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Short-circuiting effects of K^+ currents on electrical responses of type-1-like astrocytes from mouse cerebral cortex

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The membrane potential and membrane input resistance of cortical astrocytes from newborn mice were recorded with and without exposure to 1 mM barium. Barium treatment drastically decreased the membrane response to 0 and 35 mM K^+. It also revealed an electrogenic component of the Na^+,K^+-ATPase as evident by a biphasic depolarization as a response to ouabain, which was monophasic without barium presence. Untreated mouse astrocytes reacted with small monophasic depolarizations to GABA and glutamate exposure. Barium-treated astrocytes exhibited additional transient responses to both transmitters, similar to those responses of rat astrocytes as found in the literature. The transmitter responses were not changed by exposure to uptake blockers for both transmitter substances. Thus, this electrophysiological study confirms earlier studies with radioactive K^+ fluxes in showing that astrocytes derived from mouse brain are capable of short-circuiting electrogenic components and transmitter responses. This extreme high K^+ permeability resembles the one reported for endfeet of retinal Muller cells and dissociated astrocytes from optic nerve.

INTRODUCTION

It has been shown that cultured mouse and rat astrocytes differ from one another in one of the most important features of glial cells: the channel-mediated K^+ flux[25]. The unidirectional K^+ flux in mouse astrocytes is about 20 times higher than in rat astrocytes and it seems culture conditions cannot be selected to overcome this difference in a major way[27]. However, if mouse astrocytes are exposed to barium, their K^+ fluxes decrease by 95% and are in the same range as the fluxes of rat astrocytes. Moreover, it was found that activation of the Na^+,K^+-ATPase in rat astrocytes leads to an electrogenic component of the membrane potential of up to −30 mV[3,15]. In contrast, mouse astrocytes do not show any electrogenic component, even under conditions of Na^+ loading[30]. The different specific membrane resistances of mouse and rat astrocytes in culture could be responsible for this phenomena. Since the K^+ permeability of glial cells is the most crucial property of the spatial buffer theory for K^+ regulation by glial cells[20], such differences cannot be overlooked and have to be addressed. In electrophysiological studies to date rat astrocytes were used almost exclusively, with many authors being unaware that such an apparent species difference exists and therefore ignoring the importance for the interpretation of the results. In this study, we use type-1-like cortical astrocytes from mice and investigate some basic electrical responses with and without blocking barium-sensitive K^+ channels. We then compare the results with known properties of rat astrocytes in culture.

MATERIALS AND METHODS

Cell culture preparation

Astrocytes from newborn mice were cultured as described by ref. 4 in MEM growth medium. For the first week the horse serum concentration was 20%. Thereafter a 10% serum concentration was used. The cells were kept on glass coverslips in 60-mm-diameter culture dishes with 3 ml MEM medium. Twice a week the medium was changed. After 14 days the cultures were confluent monolayers. The cultures used in these experiments were 2–4 weeks old. Before an experiment the coverslip was broken into smaller pieces and incubated for 2–8 h in physiological salt solution containing 1 mM dbcAMP (37 °C). This treatment is necessary in order to round the cells up for impalement[30].

Cell identification

In tissue derived from mouse brain, A2B5 cannot be used for identification of type-1-like and type-2-like astrocytes[6]. In rat tissue, cultures yielding almost exclusively type-1-like astrocytes are gained from newborn rats, whereas type-2-like astrocytes are gained from 3-day-old rats. Rat type-1-like astrocytes ($A2B5^-$) are flat cells and react to dbcAMP with contraction so that they appear bipolar. In contrast rat $A2B5^+$ astrocytes (type-2-like) have at least 5 processes and do not react to dbcAMP treatment (≤8 h) with morphological changes. Cells were impaled that were GFAP-positive and after dbcAMP treatment had a bipolar nature and no pro-

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cesses. In addition these glial fibrillary acidic protein (GFAP)-positive bipolar mouse cells did after 1 h exposure to 10^{-4} M GABA not stain positive for GABA, whereas cells from mouse cultures which are GFAP-positive and have at least 5 processes do stain positive for GABA after 10^{-4} M exposure (Magoski, Walz and Juurlink, in preparation). This is in keeping with the observation by Levi et al.[13] that type-2-like rat astrocytes take up GABA and store it, whereas type-1-like rat astrocytes take up GABA and metabolize it, keeping the internal GABA concentration below the detection limit. Thus, we are confident that the bipolar, dbcAMP-sensitive, GFAP-positive cells are type-1-like cortical astrocytes, according to the definition by Raff[21].

