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Glutamate-Gated Inhibitory Currents of Central Pattern Generator Neurons in the Lobster Stomatogastric Ganglion

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Inhibitory glutamatergic neurotransmission is an elemental "building block" of the oscillatory networks within the crustacean stomatogastric ganglion (STG). This study constitutes the initial characterization of glutamatergic currents in isolated STG neurons in primary culture. Superfusion of 1 mM L-glutamate evoked a current response in 45 of 65 neurons examined. The evoked current incorporated two kinetically distinct components in variable proportion: a fast desensitizing component and a slower component. The current was mediated by an outwardly rectifying conductance increase and reversed at −48.8 ± 5.3 mV. Reducing the external chloride concentration by 50% deflected the glutamate equilibrium potential (E_{glu}) by +14 mV, while increasing external potassium threefold shifted E_{glu} by up to +6 mV. Ibotenic acid fully activated both components of the glutamate response. Saturating concentrations of glutamate completely occluded neuronal responses to ibotenic acid, indicating that ibotenic acid was activating the same receptor(s) as glutamate. Millimolar concentrations of quisqualic acid, kainate, AMPA, and NMDA each failed to evoke any response. Picrotoxin (10^{-4} M) completely blocked the glutamate response. Niflumic acid (100 μM) blocked >80% of the desensitizing component and ~50% of the sustained component. Reduction or elimination of extracellular calcium did not abolish the response.

This study extends the ionic and pharmacological analysis of glutamatergic conductances in STG neurons. The currents described are consistent with glutamatergic inhibitory synaptic and agonist-evoked responses previously described *in situ*. We discuss their pharmacology, ionic mechanisms, and functional significance.

[Key words: niflumic acid, lobster, stomatogastric, crustacean, reciprocal inhibition, glutamate, chloride current, potassium current, central pattern generator, voltage dependence]

The crustacean stomatogastric ganglion (STG) contains neural elements of at least two central pattern generator (CPG) circuits (Fig. 1*A*), which control rhythmic movements of the foregut (Johnson and Hooper, 1992), and have served as model systems for understanding the function of biological neural networks (Kristan, 1980; Getting, 1989). Inhibitory glutamatergic synaptic transmission is a major building block of the reciprocal inhibitory pairs and recurrent cyclic inhibitory chains of the neurons that comprise these oscillatory CPGs (Marder and Paupardin-Tritsch, 1978; Marder and Eisen, 1984). The chemical modulation of these glutamatergic synapses contributes heavily to control of oscillatory phase relationships among the participating neurons (Johnson et al., 1994), and changes in such phase relationships in turn directly mediate changes in behavioral output (Heinzel et al., 1993).

The glutamatergic IPSP *in situ* is primarily dependent on chloride, but also sometimes exhibits a smaller potassium dependence which has not been separable from the chloride-mediated response in *Panulirus interruptus* (Marder, 1987). These "fast" glutamatergic IPSPs are blocked by picrotoxin (Eisen and Marder, 1982; Marder and Eisen, 1984), and are similar in reversal potential and ionic dependence to the responses elicited by iontophoretically applied glutamate (Marder and Paupardin-Tritsch, 1978; Bidaut, 1980; Eisen and Marder, 1982). The ionic and pharmacological profile of this receptor clearly distinguish it from the major vertebrate glutamate receptor families; however, the chloride-mediated current resembles the currents mediated by glutamate-gated chloride channels in *Aplysia* neurons (Ikemoto and Akaike, 1988; Sawada et al., 1984; King and Carpenter, 1989), extrajunctional glutamate receptors in locust and American lobster muscles (Cull-Candy, 1976; Lingle and Marder, 1981), and the cloned GluCl ionotropic glutamate receptor from *C. elegans* (Cully et al., 1994).

This study describes the glutamate-gated membrane currents in isolated, cultured STG neurons under two-electrode voltage clamp in order to lay the groundwork for detailed studies of the glutamate receptor and its modulation at the cellular and network levels. Cultured neurons are advantageous for these studies in that they are electrotonically compact, isolated from other neurons which might evoke secondary effects, and accessible to manipulations such as focal perfusion. In this initial characterization, we describe receptor pharmacology and ionic dependencies, as well as an intrinsic voltage dependence of receptor activation which carries possible implications for its role in the stomatogastric neural network.

Some of these data were previously published in abstract form (Cleland and Selverston, 1994).

Materials and Methods

Cell culture. Adult Pacific spiny lobsters, *Panulirus interruptus*, were captured wild and kept in running seawater until use. The stomatogastric ganglion (STG) was removed, desheathed, pinned to a Sylgard-lined

