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THE ACTION OF 5-HYDROXYTRYPTAMINE AND RELATED COMPOUNDS ON NEUROMUSCULAR TRANSMISSION IN THE LOCUST *SCHISTOCERCA GREGARIA*

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At the present time the nature of the junctional transmitter in insects is not known. Substances such as acetylcholine or 5-hydroxytryptamine, known or suspected to be neurohumours in other groups of animals are not, in general, definitely known to have an action on insect neuromuscular transmission. Harlow (1958) found that acetylcholine (ACh) did not interfere with the response of the extensor tibialis of the hind leg of the locust *Locusta migratoria* to stimulation by the crural nerve, although perfused ACh could produce a tetanic extension of the whole leg. The nature of the action, on a locust neuromuscular junction, of an active amine such as 5-hydroxytryptamine (5-HT) which is generally (Welsh, 1957*a*) although not always (Welsh, 1957*b*) found to have an opposite effect from that of ACh, should be of interest. Twarog & Roeder (1957) found that, although highly active on the heart of the cockroach, 5-HT was effective only at high concentrations on the cercal synapse of the desheathed last abdominal ganglion. Since we too found that 5-HT must be applied in high concentrations, ACh in the same concentrations was applied as a check against non-specific effects. Since tryptamine has been found to be far less active than 5-HT on preparations where 5-HT is thought to be a neurohumour (Hill, 1958; Greenberg, 1960), we also employed tryptamine as a test of the specificity of 5-HT.

Davey (1960) has suggested that an *o*-dihydroxyindolalkylamine may have an excitatory role in the insect peripheral nervous system, and that its action may be inhibited by 5-HT, through a blocking of receptor sites by the structurally similar 5-HT. This suggested to us the use of a dimethoxyindolalkylamine in the search for an antagonist to 5-HT on our preparation. We also tested as possible antagonists lysergic acid diethylamide (LSD) and bromolysergic acid diethylamide (BOL) which are known to act as antagonists to 5-HT in other invertebrate preparations (Welsh, 1957*a*), probably by virtue of irreversibly imitating 5-HT. 3-(pyrrolidinomethyl)-thionaphthene is known to be one hundred times as active as

LSD as an antagonist of 5-HT for the rat uterus preparation (personal communication from Mr J. J. Lewis), and this compound also was tested on our preparations.

METHODS

Activity of the neuromuscular junctions associated with the 'fast' responses of the metathoracic extensor tibialis and flexor tibialis to stimulation of the crural nerve was studied during the application of 5-HT and other compounds, in the locust *Schistocerca gregaria*.

Tension development in a muscle was recorded by an RCA 5734 mechano-electronic transducer, of which the plate shaft was attached to the femur or (for more isometric conditions) directly to the apodeme of the muscle studied. Simultaneously, the resting potential and action potential of a single muscle cell were recorded internally with a glass micro-electrode filled with 3M-KCl solution. The crural nerve was stimulated by supra-maximal square pulses, of variable strength, delay, and duration, through chlorided silver hooks placed on the nerve trunk near the metathoracic ganglion, from which it had been severed. External recording from the crural nerve and from the extensor tibialis was accomplished by similar electrodes.

A double micro-electrode technique similar to that described by del Castillo, Hoyle & Machne (1953) was used to record the membrane response of single muscle cells to internally applied cathodal and anodal pulses, and to measure membrane resistance.

Drugs were applied in solution, in locust saline made according to the formula of Hoyle (1954). Those used were acetylcholine iodide, tryptamine hydrochloride (both Eastman), 5-hydroxytryptamine creatinine sulphate, lysergic acid diethylamide, bromolysergic acid diethylamide, 5,6-dimethoxytryptamine (all Sandoz), and 3-(pyrrolidinomethyl)-thionaphthene (Pharmacology Department, University of Glasgow).

Experiments were carried out in a room thermostatically controlled at 21° C.

RESULTS

Effects of 5-HT and related compounds on the response of muscle fibres to presynaptic stimulation. The normal mechanisms of the neuromuscular junction studied have been established by Hoyle (1955) in the related *Locusta migratoria*. The twitch of the metathoracic extensor tibialis or flexor tibialis resulting from a single supra-maximal shock to the fifth thoracic nerve is accompanied by an electrical response in every innervated fibre. This action potential consists of a large e.p.p.-like response plus a spike which often overshoots the zero potential base line.

