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Glial potassium uptake following depletion by intracellular ionophoresis

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Abstract. The K^+ uptake processes of immunologically identified oligodendrocytes from embryonic mouse spinal cord were studied in primary culture by injecting ions and recording membrane potential changes and, in some experiments, K^+ ion activity with intracellular electrodes. When Na^+ was injected $[K^+]_i$ decreased. Immediately before and after current injection the membrane potential was close to the K^+ equilibrium potential (E_K) and this finding was used to study K^+ uptake following its depletion by intracellular ionophoresis. The uptake of K^+ following Na^+ injection was blocked by ouabain and unaffected by removal of extracellular Cl^- or Cl^- transport blockers. This suggests that recovery comes about mostly through the activity of the Na^+/K^+-ATPase stimulated by either the increase in $[Na^+]_i$ or the decrease in $[K^+]_i$. Pump current could be determined by clamping at different membrane potentials and was found to increase in proportion to the depolarization of the cell resulting from $[K^+]_i$ depletion. The time course of recovery of membrane potential following either Li^+ or tetramethylammonium (TMA^+) injection was similar to that after Na^+ injection, indicating that injection of these ions to produce a comparable decrease in $[K^+]_i$ leads to a similar stimulation of the Na^+/K^+-ATPase. In addition, the recovery of membrane potential following injection of TMA^+, but not of Na^+ or Li^+, was blocked when the external Na^+ was removed. Internal Na^+ or Li^+ appears necessary for Na^+/K^+-ATPase-activity, but under conditions of normal or low $[Na^+]_i$ the rate of Na^+/K^+-ATPase activity seems to be sensitive to $[K^+]_i$ and/or membrane potential.

Key words: Cell culture – Mouse – Nervous system – Oligodendrocyte – Potassium – Ion regulation

Introduction

K^+ is lost from active neurons and accumulates in the extracellular space separating neurons from glia (Dietzel et al. 1980; Sykova 1983). This increase in extracellular K^+-concentration ($[K^+]_o$) can modify neuronal signalling and eventually lead to a block of activity (for review, see Sykova 1983). Glial cells are thought to play a role in K^+ homeostasis in the central nervous system by a variety of mechanisms, both active and passive (Kettenmann 1987; Nicholson 1980; Treherne 1981). The processes might include a net uptake of K^+ by glia through both Na^+/K^+-ATPase activity as well as activity of a KCl symport (Walz and Hinks 1985). The passive processes include KCl uptake predicted by the Donnan relation and space-dependent spatial buffering (Coles and Orkand 1983; Gardner-Medwin 1983; Kettenmann et al. 1985a). The latter process involves the movement of K^+ down its electrochemical gradient such that K^+ enters glial cells in regions where $[K^+]_o$ is raised and leaves the cells where $[K^+]_o$ is low.

Mammalian oligodendrocytes in culture have a number of advantages for the study of glial membrane properties (Kettenmann et al. 1983a, b). First, they may be penetrated under visual control with multiple electrodes for recording membrane potential and ion activities as well as for injecting ions. Second, the ionic environment of the cells can be better controlled than in vivo; in monolayer cultures, the surface of the cells is exposed to the culture medium. Furthermore, they can be identified immunocytochemically at several developmental stages before or after electrical recording (Kettenmann et al. 1985b; Sommer and Schachner 1981).

We have previously found that oligodendrocytes are selectively permeable to K^+ (Kettenmann et al. 1983b). The present study was designed to investigate the regulation of intracellular K^+ concentration in glial cells, and the processes responsible for its reuptake following depletion by ionophoresis.

