LOCALIZATION OF GABA MEDIATED PERIPHERAL INHIBITION IN SPIDER MECHANORECEPTOR NEURONS

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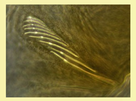
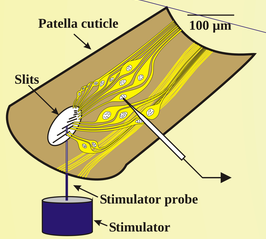
INTRODUCTION

Spider cuticular mechanosensory neurons receive extensive efferent innervation onto their sensory dendrites, somata and axons. The majority of these synapses in the tropical wandering spider, *Cupiennius salei,* are GABAergic. Muscimol and GABA, agonists of ionotropic GABA receptors, inhibit sensory neuron responses to electrical stimuli (Fabian-Fine et al. 1999; Panek et al. 2002). The response to these agonists involves an increase in the membrane conductance (shunting) and membrane depolarization similar to the primary afferent depolarization (PAD) that occurs in the axon terminals of mechanosensory afferents of other animals. We have previously shown that GABA induced inhibition in the spider mechanosensory neurons occurs even when the PAD is prevented, suggesting that membrane shunting is the primary cause of inhibition. Here, we used intra- and extracellular recordings to see if responses to mechanical stimuli are also inhibited by the same agonists, and to determine if agonist sensitivity is localized to different cell regions.

INTRACELLULAR RECORDINGS

Male Cupiennius Salei

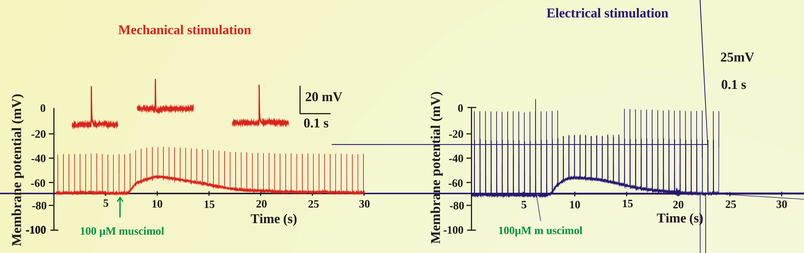




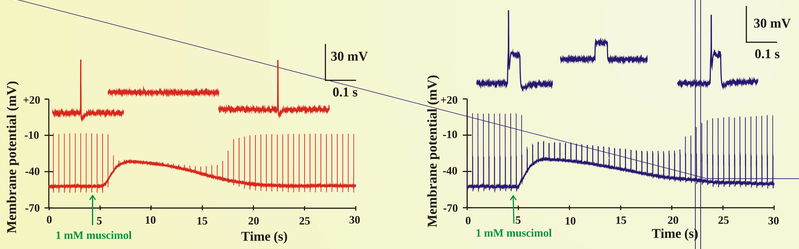
Outside view of the lyriform slit sensilla VS-3 on the spider patella

For intracellular recordings a small piece of cuticle was dissected and the muscles detached, leaving the sensory neurons attached to the cuticle. The preparation was superfused continuously with spider saline (in mM: 223 NaCl, 6.8 KCl, 8 $CaCl_2$, 5.1 $MgCl_2$, 5 sucrose, and 10 HEPES, pH 7.8). GABA or muscimol were injected into the bath solution. Intracellular recordings were performed using the discontinuous single-electrode current- and voltage-clamp methods with a SEC-05LX amplifier (NPI Electronic, Germany). The neurons were stimulated either electrically using positive current pulses via the recording electrode or mechanically using a piezoelectric stimulator (PZT-Servocontroller and P-841.10 Actuator, Physic Instrumente, Germany) that pushed a glass probe against the slits from below. Movements of 1-3 µm were usually adequate to evoke action potentials in sensory neurons. Intracellular microelectrodes were filled with 3 M KCl and their resistances were 40-80 MΩ in solution. For voltage clamp recording of the mechanically activated currents the inward sodium currents were blocked with 1 µM tetrodotoxin (TTX). All experiments were controlled by an IBM compatible computer using custom written software.

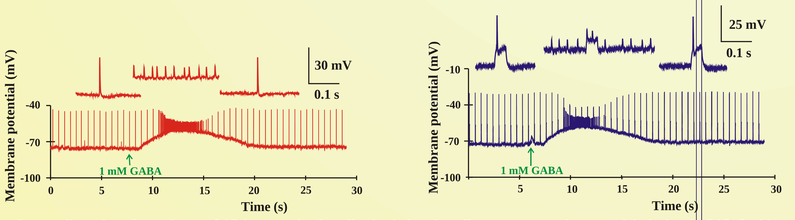
Muscimol and GABA effects on the action potential discharge during mechanical and electrical stimulations



Application of muscimol or GABA always induced a membrane depolarization (PAD), but in most recordings the neuron continued to fire normal size or slightly smaller action potentials when they were stimulated mechanically. When electrical stimulation was used, the neuron usually stopped firing at the crest of the depolarizing response. The PAD amplitudes in these two recordings were 13 mV and at its peak the membrane potential was -57 mV.



