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Characterization of Glutamate-Gated Chloride Channels in the Pharynx of Wild-Type and Mutant *Caenorhabditis elegans* Delineates the Role of the Subunit GluCl-α2 in the Function of the Native Receptor

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ABSTRACT

Glutamate-gated chloride (GluCl) channels are the site of action of the anthelmintic ivermectin. Previously, the *Xenopus laevis* oocyte expression system has been used to characterize GluCl channels cloned from *Caenorhabditis elegans*. However, information on the native, pharmacologically relevant receptors is lacking. Here, we have used a quantitative pharmacological approach and intracellular recording techniques of *C. elegans* pharynx to characterize them. The glutamate response was a rapidly desensitizing, reversible, chloride-dependent depolarization (EC_{50} = 166 μM), only weakly antagonized by picrotoxin. The order of potency of agonists was ibotenate > L-glutamate > kainate = quisqualate. Ivermectin potently and irreversibly depolarized the muscle (EC_{50} = 2.7 nM). No further depolarization was seen with coapplication of maximal glutamate during the maximal ivermectin response, indicating that ivermectin depolarizes the muscle by the same ionic mechanism as glutamate (i.e., chloride). The potency of ivermectin on the pharynx was greater than at any of the GluCl subunits expressed in *X. laevis* oocytes. This effect of ivermectin was abolished in the mutant *avr-15*, which lacks a functional GluCl-α2 subunit. However, a chloride-dependent, nondesensitizing response to glutamate persisted. Therefore, the GluCl-α2 subunit confers ivermectin sensitivity and a high-affinity desensitizing glutamate response on the native pharyngeal GluCl receptor.

The avermectins are a class of insecticides and anthelmintics that potently activate glutamate-gated chloride (GluCl) channels. The channels are apparently unique to the invertebrate phyla and play vital roles in animal function, thus accounting for the selective toxicity of these drugs. For example, Ivermectin (22,23-dihydroavermectin B1a) paralyzes both the somatic (Kass et al., 1980) and pharyngeal (Geary et al., 1993; Brownlee et al., 1997) musculature of nematodes with exceptional potency. The molecular identity of the target for ivermectin has been pursued in the nonparasitic nematode *Caenorhabditis elegans*, as a model genetic animal. This has led to the identification of a family of genes that encode subunits for glutamate-gated chloride channels (Cully et al., 1994, 1996; Dent et al., 1997, 2000; Laughton et al., 1997a; Vassilatis et al., 1997). The family consists of at least two classes of subunit, α and β, that may coassemble to form either homomeric or heteromeric ligand-gated chloride channels. The properties of some of these have been determined in the *Xenopus laevis* oocyte expression system (summarized in Table 1).

To date, only two of these genes have been shown to be expressed in *C. elegans* pharynx and may therefore contribute to the properties of the native channel that regulates feeding. *avr-15*, encodes for GluCl-α2, which in *X. laevis* oocytes forms a homomeric chloride-channel, gated by glutamate and high concentrations of ivermectin (10 μM). *ad1051* is proposed to be a null allele of *avr-15* (Dent et al., 1997). In the same study, behavioral analysis demonstrated that pharynxes dissected from these worms continue to pump in the presence of ivermectin and that the pharyngeal response to iontophoretic application of glutamate was abolished. The GluCl-β subunit is also present in pharynx (Laughton et al., 1997b). In *X. laevis* oocytes, this also forms a homomeric chloride-channel (Cully et al., 1994). However, in contrast to GluCl-α2, this channel is gated by glutamate, but not by ivermectin.

Despite this progress in identifying putative GluCl genes, the properties of the native receptors have not yet been determined, nor has the contribution of genes such as *avr-15* to receptor function been assessed. Therefore, we have used

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ABBREVIATIONS: GluCl, glutamate-gated chloride channel; PDC, *trans*-4-carboxy-L-proline/L-*trans*-pyrrolidine-2,4-dicarboxylic acid.

TABLE 1

A comparison of the pharmacological properties of GluCl channels.

The individual subunits, or *C. elegans* mRNA, were characterized in the *X. laevis* oocyte expression system. 'Pharynx' refers to the native pharyngeal GluCl channels. GBR-2A and GBR-2B have been named α3A and B by Dent et al. (2000).

