Actions of acetylcholine and GABA on spontaneous contractions of the filariid, *Dipetalonema viteae*

¹Daryl Christ, ²*Michael Goebel & *Howard J. Saz

South Bend Center for Medical Education, Indiana University School of Medicine, Notre Dame, Indiana 46556 and

*Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana 46556, U.S.A.

- 1 Isotonic contractions were recorded from the filarial nematode, *Dipetalonema viteae* (*Acanthocheilonema viteae*), in an isolated tissue chamber.
- 2 Nicotine (10^{-6} M) and pilocarpine (10^{-5} M) increased the spontaneous contractions in the intact filariid, but acetylcholine (ACh, 10^{-4} M) and muscarine (10^{-5} M) were inactive.
- 3 When ACh was applied to an opened *D. viteae*, it was 10,000 times more potent. This indicates that the cuticle is an effective barrier to the penetration of ACh to the muscle cells.
- 4 The effects of ACh on the opened *D. viteae* were not affected by hexamethonium (10^{-3} M) or atropine (10^{-5} M) and were only partially reduced by (+)-tubocurarine (10^{-4} M).
- 5 γ -Aminobutyric acid (GABA, 10^{-3} M) reduced the spontaneous activity of the intact *D. viteae*; however, the effect of GABA had a slow onset and recovery. Muscimol (10^{-5} M) was more potent than GABA and had a more rapid onset and recovery.
- 6 GABA was 1,000 times more potent on the opened *D. viteae* than on the intact *D. viteae*. Baclofen (10^{-3} M) was inactive on both preparations.
- 7 The effect of GABA was not antagonized by bicuculline (10^{-4} M), picrotoxin (10^{-5} M) or penicillin G (10^{-3} M).
- 8 It is concluded that the filariid cuticle acts like a lipid structure and blocks the penetration of polar substances, such as ACh and GABA. Also, due to the lack of efficacy of the ACh and GABA antagonists, it was concluded that the nematode receptors are somewhat different from the mammalian ACh and GABA receptors.

Introduction

Filarial infections due to *Onchocerca*, *Wuchereria*, *Loa loa* and *Brugia* are world health problems, especially in the tropical climates (Dorozynski, 1976). However, the basic pharmacology of the filariids has not been adequately explored. There are only a few models of the human filariids. The most common pharmacological model is the intestinal nematode, *Ascaris*. Due to its intestinal localization, the clinical treatment of this nematode is quite different from the treatment of the filariids, which are localized subcutaneously or in the lymphatic system.

The body wall musculature of nematodes is involved in many essential processes, including locomotion, intestinal function, reproduction and structural support; thus disruption of muscle function would appear to be an important site of drug action. The muscle cells in the body wall of *Ascaris suum* are obliquely striated (Rosenbluth, 1965a); however, there are many physiological differences from mammalian skeletal muscle (DeBell *et al.*, 1963). Contractions appear to be initiated myogenically and the myogenic activity is probably modulated by an excitatory neurotransmitter, acetylcholine (ACh) and an inhibitory neurotransmitter, γ -aminobutyric acid (GABA) which are released by the neurones in the nerve cord (del Castillo *et al.*, 1963; 1964; Johnson & Stretton, 1985; 1987). In many ways, nematode muscle is similar, physiologically, to mammalian smooth muscle.

One filariid model is *Dipetalonema viteae* (*Acanthocheilonema viteae*). Rohrer *et al.* (1988) have described the physiological properties of single muscle cells in *D. viteae*, by use of intracellular recording methods. There were many similarities to *Ascaris*. ACh depolarized the muscle cells and increased the frequency of muscle action potentials and GABA reduced the frequency of the muscle action potentials.

The depolarization by ACh, however, was not reduced by (+)-tubocurarine. Curare has been reported to reduce the responses to ACh in *Ascaris* (Norton & deBeer, 1957; del Castillo *et al.*, 1963).