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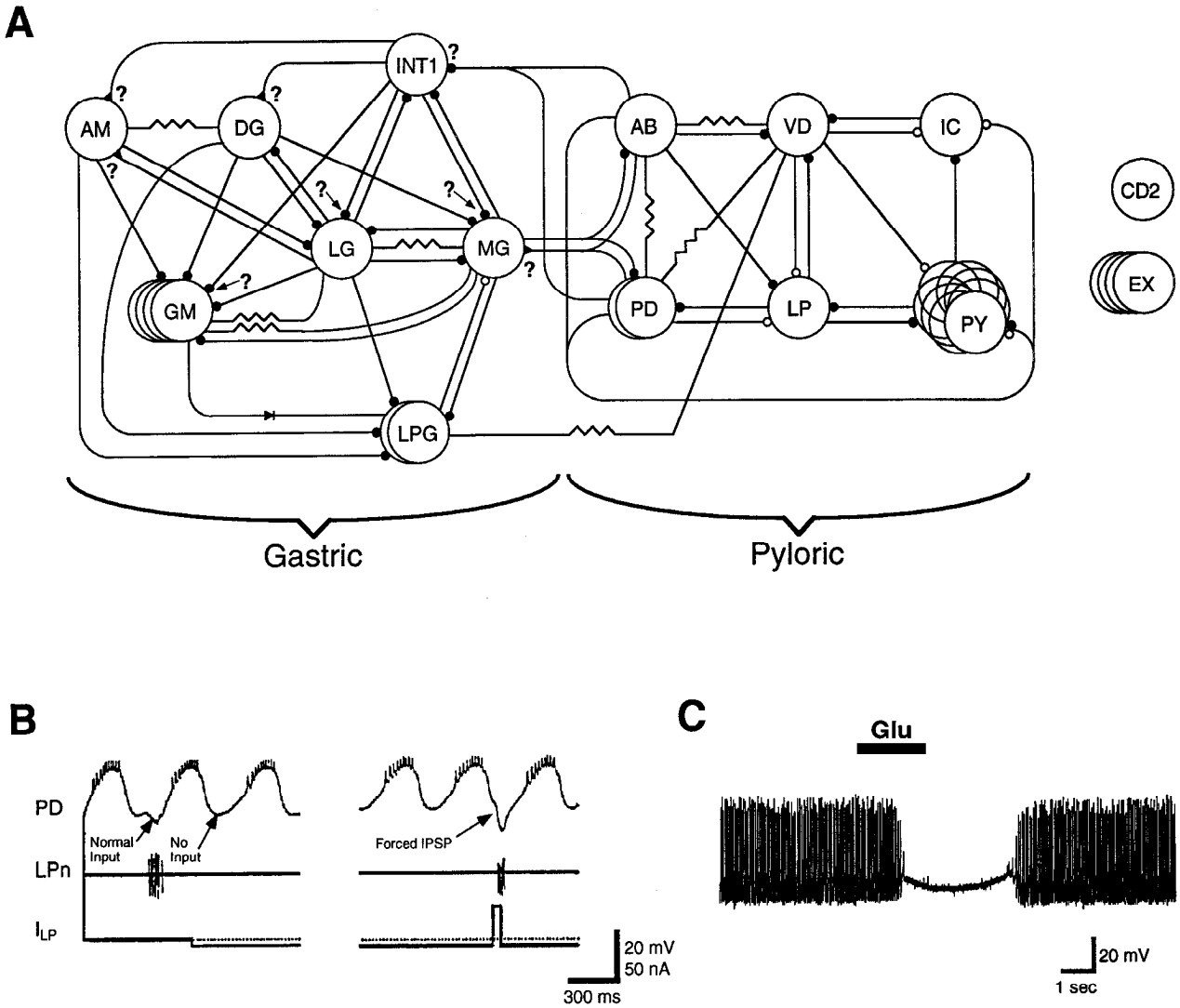

Figure 1. Functional role of the inhibitory glutamate receptor. *A*, Circuit diagram of the gastric and pyloric networks and other cells of the stomatogastric ganglion (STG). Most neurons in this central pattern generator are glutamatergic (*AB, IC, PY, LP, LG, MG, DG, AM,* and probably *Int1*). All pyloric neurons and nearly all gastric neurons receive glutamatergic inhibitory synapses from other STG neurons *in situ*. *Solid circles* indicate inhibitory glutamatergic synapses; *open circles* indicate inhibitory cholinergic synapses. *Question marks* indicate presumed glutamatergic synapses, both inhibitory (*circles*) and excitatory (*triangles*). Electrotonic synapses are depicted by the resistor symbol; rectifying electrotonic synapses by the diode symbol. Note that there are two PD, two LPG, four GM, and eight PY neurons within the STG. The stomatogastric ganglion also contains one CD2 neuron (a member of the interganglionic cardiac sac network) and an estimated four EX neurons which are not well understood. *B*, Recording from an *in situ* ganglionic preparation from *Panulirus interruptus* demonstrating the effect of a glutamatergic synapse between identified neurons in the STG. The LP neuron (depicted by the spikes recorded from its output nerve, the LPn) normally inhibits the PD neuron phasically via a glutamatergic synapse. This inhibition can be removed by hyperpolarizing LP (note the injection of hyperpolarizing current in the I_{LP} trace), and a depolarizing pulse into LP can evoke a glutamatergic "forced IPSP" in PD (figure modified from Herterich, 1992). *C*, Glutamate application hyperpolarizes a tonically spiking, isolated, cultured STG neuron, thereby terminating action potential generation. A steady current injection of +2 nA was required in order to elicit tonic spiking in the neuron depicted.

dish, and incubated at room temperature for 1 hr in 2 mg/ml subtilisin (Subtilisin Carlsberg, a nonspecific protease; Sigma) dissolved in *Panulirus* saline. After proteolysis, the ganglion was washed for 1–2 hr in maintenance medium or *Panulirus* saline, at 15°C or room temperature. These variations in wash parameters did not correlate with any observed variability of cellular properties. Individual neurons were removed from the ganglion by gentle suction and plated individually into 35 mm Falcon Primaria culture dishes in sterile maintenance medium. Recordings were made from cells after 3–4 d in culture, by which time cells had adhered securely to the substrate and most had extended short processes from the neurite stump, or occasionally from the soma. Incubation temperature (15°C or room temperature) did not noticeably influence glutamate response properties.

Media. Panulirus saline was prepared as described in Mulloney and Selverston (1974). Standard *Panulirus* maintenance medium (PMM) was prepared from full-strength Leibovitz-15 medium (Sigma powder) with all common ions supplemented to *Panulirus* saline concentrations, except NaCl which was slightly reduced (to 446 mM) from its *Panulirus* saline concentration in order to osmotically balance the added ingredients of L-15. The final recipe for 1 liter of PMM was: 14.8 gm L-15 powder, 18.07 gm NaCl, 0.55 gm KCl, 1.82 gm $CaCl_2 \cdot 2H_20$, 0.612 gm $MgSO_4$, 0.56 gm Na_2SO_4, 1.19 gm HEPES acid, 1.15 gm TES, and 3 gm D-glucose. Final salt concentrations (including the contributions from L-15 powder) were 446 mM NaCl, 12.7 mM KCl, 13.7 mM $CaCl_2 \cdot 2H_20$, 5.9 mM $MgSO_4$, 3.91 mM Na_2SO_4, 5.0 mM HEPES acid, 5.0 mM TES, and 16.7 mM D-glucose. Penicillin–streptomycin (final concentrations: 100 U/ml penicillin, 0.1 mg/ml streptomycin) was also added to the stock solution. PMM was brought to pH = 7.5 with HCl/NaOH, filter-sterilized through Corning 0.22 μM nylon filters, and refrigerated until use.

