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Pyrantel resistance alters nematode nicotinic acetylcholine receptor single-channel properties

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Abstract

Resistance to the anthelmintics pyrantel ((*E*)-1,4,5,6-tetrahydro-1-methyl-2-[2-(2thienyl)ethenyl]pyrimidine) and levamisole ((*S*)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]thiazole) is an increasingly widespread problem in gastro-intestinal nematode infestations. Both compounds act on the nicotinic acetylcholine receptors on the surface of nematode somatic muscle. The patch-clamp technique was used to measure nematode nicotinic acetylcholine receptor properties at 75, 50, −50 and −75 mV in a pyrantel-resistant isolate of *Oesophagostomum dentatum*. Patch pipettes contained 30 μM levamisole as agonist. We found that 28.1% of membrane patches contained active receptors. At −50 mV, the single-channel conductance was 36.2 ± 1.4 pS, the mean open-time (τ) was 1.45 ± 0.14 ms and the mean probability of opening (P_o) was 0.004 ± 0.002. We compared these results with previous work on an anthelmintic sensitive isolate and a levamisole-resistant isolate [Robertson, A.P., Bjorn, H.E., Martin, R.J., 1999. Levamisole resistance resolved at the single-channel level. FASEB J. 13, 749–760.]. We found that pyrantel-resistant parasites had a reduced percentage of active patches and a reduced P_o value when compared to anthelmintic sensitive worms. We concluded that pyrantel resistance is associated with a modification of the target nicotinic receptor properties.

Keywords: Patch-clamp; Anthelmintic resistance; Parasitic nematode; Nicotinic receptor

1. Introduction

Levamisole ((*S*)-2,3,5,6-tetrahydro-6-phenylimidazo-[2,1-*b*]thiazole) and pyrantel ((*E*)-1,4,5,6-tetrahydro-1-methyl-2-[2-(2thienyl)ethenyl]pyrimidine) are two widely used anthelmintic drugs. They belong to the class of nicotinic agonist anthelmintics. For treatment of human gastro-intestinal nematodes, pyrantel is often preferred because the oral preparations used limit drug distribution to the gut. These compounds bind to nicotinic acetylcholine receptors located on somatic muscle of parasitic nematodes. Drug binding promotes the opening of these ion channel receptors causing depolarization of the muscle cell and subsequent contraction (Harrow and Gration, 1985). At the whole parasite level, these drugs cause a spastic paralysis of the worm, preventing the parasite from maintaining its position in the host. The contrasting structures of both compounds are illustrated in Fig. 1. Levamisole is an isoquiniline compound but pyrantel is a tetrahydropyrimidine.

Current strategies for gastro-intestinal nematode control are almost entirely dependent on the use of anthelmintics. Reports of resistance to anthelmintic therapy are occurring more and more frequently, causing increasing concern (Prichard, 1994). Resistance to all classes of anthelmintic have been reported: avermectins (LeJambre, 1993); nicotinic agonists (Roepstorff et al., 1987; Bjorn et al., 1990); and benzimidazoles (Prichard, 1994).

In a previous single-channel study (Robertson et al., 1999), we have shown that levamisole resistance is caused by changes in the properties of the nicotinic acetylcholine receptors on the nematode muscle. We found that the probability of channel opening, the mean open-time, and

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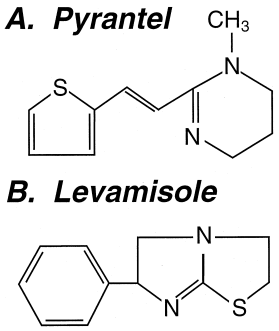

Fig. 1. Structure of (A) pyrantel ((*E*)-1,4,5,6-tetrahydro-1-methyl-2-[2-(2thienyl)ethenyl]pyrimidine) and (B) levamisole ((*S*)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]thiazole).

the overall number of functional receptors were all reduced in levamisole-resistant parasites. The combination of these factors was sufficient to explain the observed resistance to the anthelmintic. In this study we examined the response of a pyrantel-resistant isolate of the same species (*Oesophagostomum dentatum*) at the single-channel level. The aim of the present study was to determine if the mechanism of pyrantel resistance was similar to the mechanism of levamisole resistance, a possibility, as both compounds share the same site of action. We found that pyrantel resistance was associated with a reduction in the number of active channels and reduced probability of channel opening (P_o). But the pyrantel resistance, unlike levamisole resistance, was associated with an increased mean-open time. Results from levamisole-resistant and anthelmintic sensitive isolates (Robertson et al., 1999) are included for comparative purposes.

