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SHORT COMMUNICATION

RELEASE OF OCTOPAMINE FROM THE PHOTOMOTOR NEURONES OF THE LARVAL FIREFLY LANTERNS

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The biogenic amine, octopamine, has been proposed as the transmitter in the firefly light organ (Evans, 1980; Robertson, 1981). Whereas initial studies implicated adrenaline (Smalley, 1965; Carlson, 1968*a*), octopamine was found to be more potent in inducing luminescence than any amine other than synephrine (Carlson, 1968*b*). Octopamine, but not synephrine, was found in adult lantern segments (Robertson & Carlson, 1976; Copeland & Robertson, 1982) and in larval lantern tissue (Christensen, Sherman, McCaman & Carlson, 1983). Evidence that cyclic nucleotides mediate the effect of octopamine was obtained by Oertel & Case (1976). An octopamine-sensitive adenylate cyclase has been found in the larval lantern and application of exogenous octopamine strongly stimulates cyclic AMP production in adult (Nathanson, 1979) and larval (Nathanson & Hunnicutt, 1979) light organs.

The firefly photomotor neurones are dorsal unpaired median (DUM) neurones residing in the last (eighth) abdominal ganglion of the larva (Christensen & Carlson, 1982) and the last two (sixth and seventh) abdominal ganglia of the adult male (Christensen & Carlson, 1981). They are the only nerve cells in the lantern and have many features in common with DUM neurones described in locusts (Hoyle, Dagan, Moberly & Colquhoun, 1974) which have been shown to be octopaminergic (Evans & O'Shea, 1978). Synaptic profiles in adult (Smith, 1963) and larval (Peterson, 1970) photomotor nerve terminals are very similar to those in locust DUM neurone terminals (Hoyle *et al.* 1974).

To be considered an unequivocal transmitter in the photomotor neurones octopamine must be released and detected under conditions associated with light production. In this report, experiments are described which demonstrate that octopamine can be detected after synaptic activation and that this release can be accomplished only in lanterns with an intact innervation.

Photuris versicolor larvae were collected in early autumn 1984 and maintained at 10°C on moistened paper. The paired lanterns were removed from each larva and transferred to firefly saline (Carlson, 1968*a*).

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Octopamine was assayed using a modification of the procedure developed by Molinoff, Landsberg & Axelrod (1969) based upon the conversion of octopamine to [^{3}H]methyloctopamine (synephrine) by phenyl ethanolamine-*N*-methyl transferase, the [^{3}H]methyl group being donated by [^{3}H]methyl-*S*-adenosylmethionine. The assay conformed to that used by Christensen *et al.* (1983). Final incubation volumes were 85 μl. The standard curve for octopamine was linear from 0·1 to 10 μmol with an assay sensitivity of 0·05 pmol per 5 lanterns (0·01 pmol/lantern). The reaction product was identified by thin-layer co-chromatography on silica gel plates (Baker) with authentic DL-synephrine. Using a solvent system consisting of isopropyl alcohol: ammonia: water (80:10:19), 87 % of all detectable counts were located in a single spot corresponding to DL-synephrine.

Normally six lanterns were treated together. They were placed in 25 μl of the appropriate solution and observed in darkness to detect luminescence. Occasionally individual lanterns incubating in normal saline luminesced dimly. Only those placed in solutions in which sodium was replaced by potassium (high K^+ salines, 158 mmol l^{-1} K^+) glowed brightly. At the end of the 30-min incubation period the lanterns were individually transferred to a new incubating solution and 20 μl of the previous solution was collected for detection of octopamine. When the incubations were completed the lanterns themselves were collected for analysis of octopamine content.

The larval lanterns were denervated by severing the lantern nerve with a ventral cut between the sixth and seventh abdominal sternites (the lantern ganglion, which is the last abdominal ganglion, lies anterior to the transection within the sixth abdominal segment). If attempts to induce luminescence in both lanterns by mechanically irritating the animal failed, the transection was considered complete. The operated larvae were maintained on clean, moistened filter paper at 20 °C for 3 days, and tested each day to confirm that no luminescence could be induced. The dissected lanterns were tested in high K^+ saline and if no luminescence was induced under these conditions they were considered denervated (Carlson, 1968*a*).

The spontaneous release of octopamine by lanterns incubated for 30 min in normal firefly saline at 25 °C is very low, averaging just above the sensitivity of the assay of 0·015 pmol/lantern. Over four times that amount of octopamine is released by lanterns incubated for 30 min in high K^+ saline (Table 1). Furthermore, lanterns luminesce brilliantly in the latter solution and this luminescence can persist throughout the 30-min period of incubation in high K^+ saline, although at a much diminished level.

