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IONIC AND ELECTROPHYSIOLOGICAL PROPERTIES OF RETINAL MÜLLER (GLIAL) CELLS OF THE TURTLE

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SUMMARY

1. The ionic and electrophysiological properties of Müller cells, the principal glial element of the vertebrate retina, were investigated.

2. The membrane potential of enzymatically dissociated and *in situ* Müller cells was about −80 mV and depended on external K^+ concentration in a manner that was described by the Goldman–Hodgkin–Katz equation with a Na^+–K^+ permeability ratio of 0·037.

3. The current–voltage relation showed marked inward rectification, with the input resistance at the resting potential being about 30 MΩ for dissociated cells and about 3 MΩ for *in situ* cells. *In situ* Müller cells were found to be electrically coupled to each other which could explain their lower resistance.

4. We conclude that Müller cells are similar to other types of glia. In spite of a finite Na^+ permeability their membrane potential is determined mainly by K^+, they are electrically inexcitable and form an electrically coupled network in the retina.

INTRODUCTION

Wherever nerve cells occur they are usually associated with some type of glial cell. In the central nervous system glial cells are more numerous than neurones yet their function in the brain is less clearly defined. The early work of Kuffler and colleagues (reviewed by Kuffler & Nicholls, 1966) showed that glia differ from neurones in three ways. First, they neither generate action potentials nor receive synaptic inputs. Secondly, their membrane potentials are more negative than those of neurones and depend on the K^+ ion gradient in a manner accurately predicted by the Nernst equation, i.e. they behave like a K^+ electrode. Thirdly, neighbouring glial cells are electrically coupled via gap junctions.

Müller cells are a specialized form of astrocyte and the principal glial element in the vertebrate retina. They are radially directed, span the thickness of the retina, and give rise to processes that fill most of the extraneuronal space. The function of the cells is not known. Biochemical studies have shown that although they do not synthesize neurotransmitters they may participate in the inactivation of chemical transmitters and in the removal of metabolic products such as ammonia and carbon dioxide (Sarthy & Lam, 1978; Sarthy, 1983). There have been relatively few

physiological studies of Müller cells using intracellular electrodes. Miller & Dowling (1970*a*, *b*) showed that they have large resting potentials and respond to light with a slow small depolarization that resembles the b-wave of the e.r.g. in latency and wave form. On this basis it was suggested that the b-wave originates from the Müller cell. In brief, it is thought that a light-evoked increase in extracellular K^+ concentration depolarizes a portion of the Müller cell causing radial current flow and an extracellular field potential (Faber, 1969; Kline, Ripps & Dowling, 1978; Dick & Miller, 1978). Although the details of this hypothesis are not universally accepted (Oakley & Green, 1976; Karwoski & Proenza, 1978; Vogel & Green, 1980), the underlying assumption is that the Müller cell behaves as a perfect K^+ electrode, a point which has not been tested directly. It is also not known if Müller cells are electrically coupled. This issue could have bearing on hypotheses concerning the function of Müller cells and their putative role in the generation of extracellular field potentials.

Lack of basic information about the membrane properties of the Müller cell has made it difficult to assess its function. The principal goal of the present work was to investigate the electrical characteristics of the Müller cell and the ionic dependence of its membrane potential. Experiments were performed on isolated Müller cells obtained by enzymatic dissociation of the turtle retina. Such cells may be impaled, under visual control, with one or two intracellular electrodes and are free of complicating interactions with other cells. A major criticism of using enzymes to disrupt the retina is that the dissociation procedure could alter the membrane properties of the cells. We have therefore examined the effects of enzymatic dissociation by comparing the electrophysiological properties of isolated Müller cells with those in the intact retina. A preliminary account of a portion of this work has been published (Conner, Sarthy & Detwiler, 1981).

METHODS

All the experiments were performed on Müller cells obtained from the red-eared swamp turtle, *Pseudemys scripta elegans* (shells 15–30 cm long). Eyes were removed from decapitated and pithed turtles in dim room light and hemisected.

Dissociation procedures. Posterior eyecups were cut into pieces and pre-incubated in Ca^{2+}, Mg^{2+}-free Ringer solution (Table 1) for 10 min at 37 °C. The eyecup pieces were transferred to 5 ml Ca^{2+}, Mg^{2+}-free Ringer solution, containing papain (0·5 mg/ml) pre-activated with 10 mM-cysteine for 10 min at 37 °C. After a 15 min incubation (37 °C), eyecup sections were transferred to a Petri dish containing Ca^{2+}, Mg^{2+}-free Ringer solution at room temperature and the retinas were gently isolated. The isolated retinas were incubated for an additional 30 min in freshly activated papain and then moved to a 15 ml plastic, conical tube containing 2 ml standard Ringer solution (Table 1), which was supplemented with 1 % bovine serum albumin and deoxyribonuclease I (0·1 mg/ml). Using a large-bore Pasteur pipette, retinas were pipetted up and down several times to disperse them into large clumps of tissue which collected at the bottom of the tube. The supernatant was replaced by 2 ml fresh, supplemented normal Ringer solution and the tissue was dissociated by gentle trituration until a suspension of single cells was obtained. The cell suspension was transferred to a test tube containing 10 ml normal Ringer solution and this was stored on ice.

