Glutamate-gated Chloride Channels*  
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Glutamate-gated chloride channels (GluCls) are found only in protostome invertebrate phyla but are closely related to mammalian glycine receptors. They have a number of roles in these animals, controlling locomotion and feeding and mediating sensory inputs into behavior. In nematodes and arthropods, they are targeted by the macrocyclic lactone family of anthelmintics and pesticides, making the GluCls of considerable medical and economic importance. Recently, the three-dimensional structure of a GluCl was solved, the first for any eukaryotic ligand-gated anion channel, revealing a macrocyclic lactone-binding site between the channel domains of adjacent subunits. This minireview will highlight some unique features of the GluCls and illustrate their contribution to our knowledge of the entire Cys loop ligand-gated ion channel superfamily.

Despite their relative anatomical simplicity, some aspects of the neurochemistry of invertebrate nervous systems are more complex than those of vertebrate nervous systems. One example is the expanded range of transmitters that act via members of the Cys loop ligand-gated chloride channel (CysLGCC) family. In addition to the main topic of this minireview (glutamate), invertebrate CysLGCC can be gated by compounds such as histamine (1–3), serotonin (4), dopamine (5), and tyramine (6), and unlike vertebrates, acetylcholine and GABA act at both anion- and cation-gated channels (7, 8). In addition, an insect chloride channel (pHCl) inhibited by protons is a member of the CysLGCC family (9). This increased variety of ligands may be exploitable in our continuing attempts to better understand the structure-function relationships that are conserved throughout the CysLGCC family. It has also allowed them to be explored as targets for the control of agricultural pests as well as medical and veterinary pathogens.

Inhibitory glutamate-gated chloride channels (GluCls) were first described as extrajunctional receptors (the “H-receptors”) on locust muscle (10, 11) and then as postsynaptic receptors within the crustacean stomatogastric ganglion (12–14). Further studies showed that similar receptors and channels are expressed on neurons and muscle across protostome phyla, including mollusks, flatworms, roundworms (nematodes), ticks, and mites, as well as insects and crustaceans, and this has been confirmed by recent genomic and transcriptomic studies. For example, the transcriptome of the cestode *Taenia pisiformis* contains sequences similar to nematode GluCls even though the presence of GluCls has not been reported in tapeworms (15). The properties of many of these receptors were excellently summarized by Cleland (16). However, GluCls have not been found in other invertebrate taxa such as cnidarians and echinoderms.

Exploitation of GluCls

Some invertebrate species are pathogenic to mammals, including humans, and others act as disease vectors or are considered to be agricultural or domestic pests. A major stimulus to studies of invertebrate nervous systems is our desire to kill these pathogens and pests, and ion channels are the targets of many effective parasiticides and pesticides. The macrocyclic lactone (ML) group of anthelmintics, insecticides, and acaricides acts at GluCls, either activating the channels directly or potentiating their responses to glutamate (17, 18). Worldwide sales of these compounds are worth billions of dollars annually, and hundreds of millions of doses of ivermectin are given to people every year as part of the control and elimination programs for onchocerciasis and lymphatic filariasis (19, 20). Insect GluCls are also partly responsible for the insecticidal activity of fipronil, as this compound blocks these channels in addition to its action at GABA receptors (21, 22). Histamine-gated chloride channels (HisCls) are also affected by the application of MLs and may contribute to their insecticidal activity (2, 23, 24).