Although ACh and GABA increase and decrease the frequency of muscle action potentials, respectively, the effects of these substances on the contractility of *D. viteae* have not been reported. Experiments on muscle contractility would be useful to characterize further the ACh and GABA receptors in the filariid to determine whether they are similar to the receptors in *Ascaris* and in mammalian tissues. This paper describes the effects of cholinergic and GABA-receptor agonists and antagonists on the spontaneous contractions of *D. viteae*. Preliminary accounts of these findings have been presented (Christ & Saz, 1986; Christ *et al.*, 1988).

Methods

Hamsters were infected with *D. viteae* larvae at the University of Georgia and shipped to the University of Notre Dame. The hamsters were killed by cervical dislocation and were kept warm and moist, while the adult *D. viteae* (4–12 months old) were removed from the subcutaneous tissues (Rohrer *et al.*, 1988). Intact worms were mounted in an isolated tissue bath (10 ml) by tying a thread to each end of the worm. One thread was attached to an isotonic transducer (Harvard Apparatus) and the other was attached to a mounting rod at the bottom of the bath. The length of the preparation was 4–5 cm, and the tension on the preparation was approximately 25 mg. The worm was bathed in a basic filarial medium (Bueding, 1949).

Other *D. viteae* were slit open, longitudinally. The reproductive and intestinal tracts were removed, before the worm was set up for the isotonic recording. The opened worms were bathed in a *Dipetalonema* body wall saline (DBWS) (Rohrer *et al.*, 1988).

The temperature was maintained at 37°C, and the chamber was bubbled with 95% N₂:5% CO₂. Agonists were added

¹ Author for correspondence.

² Present address: University of Iowa School of Medicine, Iowa City, Iowa, U.S.A.

directly to the bath with microlitre syringes and mixing of the drugs was enhanced by the bubbling with $N_2:CO_2$. To remove the drugs, the chamber was flushed with prewarmed physiological solution. Each experiment was performed in three preparations. The magnitudes of the contractions from the cholinergic agonists were quantitated from the chart recording. Due to the rhythmic activity, it was necessary to estimate visually the peak of each response. These contractions were compared to the maximal response of each preparation, produced by either 10^{-4} M nicotine or 10^{-5} M ACh. Changes in the rhythmic contractions were not quantified. Since only small changes in length occurred with GABA and muscimol, these results were also not quantified. There were, however, marked changes in the rhythmic contractions with GABA and muscimol. Antagonists were usually added to the reservoir of solution that was used to flush the chamber.

D. viteae that were not used on the day of isolation were kept in an incubator at $37^\circ C$ with 95% $N_2:5\%$ CO_2 for use in subsequent experiments. The incubation medium was composed of 3 ml NCTC 135, 3 ml Dulbecco's modified Eagles Medium, 1 ml horse serum, 0.7 mg penicillin G and 0.7 mg streptomycin. The worms could be maintained in this medium for 3 to 4 days with little loss of responsiveness to the drugs.

The drugs used were: acetylcholine chloride, muscarine chloride, nicotine, pilocarpine hydrochloride, atropine sulphate, (+)-tubocurarine chloride, hexamethonium chloride, GABA, muscimol, baclofen, picrotoxin, bicuculline and potassium penicillin G. All were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.), except baclofen (CIBA Pharmaceutical, Summit, NJ, U.S.A.) and hexamethonium chloride (U.S. Biochemical Corp., Cleveland, OH, U.S.A.).

Results

Cholinergic agonists on the intact *D. viteae*

The filariid contracted spontaneously and the magnitude of the contractions for each worm was consistent and stable for several hours in the tissue chamber. The worms that were

used 1 to 2 days after isolation from the hamsters were much more active than those used 4 to 5 days after isolation.

When ACh was added to the solution bathing the intact filariid, it had no effect at concentrations up to 10^{-4} M (Figures 1 and 3). ACh, 10^{-3} M, usually induced a very small contraction. Muscarine, a muscarinic cholinergic agonist, was also inactive (Figure 1). ACh is considered to be the neurotransmitter that is liberated from the nerve cord neurones of nematodes and it increases the excitability of the muscle cells in *Ascaris* and *D. viteae* (del Castillo *et al.*, 1963; Rohrer *et al.*, 1988); thus it should have increased the contractility in this preparation. The weak response to ACh could be due to high cholinesterase activity (Bueding, 1952). Neostigmine (10^{-6} M) and physostigmine (10^{-6} M), however, did not enhance the actions of ACh, indicating that the lack of effect was not due to rapid metabolism of ACh by cholinesterases.

