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Inhibitory Glutamate Receptor Channels

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Abstract

Inhibitory glutamate receptors (IGluRs) are a family of ion channel proteins closely related to ionotropic glycine and γ-aminobutyric acid (GABA) receptors; They are gated directly by glutamate; the open channel is permeable to chloride and sometimes potassium. Physiologically and pharmacologically, IGluRs most closely resemble GABA receptors; they are picrotoxin-sensitive and sometimes crossdesensitized by GABA. However, the amino acid sequences of cloned IGluRs are most similar to those of glycine receptors. Ibotenic acid, a conformationally restricted glutamate analog closely related to muscimol, activates all IGluRs. Quisqualate is not an IGluR agonist except among pulmonate molluscs and for a unique multiagonist receptor in the crayfish *Austropotamobius torrentium*. Other excitatory amino acid agonists are generally ineffective. Avermectins have several effects on IGluRs, depending on concentration: potentiation, direct gating, and blockade, both reversible and irreversible. Since IGluRs have only been clearly described in protostomes and pseudocoelomates, these effects may mediate the powerful antihelminthic and insecticidal action of avermectins, while explaining their low toxicity to mammals.

IGluRs mediate synaptic inhibition in neurons and are expressed extrajunctionally in striated muscles. The presence of IGluRs in a neuron or muscle is independent of the presence or absence of excitatory glutamate receptors or GABA receptors in the cell. Generally, extrajunctional IGluRs in muscle have a higher sensitivity to glutamate than do neuronal synaptic receptors. Some extrajunctional receptors are sensitive in the range of circulating plasma glutamate levels, suggesting a role for IGluRs in regulating muscle excitability.

The divergence of the IGlu/GABA/Gly/ACh receptor superfamily in protostomes could become a powerful model system for adaptive molecular evolution. Physiologically and pharmacologically, protostome receptors are considerably more diverse than their vertebrate counterparts. Antagonist profiles are only loosely correlated with agonist profiles (e.g., curare-sensitive GABA receptors, bicuculline-sensitive AChRs), and pharmacologically identical receptors may be either excitatory or inhibitory, and permeable to different ions. The assumption that agonist sensitivity reliably connotes discrete, homologous receptor families is contraindicated. Protostome ionotropic receptors are highly diverse and straightforward to assay; they provide an excellent system in which to study and integrate fundamental questions in molecular evolution and adaptation.

Index Entries: Avermectin; molecular evolution; GABA; glycine; ibotenate; quisqualate; chloride channel; potassium channel; subunit; protostome; adaptation; homology.

Introduction

Inhibitory electrophysiological responses to glutamate have been demonstrated in neural and muscular tissues across many phyla. The known receptors underlying these responses can be grouped into three general categories: ionotropic receptors, metabotropic receptors, and a newly described family of electrogenic membrane proteins exhibiting properties of both channels and transporters. This article will deal primarily with the ionotropic and putatively ionotropic inhibitory glutamate receptors, with brief attention paid to other glutamatergic inhibitory responses mediated by slow metabotropic glutamate receptors and by glutamate transporters that resemble chloride currents.

Most ionotropic and putatively ionotropic responses to glutamate are mediated by an increase in membrane chloride permeability. These chloride-permeable glutamate receptors include extrajunctional muscle receptors, such as the "H-receptors" of locust muscle (Cull-Candy, 1976; Gration et al., 1979), and postsynaptic neuronal receptors, such as those mediating reciprocal and recurrent cyclic inhibition within the central pattern generator circuits of the crustacean stomatogastric ganglion (Marder and Paupardin-Tritsch, 1978; Bidaut, 1980; Marder and Eisen, 1984). These responses are blocked by the ionotropic GABA receptor antagonist picrotoxin. Glutamate can also evoke fast increases in potassium permeability; glutamatergic potassium currents can mediate fast inhibitory postsynaptic potentials (IPSPs), which are also picrotoxin-sensitive (Marder and Paupardin-Tritsch, 1978). Some such potassium conductances are inseparable from fast chloride conductances in the same cell, to the extent of comediating the same fast IPSPs (Bidaut, 1980; Eisen and Marder, 1982; Marder and Eisen, 1984; Elson and Selverston, 1995). Several slower glutamate-activated potassium currents have also been demonstrated, but are generally metabotropic and therefore largely outside the scope of this article.

The inhibitory glutamate receptors (IGluRs) are functionally defined as a family based on broad physiological and pharmacological criteria (described below). The evolutionary accuracy of this grouping is discussed below; ultimately, the determination will rely on molecular sequence analyses. To date, two IGluR subunits, GluClα and GluClβ, have been cloned from the nematode *Caenorhabditis elegans* (Cully et al., 1994). Sequence analyses of these two subunits have demonstrated that they are not members of the excitatory glutamate receptor gene superfamily (Darlison et al., 1993; Hollmann and Heinemann, 1994; Wo and Oswald, 1995). Rather, they are most similar in sequence to ionotropic glycine and GABA receptors (Cully et al., 1994). Assuming that the IGluRs constitute a family of orthologous receptors, the *C. elegans* data indicate that the IGluRs belong in the ionotropic GABA/glycine/ACh/5HT receptor superfamily (Schofield et al., 1987; Grenningloh et al., 1987; Barnard et al., 1987; Maricq et al., 1991; Darlison et al., 1993).

Although excitatory and metabotropic glutamate receptors appear to be present throughout the metazoa, the ionotropic IGluRs have only been convincingly described in protostomes and pseudocoelomates (the latter represented by the nematodes). In particular, there is no evidence clearly indicating their expression in vertebrates. The paucity of data from invertebrate deuterostomes, platyhelminths, and radiates prevents a thorough outgroup analysis indicating when or how many times IGluRs may have evolved, or precisely how they are related to other ionotropic receptors in their superfamily. Nevertheless, a phylogenetic description of IGluR distribution and physiological diversity provides considerable insight into the relationship among these receptor channels and suggests fruitful areas for further research. The relationship among IGluRs and related members of the ligand-gated receptor superfamily, particularly among protostomes, holds considerable promise for molecular evolutionary research.

IGluR Whole-Cell Physiology

Inhibitory responses to glutamate have been observed in both neurons and muscle cells.

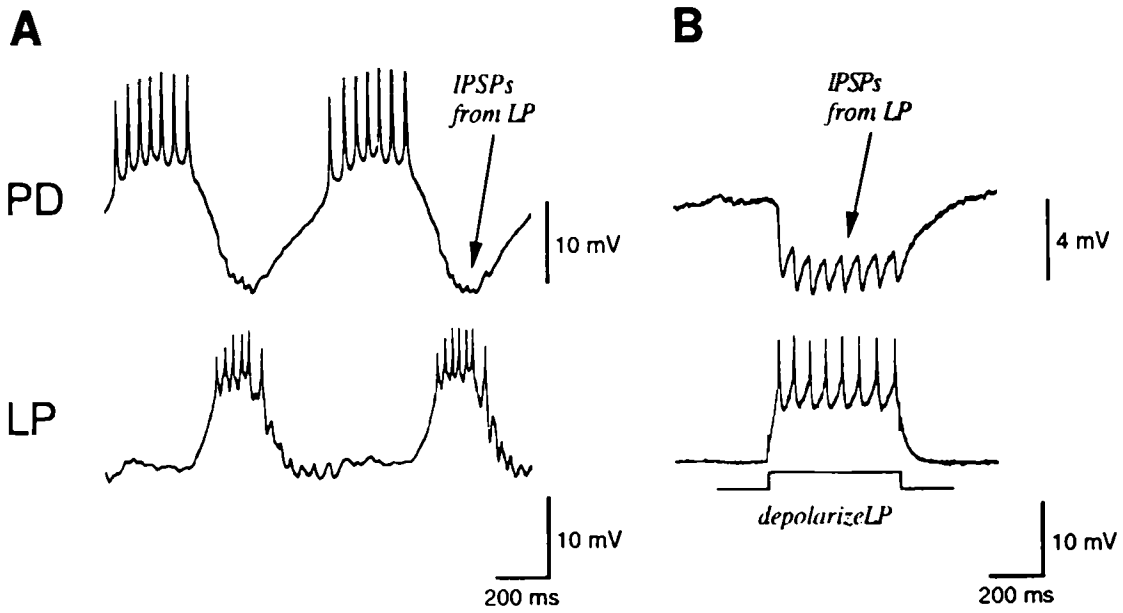


Fig. 1. In the stomatogastric ganglion of the spiny lobster, *Panulirus interruptus*, IGluRs mediate unitary IPSPs. The glutamatergic neuron LP evokes both graded inhibition and spike-mediated unitary IPSPs in the PD neuron (Graubard et al., 1983). **(A)** Fast glutamatergic inhibitory synapses contribute to rhythmogenesis. With normal modulatory inputs to the stomatogastric ganglion intact, glutamatergic IPSPs from LP helped to shape the oscillations of PD and establish the alternate-bursting pattern. **(B)** After blocking modulatory inputs, LP and PD became quiescent. Depolarization of LP evoked a barrage of spike-mediated glutamatergic IPSPs in PD. The membrane potential of PD was approx -50 mV. Figures were provided by Robert Elson.

Application of L-glutamate to the extracellular surface of a cell expressing IGluRs rapidly increases membrane permeability to chloride and/or potassium ions. This response is generally inhibitory, since decreased cellular input resistance can prevent action potential generation (Walker et al., 1971; Quinlan and Murphy, 1991; Cleland and Selverston, 1995), or block endplate potentials and muscular contractions (Carlyle, 1974; Fritz et al., 1979). Furthermore, although many invertebrate neurons exhibit a high resting permeability to chloride, such that the chloride reversal potential (E_{Cl}) remains close to the resting potential (Usherwood and Grundfest, 1965; Lea and Usherwood, 1973a; Marder and Paupardin-Tritsch, 1978), IPSPs were still visible in several preparations (Fig. 1). Across diverse protostome phyla, IGluRs show considerable physiological and pharmacological similarities, uniting them as a putative receptor family; however, they exhibit distinct differences in physiological characters, such as agonist profile and desensitization characteristics. When directly compared, IGluRs in isolated neurons exhibited similar properties to IGluRs studied *in situ* (King and Carpenter, 1987). Species-specific similarities and differences in receptor properties are depicted in Table 1.

Patterns of Expression

In striated muscle tissues expressing glutamate-gated inhibitory currents, IGluRs are extrajunctional and do not appear to be concentrated at the neuromuscular junction (Lea and Usherwood, 1973a,b; Cull-Candy, 1976; Lingle and Marder, 1981). In contrast, the depolarizing, cation-permeable glutamate receptors, which are responsible for the initiation of muscular contraction in glutamatergic muscles, exhibit substantial clustering at the postjunctional membrane (Takeuchi and Takeuchi, 1964; Cull-Candy, 1978); note that this clustering disappears following denervation in some muscle

Table 1
Pharmacology of IGluRs Expressed in Various Taxa

	Agonist sensitivity[a]							
Genus/species	L,D-Asp	L,D-α-amino adipate	L,D-α-amino pimelate	L-α-amino suberate	D-Glu	Cysteate	Homo-cysteate	L-Glutamine
Radiata								
Actinia	No	No	Yes	No	Yes (1x)	No	Yes (15x)	0.05x
Pseudocoelomata								
Caenorhabditis	No (0.1x)				No			
Mollusca								
Pulmonata								
Onchidium								
Helix[b]	Yes (0.66x)	Yes (0.66x)	No	No	Yes (0.1x)	Yes (0.5x)	Yes (1.0x)[b]	No
Helisoma			Weak					
Planorbarius								
Opisthobranchia								
Aplysia californica	Yes[c,d]							
Aplysia kurodai	No				No			
Annelida								
Hirudo								
Arthropoda								
Chelicerata								
Limulus	No (0.09x)		Weak		No (0.07x)		No	
Insecta								
Periplaneta	Yes					No	No	
Locusta	Yes					No		
Schistocerca	Yes							
Crustacea								
Cancer			No		No			
Panulirus								
Homarus	*c*	No	No		No	No	No	
Austropotamobius								
Astacus								
Procambarus								

	Agonist sensitivity[a]							
Genus/species	NMDA	NMD-glutamate	Glutarate	Glycine	β-alanine	GABA	β-GP	Taurine
Radiata								
Actinia		No	No					No
Pseudocoelomata								
Caenorhabditis	No			No		No		
Mollusca								
Pulmonata								
Onchidium						No		
Helix[b]	No	No (0.001x)	No	No	No	No		No
Helisoma	No			No		No		No
Planorbarius	No							
Opisthobranchia								
Aplysia californica	No					Yes[e]		
Aplysia kurodai	No					Yes[e]		

Table 1 *(continued)*

Genus/species	Agonist sensitivity[a] NMDA	NMD-glutamate	Glutarate	Glycine	β-alanine	GABA	β-GP	Taurine
Annelida								
Hirudo								
Arthropoda								
Chelicerata								
Limulus	No					Maybe[f]		
Insecta								
Periplaneta	No			No	No[g]	No		No[g]
Locusta				No		No		No
Schistocerca	No					No		
Crustacea								
Cancer						No	No	
Panulirus	No			No		No		
Homarus	No					No	No	
Austropotamobius				Weak		Yes	Weak	
Astacus								
Procambarus						Yes	Yes	

	Agonist sensitivity[a] Acetyl-choline	QA	Ibotenate	IVM	Antagonist sensitivity PTX	Bicuc-culine	Strych-nine	Niflumate and flufenamate
Radiata								
Actinia								
Pseudocoelomata								
Caenorhabditis		No	Yes (4x)	Yes	Yes	No	No	Yes
Mollusca								
Pulmonata								
Onchidium								
Helix[a]			Yes (1.0x)	Yes				
Helisoma	No							
Planorbarius	No							
Opisthobranchia								
Aplysia californica			Yes					
Aplysia kurodai		No						
Annelida								
Hirudo			Yes		No			
Arthropoda								
Chelicerata								
Limulus		No	Yes (1x)		Yes			
Insecta								
Periplaneta		No			Yes			
Locusta								
Schistocerca	*h*	No	Yes	Yes	Yes			
Crustacea								
Cancer					Yes	No		
Panulirus		No	Yes		Yes		No	Yes
Homarus		No			Yes	No	No	
Austropotamobius	Yes	Yes			Yes			
Astacus								
Procambarus								

(continued)

