Verh. Dtsch. Zool. Ges. 82, 153–163 (1989)

Activity-related extracellular pH transients in spinal cord

Aktivitätsabhängige extrazelluläre pH-Veränderungen im Rückenmark

Eva Syková

Institute of Physiological Regulations, Czechoslovak Academy of Sciences, Bulovka Pavillon 11, 180 85 Prague 8, Czechoslovakia

Abstract

The changes in extracellular pH (pH_e) in spinal cords of rats and in isolated spinal cords of frogs were studied by means of pH-sensitive microelectrodes. 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. about 0.2 pH units lower than that measured in the cerebrospinal fluid. 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. Chemical or thermal injury produced a long-term decrease in pH_e base line in the lower dorsal horn of about 0.05–0.1 pH units.

Electrical nerve stimulation (10–100 Hz, 20–60 s) elicited triphasic alkaline-acid-alkaline or alkaline-acid-acid changes in pH_e which have a similar depth profile as a concomitantly recorded increase in $[K^+]_e$. The pH_e changes were reduced by 65–75% after the blockade of synaptic activity demonstrating that depolarized neurons are responsible for most of the changes. The initial alkaline shift of about 0.01 pH units as well as the dominating acid shift of about 0.1–0.2 pH units were accelerated and increased by acetazolamide (a carbonic anhydrase inhibitor), showing that the high buffering capacity of the extracellular fluid may hamper th resolution of acid-base perturbations. The initial alkaline shift was significantly decreased by La^{3+}, a H^+ channel blocker. Stimulation-evoked acid shifts were blocked by amiloride, SITS, DIDS, furosemide and La^{3+} and, therefore, have a complex mechanism which include Na^+/H^+ exchange, Cl^-/HCO_3^- exchange, and/or $Na^+/Cl^-/H^+/HCO_3^-$ antiport, and/or Na^+-HCO_3^- cotransport, K^+-Cl^- cotransport, and H^+ efflux through voltage sensitive H^+ channels. Non of the inhibitors with exception of La^{3+} significantly depressed acid shifts evoked in the isolated spinal cord by 10 mmol/l K^+. The poststimulation alkaline shift (alkaline undershoot) was blocked by ouabain and reflects coupled clearance of K^+ and H^+ by active transport processes.

Introduction

pH, although considerably well regulated both in intracellular and extracellular microenvironments in excitable tissues, has been shown to change transiently under a variety of physiological and pathological conditions. The use of pH-sensitive microelectrodes enabled us to follow the dynamic changes in pH in nervous tissue resulting from neuronal activity. A large number of studies indicate that the neuronal activity gives rise to changes in intracellular and extracellular pH (for review see Thomas 1988a).

It has been found that polarized neurons appear to change its intracellular as well as surface pH (Thomas 1988b). Transient changes in extracellular pH (pH_e) were found in cortex, hippocampus, cerebellum, spinal cord, and in peripheral nerves during electrical stimulation of afferent input (Urbanics et al. 1978, Kraig et al. 1983, Ransom et al. 1985, Endres et al. 1986, Kirshtal et al. 1987, Chesler and Chan 1988, Chvátal et al. 1988, Rice and Nicholson 1988, Syková et al. 1988, Syková and Svoboda in press), seizures, spreading depression and ischemia (Lehmenkühler et al. 1981, Kraig et al. 1983, Harris and Simon 1984, Mutch and Hansen 1984, Somjen 1984, Siesjö et al. 1985).

The alkaline and acid shifts have been recorded under different experimental conditions, in various animals *in vivo*, as well as *in vitro* preparations. No simple monophasis changes,

however, occurred in all these studies during neuronal activity. Alkaline and acid transients, ranging from 0.01 to 0.4 pH units, have been found to occure in different sequences and in latencies ranging from 10 ms to more than 1 min. The time course and mechanisms of the activity-related transient changes are, therefore, not simple to study and their mechanisms are today far from clear.

I review here the results of our studies about transient pH_e changes in spinal cord and compare it with findings in brain and peripheral nerves. I summarize our knowledge and perspectives about the origin, mechanisms, and physiological significance of the so far described pH_e changes in nervous tissue.