Isolated cell recording. About 0·5 ml cell suspension was transferred to a thin nearly jelled agarose bed (4 mg/ml) which lined the floor of a Plexiglas recording chamber. Many of the isolated cells settled on the agarose surface where they were immobilized but not embedded to a depth where the hardened agarose would interfere with the manoeuvring of the recording electrodes. In early experiments, the recording chamber was connected to a gravity-fed perfusion system. In this case, the perfusate, normal turtle Ringer solution (Table 1), was chilled to 15 °C and bubbled with 5 % CO_2/95 % O_2 (pH 7·7). For experiments on the ionic basis of the membrane potential, Müller cells

were not perfused. They were equilibrated in test solutions at room temperature and then placed in the recording chamber. No differences were noticed between the results obtained in these different recording conditions except that cells survived longer (about 3 h *vs.* 1 h) when perfused. The recording chamber was mounted on the stage of a Zeiss compound microscope, where single cells could be visually identified. Müller cells were recognized by their large size, cylindrical profile, and their root-like apical processes. The central regions of Müller cells were impaled with one or two intracellular micro-electrodes by advancing them until the cell membrane appeared to dimple. Making the electrometer amplifier oscillate caused a small mechanical vibration of the tip of the electrode which facilitated abrupt penetration of the cell membrane.

TABLE 1. Composition of solutions (mM)

	Na^+	K^+	Free Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	$H_2PO_4^-$	SO_4^{2-}	Sucrose	Relative osmolality
Standard Ringer	127·5	3·3	2·4	2	93·3	42	1·5	1	0	1
Ca^{2+}, Mg^{2+}-free	127·5	3·3		0	93·3	42	1·5	1	0	1
A	126·2	0	3·7	2	0	40·7	1·5	52	46	0·98
B	42·2	84	3·7	2	0	40·7	1·5	52	46	0·98
C	42·2	114·6	3·2	2	0	40·7	1·5	67·3	0	0·92
D	0	156·8	3·2	2	0	40·7	1·5	67·3	0	0·92
E	42·2	0·3	5·4	2	0	40·7	1·5	10·2	171·5	1·10

All solutions contained 10 mM-glucose and 2·8 HEPES with pH adjusted to 7·7 in all cases except Ca^{2+}, Mg^{2+}-free where it was 7·0. The total Ca^{2+} in solutions A–E was 8 mM; free Ca^{2+}, which was measured with an ion-sensitive electrode (see Methods), varied because of binding to SO_4^{2-}. There was an insignificant difference between total and free Ca^{2+} in standard Ringer solution.

Intact retina experiments. The posterior half of the eye was drained of vitreous humour, placed in a recording chamber at room temperature, and maintained under a stream of moist 5 % CO_2/95 % O_2. Electrodes were advanced into the retina from the vitreal surface at an angle of about 45 deg. Close to the inner surface of the retina and in the vicinity of the inner nuclear layer the electrodes often impaled cells that had what Baylor & Hodgkin (1973) called 'silent potentials'. These cells were identified as Müller cells because they had a large resting potential (up to −90 mV), gave exceptionally quiet recordings, and responded to a brief flash of light with a small slow depolarizing potential change. Light stimuli were delivered to the eyecup by an optical bench which projected the reduced image of a variable field aperture onto the retina. The aperture was mounted on micrometer-driven cross-carriages and its position monitored by vertical and horizontal dial indicators. For experiments involving two electrodes the distance between their positions on the surface of the retina was estimated from the dial indicator coordinates of a small spot centred on each electrode.

Electrical recording. Intracellular micro-electrodes, pulled on a Livingston-type horizontal puller from Omega Dot capillary tubing were filled with 4 M-potassium acetate and had resistances in the range of 100–300 MΩ. They were connected to a high impedance, negative capacitance micro-electrode electrometer amplifier that allowed current to be injected through the recording electrode. Injected current was measured from the voltage drop across a 50 MΩ feed-back resistance in a virtual ground circuit. Capacitative interactions between pairs of electrodes were reduced by shielding one of the electrodes with a grounded stainless-steel sleeve. Intracellular signals were displayed on an oscilloscope and stored on magnetic tape using a FM tape recorder.

Bathing solutions. The ionic composition of the solutions is given in Table 1. Cl^--based solutions containing 0·3–30 mM-K^+ were made from standard Ringer solution in which equimolar Na^+ replaced K^+. Sulphate-based, Cl^--free, solutions with K^+ varying between 0 and 84 mM were made by mixing appropriate amounts of solutions A and B as described by Hodgkin & Horowicz (1959). In different solutions the concentrations of free Ca^{2+}, measured with a Ca^{2+} electrode (Model 93-20, Orion Research, Inc., Cambridge MA, U.S.A.) varied by 3 mM. This along with small differences in osmolality, measured with a vapour pressure osmometer (Model 5100C, Wescor, Inc., Logan, VT, U.S.A.) were assumed to have an insignificant effect on the relationship between $[K^+]_o$ and membrane potential.

RESULTS

Morphology

Enzymatically dissociated Müller cells have all the morphological features of Müller cells from the intact turtle retina (Sarthy & Bunt, 1982). As shown in Pl. 1 they are roughly cylindrical with extensive membrane specializations at each end. The apical region appears as an expanded tuft, consisting of a large number of fine microvilli extending beyond the external limiting membrane in the intact retina. The opposite end of the cell gives rise to a number of descendent processes that swell to form bulbous terminations which *in vivo* would adjoin the internal limiting membrane of the retina. Between the two ends the cell is more cylindrical with a variable number of very fine lateral processes.

