Group I, II, and III mGluR Compounds Affect Rhythm Generation in the Gastric Circuit of the Crustacean Stomatogastric Ganglion

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Krenz, Wulf D., Don Nguyen, Nivia L. Pérez-Acevedo, and Allen I. Selverston. Group I, II and III mGluR compounds affect rhythm generation in the gastric circuit of the crustacean stomatogastric ganglion. J. Neurophysiol. 83: 1188–1201, 2000. We have studied the effects of group I, II, and III metabotropic glutamate receptor (mGluR) agonists on rhythm generation by the gastric circuit of the stomatogastric ganglion (STG) of the Caribbean spiny lobster Panulirus argus. All mGluR agonists and some antagonists we tested in this study had clear and distinct effects on gastric rhythm generation when superfused over combined oscillating or blocked silent STG preparations. A consistent difference between group I agonists and group II and III agonists was that group I agonists acted excitatory. The group I-specific agonists (R,S)-3,5-dihydroxyphenylglycine, as well as the nonspecific agonist (1S,3R)-1-amino-2-cyclopropyl-1,3-dicarboxylic acid accelerated ongoing rhythms and could induce gastric rhythms in silent preparations. The group II agonist (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-I) and the group III agonist L(+)-2-amino-4-phosphonobutyric acid (L-AP4) slowed down or completely blocked ongoing gastric rhythms and were without detectable effect on silent preparations. The action of L-CCG-I was blocked partially by the group-II-specific antagonist, (RS)-1-amino-2-cyclopropyl-1,3-dicarboxylic acid [(RS)APICA], and the group-III-specific antagonist (RS)-α-methyl-4-phosphonophenylglycine completely blocked the action of L-AP4. Besides its antagonistic action, the group-II-specific antagonist (RS)APICA had a remarkably strong apparent inverse agonist action when applied alone on oscillating preparations. The action of all drugs was dose dependent and reversible, although recovery was not always complete. In our experiments, the effects of none of the mGluR-specific agonists were antagonized or amplified by the N-methyl-d-aspartate (NMDA) receptor-specific antagonist (−)-2-amino-5-phosphonopentoenic acid, excluding the contamination of responses to mGluR agonists by nonspecific cross-reactivity with NMDA receptors. Picrotoxin did not prevent the inhibitory action of L-CCG-I and L-AP4. We conclude that mGluRs, probably similar to those belonging to groups I, II, and III described in mammals, may play a role as modulators of gastric circuit rhythm generation in vivo.

INTRODUCTION

Work over the last decade has shown that in the mammalian brain, glutamate not only acts on ionotropic glutamate receptors (iGluR) but also on metabotropic glutamate receptors (mGluR). mGluRs are seven-transmembrane domain proteins. They are coupled via G proteins to cyclic nucleotide and phosphoinositide intracellular messenger pathways and are capable of inducing a variety of cellular changes (Conn and Pin 1997; Pin and Duvoisin 1995; Saugstad et al. 1995). Depending on their pre- or postsynaptic localization, they may modulate neurotransmitter release (Cochilla and Alford 1998) and/or intrinsic membrane properties of neurons (Del Negro and Chandler 1998; Lüthi et al. 1997; Morisset and Nagy 1996; Russo et al. 1997) in a time- and activity-dependent way (Rodríguez-Moreno et al. 1998). mGluRs have been shown to be involved in motor pattern generation (Krieger et al. 1998). Three groups, I, II, and III, have been identified for mammalian mGluRs. This classification is based on specific pharmacological profiles, amino acid sequence similarity, and distinct intracellular messenger coupling (Conn and Pin 1997). The functional significance of their role in fine tuning the electrical behavior of nerve cells and synapses, as well as the complexity of their dynamic interactions with other receptors and ion channels, is only beginning to emerge.

In invertebrate animals, glutamate receptors that are activated by mGluR agonists have been identified in Aplysia neurons (Katz and Levitan 1993) and in leech glial cells (Lohr and Deitmer 1997). In the neuromuscular junction (NMJ) of lobster and crayfish, where glutamate is the principal excitatory neurotransmitter (Krivitz et al. 1970; Takeuchi and Takeuchi 1964), presynaptic autoreceptors are known to modulate the release of glutamate and γ-aminobutyric acid (GABA) (Miwa and Kawai 1986; Miwa et al. 1990, 1993). Experiments done in lobster suggest that at least some of these GluRs are coupled to G proteins (Miwa et al. 1987, 1990). In the crayfish NMJ, the nonspecific mGluR ligands (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD, agonist) and (RS)-α-methyl-3-carboxymethylphosphineglycine (MCPG, antagonist) both modulate the quantum content of postsynaptic currents (Schramm and Dudel 1997). The available data suggest that mGluRs may be as important for the function of the invertebrate CNS and peripheral nervous system as they are in mammals and other vertebrates.

Important questions about mGluR function that remain difficult to address in more complex mammalian preparations are under what conditions are the different types of mGluRs activated and what are the consequences of their activation for neuronal circuits. To determine the consequences of mGluR activation for neuronal network function, we have started to investigate mGluRs in a small defined, rhythmically active circuit of ~30 neurons, the stomatogastric ganglion (STG) of decapod crustaceans. All neurons of the STG, along with their synaptic and electrical connections, are identified and can be studied at the level of the whole network, as reduced subcircuits, or as isolated cell-pairs or single neurons. Two main central pattern generator (CPG) circuits have been described in the STG, the pyloric and the gastric CGPs (Fig. 1), which
produce the cyclic motor patterns that drive the muscles of the foregut. The activities of both are controlled by a large number of modulatory substances that either are released into the STG by neurons projecting from the commissural or esophageal ganglia or act via the bloodstream (Harris-Warrick et al. 1992; Marder 1987; Marder et al. 1995).

