Br. J. Pharmac. (1982), **77**, 255–265

ELECTROPHYSIOLOGICAL EFFECTS OF PIPERAZINE AND DIETHYLCARBAMAZINE ON *Ascaris suum* SOMATIC MUSCLE

R.J. MARTIN

Department of Veterinary Pharmacology, Royal (Dick) School of Veterinary Studies, Summerhall, University of Edinburgh

1 Electrophysiological recordings were made from the bag region of *Ascaris suum* muscle. Membrane potential and input conductance or membrane current under voltage clamp were measured.

2 In high-Cl^- Ringer, bath-applied piperazine, at concentrations greater than 10^{-4} M, produced a dose-dependent and reversible increase in input conductance associated with a hyperpolarizing potential. The increase in input conductance was reduced when the preparations were bathed in low-Cl^- Ringer. γ-Aminobutyric acid (GABA) and piperazine reversal potentials were measured with a voltage clamp on the same cells using iontophoretic application of the agonists. The reversal potentials were the same and close to the predicted Nernst Cl^- potential (−65 mV). When GABA and piperazine were applied simultaneously piperazine reversibly reduced the amplitude of the control outward GABA current response. It was concluded that piperazine acts as a GABA agonist of low potency on the extra-synaptic GABA receptors of the bag, mediating an increase in Cl^- conductance.

3 Acetylcholine was applied iontophoretically within 100 μm of the bag region while the preparation was bathed in a low-Ca^{2+}, low-Cl^- Ringer. The response under voltage clamp was a dose-dependent inward current associated with an increase in input conductance. This response was reversibly antagonized by 3×10^{-5} M tubocurarine, high concentrations of diethylcarbamazine (10^{-3} to 10^{-2} M) but not high concentrations of piperazine (10^{-3} to 10^{-2} M). It was concluded that there are extra-synaptic acetylcholine receptors on the bag region of *Ascaris* muscle and that diethylcarbamazine but not piperazine acts as an antagonist.

4 Bath-applied diethylcarbamazine (10^{-4} to 2×10^{-3} M) produced a reversible dose-dependent depolarization of the membrane potential which was associated with an increase in the amplitude and frequency of spontaneous depolarizing potentials in active preparations at 32°C to 35°C in high-Cl^- Ringer. The excitatory action of diethylcarbamazine was not blocked by 3×10^{-5} M tubocurarine. Diethylcarbamazine (10^{-4} to 10^{-3} M) had no effect on the outward current response to GABA iontophoresis. Diethylcarbamazine (10^{-4} to 10^{-2} M) reversibly antagonized in a dose-dependent manner the delayed rectification of the bag membrane. In a low-Ca^{2+}, low-Cl^- Ringer, diethylcarbamazine (10^{-4} to 2×10^{-3} M) reversibly antagonized the voltage-sensitive outward current of the bag. This effect was mimicked by high-K^+ Ringer or perfusion with 4-aminopyridine (10^{-3} to 2×10^{-3} M). It was concluded that diethylcarbamazine did not react with the GABA receptor but antagonized a voltage-sensitive K^+ conductance.

Introduction

Nematode infections of intestinal parasites are common in both man and animals. For example, Standen (1975) estimated that 1 in 4 to 1 in 6 of the world's human population are infected with intestinal nematodes. In domestic animals infestations are so common that routine treatment with anthelmintics is required.

Piperazine and a derivative diethylcarbamazine are commonly used for the control of nematode infestations but their mode of action is not clear. It has been reported that piperazine mimics the action of γ-aminobutyric acid (GABA) and brings about hyperpolarization of *Ascaric* somatic muscle cells and that this is associated with relaxation (Del Castillo, De Mello & Morales, 1964a,b; Aubry, Cowell, Davey & Shevde, 1970). Despite these observations the exact mode of action of piperazine in *Ascaris* is still unknown. For example, it has been reported that iontophoretic application of piperazine on to the bag region of *Ascaris* muscle has no effect on membrane

0007-1188/82/100255-11 $01.00

potential (Del Castillo *et al.*, 1964a) but this region is known to contain extra-synaptic GABA receptors (Martin, 1980). Aubry *et al.* (1970) were unable to further the argument as to whether piperazine should best be considered to act as an inhibitory transmitter or as a cholinoceptor antagonist as originally suggested by Norton & De Beer (1956). Further, Phillips, Sturman & West (1976) have suggested that piperazine acts by increasing the absorption of histamine by the parasite and that histamine may be an inhibitory transmitter. Added to this, Broome (1962) has reported that the piperazine derivative, diethylcarbamazine, has an excitatory not an inhibitory action on the contractility of the *Ascaris* nerve muscle preparation. In a recent review of the actions of diethylcarbamazine, Hawking (1979) reported little new information on the mode of action in nematodes but suggested that the mode of action in *Ascaris* may be due to the piperazine ring of diethylcarbamazine.

It was therefore decided to examine the electrophysiological effects of piperazine and diethylcarbamazine in the large nematode intestinal parasite of the pig, *Ascaris suum*, and particularly to examine possible interactions with GABA and acetylcholine receptors. This paper describes the results of these experiments which show that piperazine but not diethylcarbamazine has a GABA agonist action increasing Cl^- conductance; piperazine did not produce a curariform block: diethylcarbamazine antagonized a voltage-sensitive K^+ conductance.