Methods

Explant cultures of spinal cord from 13-day-old mouse embryos were maintained for 1–3 months in Eagles basal medium with Earle's salts supplemented with 10% calf serum (Kettenmann et al. 1983a, b). Oligodendrocytes located on a monolayer of astrocytes in the outgrowth zone of the explants were chosen for recordings and identified by morphological criteria established through the use of cell-type-specific monoclonal antibodies (Sommer and Schachner 1981). Culture dishes were mounted on the stage of a Zeiss inverted microscope at about 30° C in a 2–5% CO_2 atmosphere sufficient to maintain pH 7.2–7.4 (Kettenmann et al. 1983a, b). The normal bathing solution contained in mmol · l^{-1}: NaCl 116.5; KCl 5.4; Na_2HPO_4 1; $MgSO_4$ 0.8; $CaCl_2$ 1.7; $NaHCO_3$ 26.2; D-glucose 5.6. SITS (10^{-3} mol · l^{-1}), DIDS (10^{-3} mol · l^{-1}, furosemide (10^{-1} mol · l^{-1}), ouabain (10^{-4} and 10^{-5} mol · l^{-1}) were added to the salt solution. For Na^+-free solution NaCl, Na_2HPO_4 and $NaHCO_3$ were replaced in equimolar concentrations by choline chloride; for Cl^--free solutions NaCl,

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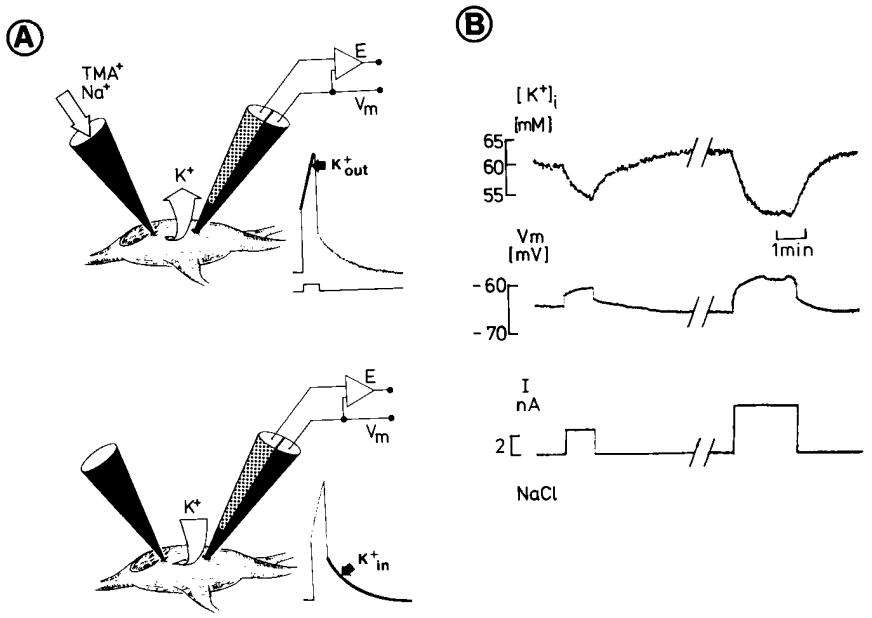

Fig. 1 A, B
Depletion of $[K^+]_i$ by ionophoresis.
A Schematic drawing of the experimental set-up. Oligodendrocytes were penetrated with two electrodes, one for injecting current (*left electrode*), the other (*right electrode*) for recording membrane potential (V_m) and, in some cases, intracellular K^+-activity ($[K^+]_i$) (*E*). During injection of TMA^+ or Na^+ (*upper record*) K^+ is driven out of the cell. The corresponding response of the membrane potential shows a continuous depolarization during the current pulse (K^+_{out}). After current injection the membrane was depolarized with regard to the original membrane potential and subsequently recovered (K^+_{in}). **B** Recording of membrane potential (V_m) and intracellular K^+-activity ($[K^+]_i$) during injection of depolarizing current (*I*) with a NaCl filled electrode. The experimental set-up is as described in **A**. During current injection the membrane potential depolarized and $[K^+]_i$ decreased and subsequently recovered after the current pulse

KCl and $CaCl_2$ were replaced by Na-isethionate, K_3PO_4 or K-acetate and $Ca(NO_3)_2$ or Ca-acetate, respectively. pH in Na^+- and Cl^--free solutions was buffered with TRIS (0.4 mmol · l^{-1}) or HEPES (5 mmol·l^{-1}). Recording microelectrodes were filled with 3 mol · l^{-1} KCl, 2 mol · l^{-1} NaCl or LiCl or 0.2 mol · l^{-1} tetramethylammonium (TMA^+) chloride. For measurement of intracellular K^+-activity ($[K^+]_i$) we used double-barrelled K^+-sensitive electrodes with the Corning exchanger 477317 in one barrel and Li-acetate in the reference barrel as previously described (Kettenmann et al. 1985a). The results are expressed not as activities, but as apparent free concentrations, that is, as if the activity coefficient in the cell was equal to that in the calibration solution. Cells were penetrated with the aid of a step motor driven micromanipulator (Sonnhof et al. 1982).

