20 min at this concentration. The tissue to medium concentration ratio for radioactivity exceeded unity after 5 min. The maximal ratio achieved was 5 after 60 min, at which time the uptake exhibited saturation kinetics. This suggests that the larval lantern has an active uptake mechanism for octopamine.

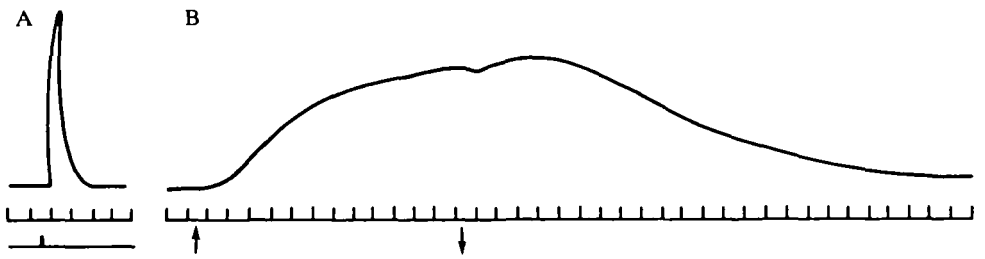

Fig. 1. Induction of luminescence in the larval firefly lantern by endogenously released and exogenously applied octopamine. (A) Light response induced by electrical stimulation of lantern nerve. Top trace: light, 0·2 mV cm^{-1}. Middle trace: time, 1 mark per 5 s. Bottom trace: stimulus, 1 V, 10 Hz frequency, 50 ms pulse duration, 500 ms train duration. (B) Light response to 4×10^{-5} mol l^{-1} octopamine. Upper trace: light, 0·05 mV cm^{-1}. Lower trace: time, 1 mark per 5 s; upward arrow, octopamine introduced; downward arrow, octopamine replaced by saline.

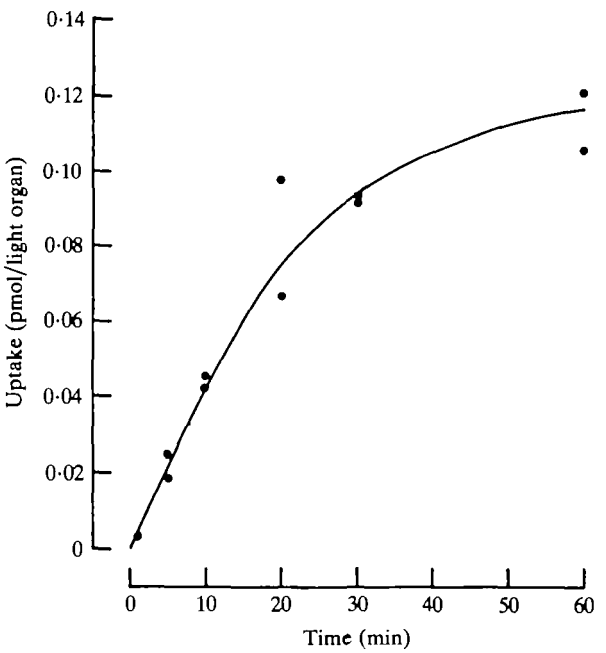

Fig. 2. Time course of uptake of octopamine by larval firefly lanterns from saline containing $4{\cdot}5\times10^{-5}$ mol l^{-1} octopamine. The results from duplicate experiments are shown.

To characterize this uptake process further we examined its concentration dependence. In this and all subsequent experiments 10-min incubation periods were used. Fig. 3A shows that the total uptake of DL-octopamine against concentration approximated to a hyperbolic curve. The nonspecific uptake of DL-octopamine was estimated as the uptake by the lanterns from ice-cold [^{3}H]octopamine-containing solutions and was linear over the concentration range examined ($1–250\times10^{-7}$ mol l^{-1}) (Fig. 3A). The specific uptake of DL-octopamine by the lanterns was estimated by subtracting the nonspecific uptake curve from the total uptake curve. Fig. 3B reveals that the specific uptake process exhibited the saturation kinetics typical of a carrier-mediated process. This was further revealed by the linear nature of a plot of V against V/S for the specific uptake (Fig. 4). The specific uptake system had a K_m of $8{\cdot}3\times10^{-6}$ mol l^{-1} and a V_m of 0·089 pmol min^{-1} light $organ^{-1}$.