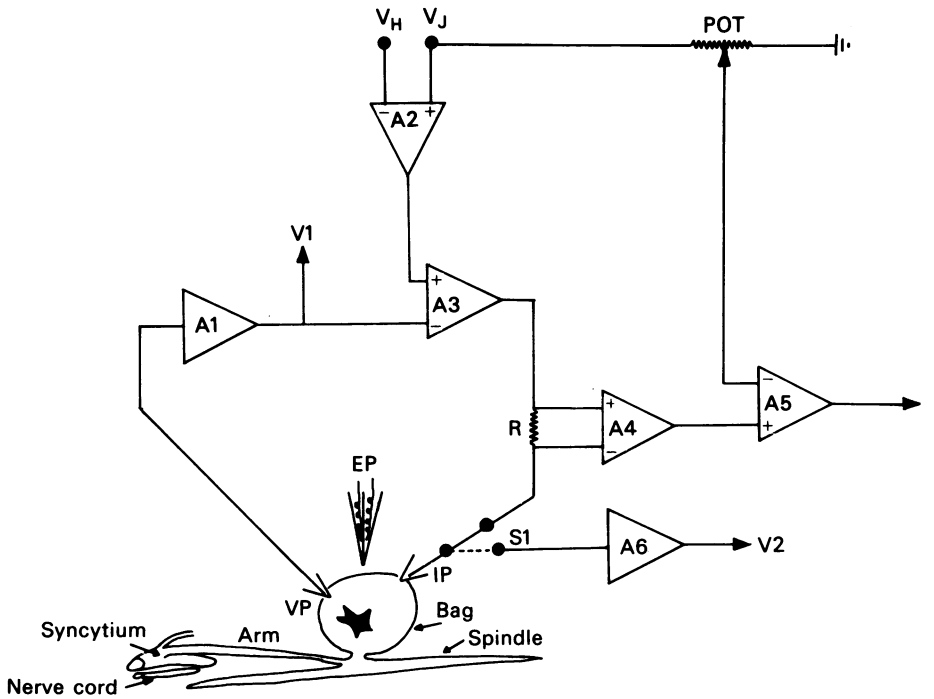

Figure 1 Diagram of preparation and recording system. Two 5 mΩ micropipettes (K^+ acetate filled) were placed intracellularly in the bag region of *Ascaris* muscle. The voltage pipette (VP) was connected to a high input impedance amplifier (A1) and the intracellular potential recorded at V1. A differential amplifier (A2) was used to determine the command potential. This allowed separate adjustment of the holding potential and voltage steps by applying different voltages to V_H for the holding potential and the V_J for the voltage steps. The control amplifier (A3) had a gain of 500. The current passed through the current pipette (IP) was determined by measuring the voltage drop across a 500 K resistor (R) using a differential amplifier (A4) with a high input impedance. A differential amplifier (A5) was used to subtract a rectangular current pulse during the voltage jumps because of the large voltage-insensitive leakage resistance of the bag. The level of the current subtraction was adjusted by using the potentiometer (POT) only at the beginning of the experiment and this was then left unaltered thereafter. The transmembrane current was recorded at the output of A5. The current pipette was placed intracellularly with the aid of a high input impedance amplifier (A6) when the switch S1 was suitably positioned to record the potential ofV_2. This amplifier (A6) was provided with a constant current injection facility. The iontophoretic pipettes (EP) were placed close (within 100 μm) to the bag membrane between the pipettes VP and IP.

Methods

The preparation

Specimens of *Ascaris suum* were collected from the local slaughter house and maintained in Locke solution (replaced daily), at 37°C in a water bath. The *Ascaris* were used for experiments within 4 days. A 2 cm flap preparation, made from the anterior region of the worm, was pinned on to sylgard in an experimental chamber and surrounded by a water jacket. The temperature of the preparation was maintained at 22° to 25°C except for experiments on the effects of diethylcarbamazine on the membrane potential where the temperature was raised to 32° to 35°C to permit spontaneous electrical activity. The anatomy of the muscle cell is illustrated in Figure 1. Further details of the preparation are available (Martin, 1980).

Ringer solution

The preparation was perfused at a constant rate of 5 ml/min. Four types of Ringer were used. A high-Cl^- Ringer containing (mM): NaCl 135, KCl 3.0, $CaCl_2$ 3.0, $MgCl_2$ 15.7, glucose 3.0 and Tris 5.0, adjusted to pH 7.6 with maleic acid, was used for the examination of Cl^- mediated events. A low-Cl^- Ringer (as above but in which the Cl^- was replaced isotonically with acetate) was used to examine the effects of Cl^- removal on the piperazine-induced conductances. A low-Ca^{2+}, low-Cl^- Ringer (in which the Ca^{2+} was omitted and the Cl^- replaced isotonically with acetate) was used to reduce the Cl^- currents and the voltage-sensitive Ca^{2+} spikes (Weisblat, Byerly & Russel, 1976). The low Ca^{2+}:Mg^{2+} ratio of this Ringer also blocks chemical synaptic transmission. A high-K^+ Ringer containing, either 19 or 35 mM K^+ was prepared by the addition of K^+ acetate to the low-Ca^{2+}, low-Cl^- Ringer.

Recording

Two micropipettes filled with 4 M K^+ acetate (pH 7.6) and 5 MΩ resistance were placed in the bag region of the muscle cell for conductance measurements or voltage clamping, Figure 1. One micropipette was used for voltage recording and the other for current injection. They were connected via Ag/AgCl wires to high input impedance amplifiers. This system permitted the selection of either current clamp (using a 1GΩ resistor as a constant source) or voltage clamp facilities. The time constant of the bag was about 6 ms so that under current clamp rectangular current pulses of greater than 250 ms were used. Under voltage clamp the gain of the control amplifier was set at × 500. During the voltage steps, the bag potential changed rapidly to the new potential in less than 1 ms but the transmembrane current took longer (less than 3 ms) before adjusting to a new level because of the capacitance currents of the membrane. The voltage-insensitive leakage current of the bags was relatively large compared to the voltage-sensitive current or agonist-induced currents. Its effect was reduced by subracting the rectangular current pulse of up to 90 nA with a differential amplifier, see Figure 1. The amplitude of the subtracted current pulse was adjusted using a potentiometer at the beginning of the experiment and this then remained unaltered afterwards. The effect of the series resistance (about 10 K) was expected to be small during the voltage clamp studies.

Responses were monitored on a Tectronix 5103N oscilloscope. Permanent records were made either by photographing the oscilloscope or on a Devices MX212 two channel pen recorder.

Iontophoresis

A third single or double barrelled micropipette was usually placed over the bag, extracellularly between the intracellular voltage and current micropipettes. This pipette was placed within 100 μm of the bag but positioned so that the use of large ejection currents did not cause a d.c. artefact on the electrical responses of the bag. Pipettes were filled appropriately with 0.5 M GABA pH 2.5, 0.5 M piperazine citrate, 0.5 M diethylcarbamazine citrate or 2 M acetylcholine chloride. The agonists were ejected as cations by micro-iontophoresis programmers Model 160 (W-P instruments INC). Retaining currents of 5–10 nA were used. The effect of ejection currents of the opposite polarity were routinely checked as a control but were found to be without effect.