	EC_{50} Glutamate	EC_{50} Ivermectin	Ibotenate Effect	IC_{50} Picrotoxin
α1	No effect at 1 mM[a,b]	140 nM[a,b] 970 nM[d,e]	N.D.	59 μM[c]
α2A	2 mM[f]	N.D.	Agonist[f]	N.D.
α2B	208 μM[g]	108 nM[b,g]	N.D.	>100 μM[g]
β	380 μM[a] 800 μM[d]	No effect at 1 μM[a,b]	N.D.	77 nM[c]
$\alpha1\beta$	1.36 mM[a] 1.8 mM[b]	190 nM[a,b] 500 nM[d,e]	Agonist[a]	42 μM[c] 18.5 μM[b]
$\alpha2\beta$	62 μM[g]	103 nM[b,g]	N.D.	>100 μ[g]
GLC3	1.9 mM[h]	>100 nM	Partial agonist	> 1 mM[h]
GBR2A	No response[i]	No response[i]		
GBR2B	No response to 10 mM[i]	Response to 10 μM[i]		
mRNA	300 μM[k]	90 nM[b,j]	4.5× More potent than glutamate[k]	180 μM[k]
Pharynx	166 μM[l]	2.7 nM[e,l]	10× More potent than glutamate[l]	>100 μM[l]

N.D., not determined.
[a] Cully et al. (1994).
[b] Using ivermectin phosphate.
[c] Etter et al. (1999).
[d] Bush (1998).
[e] Using ivermectin.
[f] Dent et al. (1997).
[g] Vassilatis et al. (1997).
[h] Horoszok et al. (1999).
[i] Dent et al. (2000).
[j] Arena et al. (1991).
[k] Arena et al. (1992).
[l] Current study.

intracellular recording techniques from the pharyngeal muscle for a quantitative pharmacological study of both wild-type and *avr-15* (*ad1051*), to enable a comparison with the properties of GluCl channel subunits expressed in *X. laevis* oocytes. This reveals that the native receptor has a higher affinity for ivermectin than at any of the GluCl subunit combinations tested so far in *X. laevis* oocytes and provides further insight into the subunit stoichiometry of native pharyngeal GluCl channels.

Materials and Methods

These studies were performed on semi-intact preparations of *C. elegans*, consisting of the pharynx, enteric nervous system, and nerve ring, in which the anterior region of the adult hermaphrodite was sectioned from the body at the level of the pharyngeal-intestinal valve, and placed in a perfusion chamber. Intracellular electrophysiological recordings were made from the terminal bulb region of the pharynx (Fig. 1). Stable recordings could be achieved from the muscle for up to 1 h.

Dissection Procedures. *C. elegans* (N2 Bristol strain) were cultured and adult hermaphrodite animals picked from 3- to 5-day-old plates. The worms were placed in saline (70 mM NaCl, 70 mM NaIsethionate; 6 mM KCl, 1 mM $MgCl_2$, 3 mM $CaCl_2$, 10 mM HEPES; 10 mM D-glucose, pH 7.4) and transiently cooled to immobilize them. The anterior region was sectioned from the rest of the body at the level of the pharyngeal intestinal valve and transferred to a custom-built, disposable perfusion chamber (volume, 500 μl) on a glass cover slip. After experiments with ivermectin the perfusion tubing and chamber were routinely replaced.

Electrophysiological Recordings. The recording chamber was mounted on an Axiovert inverted microsope (Carl Zeiss, Oberkochen, Germany) and perfused via gravity feed with saline at a rate of 5 ml/min. The preparation was secured by means of a suction electrode applied to the terminal bulb region of the pharynx and impaled with an aluminosilicate glass microelectrode (1.0-mm outer diameter; 60–80 MΩ, 4 M KAcetate, 10 mM KCl) connected to an Axoclamp 2B recording amplifier (Axon Instruments, Foster City, CA). The reference electrode was a silver chloride coated silver pellet in 3 M KCl connected to the recording chamber by an agar bridge. All drugs were applied by addition to the perfusate and rapid concentration changes were achieved by a method adapted from Slater et al. (1984); the duration of application was typically 30 s to allow maximal responses to develop. Antagonists were applied 3 min before and concurrently with agonists. Data were acquired and analyzed using pCLAMP 7 (Axon Instruments). A hard copy of the data, membrane potential, and spike frequency was obtained on a Gould chart recorder. The response that was measured was the maximum change in membrane potential from the baseline. In some experiments, drug-induced depolarization triggered spikes, especially during the early phase of the response. In these cases, the peak change in membrane potential was measured from the resting membrane potential before drug addition to the inflection point before spike initiation.

Analysis of Data. Concentration-response curves were fitted to the modified logistic equation (Prism Ver. 2, GraphPAD, San Diego, CA) to enable determination of EC_{50} values and maximal responses. Values are shown as either the mean with 95% confidence limits in brackets (for data pooled from n experiments) or the mean ± SEM. Statistical analyses were performed using either the paired or unpaired two-tailed Student's t test (as appropriate), with a significance level of $P < 0.05$.

Drugs and Supplies. *trans*-4-Carboxy-L-proline/L-*trans*-pyrrolidine-2,4-dicarboxylic acid (PDC), quisqualic acid, kainic acid, and ibotenic acid were purchased from Tocris Cookson (Bristol, UK). All other drugs and chemicals were obtained from Sigma (Poole, UK). The sodium salt of L-glutamate was used. N2, Bristol strain *C. elegans* were provided by the Sanger Center (Cambridge, UK) and the mutant strain *avr-15* (*ad501*) was provided by Joe Dent (McGill University, Montreal, Canada). Hydrophobic drugs were dissolved in ethanol and subsequently diluted in saline to a final ethanol concentration of 0.1%. This vehicle had no detectable affect on the properties of the pharyngeal muscle.

