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A COMPARISON OF TRANSMITTER AND SYNEPHRINE ON LUMINESCENCE INDUCTION IN THE FIREFLY LARVA

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INTRODUCTION

Smalley (1965) demonstrated that the adult firefly lantern can be classified as adrenergic with respect to a number of criteria established for vertebrate sympathetic junctions. The lantern luminesces in response to adrenaline and its analogues. Amphetamine acts indirectly to induce luminescence and fails in denervated lanterns. Reserpinized lanterns respond to adrenaline, but no longer to neural stimulation.

Carlson (1968*a*) demonstrated that the lanterns of the larval firefly respond in similar fashion to the adult lantern. In this system the lanterns were immersed in oxygenated saline and luminiscence could be induced by introduction of adrenaline and its analogues. Synephrine (*p*-hydroxy-α-[(methylamino) methyl]benzyl alcohol) was found to have the highest potency, initiating luminescence at a concentration of 10^{-6} M (Carlson, 1968*b*) and it acted in identical fashion to adrenaline, which was 45 times less potent. Transmitter could be released to act either by neural stimulation or by immersion of the lantern in K^+ saline (Carlson, 1968*a*). It now becomes possible to compare the actions of synephrine and transmitter in luminescence induction. This promises to allow one to determine whether these substances differ qualitatively in their pharmacological actions. This is a report of a comparative study of the effects of transmitter and synephrine. It should be emphasized at the outset that these substances are applied in somewhat different fashion and this in itself may have produced qualitative differences in their luminescence inducing actions.

MATERIALS AND METHODS

The methods used in this investigation were similar to those described in Carlson (1968*a*). The lantern of the *Photuris* larva was electrically stimulated via a square-wave stimulator, the electrodes being two wires whose uninsulated ends were closely adjacent to the lantern.

Drugs used were: synephrine, *p*-methyl-α-tyrosine and dichloroisoproterenol (D.C.I.) (Sigma Chemical Co.), iproniazid (Aldrich Chemical Co.), harmine (K & K Laboratories), and chlorpromazine (through the courtesy of Smith, Kline and French).

In a dimly glowing lantern removal and replacement of solution causes a sudden reduction of light intensity, followed by an equally sudden return to the original intensity level (see Fig. 4). This effect is due to a difference in the refractive index of

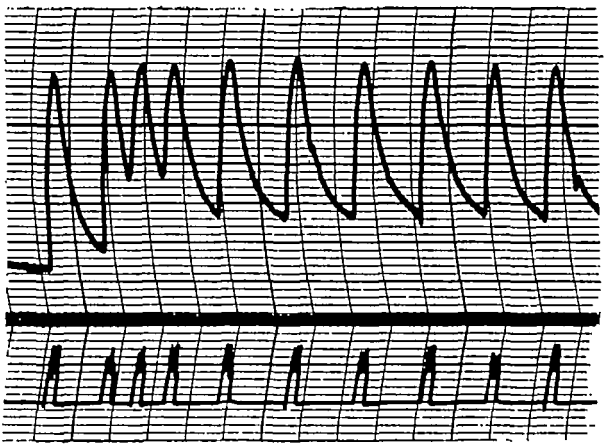

Fig. 1. Electrical stimulation of a larval lantern to induce luminescence. Upper trace: photomultiplier output. Middle trace: time base, 1 mark/sec. Lower trace: stimulus, intensity 20 V, frequency 20/sec, pulse duration 1 msec; stimulus duration 1 sec.

air versus water. Latency of the lantern response was measured from the sudden return of the glow during immersion of the lantern in synephrine or in high K^+ saline to the onset of the luminescence induced by synephrine or K^+. Chart speeds for this measurement were 100 mm/sec. Latency of the lantern response to electrical stimulation was measured from the first stimulus to the onset of the glow.

The pH of the saline containing chlorpromazine was lowered from 7·2 to 6·9 to aid in dissolving the drug. At this more acid pH the lantern response to synephrine and electrical stimulation was successively diminished even in saline. For each lantern in chlorpromazine another was simultaneously tested in saline of pH 6·9 as a control for this effect of acid.