Drugs

The drugs used were GABA (Sigma), piperazine citrate (Sigma), piperazine hexahydrate (Sigma), diethylcarbamazine citrate (Wellcome), acetylcholine chloride (BDH), diethylcarbamazine base and tubocurarine (Sigma). Bath applications of piperazine hexahydrate were used for the study on conductance changes. Diethylcarbamazine base was prepared from the citrate salt by dissolving it in a small volume of 2.5 M NaOH and extraction in chloroform followed by evaporation under vacuum.

Results

The following experiments were carried out to determine the effects of piperazine and diethylcaramazine on the electrical responses of *Ascaris*

muscle. The experiments described here were carried out on the muscle cells of 70 preparations. Except where stated, each experiment was carried out on at least 4 preparations with essentially similar results. Except for experiments on the depolarizing action of diethylcarbamazine on the membrane potential the experiments were carried out at a temperature of 22° to 25°C to block the spontaneous depolarizing potentials and to allow the recording of stable membrane potentials. Recordings were made from the bag region of the muscle cells with a conductance of less than 4 μS and resting membrane potentials of 35–40 mV.

Action of piperazine on bag input conductance

In a high-Cl^- Ringer, bath applications of piperazine in concentrations greater than 10^{-4} M resulted in a reversible increase in the input conductance of the bag together with a hyperpolarization of the membrane potential. This is illustrated in Figure 2a and b where it can be seen that 5×10^{-3} M piperazine reduces the amplitude of the electronic potential produced by the injection of a constant rectangular current pulse. There is an increase in the resting conductance (determined from the amplitude of the electrotonic potential) from 3.6 μS to 5.3 μS. The piperazine induced conductance change was dose-dependent. This is illustrated by dose-response relationship seen in Figure 2c, obtained by the application of increasing concentrations of piperazine with washing between applications. No desensitization was apparent.

Del Castillo *et al.* (1964a) have already illustrated the involvement of Cl^- in the effect of piperazine on membrane potential. The same effect on the piperazine-induced conductance change was examined when the preparations were perfused with a low-Cl^- Ringer. The conductance increases produced by piperazine (10^{-3} M) were dramatically and reversibly reduced to less than 15% of the control levels after perfusing the preparation with low-Cl^- Ringer for 30 min and were undetectable after 1.5 h (not shown). The piperazine conductance changes returned to near control levels after replacement of the Cl^-.

Iontophoresis of piperazine

It has already been pointed out that extra-synaptic GABA receptors are located on the bag region of *Ascaris* muscle cells (Martin 1980). It seemed reasonable to expect that these same receptors would be stimulated during the bath-application of piperazine to produce the recorded increase in input conductance in the bag. Piperazine was therefore applied iontophoretically to the bag region and the transmembrane current responses recorded under voltage clamp. It was found that the application of piperazine within 100 μm of the bag region (and in regions further than 1 mm from the syncytium) produced a dose-dependent outward current at −35 mV in high-Cl^- Ringer. This observation contrasts with the earlier observations of Del Castillo *et al.* (1964a) and shows that piperazine does in fact stimulate extra-synaptic receptors. The results of an iontophoresis experiment is shown in Figure 3. GABA

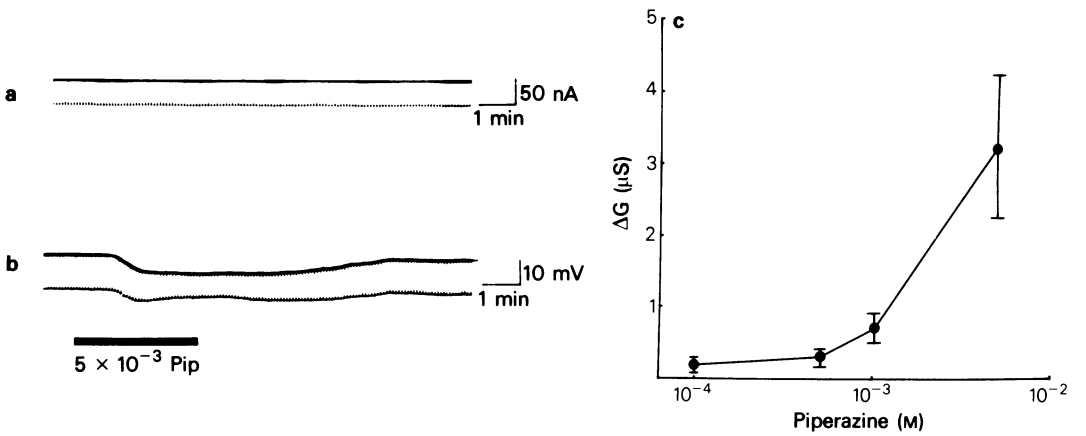

Figure 2 Effect of piperazine on bag input conductance in high Cl^- Ringer: (a) and (b) show a recording of a piperazine-induced conductance change. (a) Constant hyperpolarizing rectangular current pulses of 500 ms. (b) voltage recording during current injection and bath application of 5×10^{-3} M piperazine during the period indicated by the horizontal bar. The piperazine produced a reversible increase in input conductance of 1.7 μS associated with a hyperpolarizing potential. (c) Piperazine dose-response relationship. Ordinate scale: increase in input conductance. Abscissa scale: piperazine molar concentration. Each point shows the mean of 4 observations; vertical lines indicate s.e.mean.

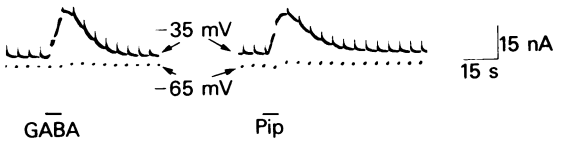

Figure 3 γ-Aminobutyric acid (GABA) and piperazine reversal potentials determined under voltage clamp conditions. The record shows the transmembrane currents (outward currents displayed upwards). The bag was held at −35 mV while hyperpolarizing steps to −65 mV of 1 s duration were made. GABA and piperazine were applied iontophoretically to the same preparation during the period indicated by the horizontal bar. 50nA was used for the ejection of GABA; 800 nA was used for the ejection of piperazine. The transmembrane current record shows that at −35 mV, GABA and piperazine produced an outward current. The envelope of this current record shows that there is little current at −65 mV, which is known to be near the GABA reversal potential. The current steps increase during the application of GABA and piperazine, indicating an increase in conductance of the bag membrane.