The finding that the avermectins, one of the major families of ML anthelmintics, insecticides, and acaricides (25), modulate the activity of GluCls from the nematode *Caenorhabditis elegans* (26) led to the isolation of two cDNAs encoding channel subunits from this organism (17). The sequences of the two subunits, then called GluClα and GluClβ, but now referred to as GLC-1 and GLC-2, clearly showed that they belonged to the CysLGCC family, confirming earlier pharmacological and physiological evidence such as their sensitivity to the chloride channel blocker picrotoxin (16). Further genetic and molecular studies have since expanded the *C. elegans* GluCl gene family to six members: *avr-14* (altered avermectin sensitivity), *avr-15*, *glc-1* (glutamate-gated chloride channel), *glc-2*, *glc-3*, and *glc-4* (17, 27–31). These studies have been extended to other nematode species, especially the animal and human parasites, and have revealed that the size and composition of the GluCl family vary between species, with *avr-14* and *glc-2* orthologs present in all genomes studied to date (32–34). A single GluCl gene, GluClα, is present in most insects, although its transcripts are extensively modified by mRNA splicing and editing (35–37), along with two genes encoding HisCl subunits and a single pHCl gene (36–38). In *Drosophila melanogaster*, GluClα mediates sensitivity to ivermectin and nodulisporic acid (18, 39), suggesting that the avermectin drug target is the same throughout the Ecdysozoa. The aphid *Acrithosiphon pisum* has two pHCl genes and only a single HisCl gene (40). Two distinct GluCl currents have been detected in cockroach thoracic gan-
glion pyriform neurons, one desensitizing and the other not. Pharmacologically, they are distinguished by the effects of the chloride channel blockers fipronil and picrotoxinin (21, 41), but the genetic basis of the two channels is unknown. Two GluCl genes have been described in the mollusk *Aplysia californica* (42), six in the mite *Lepeophtheirus salmonis* (44), and one in the sea ouse *Lepeopethirinus salmonis* (44).

The effects of the MLs on GluCls have been extensively studied, often following expression of cloned channel cDNAs in the *Xenopus* oocyte. The MLs may directly open the ion channels, or they may potentiate the effects of submaximal concentrations of the normal agonist (17). Direct activation of the channels is much slower than that seen with classical transmitters, but once open, the channels remain in this state for a very long time, essentially irreversibly in the time frame of electrophysiological recordings. The influx of chloride ions normally results in a hyperpolarization of the neuron or muscle, and the long-term change in the membrane potential of the cell means that it is essentially silenced.

Because vertebrates do not possess GluCls, there have been attempts to develop them as tools for the specific silencing of defined neurons by adding low concentrations of ivermectin (21, 41), but the genetic basis of the two channels is unknown. Two GluCl genes have been described in the mollusk *Aplysia californica* (42), six in the mite *Lepeophtheirus salmonis* (44), and one in the sea ouse *Lepeopethirinus salmonis* (44).

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Because vertebrates do not possess GluCls, there have been attempts to develop them as tools for the specific silencing of defined neurons by adding low concentrations of ivermectin (45). Expression of the *C. elegans* GLC-1 and GLC-2 subunits in mammalian neurons using recombinant virus vectors results in the formation of functional GluCls in mammalian neurons, which can then be selectively silenced by injecting ivermectin. The subunits have been modified to encode GFP as a marker which can then be selectively silenced by injecting ivermectin. Such methods have been used in the identification of an aggression locus in the mammalian hypothalamus by injecting two adeno-associated virus vectors encoding the modified GLC-1 and GLC-2 into murine neurons of the ventrolateral subdivision of the ventro-medial hypothalamus (47). A single intraperitoneal injection of 10 mg/kg is sufficient to electrically silence the infected neurons (48) and reduce the level of intermale aggression.

**Evolutionary Relationships**

The GluCls, along with the HisCl, pHCl, and the vertebrate glycine receptors, belong to the subfamily of CysLGCC that possess two Cys loops in the N-terminal extracellular domain (42, 49) and are thus distinct from the GABA-gated channels present throughout the vertebrates and invertebrates. The close evolutionary relationship between GluCls and glycine receptors may be mirrored in the apparent ability of glutamate to potentiate glycine receptor currents (50). One obvious possibility was that the GluCls are the invertebrate orthologs of the glycine receptors, but a more detailed phylogenetic analysis (42), together with the discovery of genuine glycine transmission and receptors in cnidarians (51, 52), has revealed the situation to be more complex. The GluCls seem to have evolved twice, as the lophotrocozan clade is completely distinct from and independent of the GluCls from Ecdysozoa, so either convergent evolution has resulted in the appearance of a glutamate-binding site in the two families or there is a very deep-rooted ancestral GluCl sequence that may have been lost during the evolution of the cnidarians and deuterostomes (42). An alignment of the amino acid sequences of the loops that make up the ligand-binding site in the nematode, insect, and mollusk GluCls (Fig. 1) shows that although many of the residues are conserved, there are significant differences in loops C and F between the *Aplysia* sequences and those from nematodes and insects, and indeed, it was difficult to get a good alignment of loop F.

