MECHANOTRANSDUCTION IN THE SPIDER LYRIFORM ORGAN VS-3

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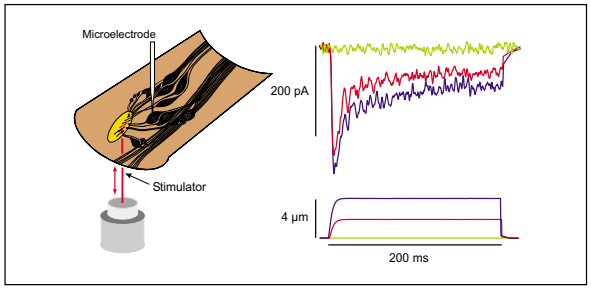
Introduction

In comparison with sensory modalities such as phototransduction and chemotransduction, little is known about the fundamental mechanisms of mechanotransduction. The small size of the sensory neurons in mechanoreceptors, their concealed locations in the tissues, and their interaction with extracellular auxiliary structures make studies at the cellular level in intact mechanoreceptors very difficult. However, it is generally accepted that mechanically-activated membrane channels (MACs) are the fundamental mediators of mechanoreception. MACs have been observed by single channel recording in many cell types, with single channel conductances ranging from 10 - 2000 pS (for reviews see: French (1992) *Annu Rev Physiol* 54:135-152, Morris (1990) *J Membrane Biol* 113:93-107). However, these studies were mainly done in cells that are not specialized for mechanoreception. Little is known about the MACs of mechanoreceptors because the transduction sites are inaccessible to single channel recording.

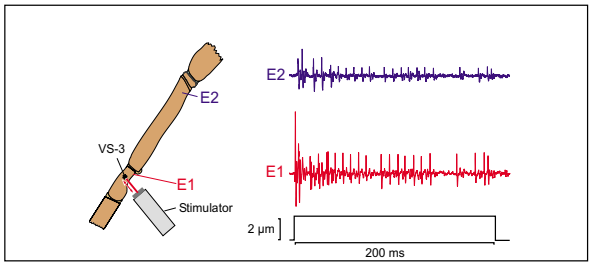
The lyriform slit-sense organ VS-3 detects mechanical strain in the exoskeleton of the Central American hunting spider *Cupiennius salei* by compression of its cuticular slits. This mechanoreceptor organ provides a useful model for studying mechanoreception in an isolated but mechanically intact preparation with natural (i.e. mechanical) stimulation. However, anatomical restrictions currently prevent direct single channel measurements from the mechanosensory dendrites in this preparation. Therefore, we used alternative methods to determine the properties of MACs in the VS-3 lyriform organ, including ion selectivity, open probability, single channel conductance, density, and activation energy.

Methods

The lyriform organ VS-3 is a compound slit sense organ located in the patellar cuticle of the spider *Cupiennius salei*. We have developed methods for studying this mechanoreceptor organ without disturbing its functional integrity, both *in situ* and in an isolated preparation.

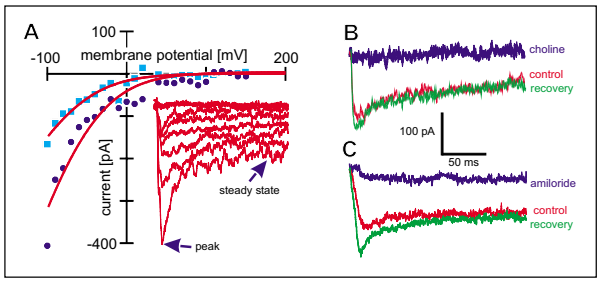


To perform intracellular recording from the receptor neurons, a piece of cuticle from the patella was dissected that carried the complete and intact sensory apparatus, i.e. the cuticle slits and the sensory neurons. With this isolated cuticle preparation mounted in a custom designed holder, the cell body of an individual neuron was impaled by a sharp glass microelectrode from above. Pushing the outside of the cuticle slits from below with a piezoelectric stimulator evoked an electrical response in the recorded neuron. This consisted of a receptor potential and action potentials when the single electrode current clamp technique was used, and a receptor current when the single electrode voltage clamp technique was used. Intracellular recordings in this preparation were sufficiently stable to allow the exchange of extracellular saline to test the effects of different drugs and ions on the receptor current. We also examined the effects of temperature on the mechanically-induced receptor current.



