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PHARMACOLOGICAL ANALYSIS OF A MONOSYNAPTIC REFLEX IN THE COCKROACH, *PERIPLANETA AMERICANA*

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SUMMARY

1. The monosynaptic connexion between the trochanteral hairplate afferents and the motoneurone D_8 was investigated pharmacologically.
2. Electrical stimulation of the hairplate produced a 1:1 reflex activation of D_8 which was blocked by saline containing 50 mM-Mg^{2+}.
3. Changes in the hairplate-to-D_8 reflex and in the tonic activity of D_8 were monitored during perfusion of drugs known to affect cholinergic transmission.
4. The reflex response was blocked by cholinergic antagonists (atropine, *d*-tubocurarine, α-bungarotoxin, hexamethonium, decamethonium and TMA); and by an inhibitor of ACh synthesis (hemicholinium-3); it was blocked and desensitized by agonists (carbachol and nicotine) and depolarized and blocked by an AChE inhibitor (eserine).
5. These results are consistent with the hypothesis that ACh is a sensory transmitter in the insect CNS.

INTRODUCTION

The actions of pharmacological agents on synaptic transmission in the central nervous system (CNS) of insects have been reviewed by several authors (Colhoun, 1963; Boistel, 1968; Pitman, 1971; Callec, 1974; Gerschenfeld 1973; Pichon, 1974). The most thoroughly studied putative synaptic transmitter in the insect CNS is acetylcholine (ACh), for which a functional role is postulated at the afferent terminals (Pitman, 1971; Gerschenfeld, 1973).

The only known electrophysiologically identifiable and pharmacologically investigated synapse in the insect CNS is the connexion from the cercal afferents to the abdominal giant axons (c.n.-to-g.a.) in the cockroach (Pumphrey & Rawdon-Smith, 1937). During the past six years this synapse has been well studied using the whole ganglion perfusion technique. Callec (1974) summarized the evidence on the nature of the c.n.-to-g.a. synapse in the cockroach: it is monosynaptic and chemical; it is

blocked by cholinergic antagonists; iontophoresis of ACh causes depolarization of the giant fibres; and the ACh agonists, nicotine and acetyl-β-methylcholine, produce a depolarizing effect on the whole ganglion, A_6. These studies strongly indicate that ACh may be the transmitter of the cercal afferent terminals (Shankland, Rose & Donniger, 1971; Callec, 1974; Satelle *et al.* 1976).

Recently, Wong and Pearson (1976) used extracellular recording methods to demonstrate a monosynaptic pathway in the metathoracic ganglion of the cockroach. The pathway consists of afferent fibres arising from a sensory organ, the trochanteral hairplate, and an identified motoneurone, D_8, which produces femoral extension during walking. Pearson, Wong & Fourtner, (1976) used intracellular recording techniques to show that stimuli applied to the hairplate will produce short-latency EPSPs in the neurites of D_8 ($\sim$ 0·4–0·5 ms delay between afferent terminal spike and onset of EPSP). In addition, they demonstrated that the amplitude of the EPSP was increased by the passage of hyperpolarizing current and was decreased following high-frequency stimulation; these results indicate that the EPSPs are probably chemical in nature (Pearson *et al.* 1976).

Pharmacological studies of the thoracic ganglion of the cockroach have demonstrated high concentrations of ACh, 95 μg/g (Colhoun, 1958), acetylcholinesterase and choline acetyltransferase (for review see Pitman, 1971; Gerschenfeld, 1973). A few studies, in which ACh was applied to a thoracic ganglia and directly onto unidentified nerves, have demonstrated that ACh may have an excitatory effect on some central elements (Yamasaki and Narahashi, 1958; Kerkut, Pitman & Walker, 1969*a*, *b*; Pitman and Kerkut, 1970; Pitman, 1971); however the synaptic input to these elements is totally unknown.

In the present study we have examined the cholinergic pharmacology of the synapse between the trochanteral hairplate and the motoneurone, D_8. A preliminary account has been published elsewhere (Carr & Fourtner, 1978).

Anatomy

The trochanteral hairplate is a sense organ formed by a group of 50–60 hair sensilla and is located close to the ventral coxa-trochanteral condyle (Pringle, 1940). The hair sensilla rest on the intersegmental membrane of the joint and are displaced by a fold of the membrane during flexion. The axons from the hair sensilla run in nerve 5r5b (notation of Nijenhuis & Dresden, 1952), which joins nerve 5 in the trochanter near the coxa-trochanter-femoral joint (Wong & Pearson, 1976).

The femoral extensor muscles 177D, 177E, 178 and 179 (notation of Carbonell, 1947) are innervated by nerve 5r1 which contains five different motor axons, four of which are spontaneously active (Pearson & Iles, 1970). The largest of the four has been identified by Pringle (1940), and Pearson and Iles (1970) as axon D_8, the slow extensor motoneurone. The other three axons are about equal in size and are inhibitory (Iles & Pearson, 1969). The largest motor axon (D_f) is not spontaneously active, but occasionally spikes can be elicited by strong stimulation of the ipsilateral cercus. Therefore, as demonstrated by Pearson & Iles (1970) it is quite easy to identify the excitatory motor axons in nerve 5r1.

