GLIA 4:56–63 (1991)

Role of Glia in K^+ and pH Homeostasis in the Neonatal Rat Spinal Cord

PAVLA JENDELOVÁ AND EVA SYKOVÁ
Laboratory of Neurohumoral Regulation, Institute of Physiological Regulations, Czechoslovak Academy of Sciences, 180 85 Prague 8, Czechoslovakia

ABSTRACT Stimulation-evoked transient changes in extracellular potassium ($[K^+]_e$) and pH (pH_e) were studied in the neonatal rat spinal cords isolated from 3–13-day-old pups. In unstimulated pups the $[K^+]_e$ baseline was elevated and pH_e was more acid than that in Ringer's solution (3.5 mM K^+, pH 7.3–7.35). The $[K^+]_e$ and pH_e in 3–6-day-old pups was 3.91 ± 0.12 mM and pH_e 7.19 ± 0.01, respectively, while in 10–13-day-old pups it was 4.35 ± 0.15 mM and 7.11 ± 0.01, respectively. The $[K^+]_e$ changes evoked in the dorsal horn by a single electrical stimulus were as large as 1.5–2.5 mM. Such changes in $[K^+]_e$ are evoked in the adult rat spinal cord with stimulation at a frequency of 10–30 Hz. The maximal changes of 2.1–6.5 mM were found at a stimulation frequency of 10 Hz in 3–6-day-old animals. In older animals the $[K^+]_e$ changes progressively decreased. The poststimulation K^+-undershoot was found after a single stimulus as well as after repetitive stimulation.

In 3–8-day-old pups, the stimulation evoked an alkaline shift, which was followed by a smaller poststimulation acid shift when the stimulation was discontinued. In pups 3–4-days-old the stimulation evoked the greatest alkaline shifts, i.e., by as much as 0.05 pH units after a single pulse and by about 0.1 pH units during stimulation at a frequency of 10 Hz. In 5–8-day-old pups, the alkaline shift became smaller and the poststimulation acid shift increased. Stimulation in 10–13-day-old pups produced an acid shift of 0.03–0.07 pH units, which was preceded by a scarcely discernible alkaline shift. $MgCl_2$ (20 mM) reversibly reduced the alkaline but not the acid shifts by 50–60%. Bath application of the carbonic anhydrase inhibitor acetazolamide had no effect on the alkaline shift, while the acid shift decreased by 70–80%. The superfusion of the cord with 10 mM KCl resulted in acid shifts of 0.10–0.14 pH units.

We conclude that the $[K^+]_e$ ceiling level and the character of pH_e transients in the spinal cord are closely related to gliogenesis. Our results suggest that glial cells buffer the activity-related $[K^+]_e$ increase and alkaline pH_e shifts in the extracellular space.

INTRODUCTION

Neuronal activity is accompanied by long-term ionic and volume changes in the neuronal microenvironment (for review see Ballanyi and Grafe, 1988; Nicholson, 1980; Syková, 1983, 1989). There is evidence that glial cells play an important role in extracellular ion homeostasis, as suggested by Hertz (1965) and Orkand et al. (1966), particularly by buffering activity-related extracellular K^+ changes ($[K^+]_e$) (for review see Walz, 1989). Despite many studies of ion and volume changes using ion-selective microelectrodes, the role of glia in the regulation of other ionic shifts is far from clear. Recent findings of stimulation-evoked extracellular pH changes (pH_e) in the brain led to investigation of the role of glia in the regulation of pH_e. Activity-related variations in pH_e have been demonstrated in the cerebral cortex (Urbanics et al., 1978), in the cerebellum (Chesler and Chan, 1988; Kraig et al., 1983), in the hippocampus (Krishtal et al., 1987; Somjen, 1984), in the spinal cord (Chvátal et al., 1988; Syková and Svoboda, 1990), and in the optic nerve during development (Ransom et al., 1985b). The changes found in the brain or in the spinal cord were not simple monophasic shifts. In the spinal

Received March 28, 1990; accepted June 27, 1990.

Address reprint requests to Dr. Eva Syková, Institute of Physiological Regulations, Czechoslovak Academy of Sciences, Bulovka, Pavilon 11, 180 85 Prague 8, Czechoslovakia.

cord the changes are triphasic—alkaline-acid-acid in the isolated frog spinal cord (Chvátal et al., 1988) and alkaline-acid-alkaline in the adult rat spinal cord in vivo (Syková and Svoboda, 1990). The initial alkaline shift is often masked by the dominating acid shift.

In this study, we attempted to investigate the role of glia in the regulation of pH_e. The enzyme carbonic anhydrase (CA), which is mainly located in glial cells and myelin (Cammer and Tansley, 1988; Giacobini, 1962; Rousel et al., 1979) and which catalyzes the reversible conversion of CO_2 and water to HCO_3^- and H^+, can be expected to play an important role in pH_e regulation. Moreover, the findings that glial cell membranes are highly permeable to HCO_3^- led to the suggestion that glial cells can buffer transient changes in acid-base balance in the extracellular space at the expense of their intracellular pH (Astion and Orkand, 1988; Kettenmann and Schlue, 1988). Since glial proliferation, maturation, and myelinization occur to a great extent postnatally (Gilmore, 1971; Gilmore et al., 1982; Sims et al., 1985; Yamate and Ransom, 1985) and CA activity likewise increases postnatally (Davis et al., 1987), important developmental changes in pH and K^+ homeostasis were expected in the spinal cord of mammals. We studied such changes in isolated neonatal rat spinal cord from 3–13-day-old pups.

MATERIALS AND METHODS

Preparation

Experiments were performed on 3–13-day-old rat pups (Wistar). Animals were decapitated and the lumbosacral spinal cord was dissected in a chamber with cold (9–11°C) modified Ringer's solution of the following composition (in mM): NaCl 113.0, KCl 3.5, $CaCl_2$ 2.0, Na_2HPO_4 2.0, $NaHCO_3$ 28, glucose 1 g/l. The isolated cord was placed in a small chamber and the preparation was continuously perfused with Ringer's solution. During 1–2 h the temperature was increased to 21–23°C. The solution was saturated with 95% O_2 and 5% CO_2 (pH 7.3–7.35). In some experiments the oxygenation of the preparation was enhanced by addition of hydrogen peroxide (Walton and Fulton, 1983); however, the results from spinal cords perfused with a solution not containing hydrogen peroxide were no different. Solutions with 20 mM $MgCl_2$ or 5 mM acetazolamide (Ciech) had a reciprocally reduced Na^+ concentration.

