Dorsal Unpaired Median Neurons of *Locusta migratoria* Express Ivermectin- and Fipronil-Sensitive Glutamate-Gated Chloride Channels


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Janssen D, Derst C, Buckinx R, Van den Eynden J, Rigo J-M, Van Kerkhove E. Dorsal unpaired median neurons of *Locusta migratoria* express ivermectin- and fipronil-sensitive glutamate-gated chloride channels. *J Neurophysiol* 97: 2642–2650, 2007. First published January 31, 2007; doi:10.1152/jn.01234.2006. Together with type A GABA and strychnine-sensitive glycine receptors, glutamate-gated chloride channels (GluCls) are members of the Cys-loop family of ionotropic receptors, which mediate fast inhibitory neurotransmission. To date, GluCls are found in invertebrates only and therefore represent potential specific targets for insecticides, such as ivermectin and fipronil. In this study, we identified the functional expression of GluCls in dorsal unpaired median (DUM) neurons of the metathoracic ganglion of *Locusta migratoria* using electrophysiological and molecular biological techniques. In whole cell patch-clamped DUM neurons, glutamate-induced changes in both their membrane potentials (current-clamp) and currents (voltage-clamp) were dependent on molecular biological techniques. In whole cell patch-clamped DUM neurons, glutamate-mediated changes in both their membrane potentials (current-clamp) and currents (voltage-clamp) were dependent on molecular biological techniques.

**INTRODUCTION**

Glutamate is the main excitatory amino acid neurotransmitter in the CNS of vertebrates, where it interacts both with ionotropic receptors, i.e., ligand-gated ion channels, and metabotropic or G-protein–coupled receptors (Hogner et al. 2002). In vertebrates, ionotropic glutamate receptors are permeable to cations and can be subdivided into three groups according to their respective main agonist: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors. In invertebrates, L-glutamate can also act as an excitatory transmitter at the neuromuscular junction (Ikeda et al. 2003; Wafford and Sattelle 1989). However, a biphasic response to L-glutamate is usually observed, consisting of a depolarization followed by a hyperpolarization. A pure hyperpolarizing effect can be obtained by applying ibotenate, a conformationally restricted structural analog of glutamate (Wafford and Sattelle 1989). These responses are mediated by another class of glutamate ionotropic receptors. These are involved in inhibitory synaptic transmission and consist of glutamate-gated chloride channels (GluCls). They were first identified in arthropods as extrajunctional glutamate receptors (“H” receptors) that cause hyperpolarization of the locust leg muscle (Cully et al. 1996; Dudel et al. 1989). The inhibitory ionotropic glutamate receptor gates a chloride-selective ion channel and belongs to the Cys-loop ligand-gated channel superfamily, together with the nicotinic acetylcholine, type A γ-aminobutyric acid (GABA), type C GABA, glycine, type 3 5-hydroxytryptamine (5-HT) receptors (Cleland 1996; Cull-Candy 1976) and the histamine-gated chloride channel (Zheng et al. 2002).

Until now, GluCls have been found only in invertebrate nerve and muscle cells (Cleland 1996). GluCl receptors thus form a potentially important target for the development of insecticides having highly selective toxic activity against insects that have become resistant to other insecticides (Raymond and Satelle 2002; Zhao et al. 2004). Chemical compounds that affect GluCl receptors include members of the avermectine/milbemycin (A/M) class of anthelmintics such as ivermectin, as well as the phenylpyrazole insecticides, including fipronil. These products are in common use and form effective prophylactic and curative treatments against endo- and ectoparasitic infestations (Bloomquist 2003; Horoszok 2001). Ivermectin, a macrocyclic lactone derived chemically from the parent macrocyclic lactone avermectin B1A, appears directly to activate GluCl or, at lower concentrations, to potentiate the effect of glutamate (Yates and Wolstenholme 2004). However, because of their close homology with GluCl, it is likely that GABA-gated channels in invertebrates are also sites of ivermectin toxicity (Kane et al. 2000). Fipronil was designated as a new GABA-gated chloride channel blocker and introduced into pest control, for instance against the Colorado potato beetle and against some cotton pests (Moffat 1993; Smith and Lockwood 2003). The blocking action of fipronil on GluCl channels was demonstrated in oocytes transfected with GluCls (Horoszok et al. 2001) and a similar degree of block was observed on chloride currents induced by glutamate and ibotenate in dorsal unpaired median (DUM) neurons of the cockroach *Periplaneta americana* and the grasshopper *Melanotis...
noplus sanguinipes (Ikeda et al. 2003; Raymond et al. 2000; Smith et al. 1999).

