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SHORT COMMUNICATION

GIGAOHM SINGLE-CHANNEL RECORDING FROM ISOLATED *HERMISSENDA CRASSICORNIS* TYPE B PHOTORECEPTORS

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Invertebrate photoreceptor electrophysiology has mainly been studied with intracellular microelectrodes. Single-channel recording has proved to be difficult, possibly because of unusual plasma membrane configurations; in *Limulus polyphemus* successful gigaohm seals were obtained only after pronase treatment and brief sonication (Bacigalupo and Lisman, 1983). Here we describe an isolated *Hermissenda* eye preparation that permits single-channel recording (Hamill *et al.* 1981) from identified type B photoreceptors; this photoreceptor sub-type has been assiduously studied with intracellular microelectrodes, under both current-clamp and voltage-clamp conditions. Associative conditioning of *Hermissenda crassicornis* (in the paired light and rotation paradigm) is attributable in part to the reduction of potassium conductances in the type B photoreceptors (Alkon, 1984, 1987; Alkon *et al.* 1985). We have used the isolated eye preparation to characterize two types of potassium channels in photoreceptor plasma membranes.

When microelectrodes are employed on *Hermissenda* photoreceptors, the preparations are treated with protease (Sigma type XXIV) to soften the connective tissue capsule that covers the photoreceptors, thus easing the insertion of microelectrodes (Alkon *et al.* 1984). Experiments with protease, collagenase and dispase showed that an appropriate enzyme cocktail for gigaohm seal formation was a mixture of protease and dispase (dispase, grade II was obtained from Boehringer Mannheim). The enzymes were dissolved in artificial sea water (NaCl 430; KCl 10; $MgCl_2$ 50; $CaCl_2$ 10; Hepes 10; pH 7.4; concentrations in $mmol\,l^{-1}$) to final concentrations of $2\,mg\,ml^{-1}$ protease and $20\,mg\,ml^{-1}$ dispase. The circumoesophageal nervous system (CONS) was removed from 2–4 cm long adult *Hermissenda*, obtained from Sea Life Supply, Sand City, CA. The animals were maintained at 14°C in sea water of defined composition (Tropic Marin, Marinus Inc., Long Beach, CA) for 3–14 days before the CONS were removed. Several CONS were incubated in the enzyme cocktail for 40–45 min at 23–25°C, then rinsed with cold (10°C) artificial sea water (ASW). The eyes were microdissected from the CONS and transferred into fresh ASW in a plastic 35 mm sterile

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Petri dish. After 10 min, the adhesion of the eyes to the surface of the dish was sufficient for the eye not to move when patch electrodes were brought up to the eye. However, adhesion was variable enough so that ripping membrane patches off the cell was not consistently possible.

The five photoreceptors and the associated lens and pigment cells are shown in Fig. 1; in most preparations two or three of the photoreceptor cells were clearly visible as hemispherical protrusions from the central dark pigmented region. Our study was directed at the type B photoreceptors. In the isolated eye we identified these cells as the three cells at the opposite pole to the lens. Distinguishing between the medial and lateral B cells was impractical; fortunately, all the B photoreceptors show a characteristic and similar electrophysiology (Alkon and Fuortes, 1972). If the lens was missing so that we could not clearly identify the cells by this criterion, the preparation was discarded. Single microelectrode studies of the preparation showed resting potentials of −45 to −55 mV. Input resistances and light responses were indistinguishable from those of intact preparations (Lederhendler *et al.* 1990). Whole-cell patch-clamp gave resting potentials between −58 and −60 mV.

