A.C.H. Yu, L. Hertz, M.D. Norenberg, E. Syková and S.G. Waxman (Eds.)
Progress in Brain Research, Vol. 94

CHAPTER 4

Role of astrocytes in ionic and volume homeostasis in spinal cord during development and injury

E. Syková, J. Svoboda, Z. Šimonová and P. Jendelová

Laboratory of Cellular Neurophysiology, Institute of Experimental Medicine, Czechoslovak Academy of Sciences, Bulovka, Pav. 11, 180 85 Prague 8, Czechoslovakia

Introduction

Our modern conception of the neuronal microenvironment has been developed recently and is based on research results of the last 25 years. During this time there have been major contributions towards the understanding of: (1) the composition and structure of the extracellular space (ECP); and (2) the properties and function of glial cells. The findings gave rise to the view that the ECS functions as a communication and modulation channel, whose ionic and chemical composition and anatomical structure (size and tortuosity) depend on neuronal activity and glial cell function. Equally important is the fact that ECS composition and structure significantly influence the complex function of the neurons and glial cells and modify nervous tissue excitability. The relationship between neurons, glia and ECS is therefore vitally important for normal neural functioning. Consequently it is of great interest to find out how the relationship differs during CNS development and how it might be impaired during various disease states and injury.

This review deals with activity-related ionic and volume changes in spinal cord of adult rats and during development, with transient changes during peripheral stimulation and peripheral injury, in a model of central injury (X-irradiation) and in a model of multiple sclerosis (experimental autoimmune encephalomyelitis, EAE). It focuses on their mechanisms, functional implications and on possible roles of glial cells in altered K^+, pH_e and ECS volume homeostasis.

K^+ and pH_e changes in the ECS

Activity-related changes in $[K^+]_e$ and pH_e have been found in brain, spinal cord, peripheral nerves and receptor organs (for reviews, see Somjen, 1979; Nicholson, 1980; Syková, 1983, 1991, 1992; Chesler, 1990). Almost all kinds of stimulation – artificial or natural – lead to transient increase in $[K^+]_e$ and alkaline-acid shifts in pH_e.

The main source of changes in $[K^+]_e$ are stimulated neurons, unmyelinated fibers and unmyelinated terminals of axons. An increase of 0.1 – 0.2 mM has been found in brain and spinal cord after a single electrical stimulus applied to peripheral nerves or after a single adequate stimulus (e.g., light touch or pinch) applied to the skin (Fig. 1) of various adult animals including cat, rat, mouse, golden hamster, guinea pig, chick, frog, turtle, etc. A long-term increase in $[K^+]_e$ in the spinal dorsal horn of the rat was evoked by chemical and

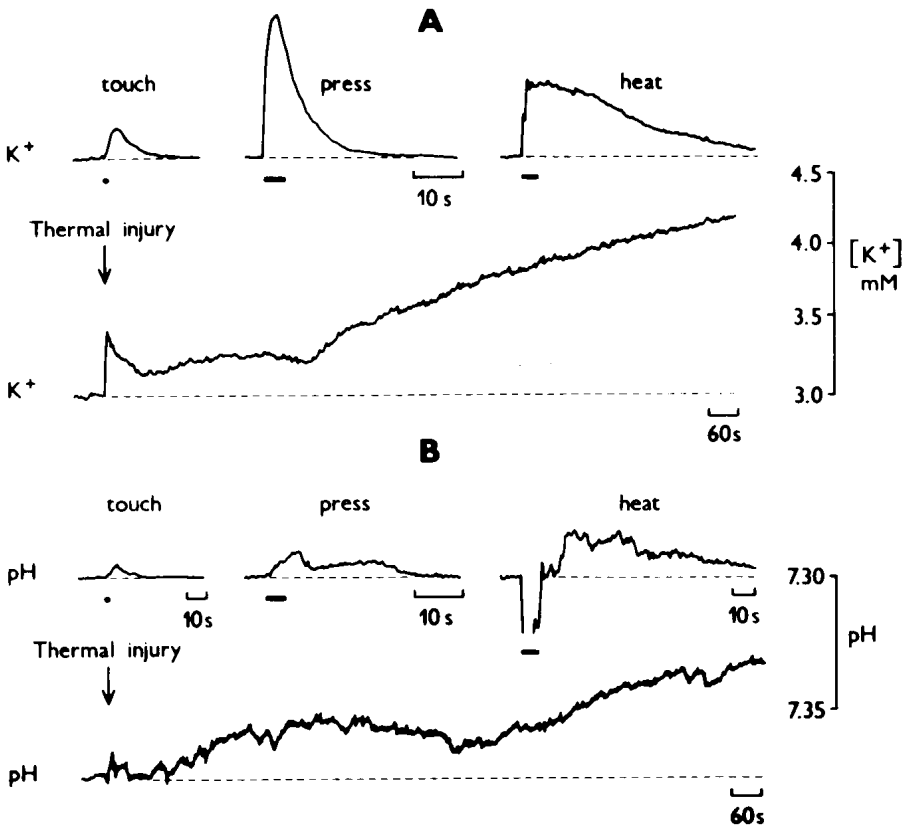

Fig. 1. Transient increase in $[K^+]_e$ (*A*) and decrease in pH_e (*B*) in the L_4 spinal segment in response to adequate stimuli applied to the plantar region of the ipsilateral hind paw. The K^+- and pH-sensitive microelectrodes were inserted to a depth of 600 μm from the dorsal surface. The upper curves represent responses to light touch, strong pressure and application of about 1 ml of hot water onto the hind limb (heat). Horizontal bars indicate stimulus duration. Bottom records in *A* and *B* show changes elicited by application of 2 ml of hot oil onto the hind limb (thermal injury) with typical long-lasting increase in $[K^+]_e$ and decrease in pH_e which persist longer than 20 min. Note different time scale. $[K^+]_e$ and pH_e data are from different experiments.