At the molecular and functional level, GluCls remain poorly characterized. Until now, cloning studies led to the identification of a small family of six GluCl genes in the Pieris napi (Ikeda et al. 2003; Raymond et al. 2000; Smith et al. 1999), a flock species used for insecticide screening (Cully et al. 1996). Electrophysiological studies of P. americana DmGluCl, expressed in oocytes, revealed direct activation by the acaride nodusporic acid and by iermein, as well as by the endogenous ligand glutamate (Kane et al. 2000).

The neurobiology of the locust has been extensively investigated and the action of glutamate on muscles of this insect has been well studied (Grolleau and Lapied 2000; Heidel and Pfüger 2006; Wicher et al. 2001). However, in the nervous system of L. migratoria, relatively little is known about the effect of glutamate on efferent DUM neurons. In the locust, efferent DUM neurons extend symmetrical pairs of axons to flight and leg (tibia) muscle, where they exert myo- and/or neuromodulatory effects. In other arthropod species, such as P. americana, GluCls seem to have a modulatory effect on the excitability of the DUM neurons and could play a substantial role in muscle and flight control (Dubas 1991; Washio 2002). It is thus tempting to postulate that GluCls fulfill the same role in DUM neurons of L. migratoria and that they might well therefore also represent a useful target for insecticide pest control in this species.

In the present study, we looked for the presence of a glutamate-gated chloride channel in an ex vivo preparation of DUM neurons of the metamorphic ganglion of L. migratoria using the whole cell patch-clamp technique. Under current-clamp conditions, glutamate-induced changes in membrane potential were measured, whereas under voltage-clamp conditions, glutamate-evoked currents were pharmacologically and kinetically characterized. Finally, using molecular biology techniques, we looked for the expression of GluCl transcripts that could account for the observed glutamate responses.

### METHODS

**Isolation of metamorphic DUM neurons of the locust**

Adult migratory locusts (L. migratoria) were used in all experiments. They were taken from a crowded laboratory colony maintained at about 32°C on a 14-h:10-h light/dark cycle and on a diet of grass and oatmeal. Isolated DUM neuronal cell bodies were prepared as described previously (Bröne et al. 2003). Briefly, the dorsal median region containing the neuromeres T3, A1–A3 of the metamorphic ganglion were mechanically removed and subjected to collagenase/ dispase (2 mg/ml) treatment. The cells were centrifuged and subsequently washed three times with culture medium consisting of equal parts of Basal Medium Eagle with Hank’s salts (BME) and Grace’s Insect Medium (GIM). In the first wash, a 1% penicillin/streptomycin mixture was added to the BME/GIM culture medium. The cells were dissociated by repetitive up and down aspirations through a pipette tip, then plated on Nunc petri dishes and incubated at 28°C in a 5% CO2 atmosphere. All products were from GibcoBRL (Invitrogen, Merelbeke, Belgium), except for the Boehringer collagenase/dispase mixture (Roche Diagnostics, Brussels, Belgium). Healthy efferent DUM neurons (diameter = 40–60 μm) were recognized by their morphological characteristics under phase-contrast microscopy (Nikon Diaphot, Tokyo, Japan) (see, e.g., Bröne et al. 2003).

**Electrophysiology**

Changes in the membrane potential of and current in DUM neurons of L. migratoria were recorded at room temperature using the whole cell configuration of the patch-clamp technique both in current-clamp and voltage-clamp conditions. Locust DUM neurons were transferred onto the stage of an inverted phase-contrast microscope (Nikon Diaphot) in a recording chamber that was continuously perfused with solutions further described in Table 1. Electrodes made from 1.5 mm (OD) borosilicate glass capillary tubes, having a resistance between 800 kΩ and 1.5 MΩ, were also appropriately filled with the solutions listed in Table 1. To reduce contaminating Na+ and K+ currents through the voltage-gated ion channels, some experiments were conducted using modified intracellular and extracellular solutions (Table 1). The osmolality of these solutions (about 380 mOsm/kg), corresponding to that of L. migratoria hemolymph (Bröne et al. 2003), was attained by addition of glucose. Liquid junction (LJ) potentials were calibrated using the Junction Potential Calculator for Windows (JP-CalcW, Peter H. Barry, Dept. of Physiology and Pharmacology, Australia and Axon Instruments, Union City, CA) and were taken into account at the start of each experiment. The LJ potential between the