Norton & deBeer (1957) noted that the sensitivity to piperazine was much greater in the open *Ascaris* preparation than in the intact *Ascaris*. The cuticle apparently acted as a permeability barrier to piperazine and other polar substances in *Ascaris*. Although the filariid cuticle is quite different, morphologically, from the *Ascaris* cuticle (Fetterer, 1986); it may still function as a permeability barrier. In support of this concept, cholinergic agonists with high lipid solubilities were very active. Nicotine, a lipid-soluble nicotinic cholinergic agonist, induced an immediate contraction at concentrations greater than 10^{-7} M (Figure 1). This effect was rapidly reversed upon flushing the chamber with control solution. The effects of nicotine were concentration-dependent and appeared to reach a maximum at 10^{-4} M. Pilocarpine, a lipid soluble muscarinic cholinergic agonist, was also effective; although it had a lower intrinsic activity than nicotine (Figures 1 and 3). The maximal response was produced at 10^{-4} M pilocarpine. These results support the concept that ACh is inactive because it cannot penetrate to its site of action.

Cholinergic agonists on the opened *D. viteae* preparation

The opened filariid was surprisingly very active, spontaneously, although the contractions appeared to have a differ-

Figure 1 Effects of cholinergic agonists on the spontaneous isotonic contractions of the intact filariid, *Dipetalonema viteae*. Acetylcholine (ACh), nicotine, muscarine and pilocarpine were added to the bathing solution at the final molar concentrations indicated below each trace. The horizontal bar indicates the time of the exposure to the drug. At the end of the exposure the bath was flushed with approximately 15 ml of control solution. Additional flushes are indicated by dots. Note that nicotine and pilocarpine were very effective, however ACh and muscarine were ineffective. Calibrations: 2 mm and 2 min.

ent type of rhythmicity from those in the intact filariid. The activity was stable for several hours; in fact, the contractions often increased with time. It was not uncommon for a preparation to be totally inactive when it was first set up, and to become very active after 30 to 60 min.

ACh was much more effective on the opened preparation. Even 10^{-7} M ACh induced a rapid contraction, as well as an increase in the rhythmic activity of the preparation (Figure 2). The effect of ACh was readily reversible upon flushing the chamber. The magnitude of the contractions was reproducible and concentration-dependent. As can be seen in Figure 3, ACh was at least 10,000 times as potent on the open preparation as on the intact preparation. Muscarine was also much more potent on the open preparation. These differences probably occur because the cuticle blocks the diffusion of ACh and muscarine, both quaternary amines, to the cholinceptors in the intact preparation. According to this hypothesis, the non-quaternary, highly lipid soluble agonists should have approximately the same potency on the intact and opened filariids. Nicotine and pilocarpine increased the contractions of the opened *D. viteae*, and they did have approximately the same potency on the opened as on the intact *D. viteae*, although the maximal response to pilocarpine of the opened *D. viteae* was somewhat less than that of the intact *D. viteae* (Figures 2 and 3).

GABA agonists on the intact *D. viteae*

GABA reduced the spontaneous activity of the intact filariid but high concentrations were necessary (Figure 4). GABA, 10^{-4} M, produced a weak suppression and complete blockade of the spontaneous contractions did not occur at concentrations less than 10^{-3} M. Also, the time course of the GABA action was very slow. The time to the maximal blockade was 1 to 2 min, and full recovery often required 10 to 15 min.

Muscimol, a GABA_A receptor agonist, was much more potent than GABA on this preparation (Figure 4). Muscimol, 10^{-5} M, was very effective. Furthermore the blockade and

recovery were very rapid with muscimol. Baclofen, a GABA_B receptor agonist, was inactive at concentrations up to 10^{-3} M.