Recording and analysis. Culture dishes were mounted on a Nikon Diaphot TMD inverted microscope and the neurons were visualized with Hoffman modulation optics. Agonists were delivered by whole-cell focal superfusion. Agonists were flowed steadily past the cell from a focal superfusion pipette to a focal outflow (suction) pipette. To deliver the agonist, a hydrostatic pressure pulse was delivered to the superfusion pipette; this expanded the arc of agonist flow so as to encompass the entire cell. Fast green (0.05% by weight; added to the focal perfusant), which had no observable physiological effect at the concentrations used, was used to visually confirm agonist application. The time delay between pressure pulse initiation and complete whole-cell perfusion was somewhat variable, but generally in the range of a few hundred milliseconds (as measured by the application of high-potassium saline). The preparation was also globally perfused at a rate of 2.5 ml/min with agonist-free saline of the same ionic composition as the focal perfusant. In experiments involving glutamate-evoked current antagonists, the antagonist was present in both the bath saline and the perfusant, except for picrotoxin experiments in which only the bath saline contained the toxin. No calcium buffers or chelators were added to calcium-free saline. If cells exhibited sodium spike currents at depolarized potentials, these were blocked with a pulse of tetrodotoxin (TTX) before experimental manipulations were begun. The spike current never recovered from such a pulse during any of our experiments. Two-electrode voltage-clamp recordings were made with an Axoclamp-2A (Axon Instruments, Foster City, CA). Voltage-recording electrodes were filled with 3 M KCl and had resistances of R_e = 15–30 MΩ, while current-passing electrodes had R_e = 8–15 MΩ and were filled with a solution of 0.6 M K_2SO_4 and 20 mM KCl in order to avoid chloride-loading the cells during voltage clamp. Offset potentials generated by altered chloride concentrations in bath saline were subtracted where appropriate. Current output was Bessel-filtered at 1–2 kHz. Data were digitized directly to disk at 2 kHz and analyzed by computer. When motion artifacts due to perfusion onset were apparent in data traces, they were graphically removed. For all "X±Y" terms presented in this study: X = arithmetic mean, Y = one sample standard deviation *s*.

Suppliers. L-Glutamic acid, quisqualic acid, NMDA, niflumic acid, and picrotoxin were purchased from Sigma (St. Louis, MO). Ibotenic acid, kainic acid, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and more quisqualic acid were purchased from Research Biochemicals, Inc. (Natick, MA). Tetrodotoxin (TTX) was purchased from Calbiochem (San Diego, CA). Polyvinylpyrrolidone-25 (PVP-25), a 25 kDa chain of pyrrolidone side rings, was manufactured by Serva and purchased from Crescent Chemical Co. (Hauppage, NY). All drugs were mixed fresh from powder on the day of the experiment, except for TTX and PTX which were kept as frozen aliquots.

Results

The glutamate response is multicomponent

Within the stomatogastric ganglion (STG), all pyloric network and most gastric network neurons receive qualitatively similar "fast" glutamatergic IPSPs (Fig. 1*A,B*; cf. also Marder and Eisen, 1984); there are also a small number of neurons within the STG which are not part of these two networks. In order to study the currents underlying this common synaptic response, we superfused glutamate (1 mM) over isolated STG neurons grown in primary culture for 3–4 d. In all, 45 of 65 (69%) isolated, cultured neurons studied exhibited a glutamate response. Glutamate application was able to hyperpolarize and terminate action potential generation in tonically spiking neurons (Fig. 1*C*), an effect analogous to that of glutamatergic synapses *in situ*. Under voltage clamp in cultured neurons, glutamate evoked a biphasic response whose kinetically distinct components varied in relative amplitude such that they were sometimes dramatically distinct and sometimes largely overlapped one another (Fig. 2). The fast component, when visible, was strongly and rapidly desensitizing, while the slower component's desensitization, if any, was not discernable in our experiments. The variability of the glutamate response among different cells was consistent with the presence of two currents varying in the level of expression (i.e., total maximal membrane conductance) with respect to one another; that is, the greater the proportional expression of the faster current, the faster its relative onset and the greater its temporal distinction from the slower-onset current. Alternatively, variability in agonist application time (on the order of a few hundred milliseconds to reach full concentration) may also have been responsible for the variability of observed responses, particularly as the fast component was desensitizing. The remaining 20 neurons (31%) showed no glutamate response at all; 19 of these unresponsive neurons did display other endogenous currents, such as I_H, the hyperpolarization-activated inward current.

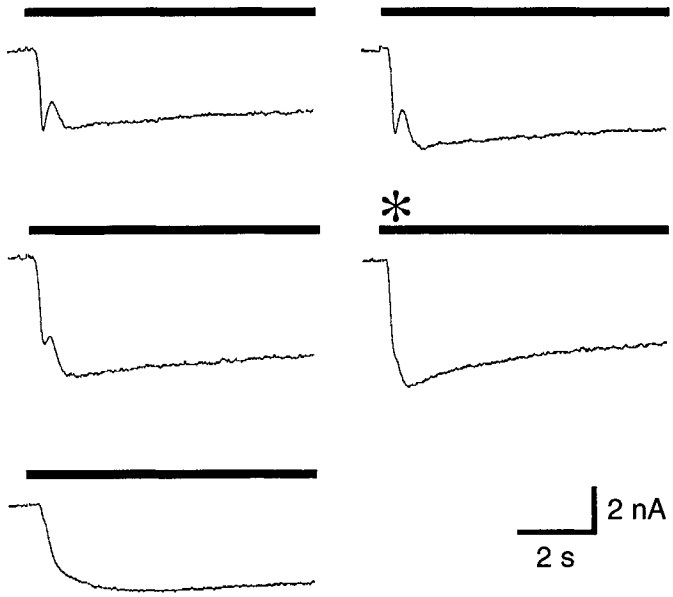

Figure 2. Variability of the glutamate response. Traces depict the currents evoked from five different voltage-clamped cells by the superfusion of 1 mM L-glutamate. Cells were clamped at a holding potential of −70 mV. Bars denote the time of application of L-glutamate. Most cells tested showed responses similar to the trace denoted with an *asterisk.*

Ion and voltage dependencies

Glutamate application elicited an increase in total membrane conductance (Fig. 3*A*). This conductance increase exhibited voltage dependence, increasing with membrane depolarization (Fig. 3*B*), and reversed at −48.8 ± 5.3 mV (n = 20). When the two components of the glutamate response were distinct, they reliably reversed within a few millivolts of each other, at −48.7 ± 5.3 mV (predominantly fast; n = 11) and −49.8 ± 5.6 mV (predominantly slow; n = 11), an insignificant difference.