Stimulation and Recording

Microelectrodes were inserted into the cord from the dorsal spinal surface. Recordings were made from lumbar segments L_4 or L_5. The dorsal root of the same segment was stimulated supramaximally (rectangular pulses of 5 V or less; duration 0.1 ms) with fine bipolar silver electrodes.

Local field potentials were recorded with the reference barrel of an ion-selective microelectrode. K^+ activity was recorded with double-barrelled K^+-selective microelectrodes filled with a liquid ion-exchanger (Corning 477317) prepared by the procedure described previously (Kříž et al., 1974). K^+-selective microelectrodes were calibrated in solutions containing 3, 4, 5, 6, 8, or 10 mM KCl in 150 mM NaCl. Basically, the same procedure was adopted to prepare the double-barrelled pH-sensitive microelectrodes (Chvátal et al., 1988; Syková and Svoboda, 1990). In principle, the reference channel was filled with 0.15 M NaCl solution while the pH-sensitive channel contained, in a siliconized tip, a 200–1,000 μm column of liquid Hydrogen Ion Ionophore II-Cocktail A (Fluka). The backfilling solution was composed of (mM): KH_2PO_4 40.0, NaOH 23.0, NaCl 15.0 (pH 7.0). Electrode sensitivity was tested in standard solutions the pH of which was 7.0, 7.2, 7.4, 7.6, 7.8, or 8.0 with a background of 150 mM NaCl and 3 mM KCl. The slope of the electrodes was about 57 mV/unit of pH change and the electrodes had a resistance of 700–1,800 MΩ. The electrical arrangements were the same as described for K^+-selective microelectrodes (Kříž et al., 1974). Each channel of a double-barrelled microelectrode was connected to one input of a differential amplifier. Microelectrodes were inserted into the spinal cord using two micromanipulators.

RESULTS

Resting Level of $[K^+]_e$ and pH_e

The resting values of $[K^+]_e$ and pH_e in the unstimulated spinal cord were established by comparing the potentials of the K^+-selective microelectrode and pH-sensitive microelectrode in Ringer's solution above the surface of the isolated cord with that in the spinal dorsal horn. The $[K^+]$ in the Ringer's solution was 3.5 mM and pH was 7.30–7.35 as measured by a pH meter. In 3–6-day-old pups the actual $[K^+]_e$ value in the spinal dorsal horn was 3.91 ± 0.12 mM (n = 14, mean ± S.E. of mean). The pH_e in the spinal dorsal horn was 7.19 ± 0.01 (n = 14), i.e., by about 0.16 pH units more acid than that in Ringer's solution. The actual values of $[K^+]_e$ in 10–13-day-old animals were significantly higher than those in 3–6-day-old animals, i.e., 4.35 ± 0.15 mM (n = 10; $P < 0.05$). The pH_e in 10–13-day-old pups was 7.11 ± 0.01 (n = 10), i.e., by 0.24 pH units more acid than in Ringer's solution, i.e., it was significantly more acid than in 3–6-day-old animals ($P < 0.01$).

Stimulation-Evoked Transient Changes in $[K^+]_e$ and pH_e

The stimulation of peripheral nerves or dorsal roots with single electrical pulses results in an increase of $[K^+]_e$ in the spinal dorsal horn of adult rats that does not exceed 0.3 mM, and in an acid shift in pH_e of about 0.005 pH unit. During repetitive electrical stimulation the $[K^+]_e$ increases by 1.0–1.5 mM at a stimulation fre-

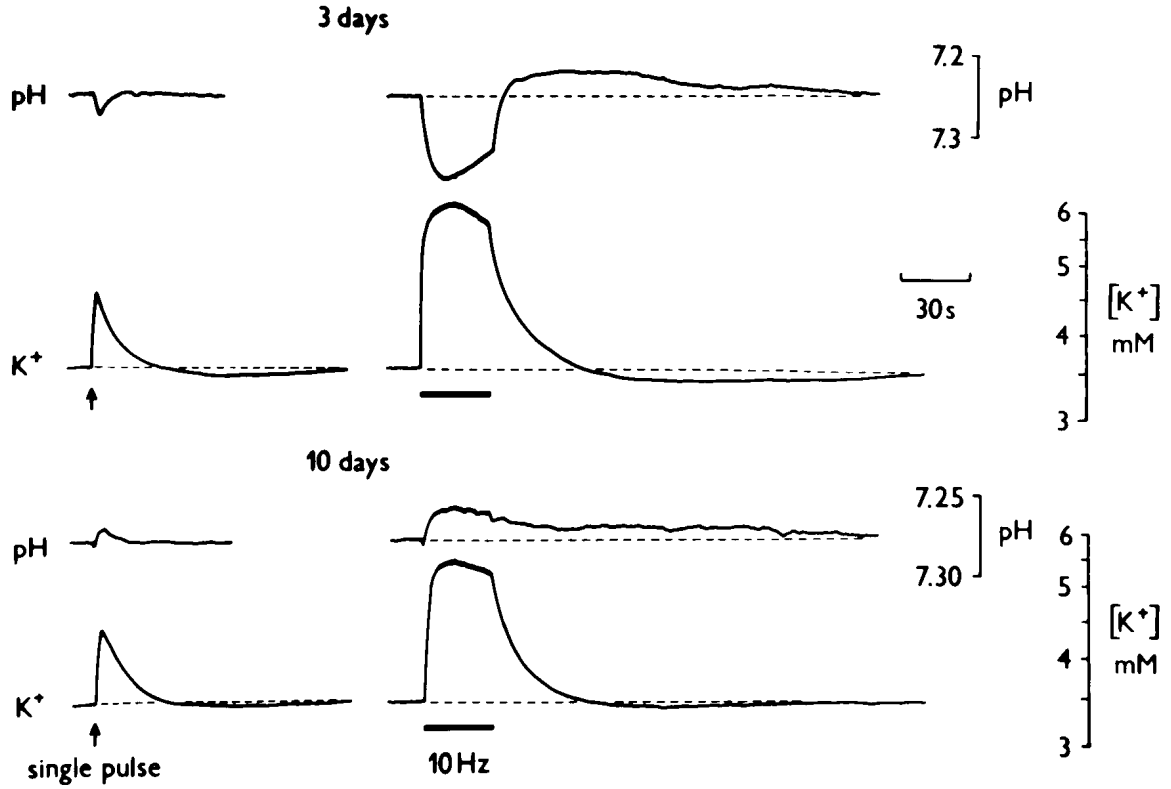

Fig. 1. Stimulation-evoked pH_e and $[K^+]_e$ changes in the spinal dorsal horn of rats 3 and 10 days old. Note that the stimulation of the dorsal root with a single electrical pulse and at a frequency of 10 Hz evoked an alkaline shift in the 3-day-old pup, which was accompanied by an increase in $[K^+]_e$; when stimulation was discontinued the poststimulation acid shift of smaller amplitude appeared, which was accompanied by a K^+-undershoot. In the 10-day-old rat the $[K^+]_e$ increase was smaller; there was a slight initial alkaline shift, which was followed by an extracellular acid shift.

