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The pharmacology of the cholinoceptor in muscle preparations of *Ascaris lumbricoides* var. *suum*

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1. The preparation of a muscle strip of *Ascaris lumbricoides* for the study of the effect of drugs *in vitro* is described.

2. Stimulant drugs which are classified as nicotine-like in mammalian pharmacology increased the isometric tension of this preparation. These drugs were, in descending order of potency: dimethylphenylpiperazinium, nicotine, acetylcholine, carbachol, decamethonium and pyridine-2-aldoxime methiodide.

3. Muscarine-like drugs (oxotremorine, methacholine, pilocarpine) had no activity.

4. Potassium and barium ions stimulated the tissue, while the anti-cholinesterases, dichlorvos and eserine, increased the resting tension of the preparation and potentiated the responses to acetylcholine.

5. Adrenaline neither stimulated the tissue nor affected the responses to nicotine-like drugs.

6. The relative potency of several blocking agents which antagonize the responses to nicotine-like drugs was assayed. These blocking agents were, in descending order of potency: mecamylamine, (+)-tubocurarine, hexamethonium, atropine and piperazine. Acetylcholine, dimethylphenylpiperazinium and pyridine-2-aldoxime methiodide apparently act on a common receptor, for each blocking agent had a similar molar inhibitory concentration against these stimulants.

7. It is concluded that the cholinoceptor in muscle preparations of *Ascaris lumbricoides* is pharmacologically similar to that of the mammalian autonomic ganglion.

An acetylcholine-like substance has been demonstrated in nematodes (Mellanby, 1955), and Guerra (1968) reported the presence of two choline esters in extracts of *Ascaris lumbricoides* which correspond chromatographically to acetylcholine and proprionylcholine. Hart & Lee (1966) demonstrated the presence of a non-specific cholinesterase in *Ascaris* which is associated with the nervous system and musculature, while Knowles & Casida (1966) found homogenates prepared from the anterior

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5 mm section of the worm to be most active in hydrolysing acetylcholine. Acetylcholinesterase appears to have a functional role in the nervous activity of the parasites (vonBrand, 1966), for histochemical evidence shows this enzyme to be localized within the elements of the nervous system, and the response of the ascarid muscle to acetylcholine is potentiated by eserine (Norton & de Beer, 1957).

One of the most extensively used ascarifuges in both human and veterinary medicine is piperazine. Its effects are specific for intestinal nematodes, and toxic side effects in the host are minimal (Saz & Bueding, 1966). Del Castillo, Morales & Sanchez (1963) concluded from electrophysiological studies that the nerve cord and syncytium of muscle cells in the ascarid have electrical properties similar to those of vertebrate involuntary muscle. Piperazine inhibits both the electrical and motor activities of *Ascaris*, and this inhibition may be reversed by washing (del Castillo *et al.*, 1963 ; Standen, 1955). The inhibition of motor activity resulting from stimulation of the " head " of the worm (Goodwin & Vaughan Williams, 1963) was shown not to be involved in the inhibitory action of piperazine (del Castillo *et al.*, 1963).

The qualitative actions of various pharmacological agents on the ascarid have been reported by Toscano-Rico (1926), Baldwin & Moyle (1949) and by Norton & de Beer (1957). Although these workers generally agree in their findings, inconsistencies in their reported results nevertheless are apparent. This may be attributed to the variety of preparations of *Ascaris* used, and to the development of knowledge of the pharmacology of the agents examined over the period separating the findings of these workers.

The following paper describes a qualitative and quantitative study to define the nature of the cholinoceptor in muscle preparations of *Ascaris lumbricoides* var. *suum*.

Methods

Ascaris worms were collected from the intestines of freshly slaughtered pigs and stored in a bathing fluid at 37° C for not more than 5 days. The bathing fluid, which contained (g/1,000 ml.) NaCl, 7·9 ; KCl, 0·175 ; $CaCl_2 \cdot 2H_2O$, 0·175 ; $MgCl_2 \cdot 6H_2O$, 0·10 ; $Na_2HPO_4 \cdot H_2O$, 0·85 and KH_2PO_4, 0·078 in distilled water (Ellison, 1959), was changed every 24 hr.