Results

Glutamate and Ivermectin Depolarized the Pharynx. The wild-type pharyngeal muscle resting membrane potential was −78 ± 1 mV (n = 16). Typically, cells generated action potentials at a frequency of 1 to 2 Hz (Fig. 1B) and these were coupled one-to-one with contractions of the terminal bulb. L-Glutamate elicited a concentration-dependent depolarization (Fig. 2A) associated with a decrease in the frequency and amplitude, and eventual cessation of, action potentials and terminal bulb contractions. A 'rebound' excitation was often observed during the washout of glutamate. Ivermectin reduced the amplitude of the pharyngeal action potentials (Fig. 2B; at 10 pM this reduction was 10 mV; $p <$ 0.05 by Student's paired t test; n = 7). This was accompanied by a slow and irreversible depolarization. Coapplication of glutamate (1 mM) during the maximal depolarization to ivermectin (1 μM) caused no further change in membrane potential (Fig. 2C; −39.3 ± 1.2 mV with ivermectin alone, and −38.0 ± 0.6 mV upon addition of glutamate; n = 3), consistent with the sharing of a common ionic mechanism by these agonists.

Quantitative Comparison of the Responses to Glutamate and Ivermectin. The response to glutamate was rapid and reversed completely on washing. In contrast, the effect of ivermectin was irreversible and the response continued to increase during the wash period, possibly because of the lipophilic nature of ivermectin and its propensity to accumulate in cell membranes. This complicated an accurate determination of an EC_{50} value. The response that was measured was the maximum depolarization from the original resting membrane potential within 1.5 min of application of a given concentration of ivermectin. Ivermectin was 5 orders of magnitude more potent than glutamate with an EC_{50}

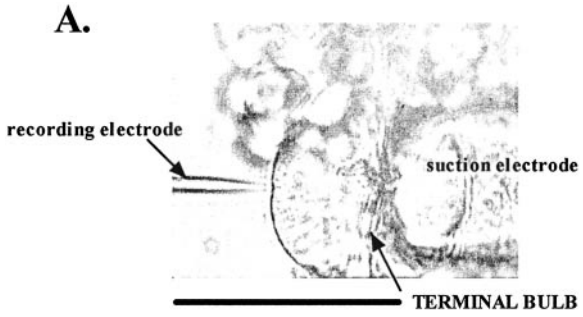

Fig. 1. The preparation for recording from *C. elegans* pharynx. A, the terminal bulb is secured with a suction electrode and impaled with an intracellular recording electrode. Scale bar is 25 μm. B, example of action potentials recorded from the terminal bulb. The resting membrane potential of this preparation was −78 mV.

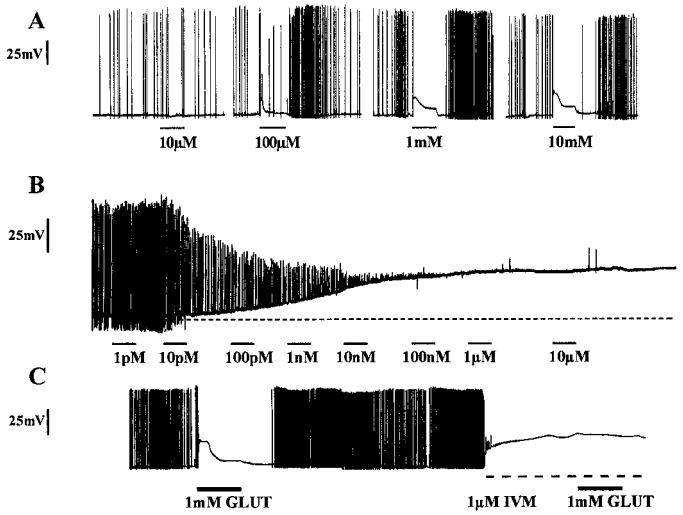

Fig. 2. The response of wild-type pharyngeal muscle to glutamate and ivermectin. A, the horizontal bars indicate the duration of application of glutamate (30 s). The resting membrane potential was −78 mV. B, the horizontal bars indicate the duration of application of ivermectin (30 s). The resting membrane potential was −75 mV. The dashed line shows the original baseline resting membrane potential from which the depolarizations were measured. C, the effect of coapplication of glutamate and ivermectin. The resting membrane potential was −79 mV. The dashed line indicates the continuous application of 1 μM ivermectin. GLUT, glutamate; IVM, ivermectin.