Glutamate is one of two major neurotransmitters in the STG (Marder 1987). It is used by the motoneurons that innervate intrinsic stomach muscles, i.e., muscles that originate and insert on the stomach wall (Govind and Lingle 1987). Glutamate activates excitatory iGluRs at the NMJ of their target muscles and inhibitory iGluRs at intraganglionic synapses that mediate the mutual interactions between neurons (Cleland and Selverston 1995, 1998; Marder 1987). Besides these well-studied, fast ionotropic receptor-mediated synaptic events, several slow postsynaptic potentials (Elson and Selverston 1995) and presumably metabotropic slow responses to glutamate, depolarizing and hyperpolarizing, have been described in crustacean STG neurons (Marder and Paupardin-Tritsch 1978; Tazaki and Chiba 1994). The second major neurotransmitter is acetylcholine (ACh) (Marder 1987). ACh within the STG also acts as a fast inhibitory transmitter between neurons and in the periphery excites a subset of stomach muscles. In addition, ACh is thought to be a powerful neuromodulator that activates both the pyloric and the gastric CPG via muscarinic receptors. In this study, we ask the questions, are mGluRs present in the gastric circuit of the STG and does their activation modulate the gastric CPG?

METHODS

Animals

Experiments were performed on STGs from 48 adult Caribbean spiny lobsters, Panulirus argus, of both sexes. Animals were provided by local fishermen and were kept in large aquaria at ambient temperature (24–25°C) before being used in experiments. Experiments were performed at the same ambient temperature.

Preparation

The combined stomatogastric nervous system (STNS) was dissected as described previously (Mulloney and Selverston 1974) and was pinned out in a silicone elastomer (Sylgard)-lined petri dish. The STG was desheathed. The stomatogastric nerve (sn) also was desheathed over a length of 5–10 mm. The STG and the desheathed portion of the sn were isolated from the rest of the bath volume in wells made from petroleum jelly (Vaseline) to enable separate superfusion. The volume of the STG well was ~500 μl and could be exchanged rapidly.

Physiological and drug solutions

Dissection and experiments were done in standard Panulirus saline, which had the following composition (in mmol/l): 479.1 NaCl, 12.7 KCl, 13.7 CaCl2, 10 MgSO4, 3.9 Na2SO4, 5 HEPES, and 5 TES, pH 7.4. In most experiments, the STG was superfused continuously with Panulirus saline.

All mGluR drugs, ACPD, (S)-3,5-dihydroxyphenylglycine (DHPG), (2S,1′S,2′S)-2-(carboxycyclopropyl)glycine (L-CGG-I), (−)-2-amino-4-phosphonobutyric acid (l-AP4), MCPG, (RS)-α-methylserine-O-phosphate (MSOP), (RS)-α-methyl-4-phosphonophenylglycine (MPPG), (RS)-1-amino-5-phosphonoindan-1-carboxylic acid [(RS)APICA], (RS)-1-aminoindan-1,5-dicarbonylic acid (AIDA), (2S,3S,4S)-2-methyl-2-(carboxycyclopropyl)glycine (MCCG), (RS)-α-methyl-4-tetrazolylphenylglycine (MTPG), as well as d(−)-2-amino-5-phosphonoentanoic acid (d-AP5), were purchased from Tocris Cookson (Bristol, UK). They were dissolved as stock solutions in 0.1 N NaOH, except DHPG, which was dissolved in H2O. L-Quisqualic acid was either from Tocris Cookson or from Research Biochemicals International (Natick, MD). It was dissolved in H2O. Aliquots of stock solutions were frozen and stored at −20°C for ≥1 mo. DHPG was dissolved directly before being used because it showed rapid loss of activity in solution. GluR

FIG. 1. Schematic diagram of cells and connections that form the gastric circuit of the Panulirus stomatogastric ganglion (STG). Int1, interneuron 1. Motoneurons are GM, gastric mill; LG, lateral gastric; MG, median gastric; AM, anterior median; DG, dorsal gastric; LPG, lateral posterior gastric. Two shaded boxes include motoneurons that participate in movement of the median or lateral teeth. Open boxes include motoneurons that fire together during the same phase of the gastric cycle. Motoneuron AM innervates muscles of the cardiac sac. Small black circles represent chemical inhibitory synapses, small white circles excitatory ones. Continuous lines represent monosynaptic connections, dashed lines nonmonosynaptic ones. Electrotonic connections are represented by resistor symbols and a rectifying electrotonic connection between GM and LPG by a diode symbol.
compounds were added to the perfusate to yield the desired concentration after adjustment of pH, if necessary. Pilocarpine was from Sigma (St. Louis, MO). It was made up as a stock solution at 10 mM in Panulirus saline and was added directly to the perfusate to yield the desired concentration. Picrotoxin (PTX) was from Sigma or Research Biochemicals International. It was dissolved as a concentrated stock in ethanol and diluted into the experimental solution.

**Drug application**

All drugs were administered by bath application. mGluR agonists were applied onto the STG for 10 min by superfusion in the Vaseline well and were washed out for 30–60 min thereafter. Antagonists were also superfused for 10 min always followed by a 60-min washout. For experiments in which agonist action was to be blocked by a specific antagonist, the antagonist was first preapplied alone for 10 min followed by coaplication for 10 min of antagonist and agonist. Washout was always 60 min in coaplication experiments. To test whether mGluR agonist action might be due to cross-reactivity with N-methyl-D-aspartate (NMDA) receptors as described in mammals (Breakwell et al. 1997; Contractor et al. 1998; Wilsch et al. 1994), we did control experiments where D-AP5, a potent NMDA receptor antagonist was preapplied and subsequently coapplied at 100–250 μM with the group I agonist L-quisqualic acid (5–10 μM) and the group II agonist L-CCG-I (50 μM) in the same protocol as used for mGluR antagonists. PTX was used in an identical procedure at 10–20 μM together with L-CCG-I (50 μM) and L-AP4 (100 μM) to test for cross-reactivity with fast glutamate-gated chloride channels.

To block modulatory inputs from commissural and esophageal ganglia, the desheathed portion of the stn was superfused in its separate well with isotonic sucrose containing 0.1–1 μM TTX, thus blocking action potential conduction. If gastric rhythms had to be induced by pilocarpine, the muscarinic agonist was superfused in the STG well at concentrations of 35–150 μM.

