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A note on the effects of some cholinergic and anticholinergic drugs on the aggressive behaviour and spontaneous electrical activity of the central nervous system in the ant, *Formica rufa*

WOJCIECH KOSTOWSKI*

The influence of various cholinergic and anticholinergic drugs on the aggressive behaviour and electroencephalogram was investigated in the ant, *Formica rufa*. Both atropine and scopolamine decreased aggressiveness and electrical activity of the brain whilst tubocurarine caused an opposite effect. The possible role of nicotinic and muscarinic receptors in the central nervous system of the ant is discussed.

THE role of acetylcholine in the central nervous system of insects is not established. Effects obtained with various cholinergic drugs might be taken to indicate the existence of cholinergic transmission mechanisms (Schallek & Wiersma, 1948, 1949; Schallek, Wiersma & Alles, 1948). On the other hand, eserine does not increase the action of acetylcholine in preparations of insect terminal abdominal ganglia (Prosser, 1940; Turner, Hagins & Moore, 1950).

Acetylcholine in the insect nervous system has been identified by various techniques (Chang & Kearns, 1955; Lewis & Smallman, 1956; Colhoun, 1958). Very high concentrations of acetylcholine (143 μg/g tissue wet weight) and acetylcholinesterase (137 μg acetylcholine hydrolysed g/tissue/hr) were obtained in the cockroach (*Periplaneta americana*) brain (Colhoun, 1958, 1959).

We have assessed the influence of some cholinergic and anticholinergic drugs on the aggressive behaviour and spontaneous electrical activity of the central nervous system in the ant, *Formica rufa*. This is an extension of previous work from our laboratory, concerned with the action of some neurohormones and psychotropic drugs on the brain of this species (Kostowski, Beck & Mészáros, 1965, 1966; Kostowski, 1966).

EXPERIMENTAL

Aggressiveness of ants. The modified test of ants attacking a beetle was used (Kostowski, 1966). 15 ants, *F. rufa*, were placed in a Petri dish (10 cm in diameter), surrounded with water. After 15 min a beetle, *Geotrupes* sp., was placed in the dish and the number of attacking ants was counted after 1, 2 and 3 min. The three counts of attacking ants were averaged and multiplied by ten to give an index of aggressiveness (Kostowski, 1966). Nicotine and (+)-tubocurarine dissolved in 0·6% saline were injected into the abdominal cavity by microsyringe in amounts of 0·5–1·0 μlitre. All other drugs were administered orally in honey.

From the Department of Pharmacology, Medical Academy of Warsaw, Warsaw, Poland.

* Present address: Istituto di Ricerche Pharmacologiche "Mario Negri", Via Eritrea 62, Milan, Italy.

Spontaneous electrical activity of ant brain. The electroencephalogram (EEG) was recorded from the optic lobes of the ant by the method of Kostowski & others (1966). Tungsten wire electrodes 40–50 μ in diameter, insulated by epoxide varnish were used. The EEG records from the optic lobe surface were made with a Biofizpribor (USSR) EEG apparatus. All drugs were injected in 0·6% saline into the abdominal cavity in 0·5 μlitre amounts with a microsyringe.

Drugs. The drugs used were: pilocarpine hydrochloride (Polfa), eserine salicylate (Vis), atropine sulphate (Polfa), scopolamine hydrobromide (Polfa), nicotine sulphate (Tescat Lab), (+)-tubocurarine chloride (Burroughs Wellcome).

RESULTS

Aggressiveness of ants. The results are in Fig. 1. In control experiments the index of aggressiveness (IA) was 67·8. Atropine or scopolamine (0·3–0·5 μg/mg body weight) decreased aggressiveness after 2–4 hr (IA = 44·8 and 47·5 respectively). Tubocurarine (0·1–0·2 μg/mg) increased the aggressiveness after 0·5–1·0 hr (IA = 76·5). A slight but not

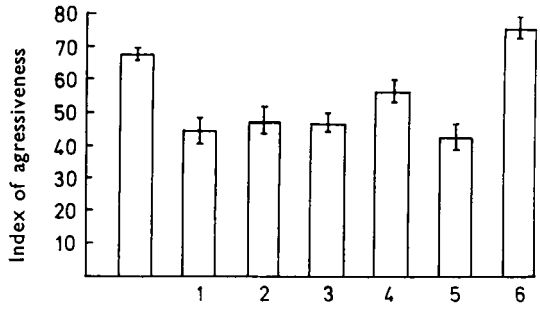

FIG. 1. The effect of drugs on aggressiveness of ants. Unlabelled column, control (40 experiments). 1, Atropine (15 exp.), 2, scopolamine (13 exp.). 3, Pilocarpine (18 exp.). 4, Eserine (25 exp.). 5, Nicotine (10 exp.). 6, Tubocurarine (10 exp.). Vertical bars represent the standard error. Statistically significant differences were calculated according to Student's *t*-test. They are: $P < 0{\cdot}02$ for 1, 2 and 3. $P < 0{\cdot}05$ for 4 and 6. Differences were significant between 5 and 6 ($P < 0{\cdot}002$), 3 and 4 ($P < 0{\cdot}005$), 4 and 5 ($P < 0{\cdot}05$), and 1 and 4 ($P < 0{\cdot}05$).

significant fall of aggressiveness was observed 2–4 hr after eserine (0·1–0·15 μg/mg) whilst both pilocarpine (0·5 μg/mg) and nicotine (0·05 μg/mg) markedly decreased aggressiveness (IA = 47·2 and 43·7 respectively) after 1–2 hr. Toxic effects such as rigor, ataxia and death were observed after larger doses of nicotine (1–2·0 μg/mg). Tremor and clonic movements of the extremities were sporadically observed after the usual doses of eserine. Aggressiveness between ants, disturbances of locomotor activity, inability to maintain an upright posture and slight ataxia occurred 0·5–1 hr after injection of tubocurarine.

