Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 67 (2004) 1019–1024
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Agonist enhancement of macrocyclic lactone activity at a glutamate-gated chloride channel subunit from *Haemonchus contortus*

Sean G. Forrester*, Robin N. Beech, Roger K. Prichard

Institute of Parasitology, McGill University, 21,111 Lakeshore Rd., Ste-Anne de Bellevue, Que., Canada H9X 3V9

Received 16 May 2003; accepted 21 August 2003

Abstract

The mode of action of ivermectin (IVM) in nematodes appears to be the opening of inhibitory ion channels, including the glutamate-gated chloride channel (GluCl). Recently, it has been shown that IVM binds with high affinity to a *Haemonchus contortus* GluCl subunit (HcGluCla) expressed in COS-7 cells, and this binding is potentiated in the presence of glutamate. To gain further insight into the potentiation of macrocyclic lactone anthlemintics we have screened various glutamatergic and nonglutamatergic ligands for their ability to enhance [^{3}H] IVM binding to HcGluCla. Of the ligands tested, only glutamate and ibotenate potentiated [^{3}H] IVM binding. Interestingly, these ligands have also been shown to open the HcGluCla channel expressed in *Xenopus* oocytes. We examined the effect of ibotenate on macrocyclic lactone binding in more detail and found that it caused a 7-fold enhancement in [^{3}H] IVM binding affinity and a 4-fold increase in [^{3}H] MOX binding affinity. In *in vivo* efficacy studies, ibotenate (up to 2 mg/kg) had no anthelmintic activity against *H. contortus* in gerbils. When 1 mg/kg ibotenate was used in combination with IVM, IVM efficacy increased by 15% ($P = 0.048$). These results demonstrate that a GluCl agonist enhances IVM activity and provides further information on the mode of action of ivermectin in parasitic nematodes.

Keywords: Glutamate-gated chloride channel; Ivermectin receptor; Moxidectin; Ibotenate; *Haemonchus contortus*

1. Introduction

IVM, a macrocyclic lactone, is a broad spectrum anthelmintic used to control nematode parasites of livestock [1]. Unfortunately, the repeated use of this drug has led to the development of IVM resistance in parasitic nematodes such as *Haemonchus contortus* [2]. The mechanism of this resistance and of IVM action is not fully understood but may involve the ligand-gated ion channels, including the GluCl [3–10]. Evidence for this has emerged mostly from studies using the free-living nematode *Caenhorhabditis elegans* as a model for parasitic nematodes where it has been shown that in this species the GluCl is a key target for IVM [4]. However, whether our current knowledge regarding the mode of action of IVM in *C. elegans* holds true for parasitic nematodes such as *H. contortus* is not known. Therefore, in order to find ways to prolong to use of IVM in economically important parasite species, further research focused on determining the mode of action of IVM in parasitic nematodes is needed.

Research by our group into the mode of action of IVM in *H. contortus* has led to the discovery of HcGluCla, a GluCl and a possible IVM target [11]. HcGluCla expressed in COS-7 cells binds IVM with high affinity (ca. 100 pM), which is consistent with the high *in vivo* potency of this drug in *H. contortus* [8,9]. Interestingly, the affinity of IVM is enhanced in the presence of glutamate [9] suggesting that the natural ligand, through its binding to a distinct site, can enhance allosterically the activity of IVM. However, the manner by which this allosteric interaction influences the paralytic action of IVM *in vivo* is not yet understood. Interestingly, glutamate was shown to reduce IVM inhibition of pharyngeal pumping in *H. contortus* at a site that is likely a GluCl [10] suggesting that the two ligands may interact *in vivo*. Nevertheless, it is not known whether glutamate directly or indirectly inhibits the effect of IVM

* Corresponding author. Present address: Department of Microbiology, University of Pennsylvania Medical Center, 202C Johnson Pavilion, 3610 Hamilton Walk, Philadelphia, PA 19104-6076, USA. Tel.: +1-215-898-3545; fax: +1-215-898-9557.

E-mail addresses: sgforrester@yahoo.ca (S.G. Forrester), roger.prichard@mcgill.ca (R.K. Prichard).

