Ionic and Volume Changes in CNS During Stimulation, Injury, Postnatal Development and Anoxia

E. Syková, J. Svoboda, A. Chvátal, J. Polák

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

It was not until the last ten years that extracellular space (ECS) came to be recognized as a dynamically changing microenvironment for the brain, with a significant influence on the function of neurones and glia (for review see Nicholson, 1980; Sykova, 1983, 1991, 1992). ECS is an communication channel between neurones and glia. ECS ionic composition, presence of neurotransmitters, neuro-peptides, enzymes and metabolic substrates changes dynamically during "normal" neuronal activity as well as during pathological states and can substantially affect function of the CNS. Any increase in neuronal activity is accompanied by transmembrane ionic and water fluxes resulting in prolonged transient extracellular ionic shifts and in extracellular space volume decrease. There is increasing evidence that glial cells play a crucial role in extracellular ion (K^+, H^+, Cl^-) and volume homeostasis (Walz, 1989; Chesler, 1990; Hertz, 1990; Sykova, 1991, 1992). Consequently it is of great interest to find out how the ionic and volume homeostasis and the relationship between neurones and glia differ during CNS development, injury and reactive gliosis.

Diffusion parameters of the ECS affects the activity-related accummulation of ions, neuroactive substances and metabolites in ECS, their movement towards the target cells and their access to capillaries. Any change in ECS diffusion parameters can therefore profoundly influence signal tramsmission and intercellular communication. It can be important factor in development of clinical signs during seizures, ischemia, anoxia, injury and migraine and for manifestation of CNS diseases (Nicholson and Rice, 1991; Sykova, 1991, 1992). The diffusion parameters of the ECS and their dynamic changes can be studied *in vivo* by the real-time iontophoretic method, which uses ion-selective microelectrodes to follow the diffusion of an extracellular marker (e.g. tetramethylammonium ion, TMA^+) applied by iontophoresis (Nicholson and Phillips, 1981; Nicholson and Rice, 1988; Svoboda and Sykova, 1991).

This article deals with activity-related ionic and volume changes in spinal cord of adult rats and during development, with transient changes during peripheral stimulation, injury, hypoxia and anoxia.

Measurements of ionic and ECS volume changes

The use of ion-sensitive microelectrodes has made it possible to record transient intracellular and extracellular changes in K^+ and pH during neuronal activity *in vivo*. K^+ activity was recorded with double-barrelled K^+-selective microelectrodes (K^+-ISM) filled with a liquid ion-exchanger (Corning 477317) and prepared by a procedure described previously (Kriz et al., 1974). The same procedure was adopted to prepare the double-barrelled pH-sensitive microelectrodes with liquid Hydrogen Ion Ionophore II-Coctail A, Fluka (Chvatal et al., 1988; Sykova and Svoboda, 1990).

Dynamic changes in the size of the ECS were studied by means of the iontophoretic administration of ions that do not cross the cell membranes and therefore remain in the ECS. Their concentration in the ECS is in inverse proportion to the size of the ECS. The ion exchanger for K^+ (Corning 477317) is highly sensitive to TMA^+, which, in small concentrations, are not toxic and do not cross cell

membranes (Nicholson and Phillips, 1981; Kriz and Sykova, 1981). TMA^+ were therefore used for testing changes in the size of the ECS. Using a current of 10 to 100 nA, these ions were administered for 5 to 80 s into the ECS with a multichannel iontophoretic pipette. The tip of the K^+-ISM with which we recorded TMA^+ concentration changes during administration was 80 to 200 µm distant from the tip of the iontophoretic pipette. The ions were administered at regular intervals (2-10 min), always with the same current. At least 3 identical control curves were obtained in succession before the stimulation was applied. The relative changes in the size of the ECS were computed from the changes in the concentration of the given ions, according to the formula:

$$\text{Decrease in ECS (in \% of control)} = \left(1 - \frac{\Delta\,[TEA^+] \text{ before activity}}{\Delta\,[TEA^+] \text{ after activity}}\right) \times 100 \qquad (1)$$

For the determination of the fraction of brain volume which is ECS, i.e. extracellular volume fraction (α) and for the determination of tortuosity (λ), which represent the increased path lenght for diffusion between two points that results from barriers (e.g. cellular membranes, extracellular matrix) the distance needs to be known exactly. The two electrodes were therefore glued together with cement (Fig. 1) and the intertip distance measured exactly with a microscope. After they had been joined together, the electrodes were introduced into the nervous tissue. For computing and determining absolute values of α and λ we iontophoresed the ions into the nervous tissue for 50 to 80 s. Before and after measurements in the nervous tissue the diffusion curve was recorded in 0.3 % agar (Fig. 1). Measurement of the concentration change in the agar gives the free-diffusion values for the relevant ion, in which $\alpha = 1$ and $\lambda = 1$. The α and λ in the spinal cord were determined by computation after Nicholson and Phillips (1981). The results of these studies show that if we incorporate factors α and λ in Fick's law,

