GABAERGIC MODULATION OF SPIDER MECHANOSENSORY AFFERENTS

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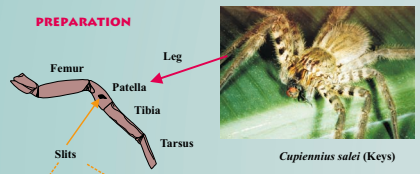
INTRODUCTION

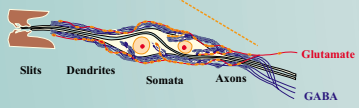
The sensitivity of a sensory system can be modified at several stages. It is now clear that transduction of mechanosensory information and its transmission to postsynaptic neurons are subject to modulation. Presynaptic inhibition of mechanosensory terminals by GABAergic efferent neurons is an ubiquitous phenomenon throughout the animal kingdom. It suppresses synaptic efficacy and reduces transmitter release from presynaptic terminals. However, ultrastructural work has shown that mechanosensory neurons of arachnids and crustaceans receive extensive GABAimmunoreactive efferent innervation not only at the axon terminals but also in the peripheral regions, including the somata and sensory dendrites, indicating very early modulation of sensory signals. Recently, Fabian-Fine et al. (1999) described an extensive and complex efferent innervation of the cuticular mechanoreceptor neurons of the spider, *Cupiennius salei* using electron microscopical and immunocytochemical approaches. One group of these, the VS-3 neurons innervating the lyriform slit sense organ on the patella of the spider, are accessible to intracellular recordings, and their mechanically- and voltage activated currents have recently been investigated using the single-electrode voltage-clamp (SEVC) method (HÖger et al. 1997; Sekizawa et al. 1999; 2000; Torkkeli et al. 2001). This research has made the VS-3 organ a preparation that can be used to investigate the presynaptic inhibition in a level that has not been possible with any other preparations before.

PREPARATION



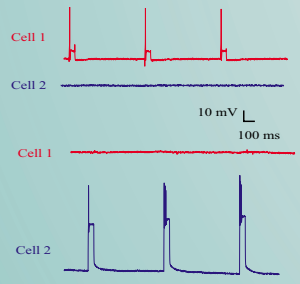
Cupiennius salei (Keys)

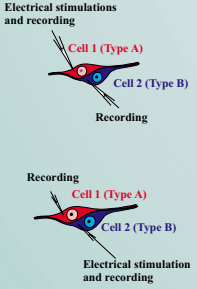
SYNAPTIC CONTACTS ON THE VS-3 NEURONS



Schematic diagram of two sensory neurons in the spider patellar VS-3 slit sense organ. Also shown are the fine efferent fibers that run parallel to the sensory neurons. Fabian-Fine et al. (1999) found three efferent fibers that show GABA-like immunoreactivity and one with glutamate-like immunoreactivity. The synaptic connections are very complex: there are at least four different types of synaptic vesicle populations, and efferent fibers form synaptic contacts with the sensory neurons, glia and other efferent neurons. In addition to simple unidirectional synapses there are reciprocal synapses, serial synapses, and convergent and divergent dyads.

NO EVIDENCE FOR DIRECT SYNAPTIC CONTACTS BETWEEN THE TWO VS-3 NEURONS IN A PAIR





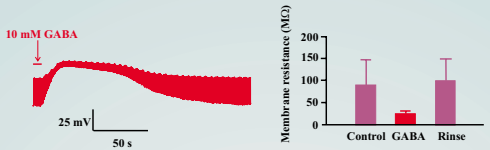
The VS-3 organ has 7 to 8 slits each innervated with a pair of mechanosensory neurons. One neuron in each pair, the Type A neuron, only fires one or two action potentials in response to a steady stimulus while the other, Type B neuron, can fire a long burst of spikes in response to similar stimulus. We performed a series of experiments where intracellular electrodes were placed in both the Type A and Type B neurons in a pair. Suprathreshold stimuli were given to one neuron while the responses of both neurons were recorded. Action potentials were only observed in the neuron that was stimulated while its partner remained silent with no change in the membrane potential. These experiments indicate that there are no direct synapses between pairs of VS-3 neurons themselves.

