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ION ACTIVITIES AND POTASSIUM UPTAKE MECHANISMS OF GLIAL CELLS IN GUINEA-PIG OLFACTORY CORTEX SLICES

BY K. BALLANYI, P. GRAFE AND G. TEN BRUGGENCATE

From the Institut für Physiologie der Universität München, Pettenkoferstrasse 12, D-8000 München 2, F.R.G.

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SUMMARY

1. Double-barrelled ion-sensitive micro-electrodes were used to measure changes in the intracellular activities of K^+, Na^+ and Cl^- (a^i_K, a^i_{Na}, a^i_{Cl}) in glial cells of slices from guinea-pig olfactory cortex during repetitive stimulation of the lateral olfactory tract.

2. Base-line levels of a^i_K, a^i_{Na} and a^i_{Cl} were about 66, 25 and 6 mM, respectively, for cells with resting potentials higher than −80 mV. During stimulation, intraglial a^i_K and a^i_{Cl} increased, whereas a^i_{Na} decreased. Within about 2 min after stimulation the ion activities returned to their base-line levels.

3. The Cl^- equilibrium potential was found to be close to the membrane potential (E_m). There was also a strong correlation between changes of E_m and a^i_{Cl}. These observations indicate a high Cl^- conductance of the glial cell membrane.

4. In the presence of Ba^{2+}, the usual depolarizing response of the glial cells to a rise of the extracellular K^+ activity (a^e_K) reversed into a membrane hyperpolarization. Furthermore, Ba^{2+} strongly reduced the stimulus-related rise of intraglial a^i_K. An additional application of ouabain blocked both the membrane hyperpolarization as well as the remaining rise of a^i_K.

5. In conclusion, our data show that glial cells in guinea-pig olfactory cortex slices possess at least two mechanisms of K^+ accumulation. One mechanism is sensitive to the K^+ channel blocker Ba^{2+} and might be a passive KCl influx. The other appears to be the electrogenic Na^+/K^+ pump, which can be activated by excess extracellular K^+.

INTRODUCTION

Enhanced neuronal activity in the central nervous system is accompanied by transient elevations in extracellular K^+ (a^e_K; Somjen, 1979; Nicholson, 1981). Alterations of a^e_K, however, can modify the activity of neurones (Sykova, 1983). Therefore, it is of interest to investigate the mechanisms of K^+ homeostasis in the brain. Glial cells surrounding the neurones seem to be involved in the regulation of a^e_K (Walz & Hertz, 1983*a*). Intracellular recordings with ion-sensitive micro-electrodes (Coles & Tsacopoulos, 1979; Coles & Orkand, 1983; Kettenmann, Sonnhof & Schachner, 1983; Schlue & Wuttke, 1983) and radiotracer studies (Walz & Hertz, 1982) have revealed the ability of glial cells to accumulate K^+ during an increase of

a_K^e. Several mechanisms such as spatial buffer (Orkand, Nicholls & Kuffler, 1966; Dietzel, Heinemann, Hofmeier & Lux, 1980, 1982; Coles & Orkand, 1983; Gardner-Medwin, 1983), Na^+/K^+ pump (Kukes, Elul & de Vellis, 1976; Walz & Hertz, 1982; Grisar, Franck & Delgado-Escueta, 1983), KCl co-transport (Walz & Hertz, 1984; Wolpaw & Martin, 1984) or Ba^{2+}-sensitive K^+ channels (Walz, Shargool & Hertz, 1984*a*) have been proposed to contribute to the K^+ uptake process. Most of the data was obtained from glial cell lines or from preparations of invertebrates. However, no recordings have been described concerning ion activities in glial cells of functionally intact mammalian cortex. Therefore, the first aim of the present study was to investigate changes in the intraglial activities of K^+, Na^+ and Cl^- (a_K^i, a_{Na}^i and a_{Cl}^i) using double-barrelled ion-sensitive micro-electrodes in guinea-pig olfactory cortex slices during stimulation of cortical afferents. The second aim was to characterize the ionic mechanisms responsible for the increase in a_K^i observed during stimulation. With the aid of Ba^{2+} and ouabain, it was possible to discriminate between two mechanisms of K^+ accumulation. One component seems to operate through K^+ channels which can be blocked by Ba^{2+}. The other component appears to be the ouabain-sensitive electrogenic Na^+/K^+ pump. Parts of the results have been presented at a meeting of the German Physiological Society (Ballanyi, Grafe & ten Bruggencate, 1985; Grafe, 1985).

METHODS

Preparation and solutions

Experiments were performed on slices of the olfactory cortex of guinea-pigs. After decapitation, the brain was rapidly removed and surface slices (approximately 500 μm thick) were cut using a plastic guide and a bow cutter. Slices were placed in a Perspex chamber (volume 2 ml) and superfused at 25 °C with a solution of the following composition (in mM): NaCl, 118; KCl, 3; $NaHCO_3$, 25; NaH_2PO_4, 1.2; $MgCl_2$, 1.0; $CaCl_2$, 1.5, and glucose, 10 (gassed with 95 % O_2, 5 % CO_2; pH 7·4). The Cl^--free solution was composed of (in mM): Na gluconate, 118; K gluconate, 3; $NaHCO_3$, 25; NaH_2PO_4, 1·2; $MgSO_4$, 1; Ca gluconate, 6·5. Drugs were added to the superfusion fluid (all chemicals in this study were purchased from Sigma; Munich, F.R.G.). Cortical neurones were activated by electrical stimulation of the lateral olfactory tract (l.o.t.) via platinum wire electrodes.

Ion-sensitive micro-electrodes

The methods used for the construction and calibration of the double-barrelled ion-sensitive micro-electrodes are described in detail elsewhere (Ballanyi, Grafe, Reddy & ten Bruggencate, 1984; Grafe, Ballanyi & ten Bruggencate, 1985). In brief, theta-capillaries were pulled with a Brown-Flaming type micro-electrode puller (Sutter Instrument Co., San Francisco, CA, U.S.A.) resulting in tip diameters below 0·3 μm. Silanization of one barrel with hexamethyldisilazane (Sigma, Munich, F.R.G.) was performed at 400 °C for 20 min. During this period, nitrogen was applied under pressure to the other barrel in order to prevent the silane from entering. Later on, a drop of liquid ion exchanger was injected into the silanized barrel, which was then backfilled with an internal reference solution. The reference barrel was filled with electrolyte solution. The following combinations of ion exchanger, backfilling solution and reference barrel solution were used: K^+-sensitive micro-electrodes (Corning 477317, 200 mM-KCl, 1 M-Mg acetate); Na^+-sensitive micro-electrodes (Fluka 71176, 200 mM-NaCl; 1 M-Mg acetate); Cl^--sensitive micro-electrodes (WP Instruments IE-170, 200 mM-KCl, 0·5 M-K sulphate). There was no significant difference in the average membrane potential of the glial cells as determined by electrodes containing 1 M-Mg acetate, 3 M-KCl (single-barrelled conventional electrodes) or 0·5 M-K sulphate in the reference barrel (see Results). This indicates that the interpretation of the data obtained with the Cl^--sensitive micro-electrodes is not seriously hindered by junction potentials of the sulphate-containing electrodes.