quency of 10 Hz and by 2.5–3.5 mM at 30 Hz when the K^+ ceiling level is achieved (Svoboda et al., 1988). The transient pH_e changes during repetitive stimulation are dominated by an acid shift of 0.1–0.2 pH units that is preceded by an initial alkaline shift of about 0.005 pH units (Syková and Svoboda, 1990).

In the neonatal rat spinal cord the stimulation-evoked changes of $[K^+]_e$ were much larger than in adult animals. We found that in 3–13-day-old pups the $[K^+]_e$ changes evoked by a single electrical stimulus are as large as those evoked in the adult rat spinal cord with repetitive stimulation at a frequency of 10–30 Hz, i.e., by as much as 1.5–2.5 mM (Fig. 1). The maximal changes were evoked in animals 3–6 days old with repetitive electrical stimulation at 10 Hz (Fig. 2), and corresponded to about 2.1–6.5 mM (3.38 ± 0.67, n = 6, mean ± S.E. of mean). In older animals the $[K^+]_e$ changes decreased, but even in 7–13-day-old pups the K^+ ceiling level was still higher than in adult rats. The stimulation frequency higher than 10 Hz did not evoke greater changes. It is therefore evident that the so-called K^+ ceiling level is achieved in neonatal spinal cords in vitro at a lower frequency than in adult animals in vivo and that the younger the rat the greater the stimulation-evoked K^+ transients.

The depth profile of $[K^+]_e$ changes was the same as in the adult rats. The greatest $[K^+]_e$ changes were found in areas of maximal field potential, i.e., in Rexed laminae III–V. The clearance of $[K^+]_e$ changes in the neonatal spinal cord was as fast as in the adult cord. The post-stimulation K^+-undershoot was found after single electrical pulses as well as after repetitive stimulation.

The stimulation-evoked pH_e transients in newborn rats differed substantially from the pH_e transients found in adult animals. In 3–8-day-old pups the stimulation-evoked dominating alkaline shift was followed by a smaller poststimulation acid shift when the stimulation was discontinued. Single pulses evoked alkaline shifts of as much as 0.05 pH units; repetitive stimulation at a frequency of 10 Hz evoked an alkaline shift of about 0.1 pH units (Figs. 1, 3, 4). The alkaline shifts were greatest in 3–4-day-old pups. In pups 5–8 days old the alkaline shift became smaller and the poststimulation acid shift was greater (Fig. 4). In some 7–9-day-old animals the changes during stimulation became biphasic, i.e., alkaline-acid shifts (Fig. 4). In 10–13-day-old pups stimulation evoked the fast acid shifts by about 0.03–0.07 pH units, which was preceded by a scarcely discernible initial alkaline shift (Fig. 1) as is the case in adult rats. However, the dominating acid shifts in these

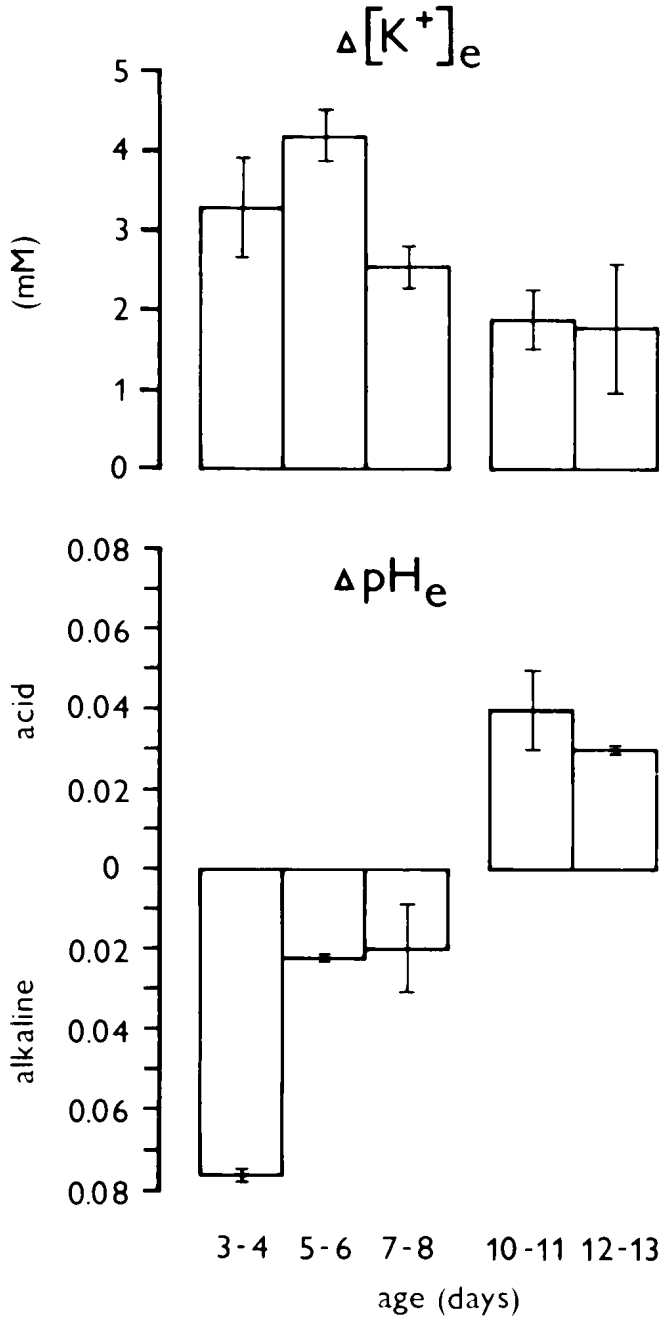

Fig. 2. $[K^+]_e$ and pH_e changes in the spinal dorsal horn in rats 3–13 days old. Each column represents data from animals 3–4 days old (n = 6); 5–6 days old (n = 4); 7–8 days old (n = 4); 10–11 days old (n = 4); and 12–13 days old (n = 3; mean ± S.E. of the mean). All recordings at a depth of about 200 μm, stimulation frequency 10 Hz.

animals were still smaller than in adult rats in vivo and were not followed by the typical poststimulation alkaline undershoot (Syková and Svoboda, 1990).