Injection into the leg of 10^{-2}M tryptamine or 5-HT reduced the response in surface fibres of the flexor tibialis to an e.p.p. in less than 1 min (Figs. 1*a* and 2*a*). As the spike became reduced its origin from the e.p.p. was progressively delayed. When the drug was washed away by injection of fresh locust saline, recovery to a full spike occurred in less than 1 min (Figs. 1*b* and 2*b*). It may be seen from Fig. 1*a* that tryptamine caused an increase in synaptic delay from which the preparation did not recover (Fig. 1*b*) as rapidly as from the diminution of the spike. 5-HT also produced an increase in synaptic delay.

Immersion for 4 min or more in 10^{-2}M tryptamine or 5-HT completely abolished the electrical response of the surface fibres, but as deeper fibres were affected more slowly the development of tension by the entire flexor tibialis was not abolished until after 30 min or more of soaking in either drug.

With decreased concentrations of 5-HT or tryptamine loss of twitch tension in the extensor tibialis proceeded more slowly (Fig. 3*a*, *b*). After

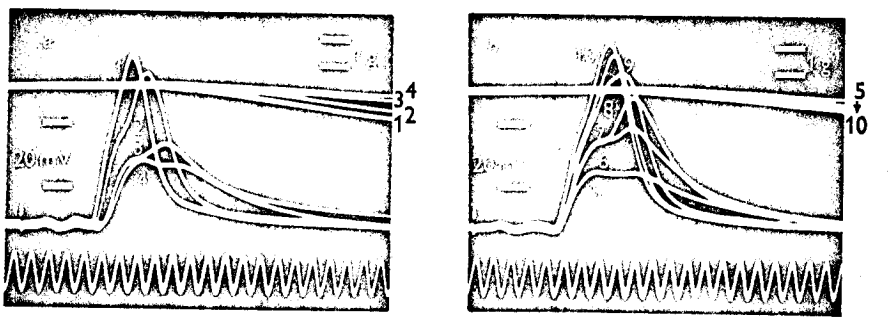

Fig. 1 (*a*) Simultaneous superimposition of tension development (upper trace) and intracellularly recorded action potentials in the flexor tibialis during inhibition by tryptamine. The first trace was photographed in normal locust saline 10 sec before application of 10^{-2}M tryptamine. Succeeding traces were photographed at 5 sec intervals after injection of the tryptamine. Note increase in synaptic delay and progressive delay in appearance of the reduced action potential from the e.p.p. Oscillations in the base line after stimulus artifact are due to impulses in coxal muscles.

(*b*) A similar record of recovery. The fifth trace was photographed in 10^{-2}M tryptamine. Succeeding traces were photographed at 5 sec intervals after injection of fresh normal locust saline. Note that synaptic delay does not recover as rapidly as does return of action potential. Time signal, 500 c/s in both.

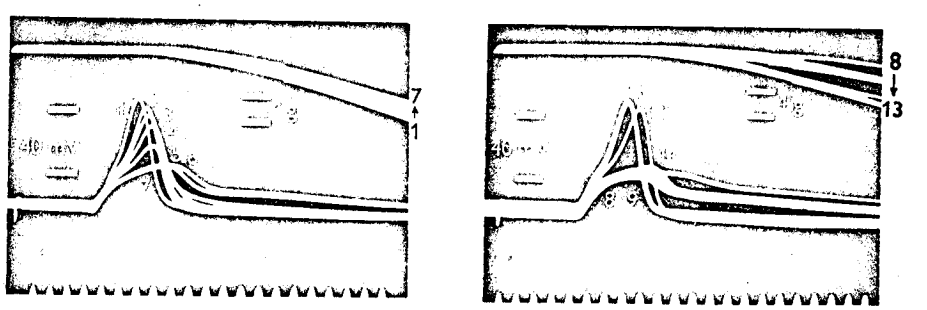

Fig. 2. (*a*) Simultaneous superimposition of tension development (upper trace) and intracellularly recorded action potentials in the flexor tibialis during inhibition by 5-HT. Increase in synaptic delay is not marked in this photograph, because not enough time had elapsed for a return to normal synaptic delay since subjection to tryptamine. Traces superimposed at 5 sec intervals.

(*b*) A similar record of recovery from 5-HT. Traces superimposed at 5 sec intervals. Time signal, 500 c/s in both.