Table 1 *(continued)*

	Antagonist sensitivity			
	Furosemide	GDEE	Curare	Refs
Radiata				
Actinia				(Carlyle, 1970a, 1974)
Pseudocoelomata				
Caenorhabditis				(Arena et al., 1991, 1992; Cully et al., 1994)
Mollusca				
Pulmonata				
Onchidium				(Oomura et al., 1974)
Helix[a]				(Walker et al., 1971; Parmentier and Case, 1972; Szczepaniak and Cottrell, 1973; Piggott et al., 1975; Walker, 1976; Bokisch and Walker, 1986)
Helisoma				(Quinlan and Murphy, 1991)
Planorbarius	Yes			(Bolshakov et al., 1991)
Opisthobranchia				
Aplysia californica			Yes	(Carpenter et al., 1977; McCreery and Carpenter, 1984; King and Carpenter, 1989; Katz and Levitan, 1993)
Aplysia kurodai		No		(Sawada et al., 1984a; Ikemoto and Akaike, 1988)
Annelida				
Hirudo			No	(Mat Jais et al., 1983)
Arthropoda				
Chelicerata				
Limulus				(Roberts and Walker, 1982)
Insecta				
Periplaneta				(Hue et al., 1979; Giles and Usherwood, 1985b; Wafford and Sattelle, 1986, 1989)
Locusta				(Dubas, 1991)
Schistocerca				Lea and Usherwood, 1973a,b; Cull-Candy, 1976; Gration et al., 1979; Scott and Duce, 1985, 1987; Duce and Scott, 1985; Dudel et al., 1989; Fraser et al., 1990)
Crustacea				
Cancer				(Marder and Paupardin-Tritsch, 1978; Sharp, 1994)
Panulirus				(Albert et al., 1986; Cleland and Selverston, 1995)
Homarus		No	No	(Lingle and Marder, 1981; Albert et al., 1986)
Austropotamobius				(Franke et al., 1986; Zufall et al., 1988, 1989)
Astacus				(Murdock, 1971; Florey and Murdock, 1974; Adelsberger et al., 1994)
Procambarus				(Shinozaki and Ishida, 1980; Pearlstein et al., 1994)

[a]Parenthetical multipliers indicate relative potency of the agonist with respect to L-glutamate, if known. The endogenous neurotransmitter molecules GABA, aspartate, and glycine are listed as IGluR agonists only when clearly shown to crossdesensitize IGlu currents; responses to these agonists (in cells expressing IGluRs) that have not been demonstrated to crossdesensitize with IGlu responses are not listed. Other molecules are listed as agonists if in the opinion of the original authors the agonist-evoked response is owing to activation of IGluRs. Rare or very weak responses are listed as no response. Any clearly positive crossdesensitization data are listed as "yes"; such data may not apply to other tissues within the same species. Since receptor characteristics can be quite tissue-dependent even within a single species or genus (*see* the *Aplysia* literature in particular), these listings should not be overinterpreted.

[b]Data are primarily derived from *Helix aspersa*. Neurons from *H. pomatia* did not respond to homocysteate (Szczepaniak and Cottrell, 1973).

[c]Aspartate potentiates and prolongs the glutamate-evoked current; this could be owing to modulation or inhibition of glutamate uptake.

[d]Distinct, glutamate-insensitive aspartate receptors have also been described.

[e]*See text* for details.

[f]This IGluR and GABA-R are both crossdesensitized by aspartate, but have not been directly tested for crossdesensitization.

[g]Response is probably owing to activation of distinct GABA receptors.

[h]The nicotinic agonist carbachol (10^{-5}M) had no effect.

preparations (Usherwood, 1969), but not in others (Frank, 1974). IGluRs are not restricted to glutamatergic muscles; in the American lobster *Homarus americanus*, IGluRs are also present on the cholinergic gm1 muscles of the foregut, which exhibit no depolarizing glutamatergic current (Lingle and Marder, 1981). Notably, although IGluRs are also present on the gm1 muscles of the crab *Cancer magister*, they are not found in the gm1 muscles of *Cancer borealis* or the spiny lobster *Panulirus interruptus* (Lingle and Marder, 1981), despite the presence of IGluRs in the stomatogastric neurons of both species (Sharp, 1994; Cleland and Selverston, 1995).

In contrast, IGluRs in neurons can directly mediate fast synaptic IPSPs, as shown in Fig. 1 (Bidaut, 1980; Eisen and Marder, 1982; Elson and Selverston, 1995). Some neurons expressing IGluRs also express excitatory glutamate receptors (Yarowsky and Carpenter, 1976; Carpenter et al., 1977; Roberts and Walker, 1982; Dubas, 1991; Pearlstein et al., 1994), resulting in biphasic responses to glutamate application. It is possible that excitatory and inhibitory glutamate receptors may be separately clustered at different postsynaptic sites, such that the same neuron may receive distinct glutamatergic excitatory PSPs (EPSPs) and glutamatergic IPSPs. Discrete clustering of distinct postsynaptic receptor populations has been demonstrated for glutamatergic excitatory receptors and GABAergic inhibitory receptors in crayfish muscle (Takeuchi and Takeuchi, 1964, 1965), but not for two discrete classes of receptor activated by a single neurotransmitter.

No ionotropic glutamate-gated inhibitory currents have been described in nonneuronal, nonmuscular tissues.

The IGluR Is an Ionotropic Receptor

The IGluR has long been presumed to be an ionotropic receptor channel, largely based on the relatively fast onset of its response to glutamate application and the visible IPSPs, which it mediates in postsynaptic neurons (Fig. 1; Bidaut, 1980; Eisen and Marder, 1982). This has been supported in several preparations by the persistence of the current after intracellular dialysis (Ikemoto and Akaike, 1988; Oyama et al., 1990; but *see* Kehoe, 1994), the isolation of single channels in outside-out or inside-out membrane patches (Franke et al., 1986; Zufall et al., 1988), and by the recent cloning and expression in *Xenopus* oocytes of two subunits for a glutamate-gated chloride channel from the nematode *C. elegans*, which share sequence similarities with ionotropic GABA and glycine receptor subunits (Cully et al., 1994).

The IGluR Is Typically Gated by Two Molecules

The Hill coefficient of a receptor represents the minimum number of agonist molecules that must bind to the receptor for it to be activated (Hill, 1909). Hill coefficients for IGluRs vary, but approximate two in most neurons (1.7 and 2.2 [Arena et al., 1992]; 1.6 [King and Carpenter, 1989]; 2.1 [Sawada et al., 1984a]; 2.5 [Wafford and Sattelle, 1989]; 1.8 [Ikemoto et al., 1988]), indicating that the simultaneous binding of at least two molecules of glutamate is required for the channel to open. Intriguingly, Hill coefficients of near 2 have also been reported for a variety of ionotropic GABA receptors in invertebrates (Takeuchi and Takeuchi, 1967; Smart and Constanti, 1986; King and Carpenter, 1989) and vertebrates (Sakmann et al., 1983; Bormann and Clapham, 1985; Akaike et al., 1986; Kaneko and Tachibana, 1986; Randle and Renaud, 1987; Smart et al., 1987). However, Hill coefficients of both 0.75 and 4.8 have been reported for the extrajunctional "H-receptor" IGluR from locust muscle (Cull-Candy, 1976; Dudel et al., 1989; Sansom and Usherwood, 1990). This discrepancy may be attributable to differences in experimental technique. Receptor desensitization will cause Hill coefficients to be underestimated; the larger Hill slope (4.8) was measured using the liquid filament switch technique (Franke et al., 1987), which minimizes desensitization.

Potentiation of the IGlu current by low concentrations of avermectin can also reduce the Hill coefficient to 1.0 (Arena, 1994).

Chloride and Potassium Permeability

Many fast, inhibitory responses to glutamate are solely dependent on chloride (Lea and Usherwood, 1973a; Roberts and Walker, 1982; Sawada et al., 1984a; Yarowsky and Carpenter, 1976; King and Carpenter, 1987; Wafford and Sattelle, 1989; Bolshakov et al., 1991). However, some fast inhibitory currents also exhibit potassium dependencies that have not been distinguished from the chloride responses temporally, pharmacologically, or by virtue of the distinct equilibrium potential of potassium (Kerkut et al., 1969; Walker et al., 1971; Szczepaniak and Cottrell, 1973; Bidaut, 1980; Eisen and Marder, 1982; Mat Jais et al., 1983; Marder and Eisen, 1984; Tazaki and Chiba, 1994; Elson and Selverston, 1995; Cleland and Selverston, 1995). Three hypotheses may account for this observation. First, in some preparations, such potassium dependencies may simply reflect some permeability of the chloride channel to potassium, as has been reported for $GABA_A$ receptors (Newberry and Nicoll, 1984), invertebrate GABA receptors (Tazaki and Chiba, 1994; Wafford and Sattelle, 1986), and background chloride channels in cultured rat hippocampal pyramidal cells (Franciolini and Nonner, 1987, 1994a,b). Smaller partial dependencies on potassium have also been observed in GABA and glycine receptor channels in cultured mouse spinal neurons (Bormann et al., 1987). However, no studies to date have observed a significant potassium permeability through single IGluR channels (Franke et al., 1986; Zufall et al., 1988; Dudel et al., 1989; Cully et al., 1994).

Second, such potassium components may represent separate populations of channels that happen to exhibit kinetics, pharmacology, and distribution similar to GluCl channels in the same cells. It is notable that in spiny lobster *(Panulirus interruptus)* stomatogastric neurons, glutamatergic IPSPs are dependent on "inseparable" chloride and potassium permeabilities (Bidaut, 1980; Eisen and Marder, 1982; Elson and Selverston, 1995), whereas in crab stomatogastric neurons *(Cancer pagurus)*, populations of chloride-permeable and potassium-permeable glutamate receptors, both picrotoxin-sensitive, are distributed differently on the neuronal membrane and are thus clearly separate populations (Marder and Paupardin-Tritsch, 1978). Furthermore, the ratio between macroscopic potassium and chloride permeabilities through fast, picrotoxin-sensitive glutamate-gated conductances in different preparations ranged from zero (Lingle and Marder, 1981; Roberts and Walker, 1982) to about 50% (Kerkut et al., 1969) to completely potassium-based (e.g., the somatic current of *C. pagurus* neurons [Marder and Paupardin-Tritsch, 1978]). Resolution of this question will require single-channel patch recordings. Even to the extent that the potassium- and chloride-dependent components are found to reflect distinct receptor populations, the similarity in the physiology and pharmacology of these currents raises the possibility that the two receptors may be closely related. It is interesting to consider that altering the relative permeabilities of potassium and chloride at postsynaptic receptor sites could regulate the strength of inhibitory synapses, owing to the different driving forces on the two ions.

A third possibility is that observed changes in reversal potential after altering the external potassium concentration were caused by Donnan equilibrium effects. However, the observation of distinct chloride and potassium conductances, both picrotoxin-sensitive, in *C. pagurus* argues against this possibility (Marder and Paupardin-Tritsch, 1978), as does the observation that changes in the glutamate reversal potential after altering $[K^+]_o$ can significantly exceed changes in the resting potential (Elson and Selverston, 1995).

Slow Glutamate-Gated Potassium Currents

Some preparations exhibit slowly activating potassium-dependent currents in response to glutamate application ("GluK" currents) that are clearly separate from fast IGlu responses in the same cell. These slow potassium currents can coexist in cells that also exhibit a potassium component integral to the fast IGlu response

(Walker et al., 1971; Szczepaniak and Cottrell, 1973; Tazaki and Chiba, 1994; Elson and Selverston, 1995) as well as those without such a fast potassium component (Yarowsky and Carpenter, 1976; Bolshakov et al., 1991; Katz and Levitan, 1993; Kehoe, 1994). Slow GluK responses are also present in some cells exhibiting no IGluR-like current at all (Watanabe and Onozuka, 1994; Rainnie et al., 1994; Premkumar and Chung, 1995). These are distinct from the faster currents in that they are generally nondesensitizing and reverse near E_K, in addition to being slower in onset and slower to decay once the agonist is removed. In contrast to IGluRs, the glutamate receptors mediating these slow responses are also generally ibotenate-insensitive and quisqualate-sensitive (Yarowsky and Carpenter, 1976; Kehoe, 1978; Ascher et al., 1986; Miwa and Kawai, 1990; Bolshakov et al., 1991; Katz and Levitan, 1993; Kehoe, 1994).

Slow, ligand-gated potassium currents are generally thought to be mediated by second messenger-coupled receptors. Slow glutamatergic potassium conductances in protostomes have been attributed to inhibition of calcium/calmodulin-dependent protein kinase II in the Japanese land snail *Euhadra peliomphala* (Watanabe and Onozuka, 1994), and to activation of G protein-coupled receptors in the Ped-9 neurons of *Planorbarius corneus* (Bolshakov et al., 1991, 1992, 1993) and in spiny lobster neuromuscular synapse (Miwa and Kawai, 1990). However, the G protein-coupled response in *Planorbarius* is not attributable to any known second messenger pathway (Bolshakov et al., 1992, 1993), and the slow GluK response in *Aplysia californica* buccal neurons B1 and B2 appears to be independent of G proteins and other soluble factors (Kehoe, 1994). This is particularly unusual in that the slow GluK response in the *Aplysia* neurons is activated by (1*S*,3*R*)-aminocyclopentane-1,3-dicarboxylic acid (ACPD) (Katz and Levitan, 1993). ACPD is considered a specific agonist for quisqualate-sensitive metabotropic glutamate receptors, the effects of which are typically mediated by soluble factors (Palmer et al., 1989; Schoepp et al., 1990).

The *Planorbarius* GluK response is clearly different from that in *Aplysia californica*. First, in *Planorbarius*, dopamine and the muscarinic agonist F-2268 activated the same potassium current as glutamate; in *Aplysia*, the slow glutamate and carbachol responses do not crossreact (Kehoe, 1994). Second, the G protein-coupled receptor mediating the GluK response in *Planorbarius* is activated by kainate as well as quisqualate and is blocked by 50 μM tetraethylammonium (TEA), whereas the *Aplysia* GluK receptor is insensitive to both kainate and TEA (Katz and Levitan, 1993). The slow GluK response in the gastric network of the crustacean stomatogastric ganglion is also TEA-resistant (Elson and Selverston, 1995).

In summary, metabotropic GluK receptors constitute a heterogeneous population about which we have insufficient data to progress beyond the descriptive stage outside of the vertebrate subphylum. When coexpressed with faster chloride- and potassium-mediated synaptic inhibitory currents, however, the slower inhibition may play a specific role in some of the same effects that IGluRs mediate (van Vreeswijk et al., 1994; Elson and Selverston, 1995), and thus merit consideration alongside any discussion of the fast inhibitory glutamate receptors.