These findings show that the activity-related increase in $[K^+]_e$, the K^+ ceiling level, and the alkaline-acid shifts in pH_e in spinal cord change during the postnatal development.

Effect of Mg^{2+}

Mg^{2+} and some other divalent cations are known to block Ca^{2+}-dependent presynaptic transmitter release. The application of 20 mM $MgCl_2$ lowered the stimulation-evoked $[K^+]_e$ increase by 50–65%. As has been originally suggested for the isolated frog spinal cord, the $[K^+]_e$ changes in high Mg^{2+} are apparently due to the K^+ release from primary afferents themselves (Syková and Vyklický, 1977). Fig. 3 shows the typical effect of Mg^{2+} on pH_e and $[K^+]_e$ changes evoked by repetitive electrical stimulation (10 Hz) in the dorsal horns of pups 3 and 10 days old. In both animals the $[K^+]_e$ changes decreased after blockade of synaptic transmission by Mg^{2+}. However, while the alkaline shift was also reduced by about 50–60% in 3–6-day-old pups, the Mg^{2+} application had no effect on the acid shifts in 10–13-day-old pups. Our results suggest that the activity-related acid shifts in the latter group of pups are not directly related to synaptic activity.

Effect of the CA Inhibitor Acetazolamide

In 3–8-day-old pups, when the dominating stimulation-evoked pH_e change was the alkaline shift, acetazolamide in a 5 mM concentration had either no effect on the alkaline shifts or it slightly enhanced them. This enhancement was apparently due to the blockade of the poststimulation acid shifts (Fig. 4). In the spinal cords of pups 10 to 13 days old the typical stimulation-evoked acid shifts decreased by 70–80% after application of acetazolamide. Acetazolamide had no effect on simultaneously recorded changes in $[K^+]_e$.

K^+-Evoked pH_e Changes

We examined the extent to which the $[K^+]_e$ changes evoked by stimulation are related to the observed alkaline or acid shifts in pH_e. In the isolated frog spinal cord (Chvátal et al., 1988) and in the rat cerebellar cortex (Kraig et al., 1983) perfusion of the tissue with Ringer's solution containing elevated $[K^+]$ results in an acid shift in pH_e of about 0.2 pH units. In 3–13-day-old pups perfusion of the spinal cord with 10 mM K^+ in Ringer's solution elevated $[K^+]_e$ at a depth of 200 μm by about 7.5–9.5 mM (Fig. 5). Elevation of $[K^+]_e$ after perfusion of the nervous tissue is known to be a function of time and is attenuated with depth (Chvátal et al., 1988; Kraig et al., 1983). Concomitantly with the $[K^+]_e$ increase, the pH_e in the dorsal horn progressively decreased (Fig. 5). The maximal evoked acid shift in the isolated cord of 3–6-day-old pups was by 0.10–0.14 pH units (n = 7). In 10–13-day-old animals the acid shift was about the same, i.e., by 0.10–0.12 pH units (n = 3). A change to the original Ringer's solution containing 3.5 mM K^+ promptly returned $[K^+]_e$ and pH_e to the original baselines.

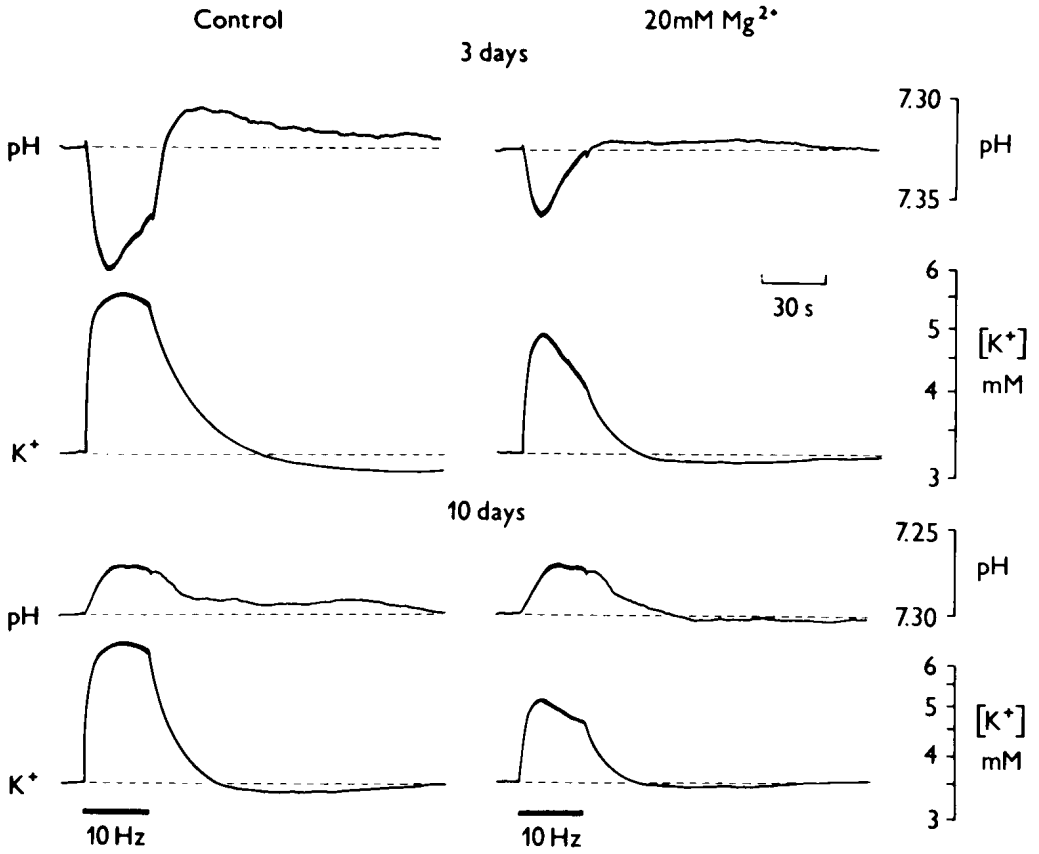

Fig. 3. Effect of 20 mM Mg^{2+} on pH_e and $[K^+]_e$ changes in rats 3 and 10 days old. Note decrease of the alkaline shift, decrease of the poststimulation acid shifts, and decrease of $[K^+]_e$ changes. Only the acid shift that occurred during stimulation in the 10-day-old rat remained unaffected.

