Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics

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SUMMARY

The macrocyclic lactones are the biggest selling and arguably most effective anthelmintics currently available. They are good substrates for the P-glycoproteins, which might explain their selective toxicity for parasites over their vertebrate hosts. Changes in the expression of these pumps have been implicated in resistance to the macrocyclic lactones, but it is clear that they exert their anthelmintic effects by binding to glutamate-gated chloride channels expressed on nematode neurones and pharyngeal muscle cells. This effect is quite distinct from the channel opening induced by glutamate, the endogenous transmitter acting at these receptors, which produces rapidly opening and desensitising channels. Ivermectin-activated channels open very slowly but essentially irreversibly, leading to a very long-lasting hyperpolarisation or depolarisation of the neurone or muscle cell and therefore blocking further function. Molecular and genetic studies have shown that there are multiple GluCl isoforms in both free-living and parasitic nematodes: the exact genetic make-up and functions of the GluCl may vary between species. The known expression patterns of the GluCl explain most of the observed biological effects of treatment with the macrocyclic lactones, though the reason for the long-lasting inhibition of larval production in filarial species is still poorly understood.

Key words: Ivermectin, *Caenorhabditis elegans*, *Haemonchus contortus*, Filaria, ionotropic receptor, chemotherapy.

THE AVERMECTIN/MILBEMYCIN ANTHELMINTICS

The avermectins and milbemycins (A/M), often also referred to as the macrocyclic lactones, are the largest selling anthelmintics in the world. As well as being widely used in veterinary medicine for the treatment of gastro-intestinal nematode infections and ecto-parasite infestations (Vercruysse & Rew, 2002), they are used in agriculture for the control of insect pests and in human medicine for the treatment of filarial nematode infections, most notably in the treatment and control of onchocerciasis (Omura & Crump, 2004). This enormous success, which has only very recently been clouded by reports of resistance in veterinary parasites in some parts of the world (Jackson & Coop, 2000; Anziani *et al*. 2001; Familton, Mason & Coles, 2001; Loveridge *et al*. 2003), is due to their rapid effects on, and specificity for, the target organisms. Though extremely effective at controlling arthropods as well as nematodes, the A/M are ineffective against flatworms and tapeworms. This article will concentrate on the nematocidal effects of these drugs.

When the A/M anthelmintics are applied to nematodes two main effects, rapid paralysis of movement and of pharyngeal pumping, are observed. As a result, the treated worms are unable to either move or to feed and, for most infections, the paralysed parasites are consequently rapidly removed from the host. The major group of nematodes refractory to A/M treatment, adult macrofilariae, may survive drug treatment because neither movement nor pharyngeal pumping are required for their continued survival in tissues. However, in this case a further effect of the drugs is revealed, a long-lasting reduction in the production of new larvae (Campbell *et al*. 1983). It is this third effect, coupled with their extreme toxicity to the motile microfilariae, which is responsible for the success of ivermectin treatment in the control of onchocerciasis. These three effects predict that the target of these drugs must have roles in the locomotion, pharyngeal pumping and reproduction of parasitic nematodes.

GLUTAMATE-GATED CHLORIDE CHANNELS

Soon after the introduction of ivermectin, the first A/M to be commercialised, in 1980, electrophysiological experiments showed that the drug caused an increase in the chloride conductance of mammalian neuronal membranes, and that this effect was blocked by picrotoxin (Kass *et al*. 1980; Supavilai & Karobath, 1981; Graham, Pfeiffer & Betz, 1982; Pong & Wang, 1982). Ivermectin has continued to be the drug used in most published studies on the A/M family, since it is both the largest selling of these compounds and also the least hydrophobic of those readily available. Given the known role of GABA receptors in nematode locomotion (McIntire *et al*. 1993) and that picrotoxin is a

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Parasitology (2005), **131**, S85–S95.
doi:10.1017/S0031182005008218 Printed in the United Kingdom

potent GABA-gated chloride channel blocker (Olsen & Tobin, 1990), it was a natural hypothesis that the A/M were acting at GABA receptors, and early data on nematode preparations offered some support for this (Holden-Dye *et al*. 1988; Martin & Pennington 1989; Holden-Dye & Walker, 1990). However, the concentrations required were rather too high for this effect to be clinically relevant and in some preparations ivermectin inhibited rather than opened GABA receptor channels (Martin & Pennington, 1988; Holden-Dye & Walker, 1990) and, though it is clear that the A/M do have effects on nematode GABA receptors (Feng *et al*. 2002), attention turned to alternative chloride channels as the likely target.

Experiments in which total mRNA isolated from *Caenorhabditis elegans* was injected into *Xenopus* oocytes revealed a glutamate-gated chloride channel (GluCl) that was sensitive to ivermectin (Arena *et al*. 1991, 1992). Fractionation of this total mRNA into smaller and smaller pools eventually resulted in the identification of two GluCl subunits, designated α and β, from *C. elegans* (Cully *et al*. 1994). Expression of the α subunit in the *Xenopus* oocyte resulted in the appearance of an ivermectin-gated channel, the β subunit produced a glutamate-gated, but ivermectin-insensitive, channel and co-expression of the two subunits resulted in an ivermectin-sensitive, glutamate-gated channel. The properties of this channel were very similar to that produced by the total mRNA preparation, and it was swiftly established that its pharmacology was very similar to that expected for the avermectin target (Arena *et al*. 1995). The genes encoding these proteins were later designated *glc-1* for the GluClα subunit and *glc-2* for the GluClβ subunit (*glc* = *gl*utamate-gated *c*hloride channel).

This rather simple picture rapidly became more complex as complementary molecular biological and genetic approaches led to the identification of two further α subunits, GluClα2A and GluClα2B, from *C. elegans* (Dent, Davis & Avery, 1997; Vassilatis *et al*. 1997*a*). These subunits produced channels gated by both glutamate and ivermectin and were products of the alternatively spliced *avr-15* (*avr* = *av*ermectin *r*esistance) gene, providing further evidence that the GluCl were indeed the *in vivo* avermectin targets. Reporter gene constructs, in which the putative promoter regions of the genes under study are attached to *lacZ* or GFP (Fire, Harrison & Dixon, 1990), demonstrated that both the GluClα2 and β subunits were expressed in pharyngeal muscle cells and, in the case of the GluClα2 subunits, more widely in the nematode motor nervous system, (Dent *et al*. 1997; Laughton, Lunt & Wolstenholme, 1997*a*) consistent with the paralysis of the pharynx and body-wall muscle observed when ivermectin is applied. The genetics of avermectin resistance suggested that this was not the end of the story, since mutations in *avr-15* alone did not cause the worms to be drug-resistant and a mutation in a second gene, *avr-14* was also necessary for moderate resistance and in a third, *glc-1*, for high level resistance (Dent *et al*. 2000). The second gene, *avr-14*, is identical to the gene also referred to, by us, as *gbr-2* (*GAB*A receptor *r*elated) (Laughton, Lunt & Wolstenholme, 1997*b*). It too is alternatively spliced and encodes two subunits, GluClα3A and GluClα3B, which share a common N-terminal half but differ in the C-terminal, channel forming, half of the protein. GluClα3B produces glutamate- and ivermectin-sensitive channels when expressed in *Xenopus* oocytes, but the GluClα3A subunit has yet to be shown to produce any functional channels (Dent *et al*. 2000; Rogers & Wolstenholme, unpublished). The complete sequencing of the *C. elegans* genome allowed bioinformatics to be added to the cDNA cloning and genetic methods previously used. This led to the identification of a fourth α-subunit encoding gene, *glc-3*: its product, GluClα4, also produced glutamate- and ivermectin-gated chloride channels when expressed in *Xenopus* oocytes (Horoszok *et al*. 2001). An additional putative GluCl-encoding gene, *glc-4*, has also been identified (Cully, Wilkinson & Vassilatis, 1996) that may also be alternatively spliced. The sequence of the predicted *glc-4* encoded subunits is rather distant from either the α or β subunits previously described, suggesting that it may belong to a different, γ class. The current list of *C. elegans* GluCl genes and the subunits they encode is given in Table 1.