Methods

Experiments were performed either on rats anaesthetized by Nembutal (pentobarbitone) or on isolated spinal cords of frogs. Rats were immobilized and ventilated with air enriched with oxygen. End-tidal CO_2 was continuously monitored and kept around 2–3%. The arterial pCO_2 was measured in blood samples by pH/Blood Gas Analyser (Radiometer), and ranged from 35–55 mm Hg. A laminectomy was performed between L_2 and L_6. The animals were mounted in a rigid frame, and a pool of skin flaps filled with paraffin oil (37° C) was made around the spinal cord. Transcutaneous electrical nerve stimulation was delivered through a pair of stainless-steel acupuncture needles inserted through the skin below the plantar surface. Stimulation parameters were adjusted at 6.0–20.0 V and 0.1–0.3 ms to obtain supramaximal responses from the cord dorsum, from field potentials, and from a maximal increase in $[K^+]_e$ in response to single electrical pulses. Adequate stimuli were applied to the most sensitive area of the receptive field by touching or light brushing the side areas of the hind paw with a cotton wick, squeezing the toes with a forcep (press), or pouring about 0.2–0.5 ml of hot water (60–80° C) within 2–5 s onto the skin (heat). To produce peripheral tissue injury and to mimic chronic pain, we injected 0.1–0.5 ml of turpentine into the plantar region of the hind paw or applied about 1–3 ml of hot oil (150–200° C) to the skin of the foot (Svoboda et al. 1988).

The drugs were dissolved and applied onto the spinal cord surface in physiological solution with 3 mmol/l K^+ the pH of which was adjusted to 7.1–7.3. We used acetazolamide (Ciech), amiloride (Merck Sharp Dohme), SITS (4-acetamido-4′-isothiocyanatostilbene- 2,2′-disulfonic acid), DIDS (4,4′-diisothiocyanatostilbene -2,2′-disulphonic acid), and ouabain (Sigma), $LaCl_3$ (BDH Chemicals Ltd.).

The dissection of the isolated spinal cord was described in detail elsewhere (Syková et al. 1976). The isolated spinal cords were mounted in a chamber and superfused with oxygenated, either bicarbonate-buffered Ringer solution (95% O_2 and 5% CO_2), or HEPES-buffered Ringer solution (100% O_2), of the following composition (in mmol/l): NaCl 114.0, KCl 3.0, $CaCl_2$ 1.8, $NaHCO_3$ 20, glucose 1 g/l, at 17–19° C, pH 7.2–7.3. When the $[K^+]$ was raised to 10.0 mmol/l the $[Na^+]$ was decreased to 107.0 mmol/l. Ringer solution, either of standard composition, or with added inhibitors, at 18–20° C, pH 7.30–7.35 (HEPES-buffered solution was titrated with NaOH, and NaCl was reduced accordingly), was used for spinal cord superfusion. Dorsal root eight or nine was stimulated supramaximally (rectangular pulses of 5 V or less and 0.1 ms) with bipolar silver electrodes.

K^+ activity was recorded by means of double-barrel K^+-sensitive microelectrodes filled with a liquid ion-exchanger (Corning 477317). Basically, the same procedure as described previously (Kříž et al. 1974, Svoboda et al. 1988), was adopted to prepare the double-barrel pH-sensitive microelectrodes with a tip diameter of 3–5 μm. The tip of the pH-sensitive barrel was siliconized with 3–5% tri-n-butyl, chlorosilane in 1-chlornaphthalene and filled up to a height of 0.3–1.0 mm with a Hydrogen Ion Ionophore II-Cocktail A (Fluka), originally developed by Ammann et al. (1981). The backfilling solution was composed of (mmol/l): KH_2PO_4 40.0, NaOH 23.0, NaCl 15.0 (pH 7.0). The electrode responded with about 58 mV/pH unit and had a resistance of 700–1800 megaohms. The microelectrodes were calibrated in standard pH solutions with a background of 150 mmol/l NaCl and 3 mmol/l KCl. Double-

barrel Ca^{2+}-sensitive microelectrodes had a tip of 2–4 μm. The ion-sensitive barrel contained a Ca^{2+}-sensitive cocktail (Fluka), the backfilling solution was composed of 150 mmol/l $CaCl_2$. All reference barrels were filled with 150 mmol/l NaCl.

The electrical arrangements were the same as described for K^+-sensitive microelectrodes (Kříž et al. 1975). Each channel of a double-barrel microelectrode was connected to one input of a differential amplifier.