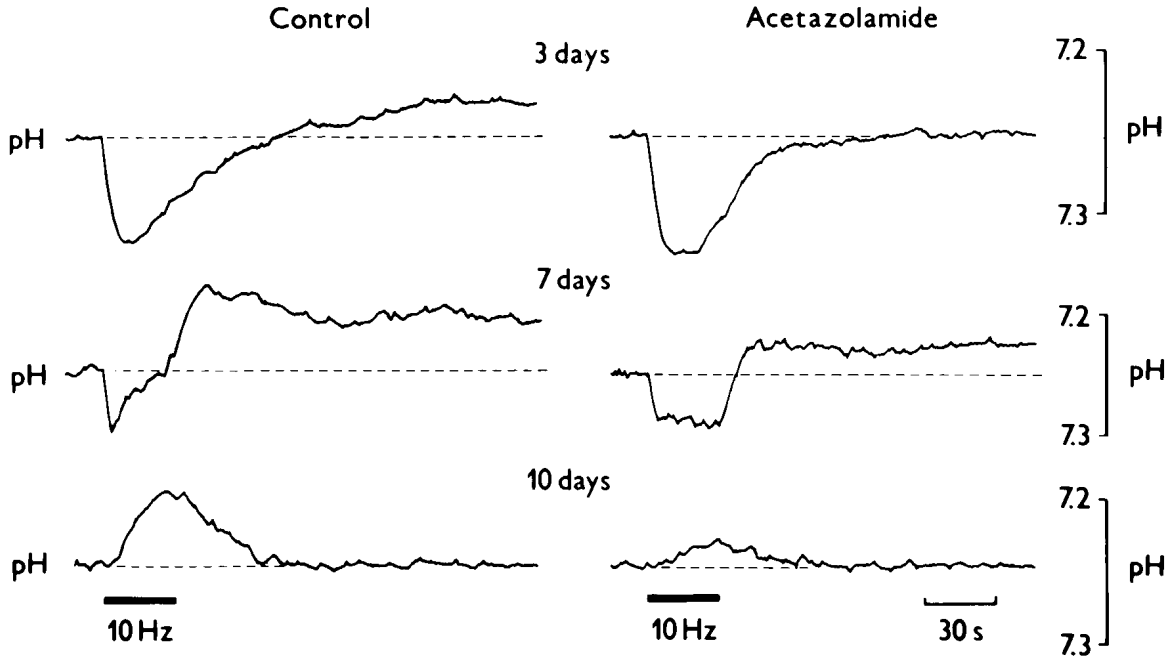

Fig. 4. Effect of acetazolamide (5 mM) on pH_e changes evoked by stimulation at a frequency of 10 Hz in 3-, 7-, and 10-day-old rats. The alkaline shift was not changed, while the acid shifts during and after stimulation were substantially decreased.

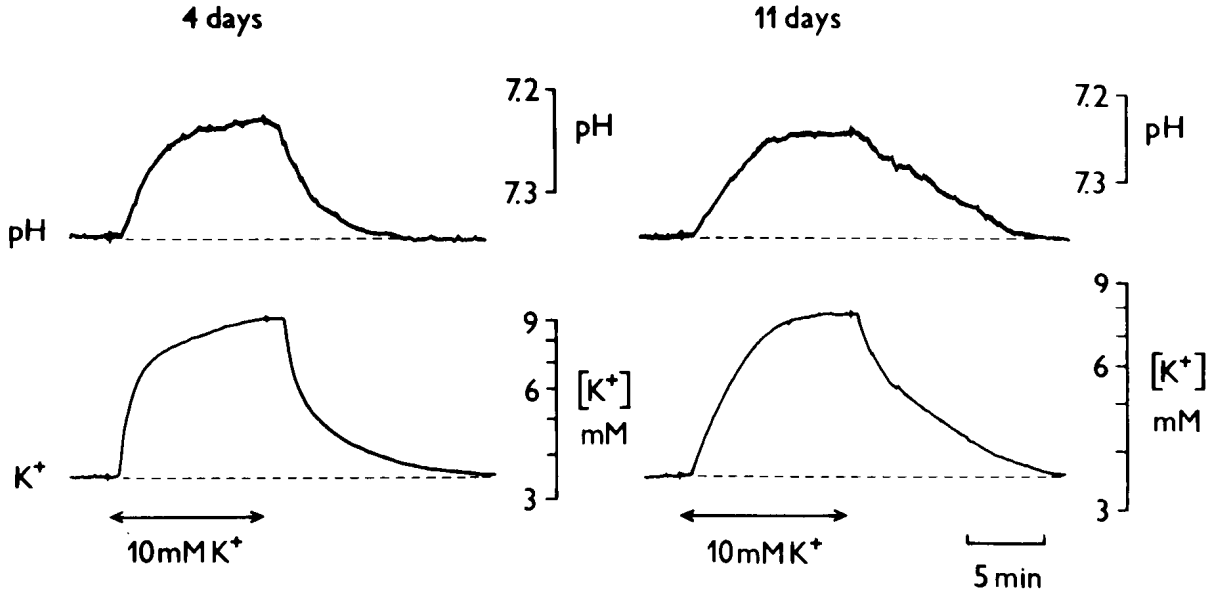

Fig. 5. Simultaneous pH_e and $[K^+]_e$ records in the dorsal horn of rats 4 and 11 days old during perfusion with a Ringer's solution containing 10 mM K^+. All recordings at depth of 200 μm.

Our results show that the KCl-evoked depolarization of neurones and glial cells in the neonatal rat spinal cord is accompanied by an acid change in the pH_e baseline.