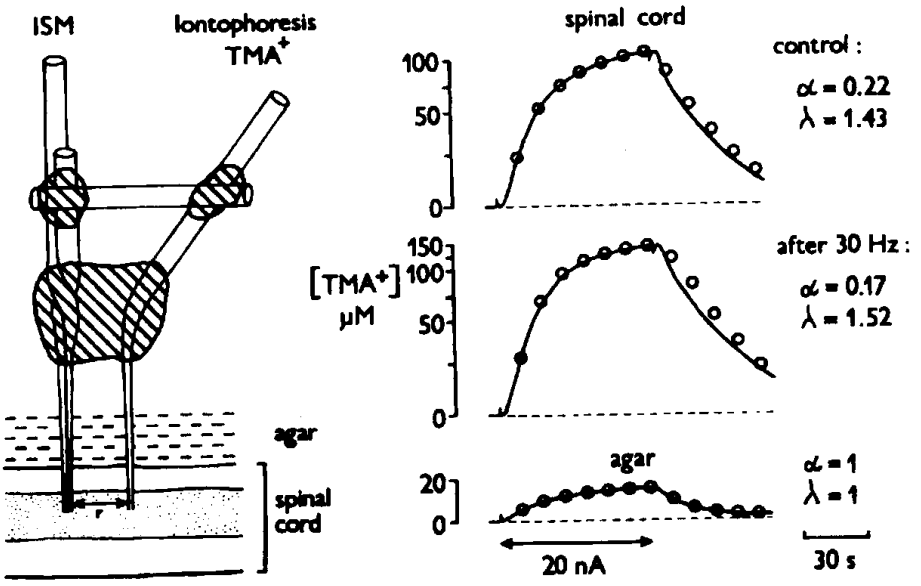

Fig. 1. Method of determining EC volume fraction (α) and tortuosity (λ) and TMA^+ diffusion curves in spinal cord *in vivo*. Left: Schematic drawing of the experimental arrangement for diffusion measurements. Two microelectrodes, the double-barrelled microelectrode sensitive to K^+ and to TMA^+ (ISM) 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 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 frequency of 30 Hz for 2 min. Diffusion curve after the stimulation is larger, due to the EC volume decrease. The volume fraction before stimulation (control) was $\alpha = 0.22$, the tortuosity $\lambda = 1.43$; after stimulation: $\alpha = 0.17$, $\lambda = 1.52$. Diffusion parameters were I = 20 nA, r = 180 μm, n = 0.308, D = 1.33 x 10^{-9} m^2s^{-1}. (Adapted from Svoboda and Sykova, 1991).

diffusion in the CNS is described fairly satisfactorily. The micropipette from which TMA^+ was released iontophoretically acts as the source Q = In/F. The appropriate solution of the diffusion equation is:

$$C = ((In\ \lambda^2) / (4\pi\ FDr\alpha))\ erfc\ (r\lambda/2\ (Dt)^{1/2}) \qquad (2)$$

where C is the concentration, I is the iontophoretic current increment, F is the Faraday's electrochemical equivalent, r is the distance of ISM from the ion source (iontophoretic pipette), D is the free diffusion coefficient, t is time of the probe ion application and erfc is the complementary error function.

TMA^+ crosses the membranes of leech glial cells and accumulates within (Ballanyi et al., 1990). Recent studies suggest that it also enters vertebrate brain cells. This can be a source of error and therefore nonspecific uptake, k' (s^{-1}), was incorporated into the diffusion equation (Nicholson, 1985). Uptake of TMA^+ is proportional to the extracellular concentration and can be a function not only of average permeability of the membranes to TMA^+ but also a function of a local volume fraction and the density of membranes in the tissue under study. The possibility that TMA^+ may cross the blood-brain barrier is not justified, since uptake values are similar in both in vivo and in vitro preparations. Therefore, assuming that loss of TMA^+ from the ECS is a linear concentration-dependent process, this factor can be incorporated into diffusion equation where apparent diffusion coefficient $D^* = D/\lambda^2$ (Nicholson, 1985; Cserr et al., 1991):

$$C(r,t) = (Q/(8\pi D^*\alpha r))\,[\exp(r(k'/D^*)^{1/2})\,\mathrm{erfc}(r/(2(D^*t)^{1/2} + (k't)^{1/2}) + \exp(-r(k'/D^*)^{1/2})\,\mathrm{erfc}(r/(2(D^*t)^{1/2}) - (k't)^{1/2})] \quad (3)$$

This equation enables the uptake k' to be determined along with the ECS volume fraction (α) and tortuosity (λ).

Peripheral stimulation and peripheral injury

Almost all kinds of stimulation - electrical or natural - lead to transient increase in $[K^+]_e$ and alkaline-acid shifts in pH_e in the corresponding spinal cord segments of rat. The main source of changes in $[K^+]_e$ are stimulated neurones, unmyelinated fibres and unmyelinated terminals of axons (for review see Sykova, 1983,