The uptake of octopamine by the firefly light organ was sensitive to the presence of the tricyclic antidepressant imipramine, as has been shown for octopamine uptake in other preparations (Evans, 1978, 1980). Fig. 5A shows that lanterns pre-incubated for 30 min in the presence of 10^{-5} mol l^{-1} imipramine before a 10-min incubation in the presence of radiolabelled octopamine plus 10^{-5} mol l^{-1} imipramine showed a reduced total uptake of octopamine. Nonspecific uptake under these conditions, however, was relatively unaffected (cf. Figs 3A and 5A). Specific uptake of octopamine on the other hand was selectively depressed (cf. Figs 3B and 5B). The inhibition of specific uptake of octopamine by 10^{-5} mol l^{-1} imipramine was much more effective at lower octopamine concentrations, being in the range of 50% at concentrations below 5×10^{-6} mol l^{-1}. Plots of V against V/S for octopamine uptake in the presence of 10^{-5} mol l^{-1} imipramine (Fig. 4) show that the inhibition of the specific uptake is competitive since the uptake curves have the same V_m in the presence and absence of imipramine. The K_i for imipramine inhibition of octopamine uptake was calculated from the equation $K_a = K_m(1+[i]/K_i)$ and was $5{\cdot}8\times10^{-6}$ mol l^{-1}. Lanterns treated with a higher concentration of imipramine ($2{\cdot}5\times10^{-4}$ mol l^{-1}) showed an almost total reduction in the specific uptake of octopamine but also showed a reduction in the nonspecific uptake. Thus imipramine at low concentrations can specifically inhibit a high-affinity uptake mechanism for octopamine in firefly light organs.

Physiological effects of imipramine on octopamine action

The physiological effects of inhibiting the active uptake of octopamine were examined in experiments where light organ luminescence was induced in the presence of imipramine. Larval lanterns were immersed in $2{\cdot}5\times10^{-4}$ mol l^{-1} imipramine-containing saline and luminescence was induced by stimulating the lantern nerve at regular intervals (500 ms stimulation at 10 Hz every 2 min). Under these conditions progressive increases in the intensity of luminescence and in the extinction time of the luminescence were observed (Fig. 6). The intensity of neurally-evoked luminescence reached a maximum at about 110 min and then declined (Fig. 7). Extinction of luminescence became more prolonged and eventually the lantern no longer responded to neural stimulation (Figs 6, 7).

Lanterns immersed in $2{\cdot}5\times10^{-4}$ mol l^{-1} imipramine were also tested with pulses of exogenous DL-octopamine. As blockage of octopamine uptake proceeded, the sensitivity of the lantern to applied octopamine progressively increased. Untreated lanterns showed just distinguishable luminescence responses to 10^{-5} mol l^{-1}

