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EFFECT OF DRUGS ON LUMINESCENCE IN LARVAL FIREFLIES

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INTRODUCTION

Smalley (1965) demonstrated conclusively that the lantern of the adult *Photinus* firefly showed a number of similarities to vertebrate adrenergic systems. Injections of epinephrine, norepinephrine, and amphetamine induced intense luminescence; but the latter drug, in typical fashion, failed to function in reserpinized or denervated lanterns. Carlson, 1968, has reported that the larval lanterns of the *Photuris* firefly also respond to adrenergic drugs in identical fashion. Because epinephrine, norepinephrine, dopamine, tyramine and isoproterenol all showed sigmoid dose-response curves typical of drug-receptor interaction it was suggested that they may operate on the photocyte membrane. The observation that norepinephrine induced luminescence equally well in sodium-free and potassium-free solutions and in solutions containing both these ions suggested that it did not operate by affecting the movement of those ions across the photocyte membrane.

This study was initiated to examine the action of monophenolic drugs related to epinephrine. Observations were also made on the effect of the metabolic poison, KCN, and the vertebrate β-receptor blocking agent, dichloroisoproterenol.

MATERIALS AND METHODS

The methods used in this investigation were similar to those described in a previous paper (Carlson, 1968). Drugs used were: synephrine, DL-octapamine hydrochloride, DL-metanephrine hydrochloride, DL-normetanephrine hydrochloride and L-phenylephrine hydrochloride (Sigma Chemical Co.); DL-epinephrine hydrochloride (K & K Laboratories, Inc.); DL-isoproterenol hydrochloride and dichloroisoproterenol (Aldrich Chemical Co.); D-amphetamine hydrochloride (Nutritional Biochemical Corp.) and reserpine phosphate (supplied through the courtesy of Ciba Pharmaceutical Co.). All drugs except reserpine phosphate were dissolved in a saline containing 9 g./l. NaCl, 0·2 g./l. KCl, 0·2 g./l. $CaCl_2$, 0·2 g./l. $MgCl_2$, 4 g./l. glucose buffered with 0·04 M Tris buffer to pH 7·3.

RESULTS

While epinephrine and its analogue norepinephrine were effective in inducing luminescence in the larval organ (Carlson, 1968), synephrine and some closely related monophenolic drugs were significantly more effective. Comparison of these drugs is

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shown in Table 1. The concentration-response curve of synephrine is sigmoidal in character and its response threshold was found to be 10^{-6} M. It was found capable of inducing intense luminescence in reserpinized lanterns which were incapable of responding to amphetamine.

Lanterns induced to glow in epinephrine or synephrine are immediately extinguished in 10^{-3} M-KCN. This is not due to rinsing out of the adrenergic drug because the lanterns are extinguished when KCN is introduced with the drugs. Lanterns extinguished in KCN recover in saline as the restoration of the luminescence indicates. These observations are illustrated in Fig. 1. 10^{-3} M synephrine applied with 10^{-3} M KCN to a darkened lantern induces luminescence which is rapidly quenched within less than 1 min.

Table 1.

Drug	Relative equivalent concentration*	Lanterns tested
DL-Synephrine	1·00	—
DL-Octapamine	6·19 ± 4·09	17
DL-Metanephrine	9·87 ± 4·78	18
DL-Normetanephrine	10·26 ± 5·32	15
DL-Epinephrine	43·66 ± 6·24	14
L-Phenylephrine	250·93 ± 125·39	15

* Concentration × 10^{-3} M at which maximum rate of intensity rise equivalent to maximum rate induced by 10^{-3} M synephrine in the same lantern.
± Standard error of mean to 99% level of confidence.

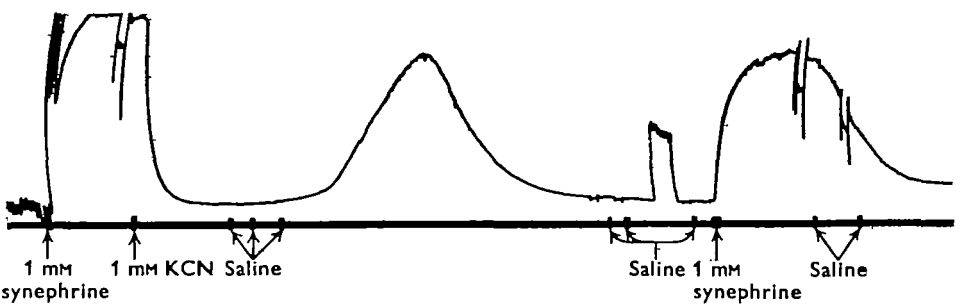

Fig. 1. Effect of 1 mM-KCN in 1 mM synephrine on luminescence induced with 1 mM synephrine. Top trace, photomultiplier output; bottom trace, time and event marker, 1 mark/sec.