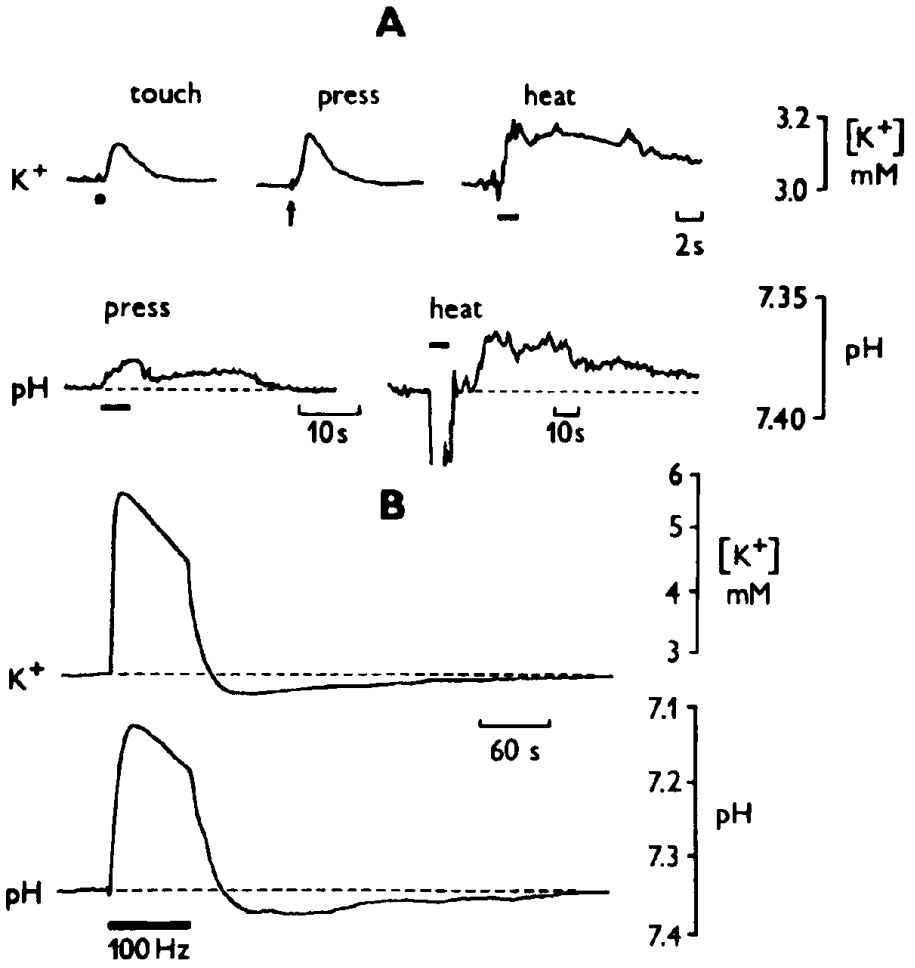

Fig. 2. A: Transient increase in $[K^+]_e$ and decrease in pH_e in the L_4 spinal segment in response to adequate stimuli applied to the plantar region of the ipsilateral hind paw. The K^+- and pH-sensitive microelectrodes were inserted to a depth of 600 μm from the dorsal surface. The curves represent responses to light touch, strong press and application of about 1 ml of hot water onto the hind limb (heat). Horizontal bars indicate stimulus duration. $[K^+]_e$ and pH_e data are from different experiments. B: $[K^+]_e$ 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. (Adapted from Svoboda et al., 1988; Sykova and Svoboda, 1990).

1992). An increase of 0.1 to 0.2 mM has been found in 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. 2A). A sustained increase in $[K^+]_e$ in the spinal dorsal horn of the rat was evoked by chemical and thermal injury of the hind paw (Svoboda et al., 1988). Repetitive electrical stimulation or prolonged 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. 2B). 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. 2B). This ceiling level (Kriz et al., 1975) is in the CNS 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 (Jendelova and Sykova, 1991).

K^+ homeostasis is ensured by two main mechanisms: 1) activation of the Na/K pump in neurones and glia, and 2) glial cell K^+ uptake or buffering of extracellular rise in $[K^+]_e$ (Sykova, 1983, Walz, 1989; Sykova, 1992). Active neurones as well as primary fibres lose K^+ and induce a change in the ionic composition of the ECS (Sykova and Vyklicky, 1977). This may be an important way in which neurones 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 active transport is strongly supported, since there is a poststimulation decrease below resting level, the so-called "K^+-undershoot" (Fig. 2B), which can be blocked by inhibitors of the Na/K pump (e.g. ouabain), or by anoxia, ischemia or anaesthetic drugs (Kriz et al., 1975; Sykova et al., 1985). Glial cells also significantly contribute to activity-related clearance of K^+ increase in ECS. There is evidence that K^+ clearance by glia include uptake of K^+ driven by the Na/K pump (Hertz, 1965) and K^+ spatial buffering (Orkand et al., 1966; Sykova et al., 1988).

Concomitantly with an increase in $[K^+]_e$ any neuronal activity results in a transient change in ECS acid-base balance. In CNS of adult animals the activity-related pH_e changes have a typical time-course: alkaline-acid shift during stimulation which is followed in mammalian spinal cord by poststimulation alkaline undershoot (Fig.2B; Sykova, 1989; Sykova and Svoboda, 1990). 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. Transient acid shifts in pH_e by 0.01-0.05 pH units were found when acute nociceptive stimuli (pinch, press, heat) were applied to the hind paw (Fig. 2A). 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 by about 0.05-0.10 pH units (Sykova and Svoboda, 1990). The decrease in pH_e began 2-10 min after injury and persisted for more than 2 hrs. Electrical nerve stimulation (10-100 Hz, 20-60 s) elicited triphasic (alkaline-acid-alkaline) changes in pH_e (Fig. 2B) which had a similar depth profile as the concomitantly recorded increase in $[K^+]_e$.

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 of water. K^+-induced swelling is mostly attributed to glia, particularly to astrocytes (Kimelberg and Ransom, 1986), but swelling of neuronal elements has also been shown (Ritchie et al., 1981; Trubatch et al., 1977; 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. With the above described method either relative changes in the ECS volume or absolute value of the ECS volume (λ) and tortuosity (α) were found in cortex, spinal cord, optic nerve and receptor organs during activity evoked by electric as well as adequate stimulation (Dietzel et al., 1980, 1982; Orkand et al., 1984;

Ransom et al., 1985; Sykova, 1987; Svoboda and Sykova, 1991) as well as during epileptic activity (Heinemann and Dietzel, 1984; Lehmenkühler et al., 1991) and during ischemia or spreading depression (Nicholson and Rice, 1988; Polak et al., in press).