RESULTS

Transmitter can be liberated to induce luminescence in the extirpated larval lantern by a number of methods. The most convenient method is by electrical stimulation of the nerves. Immersion of the lantern in high-K^+ saline or amphetamine also induces glowing indirectly. The glow induced by high-K^+ saline cannot be maintained, while that induced by amphetamine lasts for hours. The luminescence induced by all three techniques is greatly reduced or fails in lanterns suffering denervation.

Electrical stimulation of the larval lantern immersed in saline can produce uniform responses (see Fig. 1). The lanterns show the same characteristics as those stimulated in the intact larvae (Case & Buck, 1963; Carlson, 1965). Response latency was found to be virtually identical to that found by Case & Buck (1963) at 612 msec $\pm$ 24·25 S.E. A comparison of a short electrical stimulation and a tetanus is shown in Fig. 2. The extinction rates are rapid but the extinction time following tetanus is somewhat more prolonged. A comparison of the electrically induced response with that to high-K^+ saline is shown in Fig. 3.

The response obtained by immersion of the lantern in synephrine compared to that produced by electrical stimulation demonstrates the considerably different time courses of the drug and transmitter (see Fig. 4). The latency of the light response to 10^{-3} M

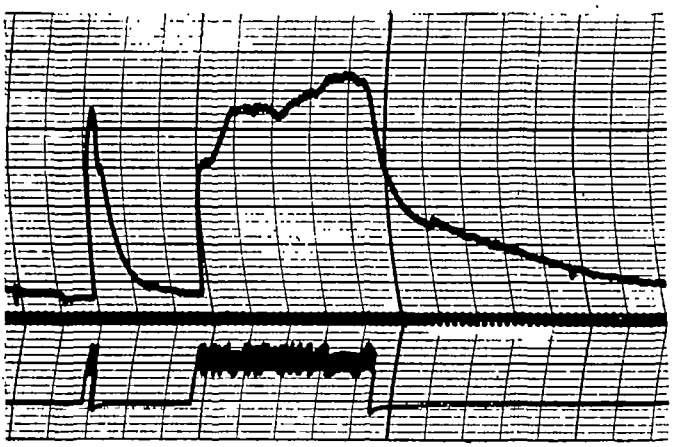

Fig. 2. Comparison of luminescence induced by electrical stimuli of short and long duration. Upper trace: photomultiplier output. Middle trace: time base, 1 mark/sec. Lower trace: stimulus; intensity of 1st stimulus train is 33 V, 2nd stimulus train is 29 V, frequency 20/sec, pulse duration 1 msec.

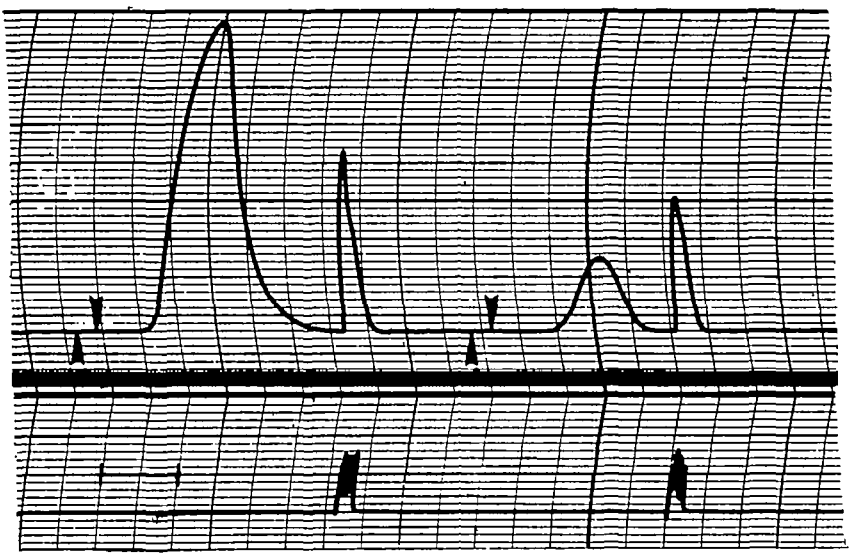

Fig. 3. Comparison of luminescence induced in high-K^+ saline and by electrical stimulation. Upper trace: photomultiplier output, arrows indicate lantern immersion in high-K^+ saline and subsequent rinsing in saline after 5 sec. Middle trace: event marker, small mark indicates immersion of lantern in high K^+ saline. Lower trace: stimulus; intensity 48 V, frequency 20·5/sec, pulse duration 1 msec, stimulus duration 5 sec. Distance between vertical lines equals 20 sec.

