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Aspects of the pharmacology of a new anthelmintic: pyrantel

M. L. AUBRY, PAULINE COWELL, M. J. DAVEY AND S. SHEVDE

Therapeutics Research Division, The Pfizer Group, Sandwich, Kent

Summary

1. The pharmacological properties of an anthelmintic, pyrantel, and some of its analogues have been described and compared with piperazine in a variety of vertebrate and helminth preparations.

2. Pyrantel and its analogues in common with nicotine and decamethonium cause spastic paralysis in chicks and contracture of the chick semispinalis and toad rectus abdominis muscles.

3. In the soleus and anterior tibialis muscles of the cat, pyrantel in large amounts caused a short-lived neuromuscular block that was preceded by initial depolarization.

4. In preparations from cat and rat, pyrantel showed properties common to both competitive and depolarizing neuromuscular blocking drugs.

5. Pyrantel blocked the contracture evoked by transmural stimulation and caused a marked contracture of the worm. Piperazine caused a gradually developing reduction in the responses to transmural stimulation and no contracture.

6. Pyrantel and its analogues caused a slowly developing contracture of strip preparations of *Ascaris*, being more than 100 times more active than acetylcholine in this respect. Piperazine caused a relaxation of *Ascaris* strip preparations and in common with (+)-tubocurarine blocked the responses to acetylcholine and pyrantel analogues on this preparation.

7. Pyrantel caused depolarization and increased spike discharge frequency in single muscle cells of *Ascaris*, these changes being accompanied by increase in tension. Piperazine, on the other hand, caused hyperpolarization and reduction in spike discharge frequency and relaxation, and antagonized the effects of pyrantel.

Introduction

Pyrantel tartrate (*trans*-1-methyl-2-[2-(2-thienyl)vinyl]-1,4,5,6-tetrahydropyrimidine) (pyrantel) was the most promising member of a new class of anthelmintics announced by Austin, Courtney, Danilewicz, Morgan, Conover, Howes, Lynch, McFarland, Cornwell & Theodorides (1966). These compounds evolved from laboratory assays using *Nematospiroides dubius* in mice and *Nippostrongylus muris* in rats. In laboratory trials, pyrantel gave good control of sheep infected with

Trichostrongylus colubriformis and substantially complete elimination of *Nematodirus battus* from experimentally infected lambs. In a trial with naturally infected sheep, pyrantel (25 mg/kg), given orally, was effective in the removal of *Ostertagia, Trichostrongylus, Nematodirus, Cooperia,* and *Chabertia* sp., but had little effect on *Trichuris.* Excellent activity has also been demonstrated against *Ascaris lumbricoides.* This nematode was chosen for use in investigations into the mechanism of action of pyrantel, being both readily available and of a sufficiently large size for experimentation. Piperazine was used throughout for comparative purposes and both piperazine and pyrantel and its analogues (U.K. 2536, U.K. 2332, U.K. 2837 and C.P. 14,445-18-B) have been examined for their pharmacological effects both in the vertebrate and helminth systems.

Methods

Isolated organ bath preparations

The isolated phrenic nerve diaphragm preparation of the rat and the isolated semi-spinalis muscle of the chick were suspended in oxygenated Tyrode solution containing a double quantity of glucose (g/l.: NaCl 8·0 ; $NaHCO_3$ 1·0 ; glucose 2·0 ; KCl 0·2 ; $CaCl_2$ 0·2 ; NaH_2PO_4 0·05 ; $MgCl_2$ 0·1) later referred to as modified Tyrode solution, following the methods of Bülbring (1946) and Child & Zaimis (1960). Changes in tension were recorded using a force-displacement transducer and Devices recorder with resting tensions of 1 g and 10 g for the chick semispinalis and rat diaphragm muscles, respectively.

The rectus abdominis muscle from the toad was suspended in oxygenated frog Ringer solution (g/l.: NaCl 6·44 ; KCl 0·14 ; $CaCl_2$ 0·12 ; $NaHCO_3$ 0·34 ; glucose 1·0) at room temperature. Responses were recorded on a smoked drum using a frontal writing lever with $\times 7$ magnification and 0·2 g tension.

Ascaris lumbricoides *preparations*

Ascaris lumbricoides suum were obtained from the slaughter house and placed in the modified Tyrode solution, which had been deoxygenated with nitrogen, at 38° C. The worms were kept in an incubator maintained at 38° C and the modified Tyrode solution changed twice a day. The worms were kept in these conditions for a maximum of 5 days and only motile worms were used.

(A) Whole worm. The spontaneous activity of whole male or female worms was measured by suspending the worm between cotton ligatures in a 150 ml organ bath, painted black because the worms are photosensitive and containing the modified Tyrode solution at 38° C. Changes in tension were recorded using either a force-displacement gauge and Devices recorder or frontal writing lever and smoked drum, the resting tension being 10 g.

(B) Electrical stimulation. The anterior 3 cm of the worm was used. Electrical stimulation across the wall was achieved by placing a platinum electrode into the lumen of the segment, the circuit being completed by a second platinum electrode placed in the bathing fluid.

(C) Ascaris *strip.* Only adult female *Ascaris* were used.

The preparation is a modification of the strip preparation of *Ascaris* described by Baldwin & Moyle (1947).

In all experiments using *Ascaris*, the modified Tyrode solution was gassed with nitrogen and the oxygen content reduced to approximately 5% saturation.