Reducing external chloride concentrations by 50% (substituted with Na-methanesulfonate) shifted the glutamate reversal potential by 10–14 mV to the positive (Fig. 4*A*); the Nernst prediction for a chloride electrode is a +16 mV shift in reversal potential, indicating that the STG glutamate response is primarily mediated by chloride. Estimated ionic activities, rather than concentrations, were used for these calculations (Robinson and Stokes, 1968; cf. Appendix 8.10, Table 10). The slope conductance of the glutamate response was found to decrease under low external chloride conditions; this suggested that Na-methanesulfonate may be effecting a slight blockade of the chloride current.

We increased external potassium concentrations with four cells and observed a shift in the equilibrium potential for glutamate of up to +6 mV in $3\times[K^+]_o$ saline (compensated with

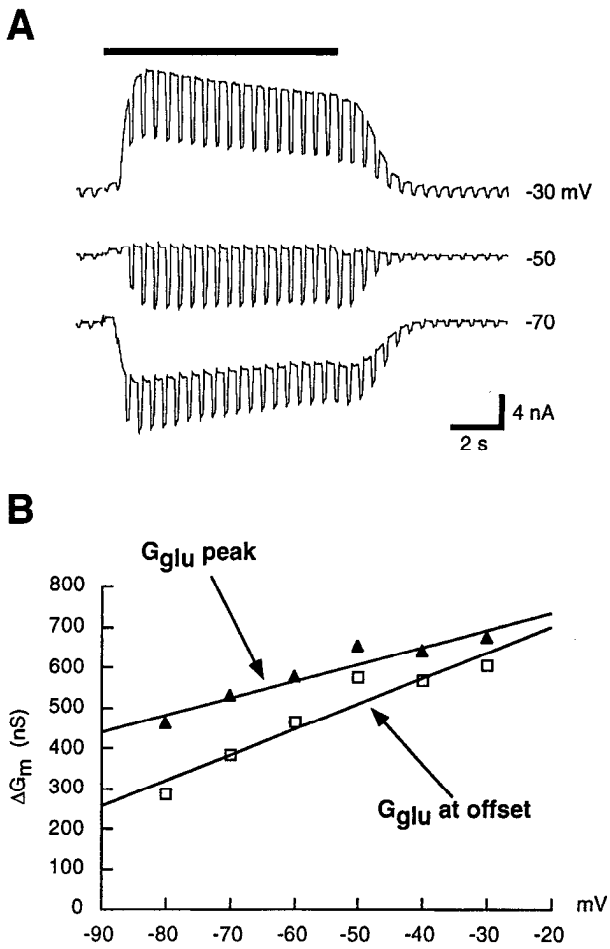

Figure 3. Outwardly rectifying membrane conductance increase in response to glutamate application. *A*, Conductance increases in response to glutamate superfusion at holding potentials of −70, −50, and −30 mV (bottom to top), as revealed by voltage steps of −10 mV applied at 2 Hz. Note that the glutamate-evoked conductance increase exhibits a voltage dependence. (n = 3). *B*, Conductance–voltage (G–V) plot of the voltage dependence of the glutamate-evoked conductance. Measurements of both peak and sustained responses are shown. Sustained currents were measured at the end of glutamate application. The lines fit to the data are intended to highlight the voltage dependence and not to imply linearity; the change in slope at −50 mV may indicate the maximum whole-cell glutamatergic conductance. (G_{glu}: glutamate-evoked membrane conductance).