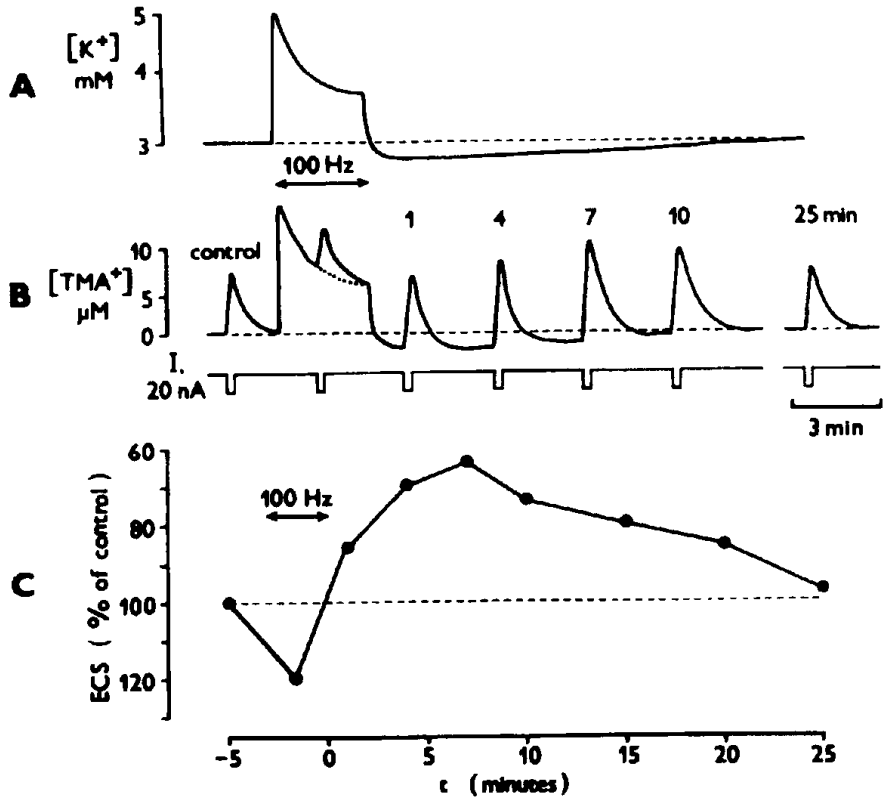

Fig. 3. Effect of repetitive electrical stimulation (3 min, 100 Hz) on the EC volume in spinal dorsal horn of adult rat. Upper trace: the record from chart recorder comprises the diffusion curves at a depth of 500 μm evoked by the regular iontophoretic application of TMA^+ (application at 3 min intervals) prior to stimulation, during the stimulation-evoked increase in $[K^+]_e$, and during the poststimulation K^+-undershoot below the original $[K^+]_e$ base line. TMA^+ applications for 15 s, iontophoretic current (I) 20 nA, and the intertip distance r = 240 μm. Dotted line represents the predicted time course of the $[K^+]_e$ curve without iontophoretic application and dashed line the prestimulation $[K^+]_e$ base line. Lower trace: graph of the EC volume changes expressed in percentage of control. (Adapted from Svoboda and Sykova, 1991).

The α in spinal cord varies in unstimulated adult animals between 20-25 % of total spinal cord volume and λ is about 1.5 (Svoboda and Sykova, 1991). These values are not significantly different from those found in brain by Nicholson and colleagues (1981, 1988). However, using the same method McBain et al. (1990) found that ECS volume is only 12% in the CA1 pyramidal cell layer of hippocampus. Therefore neuronal packing is probably an important factor that determines the EC volume. In spinal cord ECS volume was not significantly different throughout the dorsal and ventral horn gray matter.

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 (Svoboda and Sykova, 1991). However, after the end of stimulation, when the $[K^+]_e$ decreased below the original baseline (K^+-undershoot), the ECS volume decreased by 20-40 %, i.e. to α about 0.14-0.17 (Figs. 1 and 3). However, the tortuosity and uptake was not significantly changed by stimulation. The ECS volume decrease was maximal at 2-10 min after the stimulation had been discontinued, and it returned to the prestimulation value in 15-40 min (Fig. 3). The decrease in the ECS volume had the same depth profile as the ionic (K^+, Ca^{2+} and pH_e) changes evoked by neuronal activity. The higher the stimulation frequency and intensity and the greater the concomitant ionic changes, the greater was the decrease in ECS volume. The ECS volume decrease persisted after stimulation had been discontinued. It regularly lasted longer than the stimulation-evoked increase in $[K^+]_e$, its time course being related to the post-stimulation K^+-undershoot and poststimulation acid shift (Sykova and Svoboda, 1990).

The ECS volume decreased by 20-50% (but no more than to = 0.12) after injury of the ipsilateral hind paw evoked either by subcutaneous injection of turpentine or by thermal injury (Svoboda and Sykova, 1991). 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 (Fig. 4), and persisted for more than 120 min and 60 min, respectively. The tortuosity and uptake

was not significantly altered by injury. These measurements indicate that the normal volume of the ECS in adult rats occupies 1/5 - 1/4 of total CNS volume and that significant dynamic changes in the ECS volume accompany transmembrane ionic shifts resulting from neural activity.