Electrophysiological recording from muscle cells

Anterior cylindrical portions of *Ascaris* 1·5–2·0 cm long were opened along the lateral line, the gut removed and mounted horizontally in a Perspex organ bath in modified Tyrode solution. Tension was recorded with a force-displacement transducer, the resting tension being 2·5 g. The membrane potential and spike activity were recorded from medial muscle cell bellies with glass microelectrodes (20–40 MΩ) filled with 3 M KCl and via a BAK preamplifier and displayed together with tension on a cathode ray oscilloscope.

Contraction of soleus and tibialis anterior muscles of the cat

Cats were anaesthetized with chloralose (75 mg/kg) given intravenously after anaesthesia had been induced by inhalation of a mixture of halothane 3% v/v in nitrous oxide and oxygen (3 : 1). Contractions of left soleus and tibialis anterior muscles were recorded on a Devices recorder using isometric ±32 ounce strain gauges (Ether). Close arterial injections were given via a polythene cannula that had been passed to the aortic bifurcation by retrograde cannulation of the right femoral artery. The maximum injected volume including wash-in was 0·2 ml. Heart rate, arterial pressure from the right carotid and respiration were also recorded on a Devices recorder by means of a Nielson rate meter and appropriate pressure transducers respectively. All cats received atropine sulphate (0·5 mg/kg) given intravenously.

Drugs

Diethylenediamine hydrate (Piperazine hydrate, Hopkin & Williams Ltd.); acetylcholine chloride (The British Drug Houses Ltd.); (+)-tubocurarine chloride (Burroughs Wellcome & Co., London); pyrantel tartrate (Banminth, Pfizer Ltd.); physostigmine sulphate B.P.C. 1934 (Burroughs Wellcome & Co., London); nicotine hydrogen tartrate (The British Drug Houses Ltd.); decamethonium iodide, B.P. (Burroughs Wellcome & Co., London); succinylcholine chloride (Burroughs Wellcome & Co., London); 2-(2-α-thienylethyl)-5,6-dihydro-4H-1,3-thiazine fumarate (U.K. 2536); 1-methyl-2[2′-(α-thienyl)ethyl]-1,4,5,6-tetrahydropyrimidine tartrate (U.K. 2332); *trans*-1-[2-(2-thienyl)vinyl]pyridinium bromide monohydrate (U.K. 2837); *trans*-1-methyl-2[2-(3-hydroxyphenyl)vinyl]-1,4,5,6-tetrahydropyrimidine tartrate monohydrate (C.P. 14,445-18-B); atropine sulphate (The British Drug Houses Ltd.).

Results

Rat phrenic nerve diaphragm preparation

Pyrantel, U.K. 2332, U.K. 2536, U.K. 2837, C.P. 14,445-18-B and piperazine reduced and eventually blocked the twitch response of the rat hemidiaphragm to electrical stimulation of the phrenic nerve. Figure 1 shows the log dose-effect curves, and indicates the effectiveness of these compounds as compared with (+)-tubocurarine and decamethonium. (+)-Tubocurarine was 50 times more active at

blocking the twitch response of the hemidiaphragm to electrical stimulation of the phrenic nerve than decamethonium, and 100 times more effective than nicotine. Pyrantel, C.P. 14,445-18-B, U.K. 2536, U.K. 2332 and U.K. 2837 were all less active in blocking the twitch response than (+)-tubocurarine but more active than decamethonium. In contrast, piperazine was approximately 200 times less active than decamethonium at blocking the twitch response to electrical stimulation of the phrenic nerve diaphragm preparation, the minimal effective concentration of piperazine being 5 mg/ml.

After block of the responses of the hemidiaphragm to electrical stimulation of the phrenic nerve had been achieved by the incorporation of (+)-tubocurarine (8 μg/ml) in the modified Tyrode solution, pyrantel (20 μg/ml), C.P. 14,445-18-B (10 μg/ml), U.K. 2536 (20 μg/ml), U.K. 2332 (20 μg/ml), U.K. 2837 (50 μg/ml), (+)-tubocurarine (8 μg/ml), decamethonium (100 μg/ml) and nicotine (300 μg/ml) had no effect on the response to direct electrical stimulation of the diaphragm. Piperazine (5, 10 and 20 mg/ml), however, produced a dose-dependent antagonism of the responses to direct stimulation of the muscle. Because these amounts of piperazine are the same as those required to block the effects of electrical stimulation of the phrenic nerve, it is concluded that this blockade results from a direct depressant action on the muscle.

Physostigmine (10 μg/ml) restored the twitch evoked by electrical stimulation of the phrenic nerve after blockade by (+)-tubocurarine. Physostigmine (10 μg/ml) deepened the block by decamethonium, nicotine, pyrantel, U.K. 2332, U.K. 2536, U.K. 2837 and C.P. 14,445-18-B, but had no effect on the depression produced by piperazine, but tetanic stimulation surmounted the block induced by all compounds mentioned above.

Isolated chick semispinalis preparation

Pyrantel, U.K. 2332, U.K. 2536, U.K. 2837 and C.P. 14,445-18-B caused a contracture of the chick semispinalis preparation. Succinylcholine and decamethonium were more effective nicotinic agents than C.P. 14,445-18-B, nicotine, U.K. 2536, U.K. 2332, pyrantel and U.K. 2837 respectively, both the latter drugs showing weak nicotinic activity only.

Isolated toad rectus abdominis preparation

Figure 2 shows the log dose effect curves for pyrantel and its analogues, acetylcholine, decamethonium and nicotine on the toad rectus abdominis preparation.

