ACID MODULATION OF RECEPTOR CURRENT IN SPIDER MECHANORECEPTORS

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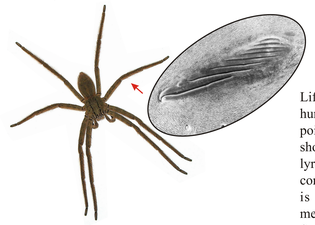
Background

Members of the epithelial sodium channel, degenerin, and acid-sensitive channel family **(ENaC/DEG/ASIC family)** share a number of functional and structural homologies. There is evidence that members of this group are involved in mechanoreception and nociception. Most of the evidence is based on analysis of genetic "Knock-Out" mutants, and immunohistochemistry. Direct electrophysiological proof of this hypothesis in mechanoreceptor neurons is still lacking. It is also unclear whether these proteins can be directly responsible for transduction, or instead play more supportive roles.

Electrophysiological analysis of mechanoelectrical transduction in the **lyriform organ VS-3**, a cuticular mechanoreceptor in the patella of the Central American spider ***Cupiennius salei***, has already shown that the spider **MACs** (**M**echanically **A**ctivated **C**hannels) have several similarities to the ENaC/DEG/ASIC family. They are highly Sodium selective and can be blocked by amiloride or Gadolinium (Höger et al., J Neurophysiol 78: 2079, 1997). Noise analysis of the receptor current has previously been used to estimate the number of transduction channels present in a VS-3 receptor cell, and to predict their single channel conductance and open probability (Höger & French, Brain Res 826: 230, 1999).

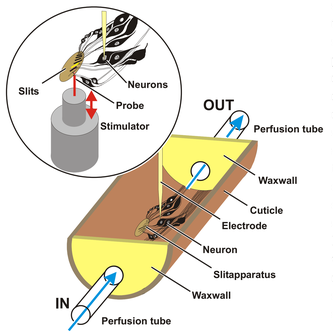
Here we recorded and analyzed the mechanically induced receptor current in VS-3 neurons at different extracellular pH values, looking for further similarities between the spider's MACs and channels of the ENaC/DEG/ASIC family. Is mechanotransduction in the spider lyriform organ VS-3 modulated by extracellular pH?



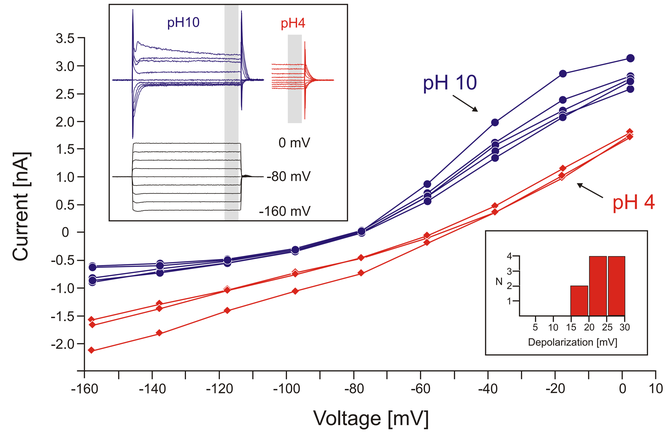
Life size adult male of the Central American hunting spider ***Cupiennius salei.*** The **arrow** points to one of the leg patella. The **insert** shows a scanning electron micrograph of a lyriform organ VS-3. This sense organ consists of 8 parallel cuticular slits. Each slit is innervated by a pair of bipolar, mechanosensory neurons (not shown, see figure below for details).

Methods

An isolated piece of patella cuticle carries the intact lyriform organ VS-3. Mounted in a custom designed preparation holder, a perfusion system allowed exchange of the extracellular medium in the cuticle segment within a few seconds. In this arrangement the cuticle slits are accessible for mechanical stimulation from below by a piezoelectric transducer, mimicking an adequate stimulus by pushing the slits with a glass probe (amplitude 1-3 µm). From above, the neurons are accessible for intracellular recording of their electrical response to mechanical or electrical stimulation with sharp glass microelectrodes. After application of 1 µM TTX to prevent the cell from firing action potentials, we recorded electrically and mechanically induced currents in identified neurons with the single electrode voltage clamp technique. During the experiment the extracellular pH was changed several times. The recorded membrane currents were sampled and stored in a computer and subsequently analyzed with custom designed software.