Muscle strips were prepared from viable female worms using the method of Norton & de Beer (1957). The anterior 5 mm was removed from the worm, and the next 2 cm anterior to the genital pore isolated. A cut was made along the left lateral line and the intestine lifted out. This procedure exposes the coelomic surface for access of the hydrophilic drugs to the muscle tissue. The muscle strip was suspended in a constant volume organ bath containing 20 ml. of bathing fluid at 37° C, gassed with nitrogen. The upper end of the muscle preparation was attached to a Grass force displacement transducer, which registered isometric contractions of the muscle on a Sargent pen recorder. The contact period and frequency of dosing was determined for each of the stimulant drugs. Acetylcholine was added at 10 min intervals and allowed to remain in contact with the tissue for 2·5 min. Pyridine-2-aldoxime methiodide (2-PAM) and dimethylphenylpiperazinium iodide (DMPP) were added at 15 min intervals with a 5 min period of contact. Blocking drugs were added to the bathing fluid 5 min before the addition of the subsequent dose of the

stimulant. The concentration of the blocking agent required to produce 50% inhibition of the response to a constant submaximal dose of stimulant was estimated by statistical analysis.

All drug solutions were prepared in the bathing fluid.

Stimulant drugs

The following drugs were examined for stimulant activity: oxotremorine sesquifumarate (Aldrich Chemical Company), pilocarpine hydrochloride, U.S.P. (Calbiochem), methacholine chloride, N.F. (Merck), acetylcholine chloride (Matheson, Coleman & Bell), nicotine sulphate (Aceto Chemical Company), carbachol (Chemicals Procurement Laboratories), decamethonium bromide (Burroughs Wellcome), diphenylmethylpiperazinium iodide and adrenaline hydrochloride (Parke, Davis), pyridine-2-aldoxime methiodide (Nutritional Biochemicals), dichlorvos (Shell Chemical Company), eserine salicylate (Mallinckrodt), potassium chloride and barium chloride (Allied Chemicals).

Blocking drugs

The following drugs were examined for blocking activity: mecamylamine hydrochloride and atropine sulphate, U.S.P. (Merck), (+)-tubocurarine chloride (Abbott), hexamethonium chloride (Warner-Lambert Research Institute) and piperazine citrate (K & K Laboratories).

Results

The effect of the stimulant drugs on the *Ascaris* muscle preparation is shown in Table 1. The drugs are classified according to recognized mammalian pharmacological convention. Drugs with a muscarine-like action were without effect on this tissue, although mammalian tissues generally respond to 1/1,000 of the concentrations used in these experiments. Drugs with a nicotine-like action produced dose-related contractions of the muscle. Acetylcholine, which has both muscarine-like and nicotine-like properties, also produced dose-related contractions. In (2+2) Latin square assays, DMPP was 3·46 times more potent than acetylcholine, while 2-PAM had only 0·018 times the stimulant activity of acetylcholine, on a molar basis. The remaining nicotine-like drugs were not assayed in this manner. The anticholinesterase compounds, dichlorvos and eserine, when added in increasing doses, produced a rise in the resting tension of the preparation without any evidence of a discrete contraction. However, the addition of the relatively high doses shown in Table 1 to the previously untreated tissue resulted in a well maintained contracture and subsequent erratic spontaneous movement in the case of dichlorvos, and spontaneity only in the case of eserine. When added to the bathing fluid at their respective 150 concentrations for *Ascaris* cholinesterase activity (Knowles & Casida, 1966) eserine, $3{\cdot}98 \times 10^{-6}$M, and dichlorvos, $2{\cdot}51 \times 10^{-7}$M, potentiated the responses of the tissue to acetylcholine.

Esterase activity in the *Ascaris* muscle preparation shortens the duration of effect of acetylcholine. This was indicated by the responses to acetylcholine coming to a maximum and starting to decline within 2·5 min. All other stimulants which are not substrates for esterase activity had slower rates of onset and longer durations of action.

The depolarizing ions, potassium and barium, produced discrete contractions or spontaneity, respectively, at the dose levels examined. Adrenaline, a catecholamine which stimulates receptors at post-ganglionic sympathetic nerve endings in mammals, neither stimulated the *Ascaris* muscle nor antagonized the contractions to acetylcholine.