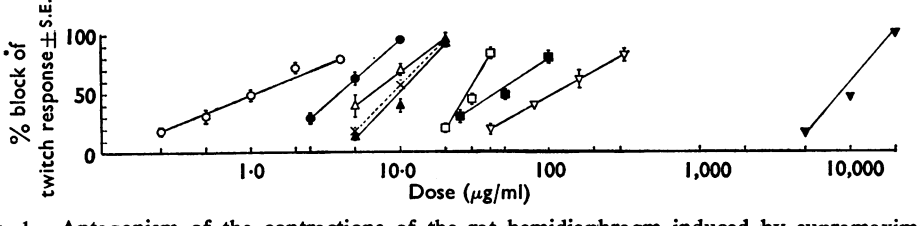

FIG. 1. Antagonism of the contractions of the rat hemidiaphragm induced by supramaximal stimulation of the phrenic nerve (1 ms duration, 8 stimuli/min) by pyrantel (×——×), U.K. 2332 (▲——▲), U.K. 2536 (△——△), U.K. 2837 (□——□), C.P. 14,445-18-B (●——●), piperazine (▼——▼), (+)-tubocurarine (○——○), nicotine (▽——▽) and decamethonium (■——■).

C.P. 14,445-18-B possessed greater nicotinic activity in this preparation than decamethonium. U.K. 2536 and U.K. 2332 showed similar activity to decamethonium and nicotine, but pyrantel and U.K. 2837 were only weakly active. In contrast, piperazine (3·2 and 6·4 mg/ml) antagonized the effects of acetylcholine in this preparation.

Intravenous administration in conscious chicks

Slow intravenous injection of pyrantel, C.P. 14,445-18-B, U.K. 2332, U.K. 2837, U.K. 2536, nicotine and decamethonium into the alar vein of 11 day old chicks produced spastic paralysis (Table 1). After cessation of the administration, recovery was immediate after pyrantel, C.P. 14,445 and U.K. 2837. Decamethonium, nicotine, U.K. 2332 and U.K. 2536 caused paralysis and death due to respiratory arrest. Intravenous injections of U.K. 2536 caused an initial head drop in the chicks, before spasticity.

In contrast to pyrantel and its analogues, piperazine caused flaccid paralysis in the chicks similar to the paralysis seen after (+)-tubocurarine. Piperazine was approximately 3,000 times less active than (+)-tubocurarine in this respect.

TABLE 1. *Paralytic potency of various compounds in conscious chicks*

Drug	No. of chicks	mg/kg i.v. for paralysis
(+)-Tubocurarine	11	0·324±0·042
Piperazine	6	1·100±0·100*
Decamethonium	9	0·032±0·002
Nicotine	5	1·17±0·12
Pyrantel	12	1·15±0·08
C.P. 14,445-18-B	5	0·071±0·005
U.K. 2837	9	1·27±0·20
U.K. 2536	8	2·73±0·22
U.K. 2332	8	1·07±0·13

* g/kg

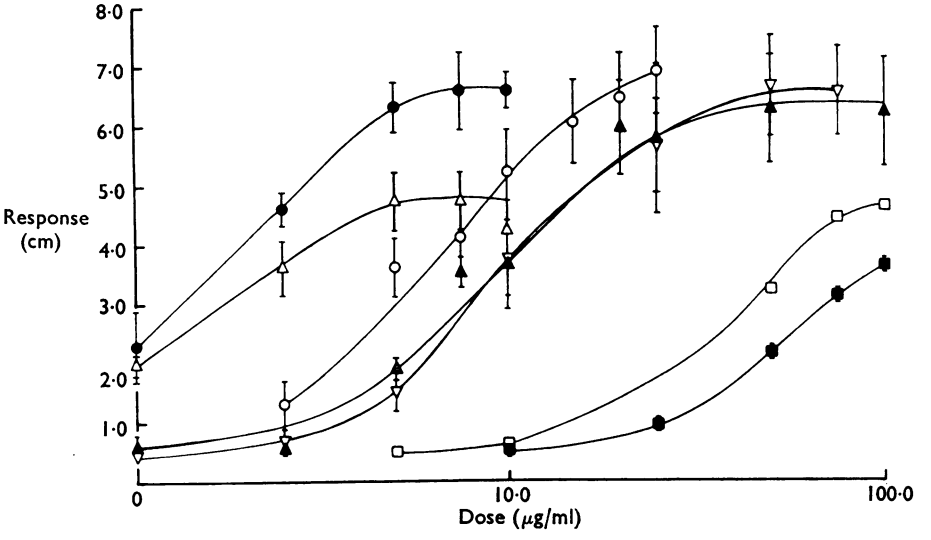

FIG. 2. Log dose-response curves for U.K. 2332 (▲——▲), U.K. 2536 (△——△), U.K. 2837 (□——□), C.P. 14,445-18-B (●——●), pyrantel (■——■), decamethonium (○——○) and nicotine (▽——▽) in the rectus abdominis muscle of the toad.

Ascaris *preparations—whole worm*

Figure 3a shows the effect of pyrantel (25 μg/ml) and Fig. 3b piperazine (20 mg/ml) on the spontaneous movements of the whole worm. After the addition of pyrantel to the bath, a sharp contraction of the worm was seen together with a cessation of spontaneous activity. In contrast to the rapid effect of pyrantel, piperazine caused a gradual paralysis of the worm over a period of several hours.