thermal injury of the hind paw (Fig. 1; Svoboda et al., 1988). Repetitive electrical stimulation or long-term adequate stimulation leads to summation of responses to a certain level, the so-called "ceiling level", from a baseline ("resting") level of about 3 – 4 mM to as much as 6 – 12 mM (Fig. 2). When stimulation continues, no further changes in $[K^+]_e$ are found because a steady state is established, which is a result of concurrent release and clearance of K^+ (Fig. 2). This ceiling level (Heineman and Lux, 1975; Kříž et al., 1975) is only broken through by pathological events, e.g., epileptic activity, anoxia, spreading depression, application of convulsive drugs and is higher in immature nervous tissue, i.e., in situations when K^+ homeostasis is impaired.

K^+ homeostasis is ensured by two main mechanisms: (1) activation of Na/K pump in neurons and glia; and (2) glial cell K^+ uptake or buffering of extracellular rise in $[K^+]_e$ (for reviews, see Syková, 1983, 1992; Walz, 1989). Active neurons lose K^+ and induce a change in the ionic composition of the ECS. This may be an important way in which neurons interact and integrate their activity; however, stability of nervous tissue function requires fast renewal of the ECS ionic composition. The conclusion that K^+ is cleared by means of ac-

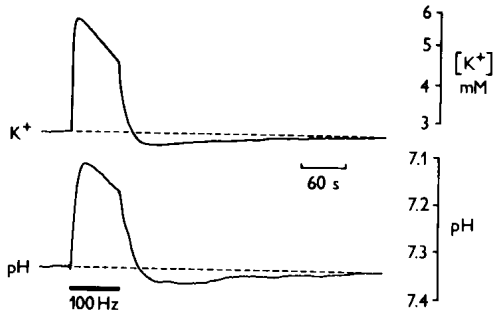

Fig. 2. $[K^+]_e$ and pH_e changes in dorsal horn of segment L_4 of rat spinal cord evoked by repetitive bipolar electrical stimulation with acupuncture needles (100 Hz, 60 sec) in plantar muscles of the ipsilateral hind paw. Horizontal bar indicates stimulus duration. Two K^+- and pH-sensitive microelectrodes were inserted separately from dorsal spinal surface. Recordings from a depth of 600 – 700 μm.

tive transport is strongly supported, since there is a post-stimulation decrease below resting level, the so-called "K^+-undershoot" (see Fig. 2), which can be blocked by inhibitors of the Na/K pump by, e.g., ouabain, or by anoxia, ischemia or anesthetic drugs. Glial cells also significantly contribute to activity-related clearance of K^+ increase in ECS. There is an evidence that K^+ clearance by glia include uptake of K^+ driven by Na/K pump (Hertz, 1965) and K^+ spatial buffering (Orkand et al., 1966).

Concomitantly with an increase in $[K^+]_e$ any neuronal activity results in a transient change in ECS acid-base balance. The use of pH-sensitive microelectrodes made it possible to record transient changes in pH_i and pH_e during neuronal activity in vivo. It has been found that the pH_e level is related to neuronal depolarization, metabolic activity, ion shifts across neuronal membranes and glial cell buffering of pH_e changes (for review, see Chesler, 1990). The time course and mechanisms of activity-related alkaline-acid shifts have been studied under physiological and pathological conditions, but the mechanisms of these shifts and how the pH_e is stabilized are not yet clear. In summary, the current research suggests that activity-related alkaline shifts are due to: (1) channel-mediated fluxes of bicarbonate; (2) channel-mediated acid influx into neurons; (3) inhibition of glycolytic acid source; and (4) ECS volume shrinkage. Acid shifts are due to: (1) classic acid extrusion membrane transport systems (Na^+/H^+ or $Na^+/HCO_3^-/Cl^-/H^+$); (2) Na^+-HCO_3^- cotransport into glial cells; and (3) efflux of lactic acid from metabolically active cells. Both the neurons and glial cells (oligodendrocytes as well as astrocytes) have been shown to possess several membrane transport systems which transport ions, including H^+ and HCO_3^-. It is reasonable to assume that glial cells regulate pH_e homeostasis at the expense of their intracellular pH.

Changes in pH_e of only a few tenths of pH units have been shown to evoke marked effects on neuronal ionic channels (Iljima et al., 1986; Konnerth et al., 1987), to affect neuronal excitability (Gruol et al., 1980; Balestrino and Somjen, 1988), cell to cell coupling (Roos and Boron, 1981) and glial cell membrane properties (for reviews, see Walz, 1989; Chesler 1990).