Though the paralytic effects of the A/M on parasitic nematodes had been observed at an early stage, and parasite preparations, especially from *Ascaris suum*, have been widely used to study the effects of the drugs on non-recombinant targets (Kass *et al*. 1980; Kass, Stretton & Wang, 1984; Holden-Dye *et al*. 1988; Martin & Pennington 1989; Holden-Dye & Walker, 1990; Martin, 1996; Adelsberger, Scheur & Dudel, 1997; Brownlee, Holden-Dye & Walker, 1997), progress in the molecular characterization of the GluCl has been much slower in parasites. The sequences of the *C. elegans* GluCl subunits were used as the basis of a reverse transcriptase-PCR approach using degenerate primers based on highly conserved regions of amino acids. The parasite species most widely studied in this way has been *Haemonchus contortus*, from which several GluCl cDNAs have been cloned (Delany, Laughton & Wolstenholme, 1998; Forrester *et al*. 1999; Jagannathan *et al*. 1999). From this work it is clear that some of these subunits are very similar to those found in *C. elegans*, including the HcGluClα3A, 3B and HcGluClβ (Hc = *Haemonchus contortus*) subunits. Others, such as the HcGluCla or α subunit, are less closely related to any particular *C. elegans* gene product (Yates, Portillo & Wolstenholme, 2003), raising the intriguing possibility that the GluCl gene family varies between nematode species. Of the various subunits

https://doi.org/10.1017/S0031182005008218 Published online by Cambridge University Press

Table 1. The GluCl genes of *Caenorhabditis elegans*, and the subunits they encode

Gene	Subunits Encoded	Properties	References
avr-14	GluClα3A GluClα3B	*avr-14* mutations cause moderate avermectin resistance in combination with *avr-15*, and very high level resistance as a triple mutant with *avr-15* and *glc-1*. GluClα3B forms glutamate- and ivermectin-gated channels: no channels have been reported for GluClα3A.	Laughton, Lunt & Wolstenholme, 1997*b*; Dent *et al*. 2000
avr-15	GluClα2A GluClα2B	In *avr-15* mutants pharyngeal pumping is insensitive to ivermectin. GluClα2 subunits form glutamate- and ivermectin-gated channels and can co-assemble with β subunits.	Dent, Davis & Avery, 1997; Vassilatis *et al*. 1997*a*; Pemberton *et al*. 2001
glc-1	GluClα1	GluClα1 forms ivermectin-gated channels: glutamate binds but does not open the channel. When expressed with GluClβ, ivermectin-potentiated, glutamate-gated channels are formed.	Cully *et al*. 1994; Etter *et al*. 1996
glc-2	GluClβ	GluClβ forms glutamate-gated channels.	Cully *et al*. 1994
glc-3	GluClα4	GluClα4 forms glutamate- and ivermectin-gated channels.	Horoszok *et al*. 2001
glc-4	?	?	Cully, Wilkinson & Vassilatis, 1996

described to date, GluClα3 has been found in the largest number of species, with sequences reported from *H. placei* (Mes, 2004), *A. suum* (Jagannathan *et al*. 1999), *Cooperia oncophora* (Njue *et al*. 2004; Njue & Prichard, 2004), *Dirofilaria immitis* (Cully *et al*. 1996; Yates & Wolstenholme, 2004), and *Onchocerca volvulus* (Cully *et al*. 1996). Mutations and polymorphisms in GluClα3 subunits have been associated with avermectin resistance in both *C. elegans* and *C. oncophora* (Dent *et al*. 2000; Njue *et al*. 2004; Njue & Prichard, 2004) which could be interpreted to suggest that they are important *in vivo* drug targets. The GluCl subunits currently known in parasitic nematodes are summarised in Table 2.

The sequence of the GluCl subunits clearly revealed them to be members of the 'Cys-loop' family of ligand-gated ion channels, closely related to vertebrate $GABA_A$ and glycine receptors, and more distantly related to the nicotinic acetylcholine receptors. In fact their closest homologues in vertebrates are probably the glycine-gated chloride channels (Vassilatis *et al*. 1997*b*). Each of the subunits has the characteristic topology of 4 membrane-spanning domains with extracellular N- and C-termini. As with other members of this family, the GluCl are predicted to be pentameric structures, with the native channels consisting of more than one subunit type. Table 1 clearly shows that in *C. elegans* there are more than five predicted subunits and this immediately implies that there must be multiple types of native GluCl, and hence avermectin targets, in this organism, and therefore presumably in other nematodes, each made up of different combinations of subunits and presumably expressed on different tissues. This molecular complexity presents a major challenge to a complete understanding of the A/M's actions, since it is likely that each of these GluCls will have different sensitivities and accessibilities to the drug, and that activation of them will have different effects on the organism. If the potential for variations in the number and expression of the individual subunits are added to the mix, then it is obvious that there is scope for considerable differences in the effects of the A/M between nematode species. The determination of the subunit composition of native GluCl is therefore of major importance, but is not trivial. One advantage may be that nematodes are anatomically rather simple, with relatively few individual cells.

Recombinant nematode GluCl are activated by L-glutamate, but not aspartate, GABA, glycine, histamine or any other amino acid or candidate neurotransmitter. Ibotenate, a conformationally constrained analogue of glutamate, is at least a partial agonist at most GluCl (Cully *et al*. 1994; Dent *et al*. 1997; Vassilatis *et al*. 1997*a*; Dent *et al*. 2000; Horoszok *et al*. 2001; Forrester *et al*. 2003; Yates & Wolstenholme, 2004). Glutamate activation of most α-subunit- containing receptors produces a rapid opening of the channel that rapidly desensitises (Fig. 1A). If the receptor is composed solely of β subunits, then the receptors do not desensitise, but do close rapidly once agonist is removed (Cully *et al*. 1994; Njue *et al*. 2004). The EC_{50} for activation of recombinant *C. elegans* GluCl by L-glutamate is usually about 1–2 mM if an α-subunit is present, although the GluClα2B is more sensitive (EC_{50} = 0·14 mM) (Cully *et al*. 1994; Dent *et al*. 1997; Vassilatis *et al*. 1997*a*; Dent *et al*. 2000; Horoszok *et al*. 2001), and those composed of β subunits are also more sensitive to the agonist (EC_{50} = 0·38 mM) (Cully *et al*. 1994). There have been fewer quantitative data published for recombinant parasite GluCl: the EC_{50} for glutamate at the *D. immitis* GluClα3B receptor is also approximately 1 mM (Yates & Wolstenholme, 2004) but, intriguingly the

https://doi.org/10.1017/S0031182005008218 Published online by Cambridge University Press

Table 2. The glutamate-gated chloride channel (GluCl) subunits of parasitic nematodes