GABA agonists on the opened *D. viteae* preparation

GABA was more than 1000 times as active on the opened filariid, as on the intact filariid. Even 10^{-7} M GABA induced a depression of the spontaneous contractions, although it was not obvious in the record in Figure 5. The concentration-response relationship was very steep, as 10^{-6} M induced a complete paralysis in most preparations. The onset of action was rapid, as was the rate of recovery, even at the high concentrations. Thus the time-course of the GABA blockade was very different from that on the intact *D. viteae*. These differences were probably due to the low lipid solubility of GABA (Yunger & Cramer, 1981).

Muscimol was also active on the opened preparation; however it had about the same potency as on the intact preparation (compare Figures 4 and 5). Baclofen was inactive (Figure 5).

ACh antagonists on the opened *D. viteae* preparation

None of the ACh antagonists, hexamethonium, (+)-tubocurarine and atropine, had consistent effects on the spontaneous contractions at concentrations that would block mammalian preparations (Figure 6). Furthermore, in most experiments the antagonists had little effect on the contractions from the cholinceptor agonists (Figure 7). Only (+)-tubocurarine reduced the contractions to ACh. This reduction was 69% in the three preparations at a concentration that would block mammalian cholinceptor responses by 100%. Hexamethonium and atropine had insignificant effects on the ACh-induced contractions.

GABA antagonists on the opened *D. viteae* preparation

The GABA antagonists, picrotoxin, bicuculline and penicillin G, had few effects on the spontaneous contractions of the

Figure 2 Effects of cholinceptor agonists on the spontaneous isotonic contractions of the opened *D. viteae*. Each agonist was applied at the time indicated by the bar and at the concentration indicated below each bar. The bath was flushed at the end of the exposure (bar) and at each dot. Note that all the agonists were effective. Calibrations: 1 mm and 2 min (acetylcholine (ACh), nicotine and pilocarpine); 2 mm and 2 min (muscarine).

Figure 3 Concentration-response relationships for the cholinergic agonists. The contractions induced by acetylcholine (ACh, intact, ■; opened, □) and nicotine (intact, ●; opened, ○) are illustrated in (a). Each contraction was measured as a percentage of the contraction to 10^{-4} M nicotine, which was the maximal response in most preparations. In (b) the results from muscarine (intact, ■; opened, □) and pilocarpine (intact, ●; opened, ○) are shown. Each point is the mean of three experiments, and the vertical bar is the s.d.

opened preparation (Figure 8). Bicuculline (10^{-4} M) did tend to increase the spontaneous activity in the preparation illustrated in this figure. The antagonists did not affect the depression of the spontaneous contractions due to GABA (Figure 9).

Discussion

These experiments demonstrate that the recording of isotonic contractions from the filariid, *D. viteae*, is an easy and reliable method for observing the acute effects of drugs. Although the filariid is very small, it can easily contract against an isotonic transducer. The nerve ring is removed during the surgery, thus the preparation is essentially cuticle, muscle and nerve cord which makes it simple and homogeneous. The magnitude of the spontaneous activity is very constant for long periods of time. The intact filariid could be useful for drug screening of anthelmintics. The open filariid is very sensitive to cholinergic and GABA receptor agonists and it would be useful for the study of basic physiological and pharmacological mechanisms of drug actions, because substances can more readily reach their sites of action.

ACh and GABA were relatively inactive on the intact preparation but were very active on the open preparation. The major difference between these two preparations is that the cuticle does not act as a barrier in the open preparation. This indicates that the cuticle can reduce the penetration of ACh and GABA into intact filariids. Both of these compounds have low lipid solubilities and high water solubilities which indicates that the cuticle acts as a lipoidal barrier, in a similar fashion to the *Ascaris* cuticle (Fetterer, 1986). In support of this conclusion, the more lipid soluble substances, nicotine, pilocarpine and muscimol, had the same activities on the intact and opened preparations. Due to their high lipid solubilities, these drugs can readily penetrate the cuticle and quickly reach their sites of action, even in the intact preparation.