Experimental set-up

The coverslips containing the astrocytes were placed in an experimental chamber which was fixed on an inverted phase contrast microscope. The volume of the bath solution in the chamber was low (300 μl) and the perfusion rate was high (3 ml/min). The bathing fluid was connected via a strip of filter paper with the bath reference electrode (Ag-AgCl pellet). The recording temperature was 25 °C.

Microelectrodes and recording conditions

Single capillaries with 1.2 diameters out of borosilicate glass and containing an inner filament were used (Clark Electromedical Instruments, Pangbourne, U.K.). They were pulled on a PE-2 Narishige puller and had a tip diameter of approx. 0.3 μm. The electrodes were filled with 0.5 M KCl. The resistance was in the range of 90–150 MΩ. The impalement was accomplished with a micropositioner using a Neurodata IR 283 preamplifier penetration system. The preamplifier was used to inject current pulses (20 ms duration). The voltage signal and the current trace was displayed on an oscilloscope and on a pen recorder. There was no electrical interference when current was injected. Tests were made for any influence of the various solutions on the potential of the bath reference electrode. No potential changes could be recorded with broken microelectrodes if the normal salt solution in the experimental chamber was replaced by any of the solutions used in the present experiments. One cell was used only for one experiment to rule out irreversible effects and desensitization reactions to neurotransmitter application. After an experiment the piece of coverslip in the experimental chamber was discarded and replaced with a new one.

Salt solutions

Physiological salt solution which was aerated with 5% CO_2 and 95% O_2 to give a pH of 7.35 had the following composition (in mM): NaCl 123, KCl 3.5, $CaCl_2$ 1.2, $MgSO_4$ 0.8, $NaHCO_3$ 26, NaH_2PO_4 0.8, glucose 10. In some experiments the K^+ concentration was increased to 35 mM by omitting Na^+ in a 1:1 molar fashion, K^+-free salt solution had KCl omitted. Solutions containing barium had $MgSO_4$ and NaH_2PO_4 removed in order to prevent precipitation as barium salts.

Drugs

All drugs used were obtained from Sigma Chemical Co. (St. Louis, MO) except ouabain which was from Boehringer Chemicals (Mannheim, F.R.G.). They were dissolved in the salt solution immediately before an experiment. Barium was used as $BaCl_2$.

Statistical analysis

Student's t-test was used with $P < 0.05$ as the criterion for significance. Mean and S.E.M. are reported throughout the text.

RESULTS

Basic electrical properties

The membrane potential of the cells used in this study

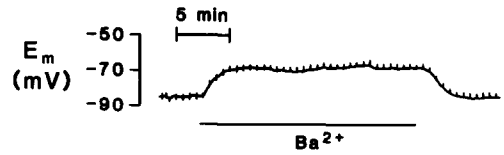

Fig. 1. Pen recording of the membrane potential of an astrocyte which was exposed for 20 min to 1 mM barium.

was −75.0 ± 1.3 mV with a range from −62 to −90 mV (n = 68). The input resistance was measured with 59 of these cells and found to be 17.2 ± 2.2 MΩ with a range of 4–50 MΩ.

Barium effects

Barium 1 mM, depolarized the membrane to a plateau value of 22.5 ± 5.2 mV (n = 6) more positive than the normal membrane potential. Fig. 1 is an example of such a barium effect. The plateau remained stable for at least 30 min. The membrane input resistance did not show any significant changes when barium was applied.