The method of analysis is based on the assumption that the glial membrane potential is a close measure of the K^+ equilibrium potential (E_K) under the conditions of these experiments (Kettenmann et al. 1983b). Figure 1 illustrates the experimental set-up and the measurements with K^+-selective electrodes to support this assumption. It can be seen that when Na^+ was injected into an oligodendrocyte, $[K^+]_i$ decreased. During the constant current pulse the membrane potential continued to decrease as E_K became less negative. Immediately after the current injection stopped, $[K^+]_i$ has a lower value than before ionophoresis and the membrane potential was close to the new E_K. During recovery, $[K^+]_i$ and the membrane potential simultaneously increased. These experiments indicate that the continuous depolarization during the current pulse is a measure for the loss of K^+ during the pulse. The membrane potential at the end of the pulse paralleld the fall in $[K^+]_i$ and the recovery of the membrane potential after the pulse indicated the recovery of $[K^+]_i$. The membrane potential measurement differs slightly from the calculated E_K determined from the measurement of $[K^+]_i$. Part of the discrepancy could be due to leakage induced by the multiple electrodes inserted. It also could reflect an uneven distribution of K^+ in the cell, since $[K^+]_i$ is measured in the center of the soma and membrane potential is determined by the K^+ gradient across and along the membrane. Therefore the membrane potential measurement is probably an averaged measure of K^+ movements across the membrane. With injection of 1–20 nA for 30 s in 25 cells, the calculated depletion of $[K^+]_i$ ranged from 5–40 mmol · l^{-1} and the initial rate of recovery ($\Delta[K^+]_i$/min) was calculated to be in the range of 5–40 mM/min. The time for complete recovery ranged from 2–15 min and varied from cell to cell. The first rapid rate of recovery within 20 s could be related to redistribution of K^+ within the cell.

In a similar experimental arrangement K^+ was injected into oligodendrocytes while membrane potential and $[K^+]_i$ were monitored (Fig. 2). In contrast to the injections of the membrane impermeable ions like Na^+ and TMA^+, depolarizing current pulses resulted in a transient increase in $[K^+]_i$. This indicates that comparable amounts of K^+ ions enter the cell through the electrode and leave the cell through membrane channels. Hyperpolarizing current pulses resulted in a substantial increase of $[K^+]_i$. These increases can be explained by an flux of K^+ across the membrane and an efflux of Cl^- from the KCl-filled electrode since part of the current is carried by Cl^-. The results further confirm that K^+ can be effectively moved across the oligodendrocyte membrane due to current injection.

Results

Mechanisms of recovery of membrane potential following Na^+ injection

Two possible processes leading to a recovery of $[K^+]_i$ following its depletion by intracellular Na^+ injection are (1) an ouabain-sensitive Na^+/K^+-ATPase activity and (2) a KCl symport which can be blocked by furosemide, SITS or DIDS (Kimelberg et al. 1979; Kimelberg 1981). Figure 3 shows that 10^{-5} mol · l^{-1} ouabain completely blocked the recovery of the membrane potential ($N = 5$) and that if extracellular Cl^- was completely replaced by isethionate, acetate or phosphate the recovery was unaffected ($N = 64$).