A drug with a high lipid solubility will have a rapid onset and high anti-filarial activity on the intact preparation. Drugs with low lipid solubilities will have low activities on the intact preparation, but they may still have anti-filarial efficacy. With long term exposure, polar drugs may eventually penetrate the cuticle and become trapped in the filariid or the filariid may ingest the drugs so they can be absorbed (Bone & Bottjer,

Figure 4 Effects of γ -aminobutyric acid (GABA) receptor agonists on the isotonic contractions of *D. viteae*. GABA, muscimol and baclofen were added to the bathing solution at the time indicated by the bar and at the concentration indicated below each bar. The bath was flushed at the end of the exposure (bar) and at each dot. Note that the onset and recovery of the GABA blockade were very slow. Calibrations: 2 mm and 2 min.

Figure 5 Effects of γ -aminobutyric acid (GABA) agonists on the isotonic contractions of the opened *D. viteae*. GABA, muscimol and baclofen were added to the bathing solution at the times indicated by the bars and at the concentration indicated below each bar. The bath was flushed at the end of the exposure (bar) and at each dot. Note that the onset and recovery of the GABA blockade were very fast in this preparation. Calibrations: 2 mm and 2 min.

1986). An advantage of drugs with low lipid solubilities is that they are less likely to penetrate the human blood brain barrier and induce CNS toxicity in the host.

The effectiveness of ACh on the opened *D. viteae* and the activity of the cholinergic agonists indicates that cholinergic receptors are present in *D. viteae*, and that activation of the cholinergic receptors can increase the muscle contractions. There are probably both nicotinic and muscarinic cholinergic receptors in *D. viteae*, since the muscarinic agonists, pilocarpine and muscarine, and the nicotinic agonist, nicotine, increased the rhythmic contractions of the opened *D. viteae*. Since the antagonists, including (+)-tubocurarine, were much less effective in blocking the actions of ACh in the filariids, the cholinergic receptors in filariids must have different properties from the cholinergic receptors in mammals. Furthermore, the shift of the ACh concentration-response curve is not indicative of a competitive interaction, which is unlike the mammalian receptors. The lack of activity of the antagonists in reducing the actions of ACh also means that the antagonists are not ideal for testing whether ACh is a neurotransmitter in *D. viteae*. del Castillo *et*

al. (1963) were able to demonstrate a blockade of the responses to exogenous ACh by tubocurarine in *Ascaris*; however, the antagonists were ineffective in reducing the spontaneous activity. On the basis of these results, they concluded that the spontaneous activity was myogenic in nature.

GABA was very effective as an agonist in *D. viteae* but the effects of GABA were not reduced by the GABA antagonists, bicuculline, picrotoxin and penicillin G. Thus the GABA receptors in this preparation are different from the classical GABA_A receptors, although they are sensitive to muscimol, a GABA_A receptor agonist. They could be classified as bicuculline-insensitive GABA_A receptors, except that muscimol was less potent than GABA. Muscimol is usually more active than GABA at mammalian GABA_A receptors. In this regard the GABA receptors in *D. viteae* are similar to those in *Ascaris* (Wann, 1987; Holden-Dye *et al.*, 1988; Holden-Dye & Walker, 1988). Of course, the antagonists had no consistent effects on the spontaneous contractions. Again, this result does not test the physiological role for GABA because the same antagonists did not reduce the effects of exogenous

Figure 6 Effects of cholinergic antagonists on the spontaneous isotonic contractions of the opened *D. viteae*. Tubocurarine, hexamethonium and atropine were applied (cumulatively) at the vertical bars at the concentrations indicated below the bars. The bath was flushed at each dot. Calibrations: 2 mm and 2 min.

Figure 7 Effects of the cholinceptor antagonists on the responses to acetylcholine (ACh) in the opened *D. viteae*. The contractions to ACh (●), ACh in the presence of 10^{-3} M hexamethonium (□), ACh in the presence of 10^{-5} M atropine (△), and ACh in the presence of 10^{-4} M tubocurarine (○) are illustrated. Each contraction was measured as a percentage of the contraction to 10^{-5} M ACh, which was the maximal response in most preparations. Each point is the mean of three experiments. The vertical line represents the s.d.