**Electrophysiological recording techniques and data processing**

Extracellular recordings from motor nerves of the STNS were made with stainless steel electrodes connected to Model 1700 differential AC amplifiers (A-M Systems, Carlsborg, WA). Intracellular recordings from STG neurons were made with glass micropipettes connected to Axoclamp 2B (Axon Instruments, Foster City, CA) and Model 1600 (A-M Systems, Carlsborg, WA) intracellular amplifiers. Micropipette resistances were 8–15 MΩ when filled with 3 M KCl. Gastric neurons were identified by correlating intracellular action potentials with the extracellular ones from the identified motor nerves. Extracellular and intracellular recordings were digitized at 11 kHz with a PCM recording adapter (Model 3000A, Vetter, Rebersburg, PA) and stored on video tape for later analysis. Data were played back on a TA6000 chart writer (Gould Instrument Systems, Valley View, OH) and processed using Kaleidagraph (Macintosh version 3.08d) and Sigmastat (SPSS Science, version 2.03) software. The significance of drug effects was determined applying one-way ANOVA using Kruskal-Wallis analysis on ranks.

**RESULTS**

All mGluR agonists we tried had clear effects that are detailed in the following text. By contrast, most mGluR antagonists we tried had no statistically significant effects on ongoing combined or pilocarpine-induced rhythms. They were also unable to block significantly the effect of agonists specific for their respective mGluR group. In the summary table, they are indicated as “no effect detected.” Except for the group-I-specific antagonist AIDA (Fig. 4), no data are shown for these negative results. For those antagonists that had clear effects on ongoing rhythms, such as (RS)APICA, or were capable of at least partially blocking the effects of mGluR agonists, such as (RS)APICA and MPPG, experimental data are also shown in the following text.

In control experiments, we tested whether the potent NMDA receptor antagonist D-AP5 interfered with mGluR agonists that were acting on the lobster gastric circuit as has been reported in mammalian systems (Breakwell et al. 1997; Contractor et al. 1998; Wilsch et al. 1994). At a concentration of 100 μM, D-AP5 had no effect on the excitatory action of 5 and 10 μM L-quisqualic acid, and 250 μM was without effect on the inhibitory action of 50 μM L-CCG-I (data not shown). At both concentrations D-AP5 had no effect on gastric rhythms when applied alone (data not shown). DL-AP5 and its analogue D-AP7 have both been shown to block crustacean NMDA receptors (Parnas et al. 1996; Pfeiffer-Linn and Glantz 1991)

**Nonspecific agonist ACPD stimulates gastric rhythm generation**

In the gastric circuit of the STG (Fig. 1.), ACPD accelerated endogenous as well as pilocarpine-induced rhythms in a concentration-dependent manner as shown in Fig. 2 for an endogenous gastric rhythm. This rhythm-accelerating effect was reproducible and could be observed at concentrations of 50 μM; however, the effect of ACPD was highly variable. In most of our experiments, the accelerating effect of ACPD decreased continuously during the 10-min superfusion period (Fig. 2E). In a total of eight experiments in which 50–100 μM ACPD was applied, ongoing oscillations were accelerated in all recorded motoneurons. In addition, in three experiments where the stn was blocked, oscillations were reversibly induced in silent [dorsal gastric (DG), gastric mill (GM), and median gastric (MG)] or in tonically firing [DG, lateral posterior gastric (LPG) and GM] motoneurons (data not shown).

**Group I mGluR agonists L-quisqualate and DHPG stimulate gastric rhythm generation**

Of the five mGluR agonists that were tried on the STG, L-quisqualic acid had the strongest activating effect, in oscillating (Fig. 3B) and nonoscillating (Fig. 3D) preparations alike (n = 14). The action of L-quisqualate was observed in all gastric motoneurons starting at concentrations as low as 5 μM. When the gastric CPG was blocked, rhythmic bursting could be induced in silent and tonically firing motoneurons by superfusion of 25 μM L-quisqualate. Characteristically, the activation of the gastric CPG by L-quisqualate was transient and decreased or ceased completely over the 10-min application period.

Figure 3A shows the results of an experiment where a vigorous gastric rhythm was induced by 35 μM pilocarpine. On application of 25 μM L-quisqualic acid, this rhythm gradually increased in frequency (Fig. 3B). Note in Fig. 3B that while the rhythm was accelerating, motoneuron AM (ann recording) also started firing during its interburst interval. This would gradually lead to tonic firing, sometimes with some gastric modulation remaining, as seen in Fig. 3C. Intracellular recordings revealed that the development of tonic firing was due to a buildup of a sustained tonic depolarization in the
electrotonically coupled motoneurons AM and DG (shown for AM in Fig. 5). In parallel with the increased tonic firing of AM and DG, the gastric rhythm often became irregular and on extended application of L-quisqualic acid became slower than initially (Fig. 3C). This figure shows that the subthreshold membrane potential oscillations recorded in neuron LPG maintained an accelerated rhythm; however, due to the failure of almost every second subthreshold potential to produce a full amplitude depolarization, an overall slower burst rhythm than the control was produced. The slower gastric rhythm can also be seen from the MG recording.

In Fig. 3D the pilocarpine and the L-quisqualate were washed out and all rhythmic gastric activity ceased. Under these conditions, motoneurons AM (amn) and GM (mgn) fired tonically, whereas LPG continued to receive some cyclic modulatory input that had neither a gastric nor a pyloric frequency. Application of 25 μM of L-quisqualic acid onto the nonoscil-lating gastric CPG resulted in a slow depolarization and a gradual increase in amplitude of subthreshold rhythmic depolarizations developed after ~40 s. The oscillations in MG were stable for 1 min at a frequency of ~0.09 Hz, which then accelerated during the following 4 min. After ~4 min, the high-amplitude oscillations ceased and also the weak bursts in amn and aln. Instead it can be seen that AM and the GMs were firing tonically at high frequencies. In Fig. 4A the development of cycle duration in a pilocarpine-induced gastric rhythm after application of 10 μM L-quisqualic acid and 200 μM of the group I-specific antagonist AIDA is plotted against the cycle number. Although 10 μM L-quisqualate reduced the cycle duration by 26% (30 ± 13.03%, mean ± SD, n = 5), AIDA alone had no significant effect and also did not block the effect of L-quisqualic acid (Fig. 4B). Figure 5 shows for motoneuron AM the slow depolarization that was characteristic for the effect of L-quisqualic acid in the electrotonically coupled motoneurons AM and DG. In most experiments, it would lead to sustained tonic firing, which then was accompanied by a slowed gastric rhythm.