In adult animals the activity-related pH_e changes have a typical time course: an alkaline-acid shift during the stimulation in mammalian spinal cord followed by a post-stimulation alkaline undershoot (Syková, 1989; Syková and Svoboda, 1990), while in the frog spinal cord a post-stimulation delayed acid shift occurs (Chvátal et al., 1988). An important fact is that the pH_e resting level recorded in various areas of adult CNS varies between 7.1 and 7.3, i.e., pH_e is slightly alkaline but more acid than that in blood or cerebrospinal fluid (CSF). In the rat regional differences in pH_e were found in unstimulated rat spinal cord. The pH_e in the lower dorsal horn was about 7.15, i.e., lower by about 0.2 pH units than that measured in the CSF. In the upper dorsal horn and in the ventral horn the pH_e base line was only slightly more acid (7.25 – 7.35) than in supraspinal fluid (7.35 – 7.40) (Syková and Svoboda, 1990). The variations in pH_e base line had a similar depth profile as the variations in $[K^+]_e$ base line in unstimulated spinal cords. An alkaline shift in pH_e base line of 0.05 – 0.10 pH units was found after blockade of synaptic activity by applying Mn^{2+} in a concentration of 4 – 5 mM onto the spinal cord surface. It has been demonstrated previously that increases in $[K^+]_e$ base line found in the

lower dorsal horn are associated with a high level of spontaneous activity of dorsal horn neurons due to continuous excitatory input from the periphery (Svoboda et al., 1988).

Transient acid shifts in pH_e of 0.01 – 0.05 pH units were found when acute nociceptive stimuli (pinch, press, heat) were applied to the hind paw (Fig. 1). Chemical or thermal injury evoked by subcutaneous injection of turpentine or by application of 1 – 3 ml of hot oil onto the hindpaw produced a long-term decrease in pH_e base line in the lower dorsal horn of about 0.05 – 0.10 pH units (Fig. 1; Syková and Svoboda, 1990). The decrease in pH_e began 2 – 10 min after injury and persisted for more than 2 h. Electrical nerve stimulation (10 – 100 Hz, 20 – 60 sec) elicited triphasic (alkaline-acid-alkaline) changes in pH_e which had a similar depth profile as the concomitantly recorded increase in $[K^+]_e$ (Fig. 2).

An initial alkaline shift of about 0.005 pH units was found to be significantly decreased by La^{3+}, an H^+ channel blocker (Syková and Svoboda, 1990). With superfusion of rat cortex with Ba^{2+} (Chesler and Kraig, 1989) the initial alkaline shift was enhanced; in spinal cord (Syková et al., 1992) Ba^{2+} enhanced the stimulation-evoked initial alkaline shift and depressed the post-stimulation acid shift. The observed effect of Ba^{2+} may be generated by acid influx into neurons through a channel-mediated pathway (Endres et al., 1986; Chesler and Chen, 1988). Superfusion of GABA elicited extracellular alkalinization in spinal cord (Syková et al., 1992) as well as in turtle cerebellum (Chen and Chesler, 1992) which was blocked by picrotoxin suggesting that gating of GABA channels in vertebrate CNS gives rise to an HCO_3^- efflux which can increase the pH_e. The relationship of the glial stimulation-evoked intracellular alkaline shifts to the initial alkaline shift is unclear. Ba^{2+} induced hyperpolarization of glia in *Necturus* optic nerve and increased Na^+-HCO_3^- cotransport into glial cells (Astion et al., 1987, 1989). Increase in Na^+-HCO_3^- cotransport cannot give rise to interstitial alkalinization but it can contribute to the observed stimulation-evoked acid shifts.

Stimulation-evoked acid shifts were blocked by amiloride, SITS, DIDS, Ba^{2+} and La^{3+} and therefore have a complex mechanism which may also include Na^+/H^+ exchange, Cl^-/HCO_3^- cotransport and/or $Na^+/Cl^-/H^+/HCO_3^-$ antiport and H^+ efflux through voltage-sensitive H^+ channels. Superfusion of isolated frog spinal cord with NaF, which block glycolytic portion of metabolic processes and production of lactate had no effect on stimulation-evoked alkaline-acid changes in ECS (Syková et al., 1988). The post-stimulation alkaline shift ("alkaline undershoot") was blocked by ouabain or during anoxia and reflects coupled clearance of K^+ and H^+ by active transport processes.

The transient changes in K^+ and pH_e homeostasis and the role of glia in K^+ and pH homeostasis were investigated during development in the spinal cord of the rat (Jendelová and Syková, 1991) as well as in hyperstriatum ventrale, neostriatum and ectostriatum of 1 – 14-day-old chicks (see Ng et al., this volume). Stimulation-evoked transient changes in $[K^+]_e$ and pH_e were studied in the neonatal rat spinal cords isolated from 3 to 13-day-old pups. In unstimulated pups the $[K^+]_e$ base line was elevated and pH_e was more acid than that in Ringer's solution (3.5 mM K^+, pH 7.3 – 7.35). The $[K^+]_e$ in 3 – 6-days-old pups was 3.91 ± 0.12 mM and the pH_e 7.19 ± 0.01 (n = 14); while in 10 – 13-day-old the values were 4.35 ± 0.15 mM and 7.11 ± 0.01 (n = 10), 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 (Svoboda et al., 1988). 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 (Fig. 3).