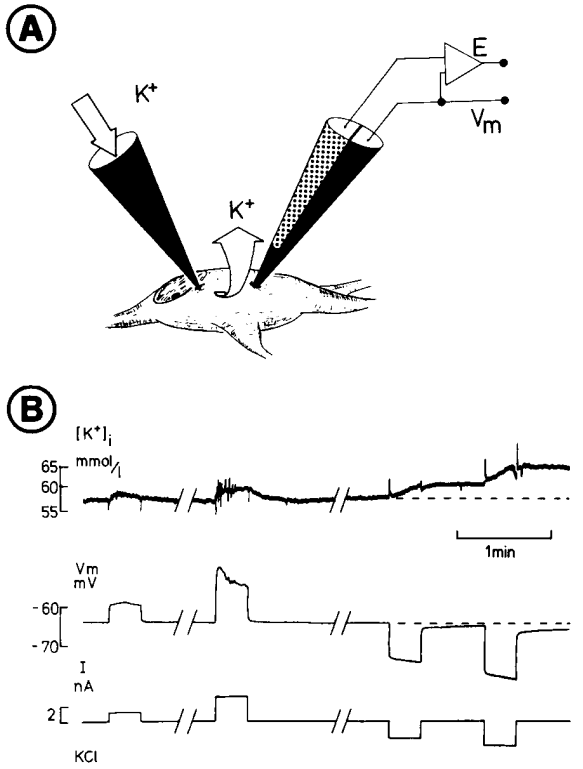

Fig. 2A, B. Response of membrane potential and $[K^+]_i$ to injection of K^+. The experimental arrangement was as described in the legend to Fig. 1 except that the current injecting electrode contained KCl. Injection of positive current resulted in a small, transient increase in $[K^+]_i$. Negative current pulses also increased $[K^+]_i$ indicating that the current is carried by K^+ and Cl^-

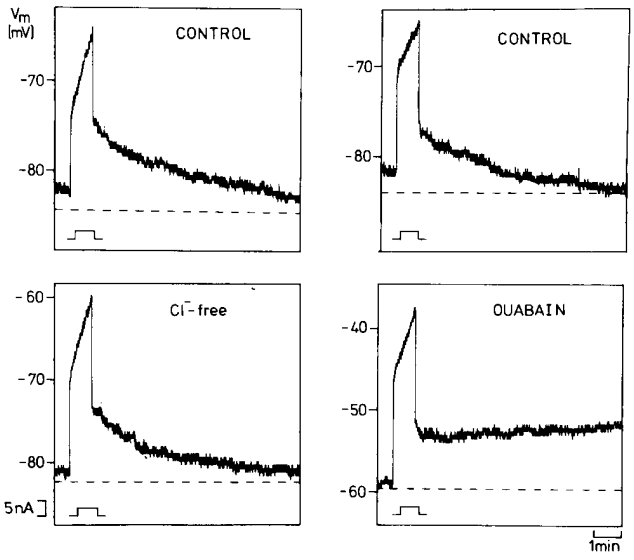

Fig. 3. Effect of Cl^--free bathing solution and ouabain on membrane potential recovery after intracellular Na^+-ionophoresis. The experimental set-up is as described in legend to Fig. 1. The recovery of the membrane potential (V_m) after a current pulse (displayed in each box) in Cl^--free solution (*left side*) and in the presence of ouabain (10^{-5} mol · l^{-1}, *right side*) were measured from one cell. While in Cl^--free bathing solution the recovery of membrane potential was not affected, it was blocked in the presence of ouabain. Since ouabain was applied 15 min before ionophoresis the membrane potential was depolarized in comparison to the control