2. Materials and methods

2.1. Preparation of adult O. dentatum

Pyrantel-resistant *O. dentatum* adults were produced at the Royal Veterinary and Agricultural School, Frederiksberg, Copenhagen (Varady et al., 1997). Ten thousand pyrantel-resistant L_3 larvae were administered by stomach tube to 25-kg Landrace × pigs. Infection was confirmed by faecal egg count 21 days later. Pigs were slaughtered by bleeding out after electrical stunning. Adult parasites were collected from the large intestine and cleaned using the agar migration technique (Slotved et al., 1996). Clean adult parasites were placed in a thermos flask containing maintenance solution at 37°C. Maintenance solution contained (in mM): NaCl, 150; KCl, 2.7; $CaCl_2$, 2; $MgCl_2.6H_2O$, 0.3; PIPES, 10; NaOH, 13; glucose, 11; penicillin 0.06 g/l; streptomycin, 0.1 g/l, pH 7.5. Clean adult parasites were air freighted overnight to Edinburgh.

In Edinburgh, the adult *O. dentatum* were removed from the thermos flask and placed in Petri dishes containing fresh maintenance solution. Parasites routinely survived between 7 and 14 days when incubated at 20°C with daily changes of maintenance solution.

2.2. Preparation of muscle vesicles

The method has been described previously (Martin et al., 1997) and is outlined in Fig. 2. Briefly, muscle flaps were prepared and treated with collagenase, causing membranous vesicles to bud from the muscle surface. The vesicles were harvested and placed in the recording chamber. After a maximum of 5 h, the vesicles were discarded and a fresh preparation used.

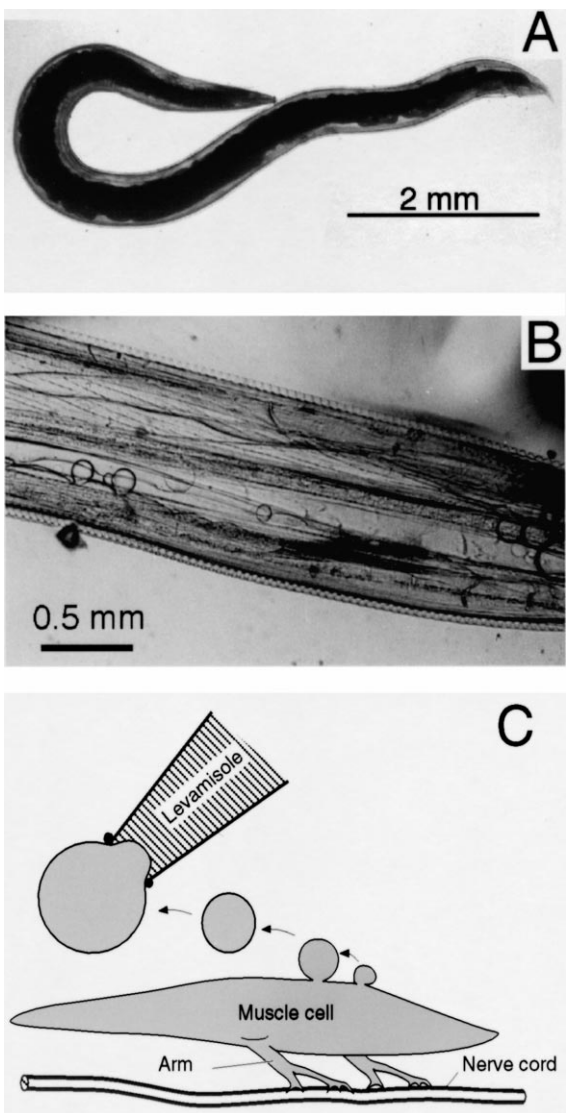

Fig. 2. (A) Photograph of an adult female *O. dentatum*. (B) Muscle flap preparation after collagenase treatment. Several muscle membrane vesicles can be seen. (C) Diagram representing vesicle formation from a somatic muscle cell after collagenase treatment. Diagram also illustrates the vesicle-attached configuration for single-channel recording with levamisole (agonist) in the pipette solution.