Spontaneous electrical activity of ant brain. The characteristic EEG pattern of the optic lobe of the ant in control experiments consisted of

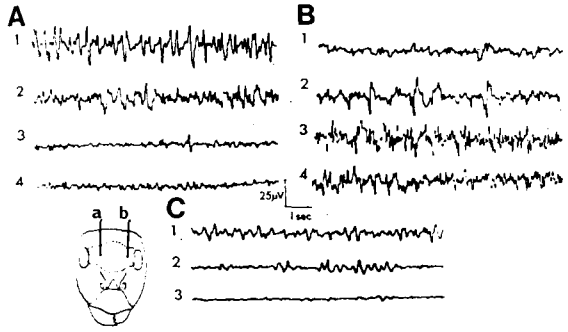

FIG. 2. EEG of ants. Points of leads marked on the diagram of the head. 1, Control. 2, 2 min; 3, 5 min; 4, 20 min after A, scopolamine or B, pilocarpine (0·1 μg/mg weight). C, 1, Control, 2, 5 min; 3, 15 min after atropine (0·2 μg/mg).

potential changes of 5–50 μV with a frequency of 3–6/sec. Of the drugs investigated, pilocarpine and tubocurarine (0·1 μg/mg) increased the amplitude and frequency of the potential changes. 5–15 min after injection of drugs, the frequency rose as high as 8–12/sec and the amplitude sporadically to 100 μV (Figs 2 and 3). This effect persisted 30 min or more. Both scopolamine and atropine (0·1–0·2 μg/mg) caused a decrease of amplitude 5–15 min after administration (Fig. 2). A similar effect

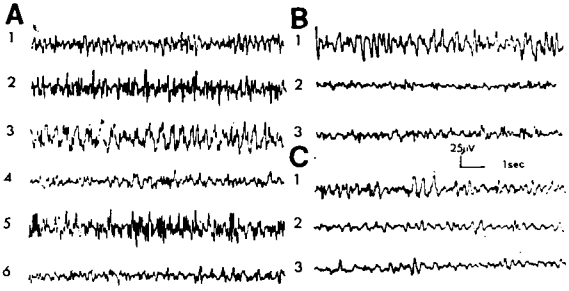

FIG. 3. EEG of ants. A 1, Control. 2, 5 min; 3, 15 min; 4, 35 min after tubocurarine (0·1 μg/mg). 5, 5 min; 6, 20 min after a further injection of tubocurarine (0·1 μg/mg). B. 1, Control; 2, 5 min; 3, 15 min after nicotine (0·05 μg/mg). C. 1, Control. 2, 5 min; 3, 15 min; after eserine (0·1 μg/mg).

was observed 5–15 min after nicotine (0·05 μg/mg). Eserine (0·1 μg/mg) did not cause a clear effect. 8–10 experiments were made with each drug. The results are summarized in Table 1.

DISCUSSION

On the basis of the results it may be possible to postulate the existence of cholinergic transmission in the central nervous system of the ant. Cholinergic blocking agents such as atropine or scopolamine decreased the aggressiveness and the amplitude of the EEG record. On the other

hand, the muscarinic stimulant pilocarpine caused an increase of amplitude and frequency of the EEG record but the effect on aggressiveness was similar to that obtained with atropine or scopolamine. The nicotinic and antinicotinic drugs caused opposite effects on aggressiveness as well as on spontaneous electrical activity of the ant brain. Decreased aggressiveness and increased amplitude of the EEG record were observed after nicotine. Tubocurarine increased aggressiveness and caused the appearance of high amplitude, fast waves in the EEG. It seems possible that

TABLE 1. THE EFFECT OF DRUGS ON AGGRESSIVENESS AND EEG PATTERN OF THE ANT, *F. rufa*.

Drug	Aggressiveness	Changes in EEG pattern	
		Amplitude	Frequency
Pilocarpine	Decreased	Increased	Increased
Eserine	Slightly decreased	Slight decrease or no change	No change
Nicotine	Decreased	Decreased	Decreased or no change
Atropine	Decreased	Decreased	No change
Scopolamine ..	Decreased	Decreased	No change
D-Tubocurarine ..	Increased	Increased	Increased

these effects might be related to the existence of both nicotinic and muscarinic receptors in the central nervous system of the ant. Since the antinicotinic drug, tubocurarine, produces excitation of the CNS of the ant we may suppose that nicotinic receptors are involved in inhibition in central pathways. Although some of the drug responses are contradictory it may be possible that muscarinic receptors in the central nervous system are involved in excitation or in excitation and inhibition but further investigation is needed.

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