synephrine is quite stable, however, averaging 3133 msec $\pm$ 428·4 S.E. and is significantly different from the electrically stimulated latency at the 0·01 level. The latency to high-K^+ saline is considerably longer and more variable, reflecting its indirect mode of luminescence induction by transmitter release.

Both transmitter and synephrine appear to stimulate ATP production. 10^{-3} M-KCN

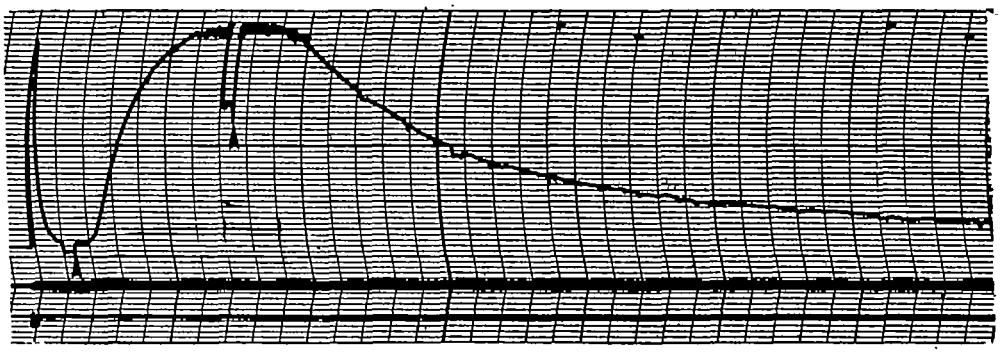

Fig. 4. Comparison of luminescence induced by electrical stimulation and by 10^{-4} M synephrine. Upper trace: photomultiplier output, first arrow notes point at which lantern immersed in synephrine and second arrow indicates initiation of saline rinse. Lower trace: stimulus; intensity 18 V, frequency 20/sec, pulse duration 1 msec, stimulus duration 5 sec. Distance between vertical lines equal 40 sec.

extinguished electrically stimulated luminescence no more rapidly at high luminescent intensities than it did at low intensities. In fact, extinction time was longer at the higher voltages of stimulation (averaging 121·0 msec) than at lower voltages (averaging 32·7 msec) and this difference was significant at only the 0·1 level. This would suggest that no ATP pool exists in the lantern and that the transmitter must induce sufficient synthesis to maintain the light reaction. A similar result was obtained for synephrine (Carlson, 1968*b*).

Both transmitter and synephrine act in similar fashion when introduced into a lantern made anoxic with nitrogen (N_2 saline). If the lantern is maintained in N_2 saline and given a 1 min immersion in an anoxic solution of 10^{-3} M synephrine, no luminescence results upon readmission of oxygenated saline 15 min later. Luminescence can be obtained if oxygen is introduced within 4 min of removal of the synephrine. If one instead stimulates the lantern for 1 min in N_2 saline and readmits oxygen 15 min later no luminescence results. Luminescence does occur if oxygen is admitted immediately after stimulation. These results conform to those found when stimulating intact larvae in nitrogen (Carlson, 1965).

In an attempt to determine whether a monoamine oxidase enzyme might be involved in destruction of transmitter, a number of enzyme inhibitors were tested. The light responses induced by electrically released transmitter in lanterns immersed in 10^{-3} M iproniazid, harmine or *p*-methyl-α-tyrosine were neither potentiated nor prolonged.