Subunit	Species in which found	Properties	References
GluClα/GluCla	*Haemonchus contortus*	Forms glutamate- and ivermectin-gated channels. Forms a high affinity ivermectin binding site.	Forrester *et al*. 1999, 2003; Cheeseman *et al*. 2001; Forrester, Prichard & Beech, 2002
GluClα3A GluClα3B	*Ascaris suum**, *Cooperia oncophora**, *Dirofilaria immitis*, *Haemonchus contortus*, *H. placei*, *Onchocerca volvulus**	GluClα3A does not express in oocytes or mammalian cell lines. GluClα3B forms glutamate- and ivermectin-gated channels and a high affinity ivermectin binding site.	Cully, Wilkinson & Vassiliatis, 1996; Jagannathan *et al*. 1999; Cheeseman *et al*. 2001; Njue & Prichard, 2004; Njue *et al*. 2004; Yates & Wolstenholme, 2004
GluClβ	*Cooperia oncophora*, *Haemonchus contortus*	Forms glutamate-gated channels. Can be co-expressed with GluClα3B. Does not form a high-affinity ivermectin binding site.	Delany *et al*. 1998; Cheeseman *et al*. 2001; Njue *et al*. 2004

* Only a single GluClα3 subunit has been identified in these species.

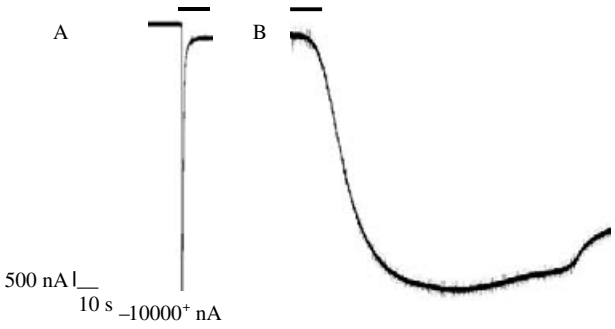

Fig. 1. Examples of chloride currents produced by activation of recombinant GluCl in *Xenopus* oocytes. HcGluClα3B cRNA was transcribed *in vitro* and microinjected into oocytes as described (Yates & Wolstenholme, 2004). Recordings were made 3–5 days later. A) shows the response to 1 mM L-glutamate and B) to 1 μM ivermectin: the horizontal bars above the recordings indicate when agonist was applied. Note the rapid onset and desensitisation of the L-glutamate-induced currents, compared to the slow onset and essentially irreversible current induced by ivermectin.

C. oncophora GluCl subunits are much more sensitive, with the α3 subunit forming a receptor with an EC_{50} of $\sim$0·03 mM and the β subunit having a EC_{50} for glutamate of $\sim$0·18 mM (Njue *et al*. 2004). This increased agonist sensitivity of the GluCl subunits may be conserved in *H. contortus*, since the HcGluClα subunit forms receptors with an EC_{50} for glutamate of only 8·4 μM (Forrester *et al*. 2003) and the HcGluClα3B receptor has a very similar quantitative pharmacology to that from *C. oncophora* (Rogers & Wolstenholme, unpublished). By analogy with the other members of the 'cys-loop' family of ligand-gated ion channels, glutamate would be expected to bind to the extracellular domains of the receptor, possibly at the subunit interface (Cascio, 2004). The agonist-binding site would be made up of six loops, three from each of the adjacent subunits. Consistent with this prediction, a mutation in the extracellular domain of the *C. oncophora* α3 subunit (L256F) reduced the efficacy of glutamate approximately three-fold and a combination of two polymorphisms in the N-terminal domain (V60A and R100H) abolished the glutamate sensitivity of the β subunit (Njue *et al*. 2004). The combination of mutations in both subunits reduced the EC50 for glutamate at the $\alpha 3\beta$ heteromeric receptor by 13-fold. It is possible to correlate the amino acid sequence of many GluClα3 subunits around this position with their sensitivity to glutamate (Fig. 2), which may indicate that this part of the subunit either forms part of the binding site or is involved in the efficient coupling of ligand-binding to channel opening.

Application of ivermectin to recombinant GluCl produces a markedly different effect from glutamate (Fig. 1B). The channels open very slowly, and the rate of channel opening may be dose-dependent (Rogers & Wolstenholme, unpublished). This channel opening is essentially irreversible, and the channels remain open even when the drug is removed (Cully *et al*. 1994; Vassilatis *et al*. 1997*a*; Horoszok *et al*. 2001; Forrester *et al*. 2003; Yates & Wolstenholme, 2004). Ivermectin has no effect on GluClβ subunits (Cully *et al*. 1994; Njue *et al*. 2004). Because of the extremely unusual properties of ivermectin and the irreversible channels it opens, it is very difficult to obtain good quantitative data with this drug, but EC_{50} values in the range of 0·1 to 10 μM have been reported for recombinant GluCl

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Subunit	Sequence from position 253	EC_{50} Glutamate
C. oncophora IVM^S	V K L L L R R	30 μM
C. oncophora IVM^R	V K L F L R R	96 μM
H. contortus	V K L L L R R	27 μM
C. elegans	V V L R L R R	2.2 mM
D. immitis	V M L L L R R	~1 mM
A. suum	V K L L L R R	ND

Fig. 2. Alignment of the partial amino-acid sequence of the GluClα3B subunits from several nematode species, correlated with their sensitivity to activation by glutamate. IVM^S and IVM^R are ivermectin sensitive and resistant, respectively. Amino-acids that are different from the ivermectin sensitive version of the *C. oncophora* subunit are shown in white on a black background.

(Arena *et al*. 1991; Cully *et al*. 1994; Vassilatis *et al*. 1997*a*; Dent *et al*. 2000; Horoszok *et al*. 2001; Forrester *et al*. 2003; Njue *et al*. 2004). These are rather higher than the concentrations at which the drug shows anthelmintic effects, and also higher than the K_d values (26–100 pM) obtained in radioligand binding experiments to membranes from mammalian cells expressing recombinant *H. contortus* GluCl (Cheeseman *et al*. 2001; Forrester, Prichard & Beech, 2002). Very recent experiments have found that the *H. contortus* GluClα3B subunit can be activated by ivermectin concentrations as low as 0·1 to 1·0 nM (Rogers and Wolstenholme, unpublished), much closer to the concentrations suggested by the binding studies.

One very interesting phenomenon is the interaction between glutamate and ivermectin. The very first paper on the cloning of the GluCl subunits from *C. elegans* (Cully *et al*. 1994) showed that concentrations of ivermectin that were too low to directly activate the channels would nonetheless potentiate the effects of simultaneously applied sub-maximal concentrations of glutamate. Similar results were obtained (Martin, 1996) using two-electrode patch clamp recordings from the pharyngeal muscle of *A. suum*, where milbemycin D caused a dose-dependent potentiation of the observed response to glutamate. A similar result has been obtained using recombinant human glycine receptors: ivermectin acted as an irreversible agonist at higher concentrations (>0·3 μM) but at lower concentrations (30 nM) it allosterically potentiated glycine-gated currents (Shan, Haddrill & Lynch, 2001). Ivermectin has similar allosteric effects at the vertebrate α7 nicotinic receptor (Krause *et al*. 1998) and here mutations in the TM2 channel domain, believed to change the conformational equilibrium that exist between the active and desensitised states of the receptor, alter ivermectin potentiation. This result was interpreted to suggest that ivermectin binding also changes this equilibrium, leading to an increased probability of channel opening. More recent experiments have also shown the reverse phenomenon, that glutamate potentiates the activity and binding of ivermectin, at least at the HcGluClα receptor (Forrester *et al*. 2002; Forrester, Beech & Prichard, 2004). Glutamate and ivermectin do not compete for the same binding site and hence have different binding sites on the receptor (Hejmadi *et al*. 2000) so these data suggest that the two sites exert complementary, and possibly additive, effects on the conformational changes needed for the channels to open. It is possible that interactions between exogenous anthelmintic and endogenous glutamate explain the extraordinary potency of the A/M for killing worms and the much lower concentrations needed to activate native GluCl (Pemberton *et al*. 2001) than those reported for recombinant channels expressed in *Xenopus* oocytes (Arena *et al*. 1991; Cully *et al*. 1994; Vassilatis *et al*. 1997*a*; Dent *et al*. 2000; Horoszok *et al*. 2001; Forrester *et al*. 2003; Njue *et al*. 2004). Binding of an A/M to a GluCl molecule will both increase the probability of that channel opening but, in addition, greatly stabilise the open state once that opening has taken place, resulting in the essentially irreversible activation observed.