2.3. Patch-clamp recordings

Patch-clamp recordings were made at room temperature. The experimental chamber was mounted on a Nikon TMS-PH3 inverted microscope and viewed under phase contrast and 300 × magnification. The vesicles were bathed in high-Cs^+ (solution 1) to minimise K^+ currents. Solution 1 contained (in mM): CsCl, 35; $C_2H_3O_2Cs$, 105; $MgCl_2$, 2; dithiothreitol, 0.1; HEPES, 10; EGTA, 1; pH adjusted to 7.2 with CsOH. Recordings were made using vesicle attached patches. Patch electrodes were pulled to a resistance of 2 MΩ from Garner (7052) capillary glass. The pipettes were coated to near the tip with Sylgard™ to improve frequency responses. Patch pipettes were filled with solution 2 which comprised (in mM): CsCl, 140; $MgCl_2$, 2; HEPES 10; EGTA 1; levamisole 0.03 (as nicotinic acetylcholine receptor agonist). Although this study was carried out on pyrantel-resistant parasites, levamisole was the nicotinic agonist used to determine nicotinic acetylcholine receptor properties. Levamisole was used to allow a comparison of the nicotinic acetylcholine receptor properties in pyrantel-resistant parasites with the receptor properties previously recorded from levamisole-resistant and anthelmintic sensitive isolates (Robertson et al., 1999). Solutions 1 and 2 were calcium-free to prevent contamination of recordings with Ca^{2+}-activated channels.

2.4. Data processing and analysis

Single-channel currents were recorded with an Axopatch 200B onto DAT tape using a Biologic recorder. Channel records were analysed with an Axon Instruments Digidata 1200 interface, an RM pentium computer and Clampex7 software. The records were digitally filtered at 1.5 kHz by the Fetchan software; the sampling time was 25 μs; and the minimum detectable opening was 0.3 ms. The duration and amplitude of channel states were measured with the threshold for channel opening set at 50% of amplitude. Mean open-time (τ) was calculated by fitting exponential curves to the histograms of the open-time distributions using a simplex maximum likelihood procedure. A maximum likelihood simplex method (using a NAG E04CCF subroutine) was used to fit Gaussian curves to the channel amplitude distributions. Probability of channel opening (P_o) was calculated by dividing the time spent in the open state by the total duration of the channel record. Single-channel conductances were obtained by linear regression of values on a current–voltage plot.

Minitab was used for statistical analysis. *t*-Tests were used to assess differences in means. The Anderson–Darling test was used to test for normality. General linear model (GLM) analysis of variance (ANOVA) was used to compare the effects on P_o, P, $\log_{10}$ P_o and τ. The P_o values were subjected to a $\log_{10}$ transformation to normalise the data.

2.5. Parasites derived from different pigs have similar properties

The adult parasites in this study were obtained from two different pigs infected using L_3 larvae from the same isolate. As a control, it was necessary to test if there were differences in receptor number and properties in the worms derived from each pig. In worms from the first pig, we examined 35 membrane patches, of which 10 contained functional receptors (28.6%); in worms from the second pig, we examined 29 membrane patches, eight of which contained functioning receptors (27.6%). There was no significant difference in the percentage of active patches between the two groups of parasite. Additionally, we compared τ values and P_o values between the two groups of parasites from separate pigs and found no significant difference ($P > 0.1$, ANOVA).

3. Results

3.1. Numbers of active receptors measured by percentage active patches

To gain an insight into receptor density on the muscle vesicle surface, we calculated the percentage of membrane patches containing functioning channels. Of the 64 membrane patches examined 18 (28.1%) contained active channels. This value is lower than 66.7% active patches observed in an anthelmintic sensitive isolate at 30 μM levamisole ($P < 0.001$, χ^2) and close to the 28.6% active patches previously observed in a levamisole-resistant isolate at the same levamisole concentration (Robertson et al., 1999). From these observations we have concluded that the pyrantel-resistant isolate has a similar number of active receptors to the levamisole-resistant isolate and both resistant isolates have a lower number of active receptors than the sensitive isolate.