RECORDING AND STIMULATION

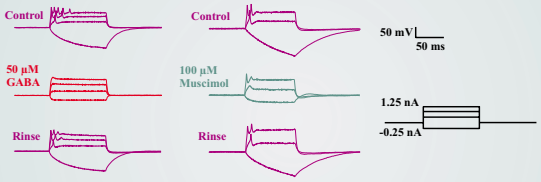
The VS-3 neurons were detached from the spider cuticle and placed in a recording chamber. The preparation was continuously perfused by spider saline (in mM: 223 NaCl, 6.8 KCl, 8 $CaCl_2$, 5.1 $MgCl_2$, 1 mM glucose, and 10 mM HEPES), into which the GABA receptor agonists and antagonists were ejected using pressure.

Current- and voltage-clamp recordings were performed with the discontinuous single-electrode method using a SEC-10 1 amplifier (NPI Electronic; Tamm, Germany). The recording electrodes were filled with 3 M KCl and the electrode resistance was 40-80 MΩ in solution.

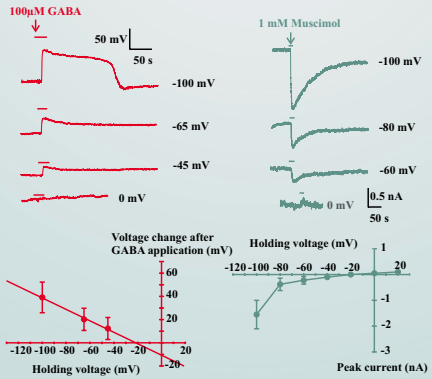
GABA EFFECTS ON VOLTAGE RESPONSE, MEMBRANE POTENTIAL AND MEMBRANE RESISTANCE



At -100 mV holding potential GABA application induced a membrane depolarization of about 40 mV. This recording was performed using short depolarizing voltage pulses (50 ms, 1 pulse/1.5 s) and it shows the change in the membrane resistance during the GABA induced depolarization. The membrane resistance decreased to about 26% of the original value after GABA application as shown in the bar graph. This change was reversible.

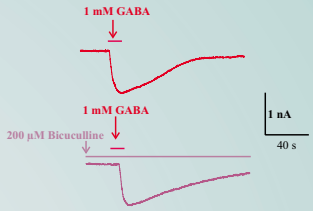


When 50 μM to 10 mM GABA or muscimol was applied to the bath solution, the threshold for firing action potentials in the VS-3 neurons became higher, the action potential amplitude became smaller or the neurons ceased firing completely. This effect was reversible.

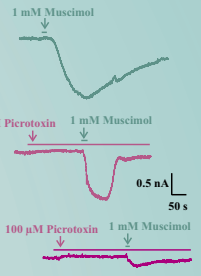


Voltage response of a VS-3 neuron to application of 100 μM GABA at four different holding voltages (*left above*) and currents induced by application of 1 mM muscimol at four different holding voltages (*right above*). Both GABA and muscimol induced a large membrane depolarization and an inward current at and below the resting potential (about -65 mV). This current reversed significantly above the resting potential. Linear fit to the data from 22 different experiments with GABA (*left below*) indicated that the reversal potential of GABA induced current was about -22 mV. The current voltage curve obtained from 6 experiments with muscimol indicated that the current reversed at about the same value (-20 mV).

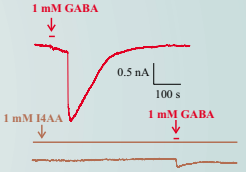
AGONIST AND ANTAGONIST EFFECTS ON THE VS-3 NEURON GABA RESPONSE



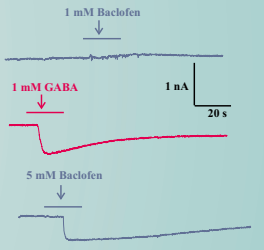
This experiment demonstrates that the vertebrate $GABA_A$ receptor blocker, bicuculline did not inhibit the VS-3 neuron's response to GABA. Both recordings were performed when the neuron was voltage-clamped to -100 mV.