In 3 – 8-day-old pups, the stimulation evoked an alkaline shift of 0.1 – 0.2 pH units which was followed by a smaller post-stimulation acid shift of 0.03 – 0.07 pH units (Fig. 3). In 10 – 13-day-old rats, however, the stimulation evoked an acid shift which was preceded by a scarcely discernible alkaline shift as is the case in adult animals (Syková

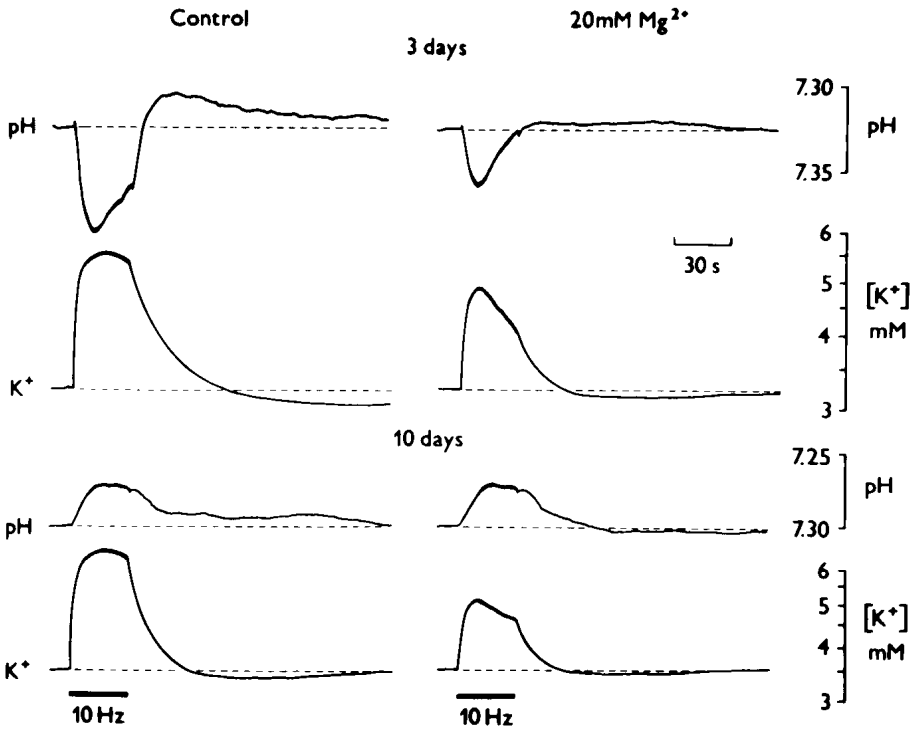

Fig. 3. Left: stimulation-evoked pH_e and $[K^+]_e$ changes in the spinal dorsal horn of rats 3 and 10 days old. Note that the stimulation of the dorsal root at a frequency of 10 Hz evoked an alkaline shift in the 3-day-old pup, which was accompanied by an increase in $[K^+]_e$; when stimulation was discontinued a post-stimulation 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. Right: effect of 20 mM Mg^{2+} on pH_e and $[K^+]_e$ in the spinal dorsal horn of rats 3 and 10 days old. Note decrease of the alkaline shift, decrease of the post-stimulation acid shifts and decrease of $[K^+]_e$ changes. Only the acid shift that occurred during stimulation in the 10-day-old rat remained unaffected (From Jendelová and Syková, 1991, with permission.)

and Svoboda, 1990). 20 mM $MgCl_2$ reversibly reduced the alkaline but not the acid shifts by 50 – 60% (Fig. 3). Bath application of the carbonic anhydrase inhibitor, acetazolamide, had no effect on the alkaline shift, while the acid shift decreased by 70 – 80% (Fig. 4). The alkaline shift was enhanced in HEPES-buffered saline and was significantly decreased by La^{3+}, a H^+ channel blocker, and by picrotoxin (Syková et al., 1992). The acid shift was blocked by amiloride and SITS and therefore has a complex mechanism which may include Na^+/H^+ exchange, $Na^+/Cl^-/HCO_3^-/H^+$ exchange and Na^+-HCO_3^-cotransport – the membrane transport systems which have been demonstrated not only in neurons but also in glia (for reviews, see Chesler, 1990; Syková, 1992). As in adult rats the post-stimulation alkaline shift was blocked by ouabain and reflects coupled clearance of K^+ and H^+ by active transport processes (Syková and Svoboda, 1990).

It has been demonstrated recently that mammalian astrocytes actively regulate their pH_i by acid extrusion membrane transport processes which are dependent on Na^+, Cl^- and HCO_3^- (see also Ransom et al., this volume). Since gliogenesis occurs during the first 10 days post-natally, the lumbosacral region of 1-day-old pups was X-irradiated (4000 R) to block gliogenesis. In irradiated pups the enhanced $[K^+]_e$ changes and the alkaline shifts persisted even in 10 – 14-day-old-pups (Syková et al.,

1992). These results suggest that glial cells buffer the activity-related $[K^+]_e$ increase and alkaline pH_e shifts.

In summary, in neonatal rat spinal cord during the first 6 post-natal days, stimulation of the afferent input evokes large alkaline changes, which are followed by slow and small acid shifts after stimulation has been discontinued. The alkaline shifts progressively decrease with development and the acid shift becomes dominant about 10 days after birth, i.e., at the same time as the $[K^+]_e$ ceiling level starts to decrease (Fig. 3). Since carbonic anhydrase and gliogenesis to a great extent occur post-natally with the peak at about 10 days later, it can be concluded that in spinal cord (Jendelová and Syková, 1991) as in the optic nerve (Ransom et al., 1985), the stimulation-evoked acid shifts are related to gliogenesis. When gliogenesis is blocked, e.g., by post-natal X-irradiation, the acid shifts are also blocked, while the alkaline shifts persist (Syková et al., 1992). We therefore suggest that activity-related alkaline shifts in pH_e, as well as the excessive increase in $[K^+]_e$, are effectively buffered by glial cells.