K^+ electrodes were calibrated in solutions containing 3, 12 and 60 mM-KCl with a constant background of 150 mM-NaCl. The mean values for the slope and the selectivity coefficient *versus* Na^+ were $53{\cdot}3 \pm 4{\cdot}6$ mV and $0{\cdot}019 \pm 0{\cdot}007$ (mean $\pm$ S.D., $n = 29$). The Cl^- electrodes were calibrated either in pure KCl solutions (15, 40, 80 and 150 mM) or in solutions containing KCl (1, 10, 40 or 100 mM) and Na glucuronate in order to keep the ionic strength constant. The average electrode slope in both cases was $50{\cdot}2 \pm 3.1$ mV ($n = 25$). Intracellular Na^+ was determined by comparing the voltage of the ion signal obtained upon withdrawal of the electrode from the cell into the bathing solution with the voltage obtained upon changing the normal Krebs solution to solutions containing different Na^+ concentrations (1, 3, 12, 30 and 60 mM) with a variable background of K^+ to keep the sum of Na^+ and K^+ at 150 mM. The Ca^{2+} concentration in these solutions was buffered to 10^{-7} M. All values of intracellular Na^+, K^+ and Cl^- are given in activities assuming an intracellular activity coefficient of 0·74 for these ions (Meier, Ammann, Morf & Simon, 1980). Intracellular impalements were achieved by means of a piezo-driven micromanipulator (built by M. Frankenberger, Munich, F.R.G.).

RESULTS

General observations

The *in vitro* slice preparation of guinea-pig olfactory cortex is well suited to investigation of neurone–glia interactions in the mammalian central nervous system. On the one hand, it is possible to maintain stable intraglial recordings either with conventional single-barrelled (Grafe, Reddy, Emmert & ten Bruggencate, 1983) or double-barrelled ion-sensitive micro-electrodes (Ballanyi *et al.* 1985). On the other hand, neuronal K^+ release can be elicited by electrical stimulation of afferent nerve fibres in the lateral olfactory tract (l.o.t.).

Membrane resting potentials (E_m) of presumed glial cells were between -75 and -95 mV (no difference between single-barrelled conventional or double-barrelled ion-sensitive micro-electrodes). Such cells were characterized by the absence of any 'injury discharge' after the impalement and by the lack of synaptic potentials, which are the typical characteristics of neurones. Furthermore, repetitive stimulation of the l.o.t. (10–50 Hz, 5–50 s) resulted in a rapid depolarization of up to 35 mV amplitude with kinetics similar to the stimulus-induced increase of extracellular K^+ concentration. Measurements from glial cells in this study were discarded if E_m was less than -75 mV, or when E_m or the ion signal were not stable for at least 10 min.

Intracellular K^+ activity

The intracellular K^+ activity (a^i_K) as measured in twelve cells with a mean E_m of $-84.4 \pm 3{\cdot}3$ mV was $65{\cdot}9 \pm 6{\cdot}6$ mM (mean $\pm$ S.D.). Stimulation of the l.o.t. (20 Hz, 15 s) in these cells resulted in an average a^i_K increase of $16{\cdot}1 \pm 6{\cdot}1$ mM. Fig. 1 gives an example of the measurement of a^i_K in a cortical glial cell. In this experiment, one K^+-sensitive micro-electrode was used to record intracellularly from a glial cell (Fig. 1*A*), whereas another was positioned extracellularly, close to the impaled cell (Fig. 1*B*). At the beginning of the intracellular recording, the base-line level of intraglial a^i_K increased, a typical observation in the 'sealing phase' of the membrane. Later on, the l.o.t. was stimulated every 3 min with pulse trains of constant frequency (30 Hz) and variable duration (5–50 s). Each of these stimuli resulted in a rise of both a^e_K as well as a^i_K. Longer-lasting stimuli induced larger increases in intraglial a^i_K. After each period of repetitive stimulation, a^i_K returned to the base line within about 2 min. Fig. 1*B* shows the recording of a^e_K as measured with the extracellularly positioned

K^+-sensitive micro-electrode. It is apparent that there is a close relationship in the kinetics of changes in a_K^e and the glial membrane potential. However, the longer-lasting stimuli (30 and 50 s) were followed by transient after-hyperpolarizations of the glial E_m, whereas this behaviour was not mirrored by an 'undershoot' of a_K^e. To analyse this discrepancy between glial E_m and a_K^e, which was observed in several preparations, a_K^i, a_K^e, glial E_m, and the calculated K^+ equilibrium potential were compared during tetanic stimulation.