Resting potentials

The central portion of isolated Müller cells extending from the apical end to the cell body was easily penetrated by one or two separate micro-electrodes. Resting potentials recorded simultaneously by two electrodes at opposite ends of the cylindrical portion of a single cell were essentially identical. The membrane potential of a sample of thirty-seven Müller cells bathed in standard chloride Ringer solution containing 3 mM-K^+ ranged from -60 to -95 mV, with a mean value of $-77{\cdot}2$ mV $\pm 1{\cdot}5$ (S.E. of the mean). These values agree with glial cell resting potentials reported from a variety of preparations (reviewed by Kuffler & Nicholls, 1966; Somjen, 1975; Stewart & Rosenberg, 1979; Picker & Goldring, 1982). They are also consistent with measurements of Müller cell membrane potentials in intact *Necturus* retina (Miller & Dowling, 1970*a*, *b*; Karwoski & Proenza, 1977, 1980). Isolated Müller cells stored at 0 °C continued to have large resting potentials for periods of time ranging from 4 to 6 h following dissociation. In many cells the potential measured immediately after penetration was small (-40 to -50 mV) but became larger and stabilized within the first 5 min of recording. Once the potential reached a steady level the intracellular recording was quiet (± 1 mV) and showed no other kinds of spontaneous changes in voltage.

Ionic basis of Müller cell membrane potential

In glial cells from the nervous systems of invertebrates, amphibians and mammals, the membrane potential (V_m) closely follows the Nernst equation for a potassium electrode:

$$V_m = \frac{RT}{F} \log_e \frac{[K^+]_o}{[K^+]_i}, \quad (1)$$

where $[K^+]_o$ and $[K^+]_i$ indicate the K^+ concentration outside and inside the cell respectively, F is Faraday's constant, R is the gas constant and T is the absolute temperature. The influence of $[K^+]_o$ on the membrane potential of isolated Müller cells was determined in the absence of Cl^- by varying the K^+ content of the bathing solution. Since mechanical movements caused by solution changes typically dislodged the recording electrode we did not monitor membrane potential while modifying the ionic composition of the bath. We measured instead stable membrane potentials from a number of representative cells bathed in different concentrations of external K^+.

Freshly dissociated Müller cells were equilibrated for 20 min or longer in the test solution before measuring their membrane potential. Fig. 1 (○) illustrates the dependence of steady-state membrane potential on $[K^+]_o$ in the absence of Cl^-. The dashed line represents the Nernst equation for a K^+ electrode and has a slope of 58 mV per decade. The Müller cell membrane potential diverged from the behaviour of a K^+ electrode as $[K^+]_o$ was reduced from 60 mM; the slope between 3 and 0·3 mM

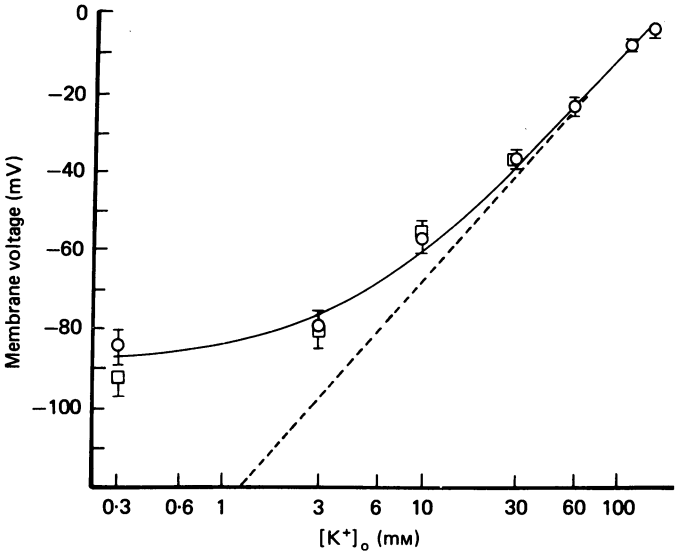

Fig. 1. Relation between Müller cell membrane potential and log $[K^+]_o$. ○, the mean (n = 16–30) steady-state potential of *isolated* Müller cells bathed in Cl^--free Ringer solution containing different concentrations of $[K^+]$. Test solutions with $[K^+]$ varying between 0·3 and 60 mM were made by mixing appropriate amounts of solutions A and B in Table 1. Solutions C and D were used to obtain measurements in 115 and 157 mM-K^+ respectively. □, mean (n = 15–39) membrane potential of *in situ* Müller cells bathed in Cl^--based Ringer solution containing different concentrations of K^+. These solutions were made from standard Ringer solution (Table 1) in which equimolar NaCl replaced KCl. Error bars represent ±S.E. of the mean.

was 6 mV per decade. The divergence can be attributed to a small Na^+ permeability (Hodgkin & Horowicz, 1959). The experimental points in Fig. 1 are reasonably well fitted by a curve drawn according to the Goldman–Hodgkin–Katz equation (Goldman, 1943; Hodgkin & Katz, 1949),

$$V_m = \frac{RT}{F} \log_e \frac{[K^+]_o + \alpha[Na^+]_o}{[K^+]_i + \alpha[Na^+]_i}, \quad (2)$$

where α is the Na^+/K^+ permeability ratio and has the value of 0·037. Since $[K^+]_i$ is much greater than $[Na^+]_i$, $\alpha[Na^+]_i$ can be neglected. The intracellular concentration of K^+ was estimated to be 155 mM from the extrapolated value of $[K^+]_o$ at zero membrane potential. Eqn. (2) would also predict that in low Na^+ solutions the resting potential would more closely correspond to the value predicted for a K^+ electrode. This was tested by recording the membrane potential of cells equilibrated in solutions

containing 0·3 mM-K^+ and 42 mM-Na^+ (Table 1, solution E). The average membrane potential of nineteen cells in this solution was $-104{\cdot}7$ mV $\pm 2{\cdot}58$ (S.E. of the mean); the predicted value is -111 mV.