DISCUSSION

K^+ Homeostasis

Our study shows that stimulation of the afferent input produces much larger changes in $[K^+]_e$ in the immature rat spinal cord than in the adult spinal cord (see also Walton and Chesler, 1988). Similar larger stimulus-evoked $[K^+]_e$ changes were found in the immature optic nerve (Connors et al., 1982; Ransom et al., 1985a) and the cerebral cortex (Hablitz and Heinemann, 1987, 1989; Mutani et al., 1974). The changes are larger in spite of the facts that the extracellular space (ECS) in immature nervous tissue is wider and that stimulation does not cause ECS shrinkage (Ransom et al., 1985b; Svoboda and Syková, 1989), which can aggravate the ionic changes. It is also of interest that the presynaptic release of K^+ is greater in the immature spinal cord than in the isolated frog spinal cord. After synaptic blockade by Mg^{2+} we still found about 35–50% of stimulation-evoked $[K^+]_e$ increase due to the K^+ release from primary afferents compared with 10–35% in the frog spinal cord (Fig. 3; Davidoff et al., 1988; Nicoll, 1979; Syková and Vyklický, 1977). Our results also show that $[K^+]_e$ is elevated to 3.91–4.34 mM K^+ in the unstimulated rat spinal cord, as in the adult rat spinal cord in vivo (Svoboda et al., 1988) and in the isolated frog spinal cord (Sykova et al., 1983) where $[K^+]_e$ is elevated due to spontaneous activity of spinal interneurones. The $[K^+]_e$ baseline in 10–13-day-old pups was significantly higher than that in 3–6-day-old pups, suggesting that the level of spontaneous activity increases with age.

The enhanced ionic changes in the ECS in immature tissues could be due to some homeostatic mechanisms not yet fully developed. It has been estimated that the $[K^+]_e$ increase per single stimulus would be about 1 mM if the processes responsible for K^+ clearance are not acting (Adelman and Fitzhugh, 1975). A variety of studies have shown that both neurones and glial cells are involved in K^+ clearance in the CNS (for reviews see Syková, 1983; Walz, 1989). A part of the K^+ that accumulates in the ECS is removed by immediate reuptake by active neurones (Kříž et al., 1975) and by diffusion in the ECS. The rest has been shown to be taken up by glial cells, either by a spatial buffer mechanism (Gardner-Medwin, 1980; Newman, 1986; Nicholson and Phillips, 1981; Orkand et al., 1966; Syková et al., 1988), or by K^+ uptake either by pumping K^+ via the Na/K pump or by channel-mediated KCl uptake (for review see Walz, 1989). Since the $[K^+]_e$ recovery after stimulation does not significantly differ in the immature and adult rat spinal cord (see K^+ undershoots in Figs. 1 and 3), it is possible that gliogenesis significantly decreases the $[K^+]_e$ ceiling level, i.e., enhances the K^+ clearance during stimulation. The higher K^+ ceiling level in immature animals and the fact that it is attained at a lower stimulation frequency therefore speak in favor of the role of glia in the regulation of $[K^+]_e$.

On the other hand, the rate of K^+ release from neurones determines the activity-related increase in $[K^+]_e$. Obviously, this factor can also change during development (Connors et al., 1982) as well as the properties, distribution, and density of K^+ and Cl^- channels (for further discussion see Walton and Chesler, 1988; Walz, 1989). Thus the postnatal alterations in the amplitude of $[K^+]_e$ increase can include several mechanisms, namely the changes in the rate of K^+ reuptake, the rate of K^+ release from neurones, the regulation of ECS

volume and maturation, and/or increase in the number of glial cells.

It is reasonable to assume that the large $[K^+]_e$ transients attained in the immature spinal cord even with a single electrical stimulus (by 1.5–2.5 mM) can modulate neuronal activity and spinal cord transmission in the neonatal rat spinal cord and may play a role in spinal cord development. The smaller $[K^+]_e$ transients in adult rats can be the result of glial cell participation in K^+ homeostasis, even when the mechanisms by which glial cells are involved are not yet completely clear. The number of astrocytes and oligodendrocytes changes, and their maturation and the myelinization of fibers occurs postnatally (Connors et al., 1982; Gilmore, 1971; Gilmore et al., 1982; Sims et al., 1985; Yamate and Ransom, 1985). The membrane properties of glial cells may also change postnatally including the density of K^+ channels or glial Na^+/K^+ ATPase sensitivity to increased $[K^+]_e$ (Grisar and Franck, 1981).

pH_e Homeostasis

During the first 8 postnatal days, stimulation of the afferent input evoked large alkaline changes in the extracellular microenvironment, which were followed by slow and small acid shifts after stimulation has been discontinued. These changes progressively decreased during further development. The acid shift became the dominating pH_e change about 10 days after birth, at the same time that the K^+ ceiling level started to decrease. Alkaline shifts evoked by stimulation have been found in the unmyelinated rat optic nerve of the 1-day-old pups, while the acid shift occurred at about 10 days and later, when $[K^+]_e$ changes became smaller (Ransom et al., 1985b). In the rat optic nerve, proliferation of astrocytes, oligodendrocytes, and myelination also occurs postnatally (Foster et al., 1982) and the time course correlates well with the observed development of the acid shift and with the decrease in the K^+ ceiling level (Ransom et al., 1985b).

The pH_e baseline in the adult rat spinal cord in vivo in the dorsal horns is about 7.15, i.e., it is more acid by about 0.2 pH units than that in the cerebrospinal fluid (Syková and Svoboda, 1990). In the immature rat spinal cord the pH_e was also more acid than that in Ringer's solution. The pH_e was significantly more acid in 10–13-day-old pups (about 7.1) than in 3–6-day-old pups (about 7.2). It has been shown in a number of studies that pH_e in the mammalian CNS is more acid than that in arterial blood and cerebrospinal fluid (Cragg et al., 1977; Kraig et al., 1983, 1986; Siesjo et al., 1985; Syková and Svoboda, 1990). The mechanism of continuous acid extrusion from cells to the interstitial fluid is far from clear. In addition to classical membrane acid extrusion mechanisms, both neurones and the glia can extrude lactic acid. Recent studies of cultured neurones and astrocytes revealed that the glia can effectively extrude lactic acid (Walz and Mukerji, 1988).

It is reasonable to assume that glial cells regulate pH_e at the expense of their intracellular pH. It has been found that astrocytes show an alkaline shift during stimulation-evoked extracellular acid shifts or during the extracellular K^+ increase (Chesler and Kraig, 1989; Walz, 1989). The mechanism of the alkaline shift in glial cells is not yet clear, but the basic transport processes that regulate pH in glia have recently been described (Astion and Orkand, 1988; Astion et al., 1989; Ballanyi and Schlue, 1990; Deitmer and Schlue, 1987, 1989; Kettenmann and Schlue, 1988). These involve amiloride-sensitive Na^+/H^+ exchange, SITS-sensitive Cl^-/HCO_3^- exchange (astrocytes and neuropile glial cells), and SITS-insensitive Na^+-HCO_3^- cotransport (oligodendrocytes).