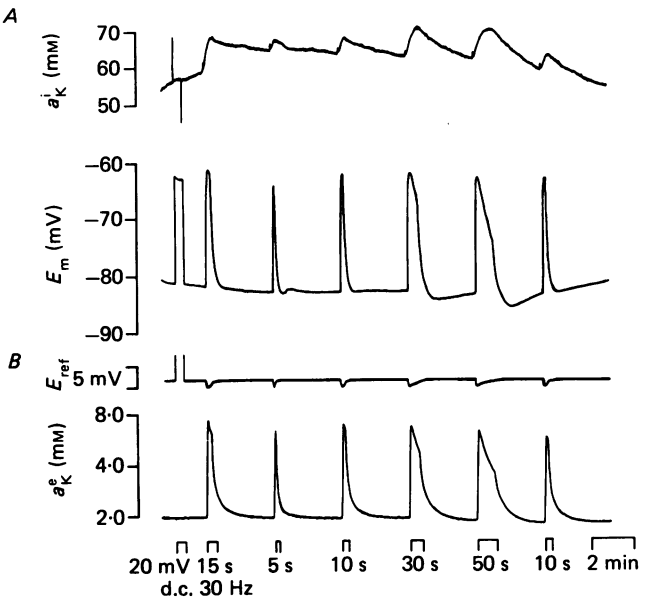

Fig. 1. Effects of repetitive stimulation on intraglial (a_K^i) and extracellular K^+ activity (a_K^e). In this Figure, the recordings of an intracellularly (*A*) and extracellularly positioned (*B*) K^+-sensitive micro-electrode are illustrated during repetitive stimulation of the lateral olfactory tract with stimulation trains of constant frequency (30 Hz) and different durations (indicated by bars). E_m is the membrane potential of the glial cell, E_{ref} the voltage reading of the reference channel of the extracellular double-barrelled electrode. The voltage deflexions on the E_{ref} trace represent extracellular field potentials. These potentials did not affect the a_K^e measurements as the a_K^e signal is the difference of the voltage reading of the reference and the K^+-sensitive barrel. At the beginning of the record, a voltage pulse of 20 mV (20 mV d.c.) was added to the ground potential in order to test the gain adjustment of the differential amplifiers.

In Fig. 2*A*, the 30 s stimulation period of Fig. 1 is illustrated on an extended time scale. First, it can be seen more clearly that the glial membrane potential shows a post-tetanic hyperpolarization, although no a_K^e undershoot as a possible cause for this behaviour was detectable. However, a calculation of the K^+ equilibrium potential (E_K) based on both changes in a_K^e as well as a_K^i, reveals a good correlation between the glial E_m and E_K. Therefore, post-tetanic glial hyperpolarization in this situation can be explained by the still elevated a_K^i in this period (see also Kettenmann *et al.* 1983). A contribution of the electrogenic Na^+/K^+ pump to the post-tetanic hyperpolarization is not necessarily involved since the behaviour of the membrane can be adequately explained on the basis of a high K^+ conductance. Application of

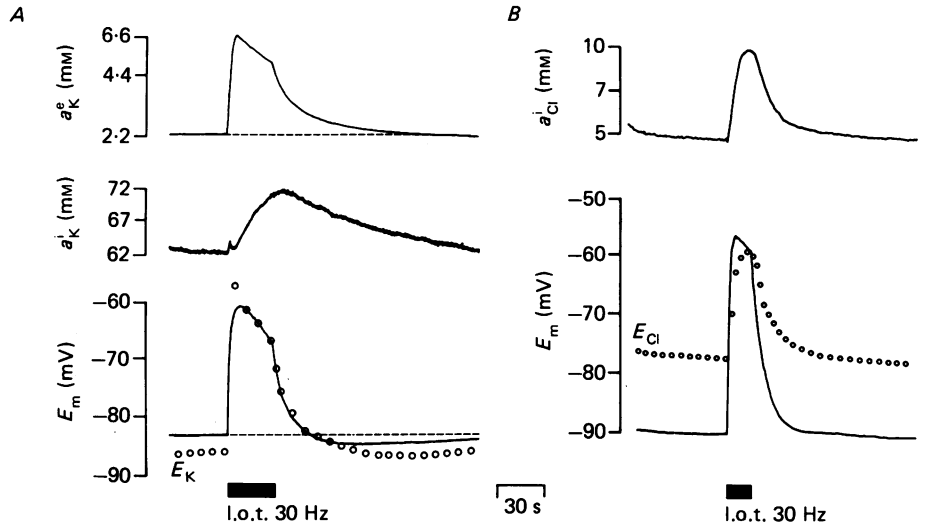

Fig. 2. Changes of ion activities and ionic equilibrium potentials. *A*, the 30 s stimulation period of Fig. 1 is illustrated on an extended time scale. Measured values of a^i_K and a^e_K were used to calculate the K^+ equilibrium potential (E_K; open circles) and to compare it with E_m. *B*, in this illustration, the Cl^- equilibrium potential (E_{Cl}; open circles) before, during, and after repetitive stimulation of the l.o.t. was calculated using the measured value of the intracellular Cl^- activity (a^i_{Cl}) and the Cl^- activity in the bathing solution. For further explanations see text.

ouabain is not a good tool to differentiate between these possibilities since it would modify both the rise of a^i_K as well as the pump-related membrane current. The good correlation between glial E_m and E_K also excludes the possibility that some other substance may have contributed to the voltage reading of the Corning ion exchanger-based K^+ electrodes. Such a situation has been shown to interfere with measurements of a^i_K in glial cells of leech ganglia (Schlue & Wuttke, 1983). Secondly, it can also be seen that a^i_K rises more slowly with respect to the increase of a^e_K. This difference might be due to the kinetics of the K^+ uptake mechanisms or due to time elapsed during intracellular diffusion. It is certainly not the result of a slow electrode response as most of the intracellularly used K^+-sensitive micro-electrodes responded very quickly when used extracellularly.

Intracellular Cl^- activity

The intracellular Cl^- activity (a^i_{Cl}) was measured in twenty-three glial cells with a mean E_m of $-86{\cdot}9 \pm 3{\cdot}8$ mV (mean ± S.D.). On the assumption of a pure Cl^- selectivity of the ion-sensitive micro-electrodes, an apparent a^i_{Cl} of $6{\cdot}0 \pm 1{\cdot}5$ mM (mean ± S.D.) and a Cl^- equilibrium potential (E_{Cl}) of $-71 \pm 5{\cdot}9$ mV was calculated. During repetitive stimulation of the l.o.t. (20–30 Hz, 15 s) a^i_{Cl} increased by $3{\cdot}6 \pm 1{\cdot}2$ mM (mean ± S.D., $n = 19$). As an example, Fig. 3 illustrates an experiment in which a Cl^--sensitive micro-electrode was used to follow changes in intraglial a^i_{Cl} when the l.o.t. was stimulated with pulse trains of constant frequency (30 Hz) and variable duration (5–50 s). Based on the known extracellular Cl^- activity and the measured a^i_{Cl}, E_{Cl} was then calculated. This calculation revealed a discrepancy