Abbreviations: PTX, picrotoxin; COS-7, African green monkey kidney cells; GluCl, glutamate-gated chloride channel; HcGluCla, *Haemonchus contortus* glutamate-gated chloride channel subunit; IVM, ivermectin; MOX, moxidectin; NMDA, *N*-methyl-D-aspartic acid; GABA, γ-aminobutyric acid.

0006-2952/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2003.08.047

at the GluCl site of action nor is it known whether such a ligand interaction has any effect on the efficacy of macrocyclic lactone anthelmintics.

GluCls are members of the nicotinic family of ligand-gated ion channels, which includes the $GABA_A$ and glycine receptors [12]. These channels once bound by ligands undergo large changes in conformation that cause the channel pore to open allowing ions to cross the membrane. Once this occurs, mechanisms are in place to allow the channel to return to its original resting state [13]. However, IVM binds irreversibly to the GluCl and causes the channel to remain locked in an open-conformational state even after unbound drug is removed [5–7]. Consistent with this, we have shown recently that *HcGluCla* expressed in *Xenopus* oocytes forms a homomeric ion channel that is gated reversibly by glutamate and ibotenate and irreversibly by IVM and MOX [14]. The mechanism of this irreversible activation is currently unknown but may involve a change in the IVM binding site after initial IVM binding. There is evidence that IVM goes through two affinity states during the binding process [15] but what changes occur in the receptor as a result of this is not known.

To further investigate the interaction between IVM and GluCls, we have examined the binding properties of a GluCl IVM-receptor subunit (HcGluCla) from *H. contortus* expressed in mammalian cells. We have shown that IVM binding affinity is enhanced only in the presence of L-glutamate and ibotenate, which are ligands known to activate GluCls. This suggests that IVM binds to the channel more readily when the channel is in an activated conformation. We have extended these findings and have shown that ibotenate enhances IVM efficacy *in vivo*. This is the first evidence that GluCl agonists enhance IVM toxicity.

2. Materials and methods

2.1. Chemicals

[^{3}H] IVM (18.6 Ci/mmol) was a gift from Merck Research Laboratories. [^{3}H] MOX (48 Ci/mmol) was a gift from Fort Dodge Animal Health. L-Glutamate, ibotenate, kainate, NMDA, quisqualate, GABA, and PTX were purchased from Research Biochemicals International (Sigma). L-Aspartate was purchased from Sigma. IVM and MOX were gifts from Fort Dodge Animal Health. All other chemicals and reagents were of highest purity commercially available. IVM drench solution was purchased from CDMV Inc.

2.2. Cell culture and transfection

COS-7 cells were maintained at 37° with 5% CO_2 in a humidified incubator. Growth medium was Dulbecco's Modified Eagle Medium (DMEM) supplemented with 0.02 M Hepes buffer (Biomedia Canada), 10% fetal calf serum, and 20 μg of gentamicin (Gibco BRL) per 500 mL bottle. For transfection experiments, a HcGluCla-pCI-neo construct [9] was transiently transfected into COS-7 cells using Lipofectamine (Gibco BRL), according to the manufacturers recommendations. Briefly, 2.5 μg of construct was mixed with 20 μL of lipofectamine and added to 10 cm dishes that were seeded with approximately 10^6 cells 12–15 hr earlier. The media was changed at 5 and 24 hr post-transfection and the cells were harvested 40–48 hr later.

2.3. Membrane preparation

The preparation of crude membrane fractions was based on the protocol outlined in Forrester *et al.* [9]. Briefly, cells were washed twice with ice-cold PBS and then incubated in a hypotonic solution (15 mM Tris–HCl, pH 7.4, 1.25 mM $MgCl_2$, 1 mM EDTA) for 10 min at 4°. The cells were scraped from the plates, centrifuged at 500 *g* for 5 min and resuspended in 5 mL of ice-cold Hepes buffer (pH 7.4) (Fisher Scientific) containing 1 mM of freshly added phenylmethylsulfonyl fluoride (PMSF) (Sigma). The cells were then lysed on ice by sonication (7 pulses of 15 s on/off) and the lysates were centrifuged at 200 *g* for 5 min. The supernatant was centrifuged at 28,000 *g* for 20 min at 4° and the resulting pellet was washed once in ice-cold Hepes and recentrifuged at 28,000 *g*. The pellet was then resuspended in 50 mM Hepes buffer (pH 7.4) and used for protein determination using the Lowry protein determination kit (Sigma). Membranes were then stored at −80° at a concentration of 5 μg/μL and used within 7 days.

