Brain Research, 498 (1989) 308–314
Elsevier

BRES 14831

Potassium conductance block by barium in amphibian Müller cells

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(Accepted 28 February 1989)

Key words: Müller cell; Glial cell; Retina; Barium; Potassium conductance; Tiger salamander

The effect of barium on Müller cell K^+ conductance was evaluated in the tiger salamander using enzymatically dissociated cells and cells in situ (retinal slice and isolated retina). Barium effects were similar in both cases. In dissociated cells, 50 μM Ba^{2+} depolarized cells 14.7 mV and raised cell input resistance from a control value of 16.0 to 133 MΩ. For cells in situ, 50 μM Ba^{2+} depolarized cells 6.9 mV and raised cell resistance from 12.5 to 50.4 MΩ. At corresponding Ba^{2+} concentrations, the resistance of cells in situ was somewhat lower than was the resistance of dissociated cells, a phenomenon that may be due to the small degree of electrical coupling present between Müller cells in situ. There was a similar positive correlation between the magnitude of Ba^{2+}-induced depolarization and input resistance in both dissociated cells and in situ cells. The magnitude of depolarizations generated by localized K^+ ejections onto Müller cells was reduced substantially by Ba^{2+}. These observations indicate that Ba^{2+} is an effective K^+ channel blocker in Müller cells in situ as well as in enzymatically dissociated cells.

INTRODUCTION

Barium is a potent potassium channel blocker in many cell systems, including neurons[13,27] and glial cells[2,19,28]. This channel-blocking property of Ba^{2+} has proved useful in investigating the role that K^+ channels play in cell function. In the vertebrate retina, Ba^{2+} has been employed in studies of the functions of Müller cells, the principal glial cells of the retina[24]. For example, several investigators have suggested that K^+ current flow through Müller cells generates the slow PIII[8,30] and b-wave[6,21,26] components of the electroretinogram (ERG). Barium, when applied to the retina, blocks the slow PIII potential[3,20], supporting a Müller cell origin of this response. Ba^{2+} has a much smaller effect in blocking the b-wave response[4,11,14,29], however, casting doubt on the hypothesis that Müller cells generate this component of the ERG.

Barium has also been used to study the effectiveness of Müller cells in regulating extracellular K^+ concentration ($[K^+]_o$) in the retina. Barium substantially reduces the transfer of K^+ from the retina to the vitreous humor, both during light stimulation[18] and when a current is applied across the retina[16,17]. These results support the hypothesis that Müller cells regulate $[K^+]_o$ in the retina through a K^+ current flow passing through Müller cells.

The experiments cited above must be interpreted with caution, however, because the effect of Ba^{2+} on Müller cell K^+ channels is unclear. In enzymatically dissociated salamander Müller cells, micromolar concentrations of Ba^{2+} effectively block cell K^+ conductance[12,23]. However, Newman[23] reported that in the retinal slice preparation of the salamander, Ba^{2+} has little effect on Müller cell resistance. Similarly, Henshel et al.[12] reported that in recordings from salamander eyecups, Ba^{2+} had little effect on cell resistance.

The present study was undertaken to clarify these seemingly contradictory results of the effect of Ba^{2+} on Müller cells. I have examined the effects of Ba^{2+}

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on Müller cell membrane potential and resistance in both dissociated cells and in cells in situ (retinal slice and isolated retina). A preliminary report of some of these results appears elsewhere[18].

MATERIALS AND METHODS

Animals

Aquatic stage tiger salamanders (*Ambystoma tigrinum*) were used. Animals were killed by decapitation and pithing.

Dissociated cells

Dissociated cells were prepared as described previously[22,25]. Briefly, isolated retinas were incubated in a Ca^{2+}, Mg^{2+}-free Ringer's solution containing 0.5 mg/ml papain for 30 min at 26 °C, rinsed in Ringer's solution and placed on ice for a 4–6-h period. The tissue was then triturated and dissociated cells plated on a slide coated with concanavalin A.