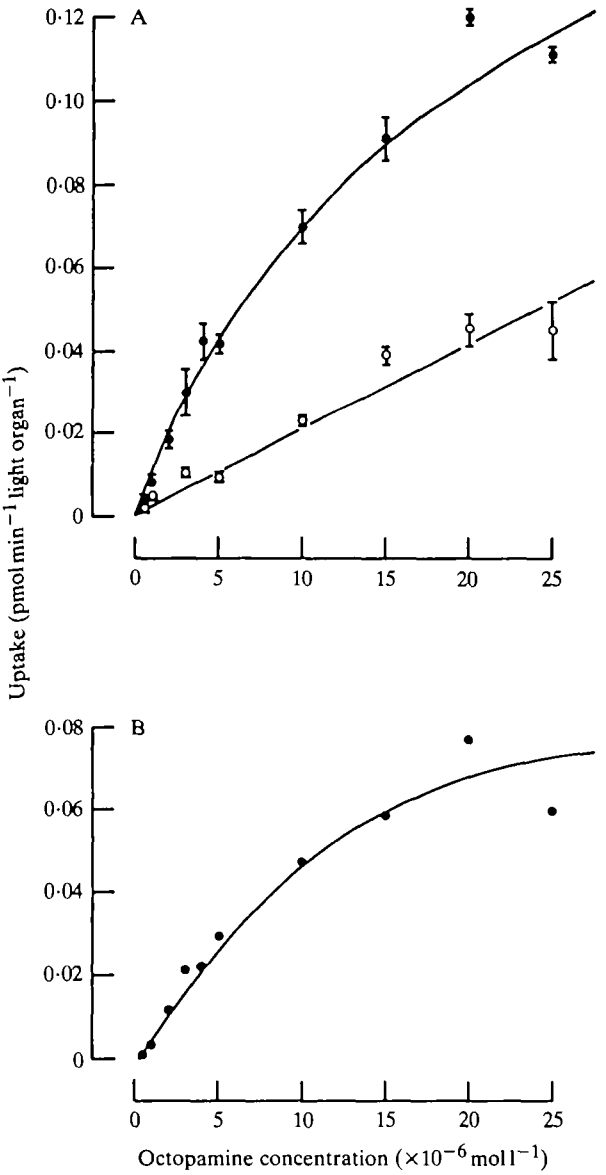

Fig. 3. (A) The rate of uptake of octopamine into firefly light organs is plotted against the concentration of DL-octopamine in the bathing medium. The filled circles (●) represent total uptake and open circles (○) represent nonspecific uptake from ice-cold bathing medium. The bars represent 2×S.E. and $N = 3$. (B). The specific uptake of octopamine into firefly light organs is plotted against the concentration of DL-octopamine in the bathing medium. The plot is obtained by subtraction of the nonspecific uptake from the total uptake at each concentration shown in Fig. 3A.

octopamine. When immersed in imipramine they became sensitive to 10^{-7} mol l^{-1} octopamine, eventually producing very bright luminescent responses to that concentration. Within 2 h after immersion in $2{\cdot}5\times10^{-4}$ mol l^{-1} imipramine the response threshold had dropped by a factor of 250 (249 ± 75 S.E., $N = 10$) to nearly 10^{-8} mol l^{-1} octopamine. The ratio by which the lantern's sensitivity to octopamine changed when treated in imipramine was calculated as in the following example. A lantern in saline gave a response of 1·0 mV luminescence intensity (arbitrary scale) to 10^{-5} mol l^{-1} octopamine. After treatment in $2{\cdot}5\times10^{-4}$ mol l^{-1} imipramine for 2 h it responded with a luminescent response of 2·5 mV to 10^{-7} mol l^{-1} octopamine. The sensitivity of the lantern to octopamine had therefore increased 250 times.

Denervation of the lanterns produced no increase in sensitivity to octopamine. Upon immersion in $2{\cdot}5\times10^{-4}$ mol l^{-1} imipramine, there was an increase in sensitivity to octopamine (270 ± 48 S.E., $N = 4$) similar to that with the nerves present.

Release of octopamine from imipramine-treated lanterns

If imipramine prevents the uptake of released transmitter, it might be expected that more octopamine would be recovered from media containing imipramine-

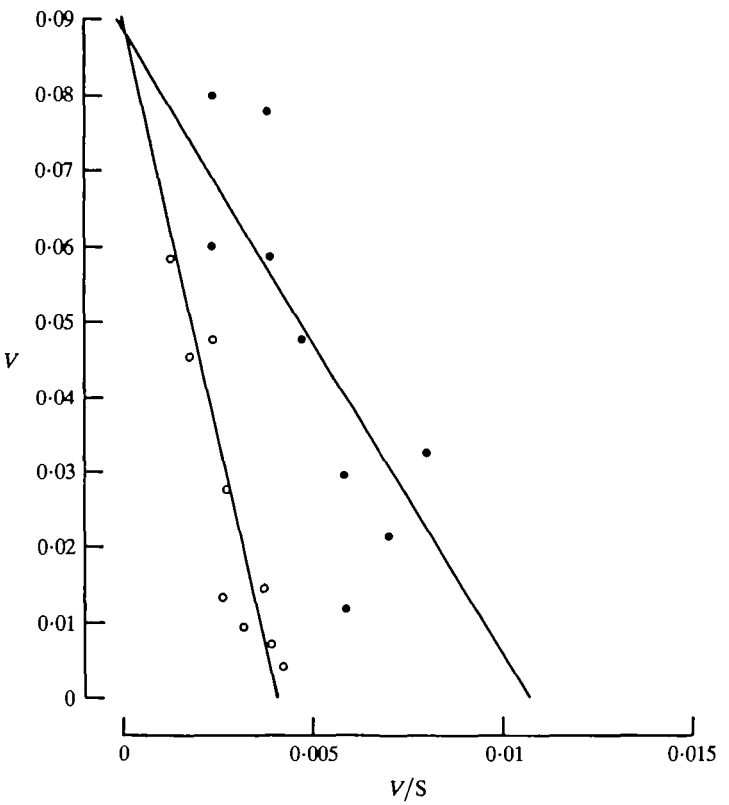

Fig. 4. Plots of V against V/S for the specific uptake component of octopamine into firefly light organs in the absence (●) and presence (○) of 10^{-5} mol l^{-1} imipramine. V is the rate of octopamine uptake (pmol min^{-1} $lantern^{-1}$) and S the concentration of octopamine (μmol l^{-1}) in the bathing medium. Lines are drawn by linear regression analysis.

treated lanterns because octopamine that was released spontaneously or in response to high K^+ saline would escape from the tissue. Although imipramine-treated lanterns spontaneously released more octopamine, the difference in release between treated and untreated lanterns was not significant (Table 1).