2.4. [^{3}H] IVM and [^{3}H] MOX binding assays

Conditions for the [^{3}H] IVM and [^{3}H] MOX binding assays were based on previously established protocols [9,15,16]. COS-7 membranes (5 μg per reaction) were incubated with a range of [^{3}H] IVM or [^{3}H] MOX concentrations in 1 mL of 50 mM Hepes buffer (pH 7.4) containing 0.02% Triton X-100 (Sigma) for 1 hr (equilibrium time) at room temperature. Reactions were terminated through Whatman GF-B glass fiber filters (Fisher Scientific) previously soaked in 50 mM Hepes buffer containing 0.1% (v/v) polyethyleneimine (PEI) (Sigma). Filters were washed three times with 5 mL of ice-cold H_2O containing 0.1% (v/v) Triton X-100. Nonspecific binding was measured in the presence of a 1000-fold molar excess of unlabelled IVM or MOX, which was added at the same time as the [^{3}H] IVM or [^{3}H] MOX. Specific binding was measured as a difference between total and nonspecific binding. Sample radioactivity was counted using a Wallac 1414 liquid scintillation counter.

2.5. Data analysis

All binding data were analyzed using a nonlinear regression analysis with the Prism 3.0 software package

(Graph Pad) according to the methods outlined in the manual. Data from saturation experiments were analyzed using the equation $Y = (B_{max}X)/K_d + X$. Comparisons of means were performed using Student's *t*-test and a *P*-value ≤ 0.05 was considered significant.

2.6. In vivo efficacy studies

Helminth free gerbils (Charles River) were fed a special diet containing 0.02% hydrocortisone, which has been shown to increase *H. contortus* establishment in this non-specific host [17]. Gerbils were infected with exsheathed third-stage (L3) *H. contortus* larvae (2000 per animal in trials 1 and 2 and 1000 per animal in trial 3), by gavage and treated with various anthelminthic drug combinations 10 days post-infection by gavage. Gerbils were killed 3 days post-treatment, the glandular portion of the stomach containing the worms was removed and the total number of worms was determined. Data were analyzed using *t*-tests. A *P*-value of ≤ 0.05 was considered significant.

3. Results

3.1. Pharmacology of macrocyclic lactone potentiation

We have previously shown that glutamate enhances [^{3}H] IVM binding to COS-7 cell membranes expressing HcGluCla with an EC_{50} of 94 nM [9]. To provide further insight into the nature of this potentiation, we examined the effect of various glutamatergic and nonglutamatergic ligands (at 320 nM; a subsaturating concentration for glutamate [9]) on [^{3}H] IVM (at 0.09 nM) binding. We used 0.09 nM of [^{3}H] IVM in order to achieve both sufficient DPMs and a large measurable difference in binding between ligand-treated and untreated experiments. L-Aspartate, quisqualate, NMDA, PTX, GABA, and kainate all had little or no effect on [^{3}H] IVM binding. Only glutamate and ibotenate potentiated [^{3}H] IVM binding ($42 \pm 7\%$ and $50 \pm 4\%$, respectively) (Fig. 1).

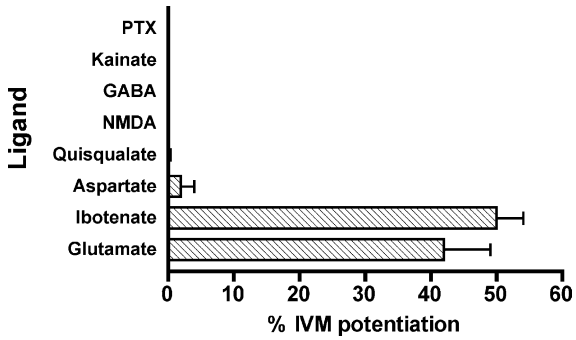

Fig. 1. The pharmacology of [^{3}H] IVM potentiation. Data from each ligand are expressed as a percent potentiation above basal [^{3}H] IVM binding [(specific binding + ligand/basal specific binding − 1) × 100]. All values are the mean ± SE of three independent experiments each performed in duplicate.