Retinal slices

Slices of salamander retina were prepared as described previously[22]. Slices were cut approximately 150 μm thick and fixed to the bottom of a perfusion chamber with the cut surface of the slices facing upwards.

Isolated retinas

Radial cuts were made along the perimeter of an isolated retina. The retina was held in place at the bottom of a perfusion chamber using a sandwich of two pieces of filter paper on either side of the retina. The retina was first placed photoreceptor side down on a piece of a translucent polycarbonate membrane filter (Nuclepore, PC Memb. 1.0 μm). Suction was applied to the filter to draw the retina flat against it. The retina was then covered with a larger piece of membrane filter (Millipore, GS, 0.22 μm) with a whole cut in its center. This sandwich was attached to a slide with petroleum jelly. The retina, with the inner limiting membrane facing upwards, was viewed by transillumination (the illumination coming through the translucent filter paper). Ganglion cell somas and surface features on the inner limiting membrane could be visualized using oblique illumination produced with a wedge prism (Melles Griot, 10.5 diopters) placed in the path of the illuminating beam.

Cell recording

Dissociated cells, retinal slices and isolated retinas were perfused with oxygenated HEPES-buffered Ringer's solution. The solution contained (in mM): NaCl 106, KCl 2.5, $MgCl_2$ 0.8, $CaCl_2$ 1.8, dextrose 10, HEPES 5, adjusted with NaOH to pH 7.4. $BaCl_2$ was added to the Ringer's solution without adjusting for osmolality.

Additional experiments on dissociated cells (not reported here) were conducted using a bicarbonate, CO_2-buffered Ringer's solution[25]. The effects of Ba^{2+} on input resistance and cell depolarization in these experiments were similar to the effects of Ba^{2+} on cells in HEPES-buffered Ringer's solution.

For dissociated cells, Müller cell potentials were recorded using suction electrodes filled with 115 mM KCl. Conventional microelectrodes were used to record from Müller cells in slices and isolated retinas. These electrodes were filled with 0.5 M potassium acetate and 0.1% Lucifer yellow CH and had a resistance of 120–160 MΩ. Lucifer was iontophoretically injected into cells and viewed using epifluorescence illumination and a video system (Dage-MTI, SIT 66) to visualize cells that were penetrated.

Cell input resistance was measured by passing anodal current pulses through the recording microelectrode and monitoring the resulting cell depolarizations. (Preliminary experiments showed that similar resistance values were obtained using either depolarizing or hyperpolarizing current pulses. In both 0 and 500 μM Ba^{2+}, resistance values obtained with cathodal pulses were within 4% of the values obtained with anodal pulses.) Current pulses ranged from 0.02 to 0.1 nA and produced cell depolarizations generally less than 7 mV in amplitude. The voltage drop across the recording electrode was balanced by the bridge circuit of the recording amplifier (Axoclamp 2A, Axon Instruments).

Localized K^+ ejections were produced by pressure-ejecting a solution containing 15 mM KCl from an extracellular pipette. For ejections in slices and in isolated retinas, the ejection pipette was positioned so as to produce large, short latency responses. The pipette was lowered onto the tissue so that it slightly

dimpled the cut surface (slices) or the inner limiting membrane (isolated retinas).

RESULTS

Dissociated Müller cells

Enzymatically dissociated salamander Müller cells in control Ringer's solution had a resting potential of −79.3 mV. The addition of Ba^{2+} to the perfusate led to an immediate and stable cell depolarization (Fig. 1). Cells were depolarized 5.5 mV in 10 μM Ba^{2+}, 14.7 mV in 50 μM Ba^{2+}, and 31.8 mV in 500 μM Ba^{2+}.