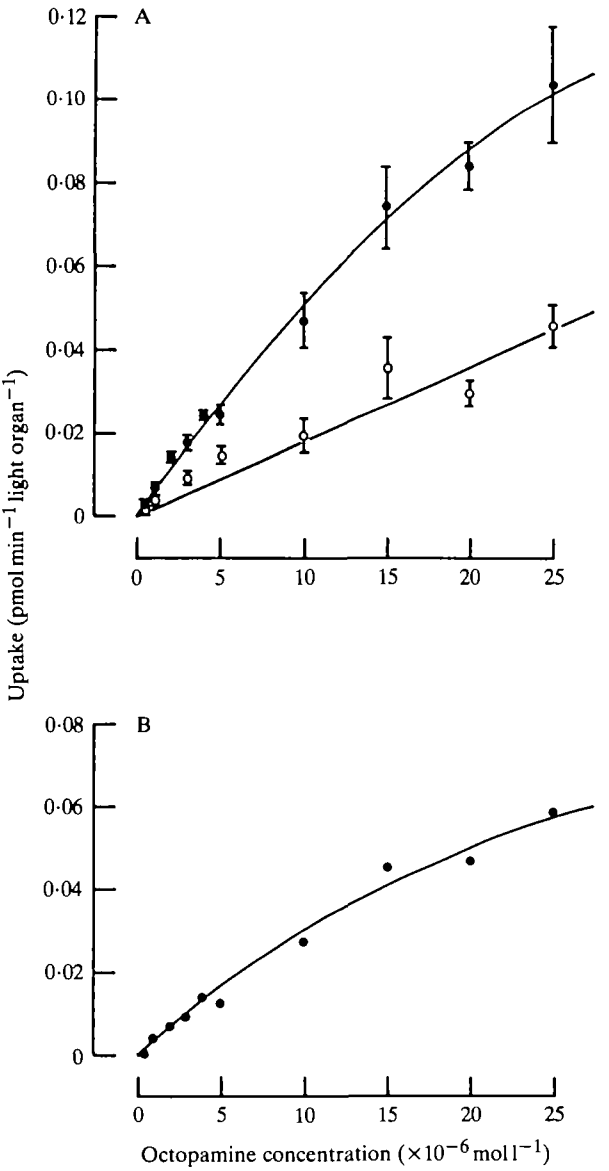

Fig. 5. (A) The rate of total uptake (●) and nonspecific uptake (○) of octopamine into firefly light organs in the presence of 10^{-5} mol l^{-1} imipramine is plotted against the concentration of octopamine in the bathing medium. The bars represent 2×S.E. and $N = 3$. (B) The specific uptake of octopamine into firefly light organs in the presence of 10^{-5} mol l^{-1} imipramine is plotted against the concentration of DL-octopamine. The plot is obtained by subtraction of the nonspecific uptake from the total uptake at each concentration shown in A.

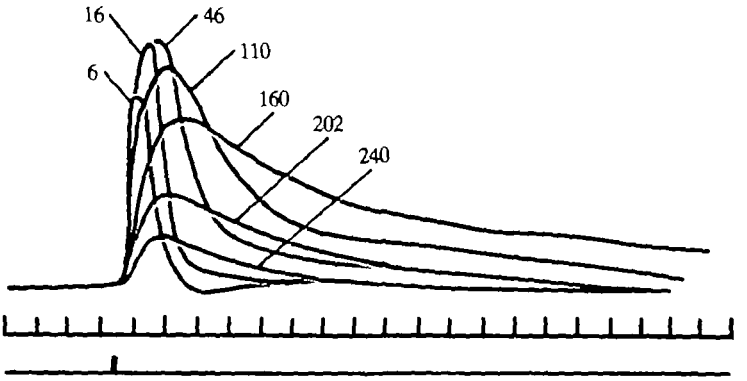

Fig. 6. Tracings of superimposed records of luminescence induced by electrical stimulation of the nerve of a lantern immersed in $2{\cdot}5\times10^{-4}$ mol l^{-1} imipramine saline. Top trace: light, 0·2 mV cm^{-1}; numbers indicate elapsed time (in min) in imipramine. Middle trace: time, 1 mark per 5 s. Bottom trace: stimulus as in Fig. 1.