For comparison purposes, we determined the effect of L-glutamate vs. D-glutamate stereoisomers on [^{3}H] MOX (at 0.09 nM) potentiation. The application of 10 μM L-glutamate enhanced [^{3}H] MOX binding by $69 \pm 15\%$ whereas 10 μM D-glutamate enhanced [^{3}H] MOX binding by only $27 \pm 5\%$ (Fig. 2a). In a dose–response experiment, the EC_{50} value of D-glutamate potentiation of [^{3}H] MOX binding was 27.2 ± 6.8 μM (Fig. 2b).

3.2. Effect of ibotenate on [^{3}H] IVM and [^{3}H] MOX binding affinity

All GluCls characterized to date are activated by L-glutamate and the conformationally restricted analog ibotenate, including those present in crustacean neurons, pulmonate molluscan neurons, annelid neurons, and expressed GluCls from nematodes [12]. To examine the effect of ibotenate on [^{3}H] IVM binding to HcGluCla in more detail we conducted [^{3}H] IVM saturation experiments in the presence or absence of 10 μM ibotenate. In the presence or absence of ibotenate, binding of [^{3}H] IVM was saturable and only a single binding site was observed. The application of ibotenate significantly increased the affinity of [^{3}H] IVM to HcGluCla approximately 7-fold from a K_d of 0.094 ± 0.006 nM (untreated) to 0.013 ± 0.002 nM

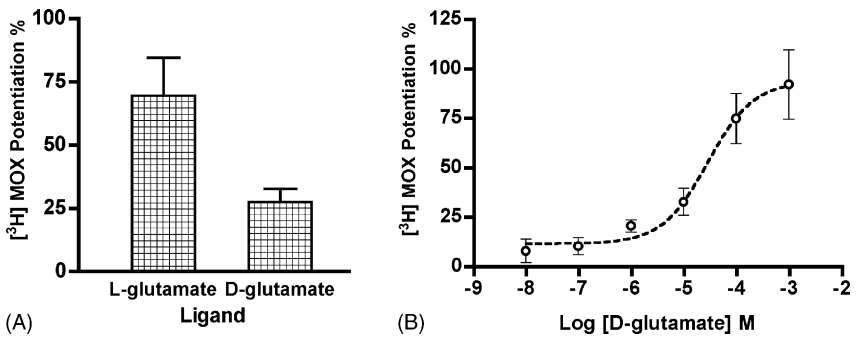

Fig. 2. Comparison of glutamate stereoisomers on [^{3}H] MOX potentiation. (A) [^{3}H] MOX potentiation using 10 μM L-glutamate vs. D-glutamate. (B) Concentration–response curve for [^{3}H] MOX potentiation by D-glutamate. Data are expressed as a percent potentiation above basal [(specific binding + D-glutamate/basal specific binding − 1) × 100] and was fitted to a single-site sigmoidal dose–response curve.

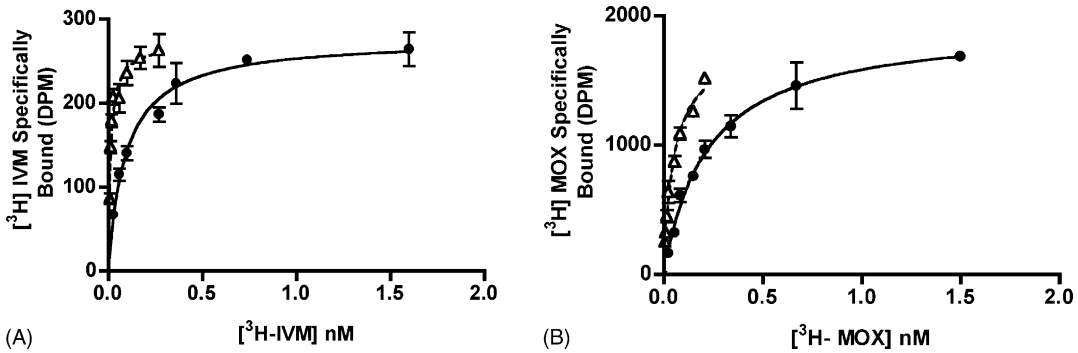

Fig. 3. The effect of ibotenate on [^{3}H] IVM and [^{3}H] MOX binding to HcGluCla transfected COS-7 cells. (A) [^{3}H] IVM saturation experiments in the presence (△) or absence (●) of 10 μM ibotenate. (B) [^{3}H] MOX saturation experiments in the presence (△) or absence (●) of 10 μM ibotenate. All values are the mean ± SE of three independent experiments each performed in duplicate.