ECS volume changes

It has been recognized that during depolarization and during neuronal activity the ECS can reversibly decrease due to the activity-related cell swelling. Experimental elevation of $[K^+]_e$ by KCl application leads to membrane depolarization of various brain cells, to an increase in the volume of the cells, and to a compensatory ECS volume decrease due to cellular accumulation of ions (particularly K^+, Na^+, Cl^-, HCO_3^-) and water. K^+-induced swelling is mostly attributed to glia, particularly to astrocytes (for review, see Kimelberg and Ransom, 1986), but swelling of neuronal elements has also been shown (Trubatch et al., 1977; Ritchie et al., 1981; Tasaki and Iwasa, 1982). These previous studies, however, did not furnish any information on the size of the ECS volume changes or on their dynamics.

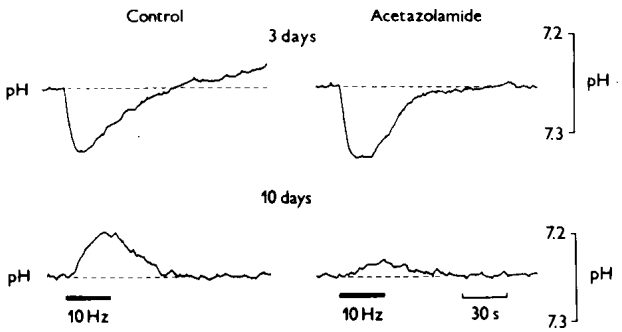

Fig. 4. Effect of acetazolamide (5 mM) on pH_e changes in the spinal dorsal horn of rats 3 and 10 days old. pH_e changes were evoked by stimulation at a frequency of 10 Hz. Note that the alkaline shift was not changed, while the acid shifts during and after stimulation were substantially decreased (From Jendelová and Syková, 1991, with permission.)

A method for measuring the dynamic changes in the ECS volume fraction (α) and tortuosity (λ, a measure of the total path length that a diffusing particle travels because of the presence of obstructions in ECS) at a specific point in a nervous tissue has been developed recently (Fig. 5; Nicholson and Phillips, 1981; Nicholson and Rice, 1988). With this method either relative changes in the ECS volume or absolute value of the ECS volume and tortuosity were found in cortex, spinal cord, optic nerve and receptor organs during activity evoked by electric or natural stimulation (Dietzel et al., 1982, 1984; Orkand et al., 1984; Ransom et al., 1985; Syková, 1987; Svoboda and Syková, 1992) as well as during epileptic activity (Heinemann and Dietzel, 1984; Lehmenkühler et al., 1991) and during ischemia or spreading depression (for review, see Nicholson and Rice, 1988).

Double-barreled tetramethyl (TEA^+) or tetrethylammonium (TMA^+) sensitive microelectrodes were used in diffusion studies with TEA^+ and TMA^+. These ions remain essentially extracellular during the measurements. Fig. 6 shows the long-term decrease in the ECS volume found in the lower dorsal horn of the frog after repetitive electrical stimulation of dorsal root (Syková, 1987) and concomitantly recorded changes in $[K^+]_e$ and pH_e. If the root or peripheral nerve was repetitively stimulated, we observed up to 30% reduction of the ECS volume in the dorsal horn of the same segment. The decrease in the ECS volume had the same depth pro-

file as the ionic changes evoked by neuronal activity. They were maximal in the depth where the maximum $[K^+]_e$ $[Ca]_e$, pH_e and field potentials were found. The higher the stimulation frequency and intensity and the greater the concomitant ionic changes, the greater was the decrease in ECS volume. The ECS decrease persisted after stimulation had been discontinued. It regularly lasted longer than the stimulation-evoked changes in $[K^+]_e$, its time course being related to the post-stimulation K^+-undershoot and post-stimulation acid shift.

The absolute values of ECS volume and its tortuosity are of great interest, particularly during physiologically evoked neuronal activity or during various disease states in which the volume regulation in CNS by astrocytes can be impaired or in which the astrocytes might not be able to ensure ECS volume stability when there is an inflammation and damage in blood-brain barrier (BBB). It is evident that changes in the ECS volume may be either the cause or the result of the long-term hypo- or hyperexcitability due to synaptic hypo- or hyperactivity in CNS (for review, see Syková, 1992).

The ECS volume fraction α and tortuosity λ were examined in brain and in the spinal dorsal horns of adult rats. The α and λ in various brain areas as well as in spinal cord vary in unstimulated animals between 20 and 25% of total brain volume and the tortuosity value is about 1.5 (Nicholson and Phillips, 1981; Svoboda and Syková, 1992). In spinal cord values were not significantly different throughout the dorsal and ventral horns. Repetitive electrical stimulation of peripheral nerves at 3 – 100 Hz increased ECS volume in Rexed laminae III – V by 15.8 ± 2.7%, i.e., during the electrical stimulation ECS volume increased apparently due to shrinkage of some neural elements. However, after the end of stimulation, when the $[K^+]_e$ decreased below the original baseline (during K^+-undershoot), the ECS volume decreased by 20 – 40%, i.e., to about 14 – 17% of total brain volume (Fig. 5). The magnitude and duration of ECS volume decrease were positively related to the stimulation frequency and duration. The ECS volume decrease was maximal at 2 – 10 min after the stimulation had been discontinued, and it returned to the pre-stimulation value in 15 – 40 min (Fig. 6). The ECS volume decreased by 20 – 50% (but no more than to 12% of total brain volume) after injury of the ipsilateral hind paw evoked either by subcutaneous injection of turpentine or by thermal injury (Fig. 7). The maximal changes were found in dorsal horn Rexed laminae III – V, 5 – 10 min after injection of turpentine and 10 – 25 min after thermal injury, and persisted for more than 120 and 30 min, respectively (Fig. 7). The tortuosity of the ECS was not significantly altered by stimulation or injury. These measurements indicate that the normal volume of the ECS occupies about one-fifth of total brain volume and that the significant dynamic changes in ECS volume accompany transmembrane ionic shifts resulting from neural activity (see Fig. 6).