Effects of barium treatment on Nernst potential and electrogenic responses

If external K^+ was removed for 15 min, the membrane hyperpolarized transiently by −55.9 ± 1.7 mV (n = 6) as shown in the example of Fig. 2A. The membrane then depolarized within 15 min to a value of 56 mV more positive than the resting potential. After reperfusion

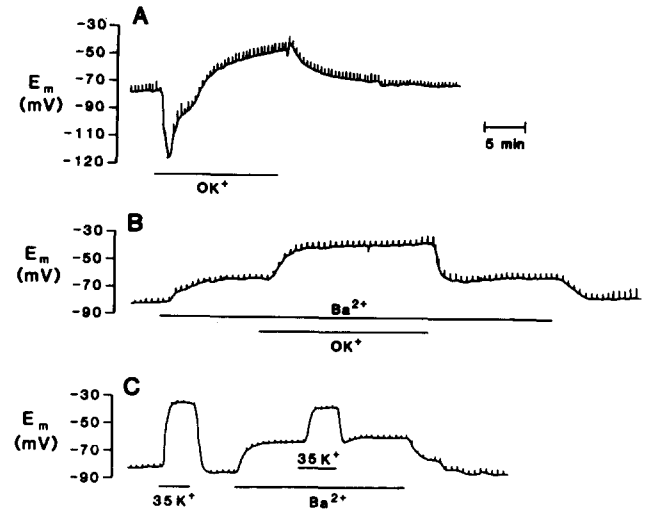

Fig. 2. Pen recordings of the membrane potential from 3 different astrocytes. A: exposure for 15 min to zero K^+-containing salt solution. B: exposure to 1 mM Ba^{2+} during the barium exposure period the K^+ of the salt solution was removed for about 20 min. C: the cell was exposed for 4 min to 35 mM K^+-containing salt solution. After repolarization the cell was exposed to 1 mM barium. During the barium exposure period the K^+ was again increased for 5 min to 35 mM.

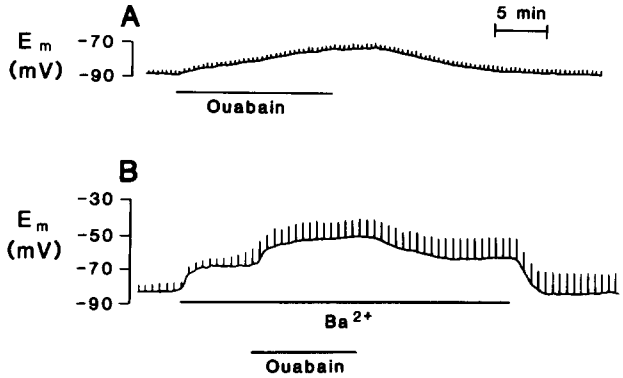

Fig. 3. Pen recordings of the membrane potential from two different astrocytes. A: the cell was exposed for about 15 min to 1 mM ouabain. B: the cell was exposed to 1 mM barium. During the barium exposure period, the experimental chamber was perfused for ca. 10 min with ouabain-containing salt solution.

with normal K^+ (3.5 mM) the membrane transiently depolarized by 6.4 ± 1.4 mV to repolarize to a final stable potential 7.0 ± 1.0 mV more positive than the original resting potential. The input resistance increased transiently by 132 ± 60% to reach control levels after the membrane repolarized to its final stable potential.

If the astrocytes were first exposed to 1 mM barium to reach a new steady level and the K^+ was removed during the presence of barium, the cells did not hyperpolarize but depolarized by 30.7 ± 2.3 mV (n = 3) within 15 min. Upon reperfusion with normal K^+ concentrations the cells repolarized to the original membrane potential. The input resistance did not change measurably during the K^+ removal. Fig. 2B illustrates a typical response of such a cell.