(ibotenate treated) ($P < 0.001$) (Fig. 3a). No effect on the B_{max} was observed (277 ± 16 DPM (untreated); 272 ± 21 DPM (ibotenate treated)) ($P = 0.89$). For comparison, we examined the effect of 10 μM ibotenate on [^{3}H] MOX binding. As with [^{3}H] IVM binding, the binding of [^{3}H] MOX in the presence of ibotenate was saturable and only a single binding site was observed. In the absence of ibotenate the affinity (K_d) of [^{3}H] MOX for HcGluCla was 0.23 ± 0.01 nM but in the presence of ibotenate the affinity significantly increased almost 4-fold to 0.06 ± 0.01 nM ($P < 0.001$) (Fig. 3b). Similar to IVM, no effect on the B_{max} was observed (1937 ± 73 DPM (untreated); 1813 ± 97 DPM (ibotenate treated)) ($P = 0.36$). In all experiments, we only observed a single [^{3}H] MOX or [^{3}H] IVM binding site. Attempts to fit a two-site model did not produce evidence of more than one site. The effect of ibotenate on [^{3}H] IVM binding was also dose dependent with an EC_{50} of 86 ± 7 nM (Fig. 4).

3.3. The effect of ibotenate on IVM efficacy in vivo

The glutamate analog, ibotenate is an agonist for GluCls [12]. It also enhances IVM and MOX binding to HcGluCla. Therefore, it is possible that ibotenate may affect the conformation of the GluCl *in vivo* and alter the efficacy of IVM. Using the gerbil model system we determined the efficacy of 0.025 mg/kg IVM on *H. contortus* in the presence and absence of 2 mg/kg ibotenate. The concentration of IVM used in our experiments has been previously demonstrated to reduce total worm numbers in the gerbil host by 50% [17]. We used a 2 mg/kg concentration of ibotenate to provide the maximal ibotenate dose *in vivo* without adverse effects to the gerbils. In our study, IVM at 0.025 mg/kg caused a 56% reduction in worm numbers compared to the water-treated control. Ibotenate alone at 2 mg/kg had no anthelmintic activity (<1%) and in combination did not enhance IVM efficacy (data not shown) (N = 10 infected animals per treatment group).

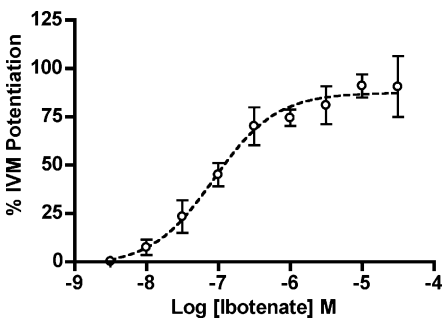

Fig. 4. Concentration–response curve for [^{3}H] IVM potentiation by ibotenate. Data are expressed as a percent potentiation above basal [(specific binding + ibotenate/basal specific binding − 1) × 100] and was fitted to a single-site sigmoidal dose–response curve.

It has previously been observed that glutamate at 10 μM potentiated the binding of IVM to HcGluCla to a greater extent than 1 mM glutamate [9]. It was therefore decided to use a dose rate of ibotenate that may provide the optimum IVM enhancement. The possibility that 1 mg/kg ibotenate would have an effect on IVM efficacy was then examined. Similar to the above experiment, total worm numbers following treatment with 0.025 mg/kg IVM alone gave a 52% reduction in total worm numbers compared to the control. However, co-administration of 0.025 mg/kg IVM + 1 mg/kg ibotenate reduced worm numbers by 67% and was significantly more effective than treatment with IVM alone ($P = 0.048$; N = 10 gerbils per treatment group). Ibotenate alone at 1 mg/kg had no effect on worm numbers compared to the control (Fig. 5a).