### TABLE 1. Composition of the solutions used in patch-clamp experiments on isolated DUM neurons of L. migratoria

<table>
<thead>
<tr>
<th>Extracellular Solutions (ES), mM</th>
<th>Intracellular Solutions (IS), mM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SES</strong></td>
<td><strong>Low Na+–ES</strong></td>
</tr>
<tr>
<td>NaCl</td>
<td>172.5</td>
</tr>
<tr>
<td>KCl</td>
<td>6.5</td>
</tr>
<tr>
<td>CaCl2</td>
<td>2.0</td>
</tr>
<tr>
<td>MgCl2</td>
<td>7.7</td>
</tr>
<tr>
<td>HEPES</td>
<td>10.0</td>
</tr>
<tr>
<td>Choline Cl</td>
<td>—</td>
</tr>
<tr>
<td>CdCl2</td>
<td>—</td>
</tr>
<tr>
<td>Tetraethylammonium Cl</td>
<td>—</td>
</tr>
<tr>
<td>4-Aminopiperidine</td>
<td>—</td>
</tr>
<tr>
<td>Tetrodotoxin</td>
<td>—</td>
</tr>
</tbody>
</table>

The free Ca2+ concentrations of the intracellular solutions were calculated for given concentrations of Ca2+, Mg2+, and chelators using the freeware Sliders v. 2.10 and WinMAXC v. 2.10 (both available at http://www.stanford.edu/~cpatton and CaBuf (at ftp://ftp.cc.KuLeuven.ac.be/pub/droogmans).
standard intracellular solution (SIS) and standard extracellular solution (SES) was $-18$ mV, between high Cl$^-$–IS and SES was $-21.8$ mV, between low Na$^+$–ES and low K$^+$–high Cl$^-$–IS was $-6.7$ mV and between low Na$^+$–ES and low K$^+$–low Cl$^-$–IS was $-19.2$ mV. The experimental protocols and data acquisition were carried out using an EPC-10 patch-clamp amplifier (HEKA Elektronik, Lambrecht, Germany) controlled by the program Pulse (HEKA Elektronik) running on a personal computer (equipped with a Pentium III processor). Capacitive and leak currents were compensated and residual capacitances and leak currents were eliminated, by means of a P/6 protocol (Moyer and Brown 2002). The series resistance was 1.5 M$\Omega$ and was compensated $\approx 53\%$. The recording of whole cell current was started 5 min after rupture of the cell membrane to allow adequate equilibration of the cell interior with the pipette solution.

Data acquisition and analysis

Data were filtered at 2.9 kHz and sampled at 20 kHz. They were stored on a computer hard disk and analyzed with the program PulseFit 8.77 (HEKA Instruments). Further analyses, including nonlinear regression, were carried out using Origin 6.0 Professional (Microcal Software, Northampton, MA).

Current–voltage relationships were fitted with the Goldman–Hodgkin–Katz (GHK) equation

\[
I_x = \frac{z^2F^2}{RT} P_x V_n \left\{ \frac{[X_0]-[X_0]e^{-\alpha V_{h}/RT}}{1-e^{-\alpha V_{h}/RT}} \right\}
\]

Desensitization kinetics of individual glutamate-evoked currents was assessed by fitting the desensitizing phase with the following equation

\[
I = I_0 e^{-\alpha V}
\]

Except where stated otherwise, results are expressed as means $\pm$ SE, where $n$ is the number of experiments. Statistical comparisons were made using the Student’s $t$-test or one-way ANOVA followed by Dunnett’s posttests. Differences were considered significant for $P \leq 0.05$.