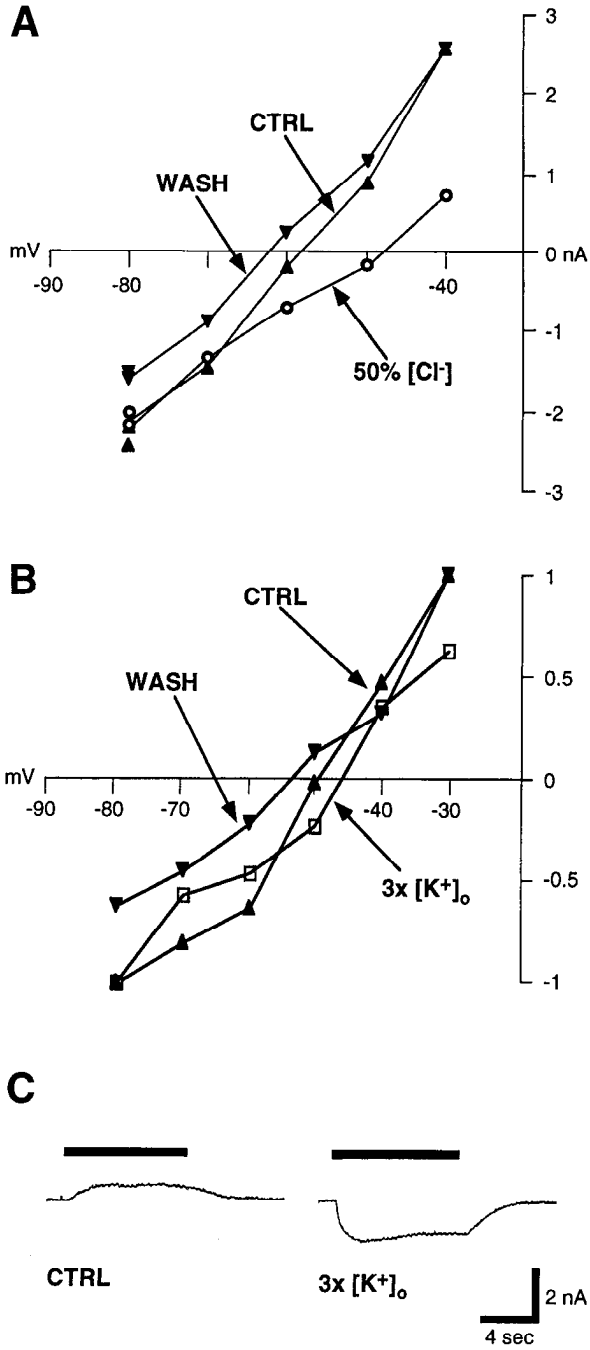

Figure 4. Ionic dependence. *A*, Reduction of external chloride by 50% (substituted by Na-methanesulfonate) shifted the glutamate reversal potential to the positive by 10–14 mV (n = 4), that is, 60–90% of the Nernst-predicted shift of +16 mV for a chloride current. Estimated chloride activity, not concentration, was used for these calculations. The decrease in slope conductance under low-chloride conditions suggests that Na-methanesulfonate may be a "slow permeator" of the glutamate-gated channels, effecting a slight blockade of the chloride current (*CTRL*, control). *B*, Increasing the external potassium concentration threefold shifted the glutamate reversal potential (E_{glu}) to the right by up to 6 mV, or approximately 21% of the shift predicted for a purely K^+-selective current. Data shown are the normalized, averaged responses from the two experiments (out of four) showing the highest degree of potassium dependence. Sustained current measurements are depicted, although peak current measurements exhibited a similar shift in E_{glu}. *C*, Currents evoked by glutamate from a holding potential of −50 mV in normal and high-potassium saline, showing a resultant shift in the equilibrium potential for glutamate.

reduced [Na^+]; Fig. 4*B*,*C*). A +6 mV shift is 21% of that predicted for a potassium electrode. The picrotoxin-sensitive glutamatergic IPSP *in situ* also exhibits a potassium dependence (Marder and Paupardin-Tritsch, 1978). However, no distinct component of the glutamate response was observed to reverse anywhere near E_K, the potassium reversal potential. The mechanisms underlying this glutamatergic potassium permeability in lobster are not yet understood.

Agonist pharmacology

Ibotenic acid induced both the peak and sustained components of the glutamate response in a manner identical to glutamate at the same concentration (Fig. 5*A*,*B*; n = 3). In order to determine whether ibotenic acid was activating the same current(s) as glutamate, summation experiments were performed. The presence of saturating concentrations (1 mM) of glutamate in the bath reversibly abolished the cells' response to ibotenate application

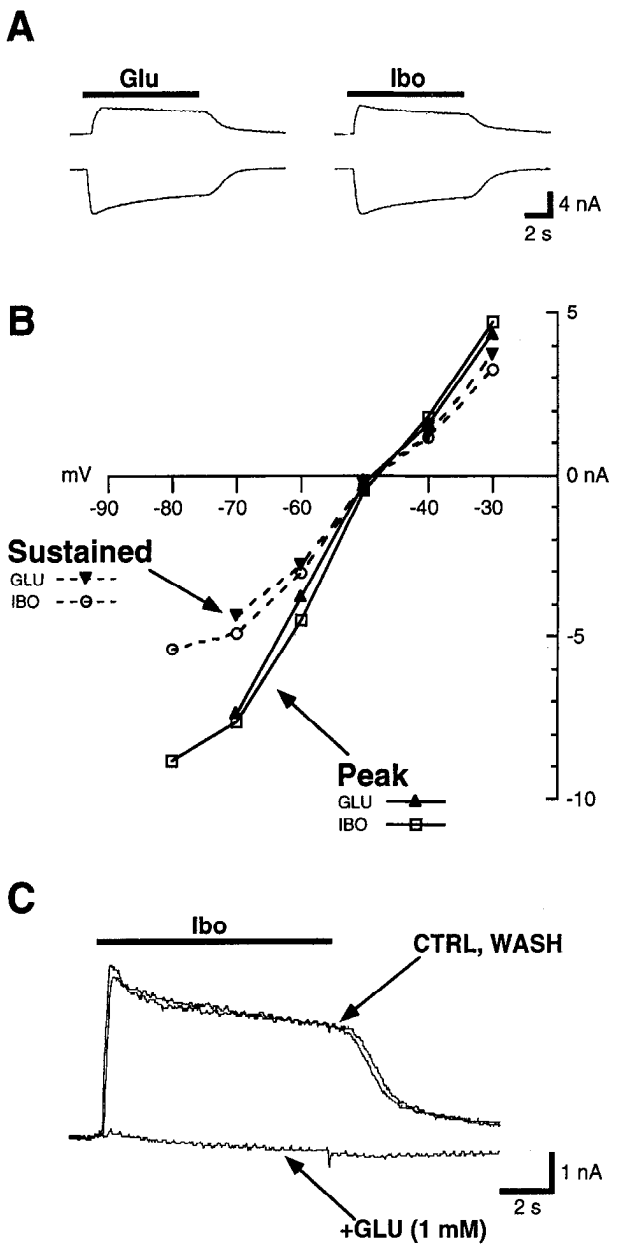

Figure 5. Effect of ibotenic acid application. *A*, Ibotenic acid (1 mM) mimics the effect of equimolar glutamate upon the same neuron. Responses from holding potentials of −40 (top) and −70 mV are shown; times of agonist application are denoted by bars. *Glu*, 1 mM L-glutamate. *Ibo*, 1 mM ibotenic acid. (n = 3). *B*, Ibotenic acid current–voltage (*I–V*) curves overlap glutamate *I–V* curves for both peak and sustained components. Sustained currents were measured at the end of agonist application. *C*, Bath-applied glutamate at saturating concentration (1 mM) reversibly occludes the ibotenic acid response, indicating that ibotenic acid acts upon the glutamate receptor. Response shown is from a holding potential of −40 mV (*CTRL*, control).

(Fig. 5*C*); if ibotenate activated different receptors than glutamate, the current(s) would have summated; 200 μM bath-applied glutamate only partially inhibited the ibotenic acid response (data not shown).

Millimolar concentrations of quisqualate (n = 4), kainate (n = 2), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA; n = 3), and NMDA; n = 2), tested in cells with robust glutamate responses, never evoked any visible response at any holding potential tested (Fig. 6).

