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RAPID PUBLICATION

Sodium-Dependent Receptor Current in a New Mechanoreceptor Preparation

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SUMMARY AND CONCLUSIONS

1. Intracellular microelectrodes recorded the receptor potential and receptor current in the neurons of spider slit sense organs during mechanical stimulation of the slits.

2. Mechanical stimulation produced two patterns of action potential discharge, corresponding to the two groups of neurons described previously by electrical stimulation.

3. Tetrodotoxin eliminated the action potentials and revealed a receptor potential with both static and adapting components. Voltage clamp gave an inward receptor current with a similar time course.

4. Replacement of sodium ions in the bath reversibly eliminated the receptor current, indicating that it is carried by sodium ions. However, this effect was comparatively slow, suggesting that the tips of the sensory dendrites lie in a chemically restricted environment.

INTRODUCTION

The transduction of movement or force into a membrane current is a widespread biological process that provides senses such as touch, vibration, and hearing, as well as the control of many vital body systems (French 1992). Mechanotransduction probably involves specialized ion channels (French 1992; Morris 1990; Sachs 1988), but the small sizes and inaccessibility of most mechanoreceptors have prevented characterization of these channels. Here, we used a new preparation for direct intracellular measurement of the receptor current in primary mechanosensory neurons, close to the site of transduction.

Lyriform slit sense organs detect strain in the exoskeleton of spider legs (Barth 1985). The anterior lyriform organ VS-3 (nomenclature of Barth and Libera 1970) of the tropical wandering spider lies on the anterio-ventral side of the leg patella. It consists of 7–8 cuticular slits, graduated in length from 15 to 120 μm, each innervated by a pair of spindle-shaped bipolar neurons. In response to intracellular electrical stimulation, Seyfarth and French (1994) found that each pair contained one neuron that produced a single action potential and one that produced a short burst of action potentials with decaying amplitudes.

Here, we recorded intracellularly from slit organ neurons while pushing on the slits. The resultant receptor potential and current did not adapt completely, although the action potential discharge adapted rapidly and completely to sustained deformations or current injections. Transduction was insensitive to tetrodotoxin (TTX) but strongly dependent on external sodium, indicating that sodium ions carry the receptor current.

METHODS

Adult female tropical hunting spiders (*Cupiennius salei* Keys., Ctenidae) were raised in the laboratory. A leg was autotomized and a piece of the patellar cuticle containing lyriform organ VS-3 was dissected and prepared as described previously (Seyfarth and French 1994). The concave piece of cuticle was fixed with beeswax onto a ring-shaped holder and filled with fresh spider saline (Maier et al. 1987) at pH 8.2, connected to a Ag/AgCl reference electrode. The holder with the preparation was mounted firmly, and mechanical stimulation was applied from below to the slits, or to the cuticle adjacent to the organ, by a custom-made stimulator (Fig. 1). To eliminate extraneous vibration, the stimulator, holder, and associated components were clamped together and mounted on an air-driven vibration isolation table. Additional passive vibration isolation was essential to eliminate extraneous vibration. The stimulator was calibrated by a semiconductor force transducer.

Intracellular single electrode current and voltage-clamp recordings were performed via glass microelectrodes that were positioned with a micromanipulator. The stimulator and recording electrode were viewed with a dissecting microscope. Electrode resistances (3 M KCl) were 25–80 MΩ with the electrode in the tissue. After dual capacitance compensation of the amplifier (SEC-1L, NPI electronic, Germany), the time constant of the electrodes, τ_e in the tissue was 2–3 μs, giving a high frequency cut-off of $\geq$60 kHz. Current-clamp and voltage-clamp measurements used the discontinuous modes with a switching frequency of 40 kHz. Voltage and current responses were filtered at 2.3 kHz, viewed on an oscilloscope, sampled at 2 kHz, digitized with a 12-bit A/D converter (DT2821, Data Translation) and stored by a digital computer for later data processing. For some experiments, tetrodotoxin was dissolved in normal spider saline (Maier et al. 1987), or sodium chloride was replaced by choline chloride.

RESULTS

Direct mechanical stimulation of the slits revealed a response pattern similar to that produced by electrical depolarization. Figure 2 shows the responses typical of a neuron that generated bursts of action potentials to both current injections and mechanical steps. Small steps produced only one action potential, but stimuli of increasing amplitude revealed a depolarizing receptor potential and produced several action potentials. The amplitudes of the action potentials in different cells varied from 50–80 mV (n = 26 cells). With larger stimuli the receptor potential saturated at a level of 6–15 mV above the resting potential (−65 to −75 mV), and the interval from the beginning of the step to the first action potential approached a minimum value of ~1 ms. However, if stimulation was applied to the cuticle adjacent to the slit, the time delay to the first action potential