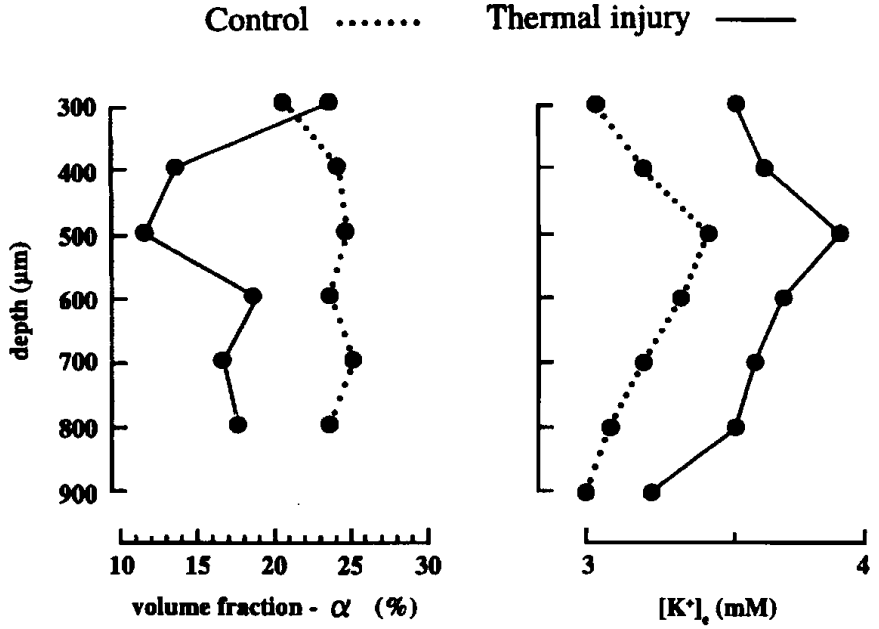

Fig. 4. Effect of thermal injury of the hind paw on the ECS volume fraction (λ) at various depth of the spinal dorsal horn. Left: typical ECS volume fraction at various depths of the spinal dorsal horn in the non-stimulated animal before injury (control) and the ECS volume fraction decrease at depth of 400-700 μm (injury). The maximal ECS volume fraction decrease from about 24% to about 12% was found at a depth of 500 μm 10-25 min after the thermal injury. Right: $[K^+]_e$ base line at various depths of the spinal dorsal horn in the control rat and in the rat after injury.

Postnatal development

In neonatal rat spinal cord the stimulation-evoked changes in $[K^+]_e$ are much larger than in adult animals. The maximal changes of 2.1-6.5 mM were found at a stimulation frequency of 10 Hz in 3

to 6 day-old animals. In older animals the $[K^+]_e$ changes progressively decreased (Fig. 5; Jendelova and Sykova, 1991). In 3 to 13 days-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 stimulation at a frequency 10-30 Hz (Svoboda et al., 1988), i.e. by as much as 1.5-2.5 mM.

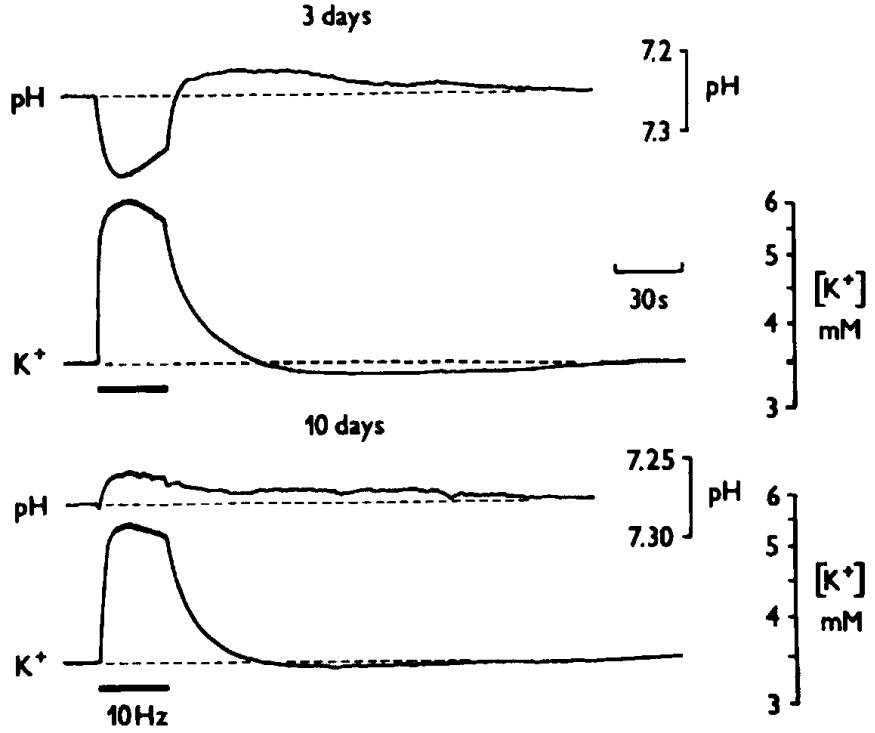

Fig. 5. Stimulation-evoked pH_e and $[K^+]_e$ changes in the spinal dorsal horn of rats 3 and 10 days-old. Note that the stimulation of the dorsal root at a frequency of 10 Hz evoked an alkaline shift in the 3 days-old pup, which was accompanied by an increase in $[K^+]_e$; when stimulation was discontinued the poststimulation acid shift of smaller amplitude appeared, which was accompanied by a K^+-undershoot. In the 10 days-old rat there was a slight initial alkaline shift, which was followed by an extracellular acid shift.