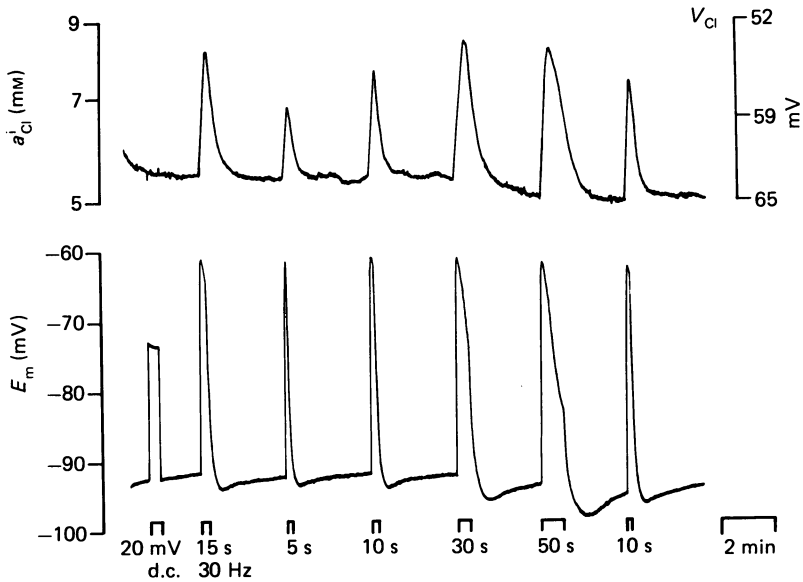

Fig. 3. Effects of repetitive stimulation on intraglial Cl^- activity (a^i_{Cl}). In this experiment, an intracellular Cl^--sensitive micro-electrode was used to measure changes in a^i_{Cl} during repetitive stimulation of the l.o.t. with stimulation trains of constant frequency (30 Hz) and different durations (indicated by bars). At the beginning of the record, a voltage pulse of 20 mV (20 mV d.c.) was added to the ground potential in order to test the gain adjustment of the differential amplifier. V_{Cl} is the difference signal of the Cl^--sensitive micro-electrode.

between resting glial E_m and E_{Cl} of around 15 mV (see Fig. 2*B*) and would indicate that either a^i_{Cl} is about 3 mM above a passive distribution or that interfering anions contribute to the voltage reading of the Cl^--sensitive ligand. In order to differentiate between these two possibilities, experiments in Cl^--free bathing solution were performed. A typical example of such an experiment is shown in Fig. 4.

In this experiment, one Cl^--sensitive micro-electrode was positioned extracellularly about 100 μm below the pial surface, and a second was placed intracellularly in an adjacent glial cell to measure a^i_{Cl}. The apparent a^i_{Cl} at the resting potential was about 7·4 mM, indicating a Cl^- equilibrium potential of −64 mV and therefore 16 mV above E_m (−80 mV). However, 8 min after the replacement of the extracellular Cl^- with gluconate, a^i_{Cl} was still at 4·2 mM in spite of a fall of a^e_{Cl} to about the same value. This observation leads us to conclude that some other anions, to which the Cl^- ligand is sensitive, result in an apparent intracellular Cl^- equivalent of about 3–4 mM. If one subtracts about 3 mM from the average apparent a^i_{Cl} of 6 mM, then an a^i_{Cl} of 3·2 mM would result in an equal E_{Cl} and E_m of −86 mV each. Direct proof of the contribution of interfering anions to the voltage reading of Cl^--sensitive micro-electrodes (liquid exchanger-based) was recently obtained in skeletal muscle by means of comparison of data obtained with recessed-tip solid-state Ag/AgCl electrodes (McCaig & Leader, 1984). It seems unlikely, although not impossible, that inwardly directed Cl^- transport mechanisms can maintain a^i_{Cl} at about 4 mM in an almost Cl^--free extracellular solution. (The almost complete absence of extracellular Cl^-

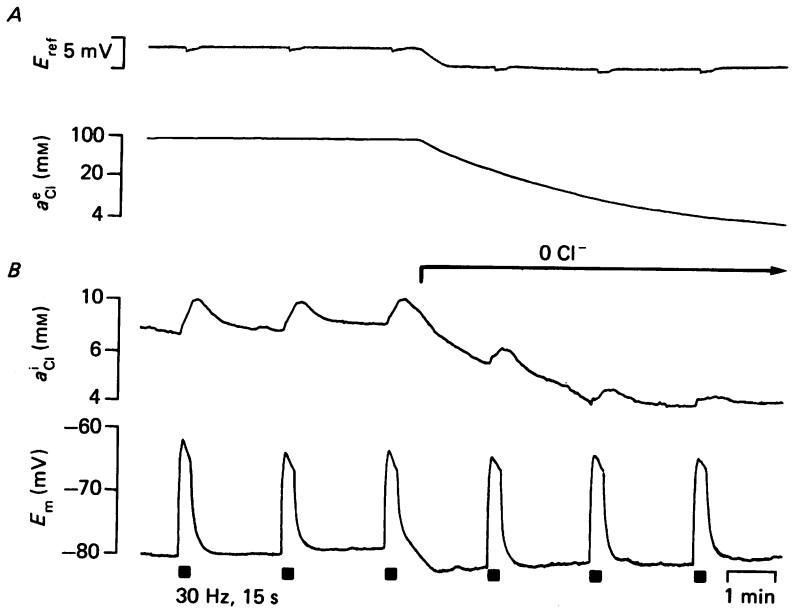

Fig. 4. Effect of a Cl^--free solution on intraglial Cl^- activity. In this experiment, the recordings of an extracellularly (*A*) and an intracellularly positioned (*B*) Cl^--sensitive micro-electrode are illustrated during three trains of stimuli in normal extracellular bathing solution and during three trains of stimuli after the extracellular Cl^- was completely replaced by gluconate. Note that the apparent a^i_{Cl} did not decrease below 4 mM at an a^e_{Cl} of about the same value. This reveals the importance of intracellular interfering anions. For further explanation see text.

around the glial cell is also indicated by the lack of the usual stimulus-related rise of a^i_{Cl}.)

Intracellular Na^+ activity

The mean intracellular Na^+ activity as measured in twelve glial cells with a mean E_m of $-83{\cdot}3 \pm 2{\cdot}8$ mV was $25{\cdot}2 \pm 4{\cdot}6$ mM (mean $\pm$ S.D.). Stimulation of the l.o.t. (20 Hz, 15 s) resulted in an average decrease of a^i_{Na} in these cells of $6{\cdot}0 \pm 1{\cdot}9$ mM. In the experiment illustrated in Fig. 5, a Na^+-sensitive micro-electrode was used to follow changes of intraglial a^i_{Na} when the l.o.t. was stimulated with pulse trains of constant frequency and variable duration (5–50 s). Each of these stimuli resulted in a decrease of a^i_{Na}. After the end of the stimulation periods, a^i_{Na} returned to its resting level within about 2 min. The mechanisms underlying the stimulus-related decrease of glial a^i_{Na} were not studied in detail in the present paper. One important factor, however, seems to be the activation of the Na^+/K^+ pump (see Discussion).