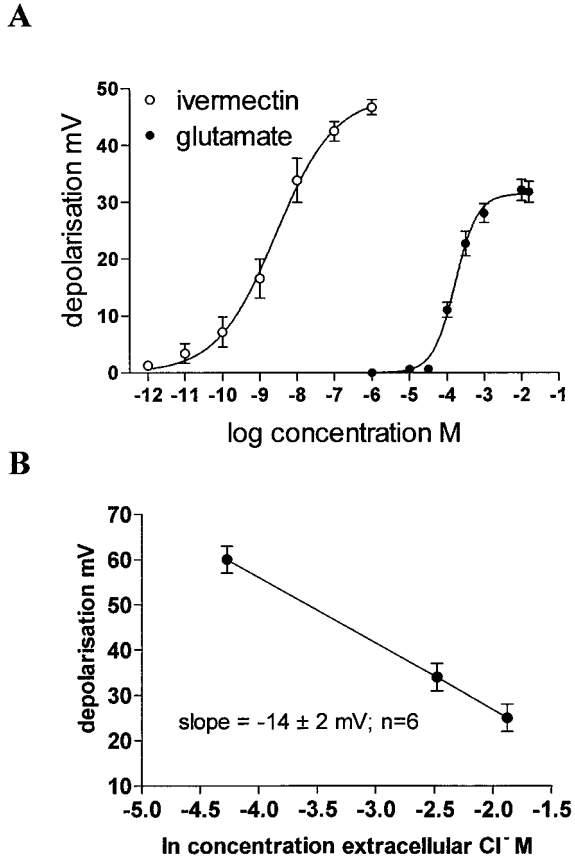

Fig. 3. A, the concentration-response relationships for glutamate and ivermectin. Each curve represents the pooled results from 16 and 8 different pharynxes, respectively, and each datum point is the mean ± SEM of at least 10 and 6 responses, respectively. B, the chloride-dependence of the glutamate response. The glutamate depolarization was inversely related to the logarithm of extracellular chloride concentration. Each datum point is the mean of six determinations. NaCl was replaced with NaIsethionate to alter extracellular chloride concentration. (The resting membrane potential did not change by more than 5 mV during the course of each experiment.)

value of 2.7 nM (1.2 to 5.8; $n = 8$; 95% confidence limits, 1.1–6.3 nM) compared with an EC_{50} value of 166 μM for glutamate (95% confidence limits, 132–207; $n = 16$; Fig. 3A). The maximal depolarization for ivermectin was greater than that for glutamate (49 mV; 95% confidence limits 42 to 55; $n = 8$, compared with 32 mV; 95% confidence limits 30 to 34; $n = 16$).

The Ionic Dependence of the Glutamate Response. The response to glutamate increased as the extracellular concentration of chloride was decreased (Fig. 3B.) For these experiments, NaCl was replaced by NaIsethionate to change the extracellular concentration of permeant anion. The replacement of extracellular chloride by isethionate resulted in a transient increase in action potential frequency but had no significant effect on resting membrane potential (for extracellular chloride concentration 154 mM, membrane potential was -77.5 ± 1.5 mV, $n = 14$; for extracellular chloride concentration 84 mM, membrane potential was -75.9 ± 1.1 mV, $n = 15$; for extracellular chloride concentration 14 mM, membrane potential was -80.4 ± 1.5 mV, $n = 8$). The glutamate depolarization was inversely related to the logarithm of the extracellular chloride concentration, consistent with the involvement of a GluCl channel (Fig. 3B).

The Pharmacology of the Glutamate Response. The order of potency of agonists was ibotenate > glutamate > quisqualate = kainate. These agonists (100 μM) elicited depolarizations of 23.2 ± 5.5 mV ($n = 6$; $p = 0.0006$ with respect to glutamate), 12.3 ± 1.5 mV ($n = 16$), 0.7 ± 0.4 mV ($n = 6$), and 0.5 ± 0.5 mV ($n = 4$), respectively. In a further series of experiments, the EC_{50} value for ibotenate was determined as 17.8 μM (Fig. 4A, B; 95% confidence limits 11.1 to 27.2 μM; $n = 10$) with a maximum depolarization of 36.5 mV (95% confidence limits 32.9 to 40.1 mV; $n = 10$). Thus, ibotenate is 10 times more potent than glutamate at eliciting a depolarization. The response to glutamate was only weakly blocked by picrotoxin (25% inhibition of 50 μM glutamate with 100 μM picrotoxin, $n = 5$). Flufenamic acid (100 μM) did not antagonize the response to 50 μM glutamate ($n = 5$).

Glutamate Receptor Desensitization. The response to glutamate and ibotenate rapidly desensitized (Figs. 2A and 4A). This was quantified by measuring the membrane potential before application of glutamate (a), the peak membrane potential (b), and the membrane potential immediately before the washout of glutamate (c). The desensitization was calculated by % desensitization $= [(b - a) - (c - a) / (b - a)] \times 100$. The desensitization was $80 \pm 6\%$ at 100 μM glutamate, $62 \pm 7\%$ at 1 mM glutamate, and $56 \pm 5\%$ at 10 mM glutamate (Fig. 5, A and B; $n = 10$).