Chlorpromazine, shown to be an adrenergic blocking agent in vertebrates, was studied for its effect on luminescence induction by transmitter and synephrine. 10^{-3} M Chlorpromazine reduced the light responses to both substances. As shown in Fig. 5, chlorpromazine reduced the response to electrical stimulation to 25% in 2·5 min and completely abolished the response within 10 min. Within 15 min chlorpromazine reduced the response to synephrine to 23·9% ± 4·6 S.E. The control response was reduced to 69·8% ± 3·02 S.E. The reduction of the control response was due mainly to the necessity to reduce the control saline pH to 6·9 to conform with the pH of the experimental saline. Still, the difference was significant to the 0·01 level.

Dichloroisoproterenol (D.C.I.), an adrenergic blocking agent in vertebrates, produced

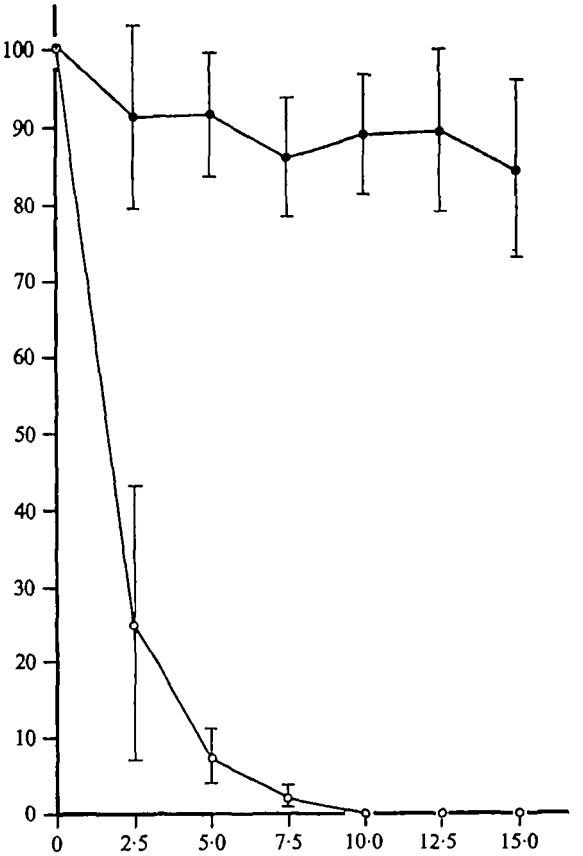

Fig. 5. Effect of chlorpromazine on luminescence induction by transmitter. Diminution of relative luminescence intensity induced by electrical stimulation in lanterns immersed in 10^{-3}M chlorpromazine (open circles) versus control lanterns in saline of pH 6·9. Vertical lines represent one standard error.

similar effects to synephrine (Carlson, 1968*b*), to electrical stimulation and to high-K^+ saline. D.C.I. induced a slow luminescence rise which reached a peak in a few minutes and then began a slow decline. Synephrine and transmitter both stimulated increased light production during the rising phase of luminescence induced by D.C.I. After the D.C.I.-induced luminescence began to decline neither method could stimulate further light output.

DISCUSSION

Before discussing the similarities and differences of the lantern response to transmitter and to synephrine it is important to point out that the route by which these substances affect the photogenic cells (photocytes) differs in important and perhaps significant details. Electrical stimulation releases transmitter from nerve ends immediately adjacent to the photocyte surface and this neuro-effector junction may have characteristics which strongly affect the luminescent response induced by the transmitter. Synephrine is provided in solution and although it has been shown to act directly on the photocytes (Carlson, 1968*b*), it may act on parts of the photocyte membrane which differs from the neuro-photocyte junction.

With the differences of administration of transmitter and synephrine in mind it is surprising to find them so similar in their luminescence-inducing characteristics. The similarities between the two substances can be summarized as follows:

(1) They both act directly on the photocyte.

(2) The response latencies of the two agents, although not identical, are quite constant. Transmitter latency averages 612 msec while synephrine averages 3133 msec. Although there is a significant difference in the latencies it is not unreasonable to assume that this is due to the differences in administration of the two substances. This is borne out by the observation that high-K^+ saline has a still longer latency of luminescence induction which varies over a wide range, reflecting the fact that it must act indirectly by liberating transmitter.