Figure 4 Simultaneous application of γ-aminobutyric acid (GABA) and piperazine. A control GABA outward current response following a brief ejection pulse (at ●, 1 μA for 400 ms) was reversibly reduced during the continuous application of piperazine (300 nA during the period indicated by the horizontal bar). Notice also that the total outward current at the peak of the GABA response during the application of piperazine is less than the control.

was first applied to the bag from a single barrelled micropipette using an ejection current of 50 nA during the period indicated by the horizontal bar. It can be seen that at −35 mV a peak outward current of 20 nA was produced. When the GABA pipette was replaced by a piperazine pipette located in the same position near the bag (and well away from the syncytium) an ejection current of 800 nA during the same time-period was required to elicit a similar response. The increase in the ejection current required for piperazine is consistent with its lower potency. Step changes in membrane potential from −35 to −65 mV, lasting 1 s were made throughout the application of the agonists to allow the simultaneous measurement of the induced currents at both potentials by using the envelope of the current record. It can be seen that at −65 mV there is little or no GABA or piperazine-induced current and that this is close to the reversal potential for GABA and piperazine. The estimations of the reversal potentials in other preparations were also similar and close to the predicted Nernst Cl^- potential of *Ascaris* muscle (Martin, 1980).

Picrotoxin will antagonize the response to GABA iontophoresis on the bag of *Ascaris* muscle (Martin, 1980). Picrotoxin in concentrations of 5×10^{-5} to 5×10^{-4} M reversibly and in a dose-dependant manner also antagonized the outward current responses to piperazine iontophoresis.

Simultaneous iontophoresis of γ-aminobutyric acid and piperazine

It is apparent from the previous experiment that piperazine mimics the actions of GABA and increases the Cl^- conductance of the bag membrane. It was decided to test the logical conclusion that piperazine and GABA act on the same receptors. This was carried out using the simultaneous iontophoretic application of piperazine and GABA from a double-barrelled pipette placed over the bag and observing the transmembrane currents at a holding potential of −35 mV. If piperazine and GABA occupy the same receptors then the amplitude of a control GABA response should be reduced during the application of piperazine. The result of a typical experiment is shown in Figure 4. A control GABA response with a peak current of 18 nA was reversibly reduced to 15 nA, then 13.5 nA during the continuous application of piperazine. In this and other preparations it was observed that the level of depression of the GABA response was dose-dependent and increased with the amount of piperazine applied iontophoretically. Notice also that the total outward current at the peak produced by piperazine and GABA in Figure 4 is less than the control. This result was only observed in this and other preparations at the higher levels of piperazine application. Such an action is expected if piperazine is a partial agonist (Iravani, 1965). Alternatively, piperazine iontophoresis at higher levels might produce an inward current due to a non-specific depolarizing effect (Del Castillo *et al.*, 1964a) in addition to the outward Cl^- current. The total piperazine current would then underestimate the activation of GABA receptors. A further study of this was not made.

Iontophoresis of acetylcholine

Bath-applied acetylcholine depolarizes *Ascaris* muscle causing contraction. The acetylcholine receptors responsible for the depolarization were believed to be exclusively located at the syncytium, the position of the neuromuscular synapse (Del Castillo *et al.*, 1963). However, Brading & Caldwell (1971) cut the

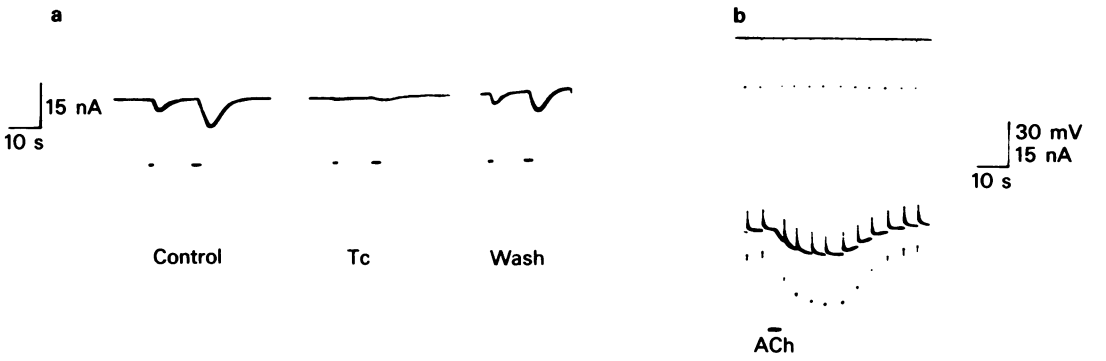

Figure 5 Effect of acetylcholine (ACh) on the transmembrane current under voltage clamp. (a) Iontophoresis of acetylcholine (500 nA for 0.5 s at the short bar and 500 nA for 2 s at the longer bar) produces a dose-dependent inward current at −35 mV when applied within 100 μm of the bag. This response was reversibly antagonized by 3×10^{-5} M tubocurarine (Tc) applied in the bath for 2 min. This antagonism was removed by washing for 3 min. (b) Iontophoresis of acetylcholine (500 nA for 2 s at the horizontal bar) during voltage steps (500 ms duration) from a holding potential of −35 mV to −65 mV. Top trace shows the voltage record and the lower trace shows the transmembrane current. Note that acetylcholine increases the current required for the voltage steps, illustrating that acetylcholine increases the input conductance of the bag membrane.

arms of the muscle cells, to separate the bag region from the syncytium and still observed the depolarizing response to bath-applied acetylcholine. It seemed likely therefore that there were extra-synaptic acetylcholine receptors on the bag. In order to test this hypothesis acetylcholine was applied iontophoretically while measuring the transmembrane current under voltage clamp at −35 mV. In most preparations a low-Ca^{2+}, low-Cl^- Ringer was used during these experiments. This had several advantages including increasing the input resistance of the bags and facilitating successful recording. The low-Ca^{2+}, high-Mg^{2+} ratio was also used to reduce the tonically released acetylcholine and its interaction with bath-applied tubocurarine (Del Castillo *et al.*, 1963). Figure 5a shows the results of a typical experiment. It was found that application of acetylcholine (within 100 μm of the bag) caused a dose-dependent inward current which was reversibly blocked by 3×10^{-5} M tubocurarine. No responses were obtained when the iontophoretic pipette was placed at greater distances from the bag (except over the arms and syncytium). Figure 5b shows that the action of acetylcholine was associated with an increase in bag conductance. Repeated voltage steps from the holding potential of −35 mV to −65 mV lasting 500 ms were made during the application of acetylcholine. It can be seen that there is an increase in the size of the transmembrane current steps during the response due to the conductance increase. Estimates of the acetylcholine-induced currents at −35 and −65 mV were made from the change in the envelope of the current records. In Figure 5b, for example, the peak of the inward acetylcholine current at −35 mV was 8.5 nA, at −65 mV it was 17 nA. Simple extrapolation suggested that the acetylcholine reversal potential was in the region of −5 mV. However, it should be pointed out that a linear current-voltage relationship was assumed for this estimation. This assumption does not hold universally.