Desensitization

Under constant glutamate superfusion, IGluRs in most preparations desensitize with a time constant on the order of hundreds of milliseconds to seconds and recover within seconds to minutes (Sawada et al., 1984a; Ikemoto and Akaike, 1988; King and Carpenter, 1989; Oyama et al., 1990; Bolshakov et al., 1991; Katz and Levitan, 1993; Cleland and Selverston, 1995). Typically, desensitization is complete or nearly complete; however, there are several examples of responses that do not desensitize (Dubas, 1991; Pearlstein et al., 1994) or desensitize only partially. In preparations exhibiting only partial desensitization to saturating glutamate concentrations, this may be due to the presence of two or more distinct glutamate-activated currents with different

desensitization characteristics, or it may reflect a persistent, agonist-bound nonzero conductance state intrinsic to the underlying channel. The former interpretation is suggested in stomatogastric neurons of the spiny lobster, in which I have observed that superfusion of glutamate onto intact, cultured neurons yielded a partially desensitizing response, whereas superfusion of the same concentration of glutamate onto multichannel outside-out membrane patches produced a fully desensitizing current with a time constant of desensitization on the order of 100 ms (unpublished data). Furthermore, the whole-cell glutamate response in this tissue could sometimes be discriminated into two distinct temporal components, both with the same reversal potential, but differing in rate of onset, desensitization, and sensitivity to niflumic acid, a chloride channel antagonist (Cleland and Selverston, 1995). In contrast, the latter interpretation is demonstrated by the partially desensitizing response to 1 m*M* glutamate exhibited by cloned *C. elegans* GluClβ homomeric channels expressed in *Xenopus* oocytes (Cully et al., 1994). It is notable, however, that when GluClα and GluClβ were coexpressed in oocytes, the net response to glutamate was nondesensitizing and slower in onset than the GluClβ homomeric current, implying that these kinetic parameters are dependent on subunit composition. IGluRs translated from extracted *C. elegans* mRNA partially desensitized when gated by 1 m*M* glutamate, although a lesser concentration, 200 μ*M*, induced almost no desensitization (Arena et al., 1992). Cully et al. (1994) suggested that other subunits besides α and β may contribute to wild-type IGluRs in *C. elegans*.

In preparations where GABA and glutamate appear to activate a common receptor channel (*see* Cross-Sensitivity to GABA), these agonists nevertheless can evoke currents with different desensitization characteristics. In *Aplysia californica* medial pleural neurons, both agonists evoked a desensitizing chloride current, although the GABA-activated current desensitized somewhat faster (King and Carpenter, 1989). In various muscles of the crayfish *Austropotamobius torrentium*, GABA activated a nondesensitizing current, whereas the glutamate-evoked current desensitized (Franke et al., 1986). In crayfish leg motoneurons, neither GABA- nor glutamate-evoked chloride currents desensitized (Pearlstein et al., 1994).

Rectification

Some IGluRs exhibited slight outward rectification, increasing their glutamate-evoked conductance levels at more depolarized potentials, whereas others had linear I–V relationships. In various *Aplysia* neurons, the glutamate-evoked chloride current was nonrectifying (Sawada et al., 1984a; Ikemoto and Akaike, 1988; King and Carpenter, 1989) or very slightly rectifying (Oyama et al., 1990). Arthropod IGluR current–voltage relations exhibited somewhat greater outward rectification; furthermore, these relations were reduced in slope above the zero-current potential, suggesting that the underlying channels passed outward current more easily than inward current, or, equivalently, that chloride permeability was greater for an influx of the anion than for an efflux (Lea and Usherwood, 1973b; Zufall et al., 1988; Dudel et al., 1989; Cleland and Selverston, 1995). In contrast, the IGlu conductances in *Aplysia* species were symmetrically permeable, except for those in identified buccal neurons BR1 and BR2 from *Aplysia kurodai*, which showed a decrease in slope conductance above the zero-current potential like the arthropod receptors (Sawada et al., 1984a).

The IGlu current exhibited by oocytes injected with *C. elegans* mRNA was slightly outwardly rectifying whether activated by glutamate or ivermectin (Arena et al., 1992), as was the IGlu current expressed when the subunit clones GluClα and GluClβ were coexpressed. GluClβ, the glutamate-sensitive subunit, outwardly rectified much more strongly when expressed alone than did the putatively heteromeric channel, whereas the avermectin-sensitive, glutamate-insensitive GluClα subunit (*see* IGluR Molecular Biology) exhibited a linear I–V relationship when gated by ivermectin (Cully et al., 1994).

Among currents activated by both glutamate and GABA, the two agonists could evoke distinct rectification properties. In isolated medial pleural neurons of *Aplysia californica*, activation of the common chloride conductance by GABA produced an outwardly rectifying current, whereas the glutamate-evoked current was nonrectifying (King and Carpenter, 1989). In contrast, in isolated pedal ganglionic neurons from *Aplysia kurodai*, GABA evoked a nonrectifying current, whereas the glutamate-evoked current exhibited a slight outward rectification (Oyama et al., 1990). Finally, both GABA and glutamate evoked similarly outwardly rectifying currents in crayfish muscle (Zufall et al., 1988).

The apparent voltage-independence of most *Aplysia* IGluRs is curious, given that the Goldman-Hodgkin-Katz current equation and rate-theory models of ion permeation through single open channels predict a degree of outward rectification, provided that the extracellular chloride concentration is greater than that inside the cell (Hille, 1992). King and Carpenter (1989) suggested that the inwardly rectifying, hyperpolarization-activated chloride currents observed in some *Aplysia* neurons may be summating with outwardly rectifying IGlu currents to yield a linear macroscopic current–voltage relationship (Chenoy-Marchais, 1982; King and Carpenter, 1989). However, observed agonist-specific differences in the rectification properties of currents gated by both glutamate and GABA are not explained by this model; a genuine difference in the voltage sensitivity of gating or the asymmetry of ion permeation through the open channel is still implied.

IGluR Pharmacology

Agonist Profiles

All IGluRs studied to date were activated by L-glutamate and its conformationally-restricted structural analog ibotenate; this was demonstrated in crustacean neurons (Roberts and Walker, 1982; Cleland and Selverston, 1995) and muscle (Lingle and Marder, 1981), insect muscle (Lea and Usherwood, 1973b; Cull-Candy et al., 1976; Dudel et al., 1989), opisthobranch molluscan neurons (Yarowsky and Carpenter, 1976; Carpenter et al., 1977; Sawada et al., 1984a, Ikemoto and Akaike, 1988; King and Carpenter, 1989; Katz and Levitan, 1993), pulmonate molluscan neurons (Kerkut et al., 1969; Walker et al., 1971; Bolshakov et al., 1991), and annelid neurons (Mat Jais et al., 1983). EC_{50} values for L-glutamate ranged from 10–500 μ*M* (Lea and Usherwood, 1973b; Lingle and Marder, 1981; Wafford and Sattelle, 1989; Cully et al., 1994). Ibotenate was roughly equipotent to L-glutamate in most preparations, although in some ibotenate was a less powerful agonist (Walker et al., 1971; Dudel et al., 1989), whereas it was considerably (4×) more effective than glutamate at gating *C. elegans* receptors expressed in oocytes (Arena et al., 1992; Arena, 1994). Excitatory glutamate receptors in protostomes are generally insensitive to ibotenate, although a few vertebrate excitatory glutamate receptors have been shown to respond to it (Johnston et al., 1968, 1974). Indeed, IGluRs were first discovered as a result of investigating the effects of ibotenate on locust muscle (Lea and Usherwood, 1973a,b).

The stereoisomer D-glutamate inhibited muscular contraction in the sea anemone *Actinia equina* as efficiently as L-glutamate (Carlyle, 1970a,b, 1974); the data are consistent with the activation of IGluRs. Neither GABA nor glycine mimicked this inhibitory effect. IGluRs in other species either showed weak responses to D-glutamate (Gorman and Marmor, 1971; Parmentier and Case, 1972; Szczepaniak and Cottrell, 1973; Piggott et al., 1975; Roberts and Walker, 1982) or were entirely insensitive to it (Marder and Paupardin-Tritsch, 1978; Lingle and Marder, 1981; Sawada et al., 1984a; Arena et al., 1992).

Varying the length of the carbon chain between the amino group of L-glutamate and its distal carboxyl group strongly influenced agonist effectiveness (Tables 1 and 2). Aspar-

Table 2
Structural Formulae for Glutamate-Like Molecules Screened for Agonistic and/or Antagonistic Activity at the IGluR[a]

Varying distance between amino group and distal carboxyl	DL-aspartate	$HOOC—CH_2—CH(NH_2)—COOH$
	DL-glutamate	$HOOC—(CH_2)_2—CH(NH_2)—COOH$
	DL-α-aminoadipate	$HOOC—(CH_2)_3—CH(NH_2)—COOH$
	DL-α-aminopimelate	$HOOC—(CH_2)_4—CH(NH_2)—COOH$
	L-α-aminosuberate	$HOOC—(CH_2)_5—CH(NH_2)—COOH$
Varying configuration at the aminated carbon	L-glutamate	$HOOC—(CH_2)_2—CH(NH_2)—COOH$
	D-glutamate	$HOOC—(CH_2)_2—CH(NH_2)—COOH$
Substitutions at distal carboxyl	Cysteate	$HO_3S—CH_2—CH(NH_2)—COOH$
	Homocysteate	$HO_3S—(CH_2)_2—CH(NH_2)—COOH$
	L-glutamine	$H_2NOC—(CH_2)_2—CH(NH_2)—COOH$
Absence of proximal carboxyl	Glycine	$HOOC—CH_2—NH_2$
	β-alanine	$HOOC—(CH_2)_2—NH_2$
	γ-aminobutyric acid (GABA)	$HOOC—(CH_2)_3—NH_2$
	Taurine	$HO_3S—(CH_2)_2—NH_2$
Substitutions at the amino group	*N*-methyl-D-aspartate (NMDA)	$HOOC—CH_2—CH(NHCH_3)—COOH$
	N-methyl-D-glutamate	$HOOC—(CH_2)_2—CH(NHCH_3)—COOH$
	Glutarate	$HOOC—(CH_2)_3—COOH$

[a]The specificity of a glutamate receptor for these systematically manipulated molecules can provide insight into the nature of the glutamate binding site(s). Adapted from Parmentier and Case (1972).

tate activated an IGlu-like current reasonably effectively in *Helix* neurons (Gerschenfeld and Lasansky, 1964; Parmentier and Case, 1972; Szczepaniak and Cottrell, 1973; Piggott et al., 1975) and insect neurons and muscle (Wafford and Sattelle, 1986, 1989; Dudel et al., 1989; Dubas, 1991), but was a weak agonist in *Limulus* (Roberts and Walker, 1982). Interestingly, IGluRs translated from insect muscle mRNA expressed in *Xenopus* oocytes were insensitive to aspartate (Fraser et al., 1990). Among crustaceans, lobster foregut muscle IGluRs were insensitive to aspartate (Lingle and Marder, 1981), whereas crayfish vas deferens muscle exhibited some sensitivity to aspartate (Murdock, 1971; Florey and Murdock, 1974). *Caenorhabditis* receptors and the *Actinia* response were largely aspartate-insensitive (Carlyle, 1970b, 1974; Arena et al., 1992).

In the opisthobranch mollusc *Aplysia kurodai*, some neurons responded strongly to aspartate and others not at all (Sawada et al., 1984a; Ikemoto and Akaike, 1988); this may have been due to differential expression of similar but distinct glutamate and aspartate receptors (*see* Yarowsky and Carpenter, 1976). In *Aplysia californica*, aspartate also crossdesensitized the IGlu current in these neurons, if present; any given neuron could exhibit one, both, or neither of these currents (Yarowsky and Carpenter, 1976). Finally, aspartate was capable of potentiating inhibitory glutamate responses; this has been attributed to blockade by aspartate of glutamate uptake (Lingle and Marder, 1981; McCreery and Carpenter, 1984), but could also be due to a direct effect of aspartate on the IGluR, as has been proposed for an excitatory GluR (Shank and Freeman, 1975; Dudel, 1977). These results imply that a heterogeneous population of receptors with varying selectivity for glutamate and aspartate exists in *Aplysia*, and further suggest that other aspartate-induced currents attributed to crossactivation of glutamate receptors should be re-examined.

Lengthening the carbon chain also affected agonist potency. Crustacean IGluRs were not activated by any molecule with a longer carbon chain than that of glutamate (Table 2), whereas neurons in the pulmonate mollusc *Helix* responded to α-aminoadipate. Interest-

ingly, the putative receptor in *Actinia* was insensitive to α-aminoadipate, which has one additional carbon in the chain compared to glutamate, and to α-aminosuberate, which has three, but responded to α-aminopimelate, which has two (Carlyle, 1970a). In some preparations, α-aminopimelate inhibited the IGlu response, presumably as a weak agonist (Roberts and Walker, 1982; Quinlan and Murphy, 1991).

Some glutamate analogs with substitutions at the distal carboxyl site evoked currents in molluscan neurons (*Helix* spp.), but could not activate IGluRs in arthropods. The putative *Actinia* receptor did not respond to cysteate, but responded to homocysteate much more strongly than to any other agonist, including glutamate. This suggests that the sulfonic group acted to increase agonist potency, but that cysteate (a substituted aspartate) may simply have been unable to span the binding site in *Actinia*. This is consistent with the observation that aspartate is also ineffective in *Actinia*. L-Glutamine, a glutamate analog with the hydroxyl group on the distal carboxyl substituted with an amino group, had no effect even on the tolerant *Helix* receptor, but did have a weak effect in *Actinia*.

Agonists lacking a proximal carboxyl group (open arrows in Fig. 2A) were generally ineffective. An interesting possible exception is GABA, a decarboxylated glutamate derivative and known inhibitory neurotransmitter in both protostomes and deuterostomes (although it may not even be present in the tissues of the anthozoan *Actinia* [Carlyle, 1971]). GABA was ineffective at most IGluRs, but crossdesensitized a few glutamate receptors and activated others, as is discussed below. Substitutions at the amino group of glutamate did not produce effective agonists. IGluRs did not respond to the excitatory glutamate receptor agonists kainate, AMPA, or NMDA (*see* Glantz and Pfeiffer-Linn, 1992 for a review of NMDA effects in invertebrates).

The response of IGluRs to quisqualic acid varied; furthermore, sufficient data were available that IGluR responses to quisqualate can be usefully mapped onto a phylogeny (Fig. 3). Opisthobranch molluscan, nematode, and a plurality of arthropod IGluRs investigated were quisqualate-insensitive, whereas IGluRs in pulmonate molluscs were activated by quisqualate (Table 1). In the annelid *Hirudo medicinalis*, the glutamate-gated chloride current was essentially quisqualate-insensitive, although occasional preparations exhibited a slight quisqualate-induced hyperpolarization (Mat Jais et al., 1984).

Arthropod IGluRs insensitive to quisqualate included those expressed in spiny lobster foregut muscle gm1 (Lingle and Marder, 1981) and stomatogastric motor neurons (Cleland and Selverston, 1995), *Limulus* central abdominal cord neurons (Roberts and Walker, 1982), cockroach leg motor neurons (Wafford and Sattelle, 1986, 1989), and locust metathoracic extensor tibiae muscles (Dudel et al., 1989). In the North American crayfish *Procambarus clarkii*, IGluRs in thoracic motor neurons (Pearlstein et al., 1994) and the opener muscle of the first walking leg (Shinozaki and Ishida, 1981) were also quisqualate-insensitive. The single exception was a glutamate-activated chloride current expressed in the stomach and leg muscles of the crayfish *Austropotamobius torrentium*, an indigenous European species once classified within the genus *Astacus*, which was also gated by quisqualate (Franke et al., 1986). However, this receptor was also extraordinarily sensitive to acetylcholine (10 n*M* threshold concentration) and activated by >10 μ*M* GABA (Zufall et al., 1988); neither of these agonists affected the quisqualate-sensitive IGluR in pulmonate molluscs. Furthermore, no similar receptor was found in a homologous stomach muscle of the closely related European crayfish species *Astacus astacus* (Adelsberger et al., 1994). The relationship of the *Austropotamobius* multiagonist receptor to other IGluRs is unclear.