Although the dependence of Müller cell membrane potential on ions other than K^+ and Na^+ was not investigated extensively, it was observed that the resting potentials of cells equilibrated in the presence and absence of chloride at constant $[K^+]_o$ were not significantly different. The effects of Ca^{2+}, Mg^{2+}, and HCO_3^- were not examined.

A recently published paper indicates that the K^+ channel density is greater in the end-feet than in other parts of the Müller cell (Newman, 1984). Regional differences in K^+ sensitivity are not dealt with in this paper but are currently under investigation and will be described in a future publication.

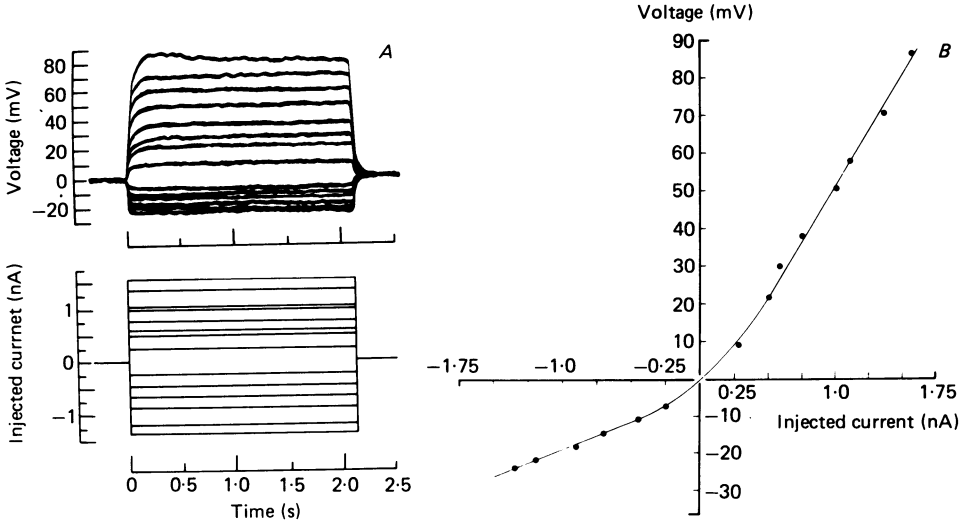

Fig. 2. Relationship between injected current and membrane voltage in an isolated Müller cell. A single dissociated cell was impaled with two intracellular electrodes. *A*, upper traces show the change in membrane voltage produced by currents (lower trace) injected through the other electrode. *B*, plot of the steady-state change in membrane voltage against injected current. Zero on voltage scales in *A* and *B* corresponds to the resting membrane potential (-80 mV). The curve was fitted to the data points by eye.

Passive properties

The passive electrical properties of isolated Müller cells were studied with separate micro-electrodes for recording voltage and injecting currents. Depolarizing currents produced larger potential changes than hyperpolarizing currents resulting in a non-linear current–voltage relation showing inward or anomalous rectification (Fig. 2). The mean input resistance, measured in eleven cells, from the slope of the current–voltage curve was 17 MΩ for potential shifts in the hyperpolarizing direction and 62 MΩ for depolarizations. Over a voltage range extending approximately ± 10 mV from the resting potential the current–voltage relation was linear, having a mean slope resistance of 29 MΩ.

Charging curves in the depolarizing and hyperpolarizing directions were different. Hyperpolarizing current steps evoked voltage shifts that followed the time course of

a single exponential process with $\tau = 11$ ms (mean of five cells). In contrast, a sum of two exponentials having nearly equal coefficients and τ values of 10 and 40 ms (mean of five cells) was needed to describe the kinetics of positive potential changes. The charging time of the passive membrane RC may be reflected in the short ($\sim$ 10 ms) time constant associated with voltage shifts in either the positive or negative directions. The slower (40 ms) exponential process evoked by only positive currents could indicate the presence of a voltage-dependent conductance. A suggestion of another kind of voltage sensitivity was apparent with passage of strong depolarizing currents. Potential shifts of +70 to +80 mV often showed an initial peak that declined by 5–10 mV over several hundred milliseconds to a maintained plateau.

Cable properties

An estimate of the cable parameters of an isolated Müller cell can be approximated by treating it as a short cylindrical cable with an infinite resistance at each end (Weidman, 1952; Jack, Noble & Tsien, 1975). The cylinder considered was given a length equal to the approximate thickness of the retina (150 μm) and a diameter representative of the Müller cells we recorded (15 μm). A specific membrane resistance (R_m) of $2{\cdot}2 \times 10^3$ Ω cm^2 was calculated from the slope resistance in the vicinity of the resting potential (mean 29 MΩ) and the surface area of the cylinder assumed above. The cell space constant (λ) was obtained by applying the standard cable equation (Hodgkin & Rushton, 1946):

$$\lambda = \left(\frac{R_m a}{2 R_i}\right)^{\frac{1}{2}}, \tag{3}$$

where R_m is as above, a is the cell radius, and R_i is the resistivity of the cytoplasm. Assuming an infinite cable with negligible external resistance and $R_i = 120$ Ω cm gave $\lambda = 829$ μm. The decrement (D_x) of a steady electrotonic potential can be calculated for a short cable of length L by the relationship (Weidmann, 1952; Jack *et al.* 1975):

$$D_x = 1 - \frac{\cosh\left[(L-x)/\lambda\right]}{\cosh\left(L/\lambda\right)}, \tag{4}$$