The recent rapid expansion of genome sequence information allows the comparison of the GluCl gene families between organisms, especially in insects and nematodes. The insect gene family is relatively simple, but that found in nematodes tends to be larger and more diverse, although there is quite a lot of variation between species, with a greater complexity in *C. elegans* and other species in clade V than in the species in clades I and III (26).

**Functions**

GluCls have a wide range of functions in invertebrate nervous systems, but these can be broadly divided into three interlinked categories: the control and modulation of locomotion, the regulation of feeding, and the mediation of sensory inputs. Treatment of nematodes with ivermectin also has effects on reproductive and fecundity, implying a role for GluCls or closely related channels in these processes, although this has yet to be directly demonstrated. Probably the best studied GluCl functions are those in nematodes, where the channels are widely expressed in sensory neurons, interneurons, and motor neurons, modulating a considerable number of different behaviors. For example, *avr-14* is expressed in a large number of *C. elegans* motor neurons and interneurons (31, 54), and mutations in this and the other GluCl genes (*avr-15, glc-1*, and *glc-3*) affect the foraging behavior of the worm (30, 54). Mutations in

**FIGURE 1. Transmitter-binding site residues of invertebrate 2-Cys loop ligand-gated anion channels.** The binding loops are shown, as described (87), for examples of nematode and insect GluCls, insect HisCls and pHCl, and mollusk GluCls. *Cel*, C. elegans; *Hco*, H. contortus; *Dmel*, D. melanogaster; *Aca*, A. californica. Amino acid residues that make contacts with bound glutamate in the GluCl structure (87) are highlighted in red; where these are substituted in other channels, they are colored purple. The alignment was made using ClustalW.

| Loop A | Loop B | Loop C | Loop D | Loop E | Loop F | ‘Loop G’ |
|--------|--------|--------|--------|--------|--------|---------|
| Cys1GLC-1 | PDSTFPN | ASYAY | CTST--INTGYYC | LTLRESW | LYSVR | QKLNGLS | LR1T |
| Cys1GLC-3 | PDSFFPN | ASYAY | CTST--INTGYYC | LTFREZW | LYSVR | QKLNGLS | IBS |
| Hco-AYR-14 | PDSTFPN | ASYAY | CTST--INTGYYC | LFTRAZW | LYSVR | QKLNGLS | LRS |
| Dmel-GlcK | PGLIFSN | ASYW | CNSE--INTGYYC | LFTRQW | LTSRI | QWQDRL | LV5S |
| Dmel-HCL | PDSFFN | ASYW | CTST--INTGYYC | VFFAQW | LYSVR | QKLNGLS | LWG |
| Dmel-HCLB | PDGFPN | ASYW | CTST--INTGYYC | IFLAGW | LTSRI | QWQDRL | LV5S |
| Dmel-pHCL | PDSTFPN | ASYAY | CTSE--INTGYYC | HLSWQQ | TANSR | LRSPLS | LLS |
| Aca-Glc1 | PVDFPN | QAAY | CNWREWTVTPFD | LYLWNT | KYSNR | DAETALT | LS |
| Aca-Glc2 | PDDFWN | QAYG | CTTW-INTGYYC | ILYQW | YTSNR | DYNGBNL | VLS |
or RNAi of avr-15, avr-15, or glc-1 increases the frequency with which the worms change direction, whereas knockdown of glc-3 expression produces the opposite effect. The expression of GFP under the control of the avr-14 promoter suggests that this gene is also expressed on a number of sensory neurons (53). AVR-15 is also widely expressed in the nervous system but, in addition, is found in muscle cells of the pharynx (29, 55) along with GLC-2 (56). Mutations in avr-15 affect gustatory plasticity (57) and, along with avr-14, mediate mechanosensory inhibition of pharyngeal pumping (58). GLC-3 is expressed in the AIY interneuron, where it receives inputs from the dorsal clock neurons (DNiS) and hence, the worm will tend to turn away. B, in Drosophila, GluCls is expressed on the LNvS neurons (75), which mediate light avoidance behavior. This is driven by the light-induced release of acetylcholine (ACh) from the visual system. Early in the day, the dorsal clock neurons (DNiS) do not release glutamate, and acetylcholine drives light avoidance behavior. At dusk, increased clock gene activity results in the release of glutamate from DNiS, which activates GluCls on LNvS and inhibits light avoidance.