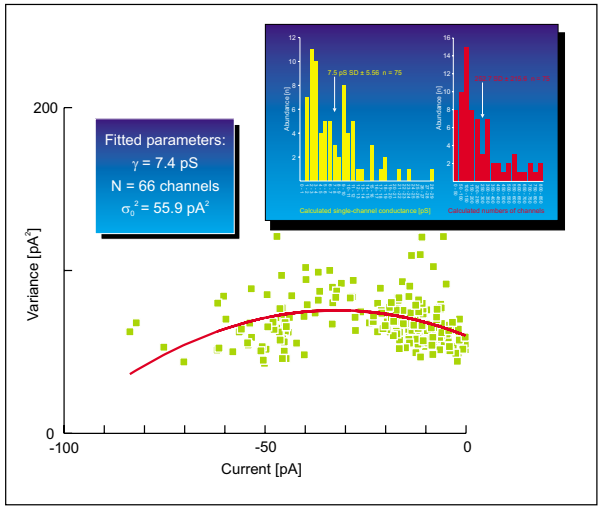
We used immobilized, but otherwise intact, spiders to make differential extracellular double recordings from the sensory nerve innervating the lyriform organ VS-3. Silver wire electrodes were implanted through the cuticle of the leg joint next to the VS-3 organ, and in the proximal femur, to record action potentials from the sensory leg nerve. A reference electrode was placed in the spider's abdomen. Pushing the cuticular slits of the VS-3 organ evoked individually identifiable action potentials in the sensory leg nerve. Time differences between identified action potentials in simultaneous double recordings were used to calculate the action potential conduction velocity in the sensory nerve at body temperatures ranging from 5 °C to 35 °C.

Na^+ -carries the receptor current



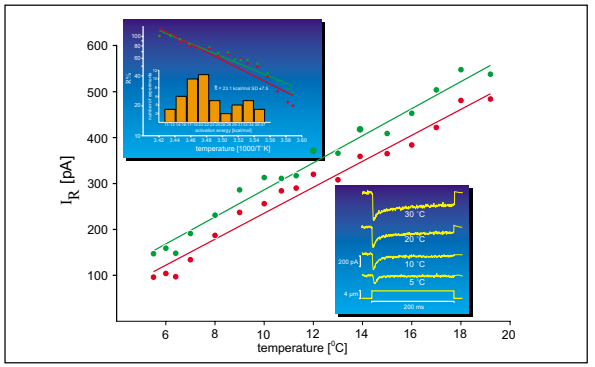
The receptor current in VS-3 neurons is carried by Na^+-ions . **A)** I-V plot of receptor current induced by a 4 µm mechanical stimulus. The receptor current decreased with more positive membrane holding potentials but failed to reverse, even at very positive membrane potentials, indicating a population of strongly sodium-selective MACs in VS-3 neurons. **Insert:** Original recordings shown as I-V plot. Amplitude readings from the current peak (arrow). **B)** Replacement of extracellular Na^+ with choline ions. Upon replacement of the regular extracellular saline with sodium free saline ($choline^+$ replaced Na^+) the receptor current (control) was eliminated. This was reversible (recovery) by washing the preparation with normal sodium-rich saline. Similar results were obtained by substituting extracellular Na^+ with Rb^+ (not shown). However, Li^+ was able to carry about 50% of the receptor current normally carried by sodium. **C)** Amiloride blocked MACs in VS-3 neurons. Application of 1 mM amiloride in normal sodium-rich saline blocked the receptor current reversibly. Similar results were obtained by application of 1mM gadolinium (not shown). Both drugs are known to block MACs in several tissues (Hamill, Lane, McBride Jr. (1992) *TiPS* 13:373-376), and amiloride is a well-known blocker of epithelial sodium channels.