To provide further evidence that 1 mg/kg ibotenate enhances IVM potency we tested whether we could enhance a sub-optimal dose of IVM. To determine this, we performed the same efficacy trial but this time using 0.001 mg/kg IVM in the presence or absence of 1 mg/kg ibotenate. The application of either 0.001 mg/kg IVM or 1 mg/kg ibotenate alone had no significant effect on worm numbers compared to the control. However, the application of both 0.001 mg/kg IVM with 1 mg/kg ibotenate reduced worm numbers by 36% compared to the control. Although a trend towards increased IVM efficacy in the presence of ibotenate was observed, mean worm numbers between the IVM and the IVM/ibotenate group and between the control group and the IVM/ibotenate group were not significantly

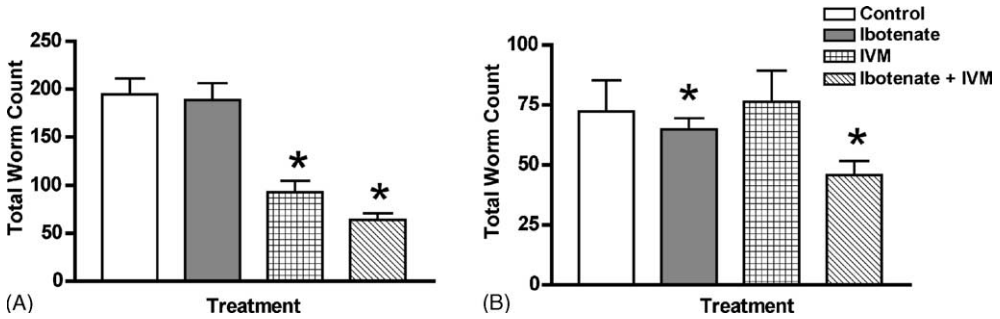

Fig. 5. The effect of 1 mg/kg ibotenate on IVM potency *in vivo*. (A) *H. contortus* infected gerbils were treated with water, ibotenate (1 mg/kg), IVM (0.025 mg/kg), or IVM + ibotenate. Bars are the mean total worm counts ± SE (N = 10 gerbils per group). The significant difference observed between the IVM treated group and the IVM/ibotenate treated group is indicated by an asterisk (*) $P < 0.05$) (B) A similar experiment using *H. contortus* infected gerbils treated with water, ibotenate (1 mg/kg), IVM (0.001 mg/kg), or IVM + ibotenate (N = 6 gerbils per group). The significant difference observed between the ibotenate treated group and the IVM/ibotenate treated group is indicated by an asterisk (*) $P < 0.05$).

different ($P = 0.059$ and 0.1, respectively; N = 6 gerbils per treatment group). However, there was a significant difference between the ibotenate-treated group and the IVM/ibotenate group ($P = 0.03$) (Fig. 5b).

4. Discussion

We have shown that IVM binds to a GluCl subunit at a higher affinity in the presence of ligands known to directly open the channel. The effect of glutamate [9] and ibotenate (present study) on IVM and MOX binding indicates that there is likely a major conformational change in the HcGluCla subunit in the presence of these agonists. This conformational change may correspond to the GluCl channel being open. Consistent with this, we have shown previously that glutamate and ibotenate open the HcGluCla channel expressed in *Xenopus* oocytes, while other ligands such as aspartate, NMDA, kainate, and GABA are ineffective [14]. Quisqualate was also ineffective at enhancing [^{3}H] IVM binding and does not activate GluCls [7] and PTX is an antagonist that has been shown to block GluCl currents [7,14]. It is possible, therefore, that IVM binds to GluCls more readily when the channel is in an open-conformational state. This may explain, in part, the two-state binding of macrocyclic lactone anthelmintics to GluCls [15]; first, channel opening then an irreversible binding that maintains the open conformation.