3.2. Probability of channel opening

Fig. 3A shows representative channel records from the pyrantel-resistant isolate at −50 mV. Example channel records from the anthelmintic sensitive and levamisole-resistant isolates are included for comparison. The records indicate that levamisole receptor channels in pyrantel-resistant parasites have a similar P_o to levamisole-resistant parasites, and that both drug resistant isolates have a lower P_o than the sensitive isolate. We measured patch P_o values at ±75 mV and at ±50 mV for the pyrantel resistant isolate. The mean P_o values (±S.E.M.) for each potential are given in Table 1. For each of the potentials tested, the relationship between isolate and P_o values was:

$$\text{anthelmintic sensitive} \gg \text{pyrantel} - \text{resistant} > \text{levamisole} - \text{resistant}.$$

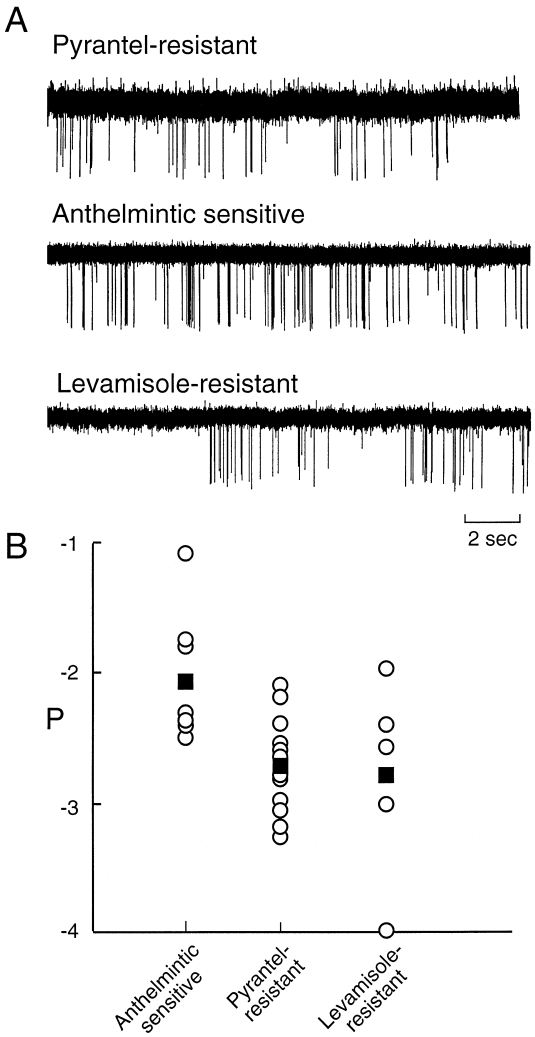

Fig. 3. (A) Representative single-channel records from a pyrantel-resistant parasite at −50 mV, sample records from anthelmintic sensitive and levamisole-resistant parasites are presented for comparison. (B) P values for the three *O. dentatum* isolates. P was calculated as $\log_{10}(P_o + 0.0001)$. The calculation was performed to normalize the data; 0.0001 was added to the original P_o values to allow experiments with $P_o = 0$ to be included in the plot. P values closest to 0 reflect the highest P_o values. Individual P values are represented by ○ while ■ represents the mean value. On analysis of variance we found a significant difference ($P < 0.001$). The pyrantel-resistant isolate has a similar mean P value to the levamisole-resistant isolate, both of which are substantially lower than the value for the sensitive isolate.

The relationship between the P_o values at −50 mV for the sensitive ($P_o = 0.0168$), levamisole-resistant ($P_o = 0.0041$) and pyrantel-resistant ($P_o = 0.0036$) isolates are illustrated in Fig. 3B (displayed as Log $[P_o + 0.0001]$ to normalise the data). Comparison of the P_o values for pyrantel-resistant with those obtained previously for sensitive and levamisole-resistant isolates over the test potential range (Robertson et al., 1999) yielded a highly significant difference for both the untransformed and log transformed data ($P < 0.001$, ANOVA). The pyrantel-resistant isolate has lower mean P_o values than the sensitive isolate but higher than the levamisole-resistant isolate. This observation was consistent for each of the potentials tested. The log plot in Fig. 3B also illustrates the individual observations of P_o at −50 mV for the three isolates as well as mean values. It is important to note that although the values are significantly different, there is a considerable amount of overlap between individual values from anthelmintic sensitive, pyrantel-resistant and levamisole-resistant. The spread in P_o values occurred between receptors on the same vesicle membrane: for example in the same vesicle produced from a pyrantel-resistant parasite, P_o was 0.0082 and 0.0002 in two separate membrane patches at −75 mV (>40-fold difference). This example shows that P_o varies between receptors from an individual vesicle and therefore even within a single muscle cell. The variation was not due to variation in experimental technique but these observations may be explained if P_o of the receptor is modulated by other, perhaps physiological, mechanisms discussed in Section 4.4.