MATERIALS AND METHODS

Adult male cockroaches (*Periplaneta americana*) were obtained from Connecticut Valley Biological Supply Co. (Southampton, Mass. 01073) and maintained in a controlled environment chamber at 25 °C.

Preparation and Recording Chamber

All experiments were carried out at room temperature (22–23 °C). The animal was pinned ventral side up in a Sylgard Chamber which was inclined 30° to allow drainage rostrally so that the animal's spiracles would not be blocked by saline. The head and

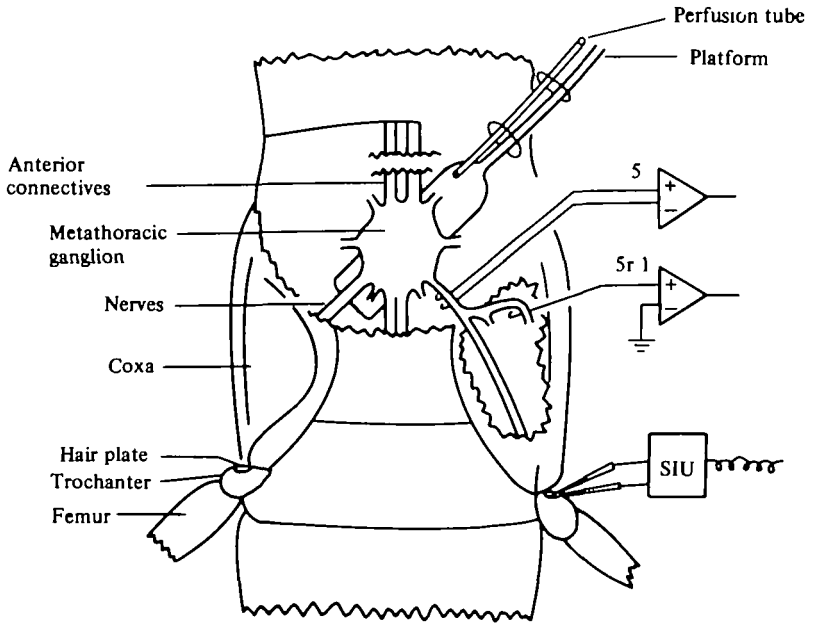

Fig. 1. Schematic diagram showing the experimental preparation and the arrangement of recording electrodes on nerves 5 and 5r1 and stimulating electrodes on the trochanteral hairplate. Ventral side up. SIU = Stimulus Isolation Unit.

the pro- and mesothoracic legs were removed. The right metathoracic leg was pinned ventral side up with the femur extended to expose the trochanteral hairplate (cf. Pearson *et al.* 1976). The cuticle above the metathoracic ganglion was removed together with the underlying fat and tissue deposits. Contralateral nerve 2 was severed so that a stainless steel platform could be placed beneath the metathoracic ganglion. The platform supported the ganglion and oriented the perfusion tube so that saline could flow directly onto the ventral surface of the ganglion (see Fig. 1). The ganglion was then elevated slightly, and the sheath was carefully removed from the entire ventral surface of the ganglion; the ventral tracheae which enter the ganglion near the ventrolateral margin were kept intact. Since the metathoracic ganglion is relatively large (600 × 900 × 400 μm) oxygenation was primarily accomplished via the organism's respiratory system; however, the perfusion fluids were saturated with oxygen as well.

Recording and Stimulating

A pair of 0·05 mm platinum extracellular hook electrodes was placed on nerve 5 to monitor both the afferent input from the hairplate and the efferent activity in D_8 (see Fig. 1). A small section of the ventral coxal cuticle and muscle 178 were removed, exposing the nerve branch 5r1, and a single platinum electrode was placed under 5r1 for *en passant* recording. Due to the long duration of the experiments, it was necessary to record directly from nerve 5r1 rather than rely on the stability of EMG recordings from the femoral extensors as had been done in previous experiments (Wong & Pearson, 1976; Fourtner, Drewes & Holzmann, 1978). Electrical signals were fed via Grass P15 a.c. preamplifiers into a Tektronix D13 storage oscilloscope and were also stored on tapes, using a Sony TC560 recorder, for later data analysis.

The hairplate was stimulated by a pair of fine insect pin electrodes as described by Fourtner *et al.* (1978). Pulses were delivered from an S44 Grass Stimulator via a Grass SIU5 stimulus isolation unit, at a frequency of 3/s throughout the course of the experiment unless otherwise noted.

Physiological saline and perfusion system

All solutions were made up in physiological saline and used within two days. The constituents of the saline were: 124 mM-NaCl, 10 mM-KCl, 8 mM-$MgCl_2$, 20 mM-$CaCl_2$, 40 mM-sucrose, 3·9 mM-3-(N-morpholino) propane sulfonic acid (sodium salt) and 3 mM-(morpholino) propane sulphonic acid (MOPS). The saline had a pH of 7·21. The high magnesium saline was adjusted to maintain osmotic pressure as follows: 50 mM-$MgCl_2$, 1 mM-$CaCl_2$ and 13 mM-sucrose. The MOPS buffer permitted a higher concentration of calcium than was possible with a phosphate buffer. The calcium concentration approximated that estimated for the extracellular space (17·6 mM; Treherne, 1962). The MOPS buffer also permitted oxygenation of the saline with little pH change (7·2–7·4) at saturation (M. Chesler & C. R. Fourtner, unpublished results).