Evidence for Tonic Glutamate Release. At concentrations above 100 μM, the glutamate uptake blocker PDC elicited a depolarization of pharyngeal muscle (300 μM PDC, 5.7 ± 1.5 mV). The effect of glutamate and PDC together was more than additive, suggesting that PDC may be synergistic with glutamate (100 μM glutamate, 10.5 ± 1.6 mV; 100 μM glutamate and 300 μM PDC, 22.0 ± 2.6 mV; $n = 6$; Fig. 6).

GluCl-α2 Contributes to the Function of the Native GluCl Channel. *avr15* (*ad1051*) is a putative null mutation for the GluCl channel subunit GluCl-α2. The resting membrane potentials of this mutant strain were indistinguishable from wild-type (-78.9 ± 1.4 mV; $n = 12$), as were the frequency and shape of the pharyngeal action potentials (Fig. 7A). However, the potency of both glutamate and ibotenate

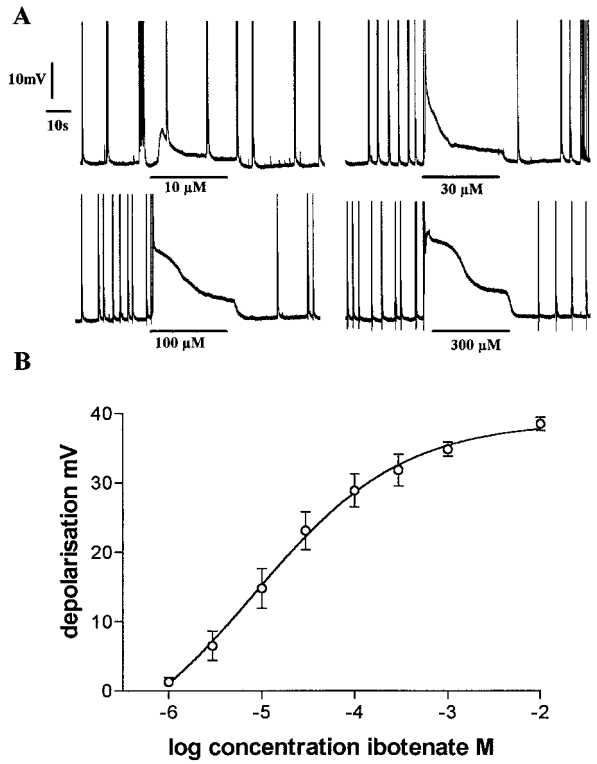

Fig. 4. The response of the pharyngeal muscle to ibotenate. A, consecutive recordings from the same cell; resting membrane potential, −80 mV. (The peak of the action potentials is truncated in these traces.) The bars indicate the duration of application of ibotenate at the concentrations indicated. B, concentration-response curves for ibotenate from 10 preparations; each point is the mean ± SEM of at least six determinations.

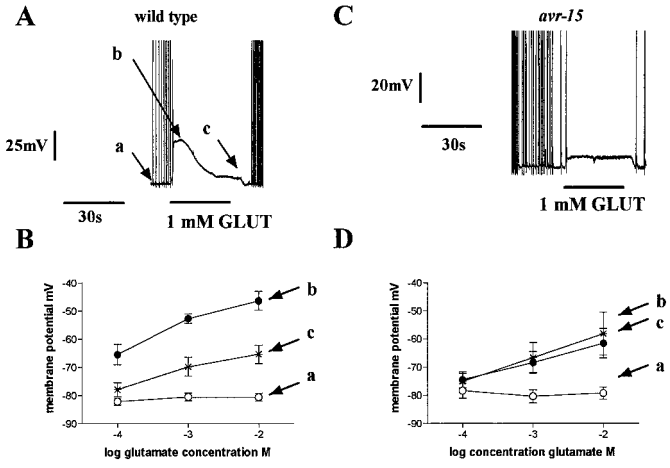

Fig. 5. Analysis of desensitization to glutamate. A, examples of a response to 1 mM glutamate (GLUT) in wild-type. The horizontal bar indicates the duration of application of glutamate. Resting membrane potential was −82 mV. Desensitization was determined by measuring the membrane potential at three points as indicated by the arrows on the response namely, at rest (a), at the peak of the response (b), and immediately before the wash period (c). B, representation of measurements taken from glutamate responses as described in A. $n = 10$. Data points are mean ± SEM. C, analysis of desensitization to glutamate in *avr-15* (*ad1051*). Examples of responses to 1 mM glutamate (glut) in *avr-15*. The horizontal bar indicates the duration of application of glutamate. Resting membrane potential was −80 mV. Desensitization was determined by measuring the membrane potential at rest (a), within 5 s of glutamate application (b), and immediately before the wash period (c). D, representation of measurements taken from glutamate responses as described in C. $n = 5$. Data points are mean ± SEM.

