Ionotropic GABA and glutamate receptors have different effects on excitability and are differentially regulated by calcium in spider mechanosensory neurons

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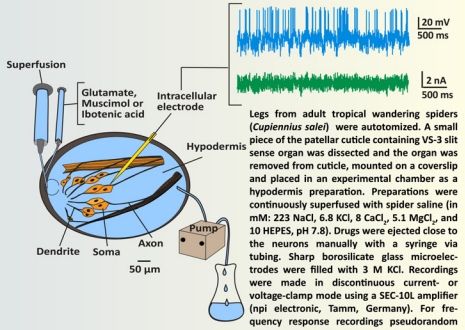
INTRODUCTION

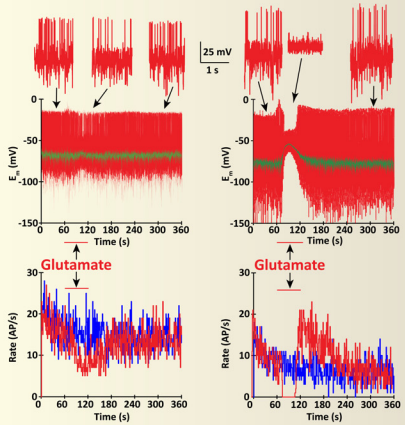
Peripheral mechanosensory neurons of the spider, *Cupiennius salei*, have GABA and glutamate receptors belonging to the ligand-gated chloride-channel family. This channel family is a primary target of insecticides and antiparasitics, so their molecular structure, pharmacology and biophysical properties have attracted significant attention. However, little is known about the physiological roles of these receptors or how they regulate neuronal excitability and animal behavior. Mechanosensory neurons of VS-3 slit sensilla in the spider patella react to the $GABA_A$-receptor agonists, GABA and muscimol, with depolarization and increase in intracellular $[Ca^{2+}]$ and, during random noise stimulation, with a mixed inhibitory-excitatory response. Here, we investigated the physiological significance of this co-existence of GluCl- and $GABA_A$-receptors in VS-3 mechanosensory neurons.

METHODS



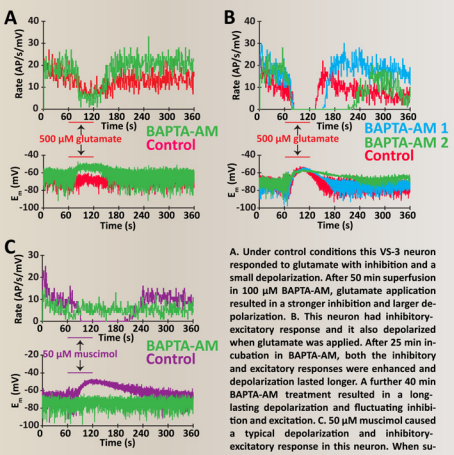
Legs from adult tropical wandering spiders (*Cupiennius salei*) were autotomized. A small piece of the patellar cuticle containing VS-3 slit sense organ was dissected and the organ was removed from cuticle, mounted on a coverslip and placed in an experimental chamber as a hypodermis preparation. Preparations were continuously superfused with spider saline (in mM: 223 NaCl, 6.8 KCl, 8 $CaCl_2$, 5.1 $MgCl_2$, and 10 HEPES, pH 7.8). Drugs were ejected close to the neurons manually with a syringe via tubing. Sharp borosilicate glass microelectrodes were filled with 3 M KCl. Recordings were made in discontinuous current- or voltage-clamp mode using a SEC-10L amplifier (npi electronic, Tamm, Germany). For frequency response recordings pseudorandom white noise stimulation was applied as current injection through the recording electrode (green trace) and resulting action potentials (blue trace) were recorded.

