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A Tetraethylammonium-insensitive Inward Rectifier K^+ Channel in Müller Cells of the Turtle (*Pseudemys scripta elegans*) Retina

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Abstract. Ion channels present in isolated glial (Müller) cells from the retina of the turtle (*Pseudemys scripta elegans*) were studied with the patch clamp technique. The predominant conductance in these cells was due to an inward rectifying potassium current. The whole-cell conductance of the inward rectifier was 20.2 ± 1.9 nS (n = 7 cells) in a standard extracellular saline solution (3 mM extracellular potassium). This conductance was dependent on the extracellular potassium concentration, with a 2.88-fold change in conductance per tenfold shift in concentration. The relative permeability sequence to potassium of the inward rectifier was found to be: potassium (1.0) > rubidium (0.7) > ammonium (0.2) > lithium (0.1) = sodium (0.1), which corresponded to the Eisenman sequence IV or V for a strong-field-strength potassium binding site on the channel. The single channel conductance measured in cell-attached patches with potassium chloride (150 mM) in the pipette was 68.5 ± 6.0 pS (n = 3 patches). The inward rectifier current was not blocked by extracellular tetraethylammonium (TEA^+, 20 mM), but was blocked by extracellular barium (5 mM) or cesium (5 mM). The TEA^+ insensitivity of the inward rectifier potassium channel in Müller cells is unusual, given that this type of channel in most excitable cells is sensitive to micromolar concentrations of this compound, and may be a characteristic of inward rectifier potassium channels that are primarily involved with extracellular potassium regulation.

Key words: Retina — Glial cell — Potassium channel — Tetraethylammonium — Patch clamp

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Introduction

Müller cells are glial cells which span the vertebrate retina from the inner to outer limiting membranes (Reichenbach, 1989). They are thought to have a variety of functions, including phagocytosis (Stolzenburg et al., 1992) and retinoic acid synthesis (Edwards et al., 1992). They are also sensitive to endogenous neurotransmitters such as glutamate (Schwartz, 1993) and γ-amino-butyric acid (Qian, Malchow & Ripps, 1993). Like most glia, Müller cells have a very highly selective membrane conductance for K^+ (Kuffler, 1967). An inwardly rectifying channel accounts for most of this K^+ conductance (Brew et al., 1986; Newman, 1993), but although this channel type is commonly associated with excitable cells, Müller cells are electrically inexcitable. This high K^+ selectivity of the Müller cell membrane has led several authors to propose that these cells play a role in regulating local extracellular K^+ concentration (Trachtenberg & Pollen, 1970; Newman, Frambach & Odette, 1984; Karwoski, Lu & Newman, 1989; Oakley et al., 1992; Reichenbach et al., 1992). A similar K^+ regulatory role has been proposed for this class of channel in nonexcitable secretory cells of the sheep parotid gland (Ishikawa, Cook & Young, 1991).

Inwardly rectifying K^+ channels have been characterized in frog skeletal muscle (Adrian, 1969) and in cardiac myocytes (Sakmann & Trube, 1984*a,b;* Josephson & Brown, 1986). In these and other cell types in which this channel has been reported (Mayer & Westbrook, 1983; Newman, 1984; Rae, Dewey & Cooper, 1989; Stelling & Jacob, 1992), blockade by extracellular tetraethylammonium (TEA^+) is one of the characteristics of this current. In other ocular tissue such as pigmented ciliary epithelium (Stelling & Jacob, 1992), this channel type exhibits marked sensitivity to micro-

molar concentrations of TEA^+. We report that the inward rectifier in Müller cells of the turtle (*Pseudemys scripta elegans*) is unusual, due to its insensitivity to TEA^+ but sensitivity to other standard inorganic blockers such as Ba^{2+} and Cs^+. Such insensitivity to TEA^+ has been reported for K^+ channels in secretory cells of the sheep parotid gland (Ishikawa et al., 1991), human macrophages (Gallin & McKinney, 1988) and starfish eggs (Hagiwara, Miyazaki & Rosenthal, 1976), and may be a characteristic of inward rectifier channels that are primarily involved with extracellular K^+ regulation.