DHPG had effects on the gastric CPG that were similar to L-quisqualate in that it reproducibly induced rhythmic discharges in silent gastric motoneurons when the stn was blocked. As with ACPD and L-quisqualate, the effect of DHPG (n = 4) was dose dependent; however, we did not observe the slow buildup of tonic firing in motoneurons AM and DG. Instead gastric rhythms were generated that appeared stable during the 10-min application period. In three experiments, these DHPG-induced rhythms were much slower in frequency (0.05–0.01 Hz) than combined gastric rhythms (~0.1 Hz). In one experiment, however, a stable rhythm was induced that looked indistinguishable from combined rhythms and had a normal gastric frequency of 0.08 Hz (Fig. 6B). Figure 6D shows the development of cycle duration over cycle number of gastric rhythms induced by different concentrations of DHPG in a silent preparation where the stn was blocked. Note the high variability at 25 μM DHPG, whereas at 50 μM, the cycle duration increased only slightly over the application period and at 100 μM, it was stable after a few cycles of adjustment.

**Group II agonist L-CCG-I inhibits gastric rhythm generation**

The effect of L-CCG-I was always inhibitory (n = 16) on both endogenous and pilocarpine-induced gastric rhythms. When a preparation in which a vigorous gastric rhythm had been induced by application of pilocarpine was superfused with L-CCG-I, rhythmic activity was either slowed or halted entirely, depending on the concentration used. Concentrations of 50 μM stopped rhythmic and tonic activity in most experiments. Figure 7A shows an example of application of 10 μM...
L-CCG-I that resulted in complete block of oscillatory activity in AM after only four gastric cycles. The intracellular recording indicates that the neuron was held hyperpolarized with the ability to generate plateau potentials removed. As can be seen in Fig. 7B, some high-frequency, rhythmic input remained intact, and although action potential threshold was reached several times, no plateau potentials were triggered over the entire application period of 10 min, although it appears from the extracellular recordings in aln, lpgn, and mgn that each action potential in motoneuron AM was correlated with a single CPG cycle in the other neurons. Under these conditions, plateau potentials could not be induced by depolarizing current injection. The extracellular recording from lpgn shows that the motoneurons LPG also stopped bursting. The extracellular recording from aln shows that the GM motoneurons started to fire action potentials tonically but with a frequency that decreased slowly over the application period without ever being completely silenced. The extracellular recording from nerve mgn shows that bursting in motoneuron MG became irregular but did not stop completely. As can be seen from Fig. 7C, this effect of L-CCG-I was completely reversible.

Figure 7D shows an almost identical experiment as in Fig. 7A, however, at a higher concentration of 50 µM L-CCG-I. Again, gastric burst firing was rapidly blocked in motoneuron AM as shown in the extracellular recording in nerve amn. DG, recorded intracellularly, behaved essentially like AM shown in Fig. 7A. It remained hyperpolarized at an apparent resting potentials after only four cycles and thereafter remained silent for the 10-min application period. At 50 µM L-CCG-I, all recorded gastric cells were silenced as can be seen from the extracellular recordings of motoneurons GM and LPG. Note also the bursting in nerves aln (GM neurons) and amn. On prolonged application of l-quisqualic acid (+4 min), action potential firing became tonic in AM, LPG, and the GMs and oscillations ceased in MG. Voltage calibration for intracellular recordings only.
recorded, although some subthreshold gastric modulation remained visible in LPG and AM continued to fire single action potentials or weak irregular bursts of few action potentials. The effect of L-CCG-I was fully reversible in combined preparations (Fig. 8C). The inhibitory action of L-CCG-I was not prevented by the chloride channel blocker PTX (Fig. 9) at concentrations between 10 and 20 μM (n = 5).

The data presented in Figs. 7 and 8 (and Fig. 9 in PTX) are representative for the action of L-CCG-I on the gastric CPG in the majority of experiments. In a total of 16 experiments at concentrations of 10–100 μM, all gastric motoneurons were affected. However, the inhibitory action was already most immediate on the return-stroke phase motoneurons AM, DG, and LPG (Fig. 1), which were always the first to be affected and were silenced completely in the majority of experiments (AM: 68.8%, n = 16; DG: 85.7%, n = 14; LPG: 80%, n = 15). This particular sensitivity of AM, DG, and LPG to the inhibitory mGluR agonist L-CCG-I matched the also exceptional sensitivity of these motoneurons to the inverse action of the group II antagonist (RS)APICA (see following text and DISCUSSION). Although the powerstroke motoneurons MG, LG, and GM were also inhibited by L-CCG-I, in all experiments, they were less frequently completely silenced (MG: 11.1%, n = 9; LG 25%, n = 8; GM 50%, n = 14). Instead the powerstroke motoneurons continued irregular or tonic firing.

Antagonistic and inverse agonist action of the group-II-specific antagonist (RS)APICA

In both endogenous and pilocarpine-induced gastric rhythms the effect of the group-II-specific antagonist (RS)APICA was always opposite to the group II agonist L-CCG-I. Figure 10 shows an experiment with a pilocarpine-induced gastric rhythm (Fig. 10A). Note that in the presence of 50 μM L-CCG-I in pilocarpine (Fig. 10B), motoneurons LPG and AM were silenced although some subthreshold rhythmic activity remained visible in LPG. The latter was correlated with the continuing activity of the gastric CPG as seen from the mgn recording and also in aln. Note, however, that the GM motoneurons recorded in aln gradually switched to continuous

FIG. 4. Acceleration of gastric rhythm by L-quisqualic acid. A: plot of cycle duration against cycle number for an experiment with a pilocarpine-induced rhythm. C, control before drug application, n = 5 cycles. L-quisqualic acid (10 μM) reduced the cycle duration by 26%. R, recovery after 30 min washing with saline, n = 9 cycles. Group-I-specific antagonist (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA) alone (200 μM) had no effect, whereas the following coapplication with 10 μM L-quisqualic acid reduced the cycle duration by 24%. R, recovery after 30 min washing with saline, n = 18 cycles. B: bar diagram of data extracted from the experiment plotted in A (means ± SD). Control: control cycles. L-quis: 16 cycles from the region of minimal cycle duration after L-quisqualate application. Recovery 1, n = 9 cycles. AIDA: 11 cycles from the region of minimal cycle duration after application of 200 μM AIDA alone. AIDA + L-quis: 15 cycles from the region of minimal cycle duration after coapplication of 200 μM AIDA with 10 μM L-quisqualate. Recovery 2, n = 18 cycles. Values for L-quis and AIDA/L-quis are significantly different (P < 0.05) from control, recoveries, and AIDA alone. Differences between control, recoveries and AIDA were not significant (P > 0.05). ↓, gap in the sequence of measured values.
firing. As described in the preceding text, the effect of L-CCG-I could be reversed completely by washing with saline (Fig. 10C). (RS)APICA (1 mM) partially blocked the action of 50 μM L-CCG-I when the two were coapplied (Fig. 10E). The recovery of oscillatory activity by the antagonistic effect of (RS)APICA was almost complete for the intracellularly recorded motoneuron LPG and a slightly irregular gastric rhythm was clearly discernible in all neurons except AM, which remained inhibited. In five different experiments, we never observed a complete block of the inhibitory action of 50 μM L-CCG-I by (RS)APICA when the antagonist was applied at a concentration of 1 mM.