In summary, *in vitro* molecular cloning and expression of recombinant subunits has provided unequivocal evidence that the GluCl are the nematode targets of the ML anthelmintics. An examination of the *in vivo* roles of these receptors should explain how the drugs exert their anthelmintic effects, and vice versa.

P-GLYCOPROTEINS AND THE A/M ANTHELMINTICS

It is apparent from the previous section that, *in vitro*, the A/M do not interact only with the invertebrate GluCl, but also with other invertebrate and vertebrate ligand-gated chloride channels. Is the *in vivo* selectivity of these compounds for the invertebrate parasites over their vertebrate hosts due solely to a higher affinity at the GluCl over these other channels, or are other factors important? An important finding has been that certain collie dogs are extremely sensitive to treatment with the A/M, and that this sensitivity is associated with mutations in the *mdr-1* gene that encodes a P-glycoprotein (Mealey *et al*. 2001; Roulet *et al*. 2003). The drugs are extremely good substrates for these pumps in vertebrates and it is likely that their reduced toxicity for host versus parasite is due to their removal from the CNS by non-specific pumps of the blood-brain barrier (Nobmann, Bauer & Fricker, 2001). The A/M are also good substrates for nematode P-glycoproteins (Kerboeuf *et al*. 2003) and it has been suggested that changes in the expression of these pumps might contribute to drug resistance (Blackhall *et al*. 1998; Sangster *et al*. 1999; Drogemuller, Schnieder

https://doi.org/10.1017/S0031182005008218 Published online by Cambridge University Press

& von Samson-Himmelstjerna, 2004). However, there are no suggestions that the anthelmintic effects of the A/M are due to their interactions with the P-glycoproteins and it seems this is solely due to their channel-opening properties.

GLUCL IN THE NEMATODE PHARYNX

The nematode pharynx is a muscular organ and its function is to take in and partially process food prior to pumping in into the gut. The structure of this organ varies widely between nematode species and is one of the most characteristic features of their morphology. It contains distinct muscle, nerve and gland cells and is almost a self-contained system (Bird & Bird, 1991). As such, it is amenable to electrophysiological recordings and pharyngeal preparations have been widely used to study the effects of the A/M. It is also easy to observe and measure the rate of pumping. Pumping is a cyclic process and includes a relaxation phase triggered by the glutamatergic inhibitory motor neurone called M3 (Avery, 1993*a*, *b*; Raizen, & Avery, 1994; Lee *et al*. 1999), which implies the presence of inhibitory glutamate receptors on the post-synaptic muscle cells.

Pharyngeal pumping in nematodes is extremely sensitive to the A/M, and in many species the pharynx is the most sensitive organ to the effects of the drugs, with reported EC50 values of 0·2 to 10 nM (Bottjer & Bone, 1985; Avery & Horvitz, 1990; Geary *et al*. 1993; Gill *et al*. 1995; Paiement *et al*. 1999; Sheriff *et al*. 2002). This may mean that the primary anthelmintic effect of the A/M is on the pharynx and that its paralysis leads to worm death, either due to starvation or to the loss of internal turgor pressure. The inhibition of pumping is due to the presence of GluCl on pharyngeal muscle cells (Fig. 3) (Martin, 1996; Adelsberger *et al*. 1997; Pemberton *et al*. 2001). The irreversible activation of these receptors by ivermectin leads to a depolarisation of the muscle (Pemberton *et al*. 2001), presumably due to high internal [Cl^-], and a cessation of pumping.

In *C. elegans*, reporter gene experiments have shown that the *avr-15* and *glc-2* genes, encoding the GluClα2 and β subunits, are indeed expressed in pharyngeal muscle cells (Dent *et al*. 1997; Laughton *et al*. 1997*a*). Pemberton *et al*. (2001) employed a pharyngeal preparation from wild type and mutant *C. elegans* to make direct recordings of the effect of glutamate and ivermectin on muscle activity. Some of the properties of these preparations were consistent with the suggestion that the pharyngeal receptor in *C. elegans* is composed of α2 and β subunits, in particular the loss of ivermectin sensitivity of this preparation in *avr-15* mutant worms (Pemberton *et al*. 2001), but some of the pharmacological details are not. The channels found in *avr-15* worms do not possess the picrotoxin sensitivity predicted for a GluClβ receptor and the sensitivity to ivermectin of the preparation is rather higher than that seen for the GluClα2 + β combination in the Xenopus oocyte. So is there a third GluCl subunit present in pharyngeal muscle and, if so, which is it? So far the only gene that can be ruled out is *avr-14*, where neither reporter gene patterns nor the physiological data supports expression in these cells (Dent *et al*. 2000; Pemberton *et al*. 2001).

For parasitic species, we have less information. The physiological experiments have been, by and large, carried out on large worms such as *A. suum* and these clearly indicate the presence of GluCl on pharyngeal muscle, but the only localisation studies have been carried out on *H. contortus* using antibodies raised against synthetic peptides corresponding to poorly conserved regions of the individual subunits. These studies have not yielded any strong evidence for expression of any *H. contortus* GluCl subunit in pharyngeal muscle cells, and in particular the HcGluClβ subunit was not found there (Delany *et al*. 1998), but have suggested the possibility that HcGluClα3B may be expressed in pharyngeal neurones (Portillo, Jagannathan & Wolstenholme, 2003). Irreversible activation of these receptors, which are presumably presynaptic, could explain the inhibition of pumping observed in this species (Geary *et al*. 1993). However, it may also be that so-far undiscovered GluCl subunits may be present on *H. contortus* pharyngeal muscle: the absence of the HcGluClβ subunit could well mean that the composition, and hence pharmacology, of any such receptor will differ from that of its *C. elegans* counterpart.

GLUCL AND THE CONTROL OF LOCOMOTION

The second major effect of the A/M on nematodes is an apparent paralysis of body-wall muscle, rendering them immobilised. However, all the evidence points to this being an indirect effect, rather than a direct inhibition of neuromuscular transmission and there are no indications that GluCl are expressed in muscle cells. Locomotion in nematodes is controlled by both excitatory and inhibitory motor neurones, organised into ventral and dorsal nerve cords, each of which innervate body-wall muscle. Waves of reciprocal excitation and inhibition pass down the body, so that the dorsal muscles are relaxed as the ventral muscles are contracted, and vice versa. This results in the characteristic sinusoidal swimming motion. Studies on *C. elegans* showed that the motor neurones are in turn controlled by command interneurones in the head of the worm that regulate the rate of locomotion and also the frequency at which the worm reverses and moves backwards (White *et al*. 1986; Zheng *et al*. 1999).