In 3 to 8 day-old pups, the stimulation evoked an alkaline shift by 0.1-0.2 pH unit which was followed by a smaller poststimulation acid shift by 0.03-0.07 pH unit (Fig. 5). In 10 to 14 day-old rats, however, the stimulation evoked an acid shift which was preceded by a scarcely discernible alkaline shift as in adult animals (Sykova and Svoboda, 1990). 20 mM $MgCl_2$ 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 alkaline shift was enhanced in HEPES-buffered saline, it was significantly decreased by La^{3+}, a H^+ channel blocker, and by picrotoxin in concentrations from 10^{-7} M (Sykova et al., in press). In concentrations 10^{-7}-10^{-6} M picrotoxin had no effect on K^+ and pH_e resting level. Superfusion of spinal cord with GABA (10^{-3} M) elicited an extracellular alkaline shift which was blocked by picrotoxin (Sykova et al., in press). In nominally HCO_3^- free solution, buffered with HEPES, GABA evoked no alkaline shift. The results suggest that the activation of GABA-gated Cl^- channels in spinal cord may give rise to an HCO_3^- efflux which can explain the pH_e alkaline shifts observed in the dorsal horn during a stimulation of an afferent input. However, the observed alkaline shifts can also be mediated by and HCO_3^- independent merchanism (see also Chen and Chesler, 1991, 1992; Kaila and Voipio, 1987; Kaila et al., 1992; Sykova et al., in press).

The acid shift in adult rats and in immature rats 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 neurones but also in glia (for review see Chesler, 1990; Sykova, 1992). 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^-. Since carbonic anhydrase and gliogenesis to a great extent occur postnatally with their peak at about 10 days, it can be concluded that in spinal cord (Jendelova and Sykova, 1991) as in the optic nerve (Ransom et al., 1985), the stimulation-evoked acid shifts are related to gliogenesis. These results suggest

that glial cells buffer the activity-related $[K^+]_e$ increase and alkaline pH_e shifts. Recent studies have therefore revealed 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.

We also studied ECS volume changes during development in rats 3 to 14 days-old, i.e. during the period of extensive gliogenesis. Similarly as in unmyelinated rat optic nerve (Ransom et al., 1985) we found that the ECS in sensorimotor cortex (Svoboda et al., in press) and the ECS in spinal cord of immature rats is larger, its varies between 30 - 40 % in 3 to 5 days-old pups and its size decreases with the animal age.

The mechanisms of cellular shrinkage and swelling during neuronal activity are discussed in several papers (for review see Kempski, 1986; Kimelberg and Ransom, 1986; Sykova, 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. 5) Increase in intraneuronal particles, produced by enhanced metabolism during excessive neuronal activity. It is evident that the glial cells, and particularly astrocytic swelling, could play the important role in activity-related ECS volume changes.

Hypoxia and anoxia

Extracellular K^+, pH and diffusion properties in spinal dorsal horn of the rat in vivo were studied during hypoxia and anoxia evoked either by decrease in blood pressure by exsanguination or by respiratory arrest (Polak et al., in press; Sykova et al., unpublished results). In normoxic conditions, prior to exposure of the rat to ischemia or anoxia, the diffusion parameters were $\alpha = 0.229 \pm 0.01$; $\lambda = 1.60 \pm 0.03$, $k' = 5.23 \pm 0.6 \times 10^{-3}\ s^{-1}$, $n = 13$ (these and subsequent values are means $\pm$ S.E.). A typical TMA^+ diffusion

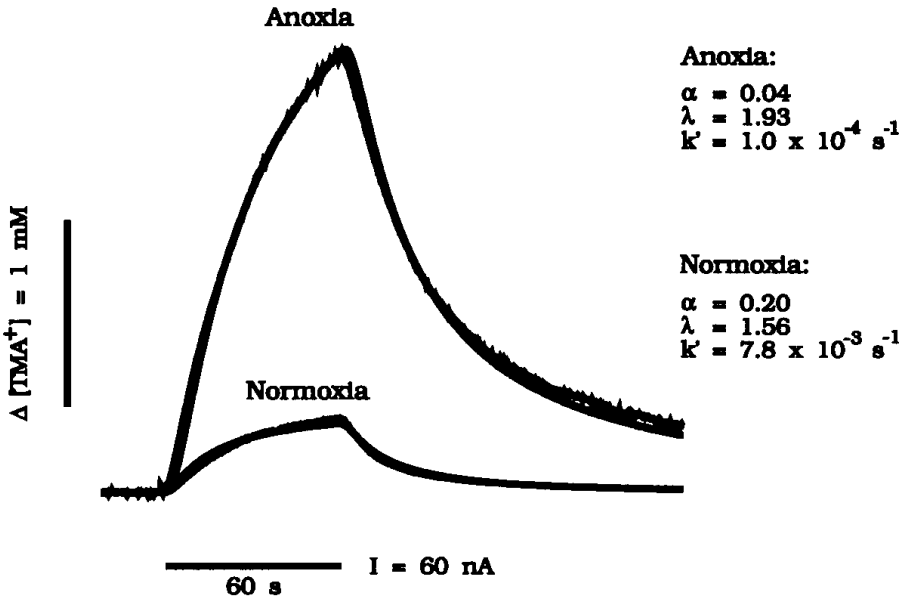

Fig. 6. TMA^+ diffusion curves obtained in the rat spinal cord in vivo in dorsal horn of segment L_4 at a depth of 750 μm from dorsal surface. In normoxic conditions $[K^+]_e$ was 3 mM, as measured by separate K^+-ISM. During terminal anoxia evoked by respiratory arrest $[K^+]_e$ rose to about 70 mM. Diffusion parameters: volume fraction (α), tortuosity (λ) and nonspecific cellular uptake (k') were determined with the use of the same electrode array with spacing between the TMA^+-sensitive microelectrode and iontophoresis pipette of 140 μm, iontophoresis transport number n = 0.21, and TMA^+ diffusion coefficient of 1.28×10^{-5} $cm^2\ s^{-1}$ at 36 °C. The accurate TMA^+ calibration was made in both 3 and 70 mM K^+ background. The curves are presented as linear concentration changes with base lines superimposed and fitted to a solution of the diffusion equation. The theoretical curve is superimposed on each experimental curve. Note that the diffusion curve during anoxia is much larger in amplitude, reflecting the decline in ECS volume fraction.