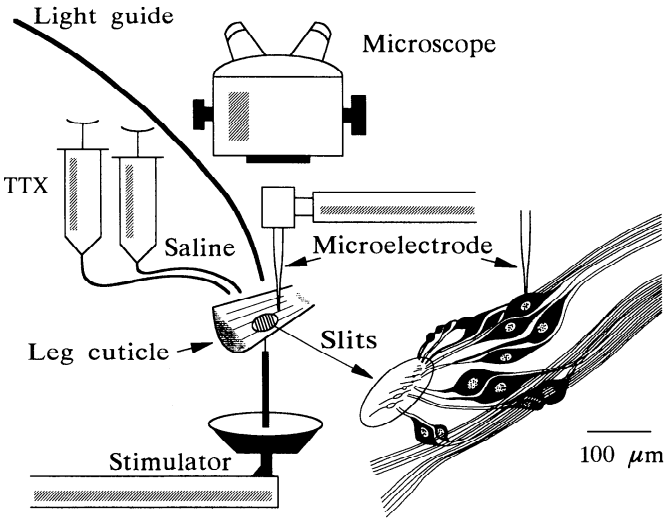

FIG. 1. Schematic drawing of the experimental arrangement for stimulating and recording from the slit organ. A magnified diagram of the slits and sensory neurons is shown at *bottom right*. Other components are not to scale. The mechanical stimulator consisted of an insect pin glued to the center of a small loudspeaker passing through a metal guide tube to the slit organ. The force produced by voltages applied to the stimulator were calibrated prior to the experiments by using a semiconductor force transducer. The force records in Figs. 2 and 3 were obtained from the voltage applied to the loudspeaker. TTX, tetrodotoxin.

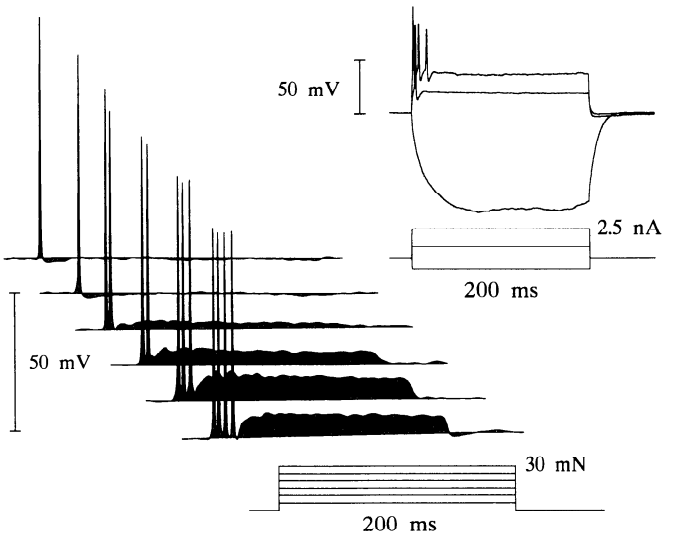

FIG. 2. Intracellular responses of a bursting neuron (neuron 3b in the nomenclature of Seyfarth and French 1994) to mechanical steps of 200-ms duration. Note the increasing numbers of action potentials and the increasing static receptor potential with stronger stimuli. *Inset*: responses of the same cell to step current injections. Note the reduction in the action potential amplitudes during the burst. The input resistance of the cell to hyperpolarizing current steps was 100 MΩ.

was always about 1 ms and the resulting receptor potentials were smaller (<4 mV). The reason for this response difference is not yet understood, but it probably involves the visco-elastic properties of the slit cuticle and the associated membranes.

The addition of 10 μM TTX to the bath solution hyperpolarized the resting potential by ~10 mV and blocked action potential production, allowing the receptor potential and current to be studied free of regenerative processes. Figure 3 shows the receptor potentials and corresponding receptor currents produced by mechanical steps of increasing amplitude applied to the slits. The peak and plateau values of these responses are also plotted versus stimulus strength in Fig. 3. The rapidly rising phase of the response suggests that the transduction process is tightly coupled to