TABLE 2. *Paralytic potency of various compounds in intact worms*

Drug	Dose (μg/ml)	Sex	Time taken to paralyse (min±S.E.)	No. of observations
U.K. 2536	1·0	♀	13·8±2·2	8
U.K. 2536	1·0	♂	19·8±3·8	5
Pyrantel	100	♀	26·3±1·4	6
Pyrantel	100	♂	23·6±4·5	5
Pyrantel	25	♀	49·0±6·0	5
Pyrantel	25	♂	49·4±12·0	5
U.K. 2332	400	♀	49·2±6·7	5
U.K. 2837	500	♀	68·6±6·0	5
C.P. 14,445-18-B	10^3	♀	95·5	4
			150	3
Piperazine	2×10^4	♀	138	5

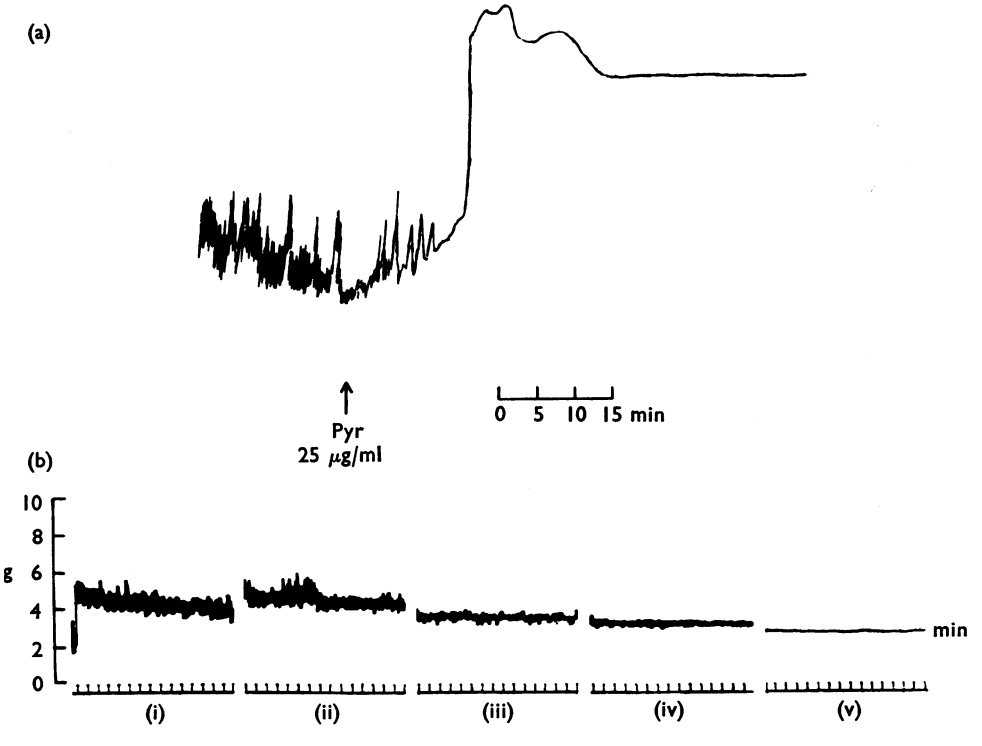

FIG. 3. (a) Effect of pyrantel (Pyr, 25 μg/ml) on the spontaneous activity of an intact male *Ascaris*. (b) Spontaneous activity of female *Ascaris*. (i) 0, (ii) 1 hr, (iii) 2 hr, (iv) 3 hr and (v) 4 hr after the addition of piperazine (20 mg/ml) to the bath.

This paralysis was not accompanied by a contraction of the worm. U.K. 2536 was approximately 100 times more effective than pyrantel, U.K. 2837, U.K. 2332 and C.P. 14,445-18-B were between 20 and 40 times less active. All caused a contraction of the worm before paralysis. Both pyrantel and U.K. 2536 were found to be as effective on female as on male worms (Table 2).

Electrically stimulated preparations of Ascaris

Figure 4a shows the effect of pyrantel (20 μg/ml) on the responses to tetanic electrical stimulation across the wall of *Ascaris*. Pyrantel (20 μg/ml) caused an immediate contracture which was accompanied by a reduction in the responses to transmural stimulation. The resting tension of the preparation was readily restored by washing the preparation with modified Tyrode solution, whereas the block of the responses to tetanic transmural stimulation was persistent and could not be removed by repeated washing of the preparation.