Muscimol, an agonist of ionotropic GABA receptors produced an inward current similar to GABA. The GABA or muscimol induced current was significantly reduced or completely blocked when the Cl^- channel inhibitor picrotoxin was applied. The recordings shown here were performed when the neurons were voltage-clamped to -100 mV.



Imidazole-4-acetic acid (I4AA) is a partial agonist of vertebrate $GABA_A$ receptors and an antagonist of $GABA_C$ receptors. When added to the perfusing solution of the VS-3 neurons it inhibited the GABA response (*above*), but rapid application of I4AA alone induced an inward current (*right*). Responses to I4AA were not consistent in all neurons, and we therefore assume that there may be more than one type of ionotropic GABA receptor on the VS-3 neuron membranes. All recordings were performed when the neurons were voltage-clamped to -100 mV.



Application of low concentrations of the vertebrate $GABA_A$ receptor agonist baclofen (*above*) did not affect a spider VS-3 neuron. Middle trace shows the response of the same neuron to GABA application. However, when a high concentration of baclofen was applied to the bath solution it sometimes produced an inward current similar to GABA (*lower trace*). This current may have been produced via an endogenous route. All recordings were performed when the neurons were voltage-clamped to -100 mV.

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SUMMARY AND CONCLUSIONS

The peripherally located somata of the spider VS-3 mechanosensory neurons receive an extensive and complex synaptic innervation. Many of the efferent axons are immunoreactive to GABA (Fabian-Fine et al. 1999). Here we show that they also respond to GABA applied via the bath solution. The major findings of this work were:

1. Bath application of GABA and the ionotropic GABA-receptor agonist muscimol induce an increase in membrane conductance and a depolarization similar to the primary afferent depolarization (PAD) that occurs in many mechanosensory afferent terminals. This depolarization inhibits the action potential discharge.

2. The inward current underlying the depolarization reverses at a potential of about -20 mV.

3. The GABA induced current is insensitive to the vertebrate $GABA_A$ receptor blocker bicuculline, but is inhibited by the chloride channel blocker picrotoxin.

4. I4AA, which is an antagonist of vertebrate $GABA_C$ receptors and a partial agonist of $GABA_A$ receptors had both of these actions on the VS-3 neurons: It acted as an agonist when applied rapidly and as an antagonist when added to the slower perfusion.

5. Baclofen, a specific agonist of $GABA_B$ receptors, was only effective when applied at very high concentrations, and only in some of the neuron, suggesting that this may have been an effect induced endogenously.

Taken together, these findings indicate that the VS-3 neuron somata have ionotropic GABA receptors that have more similarities to the vertebrate $GABA_C$ than $GABA_A$ receptors. However, based on the findings that the individual neurons responded to some agonists and antagonists in different ways, it is likely that there are several types of GABA receptors in the VS-3 neurons and their distribution may vary between neurons. Activation of these receptors by GABA will result in a complete or partial inhibition of neuronal activity with a mechanism very similar to that described in the axon terminals of other mechanosensory neurons. Based on previous voltage-clamp recordings (Torkkeli et al. 2001) the GABA induced current depolarizes the membrane adequately to keep the voltage-activated sodium current inactivated and thus prevents the cells from firing action potentials. In contrast to the presynaptic inhibition of axon terminals, which only inhibits the action of individual axonal branches, the GABA induced inhibition of the neuronal somata leads to the inhibition of all axonal branches and consequently of all postsynaptic neurons. The degree of inhibition will depend on the number and type of efferent neurons that are active and each neuron in a slit may be modulated separately, creating a very complex system for processing the mechanical information detected by the VS-3 organ.