GABA and would not be expected to reduce the effects of endogenous GABA.

From these results, one can only speculate on the sites of action for ACh and GABA. The muscle cells in nematodes have an unusual anatomy with three components, a contractile portion, a cell belly and a muscle arm (Rosenbluth, 1965b). The muscle arms extend toward the nerve cord, where they make synaptic contact with the nerve cord neurones. Gap junctions exist between many muscle arms in the region of the nerve cord. The neurotransmitters (ACh and GABA) released by nerve cord neurones probably act at receptors on the muscle arms which alters the activity of the rest of the muscle cell. The receptors on the muscle arms should be

Figure 8 Effects of γ -aminobutyric acid (GABA) blocking drugs on the spontaneous isotonic contractions of the opened *D. viteae*. Picrotoxin and bicuculline were applied (cumulatively) at the vertical bars at the concentrations indicated below the bars. The bath was flushed at each dot. Calibrations: 2 mm and 2 min.

Figure 9 Effects of the γ -aminobutyric acid (GABA) blocking drugs on the responses of the opened *D. viteae* to GABA. GABA was applied at the time indicated by the bars and at the concentration indicated below each bar. Traces (a) and (c) are the control records and traces (b) and (d) are the responses in the presence of each blocking drug. The concentrations of the blocking drugs were 10^{-4} M bicuculline and 10^{-5} M picrotoxin. Calibrations: 2 mm and 2 min.

important sites of action for exogenously applied ACh and GABA. There is also evidence that ACh and GABA can act at the cell belly. If ACh or GABA is applied by superfusion after the nerve cord is removed, or if ACh or GABA is applied by iontophoresis to the muscle belly, changes in membrane potential and conductance can be observed in the *Ascaris* muscle belly (Brading & Caldwell, 1971; Martin, 1982; 1987). The physiological importance of these receptors is unclear; however, the changes in contractility from ACh and GABA in the present experiments probably involve receptors on both the muscle bellies and muscle arms.

D. viteae is a nematode that tends to localize in the subcutaneous tissues of the host. It appears to be very similar, physiologically, to the intestinal nematode, *Ascaris*. A major difference is that the antagonists are more effective in reducing the actions of ACh in *Ascaris* (Norton & DeBeer, 1957; del Castillo *et al.*, 1963; Martin, 1982) than in *D. viteae* (Rohrer *et al.*, 1988). Such a result may indicate a slight difference in the

cholinoceptors in these two species of nematodes. Also, ACh and GABA had a much more rapid onset of action and recovery in *D. viteae*. This is probably due to the fact that the opened *D. viteae* preparation is very thin and substances can quickly diffuse in and out of the muscle. The muscle wall of *Ascaris* is very thick; and even in the opened *Ascaris* preparation, it may take considerable time for ACh and GABA to diffuse to and from their sites of action.