Noise analysis of receptor current

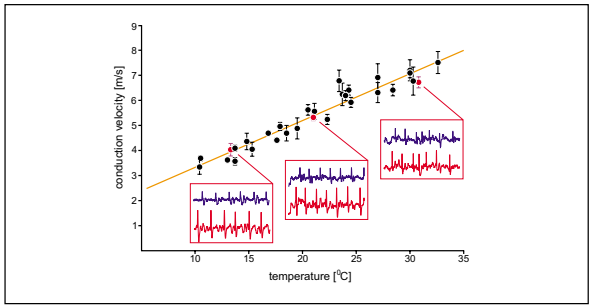


Noise analysis can be used to estimate single-channel properties in situations where the channels are not accessible to single-channel recording but the membrane current can be observed (DeFelice (1981) Introduction to Membrane Noise, Plenum Press, Traynelis, Jaramillo (1998) *TINS* 21: 137-145). A typical plot of current variance versus current from a mechanically stimulated VS-3 neuron is shown fitted with Eq. 1 (solid red line) $\sigma^2 = \sigma_0^2 + I(V-E)\gamma - I^2/N$, where σ^2 is the total variance, σ_0^2 is the background variance, γ is the single channel conductance, I is the total membrane current, V is the membrane potential, E is the reversal potential of the permeant ion (sodium), and N is the number of channels. Insert: Noise analysis of the receptor current in 75 neurons gave mean values of 7.5 pS and 253 channels/neuron. The relatively small numbers of channels and their low conductance are in good agreement with previously published estimates of 5 - 12 pS and 40 -280 channels in other mechanoreceptors (Holton, Hudspeth (1986) *J Physiol* 375:195-227, DeFelice, Alkon (1977) *Nature* 269:613-615).

Activation energy of mechanotransduction and action potential conduction



Activation energy calculated from receptor current peaks. The receptor current amplitude in VS-3 neurons was strongly temperature-dependent. The plot shows peak receptor currents induced by 3 µm (•) and 4 µm (•) stimuli (original recordings shown at lower right). For this experiment the Q_{10} values were 2.6 (4 µm) and 3.2 (3 µm). The mean Q_{10} value from 25 experiments was 3.2 ± 0.9. Upper insert: Arrhenius plot of normalized receptor currents induced by 3 µm (•) and 4 µm (•) stimuli. Solid lines are the best linear fits to the data points. The slopes of the lines correspond to activation energies of 18 kcal/mol (3µm) and 15.2 kcal/mol (4µm). The mean activation energy from the 41 experiments shown as a histogram (3kcal/mol bin width) was 23.1 kcal/mol. The relatively high activation energy for the transduction process is in good agreement with published data for other insect and vertebrate mechanoreceptors (12 - 19 kcal/mol).



Action potential conduction velocity in VS-3 neurons at temperatures between 10 °C and 35 °C. Individually identifiable action potentials in simultaneous extracellular recordings from patella and tibia were used to calculate the conduction velocity in VS-3 neurons. Each data point represents analysis of 70 - 150 individual action potentials from 5 different experiments, i.e. different VS-3 organs. Due to the obvious homogeneity of the data distribution, the 5 experiments were pooled and the solid line represents the best linear fit to all data points. At room temperature (21 °C) the mean conduction velocity was 5.2 ± 0.13 m/s. At 10 °C the conduction velocity slowed to 3.2 ± 0.28 m/s and at 32 °C it increased to 7.7 ± 0.28 m/s. The activation energy for this process, calculated from the data shown, was 6.33 ± 0.44 kcal/mol, corresponding to a Q_{10}-value of 1.43 ± 0.05. Inserts show original recordings from patella and tibia at temperatures of 13 °C, 21 °C, and 32 °C.

Conclusions

-The Na^+-dependent receptor current indicates the presence of MACs with a high selectivity for Na^+ over K^+ in the VS-3 neurons. The receptor current, and therefore the MACs, were blocked by Amiloride and Gadolinium. This suggests that VS-3 MACs may belong to the family of amiloride-sensitive, sodium-selective, ion channels normally associated with epithelia.

-Noise analysis of the receptor current predicted a relatively low single channel conductance of 7.5 pS and a low density of MACs in VS-3 neurons. Similarly low single channel conductance and densities for MACs have been found for other mechanoreceptors (DeFelice, Alkon (1977) *Nature* 269:613-615, Holton, Hudspeth (1986) *J Physiol* 375:195-227) , and these values agree with the whol cell conductance of the VS-3 neurons (Höger, Torkkeli, Seyfarth, French (1997) *J Neurophysiol* 78:2079-2085).

-Our measurements of activation energy in mechanoreceptors were the first to separate the transduction stage and action potential transmission stages. The activation energy we found for the transduction process is the highest yet published. While published data for activation energy in mechanoreceptors have been consistently high, difficulty in separating transduction and transmission may have contribute to slightly lower activation energies in previous results. However, the high activation energy observed during mechanotransduction suggests that a high energetic barrier is a common feature of the mechanotransduction process.

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