(3) Both agents stimulate ATP production. 10^{-3} M-KCN extinguished electrically stimulated and synephrine-induced (Carlson, 1968*b*) luminescence no more rapidly at high luminescent intensities than it did at low intensities. This would suggest that no significant ATP pool exists. If an ATP pool were available low-level luminescence should be maintained longer after ATP manufacture has been stopped by KCN.

(4) Neither transmitter nor synephrine appears to induce a persistent intermediate in anoxic lanterns which lasts for a significant period after removal of the agents themselves. Neither transmitter nor synephrine could induce luminescence in anoxic lanterns, and if oxygen was admitted 15 min after the agents had been presumably removed no luminescence resulted. If oxygen was admitted immediately after removal of transmitter or synephrine luminescence did appear. This latter result can most easily be explained by assuming that the agents still remain in the lantern.

(5) There does not appear to be a monoamine oxidase, at least of the vertebrate type, which destroys the released transmitter or synephrine. Neither transmitter nor synephrine showed enhanced activity in the presence of the monoamine oxidase inhibitors iproniazid, harmine or *p*-methyl-α-tyrosine.

(6) Chlorpromazine rapidly blocks the action of both transmitter and synephrine. This result does not necessarily mean that transmitter and synephrine act on the same receptor site because chlorpromazine is believed to have a number of related effects. Thoenen, Hurlimann & Haefely (1965) suggest that chlorpromazine not only blocks alpha adrenergic receptors but it also inhibits noradrenaline liberation from and noradrenaline uptake into sympathetic nerve endings.

(7) Both transmitter and synephrine show identical responses when they are delivered to a lantern in 10^{-3} M dichloroisoproterenol (D.C.I.), a β-receptor blocking agent in vertebrate adrenergic synapses. This agent induces a slow rise in lantern luminescence which reaches a peak and then declines. Transmitter and synephrine, like noradrenaline (Carlson, 1968*a*), induce further luminescence if introduced during the rising glow period caused by D.C.I. They fail to act while the D.C.I.-induced glow is declining.

Transmitter and synephrine differ in action in one significant way. Luminescence induced by transmitter is much more rapidly extinguished than that induced by synephrine (see Fig. 4). The more rapid extinction of the transmitter-induced glow may be due to the different modes of delivery of the substances. If the transmitter were inactivated by an enzyme sited at the neurophotocyte junction or by re-uptake by the nerve terminals the mode of delivery would be crucial to the luminescence extinc-

tion rate. Synephrine or other drugs delivered to the whole lantern in solution would not be restricted to the nerve-photocyte junction. This assumes that the transmitter receptor area is not restricted to the nerve-photocyte area only. On the other hand, it is possible that these substances first must penetrate the photocyte membrane and act inside the cell. This concept makes it more difficult to explain the differences in luminescence extinction time found for transmitter and synephrine. The blocking effects produced by chlorpromazine and D.C.I. point toward a membrane receptor as the active site of action. These drugs may, however, directly affect the light reaction in some way. Until more evidence is accumulated, the site of the luminescence-inducing action of the transmitter and of synephrine must remain in doubt. Further, on the basis of these observations it is not possible to say whether the transmitter differs in chemical structure from all the luminescence-inducing drugs tested that act directly on the photocyte.

SUMMARY

1. The pharmacological effects of neural transmitter and synephrine are compared with respect to induction of luminescence in extirpated larval firefly lanterns.

2. Transmitter and synephrine show many similarities of action. They are as follows:

(*a*) They both act directly on the lantern.

(*b*) Their response latencies are relatively constant.

(*c*) Both stimulate ATP production.

(*d*) Neither induces a persistent intermediate in anoxic lanterns.

(*e*) No monoamine oxidase enzyme appears to act on them.

(*f*) The luminescence-inducing action of both is rapidly blocked by chlorpromazine.

(*g*) They show identical responses in the presence of dichloroisoproterenol.

3. Luminescence induced by transmitter is much more rapidly extinguished than that induced by synephrine.

4. The possible reasons for the difference in luminescence extinction rate between the two agents are discussed and their different modes of delivery are emphasized.

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