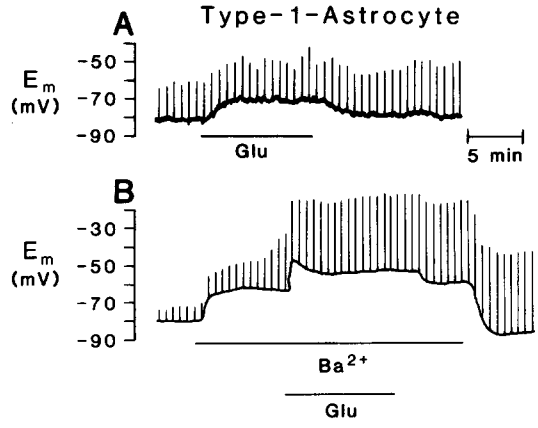

Fig. 4. Pen recordings of the membrane potential from two different astrocytes. A: the cell was exposed for 8 min to 1 mM glutamate. B: the cell was exposed to 1 mM barium. During the barium exposure period 1 mM glutamate was added for 8 min. This cell showed an exceptionally high increase in input resistance. The mean value for all 5 cells tested showed no significant difference of the input resistance during glutamate application.

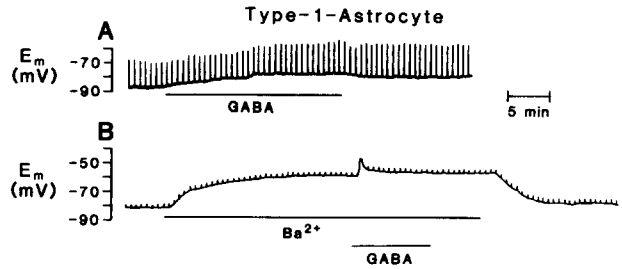

Fig. 5. Pen recordings of the membrane potential from two different astrocytes. A: the cell was exposed for 20 min to 1 mM GABA-containing salt solution. B: the cell was exposed to 1 mM barium. During the barium-exposure period 1 mM GABA was added for 8 min.

Fig. 2C represents another series of experiments. The cells were first exposed for about 4 min to a 10 times elevated K^+ concentration (from 3.5 to 35 mM). The cells depolarized by 49.5 ± 1.3 mV (n = 3), a response that was completely reversible. Thereafter 1 mM barium was introduced and after reaching a plateau value the K^+ was again increased by 10 times in the presence of barium. Under these conditions the reversible membrane depolarization evoked by the K^+ increase was 25.0 ± 0.6 mV (n = 3).

Application of ouabain (1 mM) alone results in a depolarization, which for the first 10–15 min has a constant rate of 1.2 ± 0.2 mV/min (n = 9) (see Fig. 3A). If ouabian is added in the presence of barium, as in Fig. 3B, ouabain application causes the following biphasic effects: at first there is a very rapid depolarization (10.2 ± 1.7 mV/min; n = 3) and then a second depolarization with a slower rate 1.2 ± 0.2 mV/min (n = 3). The amplitude of the first, rapid depolarization was 7.7 ± 0.9 mV (n = 3). All effects were reversible.

Effect of glutamate

If glutamate (1 mM) was applied a reversible depolarization of 9.2 ± 0.8 mV (n = 5) occurred (see Fig. 4A). The input resistance increased by 86 ± 46% (n = 5). In the presence of barium, glutamate caused a more complicated response (Fig. 4B). There was at first a transient depolarization with a maximal value of 15.4 ± 6.2 mV (n = 5). The membrane potential then reached a plateau value 9.0 ± 3.5 mV (n = 5) more positive than the potential with barium alone. This depolarization was reversed when glutamate was removed. During glutamate exposure the input resistance did not change significantly.