Materials and Methods

Preparation of Isolated Müller Cells

Müller cells from the retina of the turtle *P. scripta elegans* were isolated using methods similar to those of Tachibana (1983) for horizontal cells. Briefly, the animals were decapitated, pithed and the eyes removed. After rinsing with 70% ethanol, the eyes were hemi-sected, the vitreous humor was excised and the retina dissected free. The retina was cut into several 5 mm^2 pieces which were placed in the incubation solution and stored at 4°C in sterile 35 mm culture dishes for a maximum of four days. These conditions did not adversely affect cell viability as determined by measurement of cell membrane potentials. The cells were dissociated from these retinal pieces on each day of experiments.

The cells were dissociated in 1 ml of the incubation solution to which 15 U/ml papain and 2.5 mM cysteine were added. Following preincubation of the solution for 30 min at room temperature, pieces of retina were added and then incubated for a further 45 min at room temperature in an oscillating culture dish (2 Hz). The retinal pieces were transferred to the recording solution and triturated with a Pasteur pipette that had been fire-polished to a very fine bore. Dissociated cells were then stored at 4°C for a maximum of six hours.

For patch clamp recordings, several drops of this cell suspension were diluted in 1 ml of the recording solution in a 35 mm culture dish that had been precoated with concanavalin A (0.25 mg/ml). At least 10 min settling time was allowed before cells were viewed with a phase-contrast inverted microscope (Nikon, Japan) and recordings commenced. Recordings were made from the body of the Müller cells, which were identified by their distinct morphology.

Solutions and Chemicals

The incubation solution contained (in mM): NaCl (120), KCl (2.5), $MgCl_2$ (0.5), $MgSO_4$ (0.5), $CaCl_2$ (2.5), $NaHCO_3$ (1.0), NaH_2PO_4 (0.5), glucose (16), HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) (4.0), and bovine serum albumin (0.02 mg/ml). The pH was adjusted to 7.6 with NaOH. The recording solution contained (in mM): NaCl (125), KCl (3), $CaCl_2$ (2), $MgCl_2$ (1), $NaHCO_3$ (6), NaH_2PO_4 (0.5), and HEPES (10). The pH was adjusted to 7.4 with NaOH. The pipette solution for whole-cell experiments contained (in mM): NaCl (4), KCl (130), $MgCl_2$ (1), EGTA (ethylene glycol-bis(-aminoethyl ether) *N,N,N',N'*-tetraacetic acid) (2), and HEPES (10). The pH was adjusted to 7.4 with NaOH. The pipette solution for cell-attached experiments contained (in mM): KCl (150, 125 or 100), and HEPES (10). The pH was adjusted to 7.4 with NaOH. The relative permeability of the channel to cations was measured using whole-cell recordings with the cell bathed in solutions comprising HEPES (10 mM) and one of the following (in mM): KCl (150), NaCl (150), LiCl (150), RbCl (150), or NH_4Cl (150). These solutions were buffered to pH 7.4 with KOH. The K^+ channel blockers TEA^+, Ba^{2+}, 4-aminopyridine and Cs^+ were added to the recording solution and superfused into the culture dish by hydrostatic pressure. Excess fluid was removed by suction using a 20 ml syringe. At least 10 ml (*ca.* 10 bath volumes) were superfused to ensure complete solution changes.

Analytical grade chemicals were purchased from either Ajax Chemicals, Australia ($BaCl_2$, $CaCl_2$, glucose, HEPES, KCl, $MgCl_2$, $MgSO_4$, NaCl, $NaHCO_3$, and NaH_2PO_4) or from Sigma Chemical, St. Louis, MO (4-aminopyridine, cysteine, LiCl, NH_4Cl, papain, RbCl, and TEA chloride).