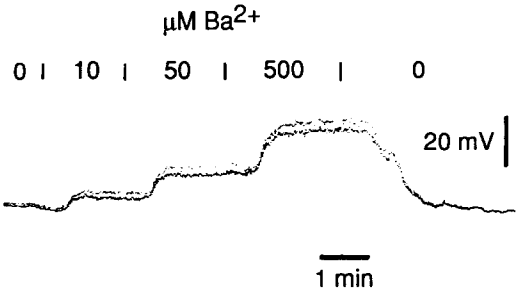

Fig. 1. Barium-induced cell depolarization in a dissociated salamander Müller cell. The cell was exposed sequentially to perfusate containing 0, 10, 50, 500 and 0 μM Ba^{2+}. Depolarizing transients produced by anodal current injection are visible on the trace. Cell resting potential was −79 mV.

Dissociated Müller cells also showed a substantial increase in cell input resistance with the addition of Ba^{2+}. Resistance increased from a control value of 16.0 to 57.7 MΩ in 10 μM Ba^{2+}, 133 MΩ in 50 μM Ba^{2+}, and 274 MΩ in 500 μM Ba^{2+}. Cell depolarization and resistance results are summarized in Table I.

Müller cells, like other glial cells, are almost exclusively permeable to K^+ (refs. 5, 22). Thus, the Ba^{2+}-induced increase in input resistance is most likely due to a reduction in the K^+ conductance of the cells. This was confirmed by monitoring cell depolarizations produced by focal ejections of a 15 mM K^+ solution onto the surface of dissociated cells. As shown previously, a focal K^+ ejection produces a transient cell depolarization which can be recorded in the soma[22]. The magnitude of this depolarization is proportional to the K^+ conductance of that portion of the cell membrane exposed to the $[K^+]_o$ increase and is inversely proportional to the total conductance of the cell[22].

Depolarizations produced by focal ejections of K^+ onto the Müller cell surface were reduced substantially in the presence of Ba^{2+} (Fig. 2). For K^+ ejections directed at the cell endfoot, the magnitude of the cell response was reduced to 39.3% of the control value by 50 μM Ba^{2+}. The cell response to K^+ ejections directed at the soma or distal process of isolated cells was similarly reduced to 38.0% of the control value. Results are summarized in Table II.

TABLE I

Input resistance, cell resting potential, and Ba^{2+}-induced depolarization in Müller cells exposed to Ba^{2+}

Values given as mean ± S.E.M. (number of cells). For each Ba^{2+} concentration, differences between dissociated cell and in situ cell values were statistically significant.

	Ba^{2+} concentration (μM)			
	0 (control)	*10*	*50*	*500*
Resistance (MΩ)				
Dissociated cells	16.0 ± 0.9 (19)***	57.7 ± 4.6 (11)	133 ± 8.4 (19)*	274 ± 16.3 (19)**
Cells in situ	12.5 ± 1.9 (29)***		50.4 ± 8.1 (17)*	207 ± 21 (5)**
Cell resting potential (mV)				
Dissociated cells	−79.3 ± 0.6 (19)			
Cells in situ	−85.5 ± 0.4 (25)			
Ba^{2+}-induced depolarization (mV)				
Dissociated cells		5.5 ± 0.4 (11)	14.7 ± 0.6 (19)*	31.8 ± 0.9 (19)*
Cells in situ			6.9 ± 1.1 (18)*	20.0 ± 2.3 (5)*

* $P < 0.001$; ** $P < 0.05$; *** $P < 0.1$.

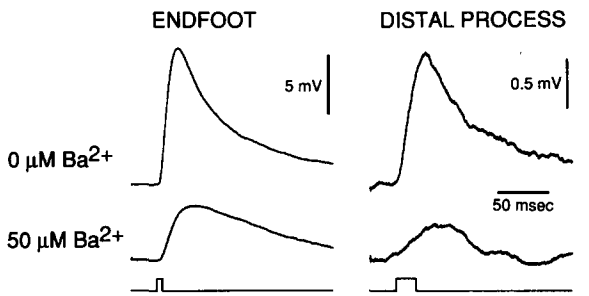

Fig. 2. Responses of dissociated Müller cells to focal K^+ ejections directed at the cell endfoot and the distal cell process. For both ejection sites, Ba^{2+} substantially reduced the amplitude of cell depolarizations. Onset and duration of the ejection pressure pulse are indicated below bottom traces. Endfoot and distal process records are from different cells. Endfoot responses are an average of 8 sweeps; distal process responses are an average of 20 sweeps.