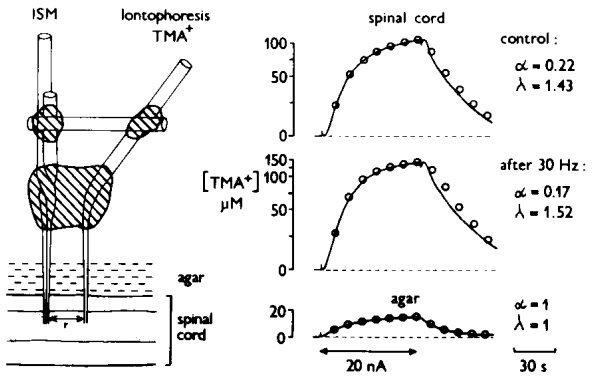

Fig. 5. Method of determining ECS volume fraction (α) and tortuosity (λ) in spinal cord in vivo. Left: schematic drawing of the experimental arrangement for diffusion measurements. Two microelectrodes, the double-barreled microelectrode sensitive to K^+ and to TMA^+ (ISM) and the micropipette for TMA^+ iontophoresis, were glued together with dental cement at the area of their shanks to stabilize the intertip distance (r) and with a glass tube bridge connecting the two microelectrodes at their upper end. Right: the diffusion curves recorded prior to (control) and 9 min after repetitive electrical stimulation at a frequency of 30 Hz (after 30 Hz). Diffusion curve after the stimulation is larger, due to the ECS volume decrease. The stimulation at 30 Hz lasted 2 min. The volume fraction before stimulation (control) was α = 0.22, the tortuosity λ = 1.43; after stimulation: α = 0.17, λ = 1.52. Diffusion parameters were I = 20 nA, r = 180 μm, n = 0.308, D = 1.33 × 10^{-9} m^2/sec.

The mechanisms of cellular shrinkage and swell-

ing during neuronal activity is discussed in several papers (for reviews, see Kempski, 1986; Kimelberg and Ransom, 1986; Svoboda and Syková, 1992). The mechanisms include: (1) extracellular ionic changes (H^+ and K^+) accompanied by uptake of Na^+, Cl^- and water; (2) increase in intraneuronal osmolarity and/or local extracellular decrease in osmolarity; (3) glial spatial buffer mechanism – glial swelling due to increase in intracellular particles accompanied by water; (4) glial swelling due to metabolic alterations such as acid – base balance; and (5) increase in intraneuronal particles, produced by enhanced metabolism during excessive neuronal activity. It is evident that the glial cells, and particularly astrocytic swelling, play the important role in activity-related changes. However, there is no direct evidence for astrocyte swelling in mammalian CNS in vivo. We therefore performed experiments on rat cortex during development (E. Syková and A. Lehmenkühler, unpublished results) and during the period of extensive gliogenesis. Similarly as in premyelinated rat optic nerve (Ransom et al., 1985) the ECS in cortex of rats in the first 14 days postnatally is larger, and its size decreases with the animal age.

Dramatic changes in the ECS volume were found during central injury and demyelinating diseases (Svoboda et al., 1992). Increase in the ECS size to 40 – 50% of total tissue volume was found in spinal cord of Lewis rats with experimental autoimmune encephalomyelitis (EAE), an experimental disease used as a model of multiple sclerosis (MS), which has been characterized by breakdown of the blood-brain barrier, edema, perivascular infiltration of inflammatory cells and by hypertrophy and proliferation of astrocytes – signs which, besides demyelination, may contribute to clinical manifestations of EAE. The increase was found only in the gray matter – in the intermediate region and in the ventral horns. The concomitantly studied demyelination, albumin leakage from the blood vessels, inflammation, impairment of ionic homeostasis and the in-