Whole-Cell Patch Clamp

Macroscopic ion currents were recorded using the whole-cell configuration of the patch clamp technique (Hamill et al., 1981). Currents were recorded using an Axopatch-1D patch-clamp amplifier (Axon Instruments, Foster City, CA), low-pass filtered at 10 kHz (−3 dB, 4-pole Bessel) and digitized at either 1.25 or 2.5 kHz using pCLAMP (Axon Instruments). Whole-cell capacitance and series resistance were corrected with the Axopatch analog circuitry. Pipettes, with impedances of 3 to 8 MΩ, were manufactured from nonheparinized borosilicate microhematocrit tubes (Vitrex, Denmark) using a Flaming-Brown micropipette puller (P-87, Sutter Instruments). Step depolarizations for 320 msec from a holding potential of −70 mV were applied at intervals of 1 sec, a rate which did not appear to inactivate the inward rectifier current. Data were analyzed offline using CLAMPFIT software (Axon Instruments), or programs written in this laboratory. Steady-state currents at each potential were measured as the average of the last 50 data points from each step depolarization, immediately preceding the repolarization transition. The predominant current in whole-cell recordings was an inward rectifier current, which was studied after subtracting an ohmic membrane leakage current. The leakage currents were calculated by estimating the minimum positive slope of the whole-cell current-voltage profile (using the method of least squares) between −80 and +50 mV. The cell resting membrane potential was measured after rupture of the membrane patch to obtain whole-cell configuration. The measured potential was corrected for the liquid junction potential (typically 4 mV) between the pipette and bath solutions. The liquid junction potentials were calculated using commercial software (JPCALC, Barry & Lynch, 1991). Whole-cell current-voltage plots are presented as command potentials from a holding potential, uncorrected for liquid junction potentials.

Cell-attached Patch Clamp

Single channel currents (Hamill et al., 1981) were recorded in cell-attached mode using the Axopatch-1D amplifier, filtered at 2 kHz and digitized at 5 kHz. Single channel currents in this paper were examined in relation to the *trans*-patch membrane potential. For cell-attached patches, this was determined from the pipette potential, measured cell resting potential and the liquid junction potential between the bath and pipette solutions. The mean cell resting potential, measured by rupturing the membrane patch to obtain a whole-cell configuration, was −76 mV in recording solution. The liquid junction potential between the bath and pipette solutions was typically 4 mV.

The single channel current amplitude was estimated as the difference between the average current level at two consecutive sojourn

levels. We did not observe any subconductance levels in the recordings. The single channel conductance was estimated as the slope of the least squares line of best fit through the single channel current between trans-patch potentials of −100 to −175 mV in 25 mV steps.

A measure of the single channel activity was obtained using the same pulse protocol, where the mean "pulse" current was obtained over the final 200 msec of fifteen 400 msec pulses at each potential. The zero-current level was estimated from a hyperpolarized potential at which there was minimal channel activity. This was scaled appropriately for each test potential and subtracted from the mean "pulse" current. The channel activity was estimated by dividing the mean "pulse" current by the single channel current (*see* Aldrich & Yellen, 1983). The activity from three patches at each potential was averaged.

The recordings were made at room temperature (21–23°C). All data are expressed as mean ± SEM unless otherwise noted.

Permeability Calculations

The permeability ratio for various ions (X^+) relative to K^+ (P_X/P_K) was determined using the Goldman-Hodgkin-Katz equation described by Katz (1966):

$$E_m = \frac{RT}{F} \ln \frac{[K^+]_o + \frac{P_X}{P_K}[X^+]_o}{[K^+]_i + \frac{P_X}{P_K}[X^+]_i}$$

where V_m is membrane potential, R is the gas constant, T is absolute temperature, F is Faraday's constant, and the other subscripts signify ion concentrations outside (o) or inside (i) the membrane. Assuming there is dialysis of the intracellular ions with the solution in the pipette, then the internal K^+ concentration will be approximately 130 mM. The extracellular K^+ concentration will be the amount of KOH required to adjust the pH of the solution to 7.4. By titration this was found to be 6.48 ± 0.07 mM (n = 3).

All procedures were approved by the Animal Ethics Committees of the University of New South Wales and Westmead Hospital, Sydney, which conformed to guidelines prepared by the Australian National Health and Medical Research Council.