Müller cells in situ

Müller cell recordings were obtained from retinal slices and from isolated retinas to determine the effect of Ba^{2+} in situ. In both preparations cells were penetrated in the soma. Müller cells were identified by their large resting potentials and by their morphology, visualized by Lucifer yellow injections. Results obtained from cells in slices and from cells in isolated retinas were similar and have been pooled for purposes of the present study.

Müller cell resting potential averaged −85.5 mV in cells from retinal slices and isolated retinas. As in dissociated cells, Ba^{2+} caused cells in situ to depolarize (Fig. 3). Fifty μM Ba^{2+} led to a 6.9 mV depolarization while 500 μM Ba^{2+} depolarized cells 20.0 mV. Ba^{2+} also caused a substantial increase in Müller cell input resistance. Cell resistance increased from a control value of 12.5 to 50.4 MΩ in 50 μM Ba^{2+} and to 207 MΩ in 500 μM Ba^{2+}. Cell depolarization and resistance results are summarized in Table I.

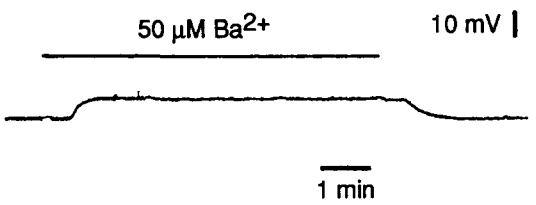

Fig. 3. Barium-induced cell depolarization in a Müller cell from a retinal slice. The cell was exposed to perfusate containing 0, 50, and 0 μM Ba^{2+}. Cell resting potential was −85 mV.

Müller cell depolarizations to focal K^+ ejections were also monitored to assess the effect of Ba^{2+} on K^+ conductance in cells in situ. In retinal slices, K^+ was ejected into the nerve fiber layer and into the outer plexiform layer. These two ejection sites correspond to the endfoot and distal process (adjacent to the Müller cell soma). In isolated retinas, K^+ was ejected into the inner limiting membrane of the retina (the endfoot of Müller cells).

As in dissociated cells, focal ejections of K^+ depolarized Müller cells in situ (Fig. 4). Ba^{2+}

TABLE II

Decrease in the magnitude of K^+ ejection responses due to Ba^{2+}

Responses in Ba^{2+} are expressed as a fraction of the control responses. Values given as mean ± S.E.M. (number of cells).

Ejection site	*Ba^{2+} concentration (μM)*	
	50	*500*
Dissociated cells		
Endfoot	0.393±0.019 (4)	–
Soma or distal process	0.380±0.058 (6)	–
In situ cells		
Retinal slice		
Nerve fiber layer	0.286±0.096 (5)	–
Outer plexiform layer	0.415±0.078 (4)	–
Isolated retina		
Inner limiting membrane	–	0.140±0.021 (3)

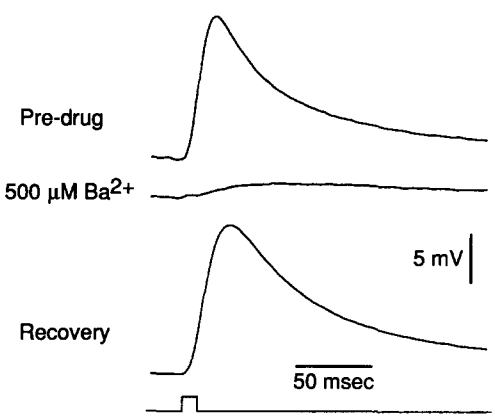

Fig. 4. Responses of a Müller cell from an isolated retina to focal K^+ ejections directed at the inner limiting membrane (Müller cell endfoot). 500 μM Ba^{2+} reduced the cell response to 9.9% of its pre-drug amplitude. Each trace is an average of 10 sweeps.