GLUTAMATE EFFECTS ON SPIKE RATE AND MEMBRANE POTENTIAL



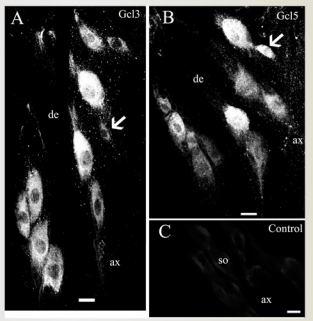
Glutamate effects on AP rate and membrane potential in two different VS-3 neurons. The upper red traces show APs elicited by pseudorandom Gaussian white noise electrical stimulus and the green traces show the membrane potentials. Insets (above) show the same recordings at different time scale before, during and after application of 500 µM glutamate. Lower traces show the original recordings converted to AP rate (AP/s) using 1-s wide bins. The blue traces are AP rates without glutamate application and red traces show the AP rate when glutamate was applied. On the left, AP rate decreased after glutamate application followed by a return to the plateau level while the membrane potential remained unchanged. On the right, the membrane depolarized significantly and AP rate declined rapidly to zero when glutamate was applied followed by an increased rate that continued after the membrane potential had returned to resting level.

BAPTA-AM ENHANCED GLUTAMATE EFFECTS BUT INHIBITED MUSCIMOL EFFECTS

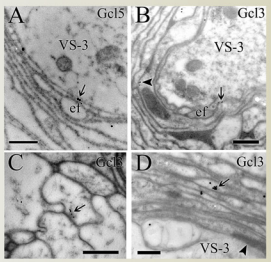


A. Under control conditions this VS-3 neuron responded to glutamate with inhibition and a small depolarization. After 50 min superfusion in 100 µM BAPTA-AM, glutamate application resulted in a stronger inhibition and larger depolarization. B. This neuron had inhibitory-excitatory response and it also depolarized when glutamate was applied. After 25 min incubation in BAPTA-AM, both the inhibitory and excitatory responses were enhanced and depolarization lasted longer. A further 40 min BAPTA-AM treatment resulted in a long-lasting depolarization and fluctuating inhibition and excitation. C. 50 µM muscimol caused a typical depolarization and inhibitory-excitatory response in this neuron. When superfused 40 min in 100 µM BAPTA-AM, muscimol did not cause depolarization or inhibition, only a small excitation.

Dm-GluClα IMMUNOLABELING

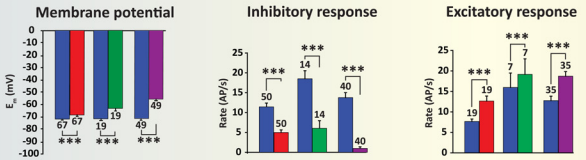


Confocal images of VS-3 organs labeled with Dm-GluClα antibodies. A. The somata of most of the ten neurons were strongly labeled with Gcl3 antibody and some labeling was also visible in the dendrites (de) and axons (ax). Some neurons were significantly less brightly labeled than others. B. In this VS-3 organ several somata were strongly labeled with the Gcl5 antibody while others had no significant labeling. Arrows indicate the smaller neurons of the pair 2, which is strongly labeled in B, but very faint in A. C. A control experiment where primary antibody was omitted and only faintly autofluorescent lipofucsin granules are visible in the VS-3 neuron somata. Scale bars 20 µm. All images are projections of fifteen 1-µm optical sections.



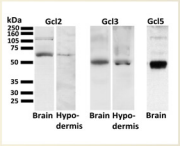
Electron microscopic images of VS-3 organs labeled with Dm-GluClα antibodies. A. Immunogold labeling for Gcl5 antibody (arrow) in a VS-3 neuron close to an efferent profile. B. Labeling for Gcl3 antibody on a VS-3 neuron (arrow) and on a glial cell (arrowhead). C. and D. show Gcl3 labeling on efferent fibers (arrows). All sections are from axo-somatic region of the VS-3 neurons. Scale bars are 500 nm in all images.