Results

Since the pH_e depends on depolarization of the neuronal membrane, on the metabolic activity of the nerve cell, and on ionic shifts across the cell membrane, neuronal activity can be expected to be accompanied by dynamic changes in the pH_e.

pH_e resting level

The pH_e resting levels studied so far in CNSs vary between 7.10–7.35 pH_e units, i.e. is slightly alkaline (Urbanics et al. 1978, Kraig et al. 1983, Mutch and Hansen 1984, Siesjö et al. 1985, Syková et al. 1988b). The pH_e base lines in unstimulated spinal cord of rat vary between 7.15–7.35 pH units (Syková and Svoboda in press). The actual values of pH_e in the spinal cord were established by comparing the potential of the pH-sensitive microelectrode in cerebrospinal fluid, or at various depths in the spinal cord, with that of a physiological solution poured onto the surface of the cord or placed in a small beaker next to the cord. The pH in the physiological solution was adjusted to 7.2–7.8 and measured by a pH-meter. The pH in supraspinal fluid measured just before microelectrode penetration into the spinal cord was 7.35–7.40. In the upper dorsal horn and in the ventral horn the pH_e base line was only slightly more acid, 7.25–7.35. The pH_e was most acid, i.e. about 7.15, in the lower dorsal horn. In the isolated frog spinal cord the pH_e resting level in lower dorsal horn was always by 0.1–0.2 pH units more acid than that of Ringer solution (pH 7.35).

The variations in pH_e base line had a similar depth profile as the variations in $[K^+]_e$ base line in unstimulated spinal cords (Svoboda et al. 1988). An alkaline shift in pH_e base line of 0.05–0.10 pH units was found after blockade of synaptic activity by Mn^{2+} in a concentration of 2–4 mmol/l. 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 (Syková et al. 1983, Svoboda et al. 1988). These findings suggest that the pH_e «resting» level in the lower dorsal horn is more acid than that in the upper dorsal horn, in the ventral horn, and in cerebrospinal fluid, respectively, because of the release of H^+ or eqivalent ions during a high level of spontaneous activity of spinal interneurons.

The pH_e base line found in the spinal dorsal horns after respiratory arrest did not fall below pH 6.4 and had a similar time course to that previously described in the rat cortex (Harris and Symon 1984). Within the first two minutes after respiratory arrest, the initial increase in blood pressure was accompanied by a pH_e decrease of about 0.1 pH unit in spinal dorsal horns, while the $[K^+]_e$ base line was not yet changed. With subsequent decreases in blood pressure there was a further reduction of pH_e by about 0.6–0.8 pH units, i.e. to pH_e 6.6–6.4. This decrease in pH_e was accompanied by an initial elevation of $[K^+]_e$ to 10–12 mmol/l. In about 6–7 min after respiratory arrest the pH_e started to increase by 0.3–0.4 pH units and this was associated with a second elevation of $[K^+]_e$ to about 20 mmol/l or more.

Stimulation-related transient pH_e changes

Single electrical pulses applied through a pair of needles inserted i.m. into the plantar region of the ipsilateral hind limb, or innocuous stimuli (touching or stroking of the lateral aspect of the hind paw skin with a brush or with cotton wick) transiently decreased pH_e in the dorsal