We thus conclude that, in the spinal cord as in the optic nerve (Ransom et al., 1985b), the occurrence of stimulation-evoked acid shifts is related to gliogenesis. In our experiments, the acid shift was effectively blocked by the CA inhibitor acetazolamide. On the other hand, the alkaline shift, but not the acid shift, was directly related to synaptic activity. It was found that when snail neurones are depolarized under voltage clamp, their surface pH_e at first increased, i.e., it became alkaline and then decreased, i.e., became acid (Thomas, 1988). In hippocampal slices, electrical stimulation with a single electric pulse induced a long-lasting extracellular alkaline shift (Krishtal et al., 1987). The initial alkaline shift, which is masked by acidification in adult animals, was unmasked in the isolated frog spinal cord during the application of acetazolamide (Chvátal et al., 1988; Syková, 1989). The alkaline shift was also found in the isolated frog spinal cord after application of excitatory amino acids (Endres et al., 1986). The alkaline changes described in the rat spinal cord (Syková and Svoboda, 1990), the cerebellum (Chesler and Chan, 1988; Kraig et al., 1983), the hippocampus (Krishtal et al., 1987), and the vagus nerve (Endres et al., 1986) were not affected by membrane transport inhibitors or by metabolic blockers, with the exception of Ca^{2+} and H^+ channel blockers. Therefore, there is a good reason to suppose that the initial alkaline shifts in adult animals and the alkaline shifts in immature nervous tissue occur in the neuronal microenvironment during neuronal and fiber activity, that the glial cells buffer the alkaline shifts, and that they are, at least partly, responsible for the long-term poststimulation acid shifts in the ECS.

REFERENCES

Adelman, W.J. and Fitzhug, R. (1975) Solutions on the Hodgkin-Huxley equations modified for potassium accumulation in periaxonal spaces. *Fed. Proc.*, 34:1322–1329.

Astion, M.L. and Orkand, R.K. (1988) Electrogenic Na^+/HCO_3^- cotransport in neuroglia. *Glia*, 1:355–357.

Astion, M.L., Chvátal, A., and Orkand, R.K. (1989) Na^+/H^+ exchange in glial cells of *Necturus* optic nerve. *Neurosci. Lett.*, 107:167–172.

Ballanyi, K. and Grafe, P. (1988) Cell volume regulation in the nervous system. *Renal Physiol. Biochem.*, 3–5:142–157.

Ballanyi, K. and Schlue, W.-R. (1990) Intracellular chloride activity in glial cells of the leech central nervous system. *J. Physiol. (Lond.),* 420:325–336.

Cammer, W. and Tansley, F.A. (1988) Carbonic anhydrase in astrocytes in the rat cerebral cortex. *J. Neurochem.,* 50:319–322.

Chesler, M. and Chan, C.Y. (1988) Stimulus induced extracellular pH transients in the *in vitro* turtle cerebellum. *Neuroscience,* 27:941–948.

Chesler, M.L. and Kraig, R.P. (1989) Intracellular pH transients of mammalian astrocytes. *J. Neurosci.,* 9:2011–2019.

Connors, B.V., Ransom, B., Kunis, D.M., and Gutnik, M.J. (1982) Activity-dependent K^+ accumulation in the developing rat optic nerve. *Science,* 216:1341–1343.

Cragg, P., Patterson, L., and Purves, M.J. (1977) The pH of brain extracellular fluid in the cat. *J. Physiol. (Lond.),* 272:137–166.

Chvátal, A., Jendelová, P., Kříž, N., and Syková, E. (1988) Stimulation-evoked changes in extracellular pH, calcium and potassium activity in the frog spinal cord. *Physiol. Bohemoslov.,* 37:203–212.

Davidoff, R.A., Hackamn, J.C., Holohean, A.M., Vega, J.L., and Zhang, D.X. (1988) Primary afferent activity, putative excitatory transmitters and extracellular potassium levels in frog spinal cord. *J. Physiol. (Lond.),* 397:291–306.

Davis, P.K., Carlini, W.G., Ransom, B.R., Black, J.A., and Waxman, S.G. (1987) Carbonic anhydrase activity develops postnatally in the rat optic nerve. *Dev. Brain Res.,* 31:291–298.

Deitmer, J.W. and Schlue, W.-R. (1987) The regulation of intracellular pH by identified glial cells and neurones in the central nervous system of the leech. *J. Physiol. (Lond.),* 388:261–283.

Deitmer, J.W. and Schlue, W.-R. (1989) An inwardly directed electrogenic sodium-bicarbonate co-transport in leech glial cell. *J. Physiol. (Lond.),* 411:179–194.

Endres, W., Grafe, P., Bostock, H., and Bruggencate, G.T. (1986) Changes in extracellular pH during electrical stimulation of isolated rat vagus nerve. *Neurosci. Lett.,* 64:201–205.

Foster, R.E., Connors, B.M., and Waxman, S.G. (1982) Rat optic nerve: Electrophysiological, pharmacological, and anatomical studies during development. *Dev. Brain Res.,* 3:371–386.

Gardner-Medwin, A.R. (1980) Membrane transport and solute migration affecting the brain cell microenvironment. *Neurosci. Res. Bull.,* 18:208–226.

Gilmore, S.A. (1971) Neuroglial population in the spinal white matter of neonatal and early postnatal rats: An autoradiographic study of numbers of neuroglia and changes in their proliferative activity. *Anat. Rec.,* 171:283–292.

Gilmore, S.A., Sims, T.J., and Heard, J.K. (1982) Autoradiographic and ultrastructural studies of areas of spinal cord occupied by Schwann cells and Schwann cell myelin. *Brain Res.,* 239:365–375.

Giacobini, E. (1962) A cytochemical study of the localization of carbonic anhydrase in the nervous system. *J. Neurochem.,* 9:169–177.

Grisar, T. and Franck, G. (1981) Effect of changing potassium ion concentration on rat cerebral slices *in vitro:* A study during development. *J. Neurochem.,* 36:1853–1857.

Hablitz, J.J. and Heinemann U. (1987) Extracellular K^+ and Ca^{2+} changes during epileptiform discharges in the immature rat neocortex. *Dev. Brain. Res.,* 36:299–303.

Hablitz, J.J. and Heinemann U. (1989) Alterations in the microenvironment during spreading depression associated with epileptiform activity in the immature neocortex. *Dev. Brain Res.,* 46:243–252.

Hertz, L. (1965) Possible role of neuroglia: A potassium mediated neuronal-neuroglial-neuronal impulse transmission system. *Nature,* 206:1091–1094.