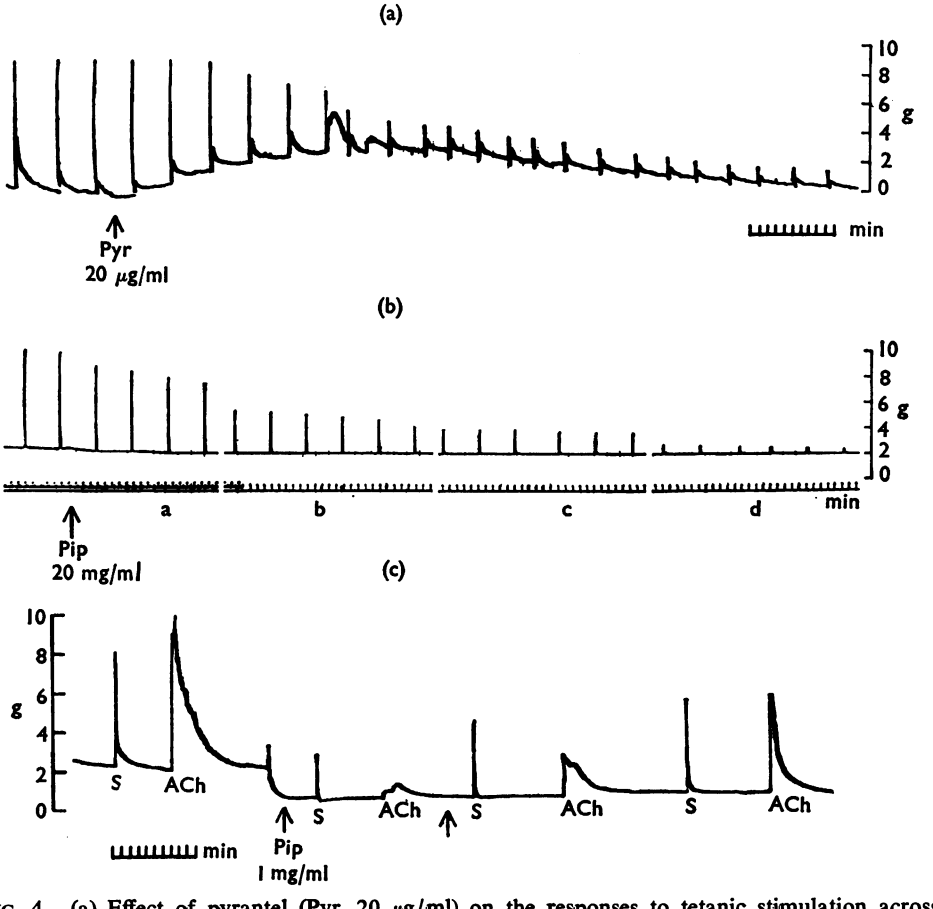

FIG. 4. (a) Effect of pyrantel (Pyr, 20 μg/ml) on the responses to tetanic stimulation across the wall of *Ascaris* (11 V for 5 s, 1 ms, 8 Hz). (b) Effect of piperazine (Pip, 20 mg/ml) on the responses to tetanic stimulation across the wall of *Ascaris* (11 V for 5 s, 2 ms, 8 Hz). Intervals of 30 min between (a) and (b), (b) and (c), and (c) and (d). (c) Effect of piperazine (Pip, 1 mg/ml) on the responses to acetylcholine (ACh, 1 μg/ml) and electrical stimulation across *Ascaris* (S) (11 V for 5 s, 1 ms, 8 Hz).

Piperazine in a concentration which produced paralysis of the whole worm over a period of 2 to 3 hr (20 mg/kg) caused a gradual reduction in responses to transmural stimulation of the worm (Fig. 4b). This block of the responses to transmural stimulation of *Ascaris* was seen at a concentration of piperazine (1 mg/ml) if the preparation was split along one lateral canal to enable free penetration of piperazine into the lumen of the worm. Figure 4c shows that piperazine (1 mg/ml) reduced the responses to both acetylcholine (1 μg/ml) and transmural stimulation after the preparation had been opened along a lateral canal. This antagonism of the effects of acetylcholine and transmural stimulation was immediate in onset and suggests that the gradual onset of paralysis seen in preparations where the cuticle was intact was probably due to slow penetration across the cuticle of the worm. This has been reported previously by Norton & De Beer (1957).

Strip preparation of Ascaris

As might be expected from the type of paralysis produced in whole worm, pyrantel and its analogues were found to have potent stimulant properties on strip preparations of *Ascaris*. In contrast to the immediate and readily reversible contracture of the *Ascaris* strip preparation produced by acetylcholine, pyrantel and its analogues caused a slowly developing prolonged contracture of the strip and repeated washing was required in order to restore resting tone. Figure 5 shows the log dose-effect curves for the contractile activity of acetylcholine and pyrantel analogues on strip preparations of *Ascaris*. All the analogues of pyrantel showed activity of the same order on this preparation, all being approximately 100 times more active than acetylcholine. Decamethonium produced a slow contracture only

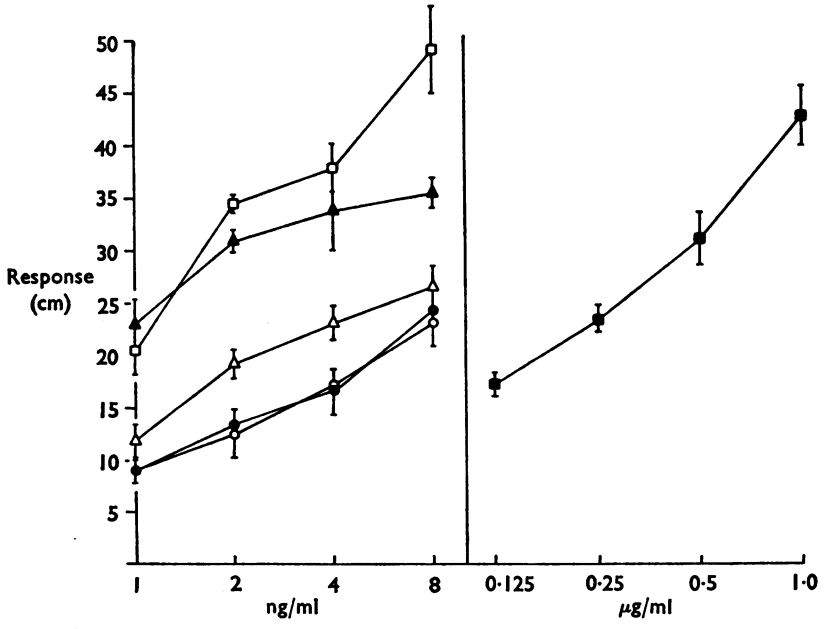

FIG. 5. Log dose effect curves for acetylcholine (■——■), pyrantel (●——●), U.K. 2536 (○——○), U.K. 2837 (△——△), U.K. 2332 (□——□) and C.P. 14,445-18-B (▲——▲) on the strip preparation of *Ascaris*.

at amounts greater than 40 μg/ml. The fact that no marked difference in activity existed between the potency of the pyrantel analogues when the muscle cell surface is exposed directly to the drugs suggests that the differences in potency seen in the whole worm and electrically stimulated *Ascaris* preparations were due to the varying abilities of the drugs to penetrate the cuticle of the worm.