Quisqualate did not activate IGluRs in neurons of the opisthobranch molluscs *Aplysia kurodai* (Ikemoto and Akaike, 1988) or *Aplysia californica* (Katz and Levitan, 1993; Kehoe, 1994), although in the latter, quisqualate did activate a pharmacologically distinct slow potassium current. Among pulmonate molluscs, quisqualate has been reported to activate

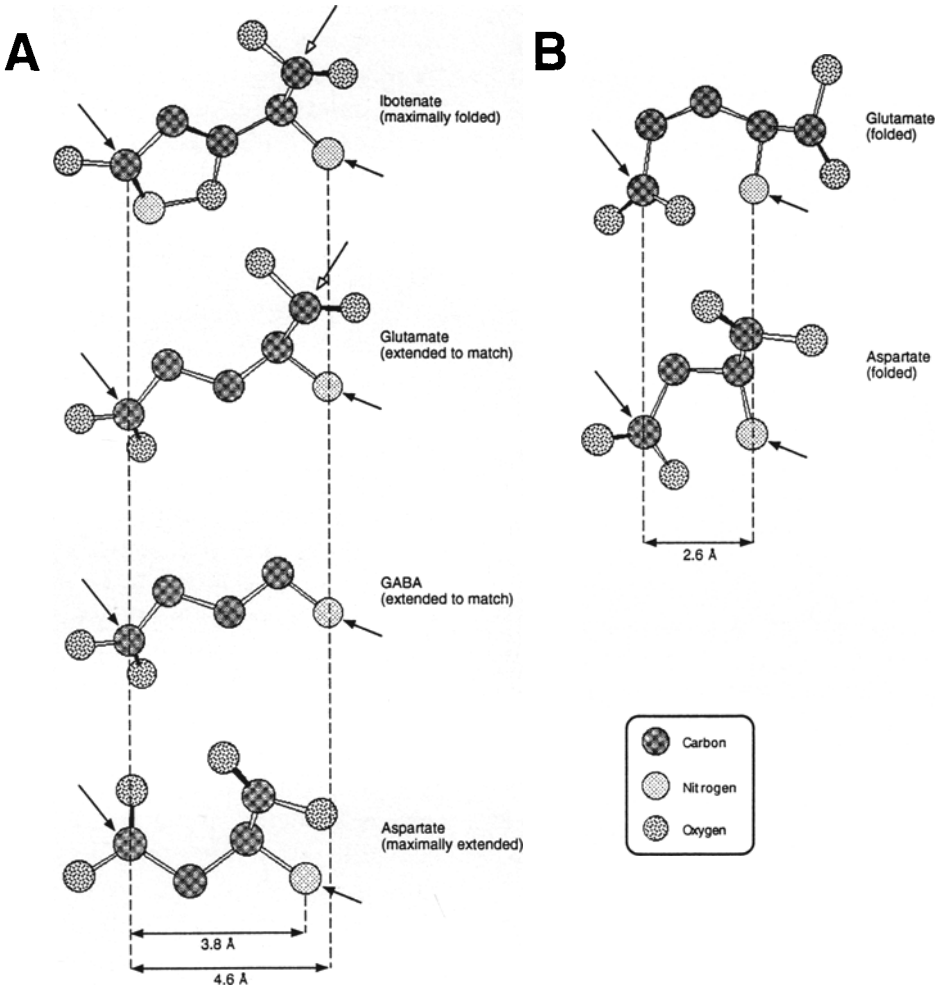


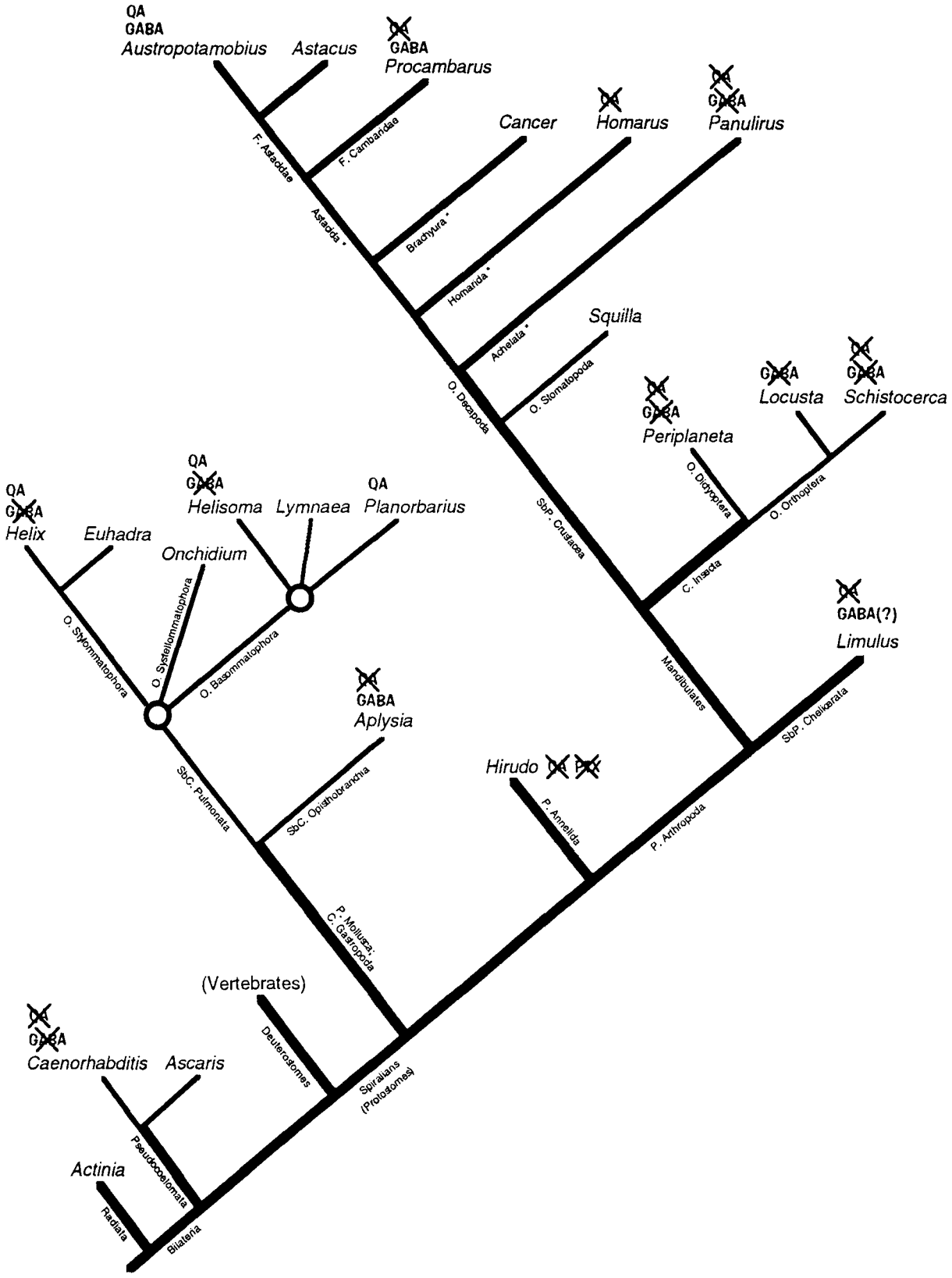
Fig. 2. Molecular conformations of selected amino acids. Single bonds are represented by open lines; solid lines indicate double bonds. Filled arrows depict charged groups common to glutamate and GABA (the amino and distal carboxyl groups); open arrows designate the proximal carboxyl group of glutamate and its analogs, which GABA and muscimol lack; a binding site for this group is implicated in the distinction between GABA and inhibitory glutamate receptors. **(A)** The conformationally restricted glutamate analog ibotenate is a strong agonist of all known IGluRs. The flexible glutamate molecule presumably adopts a similar conformation in order to bind to ibotenate-preferring receptors, such as the IGluRs. GABA is also capable of assuming this conformation, except that it lacks an amino-proximal carboxyl group (open arrows). Aspartate, even in its maximally extended conformation, is unable to position its charged groups in an ibotenate-like conformation. Aspartate-activated IGluRs, such as those in *Helix* or insects (Table 1), are presumably either more conformationally tolerant than aspartate-insensitive IGluRs or have a different receptor site involving only the amino and proximal carboxyl groups. The former is indicated in *Helix*, since a receptor that is entirely nondiscriminatory about carbon chain length should respond to long-chain glutamate analogs, such as α-aminoadipate and α-aminopimelate. **(B)** Glutamate can also assume a folded conformation in which it more closely resembles aspartate in the distribution of its charged groups; this conformation has been suggested for optimal activity at some excitatory glutamate receptors (Piggott et al., 1975). Figures are derived from the work of van Gelder (1971), Lea and Usherwood (1973b), and Piggott et al. (1975).

fast glutamate-gated chloride currents in the Ped-9 neurons of *Planarbarius corneus* (Bolshakov et al., 1991) and glutamate- and ibotenate-sensitive chloride and potassium conductances in *Helix aspersa* (Walker et al., 1971; Walker, 1976). Quisqualate also mimicked the glutamate-evoked inhibition in buccal neuron B19 of *Helisoma trivolvis* (Quinlan and Murphy, 1991); however, no ionic mechanism for this effect has been demonstrated. The authors suggested that this inhibition by quisqualate may have been mediated by activation of a CNQX-insensitive metabotropic glutamate receptor (mGluR), increasing intracellular calcium via an IP_3 cascade and consequently activating calcium-dependent potassium currents. This is certainly a plausible hypothesis; however, comparisons with closely related taxa suggest a more parsimonious mechanism. The authors noted that the quisqualate-evoked hyperpolarization in *Helisoma* neuron B19 could not be mimicked by the specific mGluR agonist ACPD (Palmer et al., 1989; Schoepp et al., 1990). ACPD is an agonist for the only well-described molluscan QA-sensitive second-messenger-coupled GluKR, on the identified buccal ganglion neurons B1 and B2 in the opisthobranch *Aplysia californica* (Katz and Levitan, 1993; Kehoe, 1994). Furthermore, although most IGluRs are quisqualate-insensitive, those expressed by pulmonate molluscs are activated by quisqualate (Fig. 3). The freshwater pulmonate *Planarbarius corneus*, which is in the same order as *Helisoma* (i.e., Basommatophora), exhibited a fast chloride current activated by quisqualate (Bolshakov et al., 1991). The more distantly related pulmonate *Helix aspersa* also exhibited a glutamate-gated increase in permeability to chloride and potassium (Kerkut et al., 1969), which was activated by ibotenate (Walker et al., 1971) and quisqualate (Walker, 1976). Thus, the physiology and pharmacology of the inhibitory glutamate response in *Helisoma trivolvis* are consistent with those of the IGluR in all other pulmonates in which it has been described (Fig. 3). Ionic substitutions and application of ibotenate and picrotoxin would address this issue more rigorously.

Antagonist Profiles

Picrotoxin at low concentrations (typically 1–100 μ*M*) blocked nearly all IGluRs, although some required up to 1 m*M* picrotoxin to block completely. (Note that some of this variability may be caused by variability in experimental technique rather than inherent differences in picrotoxin efficacy). The only picrotoxin-resistant IGlu-like current observed was expressed in leech Retzius cells, the only annelid cell for which IGluR data are available (Mat Jais et al., 1983). Some protostome GABA receptors also have reduced sensitivity to picrotoxin block (Marder and Paupardin-Tritsch, 1978; Albert et al., 1986). Chloride channel blockers, such as furosemide (Bolshakov et al., 1991) and the fenamates niflumic acid (Cleland and Selverston, 1995) and flufenamic acid (Arena et al., 1992), also blocked those IGluRs to which they were applied (typically at 100 μ*M* concentration); however, their specificity for chloride channels is in serious doubt (Lerma and del Rio, 1992; Poronnik et al., 1992; Partridge et al., 1994; Ottolia and Toro, 1994; Lee et al., 1995; Accili and DiFrancesco, 1996).

Several antagonists have demonstrated effects on the IGluR in some species/tissues but not others; unfortunately, few of these antagonists have been employed in sufficiently diverse preparations to support inferences about possible systematic differences among orthologous receptors. Glutamate diethyl ester (GDEE) had an antagonistic effect in the snail *Helix pomatia* (Szczepaniak and Cottrell, 1973), but was ineffective in other species tested (Lingle and Marder, 1981; Ikemoto and Akaike, 1988; Wafford and Sattelle, 1989). γ-D-glutamylglycine (γ-DGG; 100 μ*M*) has been reported to block the IGlu current specifically in cockroach leg motor neurons (Wafford and Sattelle, 1989). Curare blocked an IGlu current along with several other ligand-gated chloride currents in *Aplysia* neurons (Carpenter et al., 1977); however, it was ineffective at blocking the glutamate-gated chloride current in *Homarus*, as was the muscarinic antagonist atropine (Lingle and Marder, 1981).


QA
GABA
Austropotamobius
Astacus
QA
GABA
Procambarus
Cancer
QA
Homarus
QA
GABA
Panulirus
F. Astacidae
F. Cambaridae
Astacida •
Brachyura •
Homarida •
Achelata •
Squilla
O. Stomatopoda
O. Decapoda
QA
GABA
Periplaneta
GABA
Locusta
QA
GABA
Schistocerca
O. Dictyoptera
O. Orthoptera
C. Insecta
SbP. Crustacea
QA
GABA
Helix
Euhadra
QA
GABA
Helisoma
Lymnaea
QA
Planorbarius
Onchidium
O. Stylommatophora
O. Systellommatophora
O. Basommatophora
QA
GABA(?)
Limulus
Mandibulates
SbP. Chelicerata
QA
GABA
Aplysia
Hirudo
SbC. Pulmonata
SbC. Opisthobranchia
P. Annelida
P. Arthropoda
P. Mollusca;
C. Gastropoda
(Vertebrates)
QA
GABA
Caenorhabditis
Ascaris
Deuterostomes
Spiralians
(Protostomes)
Actinia
Pseudocoelomata
Radiata
Bilateria

Except for GDEE, none of the excitatory glutamate receptor blockers, such as CNQX, Joro spider toxin (JSTX), or DL-2-amino-5-phosphonovalerate (APV), affected IGlu currents (Ikemoto and Akaike, 1988; Quinlan and Murphy, 1991; Arena et al., 1991, 1992; Pearlstein et al., 1994). The glycine receptor antagonist strychnine was also ineffective (Lingle and Marder, 1981; Cully et al., 1994). The $GABA_A$ antagonist bicuculline did not block IGluRs; it also typically does not block invertebrate GABA receptors (Lingle and Marder, 1981; Roberts and Walker, 1982; Franke et al., 1986; Oyama et al., 1990; Darlison, 1992; Jackel et al., 1994a,b), although exceptions exist (Yarowsky and Carpenter, 1978a).