Both fire-polished and unpolished Sylgard (Dow–Corning) coated patch

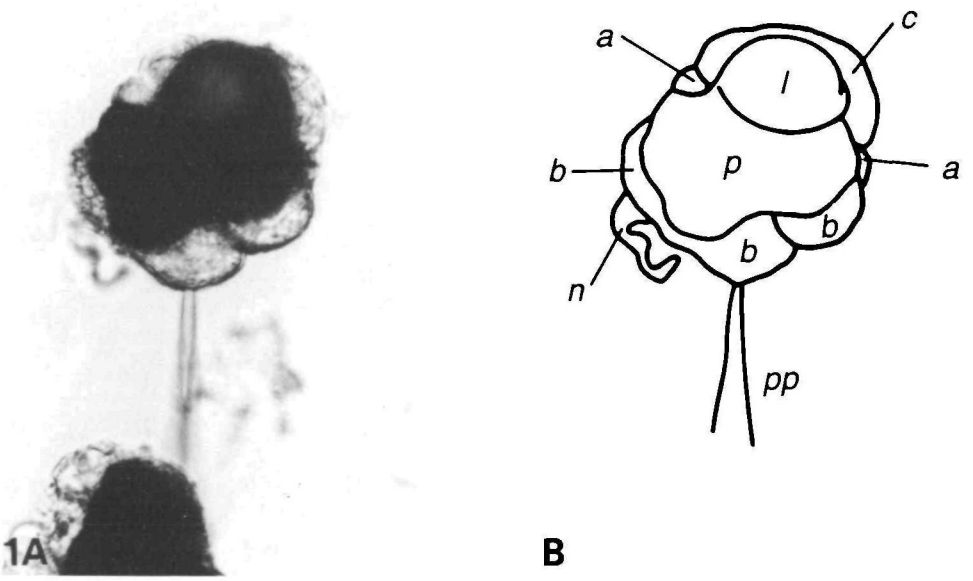

Fig. 1. Structure of the isolated *Hermissenda* eye. In A the eye is shown at 400× magnitude; in B the components of the eye are delineated. The lens (*l*) is surrounded by capsule material (*c*). The dark pigment cap (*p*) covers the central portion of the eye and largely obscures the two type A cells (*a*). The three type B cells are at the opposite pole to the lens (*b*); a patch pipette (*pp*) is apposed to one type B cell. The stump of the optic nerve (*n*) can also be seen, although out of the plane of focus.

pipettes were used. The pipettes were filled with a solution containing 350 KCl, 10 $CaCl_2$, 50 $MgCl_2$, Na-Hepes, 10, pH 7.4 (concentrations given in mmol l^{-1}) and had resistances of approximately 2 MΩ. Seal resistances greater than 5 GΩ were obtained in over 60 % of patches; high-resistance seals could be maintained for up to 1 h, but seals often failed spontaneously within a few minutes. Single-channel currents from cell-attached, inside-out and outside-out patches were measured with List EPC-7 or Axopatch-1C amplifiers and stored on a PCM-videotape recorder (d.c. to 22 kHz) for later analysis. The amplifier bandwidth was limiting at d.c. to 10 kHz. For analysis, the channel data were first low-pass Bessel filtered at cut-off frequencies of 2–5 kHz. The data were then digitized at 10 kHz sampling rates with either a Labmaster or an Axolab interface, and stored on a microcomputer, using the pClamp program suite from Axon Instruments (Foster City, CA). Event lists of channel amplitude and open and closed times were constructed with the IPROC program; amplitude histograms were obtained with pSTAT for each data set.

Two types of channel were observed in cell-attached patches and were classified by conductance as medium and large. In 27 patches with signal-to-noise ratios greater than 4:1 the medium channel was seen in 78 % of patches and the large channel was seen in 60 % of patches. In 6 of the 27 patches only the medium channel was seen and in 9 of the 27 patches only the large channel was seen.

With 350 mmol l^{-1} K^+ in the pipette the reversal for a potassium-selective channel would be close to 0 mV membrane potential; thus, the driving force for K^+ movement is the cell's resting potential, and K^+ currents are inward.

The medium channel inward currents shown in Fig. 2 are approximately 2.7 pA. When the current–voltage relationship was extrapolated (Fig. 3) the slope