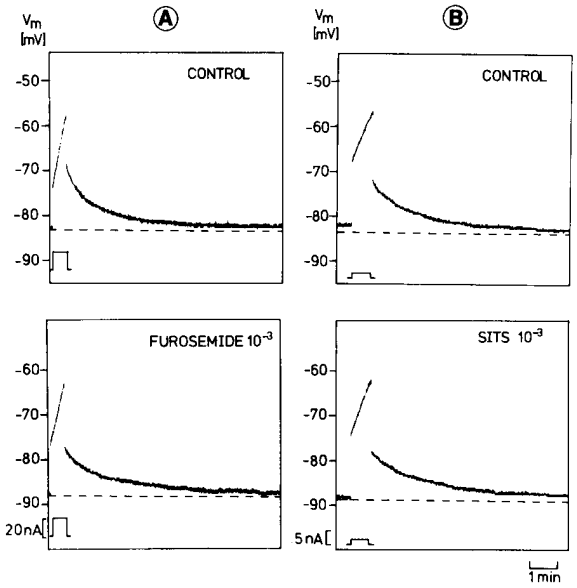

Fig. 4A, B. Effect of furosemide and SITS on membrane potential recovery after intracellular Na^+-ionophoresis. The experimental set-up is as described in legend to Fig. 1. The recovery of the membrane potential (V_m) after a current pulse (displayed in each box) in the presence of furosemide (10^{-3} mol · l^{-1}, **A**) and SITS (10^{-3} mol · l^{-1}, **B**) were measured from one cell. The blockers of Cl^--carrier mechanisms did not influence recovery of the membrane potential

The rate of depolarization after application of ouabain varied from cell to cell and was generally slower than described previously (Kettenmann et al. 1983b). This could be due to improved penetrations in these experiments in contrast to our earlier measurements. Figure 4 demonstrates that the Cl^- transport blockers furosemide (10^{-3} mol · l^{-1}, $N = 11$) and SITS (10^{-3} mol · l^{-1}, $N = 8$) were found not to affect the rate of membrane potential recovery when added to the bathing solution for 5–60 min before Na^+ injection. DIDS (10^{-3} mol · l^{-1}, $N = 2$) also did not affect the recovery (not shown). These results suggest that a Na^+/K^+-ATPase is necessary for the reuptake of K^+ following its intracellular depletion rather than KCl symport.

Characterization of the ionic requirement of the glial Na^+/K^+-ATPase

The stimulus for the pump could be either the increase in intracellular Na^+-concentration ($[Na^+]_i$) or the decrease in $[K^+]_i$ and/or membrane potential produced by the injection. When $[K^+]_i$ is decreased by 20 mmol · l^{-1}, an equimolar amount of Na^+ has to be exchanged during the recovery. It can be assumed that after injection of Na^+, $[Na^+]_i$ is increased and should return toward the base level during the recovery of membrane potential. In contrast, immediately after injection of TMA^+, $[Na^+]_i$ should essentially remain unchanged and decrease during activation of Na^+/K^+-ATPase. Cells were, therefore, penetrated with a NaCl- and a TMACl-filled electrode and recoveries of membrane potential after TMA^+- or Na^+-injection could be compared in one cell. Similarly, recoveries after Li^+ and Na^+ injections were analyzed. Recoveries after TMA^+ ($N = 24$) or Li^+ ($N = 20$) injections were similar to those resulting from Na^+ injections (Fig. 5, for TMA^+-injection). Moreover, the in-

jections of TMA^+ or Li^+ could be repeated 5–10 times without effect on rates of recovery.

In glial cells, $[Na^+]_i$ is in the range of 30–40 mmol · l^{-1} (Bührle and Sonnhof 1983; Coles and Orkand 1985) and it can be calculated that the internal pool of Na^+ is depleted when membrane potential has recovered after two or three injections of TMA^+ resulting in a decrease of 20 mmol · l^{-1} K^+ per injection. The question is whether the pump may be active without intracellular Na^+, or whether Na^+ can enter glial cells by yet unidentified channels or carrier mechanisms. As shown in Fig. 6, when Na^+ was removed from the external medium the membrane potential recovered, if the injected ion was Na^+ ($N = 13$) or Li^+ ($N = 5$), but not if it was TMA^+ ($N = 13$). Thus, some internal Na^+ appears necessary for the activity of the Na^+/K^+-ATPase and Li^+, but not TMA^+, can substitute for Na^+.

It is interesting to note that in the absence of extracellular Na^+ cell input resistance increased and the membrane potential slowly depolarized within few minutes. This depolarization has also been observed in cultured rat astrocytes (Bowman and Kimelberg 1984); the underlying mechanisms are at present unknown.