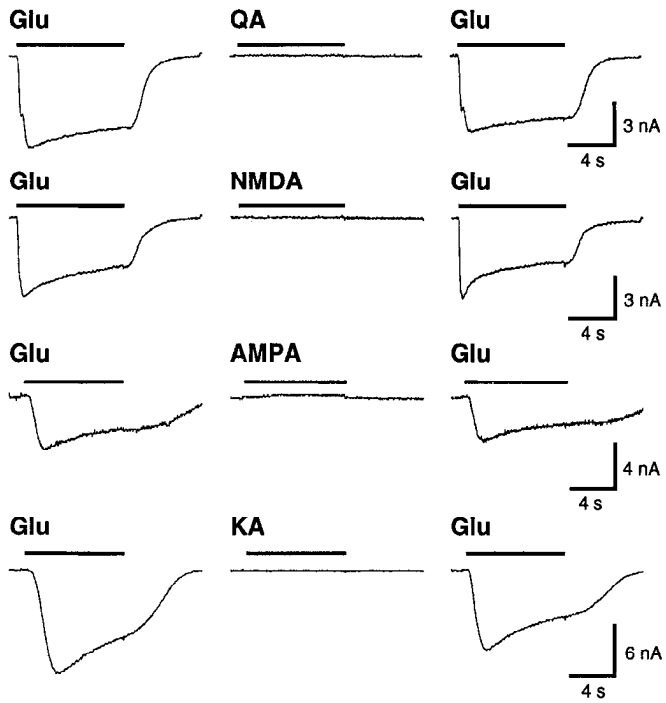

Figure 6. Agonist pharmacology. Neither 1 mM quisqualate (QA; n = 4), 1 mM NMDA (n = 2), 1 mM α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (*AMPA*; n = 3), or 1 mM kainate (*KA*; n = 2) evoked any part of the glutamate response. All cells tested exhibited robust responses to glutamate both before (*left panel*) and after (*right panel*) application of other agonists. All traces are from holding potentials of −70 mV.

Antagonist pharmacology

Picrotoxin (PTX), a chloride channel blocker (Newland and Cull-Candy, 1992; Pribilla et al., 1992), blocks inhibitory glutamatergic synapses in the STG *in situ* (Marder, 1987). We tested its effect on the glutamate response of isolated cells; 10 μM PTX attenuated the glutamate response, while 100 μM PTX abolished it (Fig. 7; n = 2). Unlike the intact STG preparation, in which PTX blockade is difficult to reverse at these concentrations, 20 min wash at 2.5 ml/min restored an appreciable fraction of the response in cultured cells.

Niflumic acid (100 μM), generally considered a chloride channel antagonist (see Discussion), blocked most of the transient

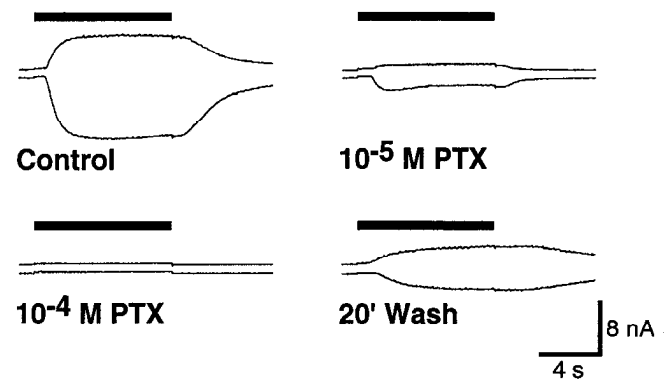

Figure 7. Effect of picrotoxin (PTX); 10 μM PTX partially blocks the glutamatergic response, while 100 μM PTX blocks it entirely (n = 2). PTX block was partially reversible in cell culture. Responses shown are from holding potentials of −40 (*top*) and −70 mV.

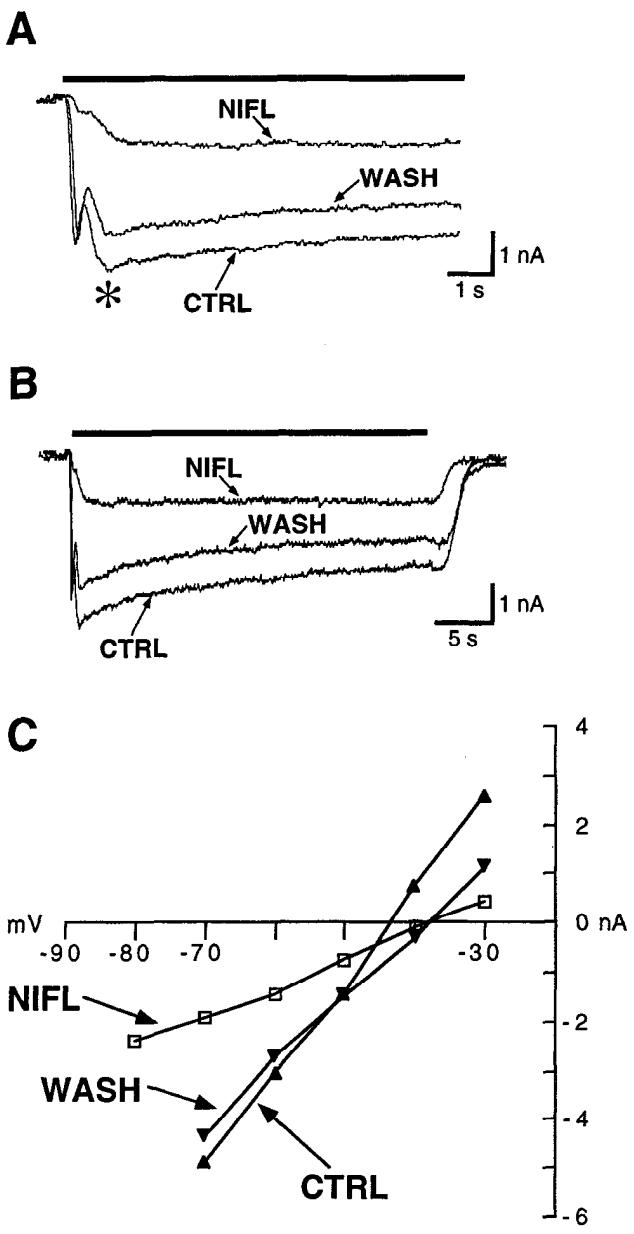

Figure 8. Blocking effect of niflumic acid. *A*, Effect of 100 μM niflumic acid on the neuronal glutamate response. Niflumic acid blocked the distinct transient component by more than 80%, and the sustained component by approximately 50%. The *asterisk* denotes the "late peak" of the evoked current, presumably incorporating both fast transient and slow current components, which is depicted in *C*. (n = 3). Responses shown are from a holding potential of −70 mV (*NIFL*, in the presence of niflumic acid; *CTRL*, control). *B*, Extended recording of the same data as in *A*. Niflumic acid reduced the degree of response desensitization from 36% to a negligible 3%. *C*, Current–voltage plot of the niflumic acid sensitivity of the late-peak glutamate response (denoted in *A* with an *asterisk*).

component of the glutamate response (>80%; Fig. 8*A*) as well as an average of about 50% of the total sustained response; it also abolished response desensitization (Fig. 8*B*). The reversal potential of the total response at the late peak, denoted by an asterisk in Figure 8*A* (at which both temporal components of the response are simultaneously active) was not affected by the niflumic acid blockade (Fig. 8*C*).