References

- BONE, L.W. & BOTTJER, K.P. (1986). Anthelmintic ingestion in nematodes: enhancement by phagostimulants. *Int. J. Parasit.*, **16**, 525–527.
- BRADING, A.F. & CALDWELL, P.C. (1971). The resting membrane potential of the somatic muscle cells of *Ascaris lumbricoides*. *J. Physiol.*, **217**, 605–624.
- BUEDING, E. (1949). Studies on the metabolism of the filarial worm, *Litomosoides carinii*. *J. Exp. Med.*, **89**, 107–130.
- BUEDING, E. (1952). Acetylcholinesterase activity of *Schistosoma mansoni*. *Br. J. Pharmacol. Chemother.*, **7**, 563–566.
- CHRIST, D., GOEBEL, M.J. & SAZ, H.J. (1988). Effects of ACh and GABA on contractions of the opened filariid, *Dipetalonema viteae*. *Soc. Neurosci. Abstracts*, **14**, 382.
- CHRIST, D. & SAZ, H.J. (1986). Actions of cholinomimetics on spontaneous contractions in *Dipetalonema viteae*. *Am. Soc. Parasitol. Abstracts*, p. 77.
- DEBELL, J.T., DEL CASTILLO, J. & SANCHEZ, V. (1963). Electrophysiology of the somatic muscle cells of *Ascaris lumbricoides*. *J. Cell. Comp. Physiol.*, **62**, 159–178.
- DEL CASTILLO, J., DE MELLO, W.C. & MORALES, T. (1963). The physiological role of acetylcholine in the neuromuscular system of *Ascaris lumbricoides*. *Arch. Int. Physiol. Biochem.*, **71**, 741–757.
- DEL CASTILLO, J., DE MELLO, W.C. & MORALES, T. (1964). Inhibitory action of γ -aminobutyric acid (GABA) on *Ascaris* muscle. *Experientia*, **20**, 141–143.
- DOROZYNSKI, A. (1976). The attack on tropical disease. *Nature*, **262**, 85–87.
- FETTERER, R.H. (1986). Transcuticular solute movement in parasitic nematodes: relationship between non-polar solute transport and partition coefficient. *Comp. Biochem. Physiol.*, **84A**, 461–466.
- HOLDEN-DYE, L., HEWITT, G.M., WANN, K.T., KROGSGAARD-LARSEN, P. & WALKER, R.J. (1988). Studies involving avermectin and the 4-aminobutyric acid (GABA) receptor of *Ascaris suum* muscle. *Pestic. Sci.*, **24**, 231–245.
- HOLDEN-DYE, L. & WALKER, R.J. (1988). ZAPA, (Z)-3-[(aminomethylthio)-2-propenoic acid hydrochloride, a potent agonist at GABA-receptors on the *Ascaris* muscle cell. *Br. J. Pharmacol.*, **95**, 3–5.
- JOHNSON, C.D. & STRETTON, A.O.W. (1985). Localization of choline acetyltransferase within identified motoneurons of the nematode *Ascaris*. *J. Neurosci.*, **5**, 1984–1992.
- JOHNSON, C.D. & STRETTON, A.O.W. (1987). GABA-immunoreactivity in inhibitory motor neurons of the nematode *Ascaris*. *J. Neurosci.*, **7**, 223–235.
- MARTIN, R.J. (1982). Electrophysiological effects of piperazine and diethylcarbamazine on *Ascaris suum* somatic muscle. *Br. J. Pharmacol.*, **77**, 255–265.
- MARTIN, R.J. (1987). The γ -aminobutyric acid receptor of *Ascaris* as a target for anthelmintics. *Biochem. Soc. Trans.*, **15**, 61–65.
- NORTON, S. & DEBEER, E.J. (1957). Investigations on the action of piperazine on *Ascaris lumbricoides*. *Am. J. Trop. Med. Hyg.*, **6**, 898–905.
- ROHRER, W.H., ESCH, H. & SAZ, H.J. (1988). Neuromuscular electrophysiology of the filarial helminth *Dipetalonema viteae*. *Comp. Biochem. Physiol.*, **91C**, 517–523.
- ROSENBLUTH, J. (1965a). Ultrastructural organization of obliquely striated muscle fibers in *Ascaris lumbricoides*. *J. Cell Biol.*, **25**, 495–515.
- ROSENBLUTH, J. (1965b). Ultrastructure of somatic muscle cells in *Ascaris lumbricoides* II. Intermuscular junctions, neuromuscular junctions, and glycogen stores. *J. Cell Biol.*, **26**, 579–591.
- WANN, K.T. (1987). The electrophysiology of the somatic muscle cells of *Ascaris suum* and *Ascaridia galli*. *Parasitol.*, **94**, 555–566.
- YUNGER, L.M. & CRAMER, R.D. (1981). Measurement and correlation of partition coefficients of polar amino acids. *Mol. Pharmacol.*, **20**, 602–608.

(Received March 27, 1990

Revised July 22, 1990

Accepted July 30, 1990)