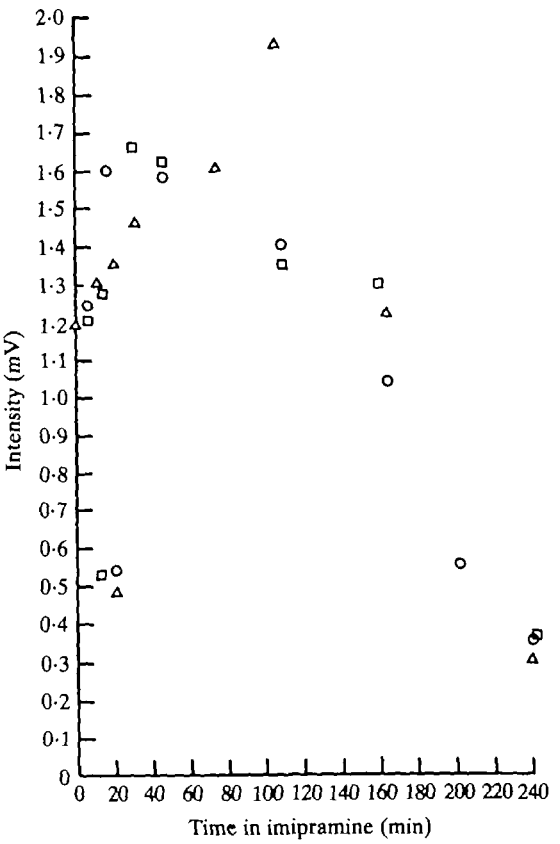

Fig. 7. Time course of the effect of $2{\cdot}5\times10^{-4}$ mol l^{-1} imipramine on the maximum intensity of luminescence induced by electrical stimulation of the nerves of three lanterns. Two lanterns were stimulated ever 2 min, one stimulated every 10 min. Stimulus parameters as described in Fig. 1.

Table 1. *Release of octopamine from larval lanterns* in normal and imipramine† salines measured by radioenzymatic assay‡*

	Saline	High K^+ saline§	Total octopamine remaining per lantern	Fractional release of octopamine in high K^+ saline
Normal saline	0·014 ± 0·004	0·138 ± 0·051	1·40 ± 0·164	0·103 ± 0·050
Imipramine saline	0·049 ± 0·016	0·222 ± 0·015	0·558 ± 0·071	0·290 ± 0·030
Significance¶	$P < 0·1$	$P < 0·1$	$P < 0·01$	$P < 0·01$

* $N = 4$, total number of lanterns = 23 for both normal saline and imipramine saline assays.
† Imipramine concentration = $2·5 \times 10^{-4}$ mol l^{-1}.
‡ Values given as pmol/lantern ± S.E.
§ Assayed for octopamine from high K^+ saline and two subsequent saline rinses.
¶ Significance measured using one-tailed Mann-Whitney U-test.

When lanterns were immersed for 30 min in high K^+ saline it was previously found that large amounts of octopamine appeared in the high K^+ saline or in the subsequent 30-min saline rinses. This suggested that octopamine sometimes diffused out of the tissue with difficulty (Carlson & Jalenak, 1986). Therefore, the release of octopamine induced by high K^+ saline was measured by combining the media from lanterns immersed in high K^+ saline with media from the two subsequent saline rinses to ensure that all released octopamine was recovered. More octopamine was recovered from the combined salines in which imipramine-treated lanterns were incubated than from salines containing untreated controls (Table 1). Again this difference was not significant. Actually, more octopamine was recovered from untreated lanterns when the high K^+ saline alone was assayed (imipramine-treated, 0·031 ± 0·009; untreated, 0·068 ± 0·024).

It appeared that imipramine prevented the uptake of released transmitter, since lanterns incubated in the presence of $2·5 \times 10^{-4}$ mol l^{-1} imipramine for 120 min had significantly lower octopamine levels than controls (Table 1). If the lower levels of octopamine in imipramine-treated lanterns was taken into account, the fractional release of octopamine by high K^+ saline and two subsequent saline rinses was significantly higher from imipramine-treated lanterns (Table 1).

The total amount of octopamine present in the lantern can be accounted for by adding up the octopamine recovered from the various media and from the lanterns themselves. Significantly less total octopamine (pmol/lantern) was recovered from imipramine-treated lanterns than from untreated controls (imipramine-treated, 0·828 ± 0·089; untreated, 1·557 ± 0·134; $P < 0·01$, two-tailed t-test).