Piperazine and diethylcarbamazine antagonism of acetylcholine

Norton & De Beer (1956) first suggested that piperazine has a tubocurarine like action in *Ascaris* and this was not ruled out by Aubry *et al.* (1976). It was possible to test this hypothesis using the response of the extra-synaptic acetylcholine receptors of the bag and observing the effect of bath-applied piperazine on preparations in which the Cl^- mediated responses had been eliminated. To eliminate the Cl^- mediated responses and tonic transmitter release the preparations were perfused as before in a low-Ca^{2+}, low-Cl^- Ringer for at least 1.5 h. After this no piperazine- or GABA-induced conductance increases were detectable. Acetylcholine was then applied iontophoretically as before while the bag was held at −35 mV. It was found that high concentrations of piperazine (10^{-3} to 10^{-2} M) had little effect on the amplitude of the acetylcholine induced current. Figure 6a shows the results of a typical experiment. This illustrates that the action of piperazine was unlike that of tubocurarine in *Ascaris* and that it did not act as a cholinergic antagonist as has been previously suggested (compare Figures 5a and 6a).

It was also of interest to examine the effects of

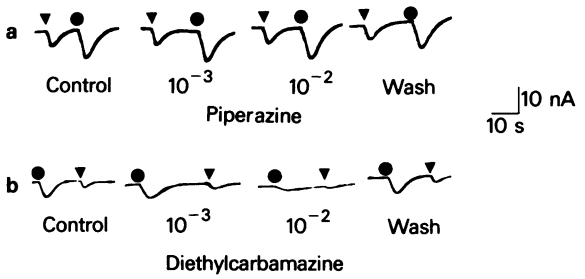

Figure 6 Diethylcarbamazine and piperazine antagonism of acetylcholine. Acetylcholine applied iontophoretically produces a dose-dependent inward current (applied with 400 nA for 0.5 s at (▼) and with 400 nA for 2.5 s at (●), bag voltage clamped at −35 mV, low-Ca^{2+}, low-Cl^- Ringer). (a) Piperazine in high concentrations up to 10^{-2} M failed to antagonize the response. Each concentration was applied for 2 min and then washed for 3 min before the final test. (b) Another preparation, diethylcarbamazine in concentrations greater than 10^{-3} M reversibly antagonized the acetylcholine responses. Each concentration was applied for 2 min and then washed for 3 min before the final test.

diethylcarbamazine under the same conditions. Bath-applied diethylcarbamazine (10^{-3} to 10^{-2} M as citrate) was used. In concentrations greater than 10^{-3} M, diethylcarbamazine produced a reversible and dose-dependent antagonism of the acetylcholine responses. Figure 6b illustrates the results of a typical experiment. It was concluded that diethylcarbamazine but not piperazine has a weak cholinoceptor antagonist action.

Effect of histamine on membrane potential and input conductance

Phillips *et al.* (1976) have reported that piperazine affects the uptake of histamine by the parasite and that histamine may be an inhibitory transmitter. Histamine was therefore applied iontophoretically to the bag and in the bath in concentrations up to 10^{-3} M with little or no effect on the membrane potential or input conductance. This was despite perfusing the preparation in a high-Cl^- Ringer in conditions under which stimulation of inhibitory nerves produces hyperpolarization (Del Castillo *et al.*, 1964a). The lack of effect of histamine does not support the suggestion that histamine is a natural inhibitory transmitter.

Effect of bath-applied diethylcarbamazine on membrane potential

Broome (1962) has described an excitatory effect of diethylcarbamazine producing bigger waves of contraction and relaxation in the *Ascaris* nerve muscle preparation. Its action was not like that of acetylcholine which produced a simple sustained contraction. Unlike acetylcholine its action was not blocked by tubocurarine. The effect of bath-applied diethylcarbamazine on membrane potential was therefore examined. A high-Cl^- Ringer was used initially to permit detection of possible interactions with GABA receptors. It was found that diethylcarbamazine (10^{-4} to 2×10^{-3} M as citrate) in active preparations at 32° to 35°C produced a reversible dose-dependent depolarization associated with an increase in the spontaneous depolarizing potentials. These potentials are produced by pacemaker activity at the syncytium. However, the effect on membrane potential at the lower temperatures of 22° to 25°C was variable and often absent. Diethylcarbamazine base (10^{-4} to 2×10^{-3} M) was also used at 32° to 35°C to test this effect further and to eliminate possible effects of citrate. Figure 7a illustrates a typical depolarizing response to diethylcarbamazine base which was associated with an increase in frequency and amplitude of the spontaneous depolarizing potentials. The effect of diethylcarbamazine base was essentially similar to that of the citrate. The dose-response relationship shown in Figure 7b was determined by applying increasing concentrations and washing between applications. At high concentrations the preparation began to contract making recording more difficult.

Broome (1962) has reported that tubocurarine has no blocking action on the excitatory effect on contraction induced by diethylcarbamazine. The effect of 3×10^{-5}M tubocurarine on the depolarizing action of diethylcarbamazine (10^{-3} to 2×10^{-3} M base) was also examined in three preparations. This concentration of tubocurarine which blocked the responses to acetylcholine iontophoresis had no antagonistic action on the diethylcarbamazine responses. From these observations it was concluded that diethylcarbamazine has a depolarizing action which is not due to a cholinergic action.