The role of the GluCls in controlling nematode pharyngeal pumping, which is required both for feeding and for maintaining the hydrostatic pressure of the worms, is relatively well understood. The glutamatergic motor neuron M3 facilitates rapid relaxation of the pharyngeal muscle (61, 62) via a chloride-dependent hyperpolarization (55), and this response is mediated by an AVR-15-containing GluCl (29, 55) expressed on the pharyngeal muscle cells. GLC-2, and possibly an additional subunit, may also be involved (56).

At present, we do not have enough information to know whether or not the multiple GluCl functions described in C. elegans are also present in other nematode species, although the inhibition of both locomotion and pharyngeal pumping seen in these worms after treatment with ML anthelmintics suggests that these two roles are widely conserved (63, 64). GLC-3 is present in at least some parasitic nematode species (32), suggesting that its role in mediating sensory processes may be conserved, and AVR-14 is also expressed in Haemonchus contortus (a gastrointestinal parasite of small ruminants) sensory neurons (65). Recently, an additional role for AVR-14 was described in the human parasitic nematode Brugia malayi; the subunit is expressed around the excretory-secretory pore of the larval microfilariae and may play a role in regulating the secretion of proteins from this structure (66). The presence of GluCls on the pharynx of parasitic nematodes is also well established, especially for Ascaris suum (67–70), and there is some evidence for the expression of GluCl subunits on pharyngeal neurons (65, 71).

Some of the earliest descriptions of GluCls were from insect muscle cells (10, 11, 16); these are extrasynaptic and are found on both glutamatergic and cholinergic muscles. GluCls are also expressed in insect neurons, including the dorsal unpaired median neurons of the locust and cockroach (72–74), along with the GABA receptor Rdl. Because these neurons innervate flight and leg muscles, GluCls are likely to influence flight and walking control in these insects. In D. melanogaster, GluCls are expressed are high levels in the central nervous system, including the larval lateral neurons, where they mediate rhythmic light avoidance behaviors (75), and on large ventrolateral neurons (LNvS) in the adult brain that control rest and arousal, where they receive inputs from the dorsal clock neurons (DNiS) (76). The proposed pathway by which GluCls inhibit fly light avoidance behavior is shown in Fig. 2B. Interestingly, ivermectin reduced the feeding of human head lice that hatched from treated eggs; this effect was independent of its lethal effects on the lice and may imply a role for GluCls in regulating feeding (77).

Histamine is the major transmitter released by arthropod photoreceptors, and HisCls containing the HCLA subunit are expressed on large laminar monopolar neurons in the insect eye (78, 79), mediating the hyperpolarization of these cells. In the eye, HCLB expression is confined to laminal glial cells (79). HisCls therefore play an important role in insect vision. Mutations in genes involved in histaminergic signaling, including those encoding HisCls, also affect temperature preference in the fly (80). Mutations in hciB affect not only Drosophila vision (24) but also responses to high temperature and anesthesia, suggesting a central role in the nervous system (81). In crayfish,
Histamine induces a chloride current in the X-organ, the main neurosecretory structure (82), and could modulate multiple physiological functions via the regulation of hormonal secretion.

**Structure**

Even though the first molecular cloning of *C. elegans* GluCl cDNAs resulted in the expression of a multimeric receptor, GLC-1 + GLC-2 (17), the majority of the GluCls that have been successfully expressed *in vitro* to date have been monomeric (18, 28–31, 83–85). Whether this reflects the subunit composition of native GluCls in those species that possess multiple subunit genes is unknown, although overlapping expression patterns and the phenotype of individual gene mutations have led to suggestions that AVR-15 and GLC-2, plus at least one other subunit, co-assemble in the *C. elegans* pharynx (55) and that GLC-5 and GLC-2 might form a receptor on the commissures of inhibitory motor neurons in the parasite *H. contortus* (65), but there is no formal proof for either of these possibilities. A report that *Drosophila* GluCl and Rdl might co-assemble to form a functional receptor, based on co-immunoprecipitation studies (86), has not been confirmed and may reflect the expression of both GluCl and the GABA receptor on the same neurons (73). Given the uncertainty about the subunit composition of native GluCl, it is no surprise that we have almost no idea of the subunit stoichiometry of heteromeric receptors, whether these are native or reconstituted.