DISCUSSION

Inactivation of octopamine in insects

The rapid extinction of luminescence in the larval light organ induced by stimulation of the lantern nerve (Fig. 1) suggests that the transmitter, most likely octopamine, is rapidly inactivated. The two principal modes of biogenic amine inactivation are enzymatic degradation and uptake of the released transmitter.

Both monoamine oxidase (MAO) and *N*-acetyltransferase have been implicated in enzymatic inactivation of biogenic amines in vertebrates (see Iversen, 1967). On the basis of biochemical assays, MAO is not found in high concentrations in insect neurones (Evans, 1980). Histochemical data, however, indicate that MAO activity appears to be concentrated in specific regions of the insect brain where it could play a role in metabolism of aminergic neurotransmitters after cellular re-uptake (Evans, 1980). There is no evidence for MAO activity in firefly lanterns: neither transmitter nor synephrine showed enhanced activity in the presence of the MAO inhibitors iproniazid, harmine or α-methyl-*p*-tyrosine (Carlson, 1972).

N-Acetyltransferase appears to play a role in the metabolism of biogenic amines in insects (Evans, 1980). It may play an important role in the inactivation of circulating octopamine released as a neurohormone. In the muscle of locusts and moths, however, it shows a low activity level which is surprising in view of the neuromodulatory role of octopamine at the locust neuromuscular junction (Evans & O'Shea, 1977; O'Shea & Evans, 1979). In the larval firefly lantern it would appear that, because of its intracellular location, *N*-acetyltransferase does not play a significant role in the initial inactivation of released octopamine.

The inactivation of octopamine by active uptake has been shown in a number of insects. In the cockroach abdominal and thoracic nerve cords, Evans (1978) has found both high- and low-affinity, sodium-sensitive uptake systems. Uptake of octopamine into locust brain has also been demonstrated (Aranyi, Hiripi & Sfoza, 1981). A removal system for octopamine has been described in locust haemolymph which may involve uptake and subsequent oxidation by MAO in the Malpighian tubules (Goosey & Candy, 1982). Uptake, therefore, appears to play an important role in octopamine inactivation in insects.

Uptake of radioactive octopamine by the larval lantern

The specific uptake of [3,5-^{3}H]octopamine by larval firefly lanterns is an active process (Fig. 2) which shows typical saturation kinetics (Fig. 3). Within 60 min after incubation in $4{\cdot}5\times10^{-7}$ mol l^{-1} octopamine the lantern was capable of concentrating that amine up to five times its concentration in the bathing medium (Fig. 2). As in the cockroach nerve cord (Evans, 1978), nonspecific uptake in the firefly lantern showed no saturation kinetics in the range of octopamine concentrations examined ($1–250\times10^{-7}$ mol l^{-1} octopamine). The stereospecificity of octopamine uptake into the firefly lantern was not studied since only racemic mixtures of [^{3}H]octopamine are available commercially. The naturally occurring isomer of octopamine in insects is the D(−) isomer (Goosey & Candy, 1980; Starratt & Bodnaryk, 1981). If the uptake of octopamine parallels the uptake of the naturally occurring L-noradrenaline into vertebrate nervous tissue it should show a marked preference for the D(−) isomer of octopamine (see Iversen, 1967).

The affinity of the uptake carriers for octopamine appears to fall between those shown by the two sodium-specific uptake systems of the cockroach nerve cord. The K_m values for the high- and low-affinity uptake systems in the cockroach were 5 and 198×10^{-7} mol l^{-1}, respectively (Evans, 1978). The firefly lantern appears to possess

a single specific uptake system with an intermediate K_m of $8{\cdot}3\times10^{-6}$ mol l^{-1} (Fig. 4). The saturation curve (Fig. 3B) does not show any obvious breaks that might indicate uptake systems with different affinities.

It is not possible accurately to compare the V_m obtained for the cockroach nerve cord (high-affinity system, $V_m = 185{\cdot}6$ pmol g^{-1} min^{-1}; low-affinity system, $V_m = 1028$ pmol g^{-1} min^{-1}) (Evans, 1978) with that found in the lantern, $V_m = 0{\cdot}089$ pmol lantern^{-1} min^{-1} (Fig. 4). Not only are the units different, but access of octopamine to the uptake sites is probably quite different as well. In the cockroach, uptake must occur initially at the cord surface, whereas in the lantern octopamine must penetrate into the photogenic tissue before uptake takes place.