Further pharmacological analysis of the nature of the cholinoceptor in *Ascaris* muscle was provided by a quantitative study of the effect of different blocking agents. Three stimulant agents were selected: acetylcholine, DMPP and 2-PAM. The amount of the different blocking agents required to inhibit constant submaximal responses to these stimulants was estimated. Each blocking agent examined produced dose-related degrees of inhibition of the contractions, and Table 2 presents the values obtained for each blocking agent against the three stimulants. The results are expressed as negative logarithms of molar concentrations. It is evident that for any one blocking agent there is a marked similarity in the values between each of the three stimulants studied. Mecamylamine, the mammalian ganglion blocking drug,

TABLE 1. *Response of muscle preparations of* Ascaris lumbricoides *to different stimulant drugs*

Stimulant	Concentration range μg/ml.	Concentration range M $\times 10^{-6}$	Contraction
Nicotine-like			
Dimethylphenylpiperazinium iodide (DMPP)	0·05–2·0	0·2–6	+
Nicotine sulphate	0·5–10·0	2–38	+
Carbachol	1·0–10·0	5–55	+
Decamethonium bromide	20·0–100·0	73–366	+
Pyridine-2-aldoxime methiodide (2 PAM)	200–800	757–3029	+
Nicotine-like and muscarinic-like			
Acetylcholine chloride	1·0–10·0	6–55	+
Muscarine-like			
Oxotremorine sesquifumarate	10·0	26	—
Methacholine chloride	100·0	511	—
Pilocarpine hydrochloride	250·0	1022	—
Anticholinesterases			
Dichlorvos	5·0	23	Contracture and spontaneity
Eserine salicylate	850·0	2056	Spontaneity
Others			
Potassium chloride	250–4000	3353–53,652	+
Barium chloride	500–2000	2401–9600	Spontaneity
Adrenaline hydrochloride	50	228	—

TABLE 2. *Negative logarithm of molar concentrations of antagonists producing* 50% *inhibition of submaximal responses to different nicotine-like drugs in* Ascaris *muscle preparations* in vitro

Mean values of two determinations

Antagonist	Stimulant: Acetylcholine	Stimulant: DMPP	Stimulant: 2-PAM
Mecamylamine	6·60	6·11	6·59
(+)-tubocurarine	5·63	5·04	5·50
Hexamethonium	3·95	3·66	3·99
Atropine base	4·15†	3·47	3·35‡
Piperazine base	2·90* (2·82)	2·70	2·52

*Calculated from results of Norton & de Beer (1957). †Mean value of three determinations. ‡ Mean value of seven determinations.

was the most potent blocking agent examined against the contractions produced by each of the stimulants used on the muscle. This was followed in potency by the somatic neuromuscular blocking agent, (+)-tubocurarine. Another ganglion blocking agent, hexamethonium, was slightly more active than the parasympathetic blocking drug, atropine. On a molar basis, piperazine, a clinically well established vermifuge which has little pharmacological activity in mammals, was the weakest blocking agent studied. Table 2 also includes a value for the inhibition of acetylcholine contractions by piperazine, following 30 min incubation, calculated from the results of Norton & de Beer (1957). The close agreement between the value derived from their results and that obtained in the present investigation indicates the extension of the incubation period from 5 min to 30 min does not appreciably increase the potency of piperazine as a blocking agent. Norton & de Beer (1957) also found graded inhibitions of the response to acetylcholine by atropine and (+)-tubocurarine. However, they reported effects at only two dose levels for each of these compounds, and it was therefore not possible to calculate their concentration for 50% inhibition of response.

Discussion

This investigation has provided a quantitative and qualitative study of the nature of the cholinoceptor in muscle preparations of *Ascaris lumbricoides* var. *suum*. From the qualitative aspect, it has been shown that the preparation contracts to stimuli which are classified as nicotine-like in mammalian pharmacology. Drugs possessing only muscarine-like properties are inactive. This confirms the earlier conclusion of Baldwin & Moyle (1949). The classification of 2-PAM as a nicotine-like drug is not unprecedented, as the methylchloride salt has been shown to induce a rise in blood pressure by autonomic ganglion stimulation on intravenous injection in anaesthetized dogs (Zarro & DiPalma, 1965). The most active stimulant examined was DMPP, a compound with a high degree of specificity for autonomic ganglia in mammals (Chen, Portman & Wickel, 1951).