Cross-Sensitivity to GABA

As fast, ionotropic, ligand-gated chloride channels, IGluRs physiologically resemble

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Fig. 3. *(opposite)* Phylogeny of genera in which putatively ionotropic inhibitory responses to glutamate have been described. Some tissue-specific differences also exist, although data describing multiple tissues within a species are scarce. For comparative purposes, selected descriptive characters of the IGlu responses in particular genera are noted on the phylogeny: QA, the IGluR is activated by quisqualate. GABA, the IGlu response is crossactivated or crossdesensitized by GABA (it does not indicate the presence or absence of a separate GABAergic current). A crossed-out symbol indicates that the effect in question is not present. The crossed-out PTX depicts the unique picrotoxin insensitivity of the *Hirudo* IGluR. The question mark denoting GABA crossreactivity in *Limulus* indicates that IGluRs and GABA receptors were both antagonized by aspartate, α-aminopimelate, and β-guanidinopropionic acid, but were not tested directly for crossdesensitization.

The thinnest lines represent grade-based relationships derived from Linnean systematic classification (Barnes, 1987). Medium lines (within Decapoda) are cladistically derived based on morphological characters (Scholtz and Richter, 1995). The thickest lines are based upon molecular phylogenetic analyses (Patterson, 1990; Boore and Brown, 1994; Boore et al., 1995; *see text* for details) and are consistent with Linnean grades. Where useful to describe phylogenetic branch points, diagonal text denotes Linnean, cladistic, or common descriptive names for taxa. Taxonomic names denoted with an asterisk are defined in Scholtz and Richter (1995). Linnean taxa are cited by grade where appropriate (P, phylum; SbP, subphylum; C, class; SbC, subclass; O, order; IO, infraorder; F, family). Specific and common names for denoted genera, with references, are as follows: *Actinia equina*, sea anemone (Carlyle, 1970a, 1974); *Caenorhabditis elegans*, free-living nematode (Arena et al., 1991, 1992; Cully et al., 1994); *Ascaris suum*, parasitic nematode (Martin and Pennington, 1989; Holden-Dye and Walker, 1990); *Onchidium verruculatum*, intertidal marine slug (Oomura et al., 1974; Kato et al., 1983); *Helix aspersa*, terrestrial snail (Kerkut et al., 1969; Walker et al., 1971; Walker, 1976); *Helix pomatia*, terrestrial snail (Szczepaniak and Cottrell, 1973); *Euhadra peliomphala*, Japanese land snail (Watanabe and Onozuka, 1994; Onozuka et al., 1994); *Helisoma trivolvis*, freshwater snail (Quinlan and Murphy, 1991; Quinlan et al., 1995); *Lymnaea stagnalis*, great freshwater pond snail; *Planorbarius corneus*, freshwater snail (Magazanik et al., 1990; Bolshakov et al., 1991); *Aplysia kurodai*, sea hare (Sawada et al., 1984a,b; Ikemoto et al., 1988; Oyama et al., 1990); *Aplysia californica*, California sea hare (Yarowsky and Carpenter, 1976; Carpenter et al., 1977; King and Carpenter, 1987, 1989); *Aplysia dactylomela*, Florida sea hare (Carpenter et al., 1977); *Hirudo medicinalis*, medicinal leech (Mat Jais et al., 1983); *Limulus polyphemus*, horseshoe crab (Walker et al., 1981; Roberts and Walker, 1982; Walker and Roberts, 1982); *Periplaneta americana*, cockroach (Wafford and Sattelle, 1986, 1989; Sattelle, 1992); *Locusta migratoria*, locust (Dubas, 1991); *Schistocerca gregaria*, locust (Cull-Candy et al., 1976; Gration et al., 1979; Scott and Duce, 1985; Dudel et al., 1989); *Squilla oratoria* (Tazaki and Chiba, 1994); *Panulirus interruptus*, Pacific spiny lobster (Bidaut, 1980; Eisen and Marder, 1982; Marder and Eisen, 1984; Cleland and Selverston, 1995); *Panulirus argus*, Florida spiny lobster (Albert et al., 1986); *Homarus americanus*, American lobster (Lingle, 1980; Lingle and Marder, 1981); *Cancer pagurus*, crab (Marder and Paupardin-Tritsch, 1978); *Cancer borealis*, rock crab, and *Cancer magister*, West coast crab (Lingle and Marder, 1981); *Procambarus clarkii*, North American crayfish (Shinozaki and Ishida, 1980; Pearlstein et al., 1994); *Astacus astacus*, European crayfish (Florey and Murdock, 1974; Adelsberger et al., 1994); *Astacus leptodactylus*, Eurasian crayfish (Murdock, 1971; Florey and Murdock, 1974); *Austropotamobius torrentium*, European crayfish (Franke et al., 1986; Zufall et al., 1988); *Austropotamobius pallipes*, European crayfish (Adams et al., 1982).

ionotropic GABA and glycine receptor channels; furthermore, the nucleotide sequences of cloned IGluRs show them to be closely related to these ligand-gated chloride channels (Cully et al., 1994). Several studies have reported that GABA and glutamate can activate the same chloride channel, perhaps via distinct receptor sites (Franke et al., 1986; King and Carpenter, 1987, 1989; Zufall et al., 1988; Oyama et al., 1990; Pearlstein et al., 1994). Other studies have reported GABAergic and inhibitory glutamatergic currents expressed in the same cell that were entirely independent of one another (Lingle and Marder, 1981; Cull-Candy and Miledi, 1981; Ikemoto and Akaike, 1988). However, the techniques and agonist concentrations used in these studies varied, limiting direct comparisons between species and tissues. The phylogenetic distribution of GABA crossreactivity is depicted in Fig. 3.

Some IGluRs, notably the multiagonist receptor channel expressed in the muscles of the crayfish *Austropotamobius torrentium* (Franke et al., 1986; Zufall et al., 1988), were simply activated by GABA as well as by glutamate, although the favored conductance state distributions differed. Other IGluRs exhibited more complex responses to the presence of GABA. Oyama et al. (1990) described two distinct GABAergic responses in isolated, internally perfused pedal ganglion neurons from *Aplysia kurodai* in a study designed to resolve differences between their own research group's earlier conclusions that glutamate-gated chloride currents in *Aplysia kurodai* neurons (of unspecified type) did not crossdesensitize with GABA-gated currents (Ikemoto and Akaike, 1988), and another group's evidence that GABAergic and glutamatergic chloride currents in *Aplysia californica* medial pleural neurons did crossdesensitize (King and Carpenter, 1987). Oyama et al. (1990) found that there was a small, transient GABAergic chloride current, insensitive to 1 m*M* glutamate, that activated with 1 μ*M* GABA and completely desensitized in the presence of 100 μ*M* GABA, and a second GABA-gated chloride current, larger and slow to desensitize, that only activated at GABA concentrations of >100 μ*M* and was fully desensitized by even 10 μ*M* glutamate. In addition, micromolar concentrations of GABA were able to reduce the rate at which the glutamate-gated chloride current recovered from desensitization; this effect occurred even in cells not expressing any transient GABA current. The authors concluded that the large, sustained GABA-gated current was caused by GABA crossactivating the IGluR, whereas the transient GABA current was mediated by a distinct receptor.

Although demonstrating that inhibitory glutamatergic currents in both species exhibited sensitivity to GABA, the results of Oyama et al. (1990) also made it clear that the ligand-gated inhibitory currents in *Aplysia kurodai* pedal ganglion neurons and *Aplysia californica* medial pleural neurons were genuinely dissimilar. First, there was no evidence of any transient GABAergic current in the *Aplysia californica* neurons, and the sustained, crossreactive GABAergic current was activated at lower GABA concentrations. Low concentrations (10 μ*M*) of glutamate abolished the entire sustained GABAergic current (1 m*M* GABA) in the *Aplysia kurodai* neurons, whereas in *Aplysia californica* neurons, the authors claimed that 1 m*M* of constitutively-applied glutamate only slightly reduced the current evoked by 50 μ*M* GABA (King and Carpenter, 1987). Whether these differences reflect tissue specificity, interspecific variability, or both is undetermined.

Conformational Specificity of Agonists

Ligand-gated receptors are commonly categorized by their differential responses to selective agonists. The power of this approach is extended by an understanding of the possible molecular conformations of such agonists, and particularly by the systematic variation of agonists according to molecular structure, such that a receptor's agonist profile generates hypotheses about the nature of the binding site. Table 2 lists the structural formu-

lae for some common glutamate and GABA agonists and antagonists.

The glutamate molecule contains three ionizable groups: the amino group and the proximal and distal carboxyl groups (Fig. 2, all arrows). While the spatial relationships among these three groups can vary substantially in the flexible glutamate molecule, they are much more restricted in the glutamate analog ibotenate, which activates IGluRs with a potency close to that of glutamate. Presumably, ibotenate-sensitive IGluRs bind glutamate in the fairly extended conformation, which resembles the conformation of ibotenate. All three ionizable groups are clearly required for optimal binding to most IGluRs, since shifts in the structural relationships among the three charged groups usually reduced or eliminated receptor gating (Tables 1 and 2).

The ubiquitous ibotenate sensitivity of IGluRs further corroborates their structural similarity to GABA receptors. Ionotropic GABA receptors are typically strongly activated by the conformationally restricted agonist muscimol, which is a decarboxylated form of ibotenate in the same way that GABA is a decarboxylated glutamate. This suggests that the agonist binding sites of the two receptor types are similar, and indicates that differences at the binding site for the amino-proximal carboxyl group (Fig. 2, open arrows) are critical for the differentiation of GABA and glutamate sensitivity.

Steric hindrance is also clearly a factor in agonist binding. Kainic acid, a preferred agonist for a class of vertebrate excitatory glutamate receptors, is structurally similar to the IGluR agonist ibotenate and can assume a similar conformation with respect to the three charged groups. However, kainate is entirely ineffective at gating IGluRs, perhaps because of its large side group (depicted in Johnston et al., 1974).

Avermectin Receptors

Avermectins are a naturally occurring class of macrocyclic (16-membered) lactones derived from the mycelia of *Streptomyces avermitilis* ([Burg et al., 1979]; reviewed in [Wright, 1986]) and demonstrated to be potent antihelminthics and insecticides with low toxicity to mammals (Campbell and Benz, 1984). Avermectin B1a (AVM), a major component, and its semisynthetic commercial analog 22,23-dihydroavermectin B1a (ivermectin, IVM; Chabala et al., 1980), have been shown to act specifically on IGluRs (Scott and Duce, 1985; Arena et al., 1992; Cully et al., 1994). They also act on some GABA receptors and can evoke picrotoxin-insensitive conductances unrelated to either receptor (Bokisch and Walker, 1986; Martin and Pennington, 1989). The terms ivermectin/IVM and avermectin/AVM will both be used herein for the sake of precision, although for present purposes they may be considered interchangeable. Avermectins have no effect on excitatory glutamate receptors (Mellin et al., 1983; Zufall et al., 1989).

Avermectin had three general effects on ligand-gated chloride channels. At low (nanomolar) concentrations, avermectin potentiated the response of *C. elegans* IGluRs to glutamate by shifting the glutamate dose–response curve to the left and slowing receptor desensitization (Arena et al., 1992). It also reduced the Hill coefficient of the *C. elegans* IGluR from approx 2 to 1.0, implying that in the presence of avermectin only one molecule of glutamate needs to bind in order to open the channel (Arena, 1994). A similar effect was observed on chick GABA receptors (Sigel and Baur, 1987). At higher concentrations, avermectin can gate IGluRs and some GABA receptors directly (Mellin et al., 1983; Scott and Duce, 1985; Duce and Scott, 1985; Zufall et al., 1989; Arena et al., 1992), although typically GABA receptors are only blocked, not activated, by avermectins. These avermectin-evoked currents are chloride-mediated and blocked by picrotoxin. In single-channel experiments on the *Austropotamobius* multiagonist receptor, avermectin evoked a pattern of conductance states characteristic of glutamate rather than of GABA (Zufall et al., 1989).

At roughly micromolar concentrations, avermectin antagonized IGlu and GABA

receptors (Holden-Dye et al., 1988; Martin and Pennington, 1989; Zufall et al., 1989; Holden-Dye and Walker, 1990; Bermudez et al., 1991). Zufall et al. (1989) noted that the open probability and mean open time of the multiagonist-gated channel both decreased when the avermectin concentration was increased. The blocking effect of avermectins may act at or near the picrotoxin binding site, since both picrotoxin and ivermectin inhibit [^{35}S]TBPS binding to locust GABA receptors (Bermudez et al., 1991), and picrotoxin can reverse some of the effects of ivermectin (Fritz et al., 1979). Ivermectin also inhibited strychnine binding to a rat glycine receptor (Graham et al., 1982). In most species examined, avermectin-induced effects became irreversible at higher concentrations. All of these effects are credible mechanisms for the antihelminthic effects of avermectins.

The threshold concentration for AVM activation of chloride currents varied widely among species. The multiagonist receptor of the crayfish *Austropotamobius torrentium* exhibited the highest affinity for avermectin (0.1 p*M*); in insects and arthropods, thresholds from 10 p*M* to 10 n*M* were found (Scott and Duce, 1985; Duce and Scott, 1985; Zufall et al., 1989). *C. elegans* IGluRs expressed in oocytes from purified mRNA had a threshold concentration of 10 n*M* avermectin for direct current activation (Arena, 1994). Heteromeric channels formed by coexpression of two glutamate-gated chloride channel subunits from *C. elegans* (GluClα, GluClβ) in *Xenopus* oocytes showed a roughly similar affinity for ivermectin. Notably, homomeric channels assembled from the GluClα subunit could be gated by IVM, but not by glutamate, whereas for homomers of the GluClβ subunit the reverse was true (Cully et al., 1994).

