VOLTAGE-DEPENDENT POTASSIUM CURRENTS IN RETINAL MÜLLER (GLIAL) CELLS OF THE SPINY DOGFISH *(Squalus acanthias)*

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Glial cells, thought until recently to be electrically inexcitable, possess several types of voltage-dependent ion channels. I have previously shown that in amphibians, Müller cells, the principal glial cells of the vertebrate retina, have Ca^{2+}-dependent K^+ channels, fast-inactivating (A) K^+ channels, inward rectifying K^+ channels and voltage-dependent Ca^{2+} channels. The present study was undertaken to determine whether Müller cells of elasmobranchs also possess multiple channel types and to determine specifically which of these channels are responsible for the resting K^+ conductance of the cell.

Whole cell patch-clamp recordings were made from freshly isolated Müller cells of the dogfish, *Squalus acanthias*. Enzymatically dissociated cells were prepared as described previously (Newman, *Glia* **1**:275-281, 1988). Cells were recorded from using patch-clamp electrodes filled with (in mM) KCl, 278; Urea, 350; trimethylamine-N-oxide (TMAO), 70. Dissociated cells were perfused with a Ringer's solution containing NaCl, 250; KCl, 4; $CaCl_2$, 4; $MgCl_2$, 2; $MgSO_4$, 1; NaH_2PO_4, 1; $NaHCO_3$, 8; Dextrose, 10; Urea, 350; TMAO, 70; bubbled with 1% CO_2 in O_2; cooled to 15°C. In high K^+ Ringer's solution the KCl concentration was raised to 254 mM and the NaCl was omitted. The access resistance of the patch electrodes was neutralized using the bridge circuity of the voltage-clamp amplifier (Axoclamp-2A, Axon Instruments) used in the continuous, single-electrode mode. This corrected for approximately 70% of the electrode resistance.

Previous reports have suggested that different types of K^+ channels are responsible for the resting K^+ conductance of Müller cells in different cell regions (Newman, *Nature* **317**:809-811, 1985). Over most of the cell surface, inward rectifying K^+ channels are thought to predominate. However, the endfoot of Müller cells may have K^+ channels which are less voltage-dependent. To test this hypothesis, the current-voltage (I-V) relations of Müller cells were determined using the voltage clamp technique. Both dissociated cells with their endfeet intact as well as cells whose endfeet had been sheared off during the dissociation process were tested. In order to accurately assess the rectifying properties of the cell membrane, cells were bathed in a 254 mM K^+ solution during voltage-clamp measurements so that the extracellular K^+ concentration was approximately equal to the intracellular K^+ concentration. The I-V relations of these cells in both 4 mM K^+ and 254 mM K^+ solutions had reversal potentials very near the respective K^+ equilibrium potentials, indicating that the measured currents were carried primarily by K^+.

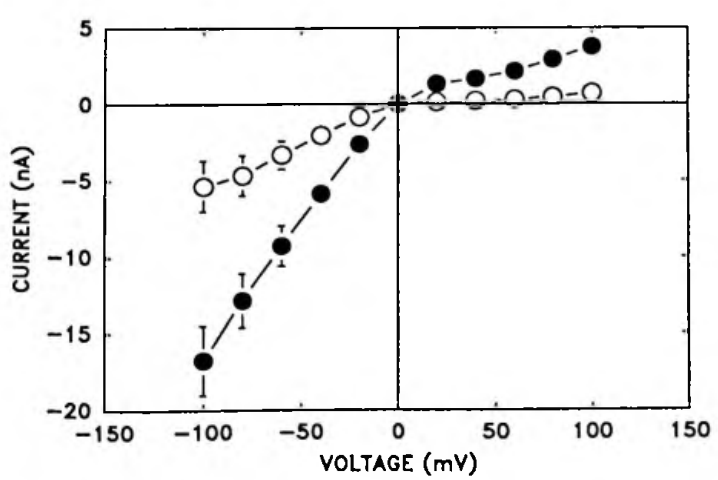

Fig. 1: Current-voltage relations of dogfish Müller cells determined by voltage-clamp. Plots show mean ± SEM for cells with endfeet intact (●, n=9) and for cells missing their endfeet (○, n=8). Cells were perfused in 254 mM K^+ Ringer's solution and had resting potentials of approximately 0 mV. Holding potential, 0 mV.

The voltage-clamp I-V relation of both intact cells and endfootless cells in 254 mM K^+ perfusate showed some degree of inward rectification. Rectification was much more pronounced in cells missing their endfeet than in cells with endfeet intact. Results in individual cells varied substantially, with some intact cells showing as much inward rectification as the least rectifying of the endfootless cells. When the I-V plots of intact cells (n=9) and endfootless cells (n=8) were averaged, however, a substantial difference in the degree of rectification was apparent (Fig. 1).