Effect of imipramine on octopamine uptake in the lantern

The tricyclic antidepressant, imipramine, has been shown to be a potent blocker of octopamine uptake in the cockroach nerve cord (Evans, 1978). Evidence obtained from both physiological and biochemical experiments suggests that imipramine also blocks the uptake of octopamine in the larval lantern.

The physiological effect of imipramine blockage is revealed in three ways. (1) In imipramine-blocked lanterns the sensitivity to transmitter increases. This results in an increased intensity of luminescence responses to electrical stimulation of constant strength (Figs 6, 7). (2) As blockage develops, the extinction of electrically stimulated luminescence is progressively prolonged. (3) The lantern blocked by imipramine exhibits a progressive increase in sensitivity to exogenous octopamine in luminescence induction. This increased sensitivity reaches the level of 250-fold. Whereas the unblocked lantern responds with luminescence to octopamine concentrations of 10^{-5} mol l^{-1} and above, the imipramine-blocked lantern lumineses in response to octopamine at concentrations between 10^{-8} mol l^{-1} and 10^{-7} mol l^{-1}. All three of these physiological effects can be explained by the assumption that the action of the endogenous transmitter or exogenous octopamine on the photocytes is enhanced as the process of octopamine inactivation by uptake is reduced.

At the locust neuromuscular junction, the response to a submaximal dose of DL-octopamine (10^{-6} mol l^{-1}) superfused onto the preparation is not increased in the presence of desipramine at concentrations up to 10^{-5} mol l^{-1}, whereas desipramine application actually produces a slight blocking of the octopamine response (P. D. Evans, unpublished data). Desipramine is a very potent antagonist of the high-affinity sodium-dependent uptake of octopamine into cockroach nerve cord (Evans, 1978). Thus the potentiation of octopamine responses by the inhibition of a high-affinity uptake system may be a property restricted to those neurotransmitter roles of octopamine where it is released at discrete transmitter sites. In contrast, the neuromodulatory actions of octopamine where it is released from blindly ending neurosecretory terminals to affect large populations of cells at a considerable distance from the release site may not be potentiated by inhibition of high-affinity uptake mechanisms.

Another possible explanation for the increased sensitivity of lanterns to octopamine in the presence of imipramine is that imipramine could potentiate the

action of octopamine on the postsynaptic receptor. This effect cannot be ruled out, but since imipramine clearly reduces uptake of octopamine in this system and other systems, transmitter inactivation alone can explain the observed results.

It is not clear why the response to electrical stimulation in lanterns blocked by $2{\cdot}5\times10^{-4}$ mol l^{-1} imipramine begins to fail in about 2 h and rapidly declines to zero. Possibly the nerve terminals become depleted of octopamine as transmitter uptake blockage progresses. This explanation is supported from two lines of evidence. Larval lanterns treated with $2{\cdot}5\times10^{-4}$ mol l^{-1} imipramine for over 30 min show a significant loss of octopamine content (Table 1). Imipramine appears to have no direct inhibiting effect on the action of octopamine on the photocytes since lanterns blocked by $2{\cdot}5\times10^{-4}$ mol l^{-1} imipramine for over 2 h still luminesce strongly in response to exogenous octopamine.

The uptake of radioactive octopamine is greatly reduced by a 30-min pre-incubation of the lantern in 10^{-5} mol l^{-1} imipramine (Figs 4, 5). The K_i for imipramine is $5{\cdot}8\times10^{-6}$ mol l^{-1}. Whereas 10^{-5} mol l^{-1} imipramine had little effect on nonspecific uptake (compare Figs 3, 5), it reduced specific uptake of octopamine strongly at octopamine concentrations below 10^{-5} mol l^{-1}. It therefore appears on the basis of physiological and biochemical investigations that the larval firefly lantern possesses a powerful octopamine uptake system which rapidly inactivates the released transmitter to terminate the light response.

Release of octopamine from imipramine-treated lanterns

If it is assumed that imipramine blocks uptake of released octopamine, then more octopamine should be recovered from the medium of lanterns treated by this antidepressant. Although imipramine-treated lanterns released elevated amounts of octopamine spontaneously and in response to high K^+ saline (Table 1), the increases were not significantly different from those of untreated controls, due probably to the high variability of the samples.

As expected, however, the octopamine content of imipramine-treated lanterns was significantly reduced compared with controls. If this imipramine-induced reduction of octopamine content is taken into account, imipramine causes a significant increase of octopamine release. The fractional release of octopamine by high K^+ saline was significantly elevated in imipramine-treated lanterns (Table 1).