30 min in 10^{-3}M 5-HT twitch tension was reduced by 25 % in most experiments, but could be restored by injection of fresh saline to wash out the 5-HT. Immersion for longer periods led to the recovery on washing with normal locust saline being incomplete, but recovery could be greatly enhanced by using saline containing excess calcium (10 mM) or entirely lacking in magnesium. Complete abolition of the twitch was obtained after 10–15 hr in 10^{-3}M 5-HT. In most preparations there was a 10 % decrease in resting potential during the first hour. The resting potential thereafter remained practically constant at its new value (presumably a

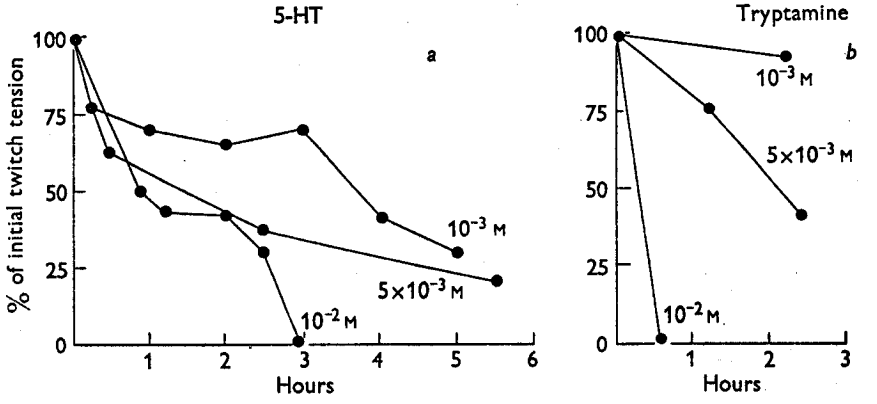

Fig. 3. (*a*) Relation of tension of single twitches of extensor tibialis to time of soaking in 5-HT.

(*b*) Relation of tension of single twitches of extensor tibialis to time of soaking in tryptamine. Numbers show molar concentration.

value reduced by electrode injury). The action potential showed a progressive decline. The initiation of the spike from the e.p.p. was progressively delayed, meanwhile becoming reduced in height. After about 5 hr in 10^{-3}M 5-HT only an e.p.p. type of response remained. This in turn diminished during about 10 hr, until finally it disappeared in all fibres at the time at which no twitch tension could be recorded. At this point the resting potentials of the fibres were still at the value reached after the first hour.

The effect of 10^{-3}M tryptamine on the electrical response of the fibres of the extensor tibialis to nerve stimulation was similar to that of 5-HT, except that there was usually a rise in the height of the action potential during the first 15 min or so, reaching about 10 % and then declining as in the same concentration of 5-HT.

Attempts to find an antagonist to 5-HT for this preparation were unsuccessful. LSD, BOL, 3-(pyrrolidinomethyl)-thionaphthene and 5,6-dimethoxytryptamine all proved to have effects on the mechanical and

electrical responses of the extensor tibialis similar to those of 5-HT or tryptamine, and in similar concentrations.

Effect of acetylcholine on response of muscle fibres to pre-synaptic stimulation. Treating the extensor tibialis with ACh for 10 hr by injection of 10^{-1}M ACh solution had no effect on isometric twitch tension. Action potentials still developed normally after stimulation through supramaximal shocks to the crural nerve.