where x is the distance from the origin of the potential at one end of the sealed cable. To calculate the decay from one end of the cable to the other, i.e. when $x = L$, eqn. (4) reduces to:

$$D_x = 1 - \frac{1}{\cosh\left(L/\lambda\right)}. \tag{5}$$

Thus, the expected attenuation of a potential spreading along the 150 μm length of the Müller cell would be about 2%. The main source of error in this calculation is in the assumed membrane surface area. Since the Müller cell membrane is infolded and the cell is branched at one end (Sarthy & Bunt, 1982), the value used for surface area is almost certainly an underestimate of the true surface area. A larger surface area would make R_m greater, giving a longer space constant, resulting in even less decrement. Our treatment of the cable properties involves several assumptions but it suggests, nevertheless, that potentials spread with negligible decrement over the entire length of the Müller cell.

Müller cells in the intact retina

In order to examine the influence of the dissociation procedure on the properties of isolated Müller cells, experiments were conducted on Müller cells *in situ*. Silent cells with large resting potentials were most commonly impaled in the proximal retina but were also recorded in the distal portion of the inner nuclear layer. These were judged to be Müller cells because they showed no signs of synaptic activity nor action potential production, and responded to a step of moderately intense white light with a slow depolarization at the 'on' and 'off' of illumination (Miller & Dowling, 1970*a*; Karwoski & Proenza, 1977). There was considerable variability in the amplitude and time course of the light responses of different Müller cells but they were typically 2–3 mV depolarizations that reached peak in about 2 s (Fig. 3). Six attempts were made to mark cells identified physiologically as Müller cells by intracellular injection of Lucifer Yellow (Stewart, 1978). Stained Müller cells were observed in three of these experiments and in one case a second Müller cell was also faintly marked. In the remaining three experiments we failed to observe any stained cells.

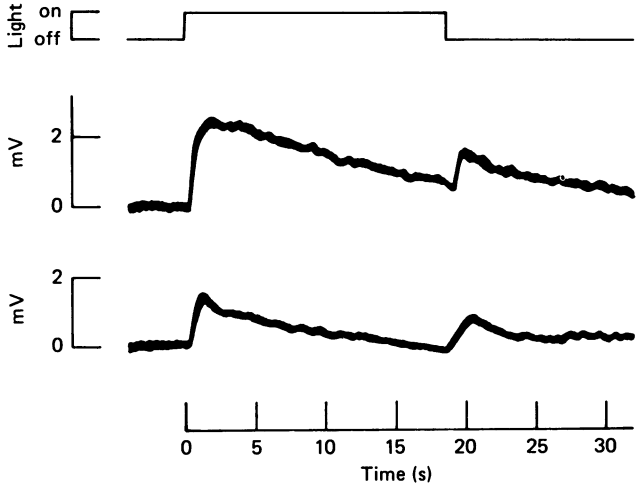

Fig. 3. The light responses of Müller cells in the intact retina. Simultaneous intracellular recordings from two different Müller cells. A step of bright white light evoked a small slow depolarization at the 'on' and 'off' of illumination. Light intensity was $4{\cdot}5 \times 10^{-5}$ J s^{-1} cm^{-2} and covered a circle 1·2 mm in diameter.

K^+ sensitivity

The mean resting membrane potential of forty Müller cells recorded from the eyecup preparation in Ringer solution containing 3 mM-K^+ was -80 mV $\pm 0{\cdot}8$ mV. The effect of extracellular K^+ on membrane potential was investigated by soaking eyecups in oxygenated Ringer solution containing different K^+ concentrations. After incubation in a test solution for 30–60 min, the eyecup was placed in an oxygenated recording chamber and membrane potentials were recorded from as many different cells as possible over the next 30–45 min. After measuring the stable resting potential of a Müller cell, the electrode was withdrawn from the retina and moved to an unexplored region of the eyecup. This ensured that a particular cell was sampled only

once and it reduced the likelihood of recording from a non-representative population of Müller cells due to possible regional variations within the retina. We felt that the incubation step did not adversely affect the retinal cells because it was possible to record normal light responses from ganglion cells, horizontal cells and cones in eyecups that had been immersed in oxygenated standard turtle Ringer solution for as long as 4 h. In fact, incubated eyecups seemed to survive in the recording chamber longer than eyecups prepared in the usual way. It is possible that incubation removes some vitreous humour and allows better oxygenation of the retina.

The light response was one of the main physiological criteria used to identify Müller cells. This set two limitations on the ionic composition of the test solutions. First, in Cl^--free solutions we found that the Müller cell light response was abolished or greatly reduced (see also Miller & Dowling, 1970*b*). For this reason the solutions used for these experiments contained Cl^- rather than SO_4^{2-} as the predominant anion. Secondly, the Müller cell light response varied inversely with K^+ concentration and could not be resolved at concentrations greater than 30 mM. Hence, the K^+ in the solutions used for these experiments were in the range 0·3–30 mM rather than 0·3–150 mM as in the experiments on isolated cells.

The relationship between Müller cell potential and K^+ concentration is the same as was seen in dissociated cells (□, Fig. 1). The points are the average values of fifteen to thirty-nine different Müller cells at each K^+ concentration. This suggests that the membrane of dissociated and *in situ* Müller cells is permeable to K^+ and to a much lesser extent, Na^+. It also indicates that leakage to Na^+ is not the result of enzymatic dissociation.