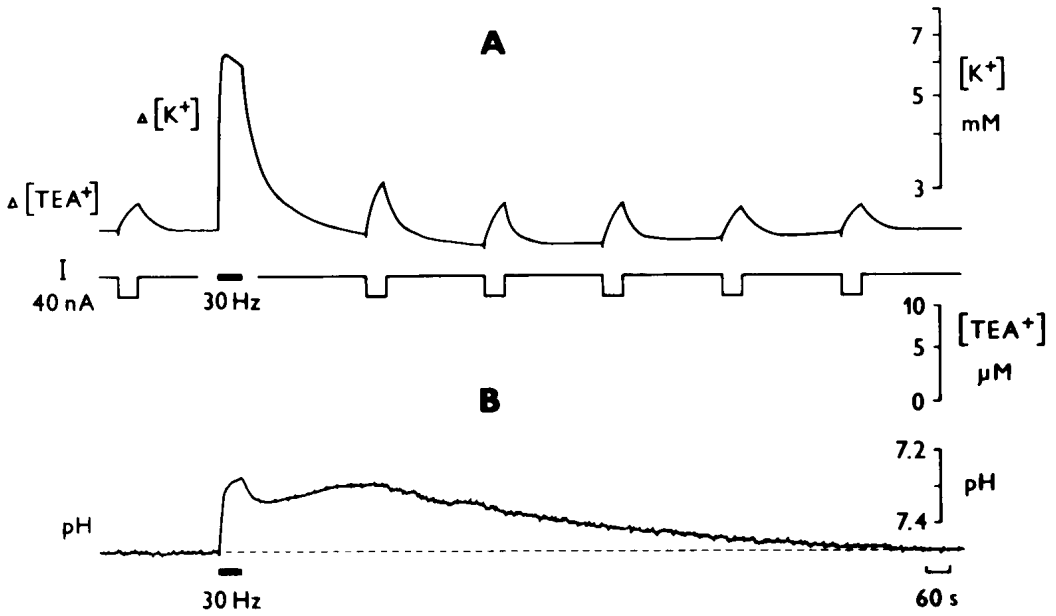

Fig. 6. *A*. Effect of repetitive electrical stimulation of dorsal root VIII (30 Hz, 60 sec) on ECS volume in segment VIII of the isolated frog spinal cord. Current (40 nA, the bottom recordings) was applied with a pipette filled with TEA^+. Top records show changes in $[TEA^+]$ during iontophoretic applications and changes in $[K^+]_e$ induced by stimulation. *B*. Concomitantly recorded pH_e changes. Three microelectrodes (iontophoretic pipette, K^+-ISM and pH-ISM) were inserted separately from the dorsal spinal surface. Recordings from a depth of about 300 μm. (From Syková, 1991, with permission.)

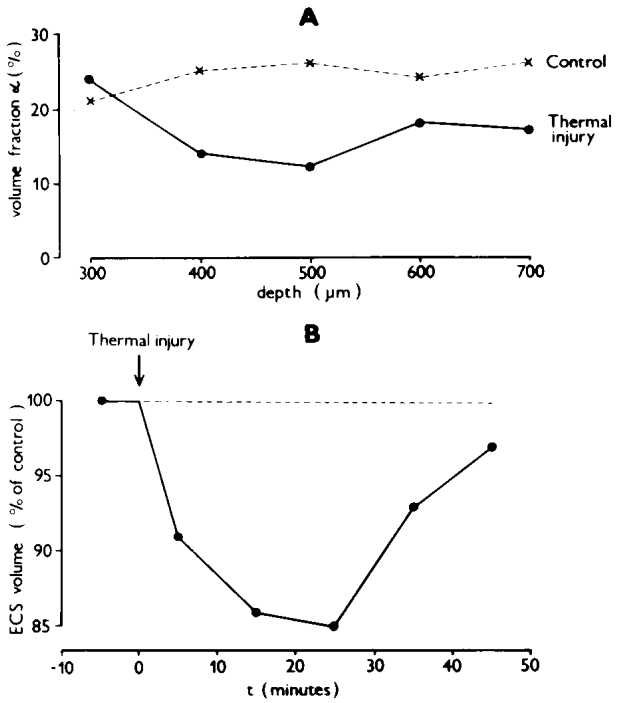

Fig. 7. *A*. Typical ECS volume fraction at various depths of the spinal dorsal horn in the non-stimulated rat (dashed line) and 10 – 25 min after the thermal injury of a small skin area on the planta evoked by application of 2 ml of hot oil (solid line). The ECS volume decrease was found at a depth of 400 – 700 μm. The maximal ECS volume decrease from 24% to 12% was found at a depth of 500 μm. *B*. Graph of the time course of the ECS volume shrinkage in spinal dorsal horn at a depth of 500 μm after the thermal injury. ECS volume decrease is expressed as a percentage of control volume (100%). Data are from two different experiments.

crease in GFAP astrocyte immunostaining revealed that the demyelination was located preferably in the dorsal horn, while the other signs were ubiquitous. The role of astrocytes in the impaired ionic and volume homeostasis needs to be studied, as well as the hypothesis that the impaired ionic and volume homeostasis may contribute to the clinical signs of EAE and MS.

Conclusions

(1) Ionic composition and the size of the ECS is significantly altered during stimulation-evoked neuronal activity, CNS development, peripheral and central injury, inflammatory and demyelinating diseases.

(2) Immature astrocytes (and possibly astrocytes during proliferation and hypertrophy) are not able to ensure K^+, pH_e and ECS volume homeostasis.

(3) Changes in ECS ionic composition and volume significantly influence the function of neurons and glia.

(4) Glial cells may play an essential role in CNS excitability by regulating the extracellular microenvironment of the neurons.

References

Astion, M.L., Coles, J.A. and Orkand, R.K. (1987) Effects of bicarbonate on glial cell membrane potential in *Necturus* optic nerve. *Neurosci. Lett.*, 76: 47 – 52.

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.

Balestrino, M. and Somjen, G.G. (1988) Concentration of carbon dioxide, interstitial pH and synaptic transmission in hippocampal formation of the rat. *J. Physiol. (Lond.)*, 396: 247 – 266.

Chen, C.Y. and Chesler, M.L. (1992) Extracellular alkalinization evoked by GABA and its relationship to activity-dependent pH shifts in turtle cerebellum. *J. Physiol. (Lond.)*, in press.