Effect of diethylcarbamazine on γ-aminobutyric acid responses

Diethylcarbamazine is a piperazine derivative and piperazine has been shown to be a GABA agonist in this preparation. A possible explanation therefore of the depolarizing action of diethylcarbamazine was that it might be acting on the same receptor as piperazine but as a GABA antagonist. To test for this, GABA responses were elicited by iontophoresis at the bag in a high-Cl^- Ringer while the effect of simultaneous iontophoresis of diethylcarbamazine or bath-application of diethylcarbamazine (10^{-4} to 10^{-3} M as citrate) on transmembrane currents was observed. It was found that neither iontophoresis nor bath-application of diethylcarbamazine had a detect-

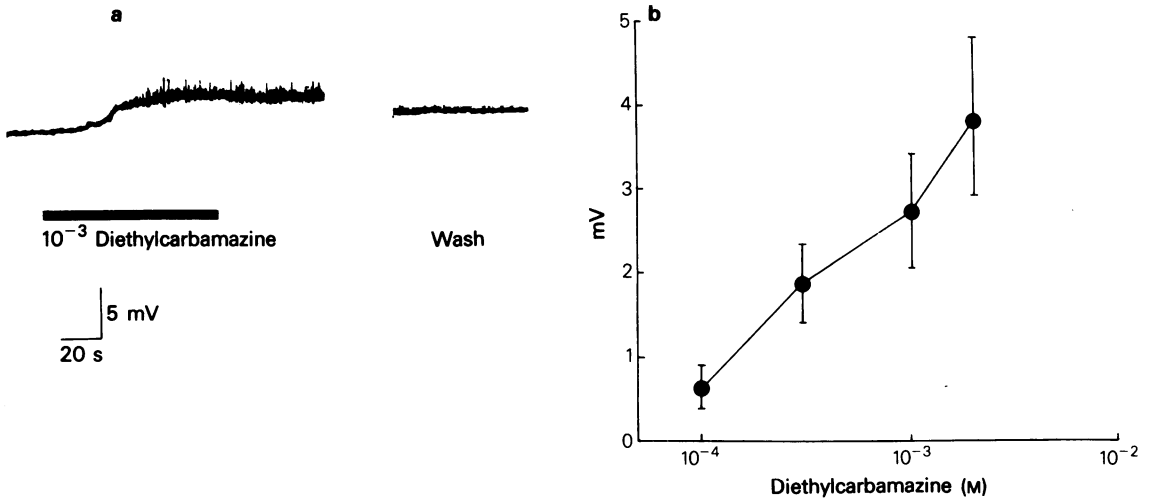

Figure 7 Depolarizing effect of diethylcarbamazine. Diethylcarbamazine base was bath-applied to preparations bathed in high-Cl^- Ringer and its effect on bag membrane potential recorded at a temperature of 32° to 35°C. (a) Diethylcarbamazine (10^{-3} M) produced depolarization associated with an increase in the frequency and amplitude of spontaneous depolarizing potentials. The membrane potential returned to the resting level after washing for 10 min, temperature 32°C. (b) Diethylcarbamazine log-dose response relationship obtained in high-Cl^- Ringer. Ordinate scale: membrane depolarization. Abscissa scale: molar concentration of diethylcarbamazine base applied in the bath. Each point shows the mean of 8 or 11 observations; vertical lines indicate s.e.mean.

able effect on the GABA currents. The result of a typical experiment is shown in Figure 8. It was concluded from these observations that diethylcarbamazine did not act as a GABA antagonist.

Effect of diethylcarbamazine on delayed rectification

The bag current-voltage relationship is characterized by the existence of delayed rectification (Martin, 1980). It was noticed during the previous experiments that diethylcarbamazine (10^{-4} to 2×10^{-3} M as base or citrate) antagonized this delayed rectification in a reversible and dose-dependant manner. This same effect was also observed at 22° to 25°C. Figure 9 shows the effects of perfusing a preparation with diethylcarbamazine base on the current-voltage relationship at 25°C.

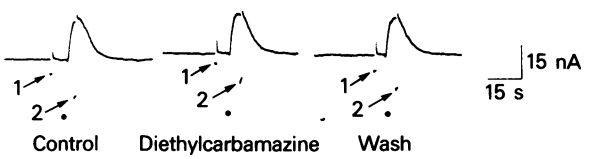

Figure 8 Effect of diethylcarbamazine on γ-aminobutyric acid (GABA) responses. In a high-Cl^- Ringer, GABA applied iontophoretically (at (●) using 1 μA for 400 ms) produced an outward transmembrane current response when the bag membrane potential was held at −40 mV. Two hyperpolarizing steps to −70 mV lasting 1 s at (1) and (2) were made. It was thus possible to estimate the GABA-induced conductance from the change in the transmembrane current steps required to hyperpolarize the bag to −70 mV. Iontophoresis of GABA (control) produced an outward current response of 20 nA at −40 mV and an increase in input conductance of 1 μS. Bath-application of 10^{-3} M diethylcarbamazine citrate had little effect on the GABA response. Occasionally, as in this preparation, there was a small increase in the resting membrane conductance during the application of diethylcarbamazine citrate.

The effect of diethylcarbamazine on the voltage-sensitive outward current in *Ascaris* was examined in a low-Ca^{2+}, low-Cl^- Ringer using depolarizing steps from −35 to −20 mV. This Ringer was used to reduce Cl^- and voltage-sensitive Ca^{2+} currents. It was found that diethylcarbamazine (as base, citrate was not used) in concentrations greater than 10^{-4} M reversibly antagonized the voltage-sensitive outward current. In addition to this effect, there was sometimes a small steady inward current during its application. The level of antagonism was dose-dependent and increased with the concentration of diethylcarbamazine within the range tested (10^{-4} to 2×10^{-3} M). Figure 10a and b shows the effects of bath-applied diethylcarbamazine. Delayed rectification in other preparations is mediated via an outward K^+ current (Hodgkin & Huxley, 1952). In order to confirm this in *Ascaris* the effects of a high-K^+ Ringer (19 mM and 35 mM) to reduce the K^+ electrochemical gradient and 4-aminopyridine (10^{-3} and 2×10^{-3} M) as a K^+ blocker were tested. It was found

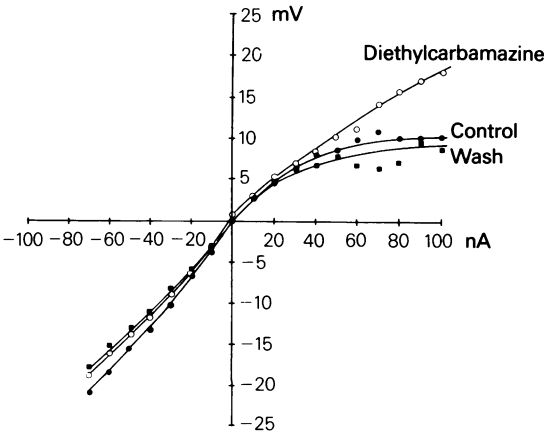

Figure 9 Effect of diethylcarbamazine in the current-voltage relationship. The current-voltage relationship was determined in a high Cl^- Ringer using constant rectangular current pulses of 500 ms duration and the change in membrane potential produced by the current measured at the end of the pulse. The control current-voltage relationship showed delayed rectification during the injection of depolarizing current. Diethylcarbamazine base 10^{-3} M bath-applied (for 5 min) produced a reversible depolarization of 2 mV and a reversible antagonism of the delayed rectification (after washing for 5 min). Temperature 25°C.

that they mimicked the action of diethylcarbamazine by decreasing the voltage-sensitive outward current. Attempts to separate effects on different voltage-sensitive K^+ currents were not made in this study. It was concluded that diethylcarbamazine has an antagonistic action on the voltage-sensitive K^+ conductance of the *Ascaris* membrane.