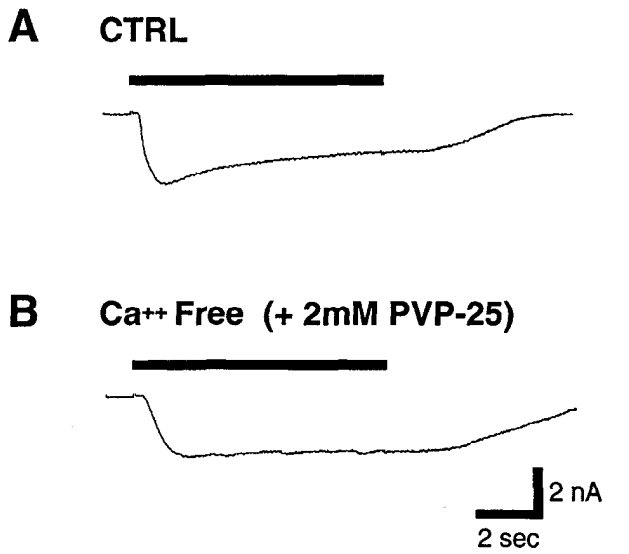

Figure 9. Influx of extracellular calcium is not necessary to evoke the glutamate response. *A*, Glutamate-evoked currents in control saline at a holding potentials of −70 mV. *B*, Glutamate-evoked currents in calcium-free saline. Due to the general toxicity of a zero-calcium environment to these neurons, 2 mM polyvinylpyrrolidone-25 (*PVP-25*) was added to the calcium-free saline in order to preserve the integrity of the plasma membrane (Raditsch and Witzemann, 1994). The absence of extracellular calcium did not abolish the glutamate-evoked response. (n = 4).

Effect of extracellular calcium

Niflumic acid is an established antagonist, in several systems, of calcium-dependent chloride channels which are dependent upon calcium influx across the plasma membrane for activation (Leonard and Kelso, 1990; White and Aylwin, 1990; Sanchez-Vives and Gallego, 1994). We removed extracellular calcium in order to assess whether this response involved calcium-dependent chloride currents dependent upon external calcium influx. As a low or zero-calcium environment was typically toxic to these neurons, 2 mM polyvinylpyrrolidone-25 (PVP-25) was added to the calcium-free saline in order to retard the breakdown of the plasma membrane (Raditsch and Witzemann, 1994). The glutamate response was not abolished in the absence of calcium, ruling out this model (Fig. 9), although the degree of apparent desensitization was reduced. The glutamate response also persisted in PVP-free saline containing 10% of the normal calcium concentration, substituted with magnesium, and including 1 mM cadmium in order to prevent calcium influx through voltage-dependent calcium channels at the more depolarized holding potentials (data not shown).

Discussion

Cell culture and identification

The stomatogastric network preparation, like most *in situ* preparations, is composed of cells with long and complex arborizations (King, 1976a,b; Graubard and Wilensky, 1994; Thuma and Hooper, 1994) which prevent reliable voltage clamping of the membrane (Graubard and Hartline, 1991; Golowasch and Marder, 1992; Hartline et al., 1993; Turrigiano and Marder, 1993). While valuable information has been gained from voltage-clamp work *in situ* (Graubard and Hartline, 1991; Golowasch and Marder, 1992), space-clamp limitations hamper the analysis of currents expressed in the neuropil. Furthermore, the application of

agonists to conductances expressed within the neuropil is hindered. Finally, while pharmacological blockade and photoinactivation techniques are powerful tools for isolating neurons (Miller and Selverston, 1979; Flamm and Harris-Warrick, 1986a,b; Hooper and Marder, 1987; Johnson et al., 1993a; Johnson et al., 1994), neuronal interactions mediated by the termini of descending inputs exist which cannot be removed by these means (Nusbaum et al., 1992). These problems are all abrogated by extracting neurons into primary culture. It should be noted that while the study of glutamatergic conductances per se is facilitated in culture, the electrotonic compactness of the cells used for voltage-clamp study eliminates the complexity and potential information content of differential receptor distribution. Furthermore, removal of neurons from their potentially instructive environment certainly could affect their membrane properties. These potential sources of error must be taken into account when reintegrating these data into systemic studies. Neurons for this study were not identified, as most neurons within the STG receive qualitatively similar glutamatergic IPSPs (Fig. 1).

The reversal potential for glutamatergic synapses in the intact ganglion is estimated at -70 ± 12 mV in *Panulirus interruptus* (Marder and Eisen, 1984), whereas our data place it at -48.8 ± 5.3 mV. This is probably due in part to the inability in the intact ganglion to accurately control the membrane potential at distal synaptic sites (Hartline and Graubard, 1992); however, it could also incorporate a genuine shift in E_{Cl}, perhaps due to a decreased ability of cultured neurons to regulate internal [Cl^-] levels at receptor sites.

Voltage dependence

The glutamate-evoked current was mediated by an outwardly rectifying conductance increase to chloride and potassium. Notably, the GluClβ clone from *C. elegans*, one of the putative subunits for a glutamate-gated chloride current, is a strong outward rectifier when expressed alone in oocytes (Cully et al., 1994). Outward rectification in an inhibitory postsynaptic receptor channel would act to increase the effectiveness of inhibitory synapses upon cells in their depolarized, plateau state, aiding in the termination of their burst of action potentials, while it would weaken the inhibition of neurons that were in their hyperpolarized interburst phase, thus facilitating their rebound into a plateau potential. This could contribute to a mechanism for phase-specific synaptic gain, which would be conducive to maintaining efficient and effective oscillatory "building blocks" based on reciprocal and recurrent cyclic inhibitory synapses.