Piperazine caused a relaxation of *Ascaris* strip preparations and blocked the responses to acetylcholine and pyrantel analogues on this preparation. Figure 6a illustrates the effect of piperazine (1 mg/kg) on the responses to acetylcholine (1 μg/ml) and pyrantel (1·5 ng/ml). Both the responses to acetylcholine and pyrantel were abolished by this concentration of piperazine, which also caused a relaxation of the strip. The responses returned immediately after the removal of the piperazine from the bath.

The responses of the *Ascaris* strip to acetylcholine (1 μg/ml) and pyrantel (1·5 ng/ml) were also blocked by (+)-tubocurarine (20 μg/ml) (Fig. 6b). An increase in the spontaneous activity of the muscle strip was seen in certain preparations after (+)-tubocurarine.

Intracellular recording from muscle cells

Microelectrode recordings from 110 medial muscle cells showed a mean resting potential of 31·4 $\pm$ 1·08 mV interrupted by rhythmical spike potentials of varying frequency and amplitude. There was a direct relationship between tension and spike frequency and an inverse relationship between tension and resting membrane potential. Acetylcholine (1 μg/ml) caused a contracture of the worm, reduction of the membrane potential and an increase in spike frequency. In six experiments acetylcholine (1 μg/ml) decreased the resting membrane potential by 8·3 $\pm$ 0·72 mV and increased spike frequency by approximately 40–50%. Pyrantel (0·025–1·0

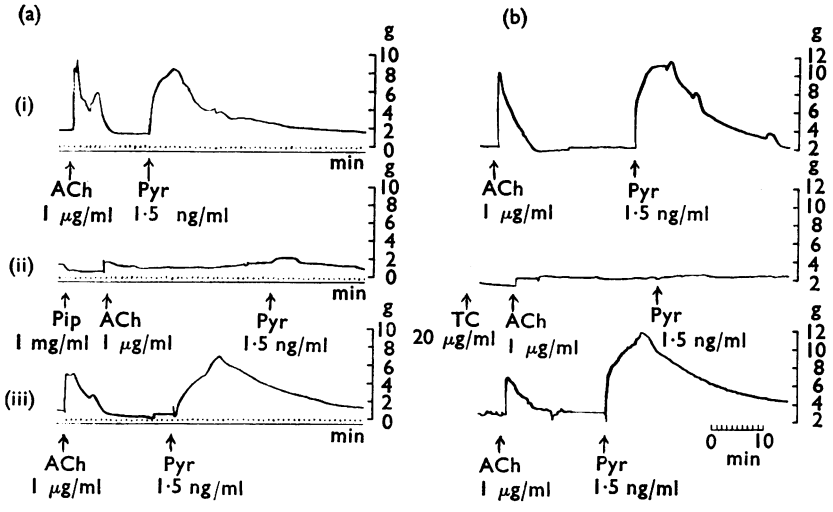

FIG. 6. Responses of *Ascaris* strip preparations to acetylcholine (ACh, 1 μg/ml) and pyrantel (Pyr, 1·5 ng/ml) (i) before and (ii) in the presence of piperazine (Pip, 1 mg/ml) in the bath and (iii) after removal of the piperazine from the bath. (b) Responses to acetylcholine (ACh, 1 μg/ml) and pyrantel (Pyr, 1·5 ng/ml) (i) before and (ii) in the presence of (+)-tubocurarine (TC, 20 μg/ml) in the bath and (iii) after removal of the tubocurarine from the bath.

μg/ml) produced depolarization, contracture and increased spike frequency (Fig. 7a). The depolarization, increase in spike discharge frequency and contracture produced by pyrantel (0·2–1·0 μg/ml) was persistent and could not be reversed by repeated washing of the tissue.

Del Castillo, De Mello & Morales (1961) reported that piperazine (600–800 μg/ml) caused a hyperpolarization of *Ascaris* muscle cells which was accompanied by a