Results

Whole-Cell Currents

The membrane potential of isolated Müller cells in recording solution measured by the whole-cell technique, uncorrected for liquid junction potentials, varied between −52 and −86 mV with an average of −75.8 ± 1.8 mV (n = 24 cells). A typical whole-cell current profile obtained from these cells is shown in Fig. 1. In the raw record there was a large inward current at potentials more negative than −75 mV, an ohmic component between −75 and +10 mV, and an outward current that started to develop at potentials more positive than +10 mV. The ohmic component was probably due to a nonspecific membrane leakage conductance, and was subtracted from the raw records. At potentials more negative than −150 mV, some cells exhibited time-dependent inactivation over the duration of the voltage pulse.

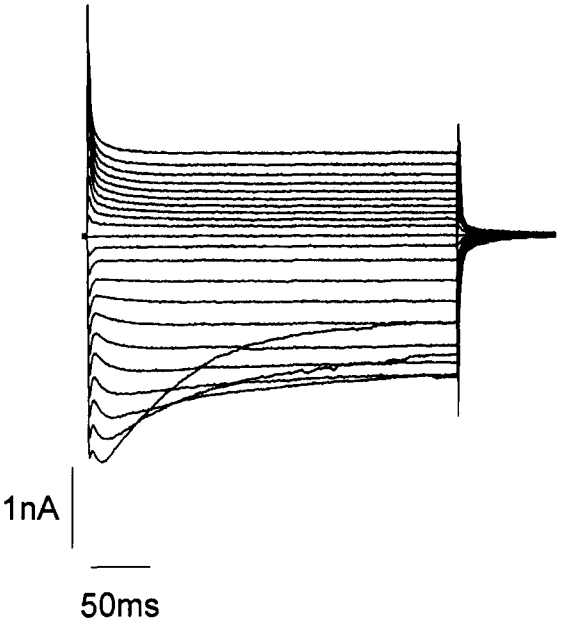

Fig. 1. Whole-cell currents from an isolated Müller cell in recording solution at room temperature. Data were filtered at 10 kHz and digitized at 1.25 kHz. The holding potential was −70 mV, with voltage jumps from −180 to +30 mV in 10 mV steps at 1 sec intervals. The resting membrane potential for this cell was −81 mV, uncorrected for the liquid junction potential of 4 mV.

The raw data from Fig. 1 are shown in Fig. 2 as a current-voltage plot. The inward rectifier component of the whole-cell currents was activated by potentials more negative than −60 mV. The reversal potential for this current was estimated by extrapolation of the least squares regression over the linear section (−150 to −80 mV) of the inward rectifier component. In recording solution, the extrapolated reversal potential corrected for liquid junction potential for this component was −77.9 ± 2.2 mV (n = 5 cells). During the first 30 msec of the voltage jumps to potentials more positive than +10 mV there appeared to be a transient outward current (I_A), which was similar to that reported previously in Müller cells of the salamander retina (Newman, 1985) but was not examined further in this study. Steady-state currents at potentials more positive than +10 mV also showed an outward K^+ current thought to be the delayed rectifier (Reichenbach et al., 1992). These currents were also not examined further in this study.

K^+ Selectivity and Permeability of the Inward Rectifier

The effect of extracellular K^+ concentrations on the inward rectifier is shown in Fig. 3. An increase in extracellular K^+ concentration was accompanied by an increase in the inward current. The reversal potential for

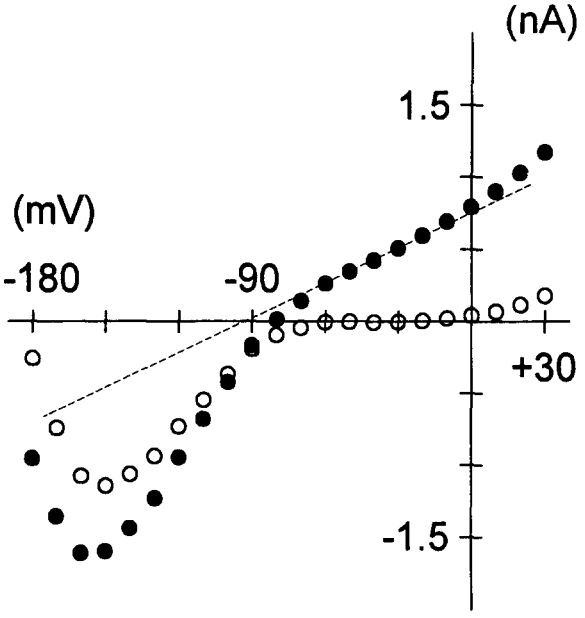

Fig. 2. Steady-state currents for the data shown in Fig. 1 as a current-voltage plot showing the raw current (●), the inward rectifier component (○) and the ohmic leak current (- - -) estimated from the minimum positive slope (between −80 and +30 mV) of the raw current curve. Steady-state currents were calculated as described in the text.