By assuming that the total octopamine content of the lantern can be assessed by adding the octopamine recovered from the various media and from the lantern directly, an unexpected observation was made. Imipramine induced a significant reduction of total octopamine content in lanterns. This suggests that imipramine may interfere with the production of octopamine or it may stimulate the destruction of the transmitter, perhaps by enzymatic action. Further experiments are required to elucidate the nature of this subsidiary effect of imipramine.

Location of octopamine uptake sites in the lantern

Where is the uptake system located within the firefly lantern? In vertebrates, inactivation of biogenic amines is by re-uptake into nerve terminals (Iversen, 1967)

and this has been proposed as the principal site of inactivation in invertebrates as well (Robertson & Juorio, 1976). An example of this is the cockroach nerve cord which contains a concentrative, sodium-dependent uptake system for octopamine (Evans, 1978).

In the firefly lantern the photomotor neurone terminals do not appear to be the principal site of octopamine uptake. The denervated lantern does not show an elevated sensitivity to exogenous octopamine as might be expected if the uptake system had been removed. The sensitivity of the denervated lantern to octopamine does increase when blocked by imipramine as does the lantern with intact innervation. That is, denervated lanterns blocked by imipramine decrease their threshold to exogenous octopamine from 10^{-5} mol l^{-1} to between 10^{-8} mol l^{-1} and 10^{-7} mol l^{-1}. If the nerves were the principal site of octopamine uptake, the denervated lantern should be unaffected by imipramine because the uptake carriers on the nerve terminals would have already been removed.

Another possible site of octopamine inactivation in the firefly light organ could be the glial cells which have been reported to surround all but the terminal portions of the nerve branches (Oertel, Linberg & Case, 1975), since they would not be removed upon denervation of the lantern. Glial cells are known to take up neurotransmitters in a variety of vertebrate and invertebrate preparations. Thus γ-aminobutyric acid (GABA) is taken up by glial cells in the optic lobe of the fly (Campos-Ortega, 1974), at the neuromuscular junction of the lobster (Orkand & Kravitz, 1971) and in rat sensory ganglia (Schon & Kelly, 1974). Glutamate is actively taken up by glial cells at the cockroach neuromuscular junction (Faeder & Salpeter, 1970) and in crab peripheral nerve (Evans, 1974). In addition, histamine has been shown to be selectively accumulated by a subpopulation of glial cells in the locust visual system (Elias & Evans, 1984). In fact it seems likely that one or more of the uptake components for octopamine into cockroach nerve cord (Evans, 1978) will be localized in the glial elements of the nerve cord. Alternatively, the principal sites of octopamine uptake in the firefly light organ may be in either the tracheolar cells or the photocytes themselves. It is hoped that autoradiographical experiments at present in progress will determine if glial cells play any role in octopamine uptake in the firefly light organ.

It should be noted that the larval nerve terminals occupy only a tiny portion of the surface of the photocytes (Peterson, 1970). However, the sensitivity of the larval lantern to exogenous octopamine (10^{-5} mol l^{-1} or above) is unusually low for a putative transmitter. In homogenized larval lantern tissue there is a marked stimulation of adenylate cyclase by octopamine at 10^{-6} mol l^{-1} (Nathanson & Hunnicutt, 1979). In adult photogenic organs the threshold of stimulation of adenylate cyclase is even lower, between 10^{-9} mol l^{-1} and 10^{-7} mol l^{-1}. Furthermore, synephrine, which is absent from the lantern (Robertson & Carlson, 1976), has a similar potency to that of octopamine in activating adenylate cyclase (Nathanson, 1979), whereas it is almost 10 times more potent than octopamine in luminescence induction (Carlson, 1968*b*). In locust oviduct, octopamine reduces the amplitude of neurally-evoked contractions at a threshold of 10^{-7} mol l^{-1} (Lange & Orchard, 1984). The apparent thresholds for

effects of octopamine on relaxation rate and frequency of myogenic rhythm in locust extensor tibialis muscle are between 10^{-9} and 10^{-8} mol l^{-1} (Evans, 1981).

Why does exogenous octopamine show such a high threshold for luminescence induction if it is the true neurotransmitter in this system? The answer may be that octopamine applied to the intact lantern is rapidly inactivated and therefore its actual concentration at the receptor on the photocytes is reduced. Perhaps octopamine receptors are present only within the highly restricted synaptic areas and uptake occurs only into nerve terminals or their associated glial cells. Alternatively, octopamine receptors could be located over both the synaptic and non-synaptic surface of the photocyte and uptake could occur into either photocytes or tracheolar cells or both.

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