Solutions were delivered by flexible polyethylene tubes from an elevated reservoir to a point 1 mm above the metathoracic ganglion (see Fig. 1) at a rate of three drops per second. All solutions were maintained in a steady, unidirectional flow over the ganglion.

The following cholinergic drugs were used in this study: Carbamylcholine chloride (carbachol), eserine, tetramethyl ammonium hydroxide, d-tubocurarine chloride, atropine H_2SO_4 and nicotine (Sigma Chemical Company); hemicholinium-3 bromide (Aldrich Chemical Company); decamethonium bromide, hexamethonium bromide and α-bungarotoxin (donated by Dr. Richard Almon and prepared according to Mebs *et al.* 1971).

RESULTS

As well as testing the effects of drugs upon the evoked hairplate-to-D_8 reflex, spontaneous activity was monitored to check whether drugs were having indirect effects on the reflex by affecting D_8 excitability. Throughout these experiments it is assumed that if spontaneous activity remains relatively constant before, during and

after drug application, then the efficacy of any synaptic input to evoke a spike in D_8 should remain constant unless the drug had a direct influence on that synaptic site.

We have used this indirect method for investigating this synapse rather than the direct intracellular method for three reasons. First, although impalement of the neurite of D_8 is plausible, it is not possible to maintain the impalement for a sufficient

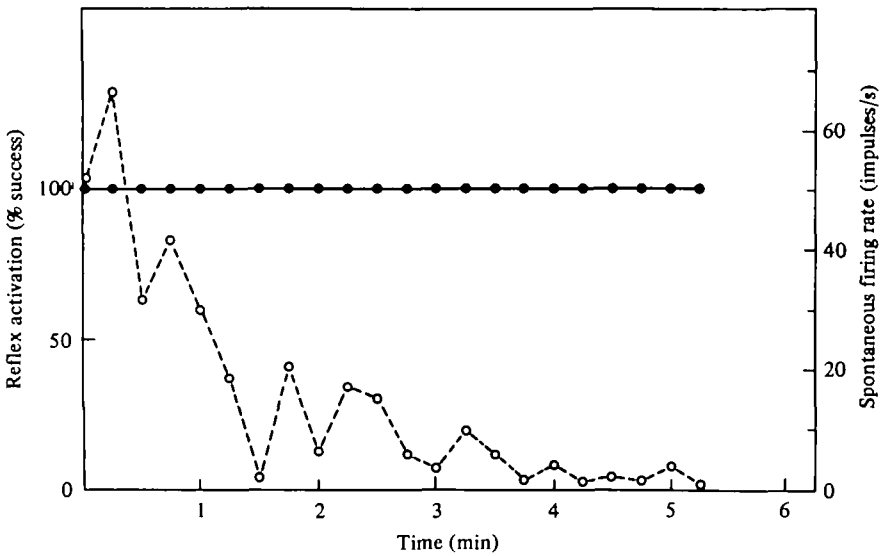

Fig. 2. Spontaneous and reflexly evoked activity in motoneruone D_8. Throughout the duration of the experiment the evoked activity (●) remains constant while the spontaneous firing rate (○) declines (n = 10 bouts).

length of time to complete a control-application-washout experiment. Second, penetration in different regions of the neurite will give different EPSP amplitudes (C. R. Fourtner, unpublished observations); therefore, the results of independent penetration at various times during the experiment would be difficult to interpret. Third, although the soma can be impaled and held for several hours, little synaptic activity can be measured in the soma.

Spontaneous Activity

The resting discharge rate of D_8 varied between 10 and 70 impulses/s, and the rates measured during the five minute periods immediately preceding and subsequent to the desheathing operation were not significantly different (4 preparations). However, the rate during the operation was increased. In five experimets the spontaneous activity was monitored for five hours with constant saline perfusion. After five hours spontaneous activity declined, but D_8 was still active in all cases (see Fig. 2, open circles).

Evoked Activity

The stability of the reflex was examined in five experiments of 5 or 6 hours duration. The hairplate was stimulated for 5 min at 15-min intervals. To determine the constancy and the efficacy of the reflex, for each 5 min of stimulation, 10 bouts of reflex

activity were analysed; each bout consisted of 10 successive stimuli. For any 5 min of stimulation 100 stimuli were analysed to determine if a D_s spike followed the compound afferent action potential. The percentage success of the reflex activation was then calculated. Examples of the reflex evoked activity have been published elsewhere (Wong & Pearson, 1976; Fourtner *et al*, 1978).

The hairplate-to-D_s reflex was demonstrated to remain fairly stable over the 5-h period (Fig. 2). In some experiments failures of D_s occurred when the spontaneous firing rate was greater than 90 impulses/s or in a few cases where the spontaneous activity was very low, less than 2 impulses/s.