Effect of GABA

GABA (1 mM) application resulted in a slow, slightly biphasic depolarization that did not recover with wash-

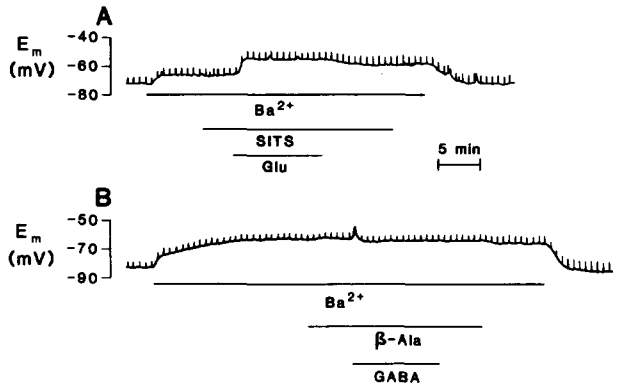

Fig. 6. Pen recordings of the membrane potential from two different astrocytes. A: the cell was first exposed to 1 mM barium, then 1 mM SITS was added. After a short period 1 mM glutamate was given in addition to Ba^{2+} and SITS. After ca. 10 min the drugs were washed out one by one in reverse order. B: the cell was first exposed to 1 mM barium, then 1 mM β-alanine was added. After a short period 1 mM GABA was given in addition to Ba^{2+} and β-alanine. After 10 min the drugs were washed out one by one in reverse order.

out (see Fig. 5A). The amplitude was 6.2 ± 1.2 mV (n = 3) for the first part of the response and the smaller, second part had an amplitude of 3.5 ± 1.3 mV. The first response was gradual (0.9 ± 0.2 mV/min) as compared to the second response (1.8 ± 0.4 mV/min). During GABA exposure the input resistance went up by 134 ± 46%. If GABA was applied in the presence of barium, this resulted in a transient, spike-like response (Fig. 5B). The spike had an amplitude of 10.3 ± 1.8 mV (n = 6). The response then reached a final potential 3.2 ± 1.1 mV (n = 6) more positive than the potential in the presence of barium alone. The membrane potential did not repolarize from this new baseline when GABA was washed out; however, when the barium was removed as well, the potential recovered to almost resting levels. The input resistance did not change significantly.

Effects of amino acid uptake inhibitors on the GABA and glutamate response

SITS (1 mM) was used in the presence of barium to investigate its effect on the glutamate response (Fig. 6A). In the presence of barium, SITS application alone had no effect. If glutamate was applied in the presence of barium and SITS, its response was not different from the one in the presence of barium alone (cf. Fig. 4B).

β-Alanine was investigated for its effects on the GABA response. In the presence of barium, β-alanine caused a small 1–1.5 mV depolarization. If GABA was now added in the presence of barium and β-alanine, the GABA response was not significantly changed (see Figs. 5B and 6B).

DISCUSSION

Barium effects on electrical properties

The cells used were GFAP-positive and had all the features which are usually attributes of $A2B5^-$ cells, although in cultures derived from mouse brain A2B5 cannot be used as criteria to distinguish between type-1- and type-2-like cortical astrocytes (see Materials and Methods). The membrane potential of –75 mV at 3.5 mM K^+ is in the range of previous literature reports for cultured astrocytes[24]. The input resistance is higher than previously reported for these cells and does not appear to be sensitive to barium[29], however this might be due to differences in the cell density, since the input resistance and its changes are mainly a measure for the extent and changes of electrical coupling between cells and not of the specific membrane resistance. We reported previously[23] that barium treatment (50 μM to 10 mM) doubled the input resistance of mouse astrocytes whereas the unidirectional transmembrane K^+ fluxes decreased to 5% of the value of untreated cultures. This is a clear sign that the input resistance cannot be used as a measure of changes in the specific membrane resistance. It is, however, useful as an estimate of changes of the extent of coupling in the culture[10,29]. The effects of barium on membrane potential and input resistance were similar to those previously reported for mouse astrocytes[23,28]. Barium application abolished the 56 mV transient hyperpolarization induced by zero K^+ and it reduced the membrane depolarization, obtained by a 10-fold increase of the external K^+ concentration from 50 to 25 mV. Previously, Walz et al.[30] have shown that the 52-mV slope they obtained for a 10-fold increase of the external K^+ concentration for their mouse astrocytes, reflects an exclusive K^+ permeability of the membrane. The deviation of the slope from the 60 mV was explained by a rapid uptake of K^+ and therefore an increase of the internal K^+ concentration. Thus, the 50-mV slope obtained in the present study using the same cells reflects this exclusive K^+ permeability combined with an increase in the internal K^+ concentration. We have previously shown that 1 mM barium does not significantly block the astrocytic Na^+,K^+-ATPase[28] and therefore should not interfere with the K^+ uptake. The fact that barium treatment abolishes the –56 mV hyperpolarization when the external K^+ concentration drops close to zero and halves the depolarization obtained by a 10-fold increase of K^+, is in keeping with an inhibitory action of barium on the permeability of a majority of K^+ channels. This reaction of barium-treated mouse astrocytes resembles rat astrocytes which, exposed to reduced K^+ concentrations hyperpolarized only by about 2–3 mV[11].