Discussion

Piperazine as a γ-aminobutyric acid agonist

The experiments described in this paper show that piperazine acts as a GABA agonist of low potency on extra-synaptic receptors on *Ascaris* muscle giving rise to hyperpolarization mediated by an increase in Cl^- conductance. No cholinergic antagonistic action of piperazine was detected despite the application of high concentrations. Thus no evidence was found to support the hypothesis of a curariform block as originally suggested by Norton & De Beer (1957) and reiterated by Sheth (1975). It is now reasonable to explain the piperazine-induced reversible paralysis of nematodes and their expulsion from the intestine during therapy (Desowitz, 1971) by its GABA mimetic action. The site of action of piperazine is not necessarily limited to the region of the neuromuscular synapse as originally described by Del Castillo *et al.* (1964a). It is clear from this study that extra-synaptic GABA receptors may also be stimulated by piperazine during therapy.

It is interesting that piperazine also acts as a GABA agonist on crayfish muscle (Iravani, 1965) and lobster muscle (Constanti & Nistri, 1976) but not on rat sympathetic neurones (Connor, Constanti, Dunn, Forward & Nistri, 1981). In fact, Nistri & Constanti (1979) have suggested the existence of at least two separate types of GABA receptor in some invertebrates and some vertebrate preparations with piperazine as a selective agonist in invertebrates. However, piperazine does have a GABA mimetic action in some areas of the vertebrate central nervous system (Perkins & Stone, 1982).

Extra-synaptic cholinoceptors

The iontophoresis of acetylcholine on to the bag region of the muscle confirms the presence of extra-synaptic receptors on the bag in addition to those already reported to be present at the syncytium (Del Castillo *et al.*, 1963). The extra-synaptic receptors were antagonized by tubocurarine and their activation increased the membrane conductance and gives rise to an inward current under voltage clamp conditions. The physiological significance of these receptors is not known since it is a region devoid of synapses. However, treatment of the host with a cholinomimetic anthelmintic may stimulate these extra-syaptic receptors, as well as the synaptic ones. For example, pyrantel tartrate, a nicotinic anthelmintic (Aubry *et al.*, 1976) is a potent agonist and acts on the acetylcholine receptors of the bag when applied iontophoretically (unpublished observations). It may have a similar action during therapy.

Effect of diethylcarbamazine on K^+ conductance

In this study it was shown that diethylcarbamazine did not mimic the actions of piperazine but antagonized a voltage-sensitive K^+ current in *Ascaris*. Diethylcarbamazine also depolarized the membrane in active preparations at 32° to 35°C, but this effect was variable and often absent at 22° to 25°C. The effect of K^+ on the resting membrane potential in *Ascaris* has been reported to be small (Brading & Caldwell, 1971). It is likely that the depolarizing action in active preparations results mainly from an action on the voltage-sensitive K^+ conductance rather than from any effect on the resting K^+ conductance. This in turn could give rise to an excitatory effect on contractility as observed by Broome (1962). The absence of any nicotinic agonist action or GABA antagonism in *Ascaris* is consistent with such a mechanism. The absence of a nicotinic action con-