The complex dose-dependent effects of avermectin (potentiation, direct gating, blockade, and the induction of irreversibility) have led several authors to propose that avermectin acts at multiple sites (Scott and Duce, 1985; Wright, 1986), although others disagree (Zufall et al., 1989). Binding studies have demonstrated that ivermectin does not act at the glutamate or GABA binding site of *C. elegans* receptors (Schaeffer and Haines, 1989; Cully and Paress, 1991). Experiments with the cloned GluClR α and β subunits from *C. elegans* have since demonstrated that ivermectin effects on the GluClR alone are sufficient to mediate both the chloride-current potentiation and the directly gated current observed in other preparations. The similar avermectin-induced effects on IGluRs and GABA receptors and ivermectin's apparent binding to a glycine receptor lend credence to the idea that these ligand-gated chloride channels retain some conformational and functional similarities, and suggest that ivermectin may be binding to a similar site or sites on each. The qualitative and quantitative differences noted between the effects of avermectin in tissues of different species, coupled with the sufficiency of the two cloned receptor subunits to mediate key effects observed in natural receptors, lend support to the proposal of Arena (1994) that avermectin may be able to act as either a positive or negative allosteric modulator in a tissue-specific manner, analogous to the effects of benzodiazepines on mammalian $GABA_A$ receptors (Olsen and Tobin, 1990; Macdonald and Olsen, 1994).

IGluR Single-Channel Biophysics

Both the locust muscle IGluR and the multiagonist receptor of *Austropotamobius* muscle have been described at the single-channel level (Table 3). Both channels exhibited a 22–25 pS open state and comparable mean open time distributions, and were highly dependent on chloride, although the crayfish multiagonist receptor was at least an order of magnitude more sensitive to glutamate than was the locust receptor. Additionally, patches containing the multiagonist receptor exhibited two higher conductance states (43 pS, 66 pS); these were unusual in that although glutamate activation of the channel favored the 22 pS state, GABA application (10 μ*M*) preferentially evoked the 43 pS state. This phenomenon was

Table 3
Single-Channel Parameters[a]

Genus	Experimental conditions		Conductance states, pS	Mean open times, ms	"Intraburst" closed times, ms	"Interburst" closed times, ms	References
Schistocerca	25 μ*M* glutamate		25	2, 12			Dudel et al., 1989
Austropotamobius	0.5 μ*M* quisqualate		22	0.6, 6			Franke et al., 1986
Austropotamobius	1 μ*M* glutamate	*(i1)*	22[b]	1, 6			Franke et al., 1986
Austropotamobius	1 μ*M* glutamate	*(i2)*	43[c]	2–3			Franke et al., 1986
Austropotamobius	1 μ*M* glutamate	*(i3)*	68	1–2			Franke et al., 1986
Austropotamobius	0.1 μ*M* glutamate[d]		22	2.5	3–4	60–70	Zufall et al., 1988
Austropotamobius	0.1 p*M* AVM		22	3.3	2.1	13.0	Zufall et al., 1989
Austropotamobius	1 p*M* AVM		22	0.5, 2.4	1.6	8.6	Zufall et al., 1989
Austropotamobius	0.1 p*M* AVM[e]		22	0.5, 2.1			Zufall et al., 1989
Astacus GABA-R	300 μ*M* GABA		35	0.3, 6.7	1[f]	7.7	Adelsberger et al., 1994
Mus GABA-R	0.5–10 μ*M* GABA		29	4, 24	2	62	Mathers, 1985
Mus GABA-R	10 μ*M* GABA	*(i1)*	12				Bormann et al., 1987
Mus GABA-R	10 μ*M* GABA	*(i2)*	19				Bormann et al., 1987
Mus GABA-R	10 μ*M* GABA	*(i3)*	30[c]				Bormann et al., 1987
Mus GABA-R	10 μ*M* GABA	*(i4)*	44				Bormann et al., 1987)

[a]Single-channel biophysical parameters of two IGluRs, compared to representative GABA receptors. The labels *i1*, *i2*, and so on, denote multiple conductance states (terminology of Franke et al. [1986]). Receptors that display mean closed time distributions fit by two dissimilar time constants exhibit bursts of activity; the two time constants are labeled "intraburst" and "interburst" accordingly. Low-calcium conditions for the *Austropotamobius* multiagonist receptor (0.1 m*M*, 1 m*M*) are with respect to a standard extracellular calcium concentration of 13.5 m*M*. The four conductance states of the mouse GABA receptor (Hamill et al., 1983) were observed under excised-patch, symmetrical chloride conditions. Under cell-attached conditions, this receptor exhibited three conductance states of 10, 17, and 28 pS, with 17 pS being the preferred GABA-evoked conductance.

[b]Also evoked by 0.5 m*M* quisqualate.

[c]Preferred conductance state when activated by GABA (10 μ*M*).

[d]Also evoked by 10 n*M* carbachol under low-calcium (0.1 mM) conditions.

[e]Low-calcium conditions (1 m*M*).

[f]An additional time constant, too fast for accurate measurement, was also indicated.

not simply the result of multiple superimposed channel openings along with a greater sensitivity of the receptor to GABA, since the 22 pS state was less probable in the presence of GABA than either the closed state or the 43 pS state.

The multiagonist receptor exhibited nearly identical mean open time distributions in response to 1 μ*M* glutamate and 10 μ*M* GABA; both could be fit with two exponentials (Franke et al., 1986). The receptor was also gated by the cholinergic agonist carbachol; the carbachol-activated open time distribution could be fit with a distinct single exponential similar to that evoked by a 10-fold lower concentration of glutamate (0.1 μ*M*). Note that these experiments were performed in low external calcium, since the carbachol-gated current was inhibited by normal external calcium concentrations (Zufall et al., 1988). The open time distributions for the higher conductance states could be fit by a single exponential. The conformationally restricted glutamatergic agonist ibotenic acid only evoked the 22 pS state, whereas its decarboxylated derivative muscimol, a GABAergic agonist, preferentially evoked the 43 pS state. Quisqualate also only evoked the 22 pS state (Zufall et al., 1989). When applied to the multiagonist receptor, picrotoxin (100 μ*M*) completely blocked the 43 and 66 pS conductance states evoked by GABA and reduced the open probability of the 22 pS state by 90%. In response to glutamate application, the same concentration of picrotoxin (100 μ*M*) was less effective, but followed the same pattern, reducing the open probability of the 22 pS state only moderately and that of the higher conductance states more strongly. A higher concentration of picrotoxin (1 m*M*) was more effective in blocking the glutamate-evoked conductances. Further analysis revealed that picrotoxin selectively blocked the longer of the two time constants of the 22-pS conductance state (Franke et al., 1986).

Low concentrations of avermectin (0.1 p*M*) activated the multiagonist receptor with a single-exponential open time distribution similar to that evoked by 0.1 μ*M* glutamate. Higher concentrations of AVM (1 p*M*) or glutamate (1 μ*M*) elicited open time distributions requiring two exponentials to fit. Interestingly, in low external calcium, the channel responded to 0.1 p*M* AVM as it did to 1 p*M* AVM under normal conditions. Avermectin activated no current when applied to the interior membrane surface, nor did it evoke any conductances in patches that were insensitive to glutamate, GABA, or carbachol; thus, it was not acting as an ionophore (Zufall et al., 1989). At higher concentrations (10 p*M*), avermectin effects became irreversible.

Closed-time distributions of the multiagonist receptor could be fit by two time constants; the longer time constant described "bursts" of channel opening. Glutamate and carbachol application both desensitized the *Austropotamobius* multiagonist receptor over time, whereas GABA did not (Franke et al., 1986). The locust IGluR desensitized to glutamate with a time constant of >1 s and recovered from desensitization with a time constant of about 300 ms (Dudel et al., 1989).

IGluR Molecular Biology

As detailed above, the IGluR has long been presumed to be an ionotropic receptor channel, largely because of its role as a classical "fast" synaptic receptor. This has been confirmed by the cloning of two glutamate-gated chloride channel subunits, entitled GluClα and GluClβ, from the nematode *C. elegans*. These IGluR clones share sequence similarity with ionotropic glycine and GABA receptors, and when expressed in *Xenopus* oocytes, form functional receptor channels with physiology and pharmacology comparable to natural IGluRs, including sensitivity to picrotoxin (Cully et al., 1994). They do not resemble the excitatory glutamate receptors. The GluClβ subunit contains a binding site for glutamate, since GluClβ homomers responded to glutamate, but GluClα homomers were glutamate-insensitive. Conversely, GluClα (but not GluClβ) homomers were gated by ivermectin. It will be

interesting to learn whether these homomeric channels are picrotoxin-sensitive.

In amino acid sequence, the cloned GluCl receptors are more closely related to (vertebrate) glycine receptors than to GABA receptors, even invertebrate GABA receptors (Fig. 4; Table 4). They also share with glycine receptors the feature of two conserved Cys-Cys pairs, whereas GABA receptors incorporate only one of these pairs. However, a tyrosine residue is found in GABA and GluCl receptors at the site corresponding to GluClβ residue 182, whereas glycine receptors incorporate phenylalanine at this site (Fig. 4, asterisk). This site is highly conserved in each receptor family; mutation of this residue alone is sufficient to change a glycine receptor into a GABA receptor (Schmieden et al., 1993). It is possible that this tyrosine residue alone is responsible for the absence of glycine sensitivity among IGluRs.

No second-messenger-mediated modulatory effects on IGluRs have been described. However, the intracellular loop of the GluClα clone between M3 and M4 contains a strong consensus sequence for phosphorylation by protein kinase C. Cully et al. (1994) suggest that wild-type IGluRs include additional subunits other than GluClα and GluClβ; any such subunits could also incorporate modulatory sites.

Physiological Roles of IGluRs

Although diverse physiological effects have been attributed to IGluRs, a role for these receptors has been demonstrated in relatively few preparations. In the crustacean stomatogastric ganglion (Harris-Warrick et al., 1992), IGluRs mediate fast synaptic inhibition, which underlies the rhythmogenic properties of this central pattern generator. Modulation of these synapses alters the oscillatory pattern of these motor neurons, and consequently directly affects behavioral output (Johnson et al., 1995). Interplay between fast IGluRs and slow, presumably metabotropic glutamate receptors is also implicated in the regulation of motor output (Elson and Selverston, 1995). Similar synaptic receptors may underlie other oscillatory neural networks; synaptic pharmacology has not been explicitly described in detail in most central pattern generator circuits.

No clear role has been demonstrated for the extrajunctional IGluRs present on some protostome muscles, although some such muscles do respond directly to modulatory agents (Dickinson, 1995). Extrajunctional IGluRs can be considerably more sensitive to glutamate than are synaptic neuronal IGluRs; it is also typical that IGluRs exhibit greater sensitivity to glutamate than do excitatory glutamate receptors expressed in the same tissue. The increased glutamate sensitivity of extrajunctional IGluRs suggests that low circulating concentrations of glutamate in the hemolymph may act to modulate muscle contractility by acting on these receptors and reducing resting input resistance. In contrast, the inhibitory, presumably junctional GABA receptor on crayfish muscle requires a considerably higher concentration of agonist in order to activate inhibitory conductances (Adelsberger et al., 1994).

Glutamate is also abundant in the circulation in several molluscan and arthropod species (Camien et al., 1951; Kerkut and Cottrell, 1962; Murdock and Chapman, 1974; Henry et al., 1991). Murdock and Chapman (1974) have measured L-glutamate concentrations in the hemolymph of three arthropod species and concluded that, in crayfish and tarantula spiders, plasma glutamate concentration was well below threshold concentrations for excitatory glutamatergic neurotransmission, whereas in locust, it was higher. However, at least in the crayfish (*Astacus* spp.), these resting glutamate levels were near the 1-μ*M* threshold for IGluR activation (measured in the closely related crayfish genus *Austropotamobius* [Franke et al., 1986]). Any increase in free plasma glutamate concentration, therefore, could have direct functional consequences. For example, it may well be adaptive for the animal to avoid muscle contraction during molt, since most behaviors in many crustaceans require that the animals' extrinsic muscles be anchored to the exoskeleton. It is known that serum levels of several

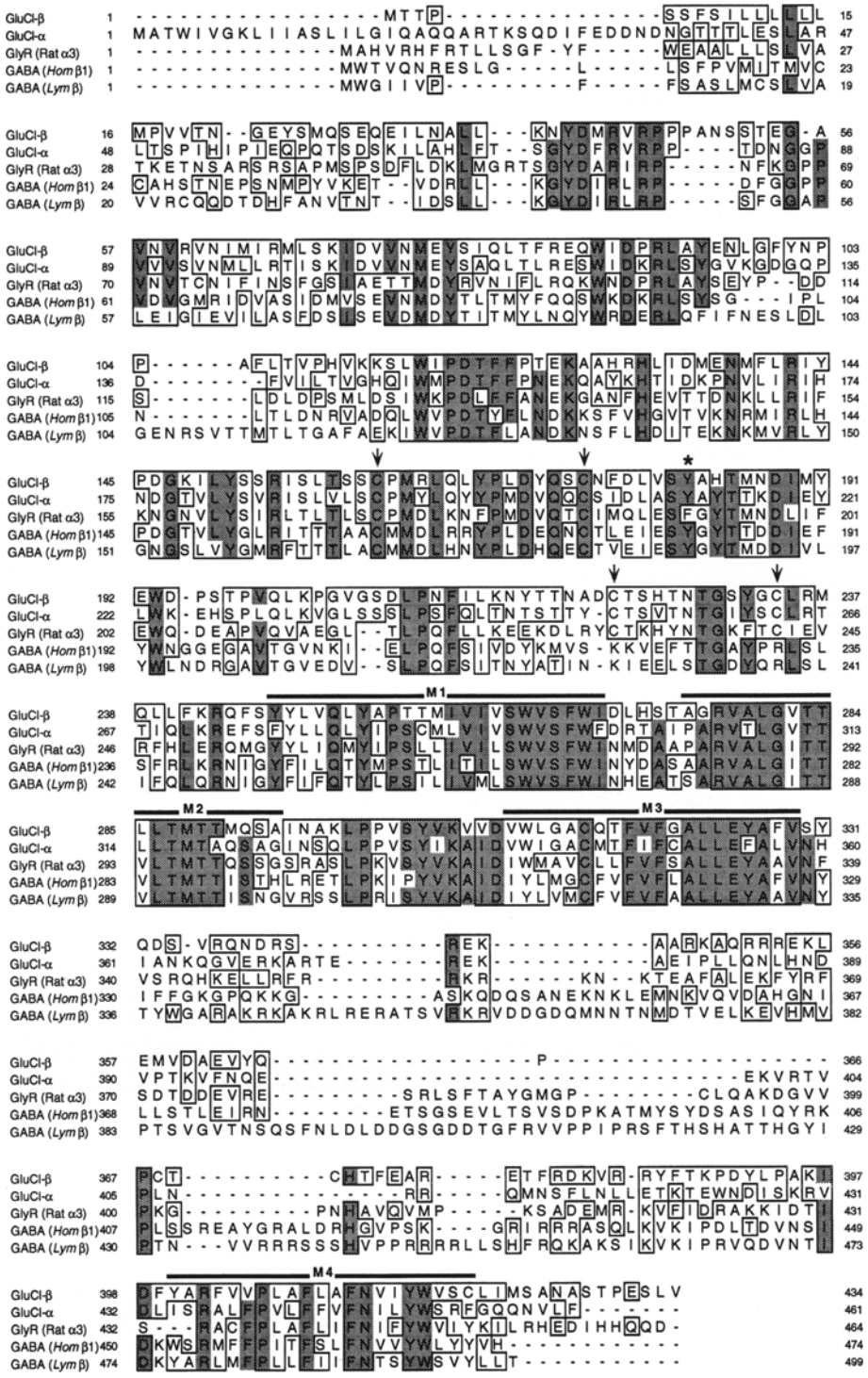

Fig. 4. Alignment of the *Caenorhabditis elegans* GluClα and GluClβ subunit protein sequences with related glycine and GABA receptor subunits, and identification of motifs common to ligand-gated chloride channels. Both GluClR subunits exhibit the greatest degree of sequence similarity to glycine receptor α-subunits; this is supported by phylogenetic analyses with a distance matrix algorithm (Cully et al., 1994). Furthermore, one pair

trace elements, including calcium, vary during the molt cycle in *Austropotamobius pallipes* (Adams et al., 1982); free amino acid levels, including glutamate, may be similarly regulated. If free plasma glutamate concentration was elevated during molt, it could inhibit muscular contractions that may be maladaptive in the absence of the rigid exoskeleton. Alternatively, Zufall et al. (1988) have suggested that the decrease in free plasma calcium levels during ecdysis may directly mediate reduced muscle fiber excitability. It may also be relevant to note that although the plasma glutamate concentration in locust was considerably higher than in crayfish, the locust muscle IGluR activation threshold was also elevated.