3.3. Mean open-time

Fig. 4 shows the mean open-time values at −50 mV for our test isolate (pyrantel-resistant) and the previously obtained results for anthelmintic sensitive and levamisole-resistant isolates at −50 mV (Robertson et al., 1999). The mean open-times for the pyrantel-resistant isolate were measured at membrane potentials of ±50 mV and ±75 mV, for example at −50 mV, $\tau = 1.45 \pm 0.14$ ms. Fig. 4 demonstrates that the mean open-time values for pyrantel-resistant are not only longer than those for levamisole-resistant ($P < 0.001$, *t*-test) but also longer than the values for the sensitive isolate ($P < 0.05$, *t*-test). The mean open-time values (±S.E.M.) for each potential tested are given in Table 1. We compared the results for pyrantel-resistant over the test potential range with data from the sensitive and levamisole-resistant isolates (Robertson et al., 1999) using analysis of variance and found a highly significant difference ($P < 0.001$, ANOVA).

3.4. Single-channel conductances

Fig. 5 gives an example of a current–voltage plot for the pyrantel-resistant isolate. For individual receptors, the

Table 1
Mean values (±S.E.M.) for mean open-time (τ) and probability of channel opening (P_o) at each of the test membrane potentials for the pyrantel-resistant isolate of *O. dentatum*

Membrane potential (mV)	Mean open-time (τ) ± S.E.M. (ms)	Mean P_o ± S.E.M.
75	1.58 ± 0.132	0.013 ± 0.002
50	1.56 ± 0.16	0.013 ± 0.003
−50	1.45 ± 0.14	0.004 ± 0.002
−75	1.09 ± 0.09	0.007 ± 0.002

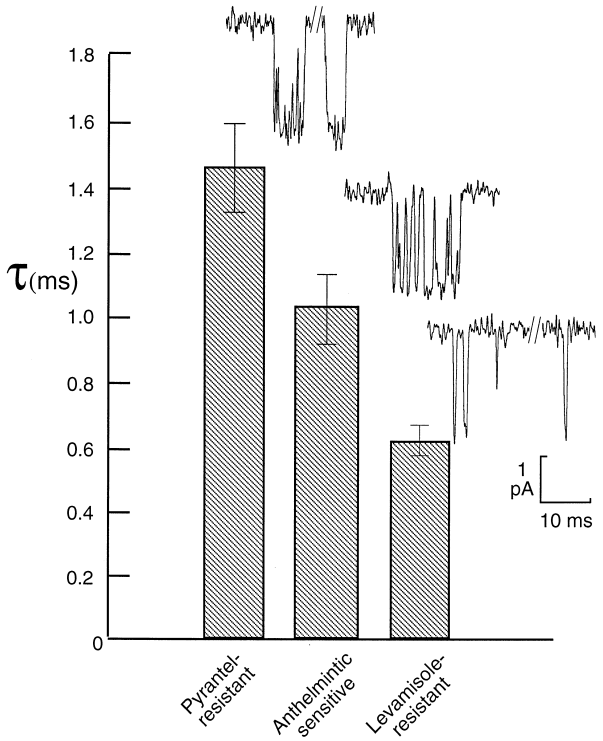

Fig. 4. Mean τ values for each isolate ($\pm$S.E.M.) at -50 mV. Figure shows that pyrantel-resistant has longer mean open-times than the sensitive isolate which in turn are longer than the levamisole-resistant isolate ($P < 0.01$, ANOVA). Numerical values are: pyrantel-resistant 1.45 ± 0.14 ms; sensitive 1.02 ± 0.10 ms and levamisole-resistant 0.60 ± 0.05 ms.

current–voltage relationship was linear over the test potential range. The mean conductance value was 36.2 ± 1.4 pS ($n = 19$). Comparison with conductance values from the sensitive isolate (mean 37.7 ± 1.1 pS) showed no significant difference between mean conductance values ($P > 0.5$, *t*-test). In the 18 pyrantel-resistant patches that contain channels, the single-channel conductances were in the range 22.7–45.2 pS. The conductance range was similar to