curve recorded in the spinal cord in normoxic conditions is illustrated in Fig. 6. As the blood pressure fell to 60-70 mmHg, K^+ rose by 10-12 mM, pH_e fell by about 0.3 pH units and volume fraction of the ECS significantly decreased, to $\alpha = 0.16 \pm 0.01$, n = 5, while the tortuosity and uptake had not yet changed. As the blood pressure fell to 20-30 mmHg, K^+ rose to 60-70 mM, pH_e fell by about

0.6 pH units and all three diffusion parameters were significantly changed: ECS volume fraction decreased ($\alpha = 0.073 \pm 0.023$, n = 6), tortuosity increased ($\lambda = 2.09 \pm 0.08$) and TMA^+ uptake decreased ($k' = 1.45 \pm 0.83 \times 10^{-3}\ s^{-1}$). Full recovery was found after re-injecting the blood or after injection of noradrenaline, if this resulted in rise in blood pressure above 100 mmHg. No further increase in $[K^+]_e$ and in the diffusion parameters of spinal cord tissue was observed after the death of the animal ($\alpha = 0.066 \pm 0.004$, $\lambda = 2.07 \pm 0.08$, $k' = 1.39 \pm 0.5 \times 10^{-3}\ s^{-1}$, n = 11; Fig. 6), while the pH_e increased by about 0.2 pH unit. These data represent the first *in vivo* measurements of diffusion parameters α, λ and k' under hypoxia and anoxia. Similar changes during hypoxia have been described in *in vitro* slices of rat neostriatum, where α fell from an average value of 0.21 to 0.13 (Rice and Nicholson, 1991). In contrast, tortuosity and k' were unchanged. These results are in agreement with those in spinal cord during hypoxia when the blood flow fell to 50-60 mmHg.

These results show that during hypoxia and anoxia the diffusion properties of the nervous tissue are substantially changed. This could affect the diffusion of various substances - ions, neurotransmitters, metabolic substances as well as various drugs used in therapy of nervous diseases. It is also evident that the excessive ionic and ECS volume changes can contribute to irreversible tissue damage.

Conclusions

Concomitantly with changes in ionic composition, the size of the ECS is significantly altered during stimulation-evoked neuronal activity, CNS development, during peripheral or central injury, hypoxia and anoxia. In immature CNS, and possibly during pathological states which are frequently accompanied by astrocytic proliferation and hypertrophy, K^+, pH_e and ECS volume homeostasis are impaired. Changes in ECS ionic composition and volume accompanied by altered diffusion parameters in ECS may significantly influence the function of neurones, glia and CNS excitability.

References

Ballanyi, K., P. Grafe, G. Serve, W.-R. Schlue: Electrophysiological measurements of volume changes in leech neuropile glial cells. Glia 3: 151-158 (1990)

Chen, J.C.T., M.L. Chesler: Extracellular alkalinization evoked by GABA and its relationship to activity-dependent pH shifts in turtle cerebellum. J. Physiol. (Lond.). 442: 431-446 (1991)

Chen, J.C.T., M.L. Chesler: Modulation of extracellular pH by glutamate and GABA in rat hippocampal slices. J. Neurophysiol. 67: 29-36 (1992)

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

Chvatal, A., P. Jenedelova, N. Kriz, E. Sykova: Stimulation-evoked changes in extracellular pH, calcium and potassium activity in the frog spinal cord. Physiol. Bohemoslov. 37: 203-212 (1988)

Cserr, H.F., M. Depasquale, C. Nicholson, C.S. Patlak, K.D. Pettigrew, M.E. Rice: Extracellular volume decreases while cell volume is maintained by uptake of ions in rat cerebral cortex during acute hypernatremia. J. Physiol. (Lond.), 442: 277-295 (1991)

Dietzel, I., U. Heinemann, G. Hofmeier, H.D. Lux: Transient changes in the size of the extracellular space in the sensorimotor cortex of cats in relation to stimulus-induced changes in potassium concentration. Exp. Brain Res. 40: 432-439 (1980)

Dietzel, I., U. Heinemann, G. Hofmeier, H.D. Lux: 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 (1982)

Heinemann, U., I. Dietzel: Extracellular potassium concentration in chronic alumina cream foci of cats. J. Neurophysiol. 3: 421-434 (1984)

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

Hertz, L.: Regulation of potassium homeostasis by glial cells. In: G. Levi (Ed.), Development and Function of Glial Cells. Alan R. Liss, New York, 1990, pp. 225-234

Jendelova, P., E. Sykova: Role of glia in K^+ and pH homeostasis in the neonatal rat spinal cord. Glia, 4: 56-63 (1991)

Kaila, K., J. Voipio: Postsynaptic fall in intracellular pH induced by GABA-activated bicarbonate conductance. Nature 330: 163-165 (1987)

Kaila, K., P. Paalasmaa, T. Taira, J. Voipio: pH transients due to monosynaptic activation of $GABA_A$ receptors in rat hippocampal slices. Neuroreport 3: 105-108 (1992)

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

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

Kriz, N., E. Sykova: Sensitivity of K^+-selective microelectrodes to pH and some biologically active substances. In: E. Sykova, P. Hnik and L. Vyklicky (Eds.), Ion-Selective Microelectrodes and Their Use in Excitable Tissues. Plenum Press, New York, 1981, pp. 25-39