Phylogenetic Distribution and Analysis

The significance of similarities and differences among biological phenomena—whether morphological characters, molecular sequences, behaviors, or physiological properties—cannot be fully understood until they are examined through the lens of their phylogenetic relationships. Incorrectly judging a pair of molecules or organs as homologous or convergent (Table 5) can lead to erroneous hypotheses about their evolution and misdirect the course of further research.

Understanding the evolutionary history of extant molecules is crucial for constructing valid hypotheses about ancestral molecules and their divergence and adaptation (Fitch, 1970). By sorting out IGluRs phylogenetically, one may be able to sort IGluRs into more functionally uniform subcategories, and thereby make sense of observed functional differences. This type of analysis has implications for the evolution of the ligand-gated receptor superfamily and highlights the great potential ability of protostome receptor proteins to answer fundamental questions in adaptive molecular evolution.

The IGluRs constitute a category based on functional (physiological and pharmacological) properties, which are unreliable criteria for homology or for inferring evolutionary relationships. Receptor agonist specificities and ionic permeabilities can be altered by single point mutations; it is quite possible that different subgroups of IGluRs evolved separately from distinct ancestral molecules. Addressing this possibility will require molecular sequencing of IGluR genes and proteins. Nevertheless, examining the distribution of characters on an

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of cysteine residues is common to GluCl and glycine receptors, but not to GABA receptors; the other pair is completely conserved among all of these ligand-gated receptors (arrows). However, the GluClRs resemble GABA receptors at the single residue responsible for determining GABA or glycine selectivity (GluClβ; Nistri, 1981; asterisk); this alone may be sufficient to explain the insensitivity of IGluRs to glycine. The four transmembrane regions are depicted by lines labeled M1–M4. Residues that are identical in at least four of the five sequences are shaded. Residues were analyzed for conservative substitutions with respect to GluClβ using the Kyte-Doolittle algorithm (Kyte and Doolittle, 1982); those that are conservatively substituted (pair-comparison values ≥85%, [Riek et al., 1995]) are boxed.

The GluCl sequences were obtained through GenBank over the World Wide Web; similar sequences were selected using the BLASTP 1.4.9MP algorithm (Altschul et al., 1990) available at the National Center for Biotechnology Information (NCBI) Web site. The rat α3 glycine receptor subunit was the most similar in sequence to both GluClR subunits; the human $GABA_A$ β1-subunit was the GABA receptor most similar to the glutamate sensitive GluClβ subunit; the *Lymnaea* GABA receptor (β-subunit) was an invertebrate subunit more closely resembling the GluClRα sequence. These five sequences were aligned using the ClustalW 1.5 algorithm provided by the Baylor College of Medicine Web server. The sequence alignments were imported into SeqVu 1.0 (shareware; Garvan Institute of Medical Research, Sydney, Australia) for assessment and depiction of identities and conservative substitutions. Transmembrane regions 1–4 were derived from the work of Cully et al. (1994). Accession numbers for these sequences are: *C. elegans* GluClβ: U14525; *C. elegans* GluClα: U14524; rat glycine receptor, α3-subunit: P24524; $GABA_A$ receptor subunit from *Homo sapiens*: P18505; ionotropic GABA receptor subunit from the pulmonate mollusc *Lymnaea stagnalis*: P26714.

Table 4
Percent Sequence Identity and Similarity[a]

	GluClα	Rat GlyR, α3	*Homo* GABA-R, β1	*Lymnaea* GABA, β
Identity				
GluClα	—	40.3%	34.4%	34.9%
GluClβ	47.3%	39.8%	35.6%	34.8%
Similarity				
GluClα	—	62.8%	57.0%	59.1%
GluClβ	64.6%	61.6%	58.5%	58.0%

[a]Sequence identities and similarities between *C. elegans* GluCl receptors and representative GABA and glycine receptors. Calculations and determinations of similarity were performed with the GAP program of the University of Wisconsin GCG program suite (version 8.1), an implementation of the Needleman-Wunsch algorithm for pairwise sequence comparisons (described in Hillis and Moritz, 1990).

Table 5
Glossary[a]

Clade: A monophyletic taxon. In the cladistic view, only monophyletic taxa are valid or natural groupings (Hall, 1994).

Homologous molecules are similar because of common evolutionary origin. "A character of two or more taxa is homologous if this character is found in the common ancestor of these taxa, or...if one [character] is directly (or sequentially) derived from the other(s)" (Wiley, 1981). Judgments about homology should be ascertained independently for different hierarchical levels of biological organization (discussed in Striedter and Northcutt, 1991; Hall, 1994).

Homoplasous molecules are similar owing to independent evolution, as by convergence (analogy); they are not homologous. "A character found in two or more species is [homoplasous]...if the common ancestor of these species did not have the character in question, or if one character was not the precursor of the other" (Wiley, 1981).

Monophyletic: A group of organisms (or taxa) is monophyletic if all its members share a common ancestor and if all the evolutionary descendants of that ancestor are included within the group. Members of a monophyletic taxon share homologous characters and an evolutionary history that are not shared with organisms outside the group. In the cladistic view, only monophyletic taxa are valid and homology is defined in terms of monophyly (Patterson, 1982; Hall, 1994).

Orthologous molecules are homologs that diverged because of speciation. The IGluRs in *Helix aspersa* and *Helix pomatia* are probably orthologs.

Paralogous molecules are homologs that diverged because of gene duplication. The GluClα and GluClβ IGluR subunits from *C. elegans* are probably paralogs.

[a]Glossary of terms, derived from Fitch (1970), Wiley (1981), Gray and Fitch (1983), Striedter and Northcutt (1991), Hillis (1994).

independently derived phylogeny (Fig. 3) can help distinguish orthologs from homoplasies (Bolker and Raff, 1996; *see also* Table 5). Homoplasous IGluRs may be tentatively identifiable by incongruous patterns of characters with respect to the distribution of characters among the IGluRs of related taxa, provided that a sufficient number of consistently defined characters have been described. The most interesting receptors can then be selected for sequencing and detailed study.

Protostome Phylogeny

Figure 3 depicts the phylogenetic relationships among the taxa discussed in this article, as well as some significant outgroups.

Although there is considerable dispute regarding the branching order of metazoan diversification, the phylogeny presented is based on the following considerations. Morphological and developmental characters have traditionally been used to depict Arthropoda, Annelida, and Mollusca as monophyletic clades, with Annelida and Arthropoda sharing the more recent common ancestor. Although this work has been carefully done, all such characters are potentially adaptive and therefore possibly convergent, such that independent confirming data are desirable.

Analyses of ribosomal DNA sequence divergences provide a powerful complement to traditional methods; sophisticated algorithms provide hypotheses of relationships that can be incorporated into cladograms (Hillis and Moritz, 1990). However, there are many factors capable of severely biasing such methods (e.g., multiple substitutions at a single site, attributed to high degrees of divergence and/or accelerated rates of sequence change [Field et al., 1988; Swofford and Olsen, 1990; Turbeville et al., 1991]), particularly across broad phylogenetic ranges. Furthermore, analyses of different sequences and/or the use of different algorithms often yield conflicting hypotheses of relatedness (Boore and Brown, 1994; Boore et al., 1995). Consequently, sequence analysis methods require careful criticism of the applicability of underlying assumptions and the robustness of the method for a particular data set (see Turbeville et al., 1991; Friedrich and Tautz, 1995), such that underdocumented reports warrant some skepticism.

Mitochondrial gene rearrangements probably constitute the strongest single basis for hypothesizing phylogeny (Boore and Brown, 1994; Boore et al., 1995). The enormous number of possible gene rearrangements render it highly improbable that the same arrangement could arise more than once independently. Gene rearrangements are not susceptible to the undetectable multiple substitutions that can bias rDNA sequence analyses; they are qualitative characters and can be meaningfully examined by outgroup analysis. Relatedness among taxa can therefore be reconstructed from the patterns of inheritance of shared-derived features.

The thickest lines within Fig. 3 are derived from cladistic analyses of mitochondrial gene rearrangement (Boore et al., 1995), and are additionally consistent with many of the hypotheses generated by other methods. The medium lines (within Decapoda) are drawn from cladistic analyses based on morphological characters (Scholtz and Richter, 1995). The thinnest lines depict relationships based on Linnean taxonomic classification (grade); although not based on cladistic analysis, most of these relationships are clear enough to reasonably hypothesize a bifurcating phylogeny (with exceptions noted in Fig. 3).

The Pseudocoelomata (represented by the nematodes) have been depicted in Fig. 3 as diverging from the other Bilateria before the protostome-deuterostome split; that is, they are not considered protostomes. Recent 18S rDNA sequence analyses (Friedrich and Tautz, 1995) and cladistic analyses based on morphology (Eernisse et al., 1992) support this phylogeny. This suggests either that the IGluR predates the common ancestor of protostomes and deuterostomes, implying a secondary loss of the IGluR in at least the vertebrate deuterostome descendants, and/or that the IGluR evolved separately in nematodes and protostomes. Glutamatergic inhibition of contraction in the muscles of the sea anemone *Actinia* lends credibility to the hypothesis of early origin. Note that some other phylogenies (e.g., Raff and Kaufman, 1983) depict the pseudocoelomates as nonspiralian protostomes; that is, they diverge from the protostome lineage after the deuterostomes, although they are not considered spiralians.

Phylogenetic Character Distribution

Inhibitory glutamate receptors have been clearly demonstrated in diverse protostome taxa. Although they possess many common properties, they also exhibit a degree of physiological and pharmacological variability. For exam-

ple, some IGluRs are activated by the glutamate analog quisqualate, although the majority are not (Fig. 3; Table 1). Since quisqualate has been considered an important diagnostic agonist for an inhibitory metabotropic glutamate receptor that activates a potassium current, as well as for the excitatory AMPA-type glutamate receptor, this has led to some confusion in the literature.

In contrast, the distribution of GABA cross-responsivity among the IGluRs is uneven. If the IGluRs constitute a single family of orthologous molecules, then GABA sensitivity must have evolved several times independently, that is, at least once for ionotropic GABA receptors themselves, once for the GABA cross-desensitization demonstrated in the opisthobranch mollusc *Aplysia* (King and Carpenter, 1987, 1989; Oyama et al., 1990), a third time for the GABA crossactivation observed in the European crayfish *Austropotamobius* (Franke et al., 1986; Zufall et al., 1988), and perhaps yet again in the chelicerate *Limulus*, in which aspartate crossdesensitized both GABA- and glutamate-gated chloride currents. Alternatively, if IGluRs are not all homologous, any of these GABA-sensitive receptors could be independently derived, perhaps from GABA receptors.

Notably, no receptor examined exhibited significant crossreactivity to glycine, even though the agonist-binding pockets of the glycine and GABA receptors are also very similar; the presence or absence of a single hydroxyl group can confer GABA responsivity onto a glycine receptor (Schmieden et al., 1993). A very low sensitivity to glycine was reported for the multiagonist receptor in *Austropotamobius* (Franke et al., 1986); other reports of IGluR crossactivation by glycine were not replicable (Laughton et al., 1995).

Several other features of IGluRs also varied significantly among taxa (Table 1); however, an insufficient breadth of data is available to support phylogenetic inferences. Note that if the inhibitory glutamate response in *Actinia* is mediated by an IGluR, then evidence for IGluRs extends to a phylogenetically more ancient root than does evidence for GABA or ACh receptors, raising the possibility that the IGluR represents the primitive condition within the ligand-gated receptor superfamily.

Implications for Molecular Evolution

The study of the phylogenetic origin of IGluRs and related receptors has promising implications for molecular evolution. Sequence analyses have demonstrated that pseudocoelomate GluClR subunit clones are most similar to (vertebrate) glycine receptors, in terms of both identical and conservatively substituted residues (Table 4; Cully et al., 1994), and both GluCl and glycine receptors are more similar to one another than either are to any GABA receptor, even though IGluRs are largely restricted to protostomes and glycine receptors have only very rarely been observed in invertebrates (Sawada et al., 1984c; Giles and Usherwood, 1985a). Furthermore, GluClRs share some potentially significant residues with glycine receptors that are lacking in GABA receptors. In addition to the pair of cysteine residues that is conserved in ligand-gated chloride channels, GluClRs (both α- and β-subunits) contain an additional pair of cysteine residues that is found in glycine receptor subunits, but not in GABA receptors (Cully et al., 1994). In contrast, IGluRs share with GABA receptors the single conserved residue distinguishing GABA sensitivity from glycine sensitivity (Fig. 4, asterisk; Schmieden et al., 1993). Nevertheless, their considerable sequence similarities suggest that glycine receptors constitute the sister group of IGluRs.