The fact that it is easy to express monomeric GluCl *in vitro* has led to a major breakthrough, the first three-dimensional structure of a eukaryotic ligand-gated ion channel, *C. elegans* GLC-1 complexed with ivermectin (87). This has revealed the detailed structure of both the glutamate- and ivermectin-binding sites. The former is in the extracellular domain of the receptor in a similar position to that of the nicotinic acetylcholine-binding sites and other members of the CysLGCC family (88), lying between adjacent subunits. The six loops that form the agonist-binding site are structurally homologous to those found in the acetylcholine-binding protein and prokaryotic channels. The residues that play a role in glutamate binding are highlighted in red in Fig. 1, which shows that they are conserved in other nematode and insect GluCls, but not always in HisCl or pHCl, in which, not surprisingly, the basic residues that confer a strongly positive electrostatic potential (Arg-37, Arg-56, and Lys-171) are not present (Fig. 1). Interestingly, these residues are also poorly conserved in the *Aplysia* GluCl sequences.

Glutamate binds to the homomeric GLC-1 receptor only after ivermectin has already bound, in contrast to almost all other GluCls, where the ligand can bind and activate the channel in the absence of the drug. The ivermectin-binding site is in the channel domain, lying between M3 and M1 of two adjacent subunits, with the drug making contacts with M2, which lines the ion channel, and the M2-M3 loop (Fig. 3). The structure confirmed the predictions of site-directed mutagenesis experi-
ments that indicated an overlap between the ivermectin-bind-
ing site and that of volatile anesthetics and other drugs that act
at mammalian GABA\textsubscript{A} and glycine receptors (89, 90). This
structure is therefore of great importance to mammalian neu-
roscience and medicine. Ivermectin binding to this site may
alter the conformation of the glutamate-binding site via its
interactions with the M2-M3 loop, which could then transmit
allosteric changes in structure, and the interactions with resi-
dues in M2 may help to keep the channel in the long-lasting
open configuration characteristic of these drugs (Fig. 3). In Fig.
3, all of the potential binding sites are shown as being occupied;
it is not known how many bound ivermectin molecules are
required to directly open the channel or to potentiate the glu-
tamate response, although the highly cooperative nature of the
responses observed in vitro may suggest that multiple mole-
cules are required (83). The mechanistic consequences of iver-
mectin binding to this site, resulting in the slow opening of
the channel, have been recently reviewed (91).

Sequence variations in glc-1 underlie natural variations in
sensitivity to the anthelmintic drug ivermectin (92) of various
strains of \textit{C. elegans}, even though the normal role of the channel
has not been described. Surprisingly, perhaps, the variation
most strongly associated with reduced sensitivity does not lie in
the binding site for either ivermectin or glutamate but is a four-
amino acid deletion toward the extreme N terminus of the sub-
unit. Unfortunately, this deletion is within a part of the subunit,
the N-terminal 40 amino acids, that had to be removed to pro-
duce the recombinant protein used for the structural studies
(87), so how this deletion might reduce the effects of ivermectin
on the channel is obscure. Mutations in the extracellular β10
strand, including the L256F mutation found in a drug–resistant
isolate of the parasite \textit{Cooperia oncophora} (85), and the M2-M3
linker region of AVR-14B affect the binding of ivermectin to the
channel (83, 93), consistent with the contacts observed between
bound ivermectin and the M2-M3 linker in GLC-1 (87).

Concluding Remarks

The GluCls are a wonderful example of the importance of
invertebrate neuroscience. Although they are probably much
less familiar than the other channels reviewed in this minire-
view series, and there are certainly far fewer papers and grants
devoted to them, studies on the GluCls and related receptors
have illustrated the many practical and academic benefits
across multiple disciplines in the basic and applied medical,
Veterinary, and agricultural sciences that result from studying
and exploiting these “simple” nervous systems.

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