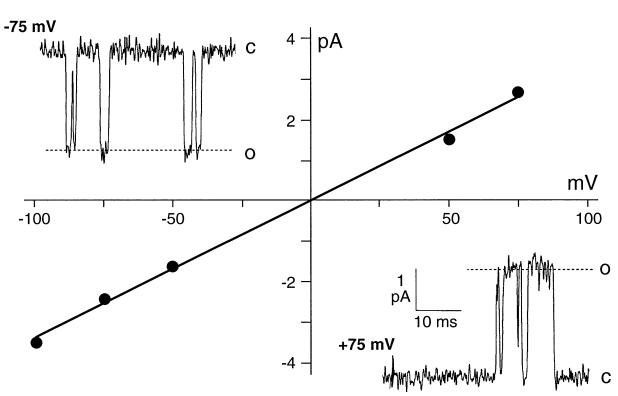

Fig. 5. Example of a current–voltage relationship obtained from a pyrantel-resistant derived membrane vesicle. The channel records illustrated gave a slope conductance of 33.8 pS. The channel records illustrated were obtained at membrane potentials of 75 mV and -75 mV; for each record **c** refers to the closed state of the channel while **o** refers to the open state.

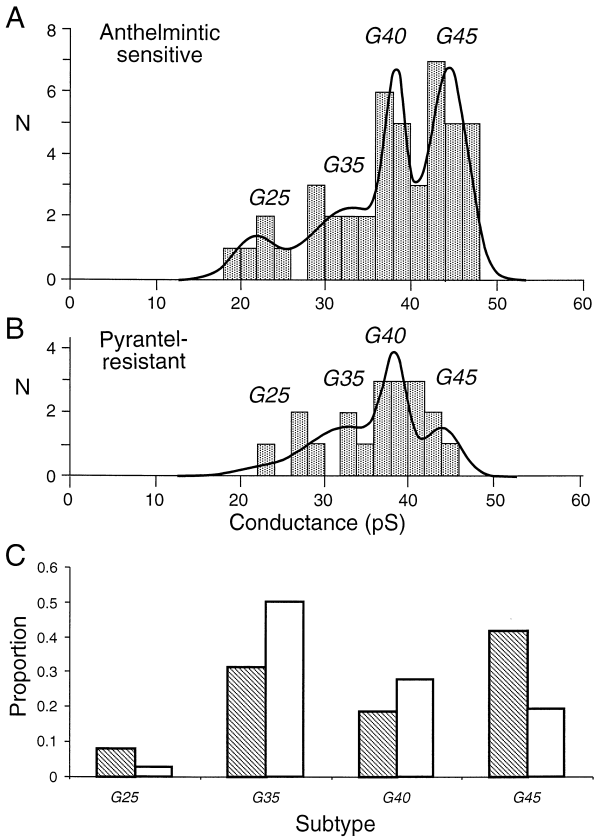

Fig. 6. (A) Conductance distribution ($n = 45$) for anthelmintic sensitive isolate (Robertson et al., 1999) fitted with four Gaussian distributions. Peaks describe the four subtypes present: G25, 21.4 ± 2.3 pS (mean $\pm$ S.D.); G35, 33.0 ± 4.8 pS; G40, 38.1 ± 1.2 pS; G45, 44.3 ± 2.2 pS. (B) Conductance distribution ($N = 19$) for the pyrantel-resistant isolate. Four Gaussians with the same mean and S.D. values for the peaks were fitted to the data to estimate the relative proportions of each subtype. (C) Proportion of total area for individual subtypes in each isolate after fitting four Gaussians. Filled bars represents sensitive islate while unfilled bars represents pyrantel-resistant. The pyrantel-resistant isolate has a higher percentage of G35 and G40 subtypes than the sensitive isolate but lower percentages of G25 and G45. The numerical values are: G25 sensitive 6%, pyrantel-resistant 0.9%; G35 sensitive 25%, levamisole-resistant 46%; G40 sensitive 16%, levamisole-resistant 28%; G45 sensitive 38%, levamisole-resistant 14%.

those previously obtained for sensitive (18.1–48.0 pS) and levamisole-resistant (15.2–47.8 pS) isolates (Robertson et al., 1999). Fig. 6 illustrates the individual conductance values for sensitive (Fig. 6A) and pyrantel-resistant (Fig. 6B).

4. Discussion

The purpose of this study was to determine the mechanism of pyrantel resistance in *O. dentatum*. We wished to determine whether pyrantel resistance was associated with altered properties of the parasite nicotinic acetylcholine receptors. Additionally, we wished to determine whether changes in receptor properties produced by pyrantel resis-

tance were similar to those produced by levamisole resistance (Robertson et al., 1999) because levamisole and pyrantel are believed to share the same site of action although they have quite different chemical structures. Additionally, it has been found, using larval development assays, that although the pyrantel-resistant isolate is resistant to levamisole, the levamisole-resistant isolate remains susceptible to pyrantel treatment (Varady et al., 1997). The results from the larval development assays suggest that although the two compounds share the same site of action, the exact mechanism of resistance in the isolates may be different.