Chemicals

Monosodium glutamate (Sigma–Aldrich Chemie, Steinheim, Germany) and avermectin B1A (Sigma–Aldrich) were dissolved in triply distilled water. Stock solutions of fipronil (PESTANAL) and picrotoxin (PTX), both from Sigma–Aldrich (Seelze, Germany), were made in ethanol (EtOH) and dimethylsulfoxide (DMSO), respectively. These stock solutions were diluted into the extracellular solutions immediately before the start of each experiment. The final concentrations of EtOH and DMSO in the solutions were $\leq 0.1\%$, at

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**FIG. 1.** Glutamate-induced membrane potential changes in locust metathoracic ganglion dorsal unpaired median (DUM) neurons, recorded with the whole cell patch-clamp technique in current-clamp mode. A: in the presence of standard intracellular and extracellular solutions (SIS–SES; see inset), application of 1 mM glutamate for 1 s hyperpolarized the DUM neuron membrane potential from $-35$ to $-61$ mV. B: when using almost identical chloride concentrations in the intracellular and extracellular solutions (high Cl$^-$–IS–SES; see inset), glutamate generated a depolarization from $-55$ to $-19$ mV.
which they had no effect on glutamate-induced currents (data not shown). L-Glutamate and ivermectin were applied topically through a valve-controlled tubing system positioned laterally above the cell at a distance of about 50–100 µm. The antagonists fipronil and PTX were applied to the bath.

Degenerate PCR and RACE

To obtain the full-length sequence of the L. migratoria GluCl channel, a Marathon RACE library was constructed using a mixture of several tissues (including muscle and neuronal tissues) as described by the manufacturer (Clontech, Hamburg, Germany). Degenerated oligonucleotides were designed and synthesized based on the predicted amino acid sequences of the conserved regions of the glutamate-activated chloride alpha channel (GluClα) after alignment of the GluClα from Drosophila melanogaster and Caenorhabditis elegans: DEG-F: 5'-GT(GC)TCATGGGT(TA)TCATT(C)TG-3' and DEG-R: 5'-TC(CG)AG(CA)AG(TG)GC(C)GGAA(AT)AC(AG)AA(G)(C)TG-3'.

The thermocycler was programmed for 35 cycles at 94°C for 30 s, 41.5°C for 45 s, and 68°C for 45 s using the thermocycler II polymerase mixture and Taq DNA polymerase (Applied Biosystems). A fruitfly cDNA was used as a positive control. PCR primers DEG-F and DEG-R were used for amplification during 40 cycles at 94°C for 30 s, 41.5°C for 45 s, and 72°C for 45 s.

Several distinctive bands were observed. The band of interest (about 40 cycles at 94°C for 30 s, 41.5°C for 45 s, and 72°C for 45 s) was directly sequenced or cloned into pGEM-T for sequencing products. The amplified PCR fragments were than separated on a 2% high-resolution agarose gel. The thermocycler was programmed for 35 cycles at 94°C for 30 s, 41.5°C for 45 s, and 68°C for 45 s using the thermocycler II polymerase mixture and Taq DNA polymerase (Applied Biosystems). A fruitfly cDNA was used as a positive control. PCR primers DEG-F and DEG-R were used for amplification during 40 cycles at 94°C for 30 s, 41.5°C for 45 s, and 72°C for 45 s. PCR products were than separated on a 2% high-resolution agarose gel. Several distinctive bands were observed. The band of interest (about 190–200 bp) was extracted from the gel using the Qiagen gel extraction kit (Qiagen), purified, and, in the case of isolated DUM neurons, subjected to a second PCR amplification using the same primers and incubation conditions. The amplified PCR fragments were either directly sequenced or cloned into pGEM-T for sequencing.

RESULTS

Glutamate elicits changes in the membrane properties of locust DUM neurons

When recorded with solutions close to physiological conditions (SIS–SES; see METHODS and Table 1), whole cell patch-clamp current-clamp locust DUM neurons had a resting membrane potential of −34.4 ± 3.8 mV (n = 6). As shown in Fig. 1A, application of 1 mM glutamate hyperpolarized the membrane to −62.6 ± 1.81 mV (n = 6). When Cl− concentrations were identical in intracellular and extracellular solutions, DUM neurons had a resting membrane potential of −58.8 ± 1.4 mV (n = 5) and, in that case, glutamate induced a depolarization of the membrane to −7.9 ± 3.4 mV (n = 5; Fig. 1B).