When superfused over the STG alone at a concentration of 1 mM (RS)APICA had a particularly dramatic effect. As can be seen in Fig. 10D, the depolarizing phases in the return-stroke motoneurons DG and LPG were remarkably prolonged (≤10 times the normal cycle duration in 1 experiment), and the correspondingly prolonged action potential bursts in the efferent nerve amn containing the AM motor axon suggest the same for this motoneuron. In a separate experiment where AM was recorded intracellularly, we confirmed that this was indeed the case under identical conditions of drug application (data not shown). The effect of (RS)APICA on combined gastric rhythms was principally identical to that on muscarinic agonist-induced rhythms, although less dramatic (Fig. 11). The prolongation of intracellular depolarizations and axonal bursts generated by motoneurons AM, DG, and LPG oscillating in an endogenous rhythm was about four times their normal duration before (RS)APICA was applied for 10 min (n = 3). Also, Fig. 11 shows clearly the augmented inhibition of LG that resulted in low-frequency oscillation. As with pilocarpine-induced rhythms (Fig. 10F), the effect of (RS)APICA was fully reversible on washing with saline (Fig. 11C).

**Effect of group-III-specific mGluR agonist L-AP4 is similar to that of L-CCG-I**

Mammalian group III mGluRs, which, like group II mGluRs, are coupled to adenylyl cyclase, are selectively activated by the agonist L-AP4. In our experiments (n = 4), we found that the action of L-AP4 on gastric rhythm generation was always inhibitory as was L-CCG-I. However, the sensitivity of all gastric cells was lower to L-AP4 than to L-CCG-I at identical concentrations. Normally concentrations of ≥100 μM of the group III agonist had to be administered to evoke effects comparable with those observed with 50 μM of the group II agonist. Figure 12 shows an experiment in which 100 μM L-AP4 was superfused over an STG preparation oscillating with a pilocarpine-induced rhythm (Fig. 12A). The figure shows clearly that L-AP4 at a concentration of 100 μM blocked
Effect of L-AP4 is blocked by group-III-specific antagonist MPPG

Figure 13 shows an experiment on a stable rhythm recorded in a combined preparation (Fig. 13A), where the inhibitory effect on the gastric CPG of 100 μM L-AP4 (Fig. 13B), was blocked by the group-III-specific antagonist MPPG at a concentration of 1 mM (Fig. 13C). Recordings were made extracellularly from motoneurons GM in nerve aln and from motoneuron LG recorded in nerve lgn. As in all experiments with antagonists, MPPG was first preapplied alone for 10 min before being coapplied with 10 μM L-AP4. Preaplication of the antagonist alone had no detectable effect on the gastric rhythm (data not shown). However, in the presence of 1 mM MPPG, the gastric rhythm was unaffected by 100 μM L-AP4 (Fig. 13C). As Fig. 13D shows, the effects of agonists and antagonists were reversible.

DISCUSSION

The principal result of our study is that agonists and antagonists that are specific for mGluR types I–III have clear and distinct effects on the gastric CPG (summarized in Table 1). This allows us to put forward the hypothesis that mGluRs of distinct groups are likely to be present in the gastric circuit of the STG and could be important for normal CPG function. Although never investigated before, the presence and modulatory role of mGluRs in the decapod STG does not come unexpectedly. Slow excitatory and inhibitory effects of L-glutamate, when ejected onto STG neurons, have been described in the past (Marder and Paupardin-Tritsch 1978; Tazaki and Chiba 1994), suggesting multiple mechanisms of action for glutamate. However, the level of detail to which the electrophysiological pharmacology of mGluR agonists in the gastric network resembles the action of these compounds in mammals, as well as the potent effect of some of them on ongoing rhythms, is remarkable and clearly suggests a role for glutamate as a modulatory substance in the STG, acting on different types of mGluRs expressed differentially in the STG. Similar data have been obtained for the pyloric circuit of the lobster STG (N. L. Pérez-Acevedo, W. D. Krenz, Y. I. Arshavsky, and A. I. Selverston, unpublished data).

Can we conclude that lobster homologs of group I–III mGluR genes are expressed in STG neurons? A definitive answer to this question will have to await cloning of Panulirus mGluR genes so that sequences and pharmacologies of individual recombinant receptors can be determined and compared with mammalian receptors. This careful consideration seems to be appropriate in the light of the sometimes unusual pharmacology of invertebrate homologs of mammalian channels and receptors (Benson 1988; Darlison 1992; Jackel et al. 1994a,b). The only invertebrate mGluR gene cloned thus far comes from Drosophila melanogaster. Amino acid sequence and pharmacology of the recombinant receptor place it closest to mammalian mGluRs in gastric rhythm generation in all gastric neurons that we recorded (Fig. 12B). The intracellularly recorded LPG neuron remained silent at its apparent resting potential and the other recorded cells fired action potentials tonically. The effect of L-AP4 was reversible (Fig. 12C). The inhibitory effect of L-AP4 was not prevented by 10–20 μM PTX (data not shown).

Figure 13 shows an experiment on a stable rhythm recorded in a combined preparation (Fig. 13A), where the inhibitory effect on the gastric CPG of 100 μM L-AP4 (Fig. 13B), was blocked by the group-III-specific antagonist MPPG at a concentration of 1 mM (Fig. 13C). Recordings were made extracellularly from motoneurons GM in nerve aln and from motoneuron LG recorded in nerve lgn. As in all experiments with antagonists, MPPG was first preapplied alone for 10 min before being coapplied with 10 μM L-AP4. Preaplication of the antagonist alone had no detectable effect on the gastric rhythm (data not shown). However, in the presence of 1 mM MPPG, the gastric rhythm was unaffected by 100 μM L-AP4 (Fig. 13C). As Fig. 13D shows, the effects of agonists and antagonists were reversible.