Input resistance

The first attempts to measure the input resistance of Müller cells in the retina were made using a bridge circuit with a single electrode to pass current and record voltage. This proved to be unsatisfactory because the potential changes across the cell membrane and the electrode resistances could not be clearly distinguished. Consequently, separate micro-electrodes were used to record voltage and inject current. Each attempt to record from the same Müller cell with two micro-electrodes started with the electrodes positioned closely enough on the proximal retina to be pulled together by the surface forces of adhering vitreous humour or Ringer fluid. Electrodes treated this way have a tendency to stay together and in some cases can be advanced for a short distance, in small parallel steps, without separating; this is a trick used, with occasional success, to impale single ganglion cells with two electrodes (P. B. Detwiler, unpublished observation). Electrodes positioned in this manner commonly recorded from what appeared, on electrical grounds, to be a single Müller cell, i.e. currents injected through one electrode were recorded as a voltage change by the other electrode. This result was obtained even when the electrodes appeared to diverge or were purposely separated to begin with. Fig. 4 shows an experiment with two electrodes placed 35 μm apart on the retinal surface. The impaled Müller cells behaved as if they were joined by a simple electrical resistance. Positive or negative currents injected through either electrode produced potential changes in the other cell having the same polarity as the applied current. These interactions disappeared when an electrode was withdrawn from either cell. Similar results were

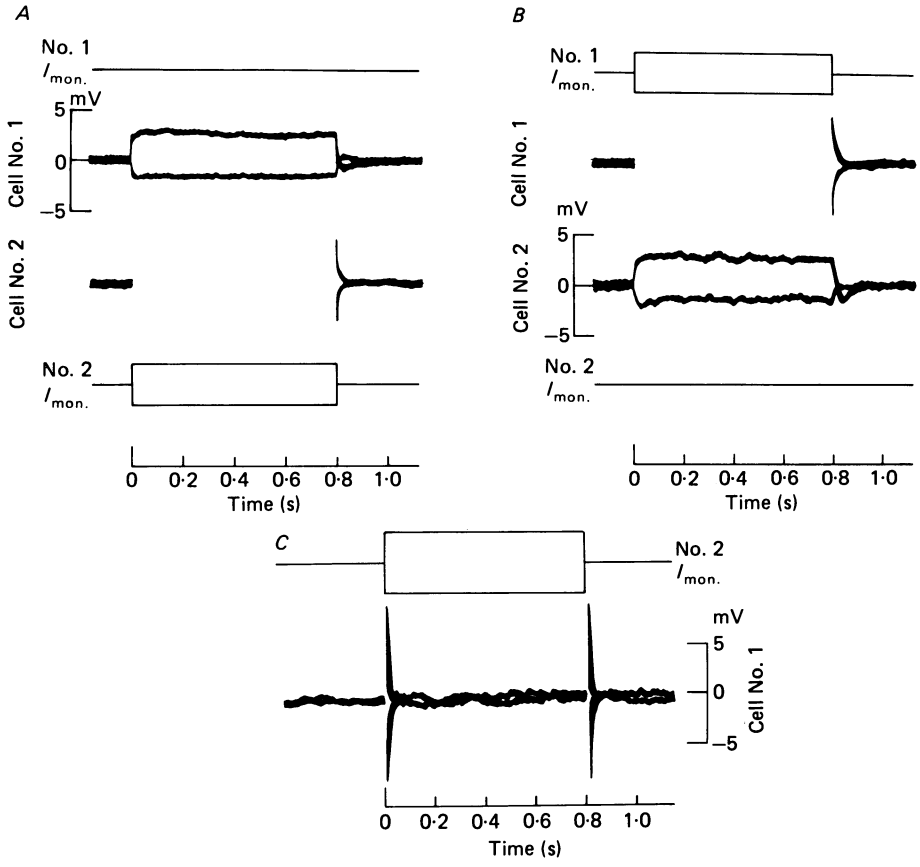

Fig. 4. Electrical coupling between *in situ* Müller cells. Simultaneous intracellular recordings from two different Müller cells. The estimated separation between cells was 35 μm. *A*, shows the voltage change in Müller cell No. 1 produced by injection of +6 and −6 nA currents into Müller cell No. 2. During passage of current the voltage trace of cell No. 2 went off scale. In *B*, current steps of +6 and −6 nA were injected into Müller cell No. 2 and the voltage change produced in cell No. 1 is illustrated, In *C*, the electrode recording from cell No. 2 was withdrawn from the cell and current steps of +10 and −10 nA were passed through electrode No. 2 into the extracellular space. The voltage trace of cell No. 1 shows capacitative artifacts at the 'on' and 'off' of the current steps but no maintained voltage drop. The light responses of Müller cells Nos. 1 and 2 are shown in Fig. 3 on the upper and lower traces, respectively. Note that the light responses are significantly different, which supports the contention that the electrodes are in separate Müller cells. The resting membrane potentials of cells No. 1 and 2 were −82 mV and −76 mV, respectively.

observed in thirteen Müller cell pairs. The strength of coupling declined with distance and was undetectable between cells separated by more than 65 μm. Not all cell pairs separated by less than this distance were electrically coupled, suggesting that either some Müller cells are electrically isolated or there is more than one network.

Both electrodes were assumed to be in the same cell when, they started as close together as possible on the retinal surface, and recorded similar resting potentials and light responses from Müller cells near the internal limiting membrane. Current–voltage curves of cells in this category showed inward rectification (Fig. 5). The slope

resistance in the vicinity of the resting potential varied in the four best cells from 2 to 6·5 MΩ (mean 3·2 MΩ). Similar values (~ 4 MΩ) have been reported for the end-foot region of Müller cells in the turtle retinal slices (Newman, 1983).