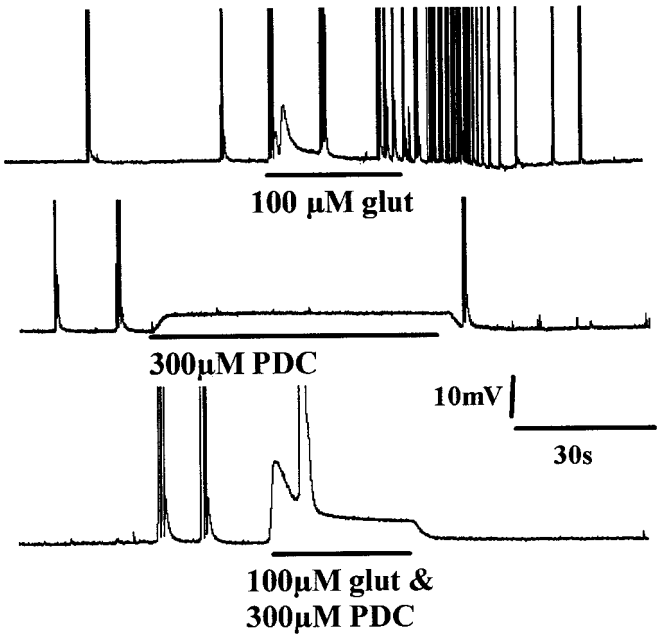

Fig. 6. The effect of the glutamate uptake blocker, PDC, on the response to glutamate. The resting membrane potential of this cell was −80 mV. (The peaks of the action potentials are truncated in these traces.) The horizontal bars indicate the duration of application of glutamate (glut) and PDC.

was reduced (Fig. 7, B and C). The EC_{50} value for glutamate was 1.15 mM for *avr-15* (95% confidence limits, 0.52 to 2.55 mM; $n = 12$; Fig. 7D). Thus, glutamate was 10 times less potent than wild-type ($p < 0.001$ at 1 mM glutamate). The maximum depolarization was not significantly different from wild-type. As in the wild-type, the response to glutamate was chloride-dependent (depolarization to 1 mM glutamate was 12.1± 1.4 mV in 154 mM chloride compared with 20.9 ± 3.2 mV in 14 mM chloride, $n = 7$). The response to glutamate was not blocked by 100 µM picrotoxin ($n = 2$), nor was it decreased in the presence of 1 mM cobalt (response to 100 µM glutamate was 9.0 ± 1.5 mV compared with 9.0 ± 2.5 mV in the presence of 1 mM cobalt; $n = 4$, mean ± SEM). This latter observation, indicates that the response is mediated by a direct effect of glutamate on the muscle and does not have an indirect, synaptically mediated action on the mutant strain. The potency of ibotenate was also reduced in *avr-15*. The EC_{50} value was 80.5 µM (95% confidence limits, 56.9 to 113.9 µM; i.e., four times less potent than in wild-type; $n = 9$; Fig. 7C, E).

The response to glutamate in *avr-15* did not desensitize at either the lowest or maximally effective concentrations of glutamate (Fig. 5, C and D). Note that the response to ibotenate also did not desensitize in *avr-15* (Fig. 7C). Ivermectin failed to elicit any response in *avr-15*. Concentrations between 1 nM and 10 µM were tested ($n = 4$).

Discussion

The physiological role of GluCl channels in the pharynx is to mediate the action of glutamate released from the pharyngeal motoneuron M3. Indirect evidence suggests that M3 is activated near the peak of the pharyngeal action potential to release glutamate and facilitate rapid relaxation of the pharynx (Avery, 1993). The effect of exogenous glutamate is an inhibition of pharyngeal pumping, which is mimicked by ivermectin. In this study, glutamate and ivermectin inhibited the pharynx by eliciting a chloride-dependent, depolarizing block. This is contrary to the previously assumed hyperpolarizing action of ivermectin (Dent et al., 2000). The depolarizing action of glutamate indicates that the equilibrium potential for chloride (E_{Cl}) is more positive than the membrane potential. If it is assumed that the maximum glutamate response approaches E_{Cl}, then an estimate of E_{Cl} is in the region of −40 mV. From a physiological viewpoint, the most important point to note is that, even in the highest extracellular chloride (154 mM), opening of GluCl channels elicits a depolarization. Therefore, E_{Cl} is more positive than the membrane potential and must be actively maintained by an inwardly directed chloride pump. Reducing extracellular chloride had only a transient effect on the resting membrane potential, indicating that the resting membrane has a low resting conductance to chloride. This is the reverse of the situation for nematode somatic body wall muscle, which has a high resting chloride conductance, and E_{Cl} is more negative than membrane potential, so that opening chloride channels causes a hyperpolarization (Del Castillo et al., 1964; Parri et al., 1991). The properties of the pharyngeal chloride pump remain to be determined, but it is likely to be a very important regulator of muscle function.