Comparative pharmacology

Ibotenic acid fully mimicked the effects of glutamate upon cultured stomatogastric neurons, while quisqualate, kainate, AMPA, and NMDA evoked no response, and picrotoxin blocked the current evoked by glutamate. This pharmacological profile is similar to those of the quisqualate-insensitive neuronal chloride current in *Aplysia* (Sawada et al., 1984; Ikemoto and Akaike, 1988; King and Carpenter, 1989), the extrajunctional glutamate receptor of *Homarus* muscle (Lingle and Marder, 1981), the extrajunctional H-current of locust muscle (Cull-Candy, 1976), and the cloned channel GluCl, a chloride-selective ionotropic glutamate receptor from *C. elegans* (Cully et al., 1994). Only one quisqualate-sensitive, chloride-mediated current has been shown, in the mollusc *P. corneus* (Bolshakov et al., 1991), although a small and rare response of this type was described in *Aplysia* (Ikemoto and Akaike, 1988), and several molluscan neurons express quisqualate-sensitive inhibitory glutamatergic currents which are mediated by potassium (Walker, 1976; Yarowsky and Carpenter, 1976; Kehoe, 1978; Katz and Levitan, 1993). In contrast, all known glutamate-gated currents in vertebrates are cation-selective and functionally excitatory (reviewed in Hollmann and Heinemann, 1994; Nakanishi and Masu, 1994). Some vertebrate retinal neurons express chloride conductances which can be activated by some glutamate analogs; these, however, may be mediated by glycine receptors (Chiba and Saito, 1994).

Implications of niflumic acid blockade

Niflumic acid, a fenamate, is a nonsteroidal antiinflammatory drug which blocks a variety of chloride currents: calcium-dependent (White and Aylwin, 1990; Hogg et al., 1994; Lamb et al., 1994; Sanchez-Vives and Gallego, 1994; Ueda and Steinberg, 1994), GABA-gated (Evoniuk and Skolnick, 1988), cAMP-gated (Hughes and Segawa, 1993), and voltage-gated (Miller and White, 1980), as well as a calcium-dependent nonselective cation current (Poronnik et al., 1992; Partridge et al., 1994) and an NMDA response (Lerma and del Rio, 1992). Pharmacologically similar flufenamic acid blocks the GluCl glutamatergic chloride channel cloned from *C. elegans* and expressed in *Xenopus* oocytes (Cully et al., 1994).

In cultured STG neurons, 100 μM niflumic acid blocks about 50% of the steady-state glutamatergic current and abolishes its desensitization (Fig. 8). This effect is consistent with the simple blockade of a fast, partially desensitizing chloride channel, with a niflumic acid–insensitive, nondesensitizing, slower current remaining. However, no membrane current clearly similar to such a chloride-selective, slower component has been described, although several other slow glutamatergic currents have been demonstrated (Miwa et al., 1990; Bolshakov et al., 1991; Nakanishi, 1994). Furthermore, fenamate pharmacology is complex and alternative hypotheses can not yet be ruled out. For example, niflumic acid and several other fenamates have been shown to potentiate GABAergic currents expressed in oocytes when agonist concentration is low, and yet inhibit currents elicited by high concentrations of GABA (Woodward et al., 1994). Noting that the crustacean glutamate-gated chloride current is physiologically and pharmacologically similar to the vertebrate $GABA_A$ receptor, if fenamates had the dual effect of preventing receptor desensitization and blocking ion permeation, with different functional activity coefficients for the two effects, the results could be consistent both with our data and that of Woodward et al. (1994). A second alternative is extended by recent evidence indicating that fenamates, including niflumic acid, can trigger calcium release from intracellular stores, probably from within mitochondria (Poronnik et al., 1992; Partridge et al., 1994). If this mechanism is activated in STG neurons, intracellular calcium release (or downstream effectors activated by calcium) could modulate the glutamate receptor channel, perhaps into a desensitized state. These two alternatives are also consistent with an alternate hypothesis that the kinetically distinct glutamatergic current components are derived from structural or modulatory variants of a single ionotropic receptor type; a hypothesis which is more consistent with the data from picrotoxin blockade.

Implications of picrotoxin blockade

The data from picrotoxin blockade contraindicate, though they do not rule out, the possibility that the slower, sustained component of the glutamate response is mediated by a G-protein-linked membrane receptor. Picrotoxin (PTX; 10^{-4} M; Fig. 6) en-

tirely blocked the glutamate response, including the sustained component. Recent work on the mechanism of action of picrotoxin upon vertebrate $GABA_A$ receptors has demonstrated a use-dependence for PTX blockade (i.e., picrotoxin affinity for the receptor is increased in its open, ligand-bound conformation), but does not favor a simple channel blocking mechanism, suggesting instead a stabilization of an agonist-bound closed state (Newland and Cull-Candy, 1992). To the extent that this mechanism of PTX blockade implies a specificity of picrotoxin for the receptor complex rather than for an epitope common to several chloride channels, this evidence suggests that the second component is either directly dependent on the faster component for activation and/or that it is mediated by a distinct receptor with some similarity to the fast-activated receptor. One possibility is that two similar ionotropic receptors are being expressed, perhaps otherwise-identical channels which are in different, durable modulatory states, or sibling receptors with different subunit compositions that affect their kinetics (Bochet et al., 1994; Gallo et al., 1994; Lerma et al., 1994), or even channels containing RNA-edited or alternatively spliced subunit isoforms (Gallo et al., 1992; Egebjerg et al., 1994).

Glutamatergic synaptic modulation

The central pattern generators of the STG are regulated by chemical modulation of their membrane and synaptic properties. About 15 endogenous modulators have been identified to date in the stomatogastric system which are capable of modulating the CPG (Marder and Weimann, 1992; Marder et al., 1994); several of these modulators have been shown to affect glutamatergic synaptic strengths within the stomatogastric network (Elson and Selverston, 1992, 1994; Johnson et al., 1993b, 1994). The complexity of these synaptic modulatory mechanisms requires study of glutamate-evoked postsynaptic currents. For example, dopaminergic modulation of (ionotropic) glutamate receptors has been observed in both invertebrates (Swann et al., 1978) and vertebrates (Krizaj et al., 1994; Maguire and Werblin, 1994; Schmidt et al., 1994), and the introduction of a postsynaptic intracellular messenger can be sufficient to potentiate a synapse (Pettit et al., 1994). This basic characterization of stomatogastric glutamatergic currents begins this work.

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