It was noted that some lanterns rinsed in normal saline for 30 min following treatment in high K^+ saline released very high amounts of octopamine into the rinse. In fact, release of octopamine in the subsequent normal saline rinse was negatively correlated with the amount of octopamine recovered from lanterns in the previous high K^+ solution. In seven groups of lanterns tested, five groups released relatively low amounts of octopamine in high K^+ saline, but high amounts in the following rinse, in two groups the reverse was the case, and in one group equal amounts were released in each saline. It is possible that octopamine was trapped in the lantern

Table 1. *Release of octopamine (pmol/lantern) from lanterns incubated for 30 min in normal and high K^+ saline*

Normal saline	High K^+ saline (8–43)*	High K^+ saline plus first rinse (7–39)*
0·015 ± 0·003†	0·065 ± 0·017 $P<0·01$	0·133 ± 0·024 $P<0·001$

In this and all subsequent tables: *(numbers of trials–total number of lanterns), †standard error of mean. Increased release compared with normal saline is significant as shown by P values obtained from t-test.

Table 2. *Release of octopamine (pmol/lantern) from lanterns incubated for 30 min in calcium-free saline*

Normal Ca^{2+}, normal K^+	Ca^{2+}-free, high K^+ (5–28)	Normal Ca^{2+}, high K^+ (5–28)
0·029 ± 0·009	0·028 ± 0·012 NS	0·082 ± 0·027 $P<0·1$

during incubation in high K^+ saline, only to diffuse out into the subsequent saline rinse. If this were true a very much larger amount of octopamine is actually released from the neurones during high K^+ saline treatment (Table 1).

Relatively little octopamine could be recovered from lanterns treated with saline in which calcium had been replaced by magnesium (Table 2). Subsequent incubation of these lanterns in calcium-free saline containing high K^+ induced no increase in octopamine release (Table 2). Upon restoration of normal calcium, these lanterns released significantly larger amounts of octopamine when incubated in high K^+ saline (Table 2).

Octopamine could not be detected in media of lanterns denervated by transection of lantern nerves 3 days prior to testing. Neither could luminescence be induced in these lanterns by high K^+ saline. In four trials totalling 17 denervated lanterns, octopamine released was below the levels of detection (0·01 pmol/lantern) in both normal and high K^+ salines. The octopamine concentration of denervated lanterns was significantly reduced compared to that of innervated lanterns (Table 3).

The results of this study fulfil an important criterion for the identification of octopamine as an unequivocal neurotransmitter in the firefly lantern. It has been demonstrated that octopamine is released in the larval lantern in response to high K^+ saline; this release induces brilliant luminescence and elevated levels of octopamine can be detected in the medium (Table 1). That this octopamine is synaptically released is suggested by the significant reduction of octopamine detected in media of lanterns incubated in calcium-free, high K^+ saline (Table 2).

Table 3. *Content of octopamine per lantern (pmol)*

Innervated lanterns	1·29 ± 0·150 (8–40)
Denervated lanterns	0·079 ± 0·036 (4–17)

The amount of octopamine found in innervated lanterns is significantly different from that found in denervated lanterns ($P<0·01$).

The photomotor neurones appear to supply the bulk, if not all, the octopamine innervation to the lantern. Not only is no detectable octopamine released from denervated lanterns in high K^+ saline, but the octopamine content of lanterns is reduced nearly 94% by denervation (Table 3).

In this study the concentration of octopamine detected in the larval lantern averaged 1·29 pmol/lantern, which conforms closely to the two values reported by Christensen *et al.* (1983) of 1·03 and 1·43 pmol. The average release of octopamine from lanterns incubated for 30 min in high K^+ saline represents only 5% of the total octopamine present in the lantern (0·064 pmol *vs* 1·29 pmol). It is possible, however, that the high release of octopamine observed from some lanterns returned to normal saline after incubation in high K^+ saline represents transmitter which had been released but had diffused into the subsequent saline rinse. If this were the case, the amounts of octopamine released due to depolarization of nerve terminals by high K^+ saline would represent a minimum of 10% of the endogenous octopamine (0·133 pmol *vs* 1·29 pmol). Actually the amount of octopamine released is probably much greater because much of the released transmitter would be lost by reuptake and enzymatic destruction.

It is also possible that this elevated amount of octopamine represents an increase of spontaneous release during the period of recovery. For instance, Morton & Evans (1984) have noted that in the presence of a prolonged pulse of high K^+ saline, release of octopamine is arrested in DUMETi of the locust, presumably by inactivation of calcium channels. This inactivation of octopamine release has also been found in lobster second thoracic roots (Evans, Kravitz & Talamo, 1976) and in the release of insect diuretic hormones in *Rhodnius* (Maddrell & Gee, 1974). If this were true, however, it would not be expected that octopamine released during recovery would vary with the amount released during incubation in high K^+ saline.

In at least four other preparations octopamine release has been demonstrated: from (1) DUMETi of the locust in response to high K^+ saline and electrical stimulation of the neurone (Morton & Evans, 1984), (2) the isotopically labelled second thoracic nerve roots and pericardial organs of the lobster, *Homarus americanus*, in response to high K^+ saline (Evans *et al.* 1976), (3) the preterminal nerve trunks of the ligamental nerve plexuses in the spiny lobster, *Panulirus interruptus*, in response to electrical stimulation (Sullivan, Friend & Barker, 1977), and from (4) the isolated half-thorax preparation of the locust in response to direct stimulation of the muscle and its neural innervation (Goosey & Candy, 1982).

The demonstration that the photomotor neurones represent probably the exclusive source of octopamine in the firefly lantern makes this preparation particularly useful for the further analysis of octopamine function.

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