Barium treatment results in an additional rapid depo-

larization phase of 8 mV as a response to ouabain. This fast ouabain response is similar to electrogenic contributions of the Na^+,K^+-ATPase of rat astrocytes which are ouabain-sensitive[3,15]. Untreated mouse astrocytes do not show any indication of a ouabain-sensitive electrogenic component. If barium-treated mouse astrocytes are exposed to 1 mM β-alanine there is a small, but statistically insignificant depolarization that could be in keeping with an electrogenic uptake of this amino acid involving cotransport with Na^+ ions. Na^+ inward flux would create such a depolarization and it is in keeping with findings by Larsson et al.[12] that astrocytes take up β-alanine with a much higher velocity than GABA. Thus, taken together, there is clear evidence that barium-treatment blocks a large part of the mouse astrocytic K^+ permeability. This results in less sensitivity of the membrane potential to changes in external K^+ and removes a part of K^+ permeability that was short-circuiting electrogenic currents. The electrical responses of barium-treated mouse astrocytes are very similar to those of rat astrocytes in culture.

Glutamate and GABA activated responses

Barium treatment transforms the glutamate response to mouse astrocytes from a monophasic 9 mV depolarization to a biphasic response with a transient maximal amplitude of 15 mV. The same biphasic responses with slightly larger amplitudes for rat astrocytes were found by other authors[3,7,9]. SITS blocks glutamate uptake into astrocytes[31] and it did not interfere with the glutamate response of barium-treated mouse astrocytes, a clear sign that this biphasic response is not due to electrogenic effects of glutamate uptake. Backus et al.[1] found that the glutamate response of rat astrocytes is due to a receptor which is activated by both kainate and quisqualate. Although we did not undertake a pharmacological receptor characterization, we assume that the same receptor response as in rat astrocytes is underlying the effects. The GABA response of the mouse astrocytes is biphasic with a maximum amplitude of about 10 mV. Barium treatment adds a spike-like component of 10 mV amplitude to this response. In rat astrocytes it was found that the GABA response is temperature-dependent yielding its peak at about 14 °C. At 25 °C the response is almost identical with the one of barium-treated mouse astrocytes[8]. β-Alanine, which blocks at a concentration of 1 mM about 50% of the GABA uptake into astrocytes[12], does not interfere with the response. Therefore a receptor activation could underlay the response. Kettenmann and Schachner[9] established that a receptor similar to the neuronal $GABA_A$ receptor is responsible for the action in rat astrocytes.