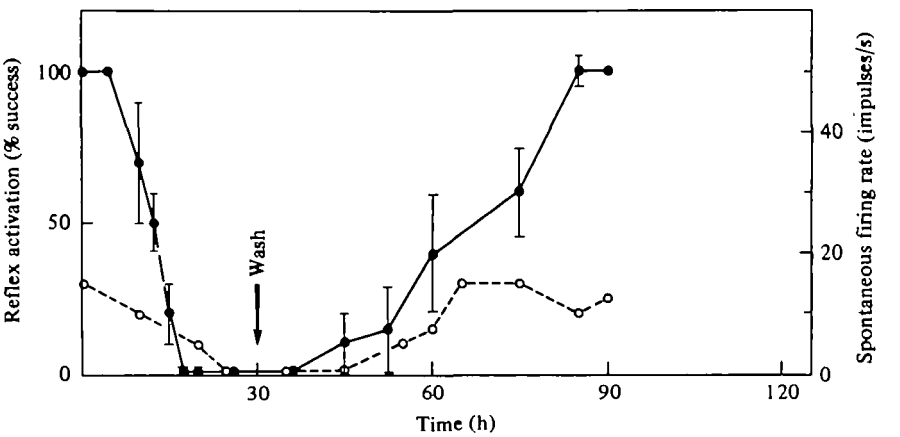

Fig. 3. Action of high magnesium saline (50 mM) on the D_s response to hairplate stimulation in one preparation (● = reflex activity; ○ = spontaneous firing rate). Bar is ±S.D., n = 10 bouts.

Block of Synaptic Transmission with High Magnesium

Pearson *et al.* (1976) provided substantial evidence that the hairplate-to-D_s reflex was monosynaptic and chemical in nature although their experiments with high magnesium (50 mM) did not support the latter (see Discussion). We therefore re-examined the effect of high magnesium on the reflex. The reflex was blocked in the three preparations tested (average time to block 19 minutes). The spontaneous activity of D_s also declined and was eventually blocked, but in all three experiments the reflex was blocked before spontaneous activity subsided (Fig. 3). Our results and the fact that the block was easily reversed (Fig. 3) support Pearson & Wong's argument for a monosynaptic and chemically mediated hairplate-to-D_s reflex.

Pharmacological Results

The actions of various cholinergic drugs on the hairplate-to-D_s reflex were tested.

Carbamylcholine (carbachol). Perfusion of the desheathed ganglion with carbachol (10^{-3} M, 4 experiments) produced an immediate increase in the spontaneous firing rate of D_s and a decline in the successful activation of a D_s spike by hairplate stimulation (Fig. 4A). Complete blockage of both the spontaneous activity and the reflex occurred within 25–30 min following the onset of perfusion. After approximately 85 min the spontaneous activity reappeared, but the reflex never recovered. Perfusion

with 10^{-2} M carbachol (3 experiments) produced reflex block (5–8 min), and an initial increase in spontaneous activity was followed by a block (Fig. 4B).

Nicotine. Perfusion with nicotine (10^{-4} M, 3 experiments; 10^{-3} M, 2 experiments) produced similar results. The spontaneous activity increased and then was depressed or blocked; the hairplate-to-D_8 reflex was blocked after 35 and 5 minutes with

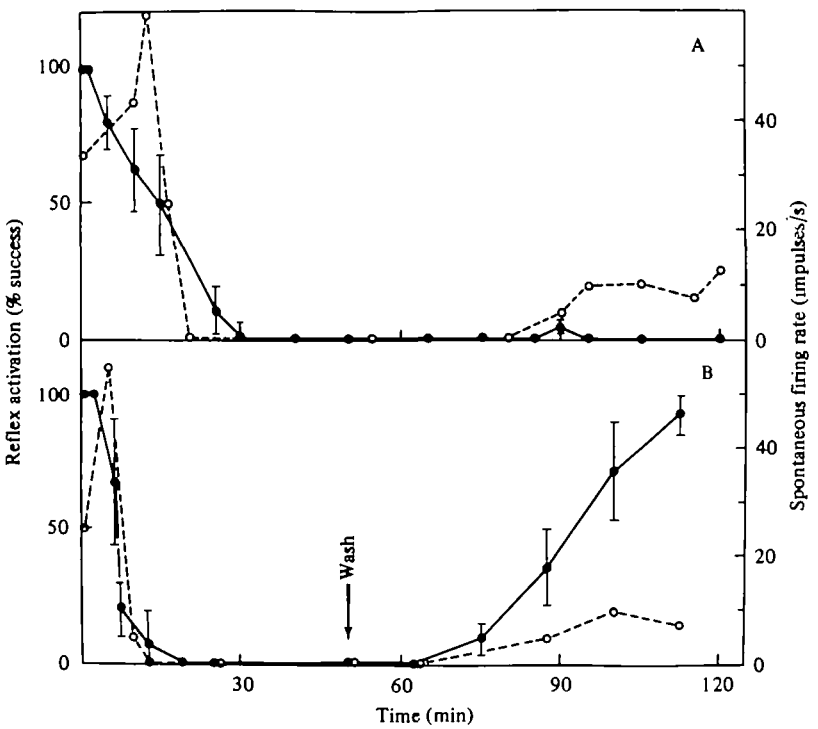

Fig. 4. Response of D_8 to hairplate stimulation during perfusion of carbachol in two different preparations. (A) 10^{-2} M carbachol. Note return of spontaneous firing in D_8 (○), although reflex response (●) does not return. (B) 10^{-2} M carbachol. Note reflex (●) returns after saline wash. Bar is ±S.D., n = 10 bouts.