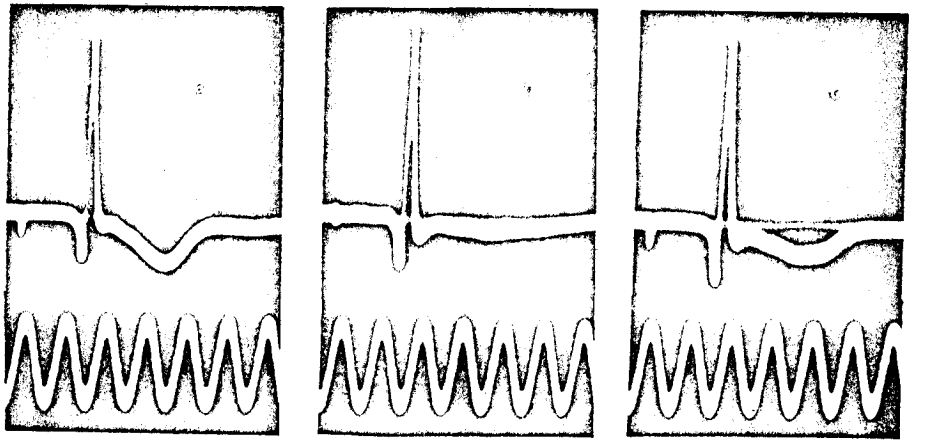

Fig. 4. Recording by external electrodes from the crural nerve trunk of nerve action potential (first deflexion) and muscle action potential (second deflexion): (*a*) in normal locust saline; (*b*) after 1 min in 10^{-2}M tryptamine; (*c*) 1 min after washing with normal locust saline. Time signal, 500 c/s.

Effects of 5-HT and related compounds on the nerve action potential. Recordings were made with external electrodes from the crural nerve trunk to test the possibility that the action reported in the preceding section could be on presynaptic nerve. Neither 5-HT, tryptamine, LSD, BOL, nor 3-(pyrrolidinomethyl)-thionaphthene inhibited the action potentials of the 'fast' nerve fibres supplying the extensor tibialis and flexor tibialis (Fig. 4). Even when all trace of tension development or electrical response in the muscle fibres was lost, the nerve impulse remained maximal.

Effect of 5-HT and tryptamine on active membrane response and on membrane resistance of muscle fibres. When cathodal pulses are applied intracellularly to fibres of the metathoracic flexor tibialis, active membrane responses appear after depolarization exceeds about 12 mV. These responses are oscillatory and resemble those found by del Castillo *et al.* (1953) in muscle fibres of *Locusta migratoria* and by Cerf, Grundfest, Hoyle & McCann (1959) in muscle fibres of *Romalea microptera.* Anodal pulses produce purely electrotonic responses, like those produced by cathodal pulses subthreshold for the active membrane response but of opposite sign.

Neither 10^{-2}M tryptamine (Fig. 5) nor 10^{-2}M 5-HT had any effect on the development of the active membrane response or on the electrotonic response to anodal pulses. Membrane resistance remained constant during immersion for up to 5 hr in 10^{-2}M 5-HT or 10^{-2}M tryptamine.

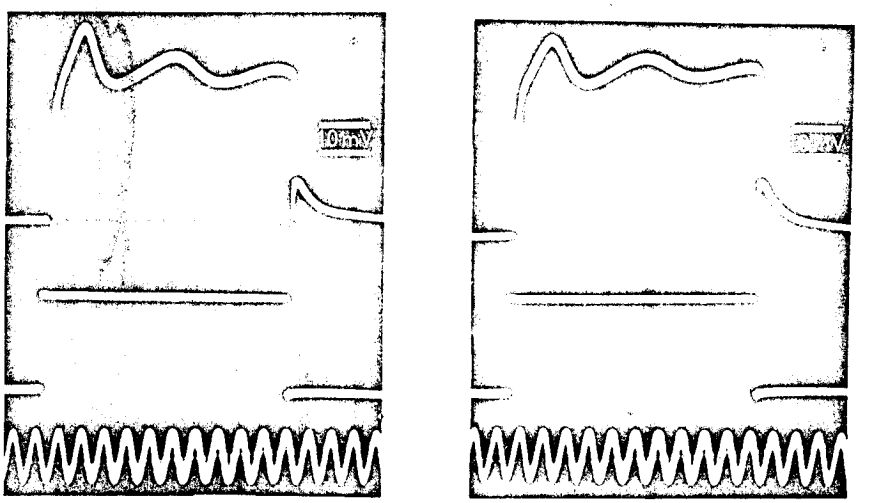

Fig. 5. (*a*) Active membrane response (upper trace) to cathodal stimulus in normal locust saline. Lower trace shows the voltage drop produced by the current flowing through the membrane across a 30 KΩ monitor resistance.

(*b*) Response of the same flexor tibialis fibre after 10 min in 10^{-2}M tryptamine. Change in oscillatory response is no greater than would occur in normal locust saline over same interval since initiation of repetitive stimulation. Time signal, 500 c/s in both.

