POTASSIUM MEMBRANE CONDUCTANCE IN RETINAL GLIAL CELLS OF TELEOST AND ELASMOBRANCH FISHES (*ALOSA PSEUDOHARENGUS AND SQUALUS ACANTHIAS*)

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Müller cells, the principal glial cells of the vertebrate retina, are selectively permeable to K^+. This K^+ conductance is not distributed uniformly over the cell surface (Newman, J. Neurosci. 7:2423, 1987). In amphibians and in mammals with avascular retinas, K^+ conductance is concentrated in the cell's endfoot. In vascularized mammalian species, high K^+ conductance is also found on and near the cell soma. The distribution of K^+ conductance in Müller cells of fishes has not been determined, although it has been predicted to resemble the pattern found in amphibians. In the present study, the K^+ conductance distribution of Müller cells was measured in two species, the alewife (*Alosa Pseudoharengus*), a teleost and the spiny dogfish (*Squalus acanthias*), an elasmobranch.

Measurements were made on freshly dissociated Müller cells, prepared by a modification of a previous dissociation procedure (Newman, J. Neurosci. 7:2423, 1987). Fish were dark adapted overnight in their holding tanks and then killed by decapitation. Pieces of retina were isolated from the back half of the eye, minced, placed in Ca^{2+}-, Mg^{2+}-free (CMF) Ringer's solution and triturated gently with a large-bore Pasteur pipette to break up the vitreous humor adhering to the retina. Retinal pieces were then incubated in CMF Ringer's solution containing 0.5 mg/ml papain and 10 mM cysteine for 30 minutes at 18°C. Retinal pieces were rinsed twice in normal Ringer's solution containing 0.1% bovine serum albumen (BSA) and placed on ice in Ringer's solution containing 0.1% BSA and 0.01% DNAase.

The retinal tissue was maintained on ice for 2 to 4 hours and then triturated using a series of Pasteur pipettes with decreasing bore size. Trituration continued until only small (approximately 1 mm) pieces of retinal tissue remained. Trituration of these small pieces yielded dissociated cells rich in Muller cells. The supernatant from the final trituration was placed in a chamber, perfused with normal Ringer's solution and viewed in a compound microscope with differential interference optics and a video system.

The composition of the Ringer's solutions used in the study is given in Table 1. The elasmobranch Ringer's solution contains more Na^+ and is somewhat hypertonic compared to elasmobranch plasma. These discrepancies are not believed to have appreciable altered the results, however, because large changes in osmolality and Na^+ concentration have little effect on Müller cell membrane properties in other species.

Isolated cells were continually perfused with a bicarbonate Ringer's solution maintained at 15 to 18°C. Cell membrane potential was recorded with suction electrodes filled with an intracellular solution of the same osmolarity as the perfusate. The distribution of K^+ conductance across the cell surface was determined by a method previously described (Newman, J. Neurosci. 5:2225, 1985). Briefly, cells were depolarized by localized increases the extracellular K^+ concentration ($[K^+]_o$) produced by pressure-ejecting an 'ejection solution' containing 15 mM K^+ from an extracellular pipette. The amplitude of cell depolarizations to such localized $[K^+]_o$ increases are directly proportional to the K^+ membrane conductance of that cell region exposed to the $[K^+]_o$ increase (Newman, J. Neurosci. 5:2225, 1985).

The membrane potential (E_m) of Müller cells of both species was high and near the K^+ equilibrium potential (E_K), indicating that the cell membrane was selectively permeable to K^+. For stable recordings, E_m of alewife cells was -71 $\pm$ 1.7 mV (mean $\pm$ SEM, n=16) and E_m of dogfish cells was -90.4 $\pm$ 1.4 mV (n=10). The values of E_K were -100 and -108 mV respectively.

Cell input impedance was measured by passing depolarizing constant current pulses through the recording electrode. Input resistance of Müller cells was 26.4 $\pm$ 1.7 MΩ (n=15) in alewife and 38.0 $\pm$ 4.5 MΩ (n=19) in dogfish. The cell time constant was 11.1 $\pm$ 1.2 ms (n=22) in alewife and 7.6 $\pm$ 0.9 ms (n=19) in dogfish.

Table 1. Composition of Ringer's solutions.

Solution	NaCl	KCl	$NaHCO_3$	$CaCl_2$	$MgCl_2$	$MgSO_4$	NaH_2PO_4	Dextrose	Urea	TMAO
Teleost										
Ringer	148	3	8	1.6		1	2.7	10		
Intra.		170								
Eject.	155	15								
Elasmobranch										
Ringer	290	4	8	5	2	1	1	10	350	77
Intra.		320							350	77
Eject.	305	15							350	77

Concentrations are given in mM. For CMF Ringer, the $CaCl_2$, $MgCl_2$ and $MgSO_4$ were omitted from the Ringer formulas. Solutions were bubbled with 1% CO_2 in O_2. Abbreviations: Intra., intracellular recording solution; Eject., ejection solution; TMAO, trimethylamine-N-oxide dihydrate.

The impedance of dogfish cells which were missing their proximal process and endfoot was also determined. The resistance of these endfoot-shorn cells was 749 $\pm$ 60.6 MΩ (n=13), indicating that almost all of the cell input conductance is localized to the endfoot and proximal cell process in this species. The cell time constant was 95.2 $\pm$ 7.4 ms (n=13). E_m of endfootless dogfish cells was -64.8 $\pm$ 3.8 mV (n=13), significantly less than E_m of cells with their endfeet intact. This difference in E_m suggests that the membrane of the soma and distal cell process is less selectively permeable to K^+ than is the membrane of the endfoot and/or proximal process in this species.

Results of the K^+ ejection experiments are summarized in Table 2. In both species, K^+ ejections onto the endfoot evoked much larger cell depolarizations than did ejections onto other cell regions. These results demonstrate that the K^+ conductance of fish Müller cells is localized, to a great extent, in the endfoot and confirm the conclusion reached from the impedance measurements in intact and endfoot-shorn dogfish cells which also suggest that most cell conductance is localized to the endfoot.

Table 2. Relative magnitudes of Muller cell depolarizations evoked by focal K^+ ejections.

	K^+ ejection location							
Species	A	B	C	D	E	F	G	H
Alewife	100	42.2±5.7	10.3±2.0	8.8±1.2	11.7±1.1	6.9±2.3		
Dogfish	100	40.7±5.3	26.9±4.3	22.9±5.3	13.0±2.1	8.6±1.1	7.0±1.0	7.8±1.0

Values represent mean $\pm$ SEM of individual K^+ responses, expressed as a percentage of the endfoot response of each cell. Ejection site locations: A, endfoot; B, C and D, proximal, mid and distal portion of proximal process; E, soma; F, G, H, proximal, mid and distal end of distal process.

It was previously predicted that in Muller cells of all species with avascular retinas the cell's K^+ conductance is largely localized to the endfoot. The present results confirm this finding for fishes, which have avascular retinas. As in other species, the non-uniform distribution of K^+ conductance may be important in regulating $[K^+]_o$ within the retina and in generating components of the electroretinogram.

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