10^{-4}M and 10^{-3} M-nicotine respectively. Upon continued perfusion with nicotine the spontaneous activity in D_8 returned (approximately 60 min).

d-Tubocurare (d-TD). Perfusion of d-TC (10^{-4} M, 5 experiments) blocked the hairplate-to-D_8 reflex in 75 min but had no effect on the spontaneous firing rate in D_8 (Fig. 5A). 10^{-3} M-d-TC blocked the hairplate-to-D_8 reflex in 5–15 min (3 experiments), and this block was accompanied by a decline in the spontaneous firing rate of D_8 (Fig. 5B). The effect of 10^{-3} M-d-TC was reversible. Reflex activity returned after 3–4 h of washing.

α-Bungarotoxin. Perfusion with α-bungarotoxin (10^{-7} M, 2 experiments) blocked the hairplate-to-D_8 reflex in 30 min whereas the spontaneous activity in D_8 was unchanged (Fig. 6A). In two additional experiments using 10^{-6} M α-bungarotoxin, the hairplate-to-D_8 reflex was blocked in 8 min and again the spontaneous activity was not altered (Fig. 6B).

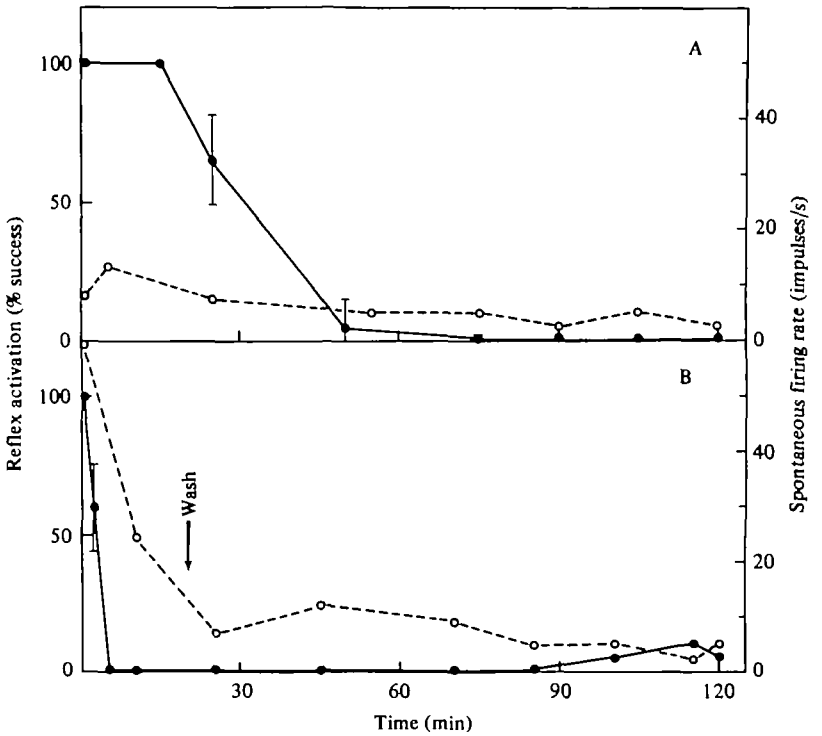

Fig. 5. Response of D_4 to hairplate stimulation during perfusion of d-Tubocurarine in two different preparations (● = reflex activity; ○ = spontaneous activity). (A) 10^{-4} M d-Tubocurarine (B) 10^{-3} M d-Tubocurarine. Bar is ±S.D., n = 10 bouts.

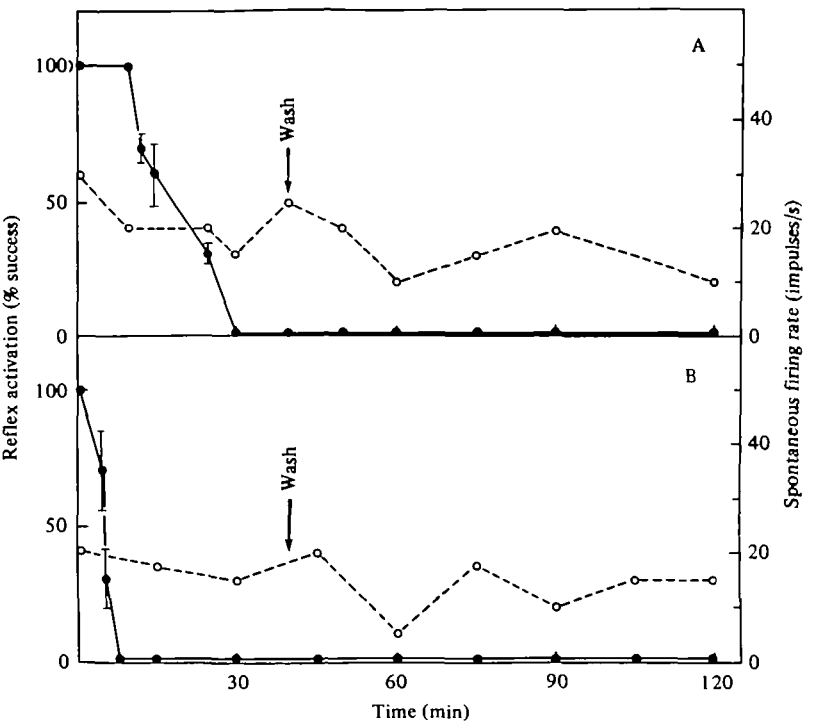

Fig. 6. Response of D_4 to hairplate stimulation during perfusion of α-Bungarotoxin. (A) 10^{-7} M α-Bungarotoxin. (B) 10^{-6} M α-Bungarotoxin. Note rapid block of reflex response (●) without a decrease in spontaneous activity (○). Bar is ±S.D., n = 10.