Chesler, M.L. (1990) The regulation and modulation of pH in the nervous system. *Prog. Neurobiol.*, 34: 401 – 427.

Chesler, M.L. and Chen, 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.

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.

Dietzel, I., Heinemann, U., Hofmeier, G. and Lux, H.D. (1982) Stimulus-induced changes in extracellular Na^+ and Cl^- concentration in relation to changes in the size of the extracellular space. *Exp. Brain Res.*, 46: 73 – 84.

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.

Gruol, D.L., Barker, J.L., Huang, L.M., McDonald, J.F. and Smith Jr., T.G. (1980) Hydrogen ions have multiple effects on the excitability of cultured mammalian neurons. *Brain Res.*, 183: 247 – 252.

Heinemann, U. and Dietzel, I. (1984) Extracellular potassium

concentration in chronic alumina cream foci of cats. *J. Neurophysiol.*, 3: 421 – 434.

Heineman, U. and Lux, H.D. (1975) Undershoots following stimulus-induced rises of extracellular potassium concentration in the cerebral cortex of cat. *Brain Res.*, 93: 63 – 76.

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

Iljima, T., Ciani, S. and Hagiwara, S. (1986) Effects of the external pH on Ca channels: experimental studies and theoretical considerations using a two-site, two-ion model. *Proc. Natl. Acad. Sci. U.S.A.*, 83: 654 – 658.

Jendelová, P. and Syková, E. (1991) Role of glia in K^+ and pH homeostasis in the neonatal rat spinal cord. *Glia*, 4: 56 – 63.

Kempski, O. (1986) Cell swelling mechanisms in brain. In: A. Baethmann, K.G. Go and A. Unterberg (Eds.), Plenum Publishing Corporation, pp. 203 – 220.

Kimelberg, H.K. and Ransom, B.R. (1986) Physiological and pathological aspects of astrocyte swelling. In: S. Fedoroff and A. Vernadakis (Eds.), *Astrocytes*, Academic Press, New York, pp. 129 – 166.

Konnerth, A., Lux, H.D. and Morad, M. (1987) Proton-induced transformation of calcium channel in chicks dorsal root ganglion cells. *J. Physiol. (Lond.)*, 386: 603 – 633.

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.

Lehmenkühler, A., Nicholson, C. and Speckman, E.-J. (1991) Threshold extracellular concentration distribution of penicillin for generation of epileptic focus measured by diffusion analysis. *Brain Res.*, 561: 292 – 298.

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

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

Nicholson, C. and Rice, M.E. (1988) Use of ion-selective microelectrodes and voltametric microsensors to study brain cell microenvironment. In: A.A. Boulton, G.B. Baker and W. Walz (Eds.), *Neuromethods: the Neuronal Microenvironment*, Humana, New York, pp. 247 – 361.

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

Orkand, R.K., Dietzel, I. and Coles, J.A. (1984) Light-induced changes in extracellular volume in the retina of the drone, *Apis mellifera*. *Neurosci. Lett.*, 45: 273 – 278.

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

Ritchie, T., Packey, D.J., Trachtenberg, M.C. and Haber, B. (1981) K^+-induced ion and water movements in the frog spinal cord and filum terminale. *Exp. Neurol.*, 71: 356 – 369.

Roos, A. and Boron, W.F. (1981) Intracellular pH. *Physiol. Rev.*, 61: 296 – 434.

Somjen, G.G. (1979) Extracellular potassium in the mammalian central nervous system. *Annu. Rev. Physiol.*, 41: 159 – 177.

Svoboda, J. and Syková, E. (1992) Extracellular space volume changes in the rat spinal cord produced by nerve stimulation and peripheral injury. *Brain Res.*, in press.

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.

Svoboda, J., Syková, E., Orkand, P. and Bernard, C. (1992) Extracellular space and volume increase in the spinal cord of the rat with experimental autoimmune encephalomyelitis. *Physiol. Res.*, in press.

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

Syková, E. (1987) Modulation of spinal cord transmission by changes in extracellular K^+ activity and extracellular volume. *Can. J. Physiol. Pharmacol.*, 65: 1058 – 1066.

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

Syková, E. (1991) Activity-related ionic and volume changes in neuronal microenvironment. In: K. Fuxe and L.F. Agnati (Eds.), *Volume Transmission in the Brain: Novel Mechanisms for Neural Transmission*, Raven Press, New York, pp. 217 – 336.

Syková, E. (1992) Ionic and volume changes in the microenvironment of nerve and receptor cells. In: *Progress in Sensory Physiology*, Springer, Heidelberg, in press.

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., Svoboda, J., Chvátal, A. and Jendelová, P. (1988) Extracellular pH and stimulated neurons. In: R.C. Thomas, (Ed.), *Proton Passage across Cell Membranes*, Wiley, Chicester, pp. 220 – 232.

Syková, E., Jendelová, P., Šimonová, Z. and Chvátal, A. (1992) K^+ and pH homeostasis in the developing rat spinal cord is impaired by early postnatal X-irradiation. *Brain Res.*, in press.

Tasaki, I. and Iwasa, K. (1982) Further studies of rapid mechanical changes in squid giant axon associated with action potential production. *Jpn. J. Phsyiol.*, 32: 505 – 518.

Trubatch, J., Loud, A.U. and Van Harreveld, A. (1977) Quantitative stereological evaluation of KCl-induced ultrastructural changes in frog brain. *Neuroscience*, 2: 963 – 974.

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