We have shown previously that [^{3}H] IVM and [^{3}H] MOX bind to the same site on the HcGluCla subunit, which is distinct from the glutamate-binding site [9]. However, in the presence of glutamate, a much smaller enhancement in [^{3}H] MOX compared to [^{3}H] IVM binding was observed (1.5-fold vs. 7-fold enhancement, respectively). Similar results were obtained using ibotenate although the difference between MOX and IVM enhancement was less dramatic (4-fold vs. 7-fold, respectively). Why such a difference in MOX binding, but not IVM, occurs between glutamate and ibotenate potentiation is not known, but further points to differences in the activity of these anthelmintics. Moreover, although the affinity of MOX to HcGluCla is lower than that of IVM ([9], present study), previous research has shown that MOX is more potent than IVM *in vivo* [17]. The differences in the pharmacokinetic behavior between the two compounds may account for some of the difference *in vivo* [18]. On the other hand, the possibility exists that MOX interacts with other targets to a greater extent than does IVM. Both IVM, which is an avermectin, and MOX, which is a milbemycin, share a 16-membered macrocyclic unit, but differ in the fact that the avermectins possess a disaccharide substituent at C-13 that is not present in the milbemycins. As well, MOX is substituted at C-23 and C-25 compared with IVM [19]. The potential affect of these differences on the mode of action of the avermectins and the milbemycins requires further elucidation.

Numerous studies have shown that the L-glutamate analog, ibotenate activates GluCls *in vitro* [6,7,14] and *in vivo* [12]. However, whether an interaction between ibotenate and a GluCl can enhance the anthelmintic activity of IVM has not previously been demonstrated. We have attempted to address this by examining the effect of ibotenate and IVM on *H. contortus* in infected gerbils. We have shown that ibotenate itself given orally to gerbils, at concentrations up to 2 mg/kg, has no anthelmintic activity. While ibotenate alone has no anthelmintic activity, low concentrations (1 mg/kg) caused a small enhancement in IVM efficacy (P-values = 0.048 and 0.059, with N = 10 and 6, respectively). However, since 1 mg/kg ibotenate produced a small enhancement in IVM efficacy and 2 mg/kg ibotenate produced no increase in efficacy, it is possible that the effect of ibotenate on IVM efficacy is concentration sensitive with any effect decreasing at higher ibotenate concentrations. This would be consistent with the effects of glutamate on IVM binding *in vitro* where a glutamate concentration of 10 μM was optimal for potentiation [9]. In other *in vivo* studies, it has been shown that high concentrations of glutamate (0.8 mM) reduced MOX inhibition of pharyngeal pumping at a target that is likely a GluCl [10]. Therefore, it appears that high concentrations

of glutamate or ibotenate may inhibit macrocyclic lactone activity, whereas lower concentrations may enhance the effect. Our results further show that in *H. contortus*, glutamate and other analogs can alter the effects of IVM and MOX, possibly at the GluCl. It is also possible that ibotenate is enhancing IVM toxicity by mechanisms not directly related to the GluCl. This requires further examination.

It is also unknown whether endogenous glutamate alters the potency of IVM *in vivo*. In a recent study, Pemberton *et al.* [20] demonstrated that *in vivo* GluCls present on the pharynx of *C. elegans* were more sensitive to IVM compared to GluCls expressed in *Xenopus* oocytes and suggested that endogenous glutamate may enhance IVM activation at the GluCl. IVM does activate GluCls expressed in *Xenopus* oocytes directly [4–7,14] suggesting that the overall toxic effect of the drug is due to IVM alone. However, whether endogenous glutamate plays a role in IVM's anthelmintic effect is unknown.

We have shown that agonists known to activate GluCls enhance IVM and MOX binding to a GluCl subunit and enhance anthelmintic activity. Further knowledge regarding the mode of action of IVM in *H. contortus* may eventually assist in our understanding of the mechanism of IVM resistance.

Acknowledgments

This work was supported by grants from NSERC, Fonds FCAR, Fort Dodge Animal Health. S. Forrester is a recipient of a Post Graduate Scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC) and a McGill Major Fellowship. Constructive suggestions by two anonymous reviewers were helpful and appreciated.