Tetramethyl ammonium (*TMA*). Perfusion of TMA (10^{-4} M, 2 experiments) blocked the hairplate-to-D_8 reflex in 90 min. The spontaneous activity showed a rapid increase immediately after perfusion began, but within 10 min was totally blocked. Thus the reflex continued to respond for about 80 min after the blockage of spontaneous activity. 10^{-3} M TMA blocked the reflex response in 5 min (3 experiments) with a concomitant increase and then block of spontaneous activity.

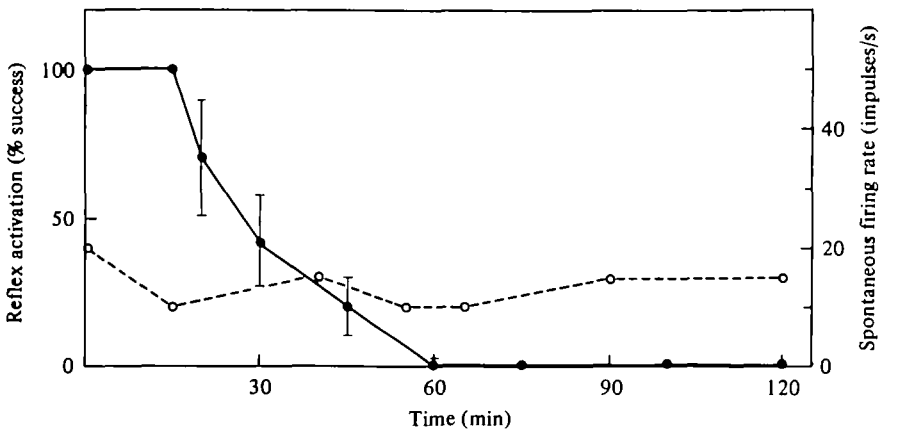

Fig. 7. Response of D_8 to hairplate stimulation during perfusion of 10^{-4} M Hemicholinium-3. Note complete blockage of reflex (●) and maintenance of spontaneous activity (○). Bar is ±S.D., $n = 10$.

Atropine. Atropine (10^{-4} M, 5 experiments) blocked the hairplate-to-D_8 reflex in 3 h whereas the spontaneous activity was not affected. 10^{-3} M-atropine blocked the reflex in 10–20 min (3 experiments). This blockage was accompanied by a decline in the spontaneous activity. The washout time to restore the reflex was 5 h.

Decamethonium (C_{10}). C_{10} at 10^{-3} M (2 experiments) blocked the reflex after 45 min and the spontaneous activity remained unchanged.

Hexamethonium (C_6). Perfusion of C_6 at 10^{-3} M (2 experiments) blocked the reflex in 30 min. The spontaneous activity showed a transient increase followed by a block which occurred at the same time that the hairplate-to-D_8 reflex was blocked.

Hemicholinium-3 (*HC*-3). It is suggested that HC-3 decreases ACh stores presynaptically by competing with choline for ACh production. Therefore the stimulation procedure for testing this drug was slightly altered. The hairplate was stimulated continuously at 3/s following the onset of perfusion. In the presence of 10^{-4} M-HC-3 we found that the reflex began to fail after 15 min of stimulation and was completely blocked after 60–75 min (Fig. 7). Spontaneous activity in D_8 remained essentially unchanged.

Eserine. The effects of 10^{-3} M and 10^{-4} M-eserine were essentially the same in five experiments. The hairplate-to-D_8 reflex was blocked within 10 min, and the spontaneous firing rate was also decreased and/or blocked within 10 min. With continued perfusion of 10^{-3} M-eserine the spontaneous activity later unblocked; however, the hairplate-to-D_8 reflex did not recover.

DISCUSSION

Wong & Pearson (1976) and Pearson *et al.* (1976) presented evidence that the reflexive connexion between the afferents of the trochanteral hairplate and the femoral extensor motoneurone was monosynaptic and chemical in nature. In the present study we provide further evidence that the synapse is chemical in nature, for the reflex is blocked after 20 min exposure of desheathed ganglia to a high magnesium concentration. Pearson *et al.* did not observe a block in such experiments, possibly because they employed too short an exposure time (not given).

Table 1. *Action of cholinergic agents on the D_8 response to hairplate stimulation*

Drug	Conc.	N	Effect on spontaneous firing rate	Mean time to block reflex (min)
d-Tubocurarine Cl	10^{-4}	5	None	75
	10^{-3}	3	Decrease	10
α-Bungarotoxin	10^{-7}	2	None	30
	10^{-6}	2	None	8
Atropine	10^{-4}	5	None	180
	10^{-3}	3	None	15
Tetramethyl ammonium hydroxide	10^{-4}	2	Block	90
	10^{-3}	3	Block	5
Hexamethonium	10^{-3}	2	Block	30
Decamethonium	10^{-3}	2	None	45
Carbachol	10^{-3}	3	Increases, then blocks	30
	10^{-2}	4	Increases, then blocks	10
Nicotine	10^{-4}	3	Increases, then declines	35
	10^{-3}	2	Increases, then blocks	5
Hemicholinium-3	10^{-4}	4	None	65
Eserine	10^{-4}	3	Increases, then blocks	10
	10^{-3}	4	Increases, then blocks	10

In this study, changes in the successful activation of the hairplate-to-D_8 reflex and changes in the level of spontaneous activity in D_8 were monitored during the perfusion of drugs known to affect cholinergic transmission. The results are summarized in Table 1.