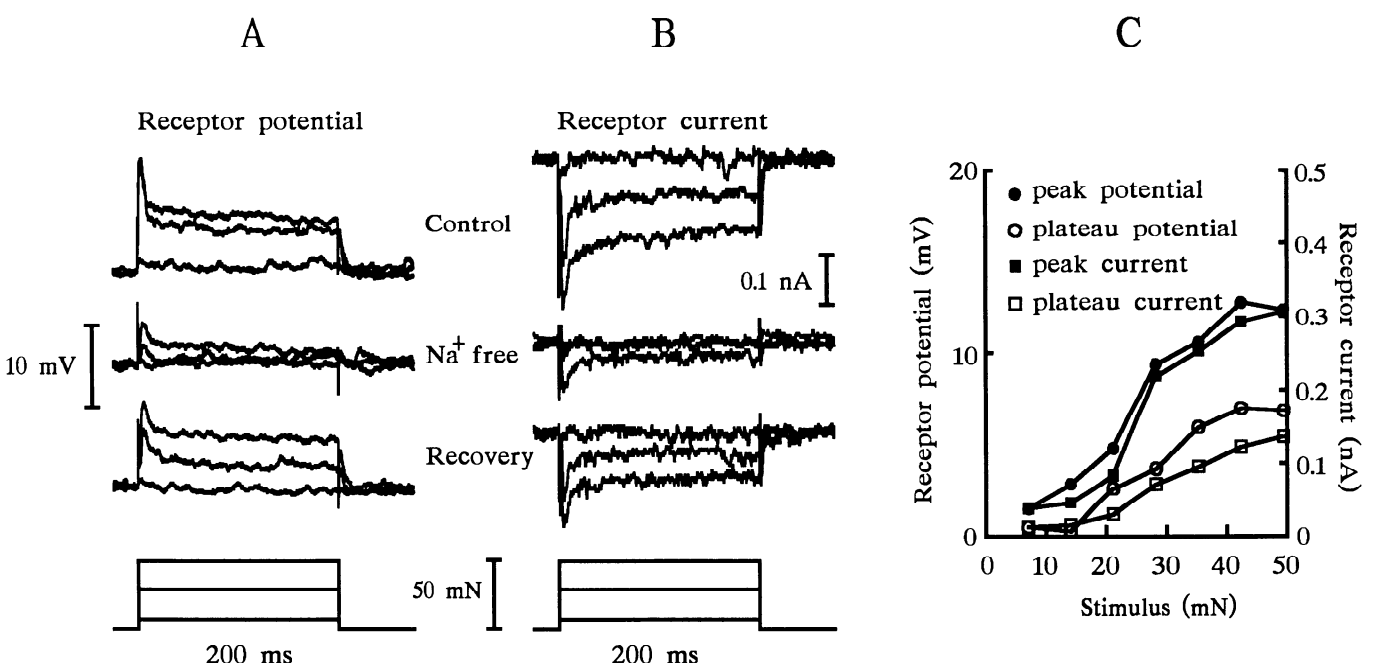

FIG. 3. Voltage and current responses of a TTX-treated mechanoreceptor neuron (neuron 2b in the nomenclature of Seyfarth and French 1994) to mechanical steps (200-ms duration) with and without Na^+ in the bath. Control voltage (*A*) and current (*B*) responses are shown after TTX-treatment but with Na^+ in the bath. Corresponding responses are shown 20 min after the replacement of Na^+ by choline, and recovery of the responses 20 min after the return to Na^+ saline. *C*: peak and plateau voltage and current responses as functions of mechanical step amplitude. Note that all of the responses saturated with the largest stimuli, indicating that the full operating range of the sensory neuron was used.

displacement of the neuronal membrane, as would be expected if the ion channels are directly affected by membrane movement. The voltage and current responses contained both adapting and static components, with the adaptation following at least two time constants. With increasing stimulus amplitude the two time constants of adaptation varied from 1.5–6 ms, and 20–60 ms. The strong static component in the response indicates that most of the rapid adaptation in the action potential discharge is produced by ionic events after transduction, such as voltage-activated or calcium-activated ion channels. There were no detectable differences in the receptor potentials or currents of the cells in the two groups of spider neurons, even though their action potential discharges are distinct (Seyfarth and French 1994). This supports the idea that action potential adaptation in these neurons occurs after sensory transduction by other ionic processes.

The sodium-dependence of the receptor current was demonstrated by replacing the sodium in the bath with iso-osmolar choline chloride (Fig. 3). The receptor current amplitude decreased gradually over a period of about 20 min. This time delay contrasts with the rapid removal of regenerative action potentials in the neurons by sodium replacement (Seyfarth and French 1994), suggesting that there is a significant chemical barrier between the extracellular solution and the region of the distal dendrite where mechanotransduction takes place.

DISCUSSION

These data provide the first direct observations of the receptor current in arthropod cuticular mechanosensilla, an important and widespread group of sensory receptors. The results show that the receptor current is carried by sodium ions, and that the rapid adaptation occurs after sensory transduction. The preparation provides an important new means of investigating mechanotransduction. In contrast to other systems, intracellular recording and voltage clamp are possible very close to the site of mechanical sensitivity and therefore to the ion channels that are responsible for transduction.

The time delay between sodium replacement and reduction of the receptor current probably results from the location of the dendrite tip in a receptor lymph space, as is normally the case for arthropod cuticular receptors (McIver 1985). Sodium replacement did not completely eliminate the receptor potential or current, and the transient initial response was reduced the least. Therefore, the receptor current could include other ionic components besides sodium, with the most obvious candidates being other mono- and divalent cations. Nevertheless, our evidence supports the idea that the receptor lymph space of spider cuticular sensilla is rich in sodium ions (Grünert and Gnatzy 1987), which carry the receptor current. This finding contrasts strongly with the situation in insects, where the receptor lymph spaces of cuticular sensilla are rich in potassium, and their receptor currents are probably carried by potassium ions (French 1992).

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