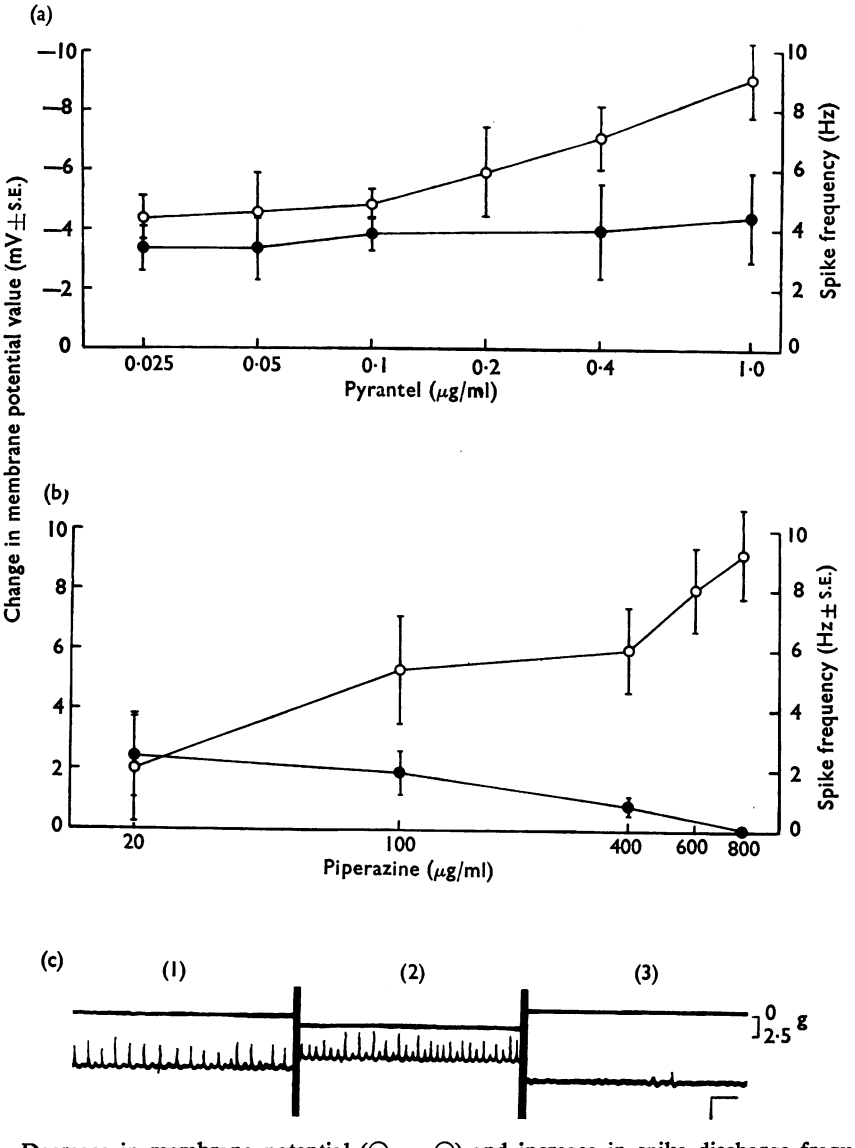

FIG. 7. Decrease in membrane potential (○——○) and increase in spike discharge frequency (●——●) induced by pyrantel in *Ascaris* muscle cells (means ± S.E.). (b) Hyperpolarization (○——○) and decrease in spike frequency (●——●) caused by piperazine in muscle cells of *Ascaris* (means ± S.E.). (c) Antagonism of the effects of pyrantel by piperazine in *Ascaris*. Calibrations: vertical, 10 mV; horizontal, 0·5 s. Top record, tension; bottom record, membrane potential. (1) Control: resting membrane potential −34 mV; spike frequency, 3·7 Hz. (2) Pyrantel (0·4 μg/ml) resting membrane potential −28 mV; spike frequency, 4·1 Hz. (3) Addition to (b) of piperazine (800 μg/ml) resting membrane potential −38 mV; spike activity absent.

decrease in tension and reduction or abolition of spike activity. Fig. 7b shows the hyperpolarization and reduction in spike discharge frequency caused by piperazine (20–800 μg/ml), thus confirming the original findings of Del Castillo *et al.* (1961). The depolarization, increased spike discharge and contracture induced by pyrantel was antagonized by piperazine (Fig. 7c).