AGONIST EFFECTS ON VS-3 NEURON ACTION POTENTIAL RATE AND MEMBRANE POTENTIAL



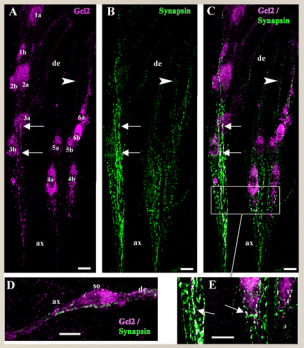
Agonist	Glutamate	Ibotenic acid	Muscimol
Inhibitory	36 (54%)	9 (47%)	12 (24%)
Excitatory	5 (7%)	2 (11%)	7 (14%)
Inhibitory + excitatory	14 (21%)	5 (26%)	28 (57%)
No change in spike rate	12 (18%)	3 (16%)	2 (4%)
Depolarization	33 (49%)	16 (84%)	47 (96%)
No depolarization	34 (51%)	3 (16%)	2 (4%)
Total number of neurons	67	19	49

SPECIFICITY OF THREE Dm-GluClα ANTIBODIES AGAINST SPIDER TISSUE



The polyclonal primary antibodies against four different regions of *Drosophila* GluClα subunit (Gcl1, Gcl2, Gcl3 and Gcl5) were generously donated by Merck Research Laboratories (Ludmerer et al., 2002). They were used in 1:200 - 1:500 dilutions. We used peroxidase-conjugated goat-anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) in 1:100,000 dilutions. Immunoreactive protein bands were visualized using an ECL plus chemiluminescent kit (Amersham Biosciences, Montreal, Quebec, Canada) .

Dm-GluClα + SYNAPSIN IMMUNOLABELING



A. Bright labeling with Gcl2 antibody in the somata of VS-3 neurons. Labeling is evident in the axonal (ax) and dendritic (de) regions (arrowhead) and on the fibers that lay on top of the VS-3 neurons (arrows). B. Synapsin labeling in the efferent neurons that surround the VS-3 neurons. C. Double exposure shows the Gcl2 labeling co-localized with the synapsin labeling on the efferent neurons (arrow). In the dendritic region the Gcl2 labeling is close to, but not in the same structure as the synapsin labeling. D. Double exposure of a tactile hair neuron showing synapsin labeling on efferent fibers and Gcl2 labeling on the hair cell. Some labeling is in the same structures. E. Higher magnification of an area in C shows co-localization of Gcl2 and synapsin labeling on the axonal region (arrows). Scale bar is 20 µm in all images. Images in A, B and C are projections of 25 1-µm optical sections, D is a projection of three sections and E is a single 1-µm section. Synapsin antibody was a generous donation from Dr. Buchner (Klagges et al. 1996).

SUMMARY AND CONCLUSIONS

Mechanosensory neurons of VS-3 slit sensilla in the patella of the spider have GABA and glutamate receptors that are ligand-gated Cl-channels. We established that:

- $GABA_A$-receptors in all VS-3 neurons are identical and their activation leads to depolarization and inhibitory-excitatory response during random noise stimulation.
- There are at least two types of glutamate receptors and some neurons do not respond to glutamate at all. Most VS-3 neurons were inhibited but not depolarized by glutamate, but some depolarized and had similar excitatory-inhibitory response to glutamate as to muscimol.
- Immunohistochemistry with antibodies against *Drosophila* inhibitory glutamate receptor α-subunit suggests that in addition to VS-3 neurons, these receptors may also be present in the efferent neurons surrounding the sensory neurons.
- The membrane permeable Ca^{2+}-chelator BAPTA-AM abolished muscimol effects but potentiated glutamate effects, indicating that GABA and glutamate receptors are differentially modulated by Ca^{2+}.
- We hypothesize that this could be achieved by different Ca^{2+} triggered phosphorylation processes at each receptor type. These findings are important for understanding the significance of Ca^{2+}-mediated regulation of transmitter receptor molecules and its role in controlling excitability.