Early reports showed that, in *A. suum*, avermectin blocked transmission between interneurones and excitatory motor neurones in the ventral cord, and

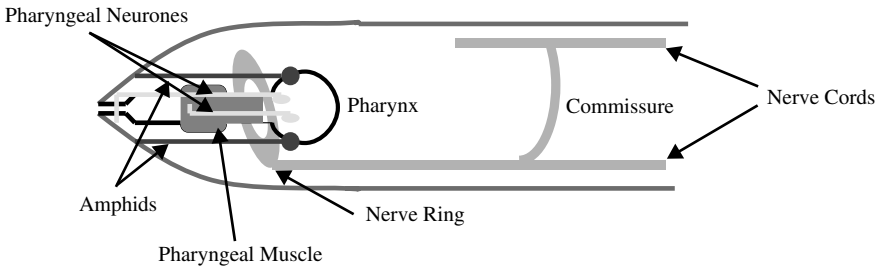

Fig. 3. Schematic representation of the distribution of GluCl in nematodes. The cuticle is outlined in grey and the pharynx in black. Structures reported to express GluCl are indicated by arrows.

also ventral inhibitory transmission (Kass *et al*. 1980, 1984). This would predict that GluCl subunits are expressed on those motor neurones and this prediction has been confirmed in *C. elegans*, using reporter gene constructs, and *H. contortus*, using antibodies (Fig. 3). Both *avr-14* and *avr-15* are expressed in *C. elegans* motor neurones (Dent 1997, 2000) and, in *H. contortus*, the HcGluClα, α3A, α3B and β subunits have all been detected on motor neurones (Delany *et al*. 1998; Jagannathan *et al*. 1999; Portillo, Jagannathan & Wolstenholme, 2003). All of the *H. contortus* subunits were detected on motor neurone commissures, structures connecting the dorsal and ventral nerve cords and which form synapses with interneurones on the lateral and sub-lateral nerve cords. The use of anti-GABA antibodies suggested that these were inhibitory motor neurons (Portillo *et al*. 2003). Application of A/M to channels on these inhibitory motor neurones would therefore presumably result in an irreversible hyperpolarisation of the cells and their consequent inability to produce action potentials. This would prevent inhibitory transmission at the neuromuscular junction and hence the abolition of the waves of muscular relaxation required for movement.

In addition, some GluCl subunits have been detected on interneurones, which might represent the command interneurones. The *C. elegans avr-14* gene is expressed in extra pharyngeal head neurones (Dent *et al*. 2000) and use of an antibody that recognised both GluClα3 subunits produced immunofluorescence in the nerve ring of *H. contortus* (Jagannathan *et al*. 1999). Direct confirmation that GluCl are present in at least some command interneurones was provided by the detection of a glutamate-gated chloride current in the AVA interneurone of *C. elegans* using *in vivo* recording techniques (Mellem *et al*. 2002). Mutations in the excitatory glutamate receptors also found in these neurones reduce the frequency at which the worms reverse direction (Brockie *et al*. 2001). We have examined the effects of mutations in several GluCl genes on this behaviour and have found the opposite phenotype, that is the worms reverse more frequently and the durations of the forward movements are reduced (N. Aptel, A. Cook, L. Holden-Dye & A. Wolstenholme, unpublished). The GluCl thus have a major role in regulating nematode locomotion and it is not surprising that their irreversible activation by A/M anthelmintics causes an effective paralysis.

GLUCL IN OTHER ORGANS

The A/M anthelmintics do not efficiently kill some tissue-dwelling adult worms, especially the macrofilariae. Nonetheless, they are effective for the treatment and prophylaxis of filarial infections such as *Onchocerca volvulus* due to their microfilariacidal effects and because they dramatically reduce the production of new microfilariae for several months (Klager *et al*. 1993; Lok *et al*. 1995). Similar effects on fecundity have been observed in other species (Petersen *et al*. 1996), implying that the GluCl have a role in reproduction. *C. elegans* carrying mutations in some GluCl genes do show a reduction in egg production (N. Aptel, V. Portillo & A. Wolstenholme, unpublished), but the nature of that role is currently unknown. No GluCl have been reported to be present on any reproductive tissue.

An intriguing recent suggestion is that GluCl may be present on sensory neurones, or involved in the pathways linking sensory stimulation to behavioural effects. Studies on *Brugia pahangi* have shown that ivermectin can block physiological responses to chemical stimuli in amphids (Perry, 2001; Rolfe, Barrett & Perry, 2001) and, in *H. contortus*, the HcGluClα3A subunit was detected in a structure that closely resembled a sensory neurone (Portillo *et al*. 2003). This is even more intriguing in light of the finding that ivermectin-resistant *H. contortus* have defects in amphid structure (Freeman *et al*. 2003): amphids are the sensory organs present in the nematode head that contain the sensory neurones. One of their functions would be expected to be to detect the stimuli that allow adult filaria to locate each other and mate and there are suggestions that ivermectin interferes with this process (Duke *et al*. 1992). In *C. elegans*, mechanical stimulation inhibits pharyngeal pumping in adult worms and it was suggested that GluCl containing the *avr-14* and *avr-15* gene products were expressed in the inhibitory pathway linking mechanosensation to pharyngeal pumping (Keane & Avery, 2003). There is therefore considerable evidence that the GluCl play a role in

https://doi.org/10.1017/S0031182005008218 Published online by Cambridge University Press

sensory signalling in nematodes, but as yet it is not clear how important this role is as a target for the A/M anthelmintics. One possibility is that the amphids act as a route of entry for the drugs into the worm.

CONCLUSIONS

It is generally accepted that the molecular targets of the avermectin/milbemycin anthelmintics are the glutamate-gated chloride channels. The number of identified GluCl subunits indicates that nematodes contain multiple forms of these channels, which may differ in their sensitivity to the current drugs, and at least some of these are expressed in the nematode neuromuscular system. The elucidation of the subunit structure of these different forms and its relationship to drug sensitivity remains a major challenge. The GluCl are widely expressed in the nematode nervous system (summarised in Fig. 2) and most of the effects of the A/M, with the possible exception of the reproductive effects, can be explained by this distribution. The implication is that these drugs can kill, or damage, nematodes in a variety of ways and, given that there are multiple forms of GluCl, the most important mechanism may vary between species. This has important implications for the development of resistance, a topic outside the scope of this review, which is becoming a major concern in veterinary parasites (Kaplan, 2004). The mechanisms of A/M resistance are still somewhat controversial (Wolstenholme *et al*. 2004), but it may be that the multiplicity of GluCl subtypes and functions may be reflected in a similar multiplicity of resistance mechanisms.

ACKNOWLEDGEMENTS

Work on the nematode GluCl in the authors' laboratory is and has been funded by the BBSRC (awards 86/GAN13134 and BBS/B/07594) and the Wellcome Trust (award 061043).

REFERENCES

ADELSBERGER, H., SCHEUR, T. & DUDEL, J. (1997). A patch clamp study of a glutamate chloride channel on pharyngeal muscle of the nematode *Ascaris suum*. *Neuroscience Letters* **230**, 183–186.