Kriz, N., E. Sykova, E. Ujec, L. Vyklicky: Changes of extracellular potassium concentration induced by neuronal activity in the spinal cord of the cat. J. Physiol. (Lond.) 238: 1-15 (1974)

Kriz, N., E. Sykova, E. Ujec, L. Vyklicky: 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 (1975)

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

McBain, C.J., S.F. Traynelis, R. Dingledine: Regional variation of extracellular space in the hippocampus. Science 249: 674-677 (1990)

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

Nicholson, C.: Diffusion from an arbitrary volume of a substance in brain tissue with arbitrary volume fraction and tortuosity. Brain Res. 333: 325-329 (1985)

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

Nicholson, C., M.E. Rice: 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, 1988, pp. 247-361

Nicholson, C., M.E. Rice: Diffusion of ions and transmitters in the brain cell microenvironment. In: K. Fuxe and L.F. Agnati (Eds.), Volume: Transmission in the Brain. Raven, New York, 1991, pp. 279-294

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

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

Polak, J., J. Svoboda, E. Sykova: Diffusion characteristics and extracellular space volume during anoxia in spinal cord of the rat. Physiol. Res. (in press)

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

Rice, M., C. Nicholson: Diffusion characteristics and extracellular volume fraction during normoxia and hypoxia in slices of rat neostriatum. J. Neurophysiol. 65: 264-272 (1991)

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

Svoboda, J., E. Sykova: Extracellular space volume changes in the rat spinal cord produced by nerve stimulation and peripheral injury. Brain Res. 560: 216-224 (1991)

Svoboda, J., E. Sykova, A. Lehmenkühler: Extracellular space volume in the rat cortex during development and central injury. Physiol. Res. (in press)

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

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

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

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

Sykova, E.: 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, 1991, pp. 217-236

Sykova, E.: Ionic and volume changes in the microenvironment of nerve and receptor cells. Progress in Sensory Physiology 13. Springer-Verlag, Heidelberg, 1992, pp. 1-167

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

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

Sykova, E., P. Jendelova, Z. Simonova, A. Chvatal: K^+ and pH homeostasis in the developing rat spinal cord is impaired by early postnatal X-irradiation. Brain Res. (in press)

Sykova, E., J. Svoboda, A. Chvatal, P. Jendelova: Extracellular pH and stimulated neurons. In: G. Bock and J. Marsh (Eds.), Proton Passage Across Cell Membranes. Wiley, Chichester, 1988, pp. 220-232

Sykova, E., I. Hajek, A. Chvatal, N. Kriz, G.I. Diatchkova: Changes in extracellular potassium accumulation produced by opioids and naloxone in frog spinal cord: Relation to changes of Na-K pump activity. Neurosci. Lett. 59: 285-290 (1985)

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

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

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

Migraine: Basic Mechanisms and Treatment

Edited by

A. Lehmenkühler, K.-H. Grotemeyer and F. Tegtmeier

Contributors

P.G. Aitken, G.L. Barkley, I.G. Böttger, J. Bures, D. Busija, A. Chvatal, G. Clincke, G. Czéh, E. De Prins, H.C. Diener, M. Fabricius, V.M. Fernandes de Lima, J. Flammer, J. Fransen, L. Friberg, A.R. Gardner-Medwin, W.D. Gerber, J. Gheuens, L. Gothe, R. Graf, K.-H. Grotemeyer, I.O. Haefliger, W. Hanke, A.J. Hansen, W.-D. Heiss, U. Heister, J.A. Helpern, O. Herreras, K.-A. Hossmann, W.J. Janssens, A.P. Jeanjean, L.H. Jensen, J. Jing, J. Kolb, R.P. Kraig, Sh. Kyuhou, G.A. Lambert, M. Lauritzen, A. Lehmenkühler, St.R. Levine, T.F. Lüscher, J.M. Maloteaux, R. Marrannes, H. Martins-Ferreira, K. Matsumoto, A. Mayevsky, J.E. Moran, M.A. Moskowitz, M. Nedergaard, C. Nicholson, K. Nozaki, Y.C. Okada, T.S. Olsen, R.D. Piper, J. Polak, B. Quistorff, N.M. Ramadan, F. Richter, G. Rosner, D. Scheller, H.P. Schlake, W.-R. Schlue, O. Schober, M.J. Sheardown, N. Shimada, G.G. Somjen, J. Sonn, D. Soyka, U. Speckenbach, T.J. Steiner, J. Svoboda, E. Syková, F. Tegtmeier, N. Tepley, K.M.A. Welch, O. Winzer, Ch. Xu, J.N. Young, N. Zarchin, A. Ziegler, M. Zimmermann

126 Figures, 30 Tables

Urban & Schwarzenberg
München – Wien – Baltimore

Die Deutsche Bibliothek – CIP-Einheitsaufnahme

Migraine : basic mechanisms and treatment ; 30 tables ;
[proceedings of the International Symposium Migraine, Basic Mechanisms and Treatment, held in Münster (Germany) from March 8 – 11, 1992] / ed. by A. Lehmenkühler ... Contributors P. G. Aitken ... - München ; Wien ; Baltimore :
Urban und Schwarzenberg, 1993
ISBN 3-541-12661-2
NE: Lehmenkühler, Alfred [Hrsg.]; Aitken, P. G.; International Symposium Migraine, Basic Mechanisms and Treatment <1992, Münster, Westfalen>

Cover design: Dieter Vollendorf, Munich
Manuscript editor: Dr. Dorothea Schneiderbanger, Munich

Printed in Germany

ISBN 3-541-12661-2