Lanterns glowing at maximum luminescence intensity induced at various concentrations of synephrine were rinsed in the same concentration of drug containing 10^{-3} M-KCN. The time required for luminescence extinction was found to be proportional to synephrine concentration. Extinction time varied over a small interval in KCN-quenched runs, however, as shown in Fig. 2.

Dichloroisoproterenol (D.C.I.) found to block adrenergic action reversibly in the vertebrate, was tested on the larval lantern. At concentrations of $2{\cdot}5 \times 10^{-3}$ M it induced luminescence which slowly increased to a high level, then slowly declined to extinction. Synephrine (10^{-4} M) introduced during this rising period caused the lantern rapidly to reach its maximum luminescence level for that concentration of synephrine. Synephrine (10^{-4} M) added during the declining period of luminescence induced by D.C.I., had little effect. Further, glows induced with D.C.I. plus 10^{-4} M

synephrine or with D.C.I. alone resisted extinction and were very slowly extinguished in saline. $2{\cdot}5 \times 10^{-3}$ M isoproterenol, an adrenergic drug structurally related to D.C.I., had a weak but normal effect. It readily induced luminescence, was easily extinguished in saline and produced no observable deleterious effects when allowed to act on the lantern for periods up to 20 min. By this time all lanterns immersed in the equivalent concentration of D.C.I. were no longer capable of responding to synephrine.

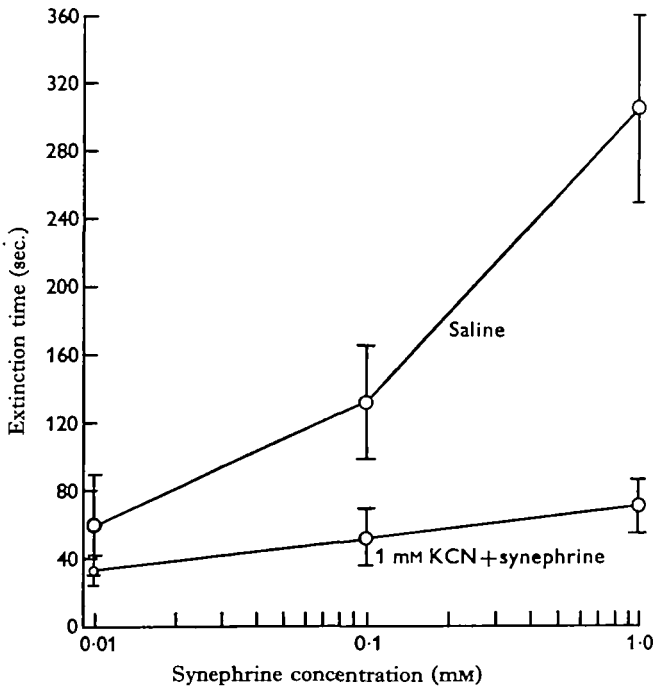

Fig. 2. Effect of synephrine concentration on the luminescence extinction time produced by 1 mM-KCN plus synephrine and by saline. Vertical lines through the points equal to standard error of the mean to 95% level of confidence.

DISCUSSION

Various chemical groups making up the structure of the catecholamines have different effects on the luminescence-inducing activity of the drugs tested. The para hydroxyl group of the catechol ring is of utmost importance, while the meta hydroxyl group actually inhibits activity. This is illustrated by the high potency of synephrine and octapamine which lack the meta hydroxyl, the moderate potency of epinephrine and norepinephrine which contain both groups, and the low potency of L-phenylephrine which possesses only a meta hydroxyl. Loss of the terminal methyl group on the amine end reduces potency significantly but not as much; compare synephrine with octapamine and epinephrine with norepinephrine (Carlson, 1968). The methoxy group in the meta position found on metanephrine and normetanephrine significantly reduces potency but not to the extent of the meta hydroxyl group. Loss of the hydroxyl group on the β-carbon dramatically reduces activity as shown by the low potency of dopamine and tyramine (Carlson, 1968). Finally, substitution of an isopropyl group

for a methyl group on the amine end greatly reduces potency. In summary, virtually all portions of the catecholamine structure play significant roles in determining the activity of luminescence induction.