The response to glutamate was rapid in onset and completely reversible, whereas the ivermectin depolarization did not reverse. Furthermore, the maximum depolarization elicited by ivermectin was greater than that for glutamate. This could not be explained by a difference in the resting membrane potentials of the pharynxes in these two sets of experiments, and may possibly be interpreted as indicating some difference in ionic mechanism between these two agonists. To test this, glutamate was applied to the muscle during the maximal ivermectin depolarization, and no further change in membrane potential was observed. Therefore, it seems most likely that the ionic mechanism for both the glutamate and ivermectin response is the same (i.e., chloride). Differences in E_{Cl}, or, more likely, an underestimate of the glutamate maximum response because of rapid receptor desensitization, therefore, could explain the difference in the maximum response between these two agonists.

Ivermectin was exceptionally potent on the pharynx, more so than at any of the GluCl subunit combinations tested to date in *X. laevis* oocytes. For example, the threshold for the effect in this study was 10 pM, compared with between 10 and 100 nM at GluCl-α1 and GluCl-α2B subunits expressed in *X. laevis* oocytes (Cully et al., 1994; Vassilatis et al., 1997). It may be that endogenous glutamate potentiates the action of ivermectin, in much the same way that ivermectin acts as a positive allosteric modulator of both *C. elegans* mRNA and the GluCl-α1:GluCl-β heteromer expressed in *X. laevis* oocytes (Arena et al., 1992; Cully et al., 1994). The observation that the glutamate uptake blocker depolarized the muscle provides evidence for tonic glutamate release in the preparation, which would be required for this to occur. In this respect, it would be interesting to test whether glutamate uptake blockers can potentiate the action of ivermectin. Alternatively, it may be that the native receptors are intrinsically more sensitive to ivermectin than at GluCl subunits expressed in *X. laevis* oocytes. Certainly, binding assays with ivermectin on *C. elegans* membranes suggest that the latter

may be the case, because the K_d value for ivermectin has been estimated as low as 3 pM (Dent et al., 2000). From this it may be deduced that the stoichiometry of the native channel comprises hitherto uncharacterized subunits or subunit combinations.

The action of agonists on the pharynx was typical for that of other invertebrate glutamate-gated chloride channels. Qualitatively, the response to ibotenate was similar to that for glutamate but it was 10 times more potent than glutamate. Quisqualate and kainate were both weak agonists. This is similar to the profile of GluCl-α1:GluCl-β heteromer expressed in *X. laevis* oocytes. Picrotoxin and flufenamic acid are chloride channel blockers that have weak blocking action on the GluCl-α1:GluCl-β heteromer (Cully et al., 1994). In the pharynx, these antagonists were weaker still: flufenamic acid was ineffective and, for picrotoxin, only a partial block of the response was observed at 100 μM.

Further insight into the role of the subunit GluCl-α2 in pharyngeal GluCl receptor function is provided by the results of recordings from the putative null mutant *avr-15* (*ad1051*). Previously, behavioral analysis of *avr-15 (ad1051)* had demonstrated that dissected pharynxes from these worms are resistant to the effects of ivermectin and glutamate (Dent et al., 1997). We found also that ivermectin did not have any effect on membrane potential or pharyngeal action potentials in *avr-15*, although the resting membrane potential and action potentials were indistinguishable from wild-type. However, glutamate did elicit a response, but at higher concentrations than in wild-type. This raises the questions: does the residual response still involve GluCl? If so, which GluCl subunits might be involved? The response to glutamate in *avr-15* was still chloride-dependent and there was a similar decrease in affinity for ibotenate, suggesting that GluCl channels are involved. Furthermore, the GluCl channel subunit, GluCl-β, is known to be expressed in the pharynx (Laughton et al., 1997b), and this subunit forms functional, homomeric, glutamate-gated channels when expressed in *X. laevis* oocytes (Cully et al., 1994). Therefore it is possible that the glutamate response in *avr-15* was caused by activation of a GluCl-β homomer, but two observations argue against this. First, the glutamate response in *avr-15* had a lower affinity for glutamate than would be expected if a GluCl-β homomer was involved in mediating the response (Cully et al., 1994). Second, the response was not blocked by 100 μM picrotoxin, whereas GluCl-β would be predicted to be blocked by nanomolar concentrations of picrotoxin, based on the pharmacology of this subunit when expressed as a homomer in *X. laevis* oocytes (Etter et al., 1999). Two possible interpretations of these data are that either the GluCl-β does not have any role in mediating this response or it is coassembled with another, as-yet-unidentified subunit, which alters its properties. The latter explanation seems most likely in view of the reported expression of GluCl-β in the pharynx. The pharyngeal GluCl receptor may therefore be a hetero-oligomer comprising GluCl-α2, GluCl-β. and at least one other isoform of GluCl subunit. This subunit would be predicted to be ivermectin-insensitive, unlike all the GluCl subunits characterized in oocytes so far (with the exception of GluCl-β). However, one putative GluCl gene, C27H5.8, remains to be pharmacologically characterized; therefore, the possible contribution of this to the pharyngeal receptor should be considered.