When muscimol was used as an agonist, the neuron occasionally stopped firing at the crest of a large PAD, even when mechanical stimulation was used. However, the duration of inhibition was always shorter than in experiments where electrical stimuli were applied to elicit action potentials. When GABA was used as an agonist the neurons never stopped firing when they were stimulated mechanically. The PAD amplitude in this experiment was 20 mV and the peak depolarization -32 mV.



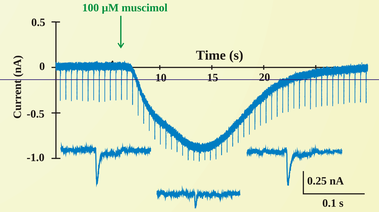
In a small number of experiments GABA or muscimol application induced small spontaneous action potentials during the PAD. In this experiment the PAD amplitude was 14 mV and the peak depolarization -59 mV.

Correlation of agonist induced peak depolarization with the effect on firing

Effect	Mechanical stimulation muscimol	Mechanical stimulation GABA	Electrical stimulation muscimol	Electrical stimulation GABA
Inhibition	-46.1 ± 10.4 mV (7)		-54.5 ± 10.6 mV (40)	-67.6 ± 4.2 mV (7)
Decreased a.p. amplitude	-55.5 ± 10.1 mV (31)			
No effect	-55.0 ± 9.7 mV (10)	-66.0 ± 5.0 mV (8)		-70.0 ± 9.0 mV (8)
Spontaneous firing	-65.3 ± 2.9 mV (3)			-59.2 ± 2.5 mV (5)

The table shows the average peak depolarization (± s.d.) with the number of experiments in parenthesis. The drug concentrations varied from 100 µM to 1 mM. The data shows that complete inhibition during mechanical stimulation required stronger depolarization than during electrical stimulation.

Muscimol effect on receptor current



Receptor currents were recorded after the sodium currents were blocked with 1 µM TTX. Each mechanical stimulus produced about 0.32 nA receptor current (insets). Muscimol application produced about 0.9 nA inward current. The receptor current amplitude decreased to 0.13 nA when the muscimol induced current was largest.

REFERENCES

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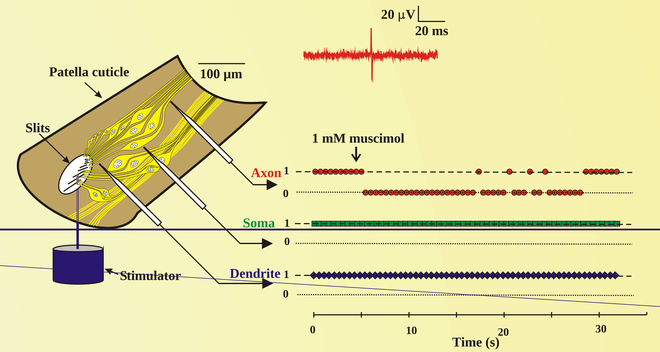
Panek I, French AS, Seyfarth E-A, Sekizawa S-i, Torkkeli PH 2002: Peripheral GABAergic inhibition of spider mechanosensory afferents. Eur J Neurosci 16: 96-104.

EXTRACELLULAR RECORDINGS



To learn which parts of the sensory neurons were inhibited by GABA receptor agonists we performed extracellular recordings. Glass capillary electrodes were placed at different locations along the sensory neurons and responses to mechanical stimuli were recorded. Extracellular electrodes were filled with spider saline and had resistances of about 1 MΩ. The same mechanical stimulator was used as for the intracellular recordings. Action potentials were recorded using a P15 AC preamplifier (Grass Instrument, U.S.A). The inset shows a typical extracellular recording of an axonal action potential. Data showing whether an action potential was recorded (1) or not (0) in response to a mechanical stimulus, before, during and after muscimol application are shown at the right. We performed twelve recordings from axons and in all the firing stopped after muscimol application. In five recordings from somata and 15 from dendrites no changes were seen in the firing after muscimol application .

SUMMARY AND CONCLUSIONS

Mechanosensory neurons of the spider lyriform slit sense organ respond to agonists of ionotropic GABA receptors with membrane depolarization (PAD) and an increase in membrane conductance. These effects usually lead to inhibition of action potential discharge when the sensory neurons are stimulated electrically via an intracellular electrode inserted into the soma. Using intra- and extracellular recordings we tested the effects of GABA receptor agonists on action potential discharge and receptor current that were induced by mechanical stimulation. We found that:

- **Complete inhibition of intracellularly recorded, mechanically induced action potentials required a significantly larger depolarization than when action potentials were induced by somatic electrical stimulation.**
- **Mechanically induced action potentials recorded extracellularly from the dendrites and somata were not affected by muscimol or GABA, but the same agonists always inhibited firing in the axons.**
- **The amplitude of intracellularly recorded receptor current was smaller during the peak of muscimol induced inward current.**
- **In some cases spontaneous action potentials were produced in response to a small PAD.**

These results suggest that:

1) As previously suggested (Gingl and French 2003) action potentials are normally initiated in the distal dendritic regions of the spider mechanosensory neurons

2) Ionotropic GABA receptors are most probably located on the axons or proximal parts of the cell bodies where they can rapidly inhibit action potential discharge.

3) GABA may have different effects depending on the amplitude of the PAD produced, varying from excitation to complete inhibition.

4) Reduction of the observed receptor current during PAD is probably an artefact due to incomplete space-clamp of the long dendrite during depolarization of the soma.

CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

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