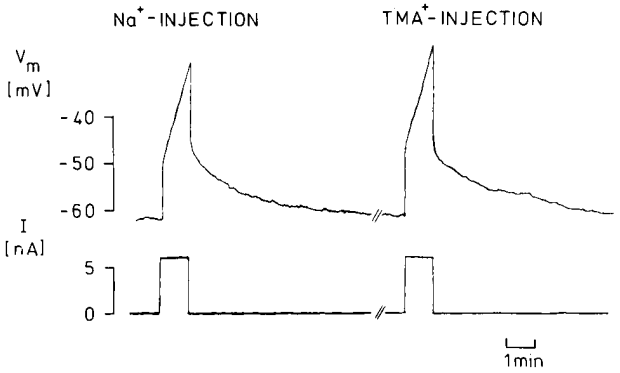

Fig. 5. Comparison of membrane potential recovery after Na^+ and TMA^+ injection. The experimental set-up is as described in legend to Fig. 1. An oligodendrocyte was impaled by a Na^+ and a TMA^+-filled electrode. Current could be injected through each of the two electrodes and the other electrode served to record the membrane potential. This experimental arrangement permitted a comparison between membrane potential recovery after Na^+ (*left traces*) and TMA^+ injection (*right traces*) on one cell

Relation between pump current and $[K^+]_i$

When Na^+ was injected the outward membrane current is predominantly carried by K^+ because the K^+ permeability is much greater (at least 50 times; Kettenmann et al. 1983b) than the Na^+ permeability. Thus, $[K^+]_i$ decreased and we assume that Na^+ increased in the cell. If the membrane continues to depolarize during injection, the outwardly directed K^+ current must exceed the inward K^+ current that results from pump activity. By adjusting a test current pulse to the level at which there is no change in membrane potential during the current injection, the outwardly directed current is equal to the inwardly directed current. Assuming that the inwardly directed current is mediated by the activity of the Na^+/K^+-ATPase one can determine the pump current during the recovery process. During the recovery phase Na^+ was injected with varying current amplitude at different membrane potential values (Fig. 7A). Large current pulses continuously decreased the membrane potential during injection. Small current pulses allowed membrane potential recovery even during current injection. At intermediate current levels the membrane potential was not altered during current injection indicating that the injected current equals the K^+-current generated by the Na^+/K^+-ATPase (Fig. 7A). Pump currents were high when the membrane potential was low and therefore decreased during the recovery phase. In Fig. 7B membrane potential and $[K^+]_i$, calculated according to the Nernst equation, were plotted against the pump current. The pump current was a linear function of the decrease in membrane potential. The extrapolated zero point of the pump current was in most cases at a membrane potential slightly more negative than resting potential (−1.8 mV, range −11.0 to +4.6, $N = 15$). The slope of the pump current versus the membrane potential is 1.8 nA/10 mV (range = 0.4 to 8.2, $N = 15$). Similar pump currents were measured after TMA^+ injection and a slope of pump current versus membrane potential of 2.4 nA/10 mV (range = 0.9 to 6.0, $N = 6$) was found. These results indicate