4.1. Similarities between pyrantel resistance and levamisole resistance

The results of this study clearly demonstrate differences in the properties of nicotinic acetylcholine receptors in a pyrantel-resistant isolate of *O. dentatum* when compared with a previously examined anthelmintic sensitive isolate (Robertson et al., 1999). In pyrantel resistance, there are fewer active receptors present on the muscle membrane (as estimated from the percentage of active patches) and the nicotinic acetylcholine receptors that are present have a reduced probability of channel opening (P_o). Previously, we estimated a resistance ratio of 10.4 for a levamisole-resistant isolate of *O. dentatum* when compared to an anthelmintic sensitive isolate at -50 mV (Robertson et al., 1999). We calculated the resistance ratio for pyrantel-resistant at -50 mV using the same equation, as follows:

$$RR = (Pr_{SENS} Po_{SENS} G_{SENS}) / (Pr_{PYRR} Po_{PYRR} G_{PYRR}),$$

where Pr_{SENS} is the proportion of patches in the sensitive isolate containing active channels; Po_{SENS} is the mean probability of channel opening in the sensitive isolate; G_{SENS} is the mean single-channel conductance of the sensitive isolate; and the subscript PYRR denotes the values for the pyrantel resistant isolate. The pyrantel-resistant isolate was found to have a resistance ratio of 10.1 for 30 μM levamisole. The resistance ratio calculation indicates that the nicotinic acetylcholine receptor population in pyrantel-resistant parasites will pass approximately 10 times less current than the receptor population in the anthelmintic sensitive isolate. The observation that both pyrantel-resistant and levamisole-resistant isolates have a reduced response to levamisole is in close agreement with a previous study on the same parasite isolates (Varady et al., 1997). We therefore have similarities in resistance to levamisole and pyrantel in *O. dentatum*: a reduction in the number of active nicotinic acetylcholine receptors present and those that are present have a reduced probability of opening. However, the nicotinic acetylcholine receptor properties in levamisole-resistant and pyrantel-resistant isolates do show some differences, the implications of which are discussed below.

4.2. Differences between pyrantel- and levamisole-resistant isolates

When the results from pyrantel-resistant parasites are compared with those from levamisole-resistant and anthelmintic sensitive parasites, some interesting features become apparent. The mean and range of single-channel conductances for each isolate are similar in value. A histogram of single-channel conductances is shown in Fig. 6. In all three isolates, there is heterogeneity of receptors. The observed heterogeneity is caused by the receptor population in each isolate being composed of several distinct subtypes. Our previous study (Robertson et al., 1999) demonstrated that the receptor population in anthelmintic sensitive parasites is heterogeneous and made up of four separate subtypes; called G25, G35, G40 and G45 on the basis of their single channel conductance (Fig. 6A). Fig. 6B illustrates that in pyrantel-resistant parasites, there is also receptor heterogeneity (more than one peak is present). The range of conductances is large enough to encompass all four subtypes found in the sensitive isolate. On the assumption that all four subtypes were present, we fitted four Gaussian distributions by restraining the fit using the mean and standard deviation values obtained from the sensitive isolate (Robertson et al., 1999). Fig. 6C shows the proportion of each receptor subtype in the pyrantel-resistant and anthelmintic-sensitive isolates. We found that the proportion of each subtype in pyrantel-resistant appeared different from the sensitive isolate. Pyrantel-resistant worms had a reduced proportion of G25 and G45 while the proportion of G35 and G40 was increased. These observations indicate that the relative numbers of each receptor subtype are altered in the pyrantel-resistant isolate. More importantly, we found that the pyrantel-resistant isolate possessed examples of the G35 subtype (Fig. 6C) that was completely absent in the levamisole-resistant isolate (Robertson et al., 1999). These observations indicate that the subtype profile in pyrantel-resistant is different from the sensitive isolate but, more interestingly, it is also different from the levamisole-resistant receptor population.

When the mean open-times of the isolates are compared, it is apparent that there is a significant difference between pyrantel-resistant and levamisole-resistant isolates with pyrantel-resistant worms having a significantly longer mean open-time than levamisole-resistant and indeed than sensitive parasites ($P < 0.01$, ANOVA). These observations indicate that the receptors in anthelmintic sensitive, levamisole-resistant and pyrantel-resistant isolates have molecular differences.