The response to glutamate and ibotenate rapidly desensitized. This was quantified for glutamate, and the greatest desensitization occurred at the lowest glutamate concentration. This argues against the possibility that this desensitization is caused by a change in the ionic gradient (e.g., for chloride) as a consequence of the opening of GluCl channels, because in this case, it would be expected that the greatest desensitization would occur at the maximally effective glutamate concentration. It is more likely to reflect an intrinsic mechanism for receptor regulation. The lack of desensitization in *avr-15* suggests a pivotal role for GluCl-α2 in this process. However, this observation does not shed any further light on which subunits are likely to be assembled in the mutant to form the low affinity receptor. In *X. laevis* oocytes, all of the subunits characterized to date (see Table 1) have exhibited receptor desensitization as homomers. The GluCl-α1; β heteromer does not seem to desensitize (Cully et al.,

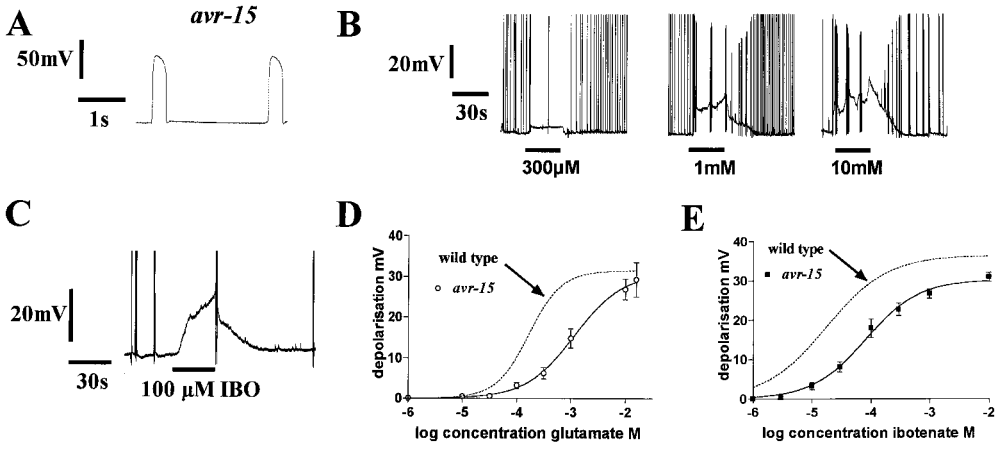

Fig. 7. Characterization of *avr-15* (*ad1051*). A, pharyngeal action potentials recorded from *avr-15*. Resting membrane potential was −78 mV. B, responses of *avr-15* to glutamate. The bar indicates the duration of application of glutamate. Resting membrane potential was −83 mV. C, the response of *avr-15* to ibotenate (IBO). The bar indicates the duration of application of ibotenate. The resting membrane potential of this cell was −85 mV. D, concentration-response curve for the response of *avr-15* to glutamate. The wild-type curve is shown as a dashed line for comparison. The curve represents the pooled results from 12 different pharynxes and each datum point is the mean ± SEM of at least eight responses. The concentration-response curve was significantly shifted to the right compared with wild-type (e.g., at 300 μM glutamate, $p < 0.0001$). E, concentration-response curve for the response of *avr-15* to ibotenate. The wild-type curve is shown as a dashed line. The curve represents the pooled results from eight pharynxes and each point is the mean ± SEM of at least five determinations.

1994), but it is unlikely that this is the 'residual' receptor in *avr-15* pharynx, because this would be expected to respond to ivermectin.

In conclusion, because the responses to glutamate, ibotenate, and ivermectin were affected by a mutation in GluCl-α2, all these agonists interact with the same population of native channels, to depolarize the pharynx and inhibit the activity of the muscle. However, whereas this subunit is essential for the effect of ivermectin, it only modulates the response to glutamate, conferring high affinity, and desensitization. The persistence of a reduced glutamate response in *avr-15* shows that the native channel must be a heteromer. The data presented here indirectly suggest it is most likely to consist of three isoforms of GluCl subunit. This receptor has a low nanomolar affinity for ivermectin. Notably, this affinity is much greater than that determined from expression of GluCl subunits in the *Xenopus* oocyte expression system. These observations will inform future studies on the GluCl subunit family aimed at resolving the stoichiometry of a therapeutically relevant receptor.

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