DISCUSSION

5-HT and related compounds evidently affect synaptic transmission, since it has been shown that the inhibition they produce cannot be due to blockage or transmission in nerve fibres or to diminution of the responsiveness of the electrically excitable component of the muscle-fibre membrane. In fact, single muscle fibres could be seen to twitch when stimulated intracellularly, even after the twitch response of the entire flexor tibialis to stimulation of the fifth thoracic nerve had been abolished by application of high concentrations of 5-HT or of tryptamine. Probably 5-HT-like compounds are either preventing release of transmitter substance from nerve terminals or blocking receptor sites at the junctional regions of the muscle cells.

The high concentrations required may have some relation to the properties of the membranes surrounding insect nerves and neuromuscular junctions. A peripheral nerve axon is directly surrounded by a loose

mantle of membranes derived from the mesaxon of a lemnoblast, until it reaches the region of neuromuscular junctions of a muscle cell. Here the axon runs in a groove, in the surface of the muscle fibre, which is covered by the merged basement membranes of the tracheoblast and the lemnoblast (Edwards, Ruska & de Harven, 1958). Hoyle (1952, 1953) and Twarog & Roeder (1956) have shown that insect nerve sheaths interfere with penetration to the axon of ions and of pharmacological agents.

O'Brien & Fisher (1958) suggest that the toxicity to insects for neuropharmacological compounds may be diminished if insect nerve sheath is relatively impermeable to ionized compounds, thus permitting only the un-ionized fraction to be effective. Similarly, if the membranes covering insect neuromuscular junctions form a barrier to the diffusion of ionized compounds, this might explain why we had to use such high concentrations. All experiments were carried out in locust saline at a pH of 6·9. If the barrier to ionized fractions is assumed to be completely effective, the

TABLE 1

Compound	pKa	Fraction un-ionized at pH 6·9
BOL	5·40	0·97
LSD	5·90	0·91
tryptamine	9·10	0·016
5,6-dimethoxytryptamine	9·12	0·017
5-HT	9·15	0·018

fraction of each compound un-ionized at pH 6·9 will correspond to the effective concentration. In Table 1 (calculated from the formula given by Albert, 1952) pKa values and fractions un-ionized at pH 6·9 are listed for five of the compounds used. From the fraction un-ionized, BOL and LSD should be nearly one hundred times as effective in penetrating as the three other compounds, but in fact there was no marked difference in effectiveness on our preparation. In any case, the effective concentration for 5-HT and tryptamine would still be very high compared to the concentrations effective in other invertebrate preparations. It might be suspected that the inhibition found was merely due to non-specific side effects, if it were not that acetylcholine, in concentrations ten times higher still, is not at all toxic.

All the compounds found to be inhibitory are indole amines or have in common a structural resemblance to indole amines. Greenberg (1960) tested the activity of a large number of tryptamine analogues on the *Venus mercenaria* heart and reported that the simplest structural requirement for 5-HT-like activity was a flat aromatic nucleus with a 2-aminoethyl side chain. This requirement is fulfilled by most of the compounds we found to be effective inhibitors of neuromuscular transmission in the

locust leg. Perhaps they act by blocking the receptor sites for a transmitter with a chemical structure just dissimilar enough to prevent the 5-HT-like compounds substituting for it in excitation.

SUMMARY

1. The effect of tryptamine analogues on the neuromuscular transmission of impulses in the jumping leg of the locust was studied by recording action potentials and active membrane responses with intracellular micro-electrodes, and by recording tension with a mechano-electronic transducer.

2. High concentrations of 5-HT, tryptamine, and a number of tryptamine analogues block the development of an action potential and of tension in fibres of the metathoracic extensor tibialis and flexor tibialis muscle.

3. High concentrations of the same compounds do not block the action potential in the fibres of the crural nerve which supplies 'fast' motor axons to the extensor and flexor tibialis.

4. Tryptamine analogues do not inhibit the active membrane response in fibres of the extensor or flexor tibialis, or alter their membrane resistance.

5. It is concluded that the tryptamine analogues tested block neuromuscular transmission.

We wish to thank Dr Graham Hoyle for advice during the course of our investigations; Mr J. J. Lewis and Dr Martin Smith for discussions on the pharmacological aspects of this paper; Mr S. T. Reid for supplying a sample of 3-(pyrrolidinomethyl)-thionaphthene; and Dr A. Hofman of Sandoz for providing the pKa values listed in Table 1 and for supplying a sample of 5,6-dimethoxytryptamine. This investigation was carried out during the tenure by one of us (R.B.H.) of a post-doctoral fellowship, HF-5211-C2, from the National Heart Institute, United States Public Health Service.

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