4.3. Basis of resistance at the molecular and biochemical level

Previously, we concluded that the simplest mechanism to explain the differences between sensitive and lev-

amisole-resistant worms was an alteration to the properties of one of the β subunits that contribute to the pentameric structure of the nicotinic acetylcholine receptor (Robertson et al., 1999). The differences in mean open-time and subtype composition between pyrantel-resistant and levamisole-resistant parasites demonstrate that the exact molecular basis of resistance in the two isolates is different. This is not surprising as levamisole and pyrantel are structurally very different. Resistance to pyrantel clearly alters the subtype profile and mean open time of the nicotinic acetylcholine receptor population, implying that the properties of one or more of the constituent subunits of the receptors has been altered. Molecular studies have so far failed to find differences in the α subunit of the receptors in resistant isolates of other nematode species (Hoekstra et al., 1997; Wiley et al., 1997). Taken with our previous results on levamisole resistance it seems likely that pyrantel resistance alters the properties of one or more of the β subunits in the receptor.

4.4. Potential strategy for management of anthelmintic resistance

We are able to conclude that P_o of nicotinic acetylcholine receptors in the pyrantel-resistant and levamisole-resistant isolates are significantly lower than the anthelmintic-sensitive isolate and contribute a large part to the observed anthelmintic resistance. Fig. 3 demonstrates that in all three isolates the P_o values can vary by more than a 100-fold between individual nicotinic acetylcholine receptors within an isolate. Additionally, the highest P_o values in each of the resistant isolates are higher than the mean P_o value for the sensitive isolate. In all three isolates, the range of P_o values cannot be explained by variation of our experimental conditions which implies that another factor/factors modulates channel opening. The probability of channel opening (P_o) describes the proportion of time an ion-channel spends in the open state: the more time a channel is open then the more current will be passed. For nicotinic acetylcholine receptors in nematodes, a high P_o value in response to levamisole would lead to more current passing through the channels and therefore more depolarization and subsequent contraction.

Studies on mammalian nicotinic acetylcholine receptors demonstrate that the phosphorylation state of the receptor plays an important role in modulating channel opening (Hoffman et al., 1994; Gurd, 1997; Hopfield et al., 1998; Khiroug et al., 1998). In nematodes, consensus sites for phosphorylation on nicotinic acetylcholine receptor subunits have been found in *C. elegans* (Flemming et al., 1997) and *Trichostongylus colubriformis* (Wiley et al., 1996). In each species these sites are located on the intracellular loop between the M3 and M4 transmembrane domains. Electrophysiological work on *Ascaris* muscle has shown that the response to levamisole and acetylcholine can be reduced by tamoxifen, a protein kinase C antagonist (Trim et al., 1998). The rate of desensitization is increased by phosphorylation in vertebrate muscle nicotinic acetylcholine receptors exposed to agonists (Hoffman et al., 1994; Hopfield et al., 1998). Conversely, in vertebrate ganglionic nicotinic acetylcholine receptors, receptor phosphorylation increases the time taken for desensitization to occur (Gurd, 1997). The effect of tamoxifen on *Ascaris* muscle is further evidence that nematode nicotinic acetylcholine receptors have properties more akin to mammalian ganglionic nicotinic acetylcholine receptors (Gurd, 1997) than vertebrate muscle nicotinic acetylcholine receptors (Hoffman et al., 1994; Hopfield et al., 1998). These results suggest that by increasing the phosphorylation state of the nematode nicotinic acetylcholine receptors, one could increase the P_o of the receptors and thus the effectiveness of the anthelmintic.

4.5. Summary

In summary, we have found that pyrantel resistance alters the nicotinic acetylcholine receptor properties in *O. dentatum* and that the change in probability of channel opening (P_o) substantially contributes to the observed anthelmintic resistance. While resistance to pyrantel and levamisole have many similar effects on the receptor population, the mean-open time results demonstrate that the exact mechanism of resistance to the two compounds is not the same. We suggest that the observed range in receptor P_o found in each isolate may be modulated by the phosphorylation state of the receptor. Additionally, compounds that promote receptor phosphorylation would be expected to increase the response to anthelmintics such as levamisole, pyrantel and morantel. Such compounds when used in association with anthelmintics could render previously resistant parasite isolates susceptible to chemotherapy. Present studies are focusing on the whole-cell response of parasites to the anthelmintics and how this response is effected by compounds known to alter receptor phosphorylation.

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