Kettenmann, H. and Schlue, W.-R. (1988) Intracellular pH regulation in cultured mouse oligodendrocytes. *J. Physiol. (Lond.),* 406: 147–162.

Kraig, R.P., Ferreira-Filho, C.R., and Nicholson, C. (1983) Alkaline and acid transients in cerebellar microenvironment. *J. Neurophysiol.,* 49:831–850.

Kraig, R.P., Pulsinelli, W.A., and Plum, F. (1986) Carbonic acid buffer changes during complete brain ischemia. *Am. J. Physiol.,* 250:R348–357.

Krishtal, O.A., Osipchuk, YU.V., Shelest, T.N., and Smirnoff, S.V. (1987) Rapid extracellular pH transients related to synaptic transmission in rat hippocampal slices. *Brain Res.,* 436:352–356.

Kříž, N., Syková, E., Ujec, E., and Vyklický, L. (1974) Changes of extracellular potassium concentration induced by neuronal activity in the spinal cord of the cat. *J. Physiol. (Lond.),* 238:1–15.

Kříž, N., Syková, E., and Vyklický, L. (1975) Extracellular potassium changes in the spinal cord of the cat and their relation to slow potentials, active transport and impulse transmission. *J. Physiol. (Lond.),* 249:167–182.

Mutani, R., Futamachi, K., and Prince, D.A. (1974) Potassium activity in immature cortex. *Brain Res.,* 75:27–39.

Newman, E.A. (1986) High potassium conductance in astrocyte endfeet. *Science,* 233:453–454.

Nicoll, R.A. (1979) Dorsal root potentials and changes in extracellular potassium in the spinal cord of the frog. *J. Physiol. (Lond.),* 290:113–127.

Nicholson, C. (1980) Dynamics of the brain cell microenvironment. *Neurosci. Res. Prog. Bull.,* 18:177–322.

Nicholson, C. and Phillips, J.M. (1981) Ion diffusion modified by tortuosity and volume fraction in the extracellular microenvironment of the rat cerebellum. *J. Physiol. (Lond.),* 321:225–257.

Orkand, R.K., Nicholls, J.G., and Kuffler, S.W. (1966) The effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. *J. Neurophysiol.,* 29:788–806.

Ransom, B.R., Yamate, C.L., and Connors, B.W. (1985a) Activity dependent shrinkage of extracellular space in rat optic nerve: A developmental study. *J. Neurosci.,* 5:532–535.

Ransom, B.R., Carlini, W.G., and Connors, B. (1985b) Brain extracellular space: Developmental studies in rat optic nerve. *Ann. N.Y. Acad. Sci.,* 481:87–105.

Rousel, G., Delaunoy, J.P., Nusbaum, J.L., and Mandel, P. (1979) Demonstration of a specific localization of carbonic anhydrase C in the glial cells of rat CNS by an immunohistochemical method. *Brain Res.,* 160:47–55.

Siesjo, B.K., von Hanwehr, R., Nergelius, G., and Ingvar, M. (1985) Extra- and intracellular pH in the brain during seizures and in the recovery period following the arrest of seizure activity. *J. Cereb. Blood Flow Metab.,* 5:47–57.

Sims, T.J., Waxman, S.G., Black, J.A., and Gilmore, S.A. (1985) Perinodal astrocytic processes at nodes of Ranvier in developing normal and glial cell deficient rat spinal cord. *Brain Res.,* 337:321–333.

Somjen, G.G. (1984) Acidification of interstitial fluid in hippocampal formation caused by seizures and by spreading depression. *Brain Res.,* 311:186–188.

Svoboda, J. and Syková, E. (1989) Activity-related extracellular K^+ and volume changes in spinal cord of the rat. *Acta Physiol. Scand.,* 136 (Suppl 582):86.

Svoboda, J., Motin, V., Hájek, I., and Syková, E. (1988) Increase in extracellular potassium level in rat spinal dorsal horn induced by noxious stimulation and peripheral injury. *Brain Res.,* 458:97–105.

Syková, E. (1983) Extracellular K^+ accumulation in the central nervous system. *Prog. Biophys. Mol. Biol.,* 42:135–189.

Syková, E. (1989) Activity-related extracellular pH transients in spinal cord. *Verh. Dtsch Zool. Ges.,* 82:153–163.

Syková, E. and Vyklický, L. (1977) Changes of extracellular potassium activity in isolated spinal cord of frog under high Mg^{2+} concentration. *Neurosci. Lett.,* 4:161–165.

Syková, E. and Svoboda, J. (1990) Extracellular alkaline-acid-alkaline transients in the rat spinal cord evoked by peripheral stimulation. *Brain Res.,* 512:181–189.

Syková, E., Kříž, N., and Preis, P. (1983) Elevated extracellular potassium concentration in unstimulated spinal dorsal horns of frogs. *Neurosci. Lett.,* 43:293–298.

Syková, E., Orkand, R.K., Chvátal, A., Hájek, I., and Kříž, N. (1988) Effects of carbon dioxide on extracellular potassium accumulation and volume in isolated frog spinal cord. *Pflugers Arch.,* 412:183–187.

Thomas, R.C. (1988) Changes in the surface pH of voltage-clamped snail neurones apparently caused by H^+ fluxes through a channel. *J. Physiol. (Lond.),* 398:313–327.

Urbanics, R., Leniger-Follert, E., and Lübbers, D.W. (1978) Time course of changes of extracellular H^+ and K^+ activities during and after direct electrical stimulation of the brain cortex. *Pflugers Arch.,* 378:43–53.

Walton, K.D. and Fulton, B.P. (1983) Hydrogen peroxide as a source of molecular oxygen for *in vitro* mammalian CNS preparations. *Brain Res.,* 278:387–393.

Walton, K.D. and Chesler, M. (1988) Activity-related extracellular potassium transients in the neonatal rat spinal cord: An *in vitro* study. *Neuroscience,* 25:983–995.

Walz, W. (1989) Role of glial cells in the regulation of the brain ion microenvironment. *Prog. Neurobiol.,* 33:309–333.

Walz, W. and Mukerji, S. (1988) Lactate release from cultured astrocytes and neurons: A comparison. *Glia,* 1:366–370.

Yamate, C.L. and Ransom, B.R. (1985) Effects of altered gliogenesis on activity-dependent K^+ accumulation in the developing rat optic nerve. *Dev. Brain Res.,* 21:167–173.