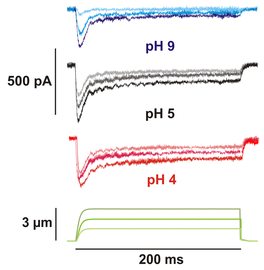


Voltage-activated membrane currents are modulated by extracellular pH



IV-plot of the voltage activated currents in a VS-3 neuron at extracellular pH 10 and pH 4. The neuron was clamped at a holding potential of -80 mV (membrane resting potential at pH 8). **Upper insert:** Current recordings at pH 10 and pH4, and the voltage step protocol used for stimulation. Gray bars indicate the part of the non-inactivating current plotted versus the step potential in the IV-plot. Previous studies showed that this current is a non-inactivating potassium current of the delayed rectifier type (Sekizawa et al., J Neurophysiol 81: 2937, 1999). **Bottom insert: S**hift in membrane resting potential caused by change in extracellular pH. The membrane resting potential at pH 10 was taken as reference potential, and depolarization caused by a change to extracellular pH 4 is plotted (n=10). In alkaline pH neurons hyperpolarized, in acid pH they depolarized. Shifts in the membrane resting potential were relatively small in the range of pH 10 to pH 6. However, extracellular pH5 or lower causes rapid depolarization of 20mV or more, as shown.

Receptor current is modulated by extracellular pH



Voltage clamp recordings of mechanically-induced receptor current in a VS-3 neuron at extracellular pH 9, pH 5, and pH 4. The neuron was clamped at a holding potential of -80 mV, the cell's resting potential at extracellular pH 9. The lower traces show the deflection of the stimulator's probe.

Acidification increased the receptor current amplitude at all times. However, changes in current amplitude were very small in the pH range between pH 10 and pH 6. The current increased dramatically below pH 6. This effect was easily reversible, and could be repeated through several pH changes.

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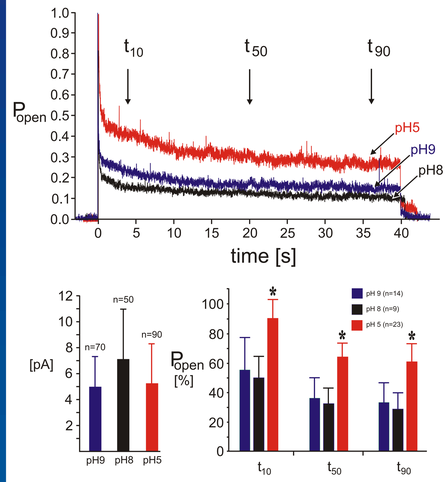
MAC open probability depends on extracellular pH

The increase in receptor current found at acid pH values (n = 18 neurons, several changes of extracellular pH each) could be due to changes in several properties of the transduction channels, including the single channel conductance, channel open probability, or the number of functioning receptor channels. We used noise analysis of the receptor current to test which of the single channel properties were altered by changing extracellular pH. Noise analysis has previously been used with this preparation to estimate single channel conductance, number of channels, and their open probability (Höger & French, Brain Res 826:230, 1999). At extracellular pH 4, recordings remained stable for only very short times. Experiments for noise analysis of the receptor current, requiring stable recordings for extended periods of time, were therefore done in a range of pH 9 to pH 5, which were tolerated by the neurons for several hours.



Single channel open probabilities (P_{open}) and single channel conductances ( ) at different extracellular pH. **Top:** open probability of MACs in one neuron at pH9, pH8, and pH5 during a 40 second mechanical stimulus. **Bottom right:** Relative MAC open probability for 23 experiments. There was no statistically significant difference at any time (t10, t50, t90) between pH 8 and pH 9, while the open probability at pH5 was significantly increased at all times. **Bottom left:** Estimated MAC single channel conductance at different extracellular pH. There was no significant effect of extracellular pH on single channel conductance. The values for single channel conductance, and the number of channels (not shown) were in good agreement with previously published results for this preparation (7.5pS; Höger & French,1999)

Conclusions

We found two major effects of acid pH (<6) on spider VS-3 neurons.

-Depolarization of the membrane resting potential by reduction of current flow through delayed rectifier type potassium channels

-Increase in MAC (mechanically activated channel) open probability during mechanical stimulation, and a resulting increase in receptor current

Our results do not indicate that acid pH itself opens MACs in the lyriform organ VS-3, which is a characteristic of ASIC channels. However, we found the most significant changes below pH6, which is the pH range in which ASIC channels are commonly activated. (e.g. Kellenberger & Schild, Physiol Rev 82: 735, 2002).

Mechanoreception in arthropods and other animals may be based on membrane channels similar to the ENaC, DEG, ASIC -family, but a range of other possibilities must continue to be considered, and more experimental work is needed to identify and characterize these important molecules.