DISCUSSION

The principal result of our study is that agonists and antagonists that are specific for mGluR types I–III have clear and distinct effects on the gastric CPG (summarized in Table 1). This allows us to put forward the hypothesis that mGluRs of distinct groups are likely to be present in the gastric circuit of the STG and could be important for normal CPG function. Although never investigated before, the presence and modulatory role of mGluRs in the decapod STG does not come unexpectedly. Slow excitatory and inhibitory effects of L-glutamate, when ejected onto STG neurons, have been described in the past (Marder and Paupardin-Tritsch 1978; Tazaki and Chiba 1994), suggesting multiple mechanisms of action for glutamate. However, the level of detail to which the electrophysiological pharmacology of mGluR agonists in the gastric network resembles the action of these compounds in mammals, as well as the potent effect of some of them on ongoing rhythms, is remarkable and clearly suggests a role for glutamate as a modulatory substance in the STG, acting on different types of mGluRs expressed differentially in the STG. Similar data have been obtained for the pyloric circuit of the lobster STG (N. L. Pérez-Acevedo, W. D. Krenz, Y. I. Arshavsky, and A. I. Selverston, unpublished data).

Can we conclude that lobster homologs of group I–III mGluR genes are expressed in STG neurons? A definitive answer to this question will have to await cloning of Panulirus mGluR genes so that sequences and pharmacologies of individual recombinant receptors can be determined and compared with mammalian receptors. This careful consideration seems to be appropriate in the light of the sometimes unusual pharmacology of invertebrate homologs of mammalian channels and receptors (Benson 1988; Darlison 1992; Jackel et al. 1994a,b). The only invertebrate mGluR gene cloned thus far comes from Drosophila melanogaster. Amino acid sequence and pharmacology of the recombinant receptor place it closest to mamma-
Drosophila mGluRs has yet been published, therefore we do not know whether native Drosophila mGluRs show any similarity with group I, II, or III pharmacology and whether intracellular messenger coupling is conserved. Interestingly, first evaluations of the recently completed Caenorhabditis elegans genome project indicate that this simple nematode has at least four genes coding for mGluRs (Bargmann 1998). It is therefore reasonable to assume that in more complex invertebrate animals mGluRs may be present in a diversity as impressive as in vertebrates.

Specificity of the responses to mGluR compounds

Any study of the action of drugs on the electrophysiology of nerve cells has to fulfill a number of criteria to be considered meaningful for the characterization of a respective receptor: the drug should be effective at low concentrations to avoid non-specific effects, the effect of the drug should be dose dependent, it should be at least partially reversible, and it should be antagonized by specific antagonists. If we take as a reference the data available from cloned and exogenously expressed mammalian mGluRs and those from the cloned Drosophila mGluR, then the effects of mGluR compounds we describe here for the gastric circuit of the Panulirus STG are specific and significant. Threshold doses for clearly identifiable effects of agonists for all three groups were at concentrations of between 5 and 50 μM. Considering that we measured effects at the network level in an intact ganglion where access of drugs to the receptor binding sites is likely to be not as immediate and rapid as for receptors expressed exogenously in cell lines or in Xenopus oocytes, these concentrations can be regarded as low. Also it is important to note that dose-response relationships obtained at the circuit level may be misleading because drugs applied to the STG act onto all susceptible elements of the circuit simultaneously, potentially leading to complex, concentration- and time-dependent interactions that may be difficult to interpret.

FIG. 8. Effect of L-CCG-I on a combined gastric rhythm. Here a stable gastric rhythm was recorded from a combined preparation (A). Extracellular recordings are from nerves aln (motoneurons GM) and dgn (motoneuron DG). Motoneurons AM and LPG were recorded intracellularly. B: on superfusion with 50 μM L-CCG-I, burst firing largely stopped in the recorded neurons although some apparent gastric modulation remained intact and AM fired irregular bursts. Effect of L-CCG-I was fully reversible on washing with saline (C). Voltage calibration for intracellular recordings only.

FIG. 9. Effect of L-CCG-I is not blocked by picrotoxin (PTX). In this experiment, a stable gastric rhythm was recorded in a combined preparation in the presence for 1 h of 10 μM PTX (A). Under these conditions, the motoneurons GM recorded in nerve aln fired tonically at low frequency (large units in aln; the small amplitude rhythmic bursts represent an artifactual pickup of the pyloric rhythm). On application of 50 μM L-CCG-I, rhythmic activity in motoneurons AM and LPG stopped (B). Effect of L-CCG-I was fully reversible on washing with saline (C). Voltage calibrations for intracellular recordings only.
A number of studies have shown that mGluR compounds have significant nonspecific cross-reactive effects on native and exogenously expressed NMDA receptors (Breakwell et al. 1997; Contractor et al. 1998; Wilsch et al. 1994). Therefore although responses to NMDA have never been described in STG neurons, we tested whether responses to mGluR compounds were antagonized by D-AP5, a widely used potent NMDA receptor antagonist. Our negative results further strengthen the case for specific mGluR-mediated modulation within the lobster STG. Similarly, PTX, a chloride channel blocker frequently used to block fast synaptic inhibition within the STG (Bidaut 1980) failed to block the inhibitory action of L-CCG-I and L-AP4. This indicates that there is no significant interaction between mGluR agonists and the fast synaptic glutamate-gated chloride channels.