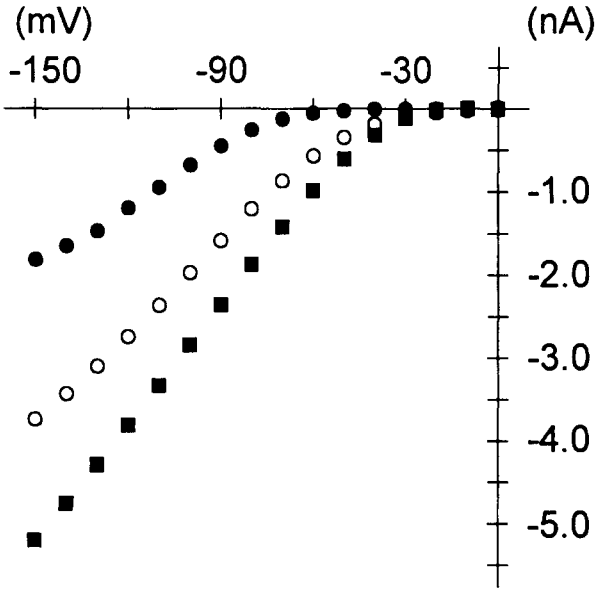

Fig. 3. Inward rectifier component of whole-cell currents from an isolated Müller cell showing the effect of increasing extracellular K^+ concentration. Data are for jumps at 1 sec intervals to potentials between −150 and +0 mV from a holding potential of −70 mV. Data are for the same cell in recording solution (●, 3 mM K^+, membrane potential −81 mV), recording solution containing 10 mM K^+ (○, membrane potential −59 mV) and recording solution containing 20 mM K^+, (■, membrane potential −49 mV). Membrane potentials are given corrected for liquid junction potentials. The whole-cell conductances for the inward rectifier for 3 mM extracellular K^+, was 23.6 nS, for 10 mM K^+ was 36.8 nS and for 20 mM K^+ was 47.9 nS. Steady-state currents at each potential were calculated as described in the text.

the inward rectifier from this cell, extrapolated from the least squares regression of the linear component (−150 to −80 mV) and corrected for liquid junction potentials was −73.9 mV for $[K^+]_o$ = 3 mM, −50.4 mV for $[K^+]_o$ = 10 mM and −44.5 mV for $[K^+]_o$ = 20 mM. These data gave a calculated shift in reversal potential of 44.6 mV per tenfold change in extracellular K^+ concentration (estimated by the method of least squares) which is similar to the Nernst relation of 58 mV/decade. The whole-cell conductance for the inward current in recording solution was 20.2 ± 1.9 nS (n = 7 cells) and followed a square-root relation as the extracellular K^+ concentration was increased. On average, the relation between whole-cell conductance (Γ) of the channel and extracellular potassium concentration ($[K^+]_o$) was: $\Gamma = 12.4 \times [K^+]_o^{0.46}$.

The permeability of the inward rectifier current was examined by changing the composition of the bathing solution. The resting membrane potentials corrected for liquid junction potentials for isolated Müller cells in these solutions were: −55.4 ± 1.5 mV (Na^+, n = 7), −54.2 ± 1.5 mV (Li^+, n = 3), −27.9 ± 1.5 mV (NH_4^+, n = 5), −5.3 ± 3.5 mV (Rb^+, n = 5), and +4.7 ± 1.2 mV (K^+, n = 3). The relative permeability sequence of the channels to these cations was K^+ (1.000) > Rb^+ (0.660) > NH_4^+ (0.245) > Li^+ (0.059) ≅ Na^+ (0.054). This followed the Eisenman sequence IV or V for a strong-field-strength site for K^+ binding on the channel (Hille, 1984).