Effects of Ba^{2+}

In order to differentiate between several possible mechanisms of K^+ uptake into cortical glia, the action Ba^{2+} was explored. Ba^{2+} is known to block K^+ channels (Hille, 1984) and has been previously used as a tool to differentiate between passive and active K^+ uptake mechanisms in skeletal muscle fibres (Sjodin & Ortiz, 1975).

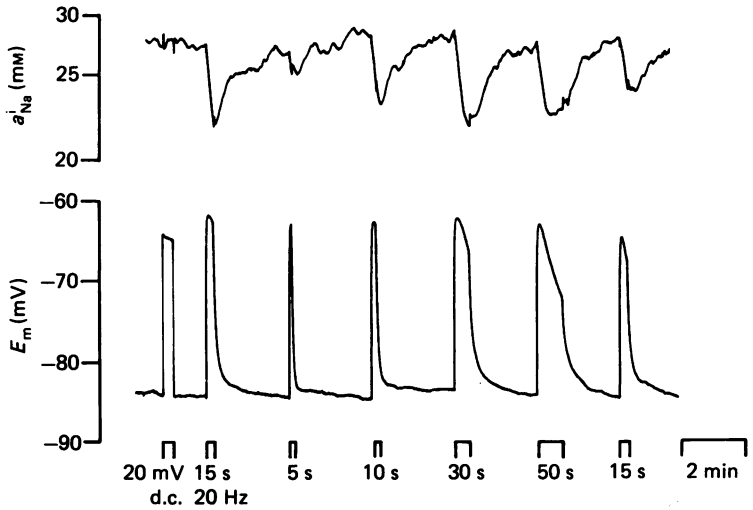

Fig. 5. Effects of repetitive stimulation on intraglial Na^+ activity (a^i_{Na}). In this recording, a double-barrelled Na^+-sensitive micro-electrode was used to measure changes in a^i_{Na} and membrane potential (E_m) during repetitive stimulation of the l.o.t. with stimulation trains of constant frequency (20 Hz) and different durations. At the beginning of the recording, a d.c. voltage pulse (20 mV) was added to the ground potential in order to test the gain adjustment of the differential amplifier.

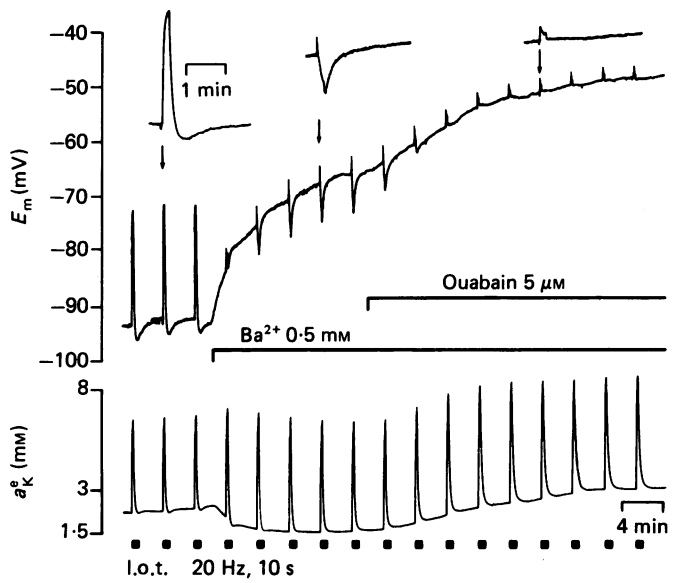

Fig. 6. Effects of Ba^{2+} and ouabain on extracellular K^+ activity (a^e_K) and glial membrane potential (E_m). After three trains of stimuli (20 Hz, 10 s) had revealed the typical relationship between changes of a^e_K and E_m, Ba^{2+} (0·5 mM) was added. This resulted in a decrease of a^e_K base-line and a glial depolarization. Stimulation under these conditions now resulted in a glial hyperpolarization (see central inset), whereas the stimulus-induced a^e_K increases were similar to the control situation. Subsequent addition of ouabain (5 μM) resulted in a rise of a^e_K, a further glial depolarization and the disappearance of the previously observed stimulus-related membrane hyperpolarization.

These ions are also known to interfere with the K^+ homeostasis of cultured glial cells (Walz *et al.* 1984*a*). Fig. 6 illustrates a typical experiment, in which the effects of repetitive stimulation of the l.o.t. on the membrane potential of a glial cell and on a_K^e were investigated in the presence of Ba^{2+}. After three trains of stimuli had revealed the typical relationship between changes of a_K^e and the glial membrane

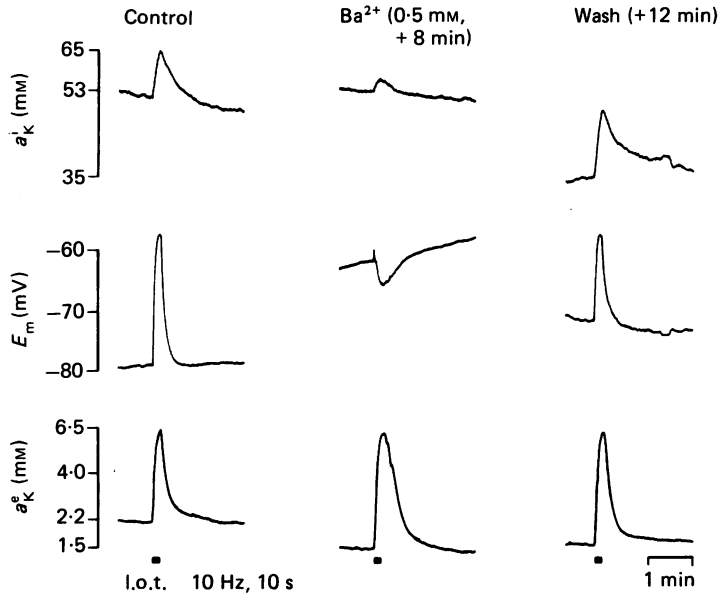

Fig. 7. Effects of Ba^{2+} on stimulus-induced increases in intracellular K^+ activity (a_K^i), extracellular K^+ (a_K^e) and glial membrane potential (E_m). The Figure shows three sections taken from a continuous recording from a single glial cell. The left column shows the typical rise of a_K^i during a stimulus-induced increase of a_K^e in normal solution. In the presence of Ba^{2+} (0·5 mM, central column), an even greater rise in a_K^e led to a much smaller increase of a_K^i, and was accompanied by a membrane hyperpolarization. Right column shows a stimulation during the recovery period from Ba^{2+}.