substantially reduced the magnitude of these K^+ responses. In retinal slices, 50 μM Ba^{2+} reduced the amplitude of depolarizations produced by ejection of K^+ into the nerve fiber layer (onto the Müller cell endfoot) to 28.6% of the control value. In isolated retinas, 500 μM Ba^{2+} reduced the response produced by K^+ ejection onto the inner limiting membrane (the cell endfoot) to 14.0% of the control value. K^+ ejection results are summarized in Table II.

DISCUSSION

Ba^{2+} block of K^+ conductance

The input resistance results reported in this study demonstrate that Ba^{2+}, even in micromolar concentrations, blocks a substantial fraction of the K^+ conductance of Müller cells in situ as well as of dissociated Müller cells. Barium-induced increases in resistance of cells in situ were similar, although not as large as, the Ba^{2+}-induced resistance increases observed in dissociated Müller cells.

The observation that Ba^{2+} depolarizes Müller cells is also consistent with a Ba^{2+} block of Müller cell K^+ channels. Normally, K^+ channels comprise a large fraction of the total cell membrane conductance. Thus, the resting potential of the cell is near the K^+ equilibrium potential[22]. However, when K^+ channels are blocked by Ba^{2+}, the cell potential will move closer to the reversal potential of other membrane-permeant ions[10]. Newman[22] has shown that Müller cells have a small but finite permeability to Na^+. Müller cells may also be permeable to Ca^{2+} (ref. 23). The positive reversal potentials of both of these ions would cause Müller cells to depolarize when K^+ channels are blocked.

If this account of Ba^{2+}-induced cell depolarization is correct, one would expect cell depolarization to be directly related to the degree of K^+ channel blockage. This indeed is the case. As shown in Fig. 5 for dissociated cells and Fig. 6 for cells in situ, larger cell depolarizations are associated with larger increases in cell input resistance in individual cells. Although there is substantial scatter in both plots, the general trend of the data indicates that there is a positive correlation between the input resistance of cells exposed to Ba^{2+} and the degree to which the cells are depolarized by Ba^{2+}.

The data plotted in Figs. 5 and 6 have been fit by linear regression (least squares). For dissociated cells, the best fit was obtained by the relation:

Cell resistance (MΩ) = 24.7 + 7.4·cell depolarization (mV)

A similar linear regression relation is obtained for data from Müller cells in situ:

Cell resistance (MΩ) = 10.8 + 8.12·cell depolarization (mV)

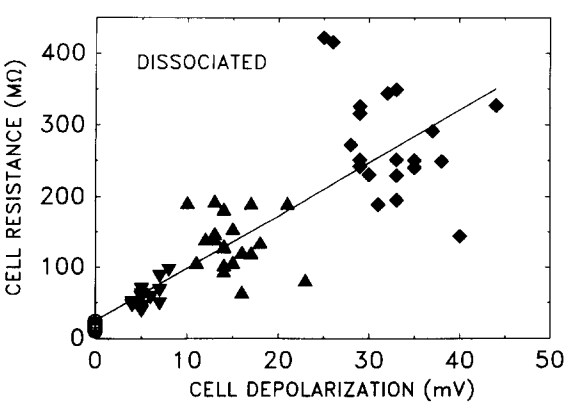

Fig. 5. Dissociated cells: cell input resistance in Ba^{2+} plotted as a function of the Ba^{2+}-induced cell depolarization. Cells were exposed to control (0 μM Ba^{2+}) perfusate (○), 10 μM Ba^{2+} (▼), 50 μM Ba^{2+} (▲) and 500 μM Ba^{2+} (◆). Solid line: least squares fit to the data (see text).