These results suggested that glutamate could activate a glutamate-gated chloride channel (GluCl) in locust DUM neurons. Such GluCls in other species are often characterized by a
produced currents. In this series of recordings, currents induced by channels (Cleland 1996), were compared with glutamate-in
clic lactone known to irreversibly and directly activate GluCl

glutamate-induced currents was chloride, the reversal potential
between applications, 

To assess whether the main ionic species carrying the glutamate-induced currents was chloride, the I–V relationships were obtained using two different intracellular solutions: one containing nearly identical intracellular and extracellular Cl− concentrations (low K+–high Cl−–IS) and a second one containing a lower Cl− concentration in the intracellular solution (low K+–low Cl−–IS). These solutions were also modified (see Table 1) to reduce contaminating currents through the voltage-dependent channels (e.g., K+, Na+, Ca2+). Currents were evoked by repeatedly applying 1 mM glutamate for 3 s and at the same time applying a voltage step ranging from −60 to +60 mV. In between applications, VH was returned to −70 mV and glutamate was washed out (Fig. 3A).

Maximum peak currents were measured at each voltage step and normalized for the cell capacitance. The reversal potential (ECl) was calculated after fitting the data with the GHK equation (see METHODS; Fig. 3B). Using nearly identical Cl− concentrations in the extracellular and intracellular solutions, glutamate-induced currents reversed in polarity at a membrane potential of +2.6 mV (n = 4), which was close to the calculated chloride equilibrium potential (ECl = −0.9 mV). At lower intracellular Cl− concentrations, ECl shifted to a more negative value (−13.6 mV; n = 3), which was in good agreement with the expected shift in the calculated Nernst equilibrium potential for chloride ions (ECl = −20 mV).

These results clearly demonstrate that the glutamate-induced currents in locust DUM neurons are mainly carried by chloride ions.

Pharmacology of glutamate-induced currents

**FIG. 3.** Current–voltage (I–V) relationship of glutamate-induced currents in locust DUM neurons recorded with the whole cell patch-clamp technique. A: currents (A) were evoked by 1 mM glutamate applications at holding potentials ranging from −60 to +60 mV (A), in the presence of almost identical Cl− concentrations and intracellular (low K−–high Cl−–IS) and extracellular solutions (low Na−–ES) modified to reduce contaminating voltage-dependent currents. Between voltage steps, VH was returned to −70 mV and glutamate was washed out. B: I–V relationship, obtained from glutamate induced currents described in A (high Cl−; closed circles). Maximum peak currents were normalized for the cell capacitance and are plotted against the holding potentials. Relation was best-fitted with the GHK equation, which yielded a reversal potential of +2.65 mV. This was close to the calculated equilibrium potential (−0.9 mV). When the intracellular chloride concentration was lowered to 83 mM ([low Cl−]; open circles), the reversal potential shifted to −13.6 mV in agreement with the theoretically expected shift (−20 mV).

1 mM glutamate in locust DUM neurons had a mean peak amplitude of −2.7 ± 0.7 nA (n = 5; Fig. 4C). In contrast, 1 µM IVM induced a current that did not desensitize and did not even fully reverse after the removal of IVM. IVM-induced currents amounted to −5.9 ± 1.5 nA (n = 6; Fig. 4C).

These pharmacological data further support the presence of a glutamate-gated chloride channel in efferent DUM neurons of L. migratoria.

Cloning of full-length LmGluClα

Because functional GluCls usually require the expression of at least GluClα subunits (Cully et al. 1996; Semenov and Pak 1999), we developed a degenerate PCR and RACE strategy in an attempt to clone the full-length sequence of L. migratoria GluClα (LmGluClα). Within the isolated cDNA, prepared as described in METHODS, a 1,362-bp open reading frame was identified encoding for a 453 amino acid protein. Typical features of GluCl subunits as in other members of the Cys-loop ligand-gated ion channel superfamily were found: a large extracellular N-terminal domain containing the ligand-binding...
site, a conserved Cys-loop and four transmembrane regions (Fig. 5). Moreover, the LmGluCl/H9251 subunit showed strong homology to orthologous sequences from *Drosophila melanogaster* (CAA05260; 82.2% identity, 87.6% similarity), *Triob lion castaneum* (XP 973383; 84.8% identity, 91.4% similarity), *Anopheles gambiae* (XP 321697; 83.8% identity, 88.6% similarity), *Apis melifera* (ABG75738; Jones and Sattelle 2006; 78.3% identity, 87.1% similarity), *Musca domestica* (BAD16657; 81.3% identity, 86.7% similarity), and *Lucilia cuprina* (AAC31949; 83.7% identity, 89.1% similarity). The cDNA sequence of the LmGluCl/H9251 subunit was deposited in GenBank under Accession Number DQ643254.