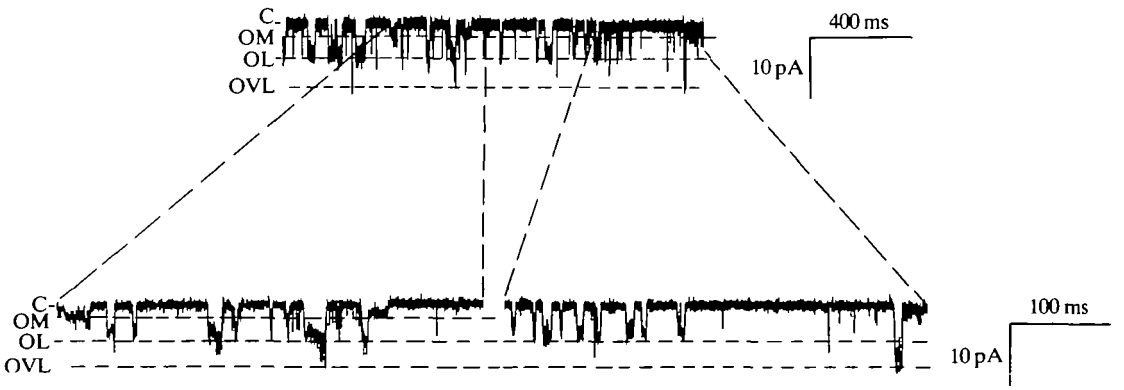

Fig. 2. Example of three types of channel activity in a single membrane patch. Downward deflections represent inward currents and channel openings. The medium channel opens to about 2.7 pA (level OM), the large channel to about 5 pA (OL) and the rare very large channel to about 10 pA (OVL). The upper trace shows 1.7 s of the recording, and two segments of this were expanded for the lower traces. The recording was low-pass at 2 kHz. C is the closed state.

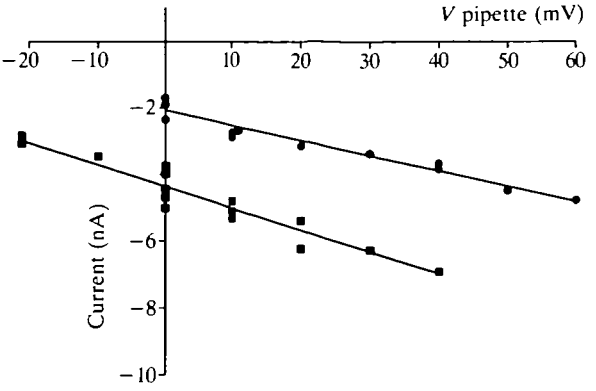

Fig. 3. Current–voltage relationships for the medium and large channels. The medium channel (●) has a conductance of 42 pS and the large channel (■) has a conductance of 64 pS. Data from five separate patches are compiled for each channel. The lines are fitted by linear regression. $V_{pipette}$, pipette potential.

conductance was estimated at 42±2 pS (mean±S.D., N=5), and the extrapolated reversal potential was estimated to be −53 mV. The larger inward currents seen in Fig. 2 had a conductance of 64±2 pS (mean±S.D., N=5) and reversed at approximately −60 mV; this is our large channel. Under these conditions of high extracellular $[K^+]$ (350 mmol l^{-1}) the expected reversal potential for a K^+-selective channel is approximately at 0 mV membrane potential or at about −60 mV pipette potential for a cell-attached patch.

Occasionally a very large inward current (approx. 10 pA) was seen in the same patches as the medium and large channels (Fig. 2); these 10 pA events were seen in only three patches, and their low frequency is not consistent with them being a superimposed pair of large channel openings. The identity of these channels remains to be established. Additionally, smaller single-channel currents were often seen. This small channel had a conductance of about 15 pS, but, as yet, we cannot unambiguously characterize this as another K^+ channel. We were able to explore the calcium dependence of the large channel in the inside-out configuration. When a membrane patch was ripped off into ASW (Ca^{2+} concentration 10 mmol l^{-1}), the activity of the large channel did not change, nor did it change when the intracellular face was perfused with ASW containing 40 mmol l^{-1} EGTA (estimated free $[Ca^{2+}]$=0.15 μmol l^{-1}, free $[Mg^{2+}]$=36 mmol l^{-1}; N=4).

The percentage open time (p_o) of the large channel was 34.8±3.2 (mean±S.E., N=7 patches) at 0 mV pipette potential. The mean open time (m_o) of 9000 events was 0.28±0.16 ms, and the mean closed time (m_c) was 1.12±1.37 ms (mean±S.D.). The medium channel had noticeably longer closed periods (at 0 mV pipette potential); for 11 patches the p_o was 12.2±3.2 (mean±S.E.). For 15 000 events m_o=0.36±0.89 ms and m_c=7.61±35.22 (means±S.D.).

In conclusion, we have developed a treatment for intact, isolated *Hermissenda* eyes that permits single-channel recording from identified photoreceptor types

and will be useful for the study of invertebrate phototransduction, photoreceptor biophysics and the cellular and molecular correlates of learning. We also describe two types of K^+ channels that are active at the resting potential. The macroscopic K^+ currents that are reduced in learning are a fast, transient current (I_A) and a calcium-dependent current. The calcium-independent large channel could be either an I_A channel or a delayed rectifier channel. The medium channel has a similar conductance to that of the calcium-dependent K^+ channel (SK channels) of *Helix* and *Aplysia* neurones (Latorre *et al.* 1989); however, we have no data on the calcium dependence of the medium channel.

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