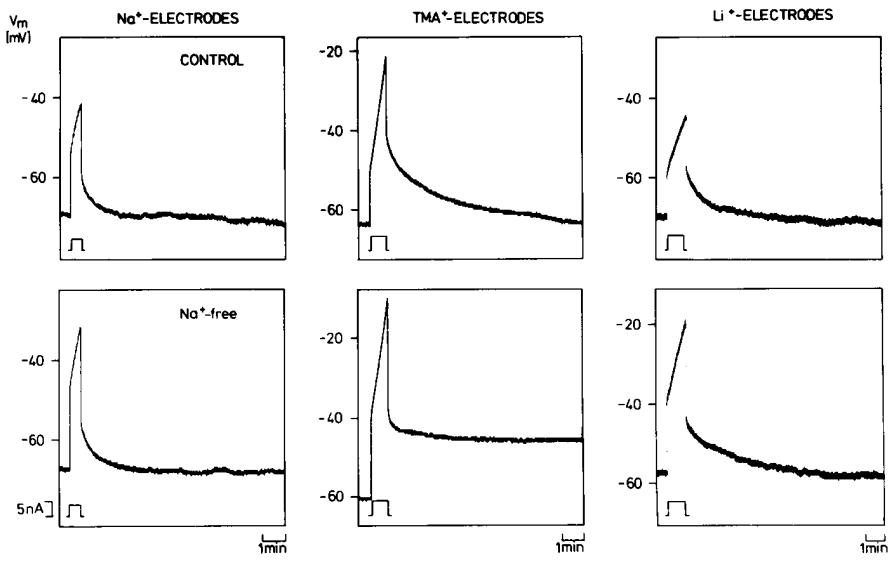

Fig. 6
Recovery of membrane potential after injection of Na^+, TMA and Li^+. The experimental set-up is as described in legend to Fig. 1. Three different cells were penetrated with two NaCl- (*left side*), TMACl- (*middle*) or LiCl- (*right side*) filled electrodes. After current injection the membrane potential recovered in all three cells when the bathing solution contained Na^+ (*upper boxes*). In Na^+-free solution (*lower boxes*) membrane potentials recovered after Na^+ and Li^+, but not after TMA^+ injection. Note that cell input resistance was increased and the membrane slightly depolarized in Na^+-free bathing solution

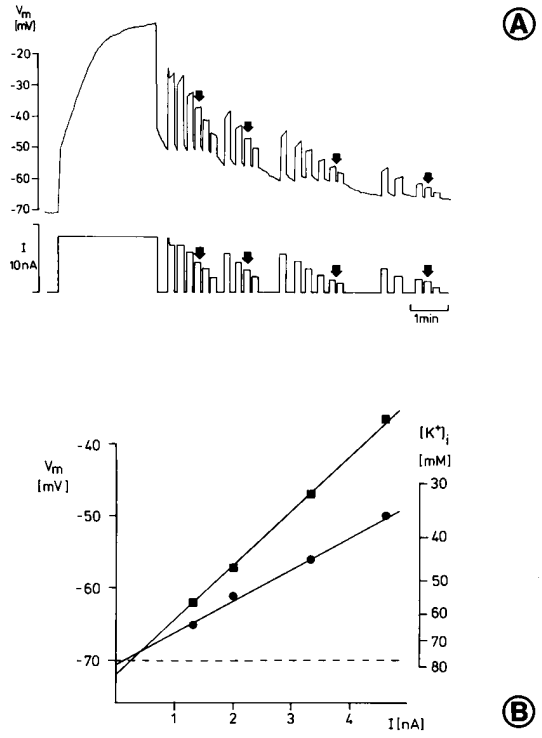

Fig. 7 A, B. Pump current as a function of membrane potential. **A** The experimental set-up is as described in Fig. 1. An oligodendrocyte was depolarized after injection of Na^+ (*I*). During the recovery brief current pulses of decreasing size were applied at four membrane potentials (V_m). Large current pulses further decreased the membrane potential. At intermediate current pulse amplitudes (*arrows*) the membrane potential was unchanged (*arrows*) during injection indicating that the injected current equals the pump activity of the Na^+/K^+-ATPase for K^+. During smaller current pulses membrane potential continued to recover. **B** The values of the current pulses (*I*) marked by *arrows* in **A** are plotted against the corresponding membrane potential during (■) and immediately after termination of current injection (●). The scale on the *right side* indicates $[K^+]_i$ calculated according to the Nernst equation based on membrane potential after termination of the current pulse and on the observation that the membrane is predominantly permeable to K^+. The calculated *regression lines* connecting the *data points* indicate that the current probably generated by the Na^+/K^+-ATPase is a linear function of membrane potential and the log of $[K^+]_i$. The current is zero at a membrane potential that is slightly more negative than the resting membrane potential (indicated by the *dotted line*)

that the Na^+/K^+-ATPase is activated when membrane potential and/or $[K^+]_i$ are decreased.