ANZIANI, O. S., ZIMMERMANN, G., GUGLIEMONE, A. A., VAZQUEZ, R. & SUAREZ, E. (2001). Avermectin resistance in *Cooperia pectinata* in cattle in Argentina. *Veterinary Record* **149**, 58–59.

ARENA, J. P., LIU, K. K., PARESS, P. S. & CULLY, D. F. (1991). Avermectin-sensitive chloride currents induced by *Caenorhabditis elegans* RNA in *Xenopus* oocytes. *Molecular Pharmacology* **40**, 368–374.

ARENA, J. P., LIU, K. K., PARESS, P. S. & CULLY, D. F. (1992). Expression of a glutamate-activated chloride current in *Xenopus* oocytes injected with *Caenorhabditis elegans* RNA: evidence for modulation by avermectin. *Molecular Brain Research* **15**, 339–348.

ARENA, J. P., LIU, K. K., PARESS, P. S., FRAZIER, E. G., CULLY, D. F., MROZIK, H. & SCHAEFFER, J. M. (1995). The mechanism of action of avermectins in *Caenorhabditis elegans* – correlation between activation of glutamate-sensitive chloride current, membrane-binding and biological-activity. *Journal of Parasitology* **81**, 286–294.

AVERY, L. (1993*a*). The genetics of feeding in *Caenorhabditis elegans*. *Genetics* **133**, 897–917.

AVERY, L. (1993*b*). Motor neuron M3 controls pharyngeal muscle relaxation timing in *Caenorhabditis elegans*. *Journal of Experimental Biology* **175**, 283–297.

AVERY, L. & HORVITZ, H. R. (1990). Effects of starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. *Journal of Experimental Zoology* **253**, 263–270.

BIRD, A. F. & BIRD, J. (1991). *The Structure of Nematodes*. New York, Academic Press.

BLACKHALL, W. J., LIU, H. Y., XU, M., PRICHARD, R. K. & BEECH, R. N. (1998). Selection at a P-glycoprotein gene in ivermectin- and moxidectin-selected strains of Haemonchus contortus. *Molecular and Biochemical Parasitology* **95**, 193–201.

BOTTJER, K. P. & BONE, L. W. (1985). *Trichostrongylus colubriformis*: effect of anthelmintics on ingestion and oviposition. *International Journal for Parasitology* **15**, 501–503.

BROCKIE, P. J., MELLEM, J. E., HILLS, T., MADSEN, T. M. & MARICQ, A. V. (2001). The *C. elegans* glutamate receptor subunit NMR-1 is required for slow NMDA-activated currents that regulate reversal frequency during locomotion. *Neuron* **31**, 617–630.

BROWNLEE, D. A., HOLDEN-DYE, L. & WALKER, R. J. (1997). Actions of the anthelmintic ivermectin on the pharyngeal muscle of the parasitic nematode *Ascaris suum*. *Parasitology* **115**, 553–561.

CAMPBELL, W. C., FISHER, M. H., STAPLEY, E. O., ALBERS-SCHÖNBERG, G. & JACOB, T. A. (1983). Ivermectin: a potent new antiparasitic agent. *Science* **221**, 823–828.

CASCIO, M. (2004). Structure and function of the glycine receptor and related nicotinicoid receptors. *Journal of Biological Chemistry* **279**, 19383–19386.

CHEESEMAN, C. L., DELANY, N. S., WOODS, D. J. & WOLSTENHOLME, A. J. (2001). High-affinity ivermectin binding to recombinant subunits of the *Haemonchus contortus* glutamate-gated chloride channel. *Molecular and Biochemical Parasitology* **114**, 161–168.

CULLY, D. F., VASSILATIS, D. K., LIU, K. K., PARESS, P., VAN DER PLOEG, L. H. T., SCHAEFFER, J. M. & ARENA, J. P. (1994). Cloning of an avermectin-sensitive glutamate-gated chloride channel from *Caenorhabditis elegans*. *Nature, London* **371**, 707–711.

CULLY, D. F., WILKINSON, H. & VASSILATIS, D. K. (1996). Molecular biology and electrophysiology of glutamate-gated chloride channels of invertebrates. *Parasitology* **113**, S191–S200.

DELANY, N. S., LAUGHTON, D. L. & WOLSTENHOLME, A. J. (1998). Cloning and localisation of an avermectin receptor-related subunit from *Haemonchus contortus*. *Molecular and Biochemical Parasitology* **97**, 177–187.

DENT, J. A., DAVIS, M. W. & AVERY, L. (1997). *avr-15* encodes a chloride channel subunit that mediates inhibitory glutamatergic neurotransmission and ivermectin

sensitivity in *Caenorhabditis elegans*. *EMBO Journal* **16**, 5867–5879.

DENT, J. A., SMITH, M. M., VASSILATIS, D. K. & AVERY, L. (2000). The genetics of avermectin resistance in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences, USA* **97**, 2674–2679.

DROGEMULLER, M., SCHNIEDER, T. & VON SAMSON-HIMMELSTJERNA, G. (2004). Evidence of P-glycoprotein sequence diversity in cyathostomins. *Journal of Parasitology* **90**, 998–1003.

DUKE, B. O. L., ZEAFLORES, G., CASTRO, J., CUPP, E. W. & MUNOZ, B. (1992). Effects of 3-month doses of ivermectin on adult *Onchocerca volvulus*. *American Journal of Tropical Medicine and Hygiene* **46**, 189–194.

ETTER, A., CULLY, D. F., SCHAEFFER, J. M., LIU, K. K. & ARENA, J. P. (1996). An amino acid substitution in the pore region of a glutamate gated chloride channel enables the coupling of ligand binding to channel gating. *Journal of Biological Chemistry* **271**, 16035–16039.

FAMILTON, A. S., MASON, P. & COLES, G. C. (2001). Anthelmintic resistant *Cooperia* in cattle. *Veterinary Record* **149**, 719–720.

FENG, X.-P., HAYASHI, P., BEECH, R. N. & PRICHARD, R. K. (2002). Study of the nematode putative GABA type-A receptor subunits: evidence for modulation by ivermectin. *Journal of Neurochemistry* **83**, 870–878.

FIRE, A., HARRISON, S. W. & DIXON, D. (1990). A modular set of LacZ fusion vectors for studying gene expression in *Caenorhabditis elegans*. *Gene* **93**, 189–198.

FORRESTER, S. G., BEECH, R. N. & PRICHARD, R. K. (2004). Agonist enhancement of macrocyclic lactone activity at a glutamate-gated chloride channel subunit from *Haemonchus contortus*. *Biochemical Pharmacology* **67**, 1019–1024.

FORRESTER, S. G., HAMDAN, F. F., PRICHARD, R. K. & BEECH, R. N. (1999). Cloning, sequencing, and developmental expression levels of a novel glutamate-gated chloride channel homologue in the parasitic nematode *Haemonchus contortus*. *Biochemical and Biophysical Research Communications* **254**, 529–534.

FORRESTER, S. G., PRICHARD, R. K. & BEECH, R. N. (2002). A glutamate-gated chloride channel subunit from *Haemonchus contortus*: Expression in a mammalian cell line, ligand binding, and modulation of anthelmintic binding by glutamate. *Biochemical Pharmacology* **63**, 1061–1068.

FORRESTER, S. G., PRICHARD, R. K., DENT, J. A. & BEECH, R. N. (2003). *Haemonchus contortus*: HcGluCla expressed in *Xenopus* oocytes forms a glutamate-gated ion channel that is activated by ibotenate and the antiparasitic drug ivermectin. *Molecular and Biochemical Parasitology* **129**, 115–121.