potential, Ba^{2+} (0·5 mM) was added to the normal bathing solution. This resulted in a decrease of the a_K^e base line (probably due to reduced resting K^+ efflux from neurones and glia; see also Nicholson, ten Bruggencate & Senekowitsch, 1976) and a glial membrane depolarization. The most striking effect, however, was a complete reversal in the relationship between stimulus-related rise of a_K^e and the behaviour of the glial membrane potential. In spite of a stimulus-induced rise in a_K^e, which normally resulted in a membrane *depolarization*, the glia now responded with a prominent membrane *hyperpolarization*. Only at the onset of the stimulus, a short-lasting, transient depolarization was visible (see central inset in Fig. 6). With the idea in mind that the K^+-induced hyperpolarization may be the consequence of an electrogenic K^+ transport mechanism, ouabain was added to the Ba^{2+}-containing bathing solution. This procedure resulted in a rise of a_K^e, a further glial membrane depolarization and a disappearance of the previously observed stimulus-related membrane hyperpolarization (see right inset in Fig. 6).

The next three Figures illustrate the behaviour of the intraglial ion activities in the presence of Ba^{2+}. The first Figure of this series (Fig. 7) illustrates that Ba^{2+}

largely diminished the stimulus-induced rise of a_K^i. This reduction was evident despite an even bigger increase in a_K^e that occurred during the l.o.t. stimulation. Additional experiments revealed that this Ba^{2+}-resistant rise of a_K^i was completely blocked by ouabain (not illustrated). Another aspect concerns the resting a_K^i base line of glial cells in the presence of Ba^{2+} (Fig. 8). In the seven cells, in which Ba^{2+} (0·5–1 mM) was tested, an increase in a_K^i of between 5 and 23 mM (15·3±6·3 mM,

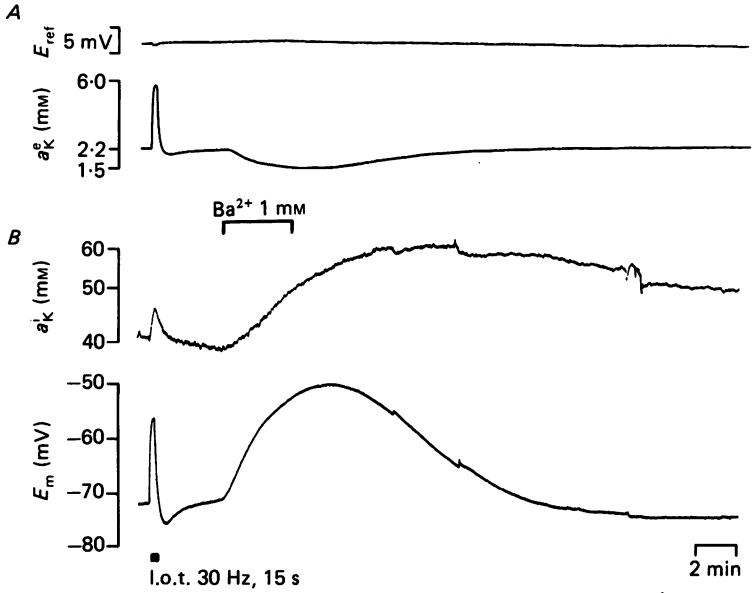

Fig. 8. Effects of Ba^{2+} on extra- and intracellular K^+ activities (a_K^e, a_K^i). In this Figure, the recordings of an extracellularly (*A*) and an intracellularly positioned (*B*) K^+-sensitive micro-electrode are illustrated. At the beginning of the recording, one period of tetanic stimulation (30 Hz, 15 s) revealed the typical glial depolarization accompanied by transient elevations in a_K^e and a_K^i. Application of Ba^{2+} (1 mM) resulted in a membrane depolarization of about 25 mV and an a_K^i increase of about 20 mM. This a_K^i increase was mirrored by a fall in a_K^e base line. Note that after wash-out of Ba^{2+}, the incomplete return of a_K^i to base line may be due to an improvement of the cell, as indicated by an increase of E_m.

mean±S.D.) was seen. A decrease in a_K^i was never observed, indicating that Ba^{2+} in the concentration of 0·5–1 mM does not inhibit the Na^+/K^+ pump of the glial cells. This observation does not support an assumption of Walz *et al.* (1984*a*), who tried to explain the Ba^{2+}-induced depolarization of glial cells by an inhibition of the Na^+/K^+ pump.

The behaviour of intraglial a_{Cl}^i in the presence of Ba^{2+} indicates the close correspondence between the glial membrane potential and a_{Cl}^i. In the experiment illustrated in Fig. 9, a Cl^--sensitive micro-electrode was used to record a_{Cl}^i and E_m of a glial cell (Fig. 9*A*), whereas a K^+-sensitive micro-electrode was positioned extracellularly to measure a_K^e (Fig. 9*B*). Two stimulus trains in normal solution at the beginning of this recording revealed the stimulus-related rise of a_K^e, the membrane depolarization and the accompanying rise of a_{Cl}^i usually seen in the glial cells. Ba^{2+} was added to the bathing solution and within a few minutes the membrane