Insufficient comparative data are available to allow us to draw strong conclusions concerning the points in phylogeny at which various members of the ligand-gated receptor superfamily arose. Nevertheless, a consideration of the properties of these receptors, along with the available sequence data, allows us to generate testable hypotheses concerning the relationships among the members of this receptor family. Two such hypotheses are depicted in Fig. 5. Since primary peptide

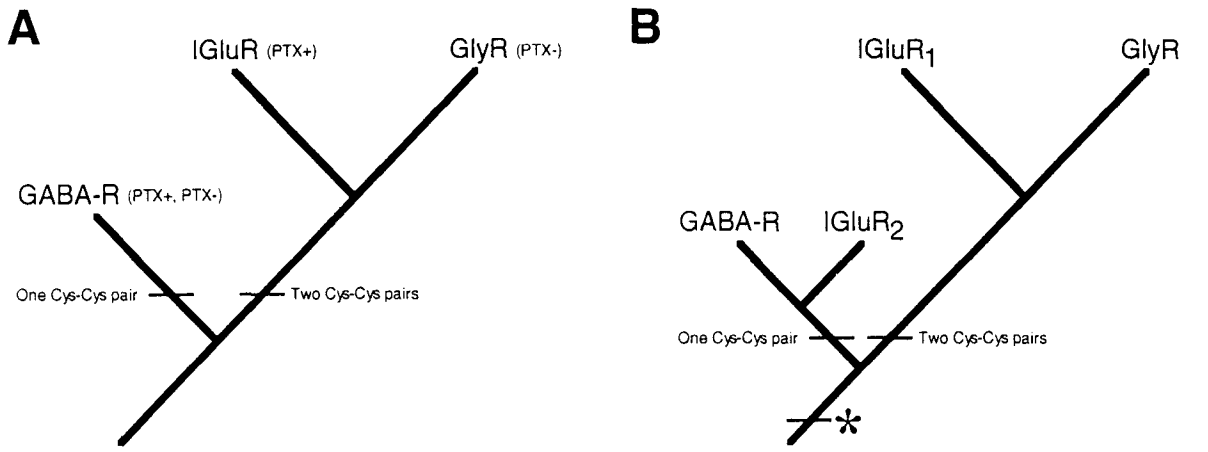

Fig. 5. Cladograms illustrating different hypotheses concerning the evolutionary origins of the IGlu receptor. **(A)** Cladogram based on receptor primary peptide sequence as the most cladistically diagnostic character and presuming homology among all extant IGluRs. Two pairs of Cys residues are characteristic of glycine receptors and the cloned GluCl subunits from *C. elegans*; GABA receptors cloned to date have only one Cys-Cys pair. The label "PTX+" indicates picrotoxin sensitivity, "PTX–" indicates insensitivity to picrotoxin, and both together indicate that the clade contains both picrotoxin-sensitive and picrotoxin-insensitive receptors. **(B)** Cladogram depicting the alternative hypothesis that distinct subgroups of functionally defined IGluRs are derived independently from different ancestral molecules (two subgroups are arbitrarily depicted). This model implies that the sequence characters exhibited by the *C. elegans* GluCl clones are not universal among IGluRs, but rather that a separate, convergent family of IGluRs evolved from an ancestor shared most recently with GABA receptors. This cladogram could also be interpreted to mean that IGluRs represent the primitive condition among ligand-gated chloride channels (asterisk), i.e., that GABA and glycine receptors are derived from a glutamate-gated ancestral receptor. There is no direct evidence for either interpretation of this alternative cladogram; subunit shuffling or convergence at the primary sequence level could also account for extant data.

sequence is a stronger criterion for homology than are pharmacological properties, IGluRs are clustered with glycine receptors in Fig. 5A, which assumes that all IGluRs are homologous. This phylogeny forces the conclusion that picrotoxin sensitivity and GABA cross-reactivity are convergent characters—i.e., that they evolved more than once independently. Furthermore, some, but not all, cation-permeable GABA receptors (Yarowsky and Carpenter, 1978; Cazalets and Harris-Warrick, 1989) and chloride-permeable acetylcholine receptors (Yarowsky and Carpenter, 1978b; Schmidt and Calabrese, 1992) are picrotoxin-sensitive, clearly demonstrating that agonist profile, picrotoxin sensitivity, and ionic selectivity are not strongly correlated. This demonstration that molecular pharmacology can be highly and repeatedly convergent opens up several intriguing possibilities about the evolutionary adaptation of receptor channel properties that cannot be resolved with the sparse extant data. Certainly the agonist specificity of any receptor should be considered as an adaptive property of the molecule, highly susceptible to convergence, rather than as a stable feature. GABA receptors, IGluRs, nAChRs, and glycine receptors should not be assumed to constitute discrete molecular taxa solely on the basis of agonist selectivity. The cladogram in Fig. 5B presents an alternative hypothesis of IGluR evolution, in which the glycine receptor-like peptide sequences of the cloned *C. elegans* IGluR subunits are only representative of the $IGluR_1$ clade. Under this phylogeny, the IGluRs either evolved independently more than once (the hypothetical $IGluR_2$ clade having evolved independently from an ancestral GABA receptor) or represent the primitive condition (Fig. 5B, asterisk).

Altering the agonist specificity and other properties of functional receptors could involve altering subunit composition rather than, or in addition to, evolutionary changes in subunit sequences. That is, functional IGluRs and GABA receptors could incorporate subunits from the same pool. This idea has been previously proposed for GABA and glycine receptors in cultured mammalian spinal neurons (Hamill et al., 1983; Bormann et al., 1987) and has been suggested for *Aplysia* ligand-gated receptors (King and Carpenter, 1989). The hypothesis offers a parsimonious explanation for the substantial diversity of receptor properties in closely related taxa, and may account for some "unusual" receptors (from a vertebrate perspective), such as curare-sensitive GABA receptors (Kehoe, 1972; Carpenter et al., 1977), excitatory GABA receptors (Arshavsky et al., 1993) that can be sodium-permeable (Yarowsky and Carpenter, 1978b; Cazalets and Harris-Warrick, 1989), avermectin-sensitive (Mellin et al., 1983), and either picrotoxin-sensitive (Cazalets and Harris-Warrick, 1989) or picrotoxin-insensitive (Yarowsky and Carpenter, 1978b), strychnine, picrotoxin, and bicuculline-sensitive chloride-permeable acetylcholine receptors (Yarowsky and Carpenter, 1978b; Schmidt and Calabrese, 1992), avermectin-sensitive inhibitory (chloride-permeable) and excitatory (sodium-permeable) acetylcholine receptors (Bokisch and Walker, 1986), and vertebrate GABA receptors that are potentiated by glutamate (Stelzer and Wong, 1989). Testing this hypothesis will require a great deal more sequencing and study of diverse invertebrate ligand-gated receptor subunits, but promises to increase our understanding of the adaptive coevolution of neurotransmitters and receptors that has produced today's considerable neurophysiological diversity. It will be interesting to see how the sequencing of these receptors will influence the implicit assumptions deriving from contemporary molecular tree analyses, which are overwhelmingly based on vertebrate receptor clones and consequently segregate receptor channel species cleanly by ionic selectivity and agonist specificity (Ortells and Lunt, 1995).

Protostome ligand-gated chloride channels and their immediate outgroups exhibit a substantial number of easily quantifiable physiological and pharmacological characters that vary semi-independently—a much greater range of character states for a relatively restricted, homologous set of molecules than is available within the phylogenetically much narrower clade of mammals, or even vertebrates. This diversity, coupled with the low cost of and ease of experimentation on invertebrate animals, presents optimal circumstances under which to study the interplay of hierarchies of biological organization—e.g., the primary sequence and physiological function of ion channels—underlying the evolutionary process (Striedter and Northcutt, 1991). The sequencing of a substantial number of protostome IGlu, GABA, nACh, and glycine receptors across a sufficiently broad phylogenetic range could contribute enormously to a practical understanding of molecular evolution.

Glutamatergic Inhibition in Vertebrates

There is no clear evidence that any IGlu-like inhibitory glutamate receptor channel is expressed in vertebrates, although there are inhibitory potassium currents activated by metabotropic glutamate receptors (Masu et al., 1991; Houamed et al., 1991; Chiba and Saito, 1994), calcium-gated potassium currents activated presumably by calcium influx through glutamate receptor channels (Nicoll and Alger, 1981), and inhibitory responses due to activation of inhibitory interneurons by glutamate. Although several studies have offered evidence for glutamate-activated chloride currents in vertebrate tissues, none provide sufficient evidence to suggest similarity to the protostome IGluR and most are clearly unrelated. A few of these inhibitory responses in vertebrates are briefly noted here.

Glutamate Transporter-Channels

Glutamate-activated chloride currents have been reported in the ON-bipolar cells of the

retinae of the white perch (Grant and Dowling, 1995) and the tiger salamander (Eliasof and Werblin, 1993; Picaud et al., 1995). Both currents exhibit a "glutamate transporter-like" pharmacology, i.e., inhibition by glutamate uptake inhibitors, such as L-trans-pyrrolidine-2,4-dicarboxylic acid (tPDC), dependence on extracellular sodium, and insensitivity to the glutamatergic agonists quisqualate, kainate, and NMDA, and the antagonists kynurenic acid and CNQX. Furthermore, the perch current was resistant to picrotoxin and strychnine blockade and was not activated by ibotenate; there are no corresponding data for tiger salamander. The salamander current is generated by a novel type of glutamate/aspartate transporter that generates an intrinsic chloride current; it has been well characterized biophysically (Larsson et al., 1996) and does not resemble the IGluR. The glutamate-generated current in perch physiologically and pharmacologically resembles that of the salamander transporter and is similarly unlike the IGluR-mediated current.

Cerebellum

An excitatory amino acid transporter protein, EAAT4, has recently been cloned from human cerebellum (Fairman et al., 1995). Its peptide sequence does not resemble those of the *C. elegans* GluClR subunits or other ligand-gated receptors. When expressed in *Xenopus* oocytes, EAAT4 strongly resembles the glutamate transporters in perch and salamander retina: L-glutamate and L-aspartate elicit a chloride current that is dependent on extracellular sodium, calcium-independent, and not blocked by the chloride channel antagonists 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid (SITS), 4,4'-diisothiocyanatostilbine 2,2'-disulfonic acid (DIDS), niflumic acid, or 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) at concentrations known to block endogenous chloride currents in oocytes. EAAT4 seems to resemble the glutamate transporters of vertebrate retina, and is clearly not related to the GluCl (IGlu) receptor family.

Another inhibitory response to glutamate has been described in guinea pig cerebellum (Yamamoto et al., 1976). It is insensitive to high magnesium concentrations, ruling out indirect synaptic inhibition via interneurons. However, the response was picrotoxin-insensitive and chloride-independent—a pharmacology more consistent with known vertebrate metabotropic glutamate receptors than with IGluR channels.

Frog Spinal Motor Neurons

In vitro preparations of spinal neurons from *Rana* species respond biphasically to glutamate, with a transient hyperpolarization followed by a sustained depolarization (Arenson and Nistri, 1982; Nistri and Arenson, 1983). The inhibitory phase of the current was not inhibited by high concentrations of magnesium, which blocks chemical synaptic transmission. It was correlated with a roughly 25% increase in membrane conductance, was unaffected by cesium, and was of the same polarity as chloride-mediated responses to GABA, suggesting that the glutamatergic inhibition was carried by chloride rather than by potassium.

The IGluR agonist ibotenate also had a hyperpolarizing effect on these neurons; however, this response was slower than the depolarizing effect and was blocked by either high magnesium or 1 μM tetrodotoxin (Nistri, 1981). Furthermore, the inhibition persisted for many minutes after removal of the agonist from the bath. A superficially similar current was also induced by ibotenate in feline spinal interneurons (Macdonald and Nistri, 1978); this effect was not antagonized by strychnine, picrotoxin, or bicuculline. These ibotenate responses (unlike those to glutamate) are likely to be mediated by secondary interneuronal inhibition; they do not resemble IGluR responses. In contrast, known IGluRs are all activated by ibotenate.

Although there is insufficient evidence so far to make a credible case that these data indicate the presence of an IGluR-like channel in vertebrates, the data allow for this possibility. If further studies support the inclusion of this

receptor among IGluRs, it will be a particularly interesting outgroup.

Notes Added in Proof

1. A cDNA clone of an IGluR subunit from *Drosophila melanogaster* heads, DrosGluClα, has recently been reported (GenBank/EBI U58776; Cully et al., 1996). Although probably less than full length, when expressed in *Xenopus* oocytes it formed homomeric channels with IGluR-like physiology and pharmacology and a Hill coefficient of 2.0. The chloride-permeable channel was activated by L-glutamate, ibotenate, and ivermectin, weakly (~5%) activated by D-glutamate and aspartate, and insensitive to quisqualate, kainate, NMDA, glycine, GABA, and histamine. Activation of homomeric DrosGluClα channels by *both* glutamate and ivermectin is novel, since glutamate and ivermectin sensitivity are mediated by different GluCl subunits in *C. elegans*. Furthermore, unlike either of the *C. elegans* subunit clones (GluClα and GluClβ), the DrosGluClα channel exhibited rapid, concentration-dependent desensitization and was only marginally potentiated by low concentrations of ivermectin. DrosGluClα's EC_{50} for glutamate was 41 n*M*, demonstrating a considerably higher affinity for glutamate than *C. elegans* GluCl heteromeric (EC_{50} = 1360 μ*M*) or β-homomeric (EC_{50} = 380 μ*M*) channels. Ivermectin activation of DrosGluClα channels was irreversible; when gated by ivermectin, the current-voltage plot was slightly inwardly rectifying and resistant to blockade by picrotoxin (nil at 100 μ*M*, 14% block at 500 μ*M* PTX). The Hill coefficient for ivermectin gating was 1.2.

   DrosGluClα shares 48% amino acid sequence identity with *C. elegans* GluClα and 43% with GluClβ. It contains the four conserved cysteine residues characteristic of GluCl and glycine receptors (Fig. 4, arrows), and a tyrosine residue at the agonist selectivity site characteristic of GluCl and GABA receptors (GluClβ 182; Fig. 4, asterisk). DrosGluClα clustered with the *C. elegans* GluCl subunits in a phylogenetic analysis of ligand-gated channels performed by the authors, and is otherwise most similar to glycine receptor α-subunits (57% similarity).

   A *Drosophila* glutamate-gated chloride channel has been previously described in larval muscle (Delgado et al., 1989). Like the multiagonist receptor of *Austropotamobius*, it is crossactivated by GABA, and the two agonists evoke different distributions of channel conductance states. In the presence of 200 μ*M* GABA, three conductance states are observed (40, 80, and 110 pS), among which the 40 pS state is most probable. In the presence of 200 μ*M* L-glutamate, only the 40 and 80 pS states are seen, with the 80 pS state the most probable. In addition, channels activated by GABA have longer mean open times than when they are activated by L-glutamate.
2. The insensitivity of *C. elegans* GluClα homomeric channels to glutamate has recently been demonstrated to be caused not by lack of a glutamate binding site, but by a deficiency in coupling ligand binding to channel gating (Etter et al., 1996). Chimeric channels consisting of a GluClα extracellular domain and a GluClβ pore domain were highly sensitive to glutamate (EC_{50} = 530 n*M*), indicating the presence of a glutamate binding site on GluClα subunits. Furthermore, a single amino acid substitution in the presumed M2 pore region could enable glutamate activation of GluClα homomeric channels. Finally, ivermectin binding to GluClα homomeric channels enabled further glutamate activation of the chloride current, presumably by inducing a conformational change that coupled glutamate binding to channel gating. All known subunits of heteromeric GluCl channels, therefore, have the potential to bind glutamate.

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