FREEMAN, A. S., NGHIEM, C., LI, J., ASHTON, F. T., GUERRERO, J., SHOOP, W. L. & SCHAD, G. A. (2003). Amphidial structure of ivermectin-resistant and susceptible laboratory and field strains of *Haemonchus contortus*. *Veterinary Parasitology* **110**, 217–226.

GEARY, T. G., SIMS, S. M., THOMAS, E. M., VANOVER, L., DAVIS, J. P., WINTEROWD, C. A., KLEIN, R., NORMAN, H. O. & THOMPSON, J. P. (1993). *Haemonchus contortus*: Ivermectin-induced paralysis of the pharynx. *Experimental Parasitology* **77**, 88–96.

GILL, J. H., REDWIN, J. M., VAN WYK, J. A. & LACEY, E. (1995). Avermectin inhibition of larval development in *Haemonchus contortus* – Effects of ivermectin resistance. *International Journal for Parasitology* **25**, 463–470.

GRAHAM, D., PFEIFFER, F. & BETZ, H. (1982). Avermectin B1a inhibits the binding of strychnine to the glycine receptor of rat spinal-cord. *Neuroscience Letters* **29**, 173–176.

HEJMADI, M. V., JAGANNATHAN, S., DELANY, N. S., COLES, G. C. & WOLSTENHOLME, A. J. (2000). L-glutamate binding sites of parasitic nematodes: an association with ivermectin resistance? *Parasitology* **120**, 535–545.

HOLDEN-DYE, L., HEWITT, G. M., WANN, K. T., KROGSGAARDLARSEN, P. & WALKER, R. J. (1988). Studies involving avermectin and the 4-aminobutyric acid (GABA) receptor of *Ascaris suum* muscle. *Pesticide Science* **24**, 231–245.

HOLDEN-DYE, L. & WALKER, R. J. (1990). Avermectin and avermectin derivatives are antagonists at the 4-aminobutyric acid (GABA) receptor on the somatic muscle cells of *Ascaris* – Is this the site of anthelmintic action? *Parasitology* **101**, 265–271.

HOROSZOK, L., RAYMOND, V., SATTELLE, D. B. & WOLSTENHOLME, A. J. (2001). GLC-3: a novel fipronil and BIDN-sensitive, but picrotoxinin-insensitive, L-glutamate-gated chloride channel subunit from *Caenorhabditis elegans*. *British Journal of Pharmacology* **132**, 1247–1254.

JACKSON, F. & COOP, R. L. (2000). The development of anthelmintic resistance in sheep nematodes. *Parasitology* **120**, S95–S107.

JAGANNATHAN, S., LAUGHTON, D. L., CRITTEN, C. L., SKINNER, T. M., HOROSZOK, L. & WOLSTENHOLME, A. J. (1999). Ligand-gated chloride channel subunits encoded by the *Haemonchus contortus* and Ascaris *suum* orthologues of the *Caenorhabditis elegans gbr-2* (*avr-14*) gene. *Molecular and Biochemical Parasitology* **103**, 129–140.

KAPLAN, R. M. (2004). Drug resistance in nematodes of veterinary importance: a status report. *Trends in Parasitology* **20**, 477–481.

KASS, I. S., STRETTON, A. O. W. & WANG, C. C. (1984). The effects of avermectin and drugs related to acetylcholine and 4-aminobutyric acid on neurotransmitters in *Ascaris suum*. *Molecular and Biochemical Parasitology* **13**, 213–225.

KASS, I. S., WANG, C. C., WALROW, J. P. & STRETTON, A. O. W. (1980). Avermectin b1A, a paralysing anthelmintic that affects interneurons and inhibitory motorneurons in *Ascaris*. *Proceedings of the National Academy of Sciences, USA* **77**, 6211–6215.

KEANE, J. & AVERY, L. (2003). Mechanosensory inputs influence *Caenorhabditis elegans* pharyngeal activity via ivermectin sensitivity genes. *Genetics* **164**, 153–162.

KERBOEUF, D., BLACKHALL, W. J., KAMINSKY, R. & VON SAMSON-HIMMELSTJERNA, G. (2003). P-glycoprotein in helminths: function and perspectives for anthelmintic treatment and reversal of resistance. *International Journal of Antimicrobial Agents* **22**, 322–346.

KLAGER, S., WHITWORTH, J. A. G., POST, R. J., CHAVASSE, D. C. & DOWNHAM M. D. (1993). How long do the effects of ivermectin on adult *Onchocerca volvulus* persist? *Tropical Medicine and Parasitology* **44**, 305–310.

KRAUSE, R. M., BUISSON, B., BERTRAND, S., CORRINGER, P. J., GALZI, J. L., CHANGEUX, J. P. & BERTRAND, D. (1998). Ivermectin: a positive allosteric effector of the $\alpha 7$

neuronal nicotinic acetylcholine receptor. *Molecular Pharmacology* **53**, 283–294.

LAUGHTON, D. L., LUNT, G. G. & WOLSTENHOLME, A. J. (1997*a*). Reporter gene constructs suggest the *Caenorhabditis elegans* avermectin receptor β-subunit is expressed solely in the pharynx. *Journal of Experimental Biology* **200**, 1509–1514.

LAUGHTON, D. L., LUNT, G. G. & WOLSTENHOLME, A. J. (1997*b*). Alternative splicing of a *Caenorhabditis elegans* gene produces two novel inhibitory amino acid receptor subunits with identical ligand-binding domains but different ion channels. *Gene* **201**, 119–125.

LEE, R. Y. N., SAWIN, E. R., CHALFIE, M., HORVITZ, H. R. & AVERY, L. (1999). EAT-4, a homolog of a mammalian sodium-dependent inorganic phosphate cotransporter, is necessary for glutamatergic neurotransmission in *Caenorhabditis elegans*. *Journal of Neuroscience* **19**, 159–167.

LOK, J. B., KNIGHT, D. H., SELAVKA, C. M., EYNARD, J., ZHANG, Y. & BERGMAN, R. N. (1995). Studies of reproductive competence in male *Dirofilaria immitis* treated with milbemycin oxime. *Tropical Medicine and Parasitology* **46**, 235–240.

LOVERIDGE, B., McARTHUR, M., McKENNA, P. & MARIADASS, B. (2003). Probable multigeneric resistance to macrocyclic lactone anthelmintics in cattle in New Zealand. *New Zealand Veterinary Journal* **51**, 139–141.

MARTIN, R. J. (1996). An electrophysiological preparation of *Ascaris suum* pharyngeal muscle reveals a glutamate-gated chloride channel sensitive to the avermectin analogue, milbemycin D. *Parasitology* **112**, 247–252.

MARTIN, R. J. & PENNINGTON, A. J. (1988). Effect of dihyroavermectin-b1a on Cl single channel currents in *Ascaris*. *Pesticide Science* **24**, 90–91.

MARTIN, R. J. & PENNINGTON, A. J. (1989). A patch-clamp study of effects of dihydroavermectin on *Ascaris* muscle. *British Journal of Pharmacology* **98**, 747–756.

McINTIRE, S. L., JORGENSEN, E. M., KAPLAN, J. & HORVITZ, H. R. (1993). The GABAergic nervous system of *Caenorhabditis elegans*. *Nature, London* **364**, 337–341.