The increase in tone of the muscle preparation and the potentiation of the responses to acetylcholine produced by the anticholinesterases, dichlorvos and eserine, confirms the presence of cholinesterase(s) in this tissue, which was originally demonstrated by Hart & Lee (1966) and by Knowles & Casida (1966). Baldwin & Moyle (1949) concluded cholinesterases to be absent from *Ascaris* preparations as they found eserine (10 μg/ml.) to have no action on the tissue, nor did it influence the responses of the tissues to acetylcholine. In the present study, eserine was found to produce spontaneity at 850 μg/ml. This concentration is of the order of 500 times the I50 concentration of eserine against the cholinesterase activity of homogenates of *Ascaris* (Knowles & Casida, 1966), whereas the concentration of eserine studied by Baldwin & Moyle (1949) was only about six times this I50 concentration. The concentration of dichlorvos which produced contracture in the present study, 5 μg/ml., was approximately 100 times the I50 concentration against the cholinesterase activity of homogenates of *Ascaris* (Knowles & Casida, 1966). The addition of either eserine or dichlorvos to the *Ascaris* muscle preparation at concentrations corresponding to the respective I50 values, and sequentially increasing these concentrations, resulted in an increase of the tone of the preparation, with the development of spontaneity, but no discrete contracture. The I50 concentrations of both anticholinesterases also potentiated responses to added acetylcholine. Con-

tracture was produced by the addition of a single dose equivalent to 100×150, only in the case of dichlorvos.

Barium and potassium ions cause depolarization of mammalian smooth muscle tissue. Whereas potassium caused the *Ascaris* muscle to contract, barium produced only spontaneity. Baldwin & Moyle (1949) reported barium ions to stimulate the tissue but not to produce a discrete contraction.

The lack of stimulant activity of adrenaline on the *Ascaris* muscle has been reported previously (Baldwin & Moyle, 1949 ; Norton & de Beer, 1957) and has been confirmed in this study. Moreover, adrenaline did not influence the responses of the tissue to acetylcholine. Therefore, there is no evidence of α or β adrenoceptors in this tissue.

Studies with antagonists show that for any one blocking agent, there is a marked similarity in the 50% inhibitory concentration against the responses to acetylcholine, DMPP and 2-PAM. This suggests that these stimulants may act at a similar receptor on the *Ascaris* muscle. The mammalian autonomic ganglion blocking drug, mecamylamine, was the most potent in inhibiting contractions elicited by each of these stimulants. Hexamethonium, which blocks mammalian autonomic ganglia, was also found to antagonize quantitatively the effects of the stimulants. Norton & de Beer (1957) reported hexamethonium to have no stimulant effect on the *Ascaris* muscle but did not examine it for blocking activity. The activity of atropine in blocking the responses to acetylcholine on this preparation is some four to five orders of magnitude weaker than it is against its muscarine-like actions on mammalian smooth muscle preparations in similar experimental conditions (Schild, 1947 ; Lockett & Bartlet, 1956). Baldwin & Moyle (1949) reported atropine to have no effect on the responses to acetylcholine in this tissue, whereas Norton & de Beer (1957) found it to inhibit these responses. The present work therefore confirms the findings of Norton & de Beer.

Piperazine, which has little pharmacological activity in mammals, has long been used as an ascarifuge. A good correlation was obtained between the molar concentration of piperazine required for 50% inhibition of acetylcholine responses in this work and that value derived from the published results of Norton & de Beer (1957). Piperazine is thought to act like (+)-tubocurarine by a competitive blockade at the neuromuscular junction of the worm (Norton & de Beer, 1957). Del Castillo *et al.* (1963) and del Castillo, deMello & Morales (1964) suggested, however, that piperazine acts as an analogue of a natural inhibitory transmitter, since the electrophysiological effects elicited by this compound are similar to those obtained on stimulation of inhibitory nerve fibres in the worm.

Nicotine-like drugs have been shown to induce flaccid paralysis in the trematode, *Schistosoma mansoni* (Barker, Bueding & Timms, 1966). Muscarine-like drugs were without activity. Paralysis also was induced with organophosphorus cholinesterase inhibitors, which was reversed with 2-PAM. Both mecamylamine and atropine, 5×10^{-5}M, reversed carbachol-induced paralysis of the schistosome, as did other non-cholinomimetic agents such as serotonin, amphetamine, reserpine, tyramine and 5-hydroxytryptophan. Barker *et al.* (1966) concluded that the cholinoceptors of schistosomes have some similarities with receptors in the autonomic ganglia of mammals.

A similar conclusion is derived from the present study. The cholinoceptor of

muscle preparations of *Ascaris lumbricoides* var. *suum* has been shown to be qualitatively similar to that of the mammalian autonomic ganglion. This conclusion has been reached on the basis of the high order of activity of both stimulant and blocking drugs in this tissue which have high specificity for the autonomic ganglia in mammals.

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