ACh antagonists

d-Tubocurarine. Curare has frequently been applied to the insect nervous system, with conflicting results, and its sites of action are still open to debate (Roeder, 1948; Flattum, Friedman & Larsen, 1967; Kerkut *et al.* (1969*a*, *b*). Curare has been shown to affect axonal conduction at high concentrations, which may account for paralysis caused by its injection into insects (Friedman & Carlson, 1970). Curare has also been shown to block giant fibre responses in the cockroach (Shankland *et al.* 1971); this effect was not observed previously by Roeder (1948), presumably due to restricted penetration into synaptic sites, and the inability to control the electrical activity in the experimental preparation (Callec, 1974). Shankland *et al.* (1971) found that 10^{-5} M d-TC produced a synaptic block in desheathed ganglia in 20–40 min of perfusion. Synaptic block of the D_8 reflex occurred at higher concentrations, similar to those

(around 10^{-5} mM) used by Kerkut *et al.* (1969*b*), who found that all activity in an isolated metathoracic ganglion preparation was abolished in 10 min.

In our results the hairplate-to-D_8 reflex was blocked in 75 min by 10^{-4} M d-TC and within 5 min by 10^{-3} M d-TC perfusion. Since the spontaneous activity in D_8 continued and the afferent volley could still be electrically evoked, the loss of the reflex could not be due to a conduction block in the afferent or efferent axons and thus most likely occurred at the synapse between the hairplate afferents and D_8.

α-Bungarotoxin. α-Bungarotoxin appears to bind specifically and tightly to nicotinic ACh receptors in vertebrates (Changeux, Kasai & Lee, 1970), and an α-bungarotoxin binding component with the expected properties of an ACh receptor has been characterized in abdominal nerve cords of the cockroach (Gepner, Hall & Sattelle, 1978). The toxin has also been shown to bind to arthropod ACh receptors (Sanes & Hildebrand, 1976).

Marder (1976) found that synaptic transmission at a cholinergic synapse in the lobster was blocked by 10^{-6} M-α-bungarotoxin, 10^{-3} M-atropine or 10^{-4} M d-TC; and Gepner *et al.* (1978) have shown that α-bungarotoxin blocks transmission at the c.n.-to-g.a. synapses in the cockroach. These results are similar to ours, and it is reasonable to assume that the synaptic block of the hairplate-to-D_8 reflex caused by 10^{-6} M and 10^{-7} M-α-bungarotoxin was due to blockage of the cholinergic receptor and that the low concentrations used were due to its high affinity for nicotinic receptors (Dudai, 1977). As with d-TC, α-bungarotoxin blocked the reflex without affecting the spontaneous activity in D_8.

Atropine. Atropine was originally thought to be as ineffective as curare in the intact insect ganglion (Roeder, 1948). However, this lack of effect was probably due to lack of penetration of the drug into the ganglion (cf. Gerschenfeld, 1973). Shankland *et al.* (1971) found 10^{-3} M-atropine blocked synaptic transmission in 30–100 min in the abdominal ganglion of the cockroach, while Kerkut *et al.* (1969*b*) found that around 3×10^{-3} M blocked the action of ACh in the metathoracic ganglion. In crustacean systems Marder (1976) found 10^{-3} M-atropine blocked cholinergic synaptic transmission. The hairplate-to-D_8 reflex was blocked by similar concentrations of atropine; 10^{-4} M-atropine blocked the reflex in 3 h, and 10^{-3} M-atropine blocked the reflex in 10 min.

TMA, C_{10}, C_6. These are depolarizing ACh blockers. Flattum and Shankland (1971) demonstrated that TMA (10^{-3} M) a nicotinic antagonist, blocked the c.n.-to-g.a. synapse in the cockroach; Marder (1976) and Ascher, Maitz & Neild, (1978) found that C_6 and C_{10} blocked cholingergic synapses in the lobster and *Aplysia*, respectively, at concentrations similar to those which blocked the hairplate-to-D_8 reflex in this study. In the present study, TMA and C_6 blocked spontaneous activity, so these two compounds did not selectively block the reflex.

In summary, these data on the effects of ACh antagonists support the hypothesis that the hairplate-to-D_8 synapse is cholinergic. The data also suggest that the ACh receptors on motoneurone D_8 may have both nicotinic and muscarinic components. This conclusion is consistent with other pharmacological studies on insect CNS (Sanes, Hildebrand & Prescot, 1976; Eldefrawi & O'Brien, 1971; Dudai, 1977; Schmidt-Nielson *et al.* 1977).

ACh Agonists. ACh analogues such as carbachol and nicotine are highly resistant to

hydrolysis by either AChE or non-specific cholinesterase. They consequently might be more potent than ACh in eliciting activity in D_s initially although continued exposure would be expected to block the response due to desensitization of D_s.