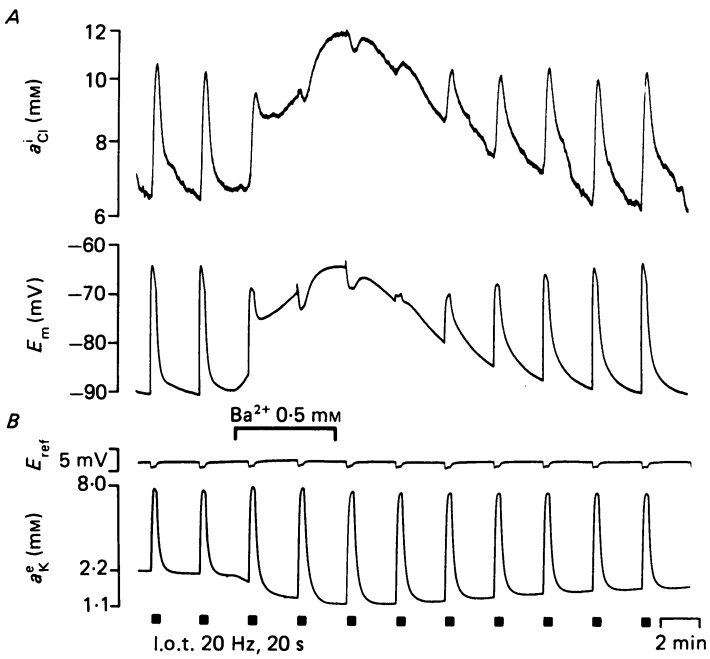

Fig. 9. Effects of Ba^{2+} on intracellular Cl^- activity (a^i_{Cl}) and extracellular K^+ (a^e_K). After two control stimulation trains, Ba^{2+} (0·5 mM) was added to the bathing solution. The typical glial depolarization in Ba^{2+} was accompanied by an increase in a^i_{Cl} base line. The Ba^{2+}-induced shift of a stimulus-related depolarization into a hyperpolarization was mirrored by a shift from an a^i_{Cl} increase to an a^i_{Cl} decrease. After the wash-out of Ba^{2+}, the membrane potential as well as a^i_{Cl} recovered to their base-line values.

depolarized by more than 20 mV and a^i_{Cl} increased to about 12 mM. Repetitive stimulation of the l.o.t. during Ba^{2+} induced a small membrane hyperpolarization and a slight fall in a^i_{Cl}. Therefore, the increase in resting a^i_{Cl} as well as the stimulus-related decrease of a^i_{Cl} clearly indicate the close relationship between the membrane potential and a^i_{Cl}. If a^i_{Cl} were to follow changes in a^e_K, then the Ba^{2+}-induced decrease of the a^e_K base line and the stimulus-related a^e_K increase should have altered a^i_{Cl} in the opposite direction.

DISCUSSION

Base-line levels of intraglial ion activities

At the beginning of the discussion, the observed resting levels of intracellular ion activities need consideration. With respect to K^+, the measured values of a^i_K and the known level of the extracellular K^+ concentration reveal an E_K close to E_m. This confirms previous findings of intraglial K^+ activity in glial cells (Kettenmann *et al.* 1983) and is closely related to the high K^+ conductance of glial cells (Orkand *et al.* 1966). The base-line level of a^i_{Na} (25 mM on an average) is higher than a^i_{Na} determined in frog motoneurones (7·5 mM, Grafe, Rimpel, Reddy & ten Bruggencate, 1982) or rat sympathetic ganglion cells (5 mM, Ballanyi *et al.* 1984). This confirms a previous report describing higher intracellular Na^+ levels in glia as compared to neurones

(Coles & Orkand, 1985). However, for the accurate determination of a^i_{Na} base-line levels, the Ca^{2+} sensitivity of the Na^+ ligand has to be taken into account. Our Na^+ electrodes were calibrated under the assumption of an intraglial free Ca^{2+} concentration of 10^{-7} M (see Methods). This Ca^{2+} background level might be too low since, very recently, Morris, Krnjević & MacDonald (1985) as well as Coles & Orkand (1985) have determined free intraglial Ca^{2+} concentrations ($[Ca^{2+}]_i$) between 10^{-6} and 10^{-5} M. If $[Ca^{2+}]_i$ would be 10^{-5} M, a calculation reveals that the voltage reading of our Na^+ electrodes would indicate an a^i_{Na} of 20 mM instead of 25 mM (calculation based on a selectivity ratio for $Ca^{2+}:Na^+:K^+ = 2{\cdot}5:1:0{\cdot}025$, background 90 mM-$K^+$, 25 mM-$Na^+$; Meier, Lanter, Ammann, Steiner & Simon, 1982). Such a result would not change the conclusion about a relatively high a^i_{Na} in the glial cells.

The Cl^--sensitive micro-electrodes revealed an intraglial a^i_{Cl} base-line level of about 6 mM and a chloride equilibrium potential slightly positive to the resting potential. However, experiments using low Cl^- concentrations in the extracellular bathing solution (see Fig. 4) indicate that other intracellular anions to which the liquid exchanger is sensitive may result in a voltage reading of the Cl^--sensitive micro-electrodes equivalent to 3–4 mM-Cl^-. The subtraction of these 3–4 mM-Cl^- equivalents reveals a nearly equal E_{Cl} and E_m of the glial cell membrane. This result is in accordance with two other studies in which ion-sensitive micro-electrodes were used to measure a^i_{Cl} in glial cells (Bührle & Sonnhof, 1983; Coles & Orkand, 1984). An almost identical E_{Cl} and E_m together with the observation of a strong correlation between changes in membrane potential and a^i_{Cl} (see Fig. 9) indicates a high Cl^- conductance of the membrane. Later in the Discussion we will use this conclusion to explain the stimulus-related rise of intraglial a^i_{Cl}. One experiment, on the other hand, apparently contradicts the view of a high Cl^- conductance. A reduction of the extracellular Cl^- concentration (see Fig. 4) was not accompanied by a transient membrane depolarization expected if Cl^- was leaving the cell via conductance pathways. Such a transient depolarization was, for example, seen in skeletal muscle fibres after reduction of the Cl^- concentration in the extracellular solution (Hodgkin & Horowicz, 1959). However, two possibilities exist to explain this apparent contradiction. First, Cl^- could possibly leave these small cells passively as fast as the extracellular Cl^- concentration decreases. During such a situation E_{Cl} would never deviate from E_m and no potential change would be visible. To test this, a very rapid extracellular Cl^- concentration change would be necessary, an experiment not possible in our slice preparations. Secondly, there are reasons to assume that the anion channels of glial cells are unusually large in size and not completely impermeable to isethionate and gluconate, the Cl^- substituents used in the present study (Sonnhof & Schachner, 1984; Gray & Ritchie, 1985). In such a situation, Cl^- leaving the cell via conductance pathways would be replaced by the other anions entering the cell passively. Again, no potential change would be visible during the transition of high to low extracellular Cl^- concentration.