In our investigation, antagonists always required much higher doses than agonists for clear effects. Concentrations of mGluRs IN GASTRIC RHYTHM GENERATION

FIG. 10. Effect of the group-II-specific antagonist (RS)-1-amino-5-phosphonoindand-1-carboxylic acid [(RS)APICA] on the gastric CPG in pilocarpine. In this experiment, a stable gastric rhythm was induced with 50 μM pilocarpine (A). Extracellular recordings were made from nerves ann (motoneuron AM), aln (motoneurons GM), and mgn (motoneuron MG and pyloric units). Motoneurons DG and LPG were recorded intracellularly. B: on superfusion with 50 μM L-CCG-I, motoneurons AM and LPG stopped burst firing and the rhythm recorded from motoneurons GM, MG, and DG was less vigorous. Gastric CPG remained active. Note that the pyloric rhythm recorded on the mgn trace also was affected. Effect of L-CCG-I was reversible on washing with saline (C). D: on superfusion with 1 mM (RS)APICA alone for 10 min, the gastric CPG was slowed dramatically. In the presence of (RS)APICA, depolarizing potentials and burst firing were drastically prolonged in the return-stroke phase motoneurons LPG, DG, and AM while the power-stroke phase motoneurons GM and MG fired antagonistic short bursts at low frequency. Effect of (RS)APICA was fully reversible (data not shown). After preapplication for 10 min of 1 mM (RS)APICA alone (data not shown), 50 μM L-CCG-I was applied in the continued presence of 1 mM (RS)APICA (E). Under these conditions, the inhibitory action of L-CCG-I was less pronounced than when applied alone. LPG continued to oscillate and DG and the motoneurons GM maintained a better defined burst rhythm than with L-CCG-I alone. Drug effects were largely reversible on washing with saline (F). Voltage calibration for intracellular recordings only.

FIG. 11. Effect of (RS)APICA on a combined rhythm. In this experiment, a stable gastric rhythm was recorded intracellularly in a combined preparation from motoneurons DG, LG, and LPG (A). On application of 1 mM (RS)APICA, the gastric CPG was slowed significantly (B). Depolarizations and burst firing were prolonged in return-stroke phase motoneurons DG and LPG. Power-stroke phase motoneuron LG was kept hyperpolarized for extended times and generated slow depolarizations and action potential bursts at low frequency and with a shortened duty cycle. Effect of (RS)APICA was reversible on washing with saline (C). Voltage calibration for intracellular recordings only.
1 mM were needed to block the action of agonists at least partially, and blocks were only obtained with (RS)APICA and MPPG. This finding is reminiscent of the effect of mGluR antagonists in many mammalian preparations where IC50 values for antagonists are frequently high (Conn and Pin 1997), mandating high concentrations for complete blockade of agonist responses. Again the observation that invertebrate receptors may have significantly different pharmacologies than their vertebrate counterparts (Benson 1988; Darlison 1992; Jackel et al. 1994a,b) offers the most likely interpretation for the inability of most antagonists to block mGluR agonists.

Intracellular messenger coupling

It is known that cyclic AMP is involved as an intracellular messenger in modulation of at least some STG neurons by biogenic amines (Flamm et al. 1987; Hempel et al. 1996). Also application of the muscarinic agonist pilocarpine induces a rise in intracellular cyclic AMP in the pyloric pacemaker neuron AB (Hempel et al. 1996). No such information is available for responses to glutamate in the STG; however, a G-protein-coupled activation cascade has been suggested for presynaptic GluRs at the crustacean NMJ (Michel et al. 1991; Miwa et al. 1987, 1990). mGluRs of type II and III are coupled to adenylyl cyclase and reduce cyclic AMP synthesis in mammalian systems. In the STG, modulatory compounds that are capable of inducing CPG activation, such as biogenic amines and muscarinic agonists, increase cyclic AMP production in pyloric neurons and the gastric neurons GM. Thus it appears reasonable to hypothesize that group II and III agonists that reduce or block gastric CPG activity, known from other systems to reduce cyclic AMP synthesis, are probably also inhibiting cyclic AMP synthesis in the STG. The particularly strong inverse agonist action on pilocarpine induced gastric rhythms would be in accordance with this notion. Clearly more research is needed to elucidate this possibility.

In mammals it has been demonstrated in several systems that activation of mGluRs, especially by group I agonists and the nonspecific ligand ACPD, results in release of calcium from intracellular stores (Geiling and Schild 1996; Linden et al. 1994; Netzeband et al. 1997). The same was found in leech glial cells (Lohr and Deitmer 1997). Caffeine, known to release calcium from the endoplasmatic reticulum in neurons, induces rhythmic discharges of the pyloric CPG in crayfish. (Hermann 1981). Because group I agonists also induce rhythmic discharges in the gastric and pyloric networks (N. L. Pérez-Azevedo, W. D. Krenz, Y. I. Arshavsky, and A. I. Selverston, unpublished data), it could be that in the crustacean STG type I mGluRs also may act via an increase in intracellular calcium concentration by release from intracellular stores. Again, more research is needed to address this question.

Functional implications of mGluRs in the STG

Studies at the network level do not give information about target cells and real dose-dependence relationships. However, the differential effects of group-specific compounds on rhythm generation suggest that different types of mGluRs may be
present in the STG, specific activation of the different types of mGluRs leads to differential, sometimes opposite actions on the gastric rhythm, and in particular the group-II-specific drugs show apparent preference for action on the return-stroke motoneurons AM, DG, and LPG (Figs. 7–11) Again, we cannot claim that the apparent preference of group-II-specific mGluR agonists and antagonists is the result of the expression of group II mGluRs in the membranes of AM, DG, and LPG or whether they are under modulatory control of other neurons that express these mGluRs. However, it can be inferred from our data that group II mGluRs could play a key role in the regulation of rhythm generation in this functional subgroup of neurons (Fig. 1). Our results make them key candidates for cellular studies.

In our experiments, the group-II-specific antagonist (RS)APICA had a very strong effect on ongoing endogenous and pilocarpine-induced rhythms (Figs. 10 and 11). It is possible, that this effect of (RS)APICA is due to its peculiar inverse agonist action as described earlier in recombinant mammalian receptors (Ma et al. 1997). An alternative explanation would be that, during rhythmic behavior, the antagonist is blocking a population of group-II-like mGluRs. This would imply that during endogenous as well as pilocarpine-induced gastric rhythms, glutamate release may be involved in the regulation of membrane conductances that determine the oscillatory behavior of the gastric CPG. According to this model, group II mGluRs would be acting in a feedback loop regulating the duration of plateau potentials. Our experimental data do not allow us to distinguish between the two possibilities. Evidently the two mechanisms of action may well be acting synergistically to produce the strong effect of (RS)APICA.