Rat–mouse astrocytes membrane properties

In this study we showed that primary cultures from rat and mouse brain differ electrically. This difference is entirely due to a large barium-sensitive K^+ conductance of the cultured mouse astrocytes, as already suggested by earlier measurement of the radioactive unidirectional K^+ flux[25]. These radioactive flux measurements suggested that in mouse astrocytes this barium-sensitive component accounts for 95% of the total unidirectional K^+ flux and that the remaining 5% are in the same magnitude as the fluxes of rat astrocytes. It is unlikely that this difference, which would greatly interfere with the spatial buffer currents of astrocytes, represents a real species difference of astrocytic function between mouse and rat brain. An obvious question is then which culture system, if any, represents the in vivo conditions, the best. Electrical responses from brain slices or in vivo preparations are very problematic to compare with cultured astrocytes, since the degree of coupling and K^+ accumulation in the ECS make such a comparison difficult. Thus, these additional effects accompanying ouabain application and K^+ depletion make it impossible to judge if these in situ systems have an electrogenic component. Astrocytes in brain slices react to glutamate and GABA application with non-spike-like depolarizations[14,26], but again changes in external K^+ concentrations make comparisons difficult. Evidence for an extremely large K^+ conductance in glial cells in situ comes from indirect measurements: Nicholson and Phillips[18] studied ion diffusion in rat cerebellum using ion-selective micropipettes and ionophoretic point sources. They found that for a variety of cations and anions the laws of macroscopic diffusion were closely obeyed assuming that the ECS occupies about 20% of the rat cerebellum. The movement of K^+ in an electrical gradient, however, behaves as if the ECS has a volume of more than 100%. The anomalous nature of K^+ migration can be solved by assuming that the ion does not remain in the ECS but is in fact the major current carrier across cell membranes. Hounsgaard and Nicholson[5] measured changes in the extracellular K^+ concentration in the vicinity of Purkinje cells in guinea pig cerebellar slices. No extracellular K^+ changes were seen when the cells were hyperpolarized with current passage or during subthreshold depolarization. Only during spike activity was there a rise in extracellular K^+. In the vicinity of glial cells, however, hyperpolarizing and depolarizing currents changed the extracellular K^+ concentration in a symmetrical manner. These experiments demonstrate that the anomalous nature of K^+ migration is mainly due to movement through glial cell membranes, which have a resting K^+ conductance similar to the neuronal one at the peak K^+ conductance during an action

potential. This point is underscored by experiments from oligodendrocytes of mouse corpus callosum slices. Whole-cell clamp measurements demonstrate that the K^+ equilibrium potential is able to shift within milliseconds to changes in the membrane potential, due to extremely high conductances[2]. The K^+ channel, which is responsible for this in situ property, was found to be barium-sensitive, and it is *not* found in cultures of oligodendrocytes. Thus, in this case a K^+ channel responsible for the extreme high K^+ conductance got lost during the culture procedure. A similar mechanism could underlie in the case of rat astrocytes. However, in all likelihood, the problem is even more complex: Mammalian Muller cells do have regional specializations[17,19,22]. Their endfeet have an extremely high K^+ conductance (360 pS per channel), whereas the soma does not. Newman[16] confirmed further, that freshly dissociated amphibia astrocytes from the optic nerve had a K^+ conductance, which is 10-fold higher at the endfeet than at the soma. The increased K^+ conductance is due to inward rectifying K^+ channels, which are barium-sensitive. So far no studies exist, that attribute regional conductance differences to astrocytes in culture. It may be speculated that culture conditions do not preserve the regional differentiations of astrocytes in situ. If so, it could well be that cultured mouse astrocytes have only endfeet properties preserved and cultured rat astrocytes have only properties of the soma membrane preserved. Obviously, such a K^+ channel will not be the only parameter that one would expect to be asymmetrically distributed, but other channels and carriers might have a similar regional variation.

Conclusions

It can be shown that mouse and rat astrocytes in culture, have similar physiological properties. However, an extremely large barium-sensitive K^+ conductance is short-circuiting these responses in cultured mouse astrocytes, whereas those of cultured rat astrocytes are not. There is no clear evidence available to clearly evaluate this problem, but there are some indirect measurements available pointing to the possibility, that an extremely large K^+ conductance plays a role in in vivo glial cells. If so, this extremely large conductance might be confined to end-feet with somata having more 'normal' K^+ conductances. There is a clear need to further evaluate this problem, considering the importance of cultured astrocytes as a model preparation in physiological research and for researchers using astrocytes in culture to be aware of the different possibilities for the interpretation of their data.

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