Blockade of the Inward Rectifier

Several K^+ channel blockers were added to the recording solution in different experiments and the results of their effect on the inward rectifier K^+ channel are shown in Fig. 4. The inward rectifier was blocked by Ba^{2+} (5 mM), or Cs^+ (5 mM). The presence of 4-aminopyridine (5 mM) did not block the inward rectifier current (*data not shown*) as would be expected from the pharmacology of this compound; 4-aminopyridine is known to block outward K^+ currents, but does not affect the inward rectifier (Rudy, 1988). The inward rectifier current was not blocked by TEA^+, at a concentration of 20 mM.

Single Channel Recordings

Single channel currents of the inward rectifier were recorded in cell-attached configuration. An example of these currents at several potentials is shown in Fig. 5. Multiple channel openings were seen in all patches with high K^+ present in the pipette. The amplitude of the channels increased with increasing hyperpolarization,

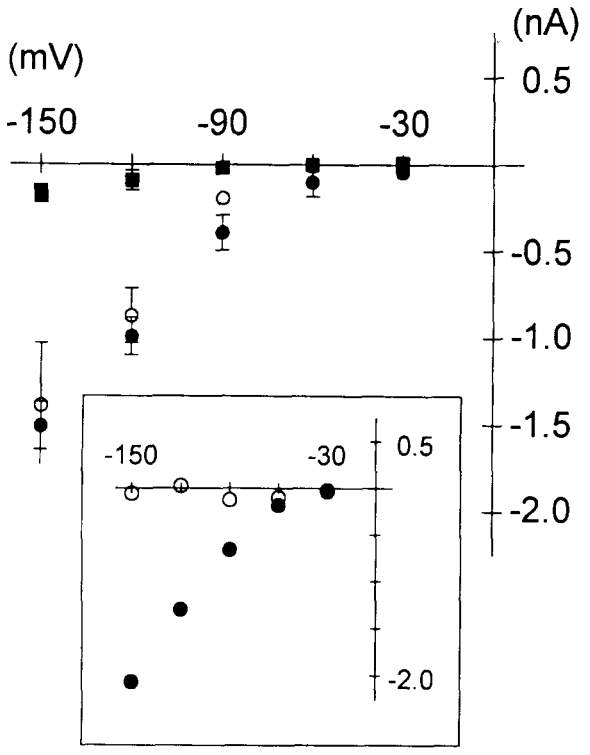

Fig. 4. Mean inward rectifier K^+ currents recorded from isolated Müller cells for voltage jumps between −150 and −30 mV from a holding potential of −70 mV. Data are paired control and test values for recording solution alone (●, $n = 6$), and recording solution containing 20 mM TEA^+ (○, $n = 3$ cells) or 5 mM $BaCl_2$ (■, $n = 3$ cells). Steady-state currents at each potential were calculated as described in the text. Data are presented as mean ± SE where larger than the symbol. For figure clarity, only selected potentials are shown. **Inset:** Inward rectifier K^+ currents from a typical Müller cell showing the effect of Cs^+. Data are steady-state currents in recording solution (●, membrane potential −84 mV) and recording solution containing 5 mM Cs^+ (○, membrane potential −75 mV). Membrane potentials are corrected for liquid junction potentials.

indicating an inward current. The single channel conductance varied with extracellular K^+ concentration. The single channel conductance (γ) was 68.5 ± 6.0 pS ($n = 3$ patches) with $[K^+]_o$ = 150 mM, 42.8 ± 5.8 pS ($n = 3$) with $[K^+]_o$ = 125 mM and 33.0 ± 7.2 pS ($n = 5$) with $[K^+]_o$ = 100 mM.