Carbachol. 10^{-3} M carbachol produced an increase in spontaneous firing rate of D_s, followed by a decline in activity. The reflex was abolished within 30 min. Perfusion with 10^{-2} M carbachol eliminated the reflex in approximately 8 min and caused a similar excitation followed by a block of spontaneous activity. These results correspond to those obtained by Sattelle *et al.* (1976) who found a rapid reduction and block of the c.n.-to-g.a. synapse of the cockroach during perfusion of carbachol. Long exposures to carbachol produced a repolarization of neural elements in the ganglion. This attenuation of the ganglionic response, expressed as a return of spontaneous activity in D_s, is an important secondary effect of ACh and its analogues. This effect is the desensitization described by Katz & Thesleff (1957), a condition where the post-synaptic receptor becomes refractory to depolarizing agents. Sattelle *et al.* noted a complete repolarization within about an hour of continuous application of 10^{-3} M carbachol, but not the return of the giant fibre EPSP. Similar results were obtained in our experiments, as the spontaneous activity returned within an hour, while the reflex response did not; therefore it is probable that synaptic desensitization occurred.

Nicotine. Flattum and Shankland (1971) examined the action of nicotine on the sixth abdominal ganglion of the cockroach and demonstrated a diphasic action with blockage of the c.n.-to-g.a. synapse, followed by desensitization.

A similar diphasic action of nicotine was observed in the hairplate-to-D_s reflex and in the spontaneous activity of D_s. At a concentration of 10^{-4} M nicotine the reflex blocked in 35 min, compared to 4–5 min observed by Flattum and Shankland (1971). This difference may be attributed to a more effective barrier to accessibility in the metathoracic ganglion or to a difference in the method of drug application (they employed a bath application), rather than a decreased sensitivity. Perfusion with 10^{-3} M nicotine blocked the hairplate-to-D_s reflex within 5 min, compared to 2 min for the c.n.-to-g.a. synapse. Desensitization was also slower in our experiments, 120 min or longer compared with 20–25 min. This would seem to indicate a restricted penetration to synaptic sites, as described by Smith and Treherne (1963).

Enzymatic Blockers

Hemicholinium-3. Hemicholinium-3 (HC-3) is known to decrease ACh stores presynaptically by competing with choline for ACh production in mammals (Birks & MacIntosh, 1961). HC-3 was used by Miller & McCann (1971) to block an EPSP in an insect motoneurone although the site of its action was unknown. Shankland *et al.* (1971) found perfusion with 10^{-3} M HC-3 reduced synaptic transmission at the c.n.-to-g.a. synapse with complete blockage occurring after 60–100 min of stimulation (3 pulses/s). The hairplate-to-D_s reflex appears somewhat more sensitive to HC-3 since 10^{-4} M completely blocks the reflex following one hour of stimulation (3 pulses/s), and suggests a more rapid turnover of transmitter in the hairplate afferent terminal than in the cercal afferent terminals.

Eserine. Roeder (1948) found that the anti-acetylcholinesterase, eserine, (10^{-4} M–10^{-5} M) blocked synaptic transmission in the c.n.-to-g.a. synapse and also caused an increase in, or facilitation of, spontaneous activity which persisted long after synaptic

transmission had been blocked. Yamasaki and Narahashi (1958) found similar effects, with 10^{-5} M eserine blocking synaptic transmission after 30–90 min by gradual depolarization of the neural elements of the ganglion. Callec (1974) found that concentrations of more than 10^{-6} M eserine depolarized the postsynaptic fibre very quickly. Barker *et al.* (1972) observed a large potentiation of an EPSP in the lobster abdominal ganglion with iontophoresis of 5×10^{-4} M-neostigmine. The perfusion of 10^{-3} M and 10^{-4} M eserine produced a desensitization of the hairplate-to-D_8 reflex in 10 min. It caused an increase in the spontaneous firing rate in D_8 which was probably due to depolarization of the post-synaptic fibre. The action of eserine suggests the presence of cholinesterase in the metathoracic ganglion, and supports the hypothesis that the transmitter at the synapse is ACh. However, it should be noted that Schmidt-Nielsen *et al.* (1977) have shown that high concentrations of eserine inhibit α-bungarotoxin binding sites in *Drosophila*, and therefore eserine may have a post-synaptic effect as well.

The results are consistent with the hypothesis that the hairplate-to-D_8 synapse is cholinergic although rather high drug concentrations were used throughout the study and the sheath surrounding the ganglion had been removed. It may be that higher concentrations of drugs are required with the perfusion method of application employed here, rather than bath application. Removal of the sheath is often necessary to allow drugs to penetrate insect ganglia (Gerschenfeld, 1973). Another valid criticism of our method is that it is not possible to determine if the drugs acted directly on the hairplate-to-D_8 synapse or indirectly at some interneuronal level presynaptic to D_8 or even presynaptic to the afferent terminals. To prove conclusively that ACh is the transmitter at the hairplate afferent to motoneurone D_8 synapse, one would have to record intracellularly from the motoneurone while concurrently stimulating the afferent terminal and applying drugs. With the present techniques and the duration of the experiments this particular approach is not feasible.

In conclusion, other results for the cockroach suggests that ACh is the specific afferent transmitter at the cercal afferent-to-giant synapse (sensory to interneurone) and our results suggest that ACh is the transmitter at the hairplate-to-D_8 synapse (sensory to motoneurone).

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