Discussion

Mechanism of K^+ reuptake following depletion

Because the oligodendrocyte permeability to K^+ is much greater than to other ions (Kettenmann et al. 1983b) the injection of positive charge into the cell leads to a decrease in cellular $[K^+]$. Our finding that the reuptake of K^+ is ouabain-sensitive suggests that an important mechanism for uptake under these conditions is the Na^+/K^+-ATPase. The failure of Cl^--free solutions or Cl^- transport inhibitors to affect the uptake indicates that in oligodendrocytes KCl uptake does not contribute substantially to $[K^+]_i$ recovery following its depletion by ionophoresis. In contrast, when extracellular K^+ is raised astrocytes have been reported to accumulate K^+ by cotransport with Cl^- (Kimelberg et al. 1979) and Cl^- is known to enter glial cells in the retina of the bee drone (Coles and Orkand 1984).

Regulation of the glial Na^+/K^+-ATPase-activity

The observation that K^+-uptake is similar, when TMA^+ or Li^+ are injected instead of Na^+ suggests that it is predominantly $[K^+]_i$ and not $[Na^+]_i$ which controls the activity of the Na^+/K^+-ATPase at levels of normal or low $[Na^+]_i$. We do not exclude that an increase in $[Na^+]_i$ under different experimental conditions could stimulate the Na^+/K^+-ATPase. However, our experiments cannot distinguish whether the membrane potential also contributes to the regulation of the Na^+/K^+-ATPase, since membrane potential is directly linked to $[K^+]_i$.

We would like to suggest, therefore, that the activity of the Na^+/K^+-ATPase in oligodendrocytes can be regulated by $[K^+]_i$ and/or membrane potential. The pump current of giant snail neurons was found to depend on membrane potential (Kostyuk et al. 1972). Since in neurons Na^+/K^+-ATPase depends predominantly on $[Na^+]_i$ (Thomas 1969) our findings imply that the Na^+/K^+-ATPase of glial cells is different from that of neurons. Indeed, two biochemically distinct forms of Na^+/K^+-ATPase have been observed isolated from brain and enriched populations of neurons and glial cells. The two molecular forms can be distinguished by their sensitivity to strophanthidin (Sweadner 1979) and their different activation by K^+-ions (Grisar et al. 1979, 1980; Franck et al. 1983).

The observation that in Na^+-free solutions recovery only followed Na^+ and Li^+ injection but not that of TMA^+ indicates that some internal Na^+ or Li^+, a substitute for Na^+ transported by the neuronal Na^+/K^+-ATPase (Ritchie and Straub 1980; Tobin et al. 1974), are necessary for Na^+/K^+-ATPase activity. In Na^+-free solutions, levels of $[Na^+]_i$ have been observed to fall below those required for the pump to function (Coles and Orkand 1985). Presumably low levels of Na^+ sufficient to trigger the intracellular enzyme site in the cell are sustained by entrance through membrane leakage, by Na^+-mediated carrier mechanisms such as Na^+/H^+-exchange (Schlue and Thomas 1985) Na^+/HCO_3^--cotransport (Deitmer and Schlue 1987) or from coupled cells (Kettenmann et al. 1983a).

Role of Na^+/K^+-ATPase in K^+ homeostasis by neuroglia

These results are difficult to relate to the mechanisms responsible for the glial uptake of K^+ when $[K^+]_i$ is normal and $[K^+]_o$ is elevated as a result of neuronal activity. Under these conditions there is a net uptake of K^+ in cultured oligodendrocytes (Kettenmann et al. 1983b), astrocytes (Hertz 1978) and in glial cells in the drone retina (Coles and Tsacopoulos 1979).

When glial cells are involved in the space-dependent clearance of K^+ via spatial buffer currents, K^+ enters the cells in regions where $[K^+]_o$ is elevated and leaves the cell at distant regions where $[K^+]_o$ is more nearly normal (Coles

and Orkand 1983; Gardner-Medwin 1983; Kettenmann et al. 1985a; Nicholson 1980). Because the transmembrane current is essentially only carried by K^+ and there are a number of other mobile ions in the cell there should be a depletion of $[K^+]_i$ in regions where K^+ leaves the cell. The present results suggest that at these regions of the cell a reuptake of K^+ as a result of increased Na^+/K^+-ATPase activity may occur.

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