The single channel activity (nP_o) was estimated from cell-attached patches, using the repetitive pulse protocol described in Materials and Methods. The transpatch potential was calculated from the voltage applied to the pipette, the mean resting membrane potential for the isolated Müller cells (−75.8 mV), and the liquid junction potential (4.0 mV). The channel activity at these potentials was 21.62 ± 4.32 (−100 mV, $n = 3$ patches), 9.55 ± 3.34 (−125 mV, $n = 3$), 1.74 ± 0.52 (−150 mV, $n = 3$), and 1.60 ± 0.36 (−175 mV, $n = 3$). The decrease in single channel activity seen at strong hyperpolarizations reflects the time-dependent inactivation seen in the whole-cell currents (*see* Fig. 1). The channel activity for the mean resting membrane potential (−79.8 mV) extrapolated from −100 to −150 mV using least squares was 28.9, with a correlation coefficient of 0.9924. Similar to the whole-cell currents, extracellular $BaCl_2$ (5 mM) added to the pipette solution blocked all channel openings. However, TEA^+ (20 mM) added to the pipette solution had no effect on the single inward rectifier channels (*data not shown*).

Discussion

We found the inward rectifier K^+ current to be the principal conductance in whole-cell recordings. Inward rectifier channels have been reported in Müller cells of the salamander (Newman, 1985, 1993) where they are thought to play a role in the maintenance of the extracellular K^+ ion concentration (Newman, 1984; Newman et al., 1984). These channels are present over the entire cell surface, but predominant at the endfeet where they are located in higher density (Brew et al., 1986). We also observed an outward delayed K^+ current in these cells, which we did not characterize further. Outward K^+ currents of this type have been reported in Müller cells from the rabbit retina (Reichenbach et al., 1992). Other channel types present in this retinal glial cell include Ca^{2+}, transient outward K^+, and Ca^{2+}-activated K^+ channels (Newman, 1985). More recently, Na^+ channels have been reported in Müller cells from the canine and feline retina (Chao et al., 1993).

The observed mean membrane potential after correction for liquid junction potentials (−80 mV) was similar to that reported by others for isolated Müller cells from a variety of preparations (−77 mV, Conner, Detweiler & Sarthy, 1985; −88 mV, Brew et al., 1986; −65 mV Reichenbach et al., 1992; −85 mV, Reichelt & Pannicke, 1993). However, this value was lower than the calculated membrane potential from the Nernst equation. This deviation could be due to cell damage during the isolation procedure. However, we observed an ohmic leak current in all cells examined, which suggested that the slightly lower membrane potential was due to membrane permeability to ions other than K^+. This ohmic current may also be a nonspecific conductance of some type, since it is sensitive to low micromolar concentrations of Ba^{2+} (Reichelt & Pannicke, 1993).

The conductance of the inward rectifier, both in whole-cell and single channel recordings, increased with increased extracellular K^+ concentration. The whole-cell conductance varied with the square root of the extracellular K^+ concentration, as has been reported for this channel in Müller cells of the rabbit (Nilius & Reichenbach, 1988) and other cell types (Sakmann &

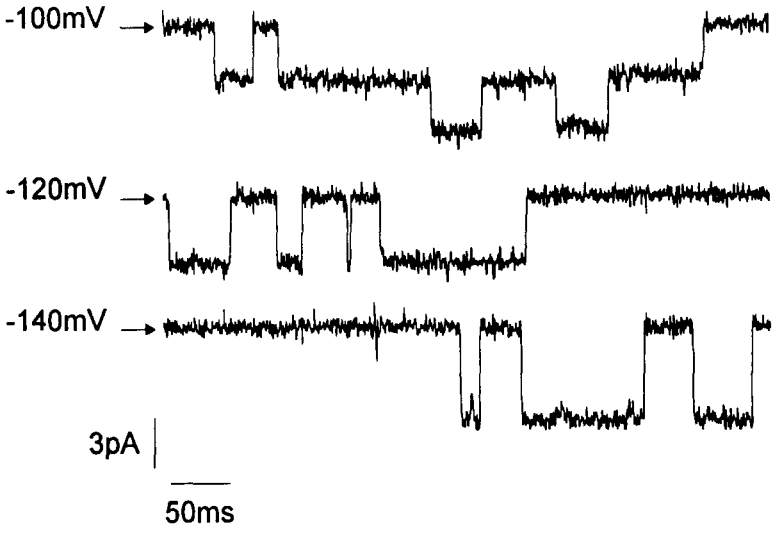

Fig. 5. Single inward rectifier K^+ channel currents in a cell-attached patch recorded from an isolated Müller cell in recording solution at room temperature. Pipette solution contained 150 mM K^+. Inward currents are shown as downward deflections, with the arrows indicating the zero current level. Data were filtered at 500 Hz and digitized at 20 kHz. Holding potentials are indicated as trans-patch potential, which was calculated using the average membrane potential for these cells (−76 mV), the liquid junction potential (4 mV), and the potential applied to the pipette.