References

[1] Campbell WC. Ivermectin and ambectin. New York: Springer-Verlag; 1989.
[2] Prichard RK. Anthelmintic resistance. Vet Parasitol 1994;54:259–68.
[3] Blackhall WJ, Pouliot JF, Prichard RK, Beech RN. *Haemonchus contortus*: selection at a glutamate-gated chloride channel gene in ivermectin- and moxidectin-selected strains. Exp Parasitol 1998;90: 42–8.
[4] Dent JA, Smith MM, Vassilatis DK, Avery L. The genetics of ivermectin resistance in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 2000;97:2674–9.
[5] Arena JP, Liu KK, Paress PS, Cully DF. Ivermectin-sensitive chloride currents induced by *Caenorhabditis elegans* RNA in *Xenopus* oocytes. Mol Pharmacol 1991;40:368–74.
[6] Arena JP, Liu KK, Paress PS, Schaeffer JM, Cully DF. Expression of a glutamate activated chloride current in *Xenopus* oocytes injected with *Caenorhabditis elegans* RNA: evidence for the modulation by ivermectin. Mol Brain Res 1992;15:339–48.
[7] Cully DF, Vassilatis DK, Liu KK, Paress PS, van der Ploeg LHT, Schaeffer JM, Arena JP. Cloning of an avermectin-sensitive glutamate-gated chloride channel from *Caenorhabditis elegans*. Nature 1994;371:707–11.
[8] Cheeseman CL, Delany NS, Woods DJ, Wolstenholme AJ. High-affinity ivermectin binding to recombinant subunits of the *Haemonchus contortus* glutamate-gated chloride channel. Mol Biochem Parasitol 2001;114:161–8.
[9] Forrester SG, Prichard RK, Beech RN. A glutamate-gated chloride channel from *Haemonchus contortus*: expression in a mammalian cell line, ligand binding and modulation of anthelmintic binding by glutamate. Biochem Pharm 2002;63:1061–8.
[10] Paiement J-P, Leger C, Ribeiro P, Prichard RK. *Haemonchus contortus*: effects of glutamate, ivermectin, and moxidectin on inulin uptake activity in unselected and ivermectin selected adults. Exp Parasitol 1999;92:193–8.
[11] Forrester SG, Hamdan FF, Prichard RK, Beech RN. Cloning, sequencing, and developmental expression levels of a novel glutamate-gated chloride channel homologue in the parasitic nematode *Haemonchus contortus*. Biochem Biophys Res Commun 1999;254:529–34.
[12] Cleland TA. Inhibitory glutamate receptor channels. Mol Neurobiol 1996;13:97–136.
[13] Unwin N. Neurotransmitter action: opening of ligand-gated ion channels. Cell 1993;72:31–4.
[14] Forrester SG, Dent JA, Prichard RK, Beech RN. *Haemonchus contortus*: *HcGluCla* expressed in *Xenopus* oocytes forms a glutamate-gated ion channel that is activated by ibotenate and the antiparasitic agent ivermectin. Mol Biochem Parasitol 2003;129:115–21.
[15] Schaeffer JM, Haines HW. Avermectin binding in *Caenorhabditis elegans*: a two-state model for the avermectin binding site. Biochem Pharm 1989;38:2329–38.
[16] Cully DF, Paress PS. Solublization and characterization of a high affinity ivermectin binding site from *Caenorhabditis elegans*. Mol Pharmacol 1991;40:326–32.
[17] Molento MB, Wang GT, Prichard RK. Decreased ivermectin and moxidectin sensitivity in Haemonchus contortus selected with moxidectin over 14 generations. Vet Parasitol 1999;86:77–81.
[18] Lanusse C, Lifschitz A, Virkel G, Alvarez L, Sanchez S, Sutra JF, Galtier P, Alvinere M. Comparative plasma disposition kinetics of ivermectin moxidectin and doramectin in cattle. J Vet Pharmacol Ther 1997;20:91–9.
[19] Shoop WL, Mrozik H, Fisher MH. Structure and activity of avermectins and milbemycins in animal health. Vet Parasitol 1995;59:139–56.
[20] Pemberton DJ, Franks CJ, Walker RJ, Holden-Dye L. Characterization of glutamate-gated chloride channels in the pharynx of wild-type and mutant *Caenorhabditis elegans* delineates the role of the subunit GluCl-alpha2 in the function of the native receptor. Mol Pharmacol 2001;59:1037–43.