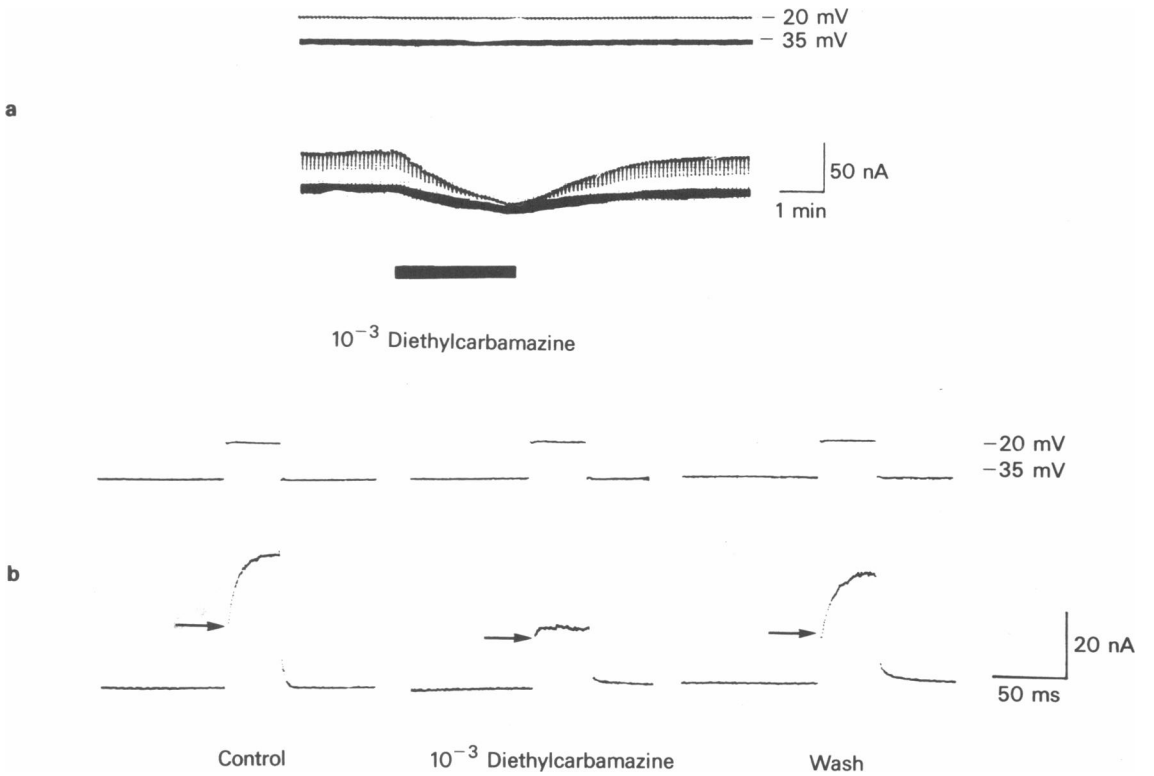

Figure 10 Effect of diethylcarbamazine antagonism on the voltage-sensitive outward current: (a) and (b) are recordings from two separate preparations bathed in low-Ca^{2+}, low-Cl^- Ringer at 25°C. Both illustrate the effect of bath-applied diethylcarbamazine base (10^{-3} M) on transmembrane currents. Bag membrane potentials were held at −35 mV with depolarizing steps to −20 mV lasting 150 ms in (a) but 50 ms in (b). The top traces display membrane potentials while the lower traces display the transmembrane currents. (a) Diethylcarbamazine applied during the horizontal bar produced a steady inward current at −35 mV and reduced the inward current required to depolarize the cell. (b) Similar conditions as (a) except that the recordings were made on a faster time scale. After the depolarizing step there was a voltage-independent step in the transmembrane current (up to arrow) due to the leakage resistance of the bag. The capacitance currents were too fast to be seen on this photograph. This current step was then followed only in the control and post-wash records by an approximately exponential increase in the outward current. The early part of the control and wash voltage-sensitive currents were retouched with dots. Note that diethylcarbamazine reversibly antagonized the voltage-sensitive outward current.

trasts with the effects of diethylcarbamazine reported in some mammalian preparations (Abaitey & Parratt, 1976; 1977). However, the interpretation of the mechanism of action of diethylcarbamazine in whole animal studies is complicated by the rapid appearance of metabolites (Hawking, 1979).

The author wishes to thank the Agricultural Research Council for financial support and Dr S. Watts of the Wellcome Foundation for the gift of diethylcarbamazine citrate.

References

ABAITEY, A.K. & PARRATT, J.R. (1976). Cardiovascular effects of diethylcarbamazine citrate. *Br. J. Pharmac.*, **55**, 219–227.

ABAITEY, A.K. & PARRATT, J.R. (1977). The effects of diethylcarbamazine citrate on smooth muscle. *J. Pharm. Pharmac.*, **29**, 428–432.

AUBRY, M.L., COWELL, P., DAVEY, M.J. & SHEVDE, G. (1970). Aspects of the pharmacology of a new anthelmintic: pyrantel. *Br. J. Pharmac.*, **38**, 332–344.

BRADING, A.F. & CALDWELL, P.C. (1971). The resting membrane potential of the somatic muscle cells of *Ascaris lumbricoides*. *J. Physiol.*, **217**, 605–624.

BROOME, A.W.J. (1962). Mechanisms of anthelmintic action with particular reference to drugs affecting neuromuscular activity. In *Drugs Parasites and Hosts* ed. Goodwin, L.G. & Nimmo-Smith, R.H. pp. 43–61. London: Churchill.

CONNOR, J.D., CONSTANTI, A., DUNN, P.M., FORWARD, A. & NISTRI, A. (1981). The efffects of piperazine on rat sympathetic neurones. *Br. J. Pharmac.*, **74**, 445–454.

CONSTANTI, A. & NISTRI, A. (1976). A comparative study of the action of γ-aminobutyric acid and piperazine on the lobster muscle fibre and the frog spinal cord. *Br. J. Pharmac.*, **57**, 347–358.

DEL CASTILLO, J., DE MELLO, W.C. & MORALES, T. (1963). The physiological role of acetylcholine in the neuromuscular system of *Ascaris lumbricoides*. *Archs Int. Physiol.*, **71**, 741–757.

DEL CASTILLO, J., DE MELLO, W.C. & MORALES, T. (1964a). Mechanism of the paralysing action of piperazine on *Ascaris* muscle. *Br. J. Pharmac.*, **22**, 463–477.

DEL CASTILLO, J., DE MELLO, W.C. & MORALES, T. (1964b). Inhibitory action of γ-aminobutyric acid (GABA) on Ascaris muscle. *Experientia.*, **20**, 145–146.

DESOWITZ, R.S. (1971). Antiparasite chemotherapy. *A. Rev. Pharmac.*, **11**, *351–368*.

HAWKING, F. (1979). Diethylcarbamazine and new compounds for treatment of filariasis. *Adv. Pharmac. Chem.*, **16**, 130–194.

HODGKIN, A.L. & HUXLEY, A.F. (1952). Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *J. Physiol.*, **116**, 449–472.

IRAVANI, J. (1965). Wechselbeziehung von Barbituraten und Piperazin mit GABA an der Membran des Krebsmuskels. *Naunyn-Schmeidebergs Arch. exp. Path. Pharmak.*, **251**, 265–274.

MARTIN, R.J. (1980). The effect of γ-aminobutyric acid on the input conductance and membrane potential of *Ascaris* muscle. *Br. J. Pharmac.*, **71**, 99–106.

NISTRI, A. & CONSTANTI, A. (1979). Pharmacological characterization of different types of GABA and glutamate receptors in vertebrates and invertebrates. *Prog. Neurobiol.*, **13**, 117–235.

NORTON,S. & DE BEER, E.J. (1957). Investigations on the action of piperazine on *Ascaris lumbricoides*. *Am. J. trop. Med.*, **6**, 898–905.

PERKINS, M.N. & STONE, T.W. (1982). Comparison of the effects of ethylinediamine analogues and γ-animobutyric acid on cortical and pallidal neurones. *Br. J. Pharmac.*, **75**, 93–99.

PHILLIPS, J.L., STURMAN, G. & WEST, G.B. (1976). The interaction between anthelmintic drugs and histamine in *Ascaris suum*. *Br. J. Pharmac.*, **57**, 417–420.

SHETH, U.K. (1975). Mechanisms of anthelmintic action. *Prog. Drug. Res.*, **19**, 147–157.

STANDEN, O.D. (1975). Chemotherapy of intestinal helminthiasis. *Prog. Drug. Res.*, **19**, 158–165.

WEISBLAT, D.A., BYERLY, L. & RUSSELL, R.L. (1976). Ionic mechanisms of electrical activity in somatic muscle of the nematode *Ascaris lumbricoides*. *J. comp. Physiol.*, **111**, 93–113.

(Received February 5, 1982.)