One of the interesting properties of mGluRs is the fact that some of the subtypes show significant desensitization (Guérineau et al. 1997; Herrero et al. 1994) on sustained presence of glutamate and specific ligands as studied in detail in recombinant mGluRs (Gereau and Heinemann 1998). Recent data from mammals provide evidence that receptor desensitization may play a role in dynamic switching of presynaptic mGluR function from facilitation to depression of neurotransmitter release at mammalian hippocampal synapses (Herrero et al. 1998; Rodriguez-Moreno et al. 1998). In our experiments, the effects

<table>
<thead>
<tr>
<th>Group Coupling*</th>
<th>Drug</th>
<th>Action in Gastric Circuit**</th>
<th>Concentration Used, µM</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>ns</td>
<td>ago ACPD</td>
<td>Excitatory, accelerating</td>
<td>10–100</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>ant MCPG</td>
<td>No effect detected</td>
<td>500–1000</td>
<td>4</td>
</tr>
<tr>
<td>I PLC+</td>
<td>ago DHPG</td>
<td>Excitatory, accelerating, starts gastric rhythm</td>
<td>25–100</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>ago L(-)-quiscalic acid</td>
<td>Excitatory, transiently accelerating</td>
<td>5–100</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>ant AIDA</td>
<td>No effect detected</td>
<td>200–500</td>
<td>3</td>
</tr>
<tr>
<td>II AC−</td>
<td>ago L-CCG-I</td>
<td>Inhibitory</td>
<td>10–100</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>ant MCCG</td>
<td>No effect detected</td>
<td>500</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>ant MTPG</td>
<td>No effect detected</td>
<td>500–1000</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>ant (RS)APICA</td>
<td>Cell-specific excitatory, blocks inhibition by L-CCG-I</td>
<td>1000</td>
<td>5</td>
</tr>
<tr>
<td>III AC−</td>
<td>ago L-AP4</td>
<td>Inhibitory</td>
<td>50–100</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>ant MSOP</td>
<td>No effect detected</td>
<td>200–1000</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>ant MPPG</td>
<td>Blocks inhibition by L-AP4</td>
<td>1000</td>
<td>4</td>
</tr>
</tbody>
</table>

Summary table of all mGluR agonists and antagonists tested in the STG and their effects. I, II, III, mGluR group I—III; ago, agonist; ant, antagonist; PLC+, positively coupled to phospholipase C; AC−, negatively coupled to adenyl cyclase; ns, nonselective; ACPD, (1S,3R)-1-amino-3-cyclopentene-1,3-dicarboxylic acid; MCPG, (RS)-α-methyl-3-carboxymethylphenylglycine; DHPG, (S)-3,5-dihydroxyphenylglycine; AIDA, (RS)-1-aminoindan-1,5-dicarboxylic acid; L-CCG-I, (2S,1′S,2′S)-2-(carboxycyclopropyl)glycine; MCCG, (2S,3S,4S)-2-methyl-2-(carboxycyclopropyl)glycine; MTPG, (RS)-α-methyl-4-tetrazolylphenylglycine; (RS)APICA, (RS)-1-amino-5-phosphonoindanol-1-carboxylic acid; t-AP4, t(+)-2-amino-4-phosphonobutyric acid; MSOP, (RS)-α-methylserine-O-phosphate; MPPG, (RS)-α-methyl-4-phosphonophenylglycine.

* As determined for mammalian mGluRs. **In pilocarpine-induced and combined rhythm and in nonoscillating preparations.

TABLE 1. Summary of effects of group I–III mGluR agonists and antagonists in the gastric circuit

Possible sources for the modulatory action of glutamate

Two possible sources for glutamate acting as a neuromodulator within the STG may be considered. These may be acting either in isolation or in combination and in a time- and/or activity-dependent manner. The first possibility would refer to extrinsic modulation (Katz 1995). Neurons in the higher ganglia or in the brain may project into the STG where they release t-glutamate exclusively or as a cotransmitter. Examples of extrinsic modulation are well documented in the STG of decapod crustaceae. Examples include the gastropyloric muscle receptor GPR (Katz and Harris-Warrick 1989; Katz et al. 1989; Kiehn and Harris-Warrick 1992) and the modulatory neuron APM, which has its cell body in the esophageal ganglion (Dickinson et al. 1988; Nagy et al. 1988). In this scenario, the targets for glutamate could be STG motoneurons directly or nerve endings that release a neuromodulator other than glutamate, which in turn would excite or inhibit target cells. Pre-synaptic inhibition has indeed been shown in the crab STG to be involved in motor pattern selection (Bartos and Nusbaum 1997). In addition, positive or negative feedback via presynaptic autoreceptors is possible as described in vertebrates (Co-chilla and Alford 1998) and also suggested for the crustacean neurons (Miwa et al. 1993; Parnas et al. 1994, 1996; Schramm and Dudel 1997). At present we cannot distinguish between these different possibilities.

A second alternative is intrinsic modulation (Katz 1995), where t-glutamate from the fast inhibitory glutamatergic synapses within the ganglion would diffuse away from the synaptic cleft and reach para- or extrasynaptic metabotropic receptors, which again may be localized on the pre- or postsynaptic
side. If located presynaptically, they could enhance or suppress transmitter release (Schramm and Dudel 1997). As has been suggested from work in vertebrates, the degree of diffusion of glutamate to mGluRs remote from the subsynaptic membrane may depend on the activity level of the synapse (Min et al. 1998). If located postsynaptically, they could act on various postsynaptic currents or modulate intrinsic electrical membrane properties leading to excitation, oscillation, or inhibition. Intrinsic modulation by glutamate is particularly interesting because much of the neurotransmitter release in the STG is graded (Graubard et al. 1983; Raper 1979). Therefore the effective output of glutamate into the synaptic cleft, and as a consequence diffusion to more remote synaptic sites or presynaptic receptors, might be under dynamic control of oscillatory cycles. Evidently extrinsic and intrinsic modulation are not mutually exclusive but instead may very well act in concert.

We clearly have shown that mGluR-specific compounds have important activating and inactivating effects on motor patterns generated by the gastric circuit of the lobster STG. Now we have to elucidate the molecular identity of the metabotropic receptors involved, their localization in specific identified motoneurons of the STG, their intracellular messenger coupling, their evolutionary relationship with mammalian mGluRs, and the conditions under which they are activated in the intact isolated STNS and further in the intact animal. The latter parameter is of particular importance when one wants to understand the modulatory role of glutamate in the STG.

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references


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