Trube, 1984*a;* Chen et al., 1991). The inward rectifier was selective for K^+ and less permeable to other monovalent ions. Our value for the relative permeability of NH_4^+ to K^+ of 0.245 agrees well with that obtained for K^+ channels of the squid axon (0.26, Binstock & Lecar, 1969), but is different to that reported by Hille (1984) for the inward rectifier of starfish eggs (0.035). Our value for the relative permeability of Na^+ to K^+ of 0.054 is similar to that obtained by others for Müller cells from the same species of turtle used in the present study (0.037, Conner et al., 1985). Our permeability sequence was identical to that for the ATP-sensitive K^+ channel in heart muscle, which also has inwardly rectifying properties (Martin et al., 1993).

Some of the whole-cell currents associated with the inward rectifier exhibited time-dependent inactivation with strong hyperpolarization. This inactivation was also reflected in decreases in single channel activity recorded in cell-attached patches at strong hyperpolarizing potentials. This inactivation has been previously reported to be due to blockade of the inward rectifier by Na^+ because the inactivation is absent in Na^+-free solutions (Standen & Stanfield, 1979). However, the inactivation may also be attributed in part to voltage-dependent changes in channel gating kinetics because the phenomenon is present in Na^+-free solutions in some preparations (Sakmann & Trube 1984*b*). Interestingly, the Na^+-dependent inactivation is absent in freshly dissociated epithelial cells, but is present in cultured cells of the same type (Wen, Lui & Steinberg, 1993). We cannot account for our observations that some Müller cells displayed the inactivation and some did not.

In the present study, the inward rectifier K^+ current was blocked by Cs^+ and Ba^{2+}. Blockade by these inorganic ions is a characteristic of the inward rectifier. For example, 1 mM Cs^+ blocks the inward rectifier from starfish eggs (Hagiwara et al., 1976), 5 mM Cs^+ abolishes the inward rectifier from Müller cells of the rabbit (Reichenbach et al., 1992), 0.5 mM Ba^{2+} blocks the inward rectifier from myocytes (Chen et al., 1991), and 1 mM Ba^{2+} blocks the inward rectifier of glial cells from the rabbit retina (Reichelt & Pannicke, 1993).

Another standard blocker of the inward rectifier is TEA^+. As well as blocking the delayed rectifier K^+ current in a variety of preparations (Rudy, 1988), this compound has also been shown to reduce the amplitude of the inward rectifier K^+ current from frog skeletal muscle (50% inhibition by 20 mM, Stanfield, 1970) and bovine pigmented ciliary epithelium (74% inhibition by 290 μM, Stelling & Jacob, 1992). In the present study, the inward rectifier from the turtle retina was shown to be TEA^+ insensitive. This insensitivity to TEA^+ has been reported in secretory cells of the sheep parotid gland (Ishikawa et al., 1991), human macrophages (Gallin & McKinney, 1988) and starfish eggs (Hagiwara et al., 1976). It is also possible that the inward rectifier K^+ current in cultured microglial cells from the rat brain may be insensitive to TEA^+, since a combination of 4-aminopyridine and TEA^+ blocked this current (Kettenmann et al., 1990). Another TEA^+-insensitive ion channel active at resting membrane potentials has also been recently reported (Koh et al., 1992). An ATP-sensitive K^+ channel in ventricular myocytes that has similar biophysical characteristics to the channel we report in Müller cells was blocked by 5 mM TEA^+ extracellularly (Martin et al., 1993). Insensitivity to TEA^+ may be a characteristic of inward rectifier channels that are primarily involved with extracellular K^+ regulation.

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