MEALEY, K. L., BENTJEN, S. A., GAY, J. M. & CANTOR, G. H. (2001). Ivermectin sensitivity in collies is associated with a deletion mutation of the mdr1 gene. *Pharmacogenetics* **11**, 727–733.

MELLEM, J. E., BROCKIE, P. J., ZHENG, Y., MADSEN, D. M. & MARICQ, A. V. (2002). Decoding of polymodal sensory stimuli by postsynaptic glutamate receptors in *C. elegans*. *Neuron* **36**, 933–944.

MES, T. H. M. (2004). Purifying selection and demographic expansion affect sequence diversity of the ligand-binding domain of a glutamate-gated chloride channel gene of *Haemonchus placei*. *Journal of Molecular Evolution* **58**, 466–478.

NJUE, A. I., HAYASHI, J., KINNE, J., FENG, X.-P. & PRICHARD, R. K. (2004). Mutations in the extracellular domain of glutamate-gated chloride channel α3 and β subunits from ivermectin-resistant *Cooperia oncophora* affect agonist sensitivity. *Journal of Neurochemistry* **89**, 1137–1147.

NJUE, A. I. & PRICHARD, R. K. (2004). Genetic variability of glutamate-gated chloride channel genes in ivermectin-sensitive and -resistant strains of *Cooperia oncophora*. *Parasitology* **129**, 741–751.

NOBMANN, S., BAUER, B. & FRICKER, G. (2001). Ivermectin excretion by isolated functionally intact brain endothelial capillaries. *British Journal of Pharmacology* **132**, 722–728.

OLSEN, R. & TOBIN, A. (1990). Molecular biology of $GABA_A$ receptors. *FASEB Journal* **4**, 1469–1480.

OMURA, S. & CRUMP, A. (2004). The life and times of ivermectin – A success story. *Nature Reviews Microbiology* **2**, 984–989.

PAIEMENT, J.-P., LEGER, C., RIBEIRO, P. & PRICHARD, R. K. (1999). *Haemonchus contortus*: effects of glutamate, ivermectin, and moxidectin on inulin uptake activity in unselected and ivermectin-selected adults. *Experimental Parasitology* **92**, 193–198.

PEMBERTON, D. J., FRANKS, C. J., WALKER, R. J. & HOLDEN-DYE, L. (2001). Characterization of glutamate-gated chloride channels in the pharynx of wild-type and mutant *Caenorhabditis elegans* delineates the role of the subunit GluCl-alpha 2 in the function of the native receptor. *Molecular Pharmacology* **59**, 1037–1043.

PERRY, R. N. (2001). Analysis of the sensory responses of parasitic nematodes using electrophysiology. *International Journal for Parasitology* **31**, 909–918.

PETERSEN, M. B., VARADY, M., BJORN, H. & NANSEN, P. (1996). Efficacies of different doses of ivermectin against male, female and L4 *Oesophagostomum dentatum* in pigs. *Veterinary Parasitology* **65**, 55–63.

PONG, S. S. & WANG, C. C. (1982). Avermectin-B1a modulation of gamma-aminobutyric acid receptors in rat-brain membranes. *Journal of Neurochemistry* **38**, 375–379.

PORTILLO, V., JAGANNATHAN, S. & WOLSTENHOLME, A. J. (2003). Distribution of glutamate-gated chloride channel subunits in the parasitic nematode *Haemonchus contortus*. *Journal of Comparative Neurology* **462**, 213–222.

RAIZEN, D. M. & AVERY, L. (1994). Electrical activity and behaviour in the pharynx of *Caenorhabditis elegans*. *Neuron* **12**, 483–495.

ROLFE, R. N., BARRETT, J. & PERRY, R. N. (2001). Electrophysiological analysis of responses of adult females of *Brugia pahangi* to some chemicals. *Parasitology* **122**, 347–357.

ROULET, A., PUEL, O., GESTA, S., LEPAGE, J. F., DRAG, M., SOLL, M., ALVINERIE, M. & PINEAU, T. (2003). MDR1-deficient genotype in Collie dogs hypersensitive to the P-glycoprotein substrate ivermectin. *European Journal of Pharmacology* **460**, 85–91.

SANGSTER, N. C., BANNAN, S. C., WEISS, A. S., NULF, S. C., KLEIN, R. D. & GEARY, T. G. (1999). *Haemonchus contortus*: Sequence heterogeneity of internucleotide binding domains from P-glycoproteins and an association with avermectin/milbemycin resistance. *Experimental Parasitology* **91**, 250–257.

SHAN, Q., HADDRILL, J. L. & LYNCH, J. W. (2001). Ivermectin, an unconventional agonist of the glycine receptor chloride channel. *Journal of Biological Chemistry* **276**, 12556–12564.

SHERIFF, J. C., KOTZE, A. C., SANGSTER, N. C. & MARTIN, R. J. (2002). Effects of macrocyclic lactone anthelmintics on feeding and pharyngeal pumping in *Trichostrongylus colubriformis in vitro*. *Parasitology* **125**, 477–484.

SUPAVILAI, P. & KAROBATH, M. (1981). *In vitro* modulation by avermectin-B1a of the GABA-benzodiazepine

receptor complex of rat cerebellum. *Journal of Neurochemistry* **36**, 798–803.

VASSILATIS, D. K., ARENA, J. P., PLASTERK, R. H. A., WILKINSON, H., SCHAEFFER, J. M., CULLY, D. F. & VAN DER PLOEG, L. H. T. (1997*a*). Genetic and biochemical evidence for a novel avermectin sensitive chloride channel in *C. elegans*: isolation and characterisation. *Journal of Biological Chemistry* **272**, 33167–33174.

VASSILATIS, D. K., ELLISTON, K., PARESS, P. S., HAMELIN, M., ARENA, J. P., SCHAEFFER, J. M., VAN DER PLOEG, L. H. T. & CULLY, D. F. (1997*b*). Evolutionary relationship of the ligand-gated ion channels and the avermectin sensitive, glutamate-gated chloride channels. *Journal of Molecular Evolution* **44**, 501–508.

VERCRUYSSE, J. & REW, R. E. (2002). *Macrocyclic Lactones in Antiparasitic Therapy*. CABI Publishing, Wallingford, UK.

WHITE, J. G., SOUTHGATE, E., THOMPSON, J. N. & BRENNER, S. (1986). The structure of the nervous system of *Caenorhabditis elegans*. *Philosophical Transactions of the Royal Society of London Series B* **314**, 1–340.

WOLSTENHOLME, A. J., FAIRWEATHER, I., PRICHARD, R. K., VON SAMSON-HIMMELSTJERNA, G. & SANGSTER, N. C. (2004). Drug resistance in veterinary helminths. *Trends in Parasitology* **20**, 469–476.

YATES, D. M., PORTILLO, V. & WOLSTENHOLME, A. J. (2003). The avermectin receptors of *Haemonchus contortus* and *Caenorhabditis elegans*. *International Journal for Parasitology* **33**, 1183–1193.

YATES, D. M. & WOLSTENHOLME, A. J. (2004). An ivermectin-sensitive glutamate-gated chloride channel subunit from *Dirofilaria immitis*. *International Journal for Parasitology* **34**, 1065–1071.

ZHENG, Y., BROCKIE, P. J., MELLEM, J. E., MADSEN, D. M. & MARICQ, A. V. (1999). Neuronal control of locomotion in *C. elegans* is modified by a dominant mutation in the GLR-1 ionotropic glutamate receptor. *Neuron* **24**, 347–361.