**DUM neurons of *L. migratoria* express LmGluClα messengers**

The expression of LmGluClα in locust DUM neurons was assessed as follows. mRNAs of isolated DUM neurons were reverse transcribed and used as template for a two-step PCR procedure. After the first PCR, a faint band was visible (Fig. 6A). This PCR band was excised, purified, and reamplified (Fig. 6B). Direct DNA sequencing confirmed the LmGluClα sequence.

**DISCUSSION**

In the work reported herein, we have clearly demonstrated that efferent DUM neurons of *L. migratoria* express functional glutamate-gated chloride channels (GluCls), using molecular biology and electrophysiological methods. In the presence of physiological solutions mimicking the hemolymph, glutamate hyperpolarized the membrane of DUM neurons as would be expected were GluCls to be present and active. Voltage-clamp experiments indicated that glutamate-induced currents in locust DUM neurons: 1) desensitize completely under continuous glutamate application, 2) are mainly carried by chloride ions, and 3) are blocked by fipronil and PTX, consistent with the properties of GluCls in other invertebrate species. Expression of GluClα subunit-containing GluCls was suggested by the sensitivity to IVM, which induced nondesensitizing persistent currents. Expression was confirmed by the RT-PCR detection of GluClα transcripts in locust DUM neurons. Finally, we also...
report the full DNA sequence of the GluCl\textsubscript{H9251} subunit of \textit{L. migratoria}.

In invertebrates, glutamate can interact with two classes of ionotropic receptors: excitatory channels, mainly permeable to cations as in vertebrates, and inhibitory anionic channels that are found only in invertebrates. When using intra- and extracellular solutions with an ionic composition close to that of the hemolymph, we observed a hyperpolarizing response on glutamate application, suggesting that locust DUM neurons express only inhibitory glutamate-activated channels (Ikeda et al. 2003; Wafford and Sattelle 1989). The chloride dependency of glutamate-induced responses was further confirmed by the shift to depolarization when \( E_{\text{Cl}} \) was changed from \(-79.6 \) mV to \(-4.1 \) mV and by the concordance of reversal potentials of glutamate-induced currents with chloride equilibrium potentials. Thus it appears that \textit{L. migratoria} DUM neurons express inhibitory ionotropic glutamate receptors only and not their excitatory counterparts. This was also observed for DUM neurons of other insect species such as the cockroach \textit{Periplaneta americana} (Washio et al. 2002) and for the stomatogastric ganglion of the lobster \textit{Panulus interruptus} (Cleland and Selverston 1998). This is in contrast to the expression of both inhibitory and excitatory glutamate receptors by muscle cells in the locust or the crayfish (Dudel and Franke 1987; Kerry et al. 1987, 1988) or by motor neurons such as the fast coxal depressor motor neuron of the cockroach thoracic ganglion (Wafford and Sattelle 1989).

In our hands, both glutamate-induced voltage and current changes were monophasic. Furthermore, under voltage-clamp conditions, glutamate-induced currents completely desensitized in about 1 s in the continuous presence of glutamate. These properties are similar to those reported for \textit{Caenorhabid-
**FIG. 6.** Expression of the GluClα channel in DUM neurons of *L. migratoria*. A: agarose electrophoresis of the first PCR using locust DUM neuron cDNA as a template (lane a2; marker = 100-bp ladder shown in lane a1). B: reamplification of the corresponding 193-bp PCR product for DNA sequencing (lane b3; marker = 50-bp ladder shown in lane b1). Positive control used was *Drosophila melanogaster* glutamate-activated chloride alpha channel (DmGluClα, lane b2).

**diits elegans** GluCls expressed in *Xenopus* oocytes (Forrester et al. 2003). In cockroach DUM neurons, on the other hand, biphasic glutamate responses were observed, consisting of an initial transient and a subsequent prolonged phase (Heckman and Dudel 1995; Raymond et al. 2000; Zhao et al. 2004a). The desensitizing and nondesensitizing components of the glutamate-induced responses seem to be attributable to the presence of pharmacologically distinct GluCls: the former sensitive and the latter insensitive to PTX or BIDN. The desensitizing glutamate-evoked current we report in locust DUM neurons is also PTX sensitive. It therefore probably corresponds to the transient current described in cockroach CNS neurons.