These results can be quantified by measuring the cell cord conductance at +20 and -20 mV. For cells with their endfeet intact, cell conductance was 130 nS for hyperpolarizing pulses and 66.5 nS for depolarizing pulses. In cells missing their endfeet, conductance was 42.5 nS for hyperpolarizing pulses and 7.3 nS for depolarizing pulses. The ratio of the hyperpolarizing cord conductance to the depolarizing cord conductance was 1.95 in intact cells and 5.82 in endfootless cells.

These results suggest that K^+ channels having different voltage dependencies are distributed in different regions of dogfish Müller cells. The non-endfoot portion of these cells shows substantial inward rectification. Thus, inward rectifying K^+ channels most likely predominate in this cell region. However, cells which have their endfeet show substantially less inward rectification. This suggests that the cell endfoot contains K^+ channels which are distinct from the channels localized over the rest of the cell.

Other interpretations of the data are possible, however. Damage inflicted to the endfootless cells when their endfeet were sheared off may have altered the voltage-dependent properties of their membrane. In addition, it was not possible to fully compensate for the access resistance of the recording electrode or to compensate at all for the internal cell resistance. These series resistances may have led to a distortion of the measured I-V curves.

The K^+ currents measured in 254 mM K^+ perfusate were largely time-independent. Currents resulting from depolarizing or hyperpolarizing command steps had fast rise times and did not inactivate. It was noted, however, that in 4 mM K^+ perfusate, depolarizing voltage steps evoked outward currents that had a transient, inactivating component. This transient current was seen in cells lacking their endfeet and was investigated in a number of experiments on endfootless cells.

The amplitude of the transient outward current (measured from the response peak to the amplitude at 1.5 s) varied as a steep function of voltage (Fig. 2). The activation threshold for the transient current was approximately -10 mV. In some cells the I-V relation continued to rise up to +120 mV while in others the I-V relation peaked at approximately +80 mV. The decay time constant (decay to 1/e peak response) of the transient current ranged from 520 to 803 ms (n=4; for depolarizations to +80 mV).

The transient current was reduced or abolished by depolarizing pre-pulses. For 5 s pre-pulses from a holding potential of -80 mV, inactivation began at -50 mV and was essentially complete at -10 mV. 50% inactivation occurred at approximately -30 mV. Pre-pulse duration also affected the degree of inactivation. A 400 ms pre-pulse to 0 mV reduced the transient current (produced by a +40 mV pulse) by half while a 4 s pre-pulse completely abolished the transient current. The transient current was blocked 70% by 10 mM tetraethylammonium (TEA) and 58% by 10 mM 4-aminopyridine (4-AP).

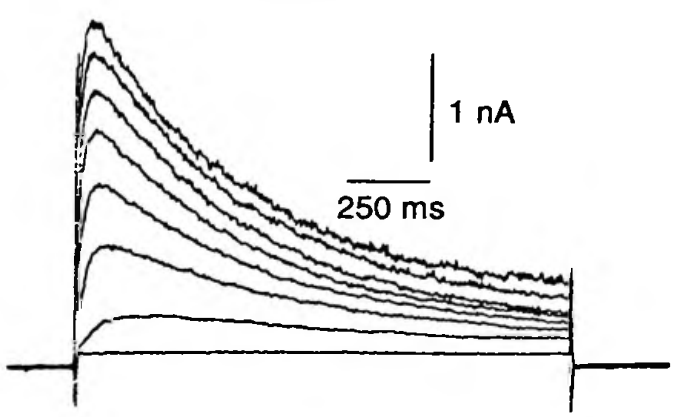

Fig. 2: Transient outward current seen in voltage-clamp records of an endfootless dogfish Müller cell. Holding potential, -80 mV. Command potentials, -20 to 120 mV, in 20 mV increments. Cell in 4 mM K^+ perfusate.

The type of K^+ channel underlying this transient outward current is uncertain. The current shares some of the same properties as the fast inactivating A current: it decays rapidly following an initial peak and is inactivated by depolarizing pre-pulses. However, both the activation and inactivation thresholds of this current are more positive than are those of typical A currents. In addition, this transient current is partially blocked by both TEA and 4-AP while the A current is blocked preferentially by 4-AP. It is possible that the transient current reported here is an A current whose properties have been modified by the unusual ionic environment of the elasmobranch Ringer's solution.

In summary, voltage clamp experiments suggest the presence of 2 and possible 3 types of K^+ channels in Müller cells of the dogfish retina. Over most of the cell surface an inward-rectifying K^+ channel is responsible for a substantial portion of the resting membrane conductance. On the cell endfoot a second K^+ channel may be responsible for cell rest conductance. Finally, an inactivating K^+ channel, which opens when the cell is depolarized, is present over most of the cell surface.

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