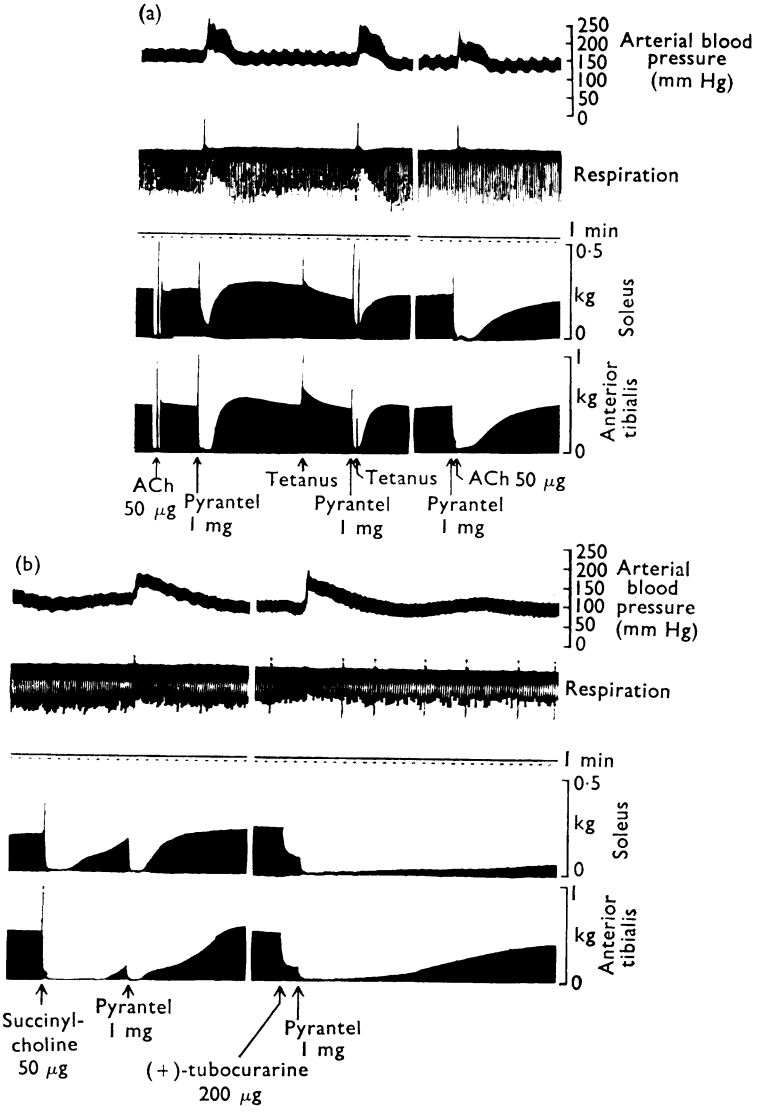

FIG. 8. (a) Cat, chloralose anaesthesia. Atropine sulphate (0·5 mg/kg) given intravenously. Arterial pressure, respiration and maximal twitches and tetanus of the left soleus and anterior tibialis muscles elicited by stimulation of the distal end of the divided left sciatic nerve with supramaximal (5 V) pulses of 1 ms duration at a frequency of 0·5 Hz or in the case of a tetanic contracture 125 Hz for 5 s. All compounds were given by close arterial injection. (b) Cat, chloralose anaesthesia. Atropine sulphate (0·5 mg/kg) given intravenously. Arterial pressure, respiration and maximal twitches of the left soleus and anterior tibialis muscles elicited by stimulation of the distal end of the divided left sciatic nerve with supramaximal (5 V) pulses of 1 ms duration at a frequency of 1·5 Hz. All compounds given close arterially.

Effects on cat soleus and anterior tibialis muscles

Close arterial injection of pyrantel (1 mg/kg), U.K. 2332 (100 μg), U.K. 2536 (200 μg) and U.K. 2837 (200 μg) caused, after an initial depolarization, short-lived neuromuscular block in both the soleus and anterior tibialis muscle. The degree of neuromuscular block was greater on the fast anterior tibialis muscle than on the slow soleus muscle; however, recovery was complete within 2–3 min on both muscles with all the compounds studied. The close arterial injection of acetylcholine (25–50 μg), during the period of impaired neuromuscular transmission induced by pyrantel, produced a deepening of the block in place of the contracture it had previously produced in both muscles (Fig. 8a). Pyrantel 1 mg given close arterially enhanced the failure of neuromuscular transmission induced by both succinylcholine (50 μg) and (+)-tubocurarine (200 μg), thus suggesting that the nature of the neuromuscular block produced by pyrantel is mixed in character (Fig. 8b). This conclusion may account for the observation that tetanus surmounted, whereas prostigmine (250 μg) deepened, the block of neuromuscular transmission caused by pyrantel.

Discussion

Preliminary experiments suggested that pyrantel and its analogues owed their anthelmintic activity to an action on neuromuscular transmission. This prompted a detailed investigation of the pharmacological effects of these compounds on both heminth and vertebrate neuromuscular preparations.

Pyrantel and its analogues showed activity characteristic of "depolarizing" neuromuscular blocking agents on the rat phrenic nerve diaphragm preparation, chick semispinalis and toad rectus abdominis muscles. With the exception of the activity on the diaphragm, all compounds were less active than decamethonium, pyrantel and U.K. 2837 in particular showing only weak "nicotinic" activity in the vertebrate system. On the other hand, piperazine showed characteristic "curare-like" activity in blocking the responses to acetylcholine on the toad rectus abdominis preparation. However, the twitch response to electrical stimulation of the phrenic nerve to the rat hemidiaphragm was blocked by piperazine only in doses which also blocked the response to direct electrical stimulation of the muscle.

Piperazine was 3,000 times less active than (+)-tubocurarine in producing flaccid paralysis in conscious chicks and was inactive in the soleus and anterior tibialis muscle preparation in the cat. Pyrantel and its analogues caused a transient neuromuscular block in these two nerve muscle preparations of the cat, and because this activity was enhanced by decamethonium, (+)-tubocurarine and prostigmine but reversed by tetanic stimulation, it is assumed that the neuromuscular block produced by pyrantel is mixed in showing some properties possessed by competitive and others possessed by those compounds which act like an excess of acetylcholine.

Evidence of the depolarizing activity of pyrantel was obtained in conscious chicks where pyrantel and its analogues produced spastic paralysis. Recovery was immediate after cessation of administration of pyrantel, C.P. 14,445-18-B, and U.K. 2837, whereas U.K. 2332 and U.K. 2536 caused paralysis and death due to respiratory arrest.

It is concluded that the side effects in the host animal due to the neuromuscular blocking activity displayed by pyrantel and its analogues should be negligible at the amounts required to show an anthelmintic effect.

The pattern of neuromuscular effects of pyrantel and its analogues and piperazine in vertebrate systems was mirrored to a certain extent by their effects on preparations *of Ascaris* in which pyrantel and its analogues showed marked persistent nicotinic properties which resulted in the spastic paralysis of the worm. Decamethonium, which produced marked neuromuscular effects in the vertebrate systems, showed only weak nicotinic activity in preparations of *Ascaris*. The depolarizing effects of pyrantel and its analogues have been confirmed by electrophysiological studies using intracellular recording from muscle cells of *Ascaris*. The present experiments have confirmed that piperazine causes a flaccid paralysis which is associated with hyperpolarization of the muscle cell membrane in *Ascaris*, but do not further the argument as to whether piperazine should best be considered as acting as an inhibitory transmitter or as competitive antagonist at the neuromuscular junction.

Piperazine and pyrantel have opposing mechanisms of action in *Ascaris*, a fact that is strengthened by the abolition of the depolarizing effect of pyrantel by piperazine in strip preparations of *Ascaris*. Both types of drugs have been shown to have a high degree of specificity for the *Ascaris* neuromuscular system, pyrantel and its analogues being of the order of 1,000 times more effective than piperazine in respect of the concentration required to produce paralysis. Since pyrantel and piperazine by virtue of their mechanisms of action can be regarded as being potentially mutually antagonistic, combination therapy with pyrantel and piperazine may well be contraindicated.

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