In locust DUM neurons, fipronil seems to be a more powerful antagonist of GluCl-mediated responses than does PTX. At 10 μM, it blocked 86% of glutamate-gated currents. This is in good agreement with the 75% block by 10 μM fipronil of the transient component of glutamate responses in cockroach DUM neurons reported by Raymond et al. (2000). To the best of our knowledge, our work is the first report of an effect of fipronil on isolated DUM neurons of *L. migratoria*. The blocking effect of fipronil on GluCls was previously proposed to explain part of the toxicity of this insecticide, which is widely used in pest control to eradicate dieldrin-resistant insects (Horoszok et al. 2001; Smith and Lockwood 2003; Tingle et al. 2003; Zhao et al. 2004b).

Ivermectin (IVM) at 1 μM generated an irreversible inward current under identical chloride concentrations in DUM neurons of *L. migratoria*. At concentrations in the micromolar range, avermectins are known activators of ligand-gated chloride channels, i.e., strychnine-sensitive glycine receptors (Shan et al. 2001), GABA receptors (Adelsberger et al. 2000; Robertson 1989), and GluCls (Cleland 1996). Because glycine receptors are not found in invertebrates, the IVM-induced current observed in DUM neurons could be the consequence of the activation of either GABA receptors or GluCls—or even both, as already demonstrated in the fruit fly (Ludmerer et al. 2002). Accordingly, the amplitude of the current induced by 1 μM IVM was significantly higher than that induced by 1 mM glutamate, a potentially saturating concentration for GluCls. This could suggest that IVM activates both GABA receptors and GluCls in DUM neurons of the locust. On the other hand, avermectin-sensitive chloride currents in *Xenopus* oocytes injected with *C. elegans* mRNA were GABA insensitive, but sensitive to glutamate (Yates et al. 2003). Further characterization of IVM-induced currents is needed to solve this issue, but that is beyond the scope of this work.

With respect to GluCls, it is usually accepted that the ability to irreversibly bind IVM is typically diagnostic for α-like subunits of GluCls (Cully et al. 1994; Li et al. 2002). This was previously demonstrated in several invertebrate species: locust (Schistocerca americana; Rohrer et al. 1994), barber pole worm (*Hemionthus contortus*; Forrester et al. 2001; Portillo et al. 2003), heartworm (*Dirofilaria immitis*; Yates et al. 2004), elegant worm (*Caenorhabditis elegans*; Dent et al. 1997, 2000; Horoszok et al. 2001), and strongylid worm (*Cooperia oncophora*; Njue et al. 2004). In contrast to GluClα subunits, GluClβ subunits were never shown to respond to IVM (Cully et al. 1994; Li et al. 2002). Note, however, in some species, splice variants of GluClα subunits were shown to be IVM resistant, e.g., DiGluClα3A from *Dirofilaria immitis* (Yates et al. 2004) or CoGluClα3 from *Cooperia oncophora* (Njue et al. 2004).

The functional expression of α-subunit–containing GluCls by locust DUM neurons suggested by their IVM sensitivity was demonstrated at the molecular level. A full-length LmGluClα channel was cloned, using a PCR/RACE strategy, and its messenger RNA in isolated DUM neurons was detected by RT-PCR. This does not rule out the expression of other LmGluClα subunits and thus does not allow any conclusion about the homo- or heteromeric nature of locust GluCls. Transfection of the cloned LmGluClα in heterologous expression systems might help to solve this question.

The functional implication of glutamate-gated chloride channels in DUM neurons of *L. migratoria* certainly deserves further attention. Because metathoracic effector DUM neurons innervate flight and leg muscles, their modulation by inhibitory GluCls is likely to influence flight control (Groleau and Lapied 2000). Furthermore, and more generally, because GluCls have been described until now in invertebrates only, a better insight into their functioning would undoubtedly contribute to the development of novel insecticides both more selective and more potent (Raymond and Satelle 2002; Zhao et al. 2004b).

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**References**