No evidence other than potency of luminescence induction has been developed to support the hypothesis that the actual transmitter is a monophenolic drug related to synephrine. A monophenolic transmitter may be necessary in order to maintain a transparent cuticle. Just as likely, the monophenolic structure may be necessary to allow transmitter destruction by a monophenol-attacking enzyme. The possibility that a catecholamine is the true transmitter which is rendered less potent than the monophenolic amines by an inactivating enzyme cannot be ruled out.

KCN has a rapid effect in extinguishing luminescence and may operate by inhibition of the production of ATP needed for the light reaction. As described by McElroy (1951) and McElroy & Hastings (1955), this ATP may either be required to form the active complex or it may be necessary to provide the pyrophosphate needed to breakdown the inactive complex and free luciferase for further activity. Synephrine and epinephrine apparently act more quickly than KCN, perhaps because they need not penetrate the photocyte membrane. This was demonstrated by the ability of the drugs to initiate luminescence when introduced simultaneously with KCN. Alternatively, this may simply indicate that a small KCN-insensitive pool of ATP is present which is rapidly exhausted.

The suggestion by Smalley (1965) that the transmitter of the adult lantern might stimulate glycogen metabolism and thereby increase ATP production is supported by another observation of synephrine action in the larval lantern. If a large ATP pool existed in the larval lantern one might expect that low-intensity luminescence, which would require a relatively small amount of ATP, could be maintained for long periods following KCN poisoning. The observation that KCN abolishes luminescence very quickly and that the extinction time is proportional to synephrine concentration, and correspondingly to luminescence intensity, suggests that only a very small ATP pool exists. If this is true, then synephrine must stimulate ATP production in order to sustain the intense luminescence obtained at high drug concentrations.

The demonstration by Rall & Sutherland (1962) that catecholamines act with adenyl cyclase to produce cyclic adenosine-3′,5′-phosphate and pyrophosphate may serve as a possible model of catecholamine and synephrine action in larval luminescence induction. It is possible that through a mechanism of this type the pyrophosphate, shown by McElroy & Hastings (1955) to be active in *in vitro* luminescence initiation, is liberated. By this process pyrophosphate would be liberated directly by the transmitter, rather than being released through a number of intermediate chemical steps as proposed for acetylcholine by McElroy & Hastings (1955). The earlier observation by Sutherland & Cori (1951) that catecholamines stimulate phosphorylase activity and glycogenolysis could suggest a way whereby the drugs tested stimulate ATP production in the larval lantern.

The action of D.C.I. on the lantern shows similarities to its action in the vertebrate. It has sympathomimetic properties which in the lantern result in luminescence induction and it also blocks the action of applied adrenergic drugs. Its blocking and luminescence-inducing effects in the firefly differ from its effects in the vertebrate in not being readily reversible. That the blockage of luminescence induction is not due to

osmotic or other physicochemical damage is suggested by the observation that its close analogue, isoproterenol, acts as a weak but readily reversible adrenergic drug.

No conclusive evidence has been obtained that D.C.I. produces its blocking effect by attachment to the sites of action of the luminescence-inducing drugs. It might block just as effectively by occupying another site which prevents the adrenergic drugs from reaching the appropriate position. That isoproterenol induces low-level luminescence suggests that it imperfectly fits the site of action and this imperfection of fit may explain the irreversible blocking action of D.C.I. It would be of interest to test a chloride analogue of synephrine such as p-chloro-(methylaminomethyl) benzyl alcohol.

SUMMARY

1. Synephrine and other related monophenolic drugs were tested for potency of luminescence induction in the extirpated lantern of larval fireflies. Synephrine was found to be the most potent drug so far tested, with a threshold concentration of 10^{-6} M.

2. Immersion of glowing lanterns in a solution containing 10^{-3} M synephrine and 10^{-3} M-KCN resulted in rapid extinction of luminescence. Luminescence extinction times in KCN were found to be proportional to synephrine concentration and suggest that only a small ATP pool exists in the lantern. It is hypothesized that synephrine must stimulate ATP production in order to maintain high luminescence intensities.

3. The vertebrate adrenergic blocking agent, dichloroisoproterenol (D.C.I.), was found to slowly induce luminescence which eventually declined to extinction. Synephrine was ineffective in luminescence induction after the declining phase of D.C.I. action began. A close analogue of D.C.I., isoproterenol, acted in a weak but similar manner to synephrine. It is suggested that D.C.I. prevents synephrine action by blocking the photocyte receptor sites.

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