The difference in the input resistance of *in situ* and dissociated Müller cells can be attributed to electrical coupling. A coupled cell will appear to have a lower input resistance because a significant fraction of injected current will flow to other cells in

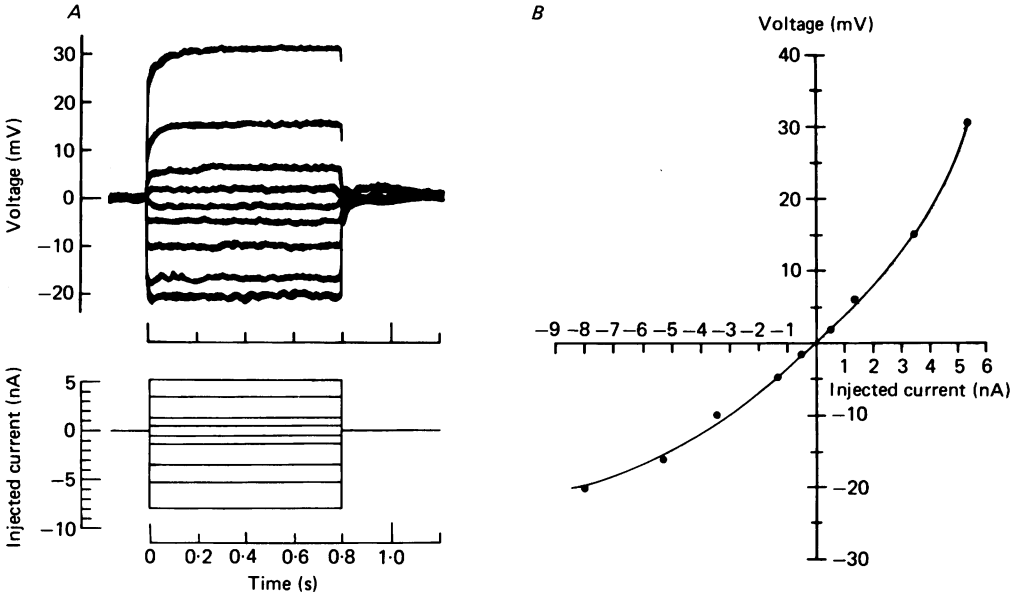

Fig. 5. Relationship between injected current and membrane voltage in an *in situ* Müller cell. A single Müller cell was impaled with two intracellular electrodes: one for passing current and the other for recording voltage. The electrodes were judged to be in the same, rather than different, Müller cells because both electrodes recorded similar resting potentials and light responses. *A*, upper traces show the change in membrane voltage produced by currents (lower trace) injected through the second intracellular electrode. *B*, plot of the steady-state change in membrane voltage against injected current. Zero on voltage scales in *A* and *B* corresponds to the resting membrane potential (−87 mV). The curve was fitted to the data points by eye.

the network rather than across the membrane of the impaled cell. The space constant for the spread of a potential in an electrical syncytium can be estimated from the ratio of the input resistances of isolated and coupled cells (Lamb & Simon, 1976). In our experiments this ratio was nearly 10 which corresponds (see Fig. 1, Detwiler & Hodgkin, 1979) to a length constant of approximately twice the mean cell separation. Since Müller cells are closely packed in the retina (Rasmussen, 1972) it is reasonable to assume a cell spacing equal to the cell diameter. This would make the network length constant equal to about 30 μm. Coupled cell input resistances of 2 and 6·5 MΩ would give network length constants of approximately 38 and 19 μm respectively.

DISCUSSION

In general Müller cells resemble other types of glia. They have a high resting potential, their surface membrane behaves electrically as a passive element predominantly permeable to K^+, and they are electrically coupled *in situ*.

The relationship between membrane potential and K^+ concentration does not follow the Nernst equation for a membrane exclusively permeable to K^+. The deviation from the behaviour of an ideal K^+ electrode can be accounted for by a permeability to Na^+ and K^+ with a ratio of 0·037. A similar relationship between K^+ concentration and membrane potential has been observed in several types of glial cells when external K^+ is equal to or reduced below its normal physiological concentration (Kuffler & Nicholls, 1964; Kuffler, Nicholls & Orkand, 1966; Dennis & Gerschenfeld, 1969; Pape & Katzman, 1972; Ransom & Goldring, 1973; Picker & Goldring, 1982). In all cases the discrepancy can be attributed to a Na^+ permeability that is approximately 1/25–1/30 the K^+ permeability.

The intracellular K^+ concentration used in fitting eqn. (2) to our data was 155 mM. This is higher than the estimated value of 100–110 mM for freely diffusible K^+ in leech and amphibian glia (Kuffler & Nicholls, 1964) and lower than the value of 200 mM used by Ransom & Goldring (1973) for mammalian glia. Our estimate of intracellular K^+ corresponds to an equilibrium potential (−97 mV) which is greater than the average resting potential (−77 mV) for dissociated cells. This suggests that Müller cells are either slowly losing K^+ and gaining Na^+ or that a steady state is maintained by a metabolically driven pump. The present experiments are consistent with either view, but the observation that both *in situ* and dissociated Müller cells are able to maintain a stable resting potential for several hours argues for the involvement of an active transport process.