It should be noted, however, that a high Cl^- conductance does not preclude the presence of an active transport of Cl^-. Rather the effects of such a system on a^i_{Cl} will be largely short-circuited through the membrane because of its high Cl^- permeability (Vaughan-Jones, 1982). Indeed, previous studies, using radiotracer methods, have revealed inwardly directed Cl^- pump mechanisms in cultures from either astroglial

cells or from a glioma cell line (Kimelberg, 1981; Walz & Hertz, 1984; Wolpaw & Martin, 1984; see also Ballanyi & Grafe, 1985). It might be possible that such Cl^- pumps are involved in the pH regulation by the coupling to the transport of HCO_3^-. However, it is unlikely that active Cl^- transport contributes significantly to the stimulus-related rise of intraglial a^i_{Cl}. This observation is better explained by the high Cl^- conductance of the membrane (see below).

Stimulus-related changes of intracellular ion activities

Our experiments demonstrate that the enhanced neuronal activity induced by the stimulation of the l.o.t. resulted in an increase in intraglial a^i_K and a^i_{Cl}, whereas a^i_{Na} was decreased. The increase of a^i_K is similar to data from other glial cells (for references see Introduction), however, less is known about the behaviour of a^i_{Cl} and a^i_{Na}. Radiotracer data from mouse astrocytes in culture show that $[Cl^-]_i$ increased and $[Na^+]_i$ decreased when the extracellular K^+ concentration was raised from 3 to 12 mM (Walz & Hertz, 1983*b*). Similar data was described for the bee retina during light stimulation (Coles & Orkand, 1984, 1985). Qualitatively, our data resemble that obtained from these studies.

What are the mechanisms underlying the stimulus-related changes of intracellular ion activities? With respect to the K^+ uptake, at least two uptake processes have to be considered, which will be discussed in detail below. Concerning the increase in a^i_{Cl}, we first explored the possibility of a 'pump'-mediated uptake, since the described exclusive K^+ conductance of the glial membrane (Kettenmann *et al.* 1983; Walz, Wuttke & Hertz, 1984*b*) apparently seems to exclude a passive increase. It should be noted, however, that in neuropile glial cells of the leech, a Cl^- permeability contributes significantly to the input conductance of the cell membrane (Walz & Schlue, 1982). Our experiments designed to reveal a K^+/Cl^- co-transport as one likely candidate failed to give a positive result. In particular, in the presence of Ba^{2+}, the usual a^i_{Cl} *increase* reversed to a small a^i_{Cl} *decrease*. Since Ba^{2+} did not inhibit the stimulus-related rise of a^e_K, which should be the important factor in activating a K^+/Cl^- co-transport, the latter result is not consistent with such a carrier-mediated increase of a^i_{Cl}. The experiments using Ba^{2+} did, on the other hand, reveal a strong correspondence between changes of E_m and a^i_{Cl}, which is good evidence for a high Cl^- conductance of the membrane (see above). Consequently, we favour the idea that stimulus-related changes of a^i_{Cl} are due to Cl^- movements via a conductive pathway.

Several possible mechanisms may explain the stimulus-related decrease of a^i_{Na}. These include activation of a Na^+/K^+ pump, a decrease of an inwardly directed driving force for Na^+ due to the membrane depolarization, a redistribution of Na^+ within the glial syncytium as a result of spatial buffer currents, and/or cell swelling during KCl uptake. At the moment, we are not able to estimate the relative contribution of these possibilities. One important factor, however, seems to be the activation of a Na^+/K^+ pump (see below).

K^+ uptake via a Ba^{2+}-sensitive mechanism

Ba^{2+} is known to block K^+ channels in a variety of excitable cells (for review see Hille, 1984) and, as recently described, also in oligodendroglia (Corey, Barres & Chun, 1985). This effect has been previously used as a tool to differentiate between passive

and active K^+ uptake mechanisms (Sjodin & Ortiz, 1975). The membrane depolarization, the partial block of K^+ uptake, and the K^+-induced membrane hyperpolarization, observed in the presence of Ba^{2+}, are understandable in view of the present knowledge regarding the effects of this ion. The membrane depolarization can be explained by the closure of K^+ channels which shifts the membrane potential towards the equilibrium potential of another ion species. Na^+ is the most likely candidate, since E_{Cl} and E_m are almost identical.

The decrease in the K^+-induced rise of a_K^i during exposure to Ba^{2+} reveals an important passive K^+ uptake into glial cells. One possibility as to how this uptake can take place is via a mechanism suggested by Boyle & Conway (1941) and Hodgkin & Horowicz (1959) to explain a passive uptake of K^+ into muscle fibres. According to these authors, during an elevation of a_K^e, a transient deviation of E_m to the more depolarized E_K (due to the Cl^- conductance of the membrane) would result in an influx of K^+ and Cl^-. Our data are compatible with this view. Another mechanism by which K^+ uptake could occur through Ba^{2+}-sensitive channels would be via spatial buffer currents (Orkand *et al.* 1966; Dietzel *et al.* 1982; Coles & Orkand, 1983; Gardner-Medwin, 1983). According to this view, K^+ would enter the glial syncytium through K^+ channels as a result of a spatial potential gradient along the membrane of the electrically coupled glia; the rise of intraglial a_{Cl}^i would be explained by a redistribution of Cl^- within the linked glial cells. Our experiments do not exclude such a mechanism. However, the existence of a passive KCl uptake would allow the K^+ to be stored in the glial cell in the immediate neighbourhood of the active neurone, where it might be the more readily available to the neurone during recovery from K^+ loss (Gray & Ritchie, 1985).

The third phenomenon observable in the presence of Ba^{2+} is a K^+-induced membrane hyperpolarization. This may be due to the activation of a K^+-induced electrogenic pump current (see below).

K^+ uptake via the Na^+/K^+ pump

Our data show that a small part of the stimulus-induced rise in a_K^i in the glial cells was Ba^{2+} insensitive. This component disappeared in the presence of ouabain. This finding is consistent with previous reports of a ouabain-sensitive, Na^+/K^+ pump-mediated K^+ uptake in glial cells from invertebrates (Tang, Cohen & Orkand, 1980; Walz, Wuttke & Schlue, 1983) and mammalian glia in culture (Kukes *et al.* 1976; Walz & Hertz, 1982). However, in contrast to other electrophysiological investigations (Tang *et al.* 1983; Walz *et al.* 1983), our results reveal that the electrogenic nature of this glial Na^+/K^+ pump is a result of excess extracellular K^+; raised intracellular Na^+ is not essential. Since the membrane hyperpolarization is only seen in the presence of Ba^{2+}, it is likely that the high membrane conductance of the glia in the normal extracellular fluid shunts the pump-mediated outward current.

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