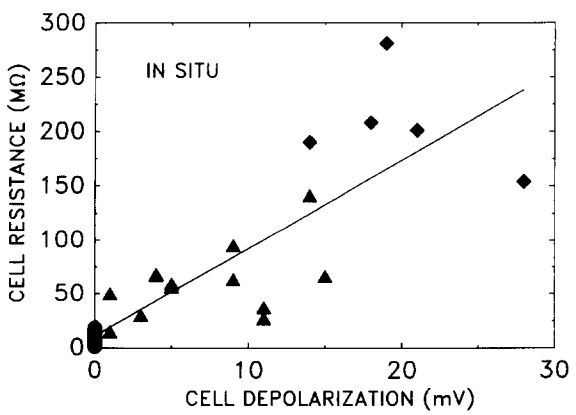

Fig. 6. Cells in situ: cell input resistance in Ba^{2+} as a function of the Ba^{2+}-induced cell depolarization. Cells were exposed to control (0 μM Ba^{2+}) perfusate (○), 50 μM Ba^{2+} (▲) and 500 μM Ba^{2+} (◆). Solid line: least squares fit to the data (see text).

The correlation coefficient for both relations was 0.87.

Results of Ba^{2+}-induced reductions in K^+ ejection responses also support the hypothesis that Ba^{2+} blocks K^+ channels in Müller cells. The amplitude of a K^+ ejection response in Müller cells is proportional to the local K^+ conductance as compared to total cell conductance[22]. In both dissociated cells and in cells in situ, depolarizations produced by local increases in $[K^+]_o$ were reduced by Ba^{2+} (Table II). This demonstrates that Ba^{2+} is blocking Müller cell K^+ channels to a greater extent than it is blocking channels to other permeant ions (Na^+ and perhaps Ca^{2+}).

The conclusion that Ba^{2+} blocks K^+ channels in Müller cells is consistent with observations in other glial cells. Barium has been shown to block K^+ fluxes in glial cells of guinea pig olfactory cortex slices[2] and in primary cultures of mouse astrocytes[19,28].

Differences between in situ and dissociated cells

Although Ba^{2+} blocks K^+ channels in Müller cells in situ as well as in dissociated cells, there was a difference in the effect of Ba^{2+} in these two preparations. For each concentration of Ba^{2+} tested, cell resistance was lower for in situ cells compared with dissociated cells. These differences were statistically significant (Table I).

This difference may arise because in situ cells, unlike dissociated cells, are coupled together by gap junctions[7]. Electrical coupling (albeit weak[9]) has been demonstrated in salamander[1] and turtle[5] Müller cells. Because of this coupling, changes in cell input resistance in cells in situ do not accurately reflect changes in resistance of the cell membrane (see discussion by Jack et al.[15]). As membrane resistance increases, the space constant of the syncytium also increases[19]. Cell input resistance then reflects the combined resistance of a greater number of cells in the syncytium, lowering the measured input resistance. Thus, differences between the resistance of in situ and dissociated cells at different Ba^{2+} concentrations may be due to the small degree of coupling which exists between cells in situ.

Earlier reports indicating that Ba^{2+} did not block K^+ conductance in Müller cells in situ were apparently erroneous. In the case of my own study[23], the observation that Ba^{2+} had little effect on cell conductance was based on indirect evidence. No direct measurements of cell resistance were made. Henshel et al.[12], also reported preliminary findings indicating that Ba^{2+} had little effect on cell resistance. However, they now find a small but significant Ba^{2+}-induced resistance increase in Müller cells of the mudpuppy eyecup (R.F. Miller, personal communication). As discussed above, electrical coupling between in situ cells can distort input resistance measurements. The negative results obtained in earlier Ba^{2+} studies[12,23] could have arisen if coupling were more extensive in those preparations than it was in the present study.

ACKNOWLEDGEMENTS

I thank Janice I. Gepner and Anthony R. Maranto for their helpful comments on the manuscript. This work was supported by National Institutes of Health Grant EY 04077.

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