The current–voltage curve of both isolated and *in situ* Müller cells showed inward rectification; membrane conductance was greater for hyperpolarizing than depolarizing current pulses. Rectification was more marked in isolated Müller cells than those *in situ*. This difference probably reflects the fact that, in a coupled network, input resistance is more a measure of the coupling resistance between cells than the membrane resistance of a single cell (Lamb & Simon, 1976; Detwiler, Hodgkin & McNaughton, 1980). The direction of rectification and the dominant K^+ permeability of the Müller cell suggest a permeability mechanism that offers more resistance to outward than inward movement of K^+. This might benefit the Müller cell by helping it to retain K^+ during a local decrease of K^+. Such a change in external K^+ concentration has been measured in the distal retina following illumination (Karwoski & Proenza, 1978; Oakley & Green, 1976).

The rectifying current–voltage curve and high input resistance (30 MΩ) of isolated Müller cells set them apart from most kinds of glial cells which generally have linear current–voltage curves and resistances of the order of a few megaohms (Kuffler *et al.* 1966; Trachtenberg, Kornblith & Hauptli, 1972). Input resistances in the vicinity of 9–13 MΩ have been reported for Müller cells enzymatically dissociated from salamander retina (Newman, 1984). These values are smaller than the resistances we measure in Müller cells isolated from the turtle. The explanation for this is not clear. It may be due to differences in species or in the way the cells were dissociated and/or the experiments performed.

Müller cells in the intact retina had nearly 10-fold smaller input resistances than isolated cells. We attribute this to the presence of electrical coupling between *in situ* Müller cells. Coupling would also account for the spread of injected currents over distances that exceed the Müller cell diameter. Although the end-feet expand to form the internal limiting membrane and may extend laterally over a large distance, the

possibility that the two electrodes recorded from different end-feet of the same cell seems unlikely since coupled pairs were recorded deeper than the internal limiting membrane and in some cases in the inner nuclear layer. Electrical coupling between Müller cells is consistent with the demonstration of coupling between glial cells in a number of mammalian, lower vertebrate and invertebrate nervous systems (reviewed by Somjen, 1975). It also agrees with the descriptions of close membrane appositions or gap junctions between Müller cells in a variety of retinas (Miller & Dowling, 1970*a*; Uga & Smelser, 1973). Cytoplasmic continuity, conferred by the intracellular channels associated with gap junctions, would permit the exchange of ions and small metabolites between cells. This could provide a route for rapidly neutralizing local changes in the concentration of a diffusible substance and might play an important role in maintaining the metabolic and ionic homoeostasis of the retina.

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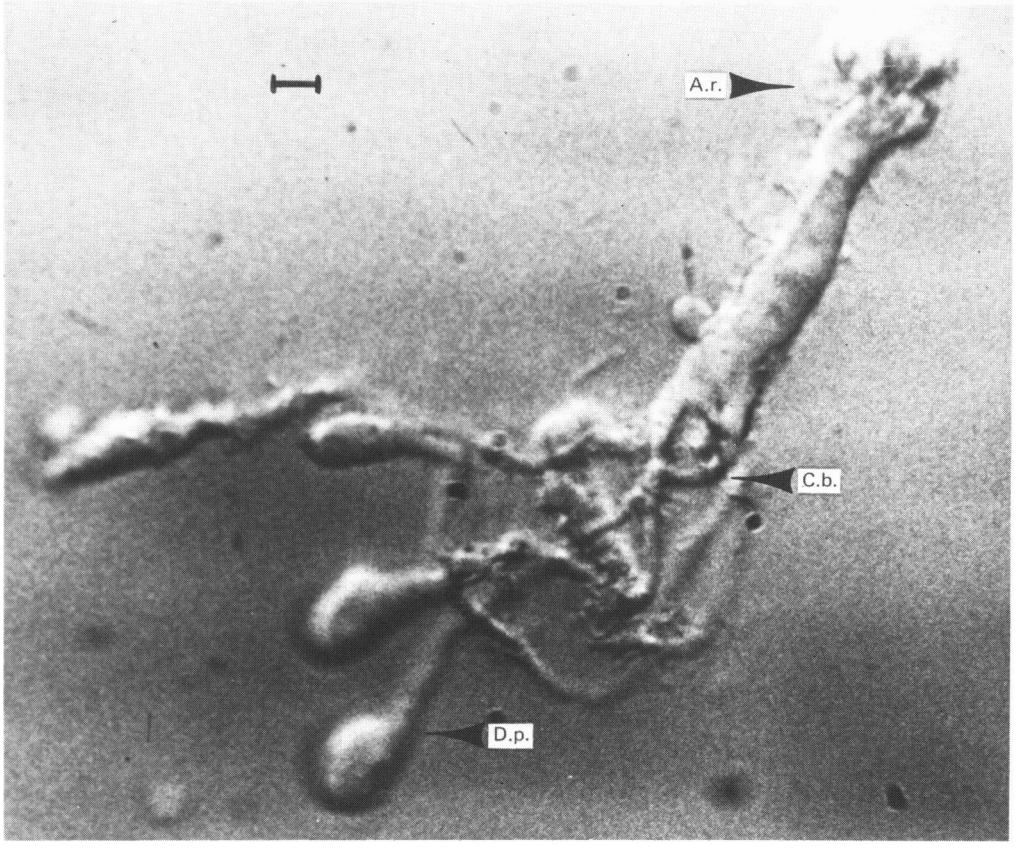
EXPLANATION OF PLATE

Bright field photomicrograph of an unfixed isolated Müller cell obtained by enzymatic dissociation of turtle retina according to the procedures described in the Methods. A.r. = apical region. C.b. = cell body. D.p. = descendant process. Calibration bar represents 10 μm.


A.r.
C.b.
D.p.