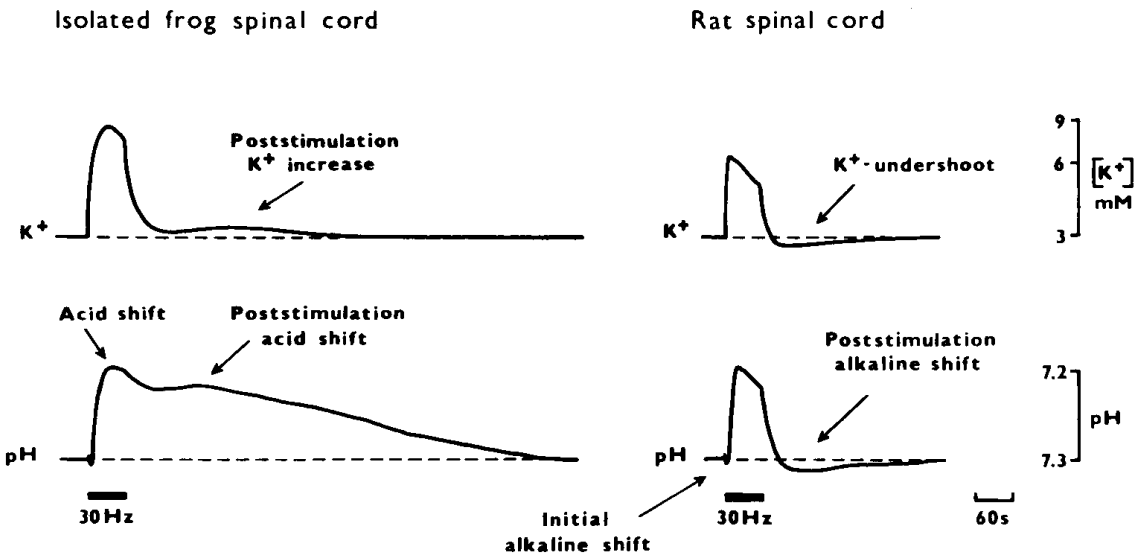

Fig. 1: Typical records of stimulation-related changes in extracellular pH and $[K^+]_e$ in the isolated frog spinal cord and in the rat spinal cord *in vivo*. Changes in pH_e and $[K^+]_e$ were produced in dorsal horns by electrical nerve stimulation at a frequency of 30 Hz applied for 60 s. Note the characteristic triphasic, alkaline-acid-acid changes in the isolated frog spinal cord, and alkaline-acid-alkaline changes in the rat spinal cord and their relation to the simultaneously recorded changes in $[K^+]_e$.

horns of rats by about 0.01 pH units. Acute nociceptive stimuli (squeezing of the toes) reduced pH_e transiently by about 0.01–0.05 pH units. Peripheral tissue injury evoked by s.c. injection of turpentine or by application of 1–3 ml of hot oil (150–200° C) onto the plantar surface of the hind paw was accompanied by a decrease in pH_e base line of 0.05–0.1 pH units (Syková and Svoboda, in press). The onset of pH_e decreases had a similar latencies and time course as the increases in $[K^+]_e$ (Svoboda et al. 1988).

Electrical nerve stimulation at a frequency of 10–100 Hz elicited typical triphasic alkaline-acid-alkaline changes in the dorsal horns of rats and alkaline-acid-acid changes in the dorsal horns of isolated spinal cords of frogs (Syková et al. 1988b) (Fig. 1). The amplitude and duration of the pH_e changes increased with the intensity, duration and frequency of stimulation. The dominating acid shift was preceded by a small initial alkaline shift of about 0.01 pH units which lasted 2–5 s. During stimulation, pH_e decreased by 0.15–0.25 pH units. When the stimulation was discontinued, pH_e transients returned to the base line and the former acid shift was followed by an alkaline shift (alkaline undershoot) of about 0.02–0.05 pH units. The alkaline undershoot recorded in the rat spinal cord *in vivo* was associated with the poststimulation K^+-undershoot, which has been shown to be due to uptake process involving Na^+/K^+ pump (for review see Syková 1983). While the poststimulation K^+-undershoot and alkaline undershoot are present regularly in the mammalian spinal cords *in vivo*, they are present only rarely in the frog spinal cord. The recovery in the isolated frog spinal cord was frequently not smooth but a second spontaneous acid shift occurred during poststimulation recovery to the original pH_e base line, which was associated with a spontaneous increase in $[K^+]_e$ with a similar time course (Fig. 1).

The depth profiles of pH_e changes in the spinal cords of rats or frogs were similar to the depth profiles of the stimulation-evoked increases in $[K^+]_e$ and decreases in $[Ca^{2+}]_e$ (Chvátal et al. 1988, Syková et al. 1988b). Maximal changes in pH_e, $[K^+]_e$ and $[Ca^{2+}]_e$ evoked by electrical as well as by adequate stimulation were found in the lower dorsal horn, i.e. in the areas of the largest amplitude of field potentials evoked by single electrical pulses.

The pH_e changes were closely related to the changes in $[K^+]_e$. Any spontaneous or stimulus-evoked increase in $[K^+]_e$ was accompanied by an acid shift in pH_e. However, the rise time of the stimulation-evoked acid shift was slower than the rise time of the increase in $[K^+]_e$. The $[K^+]_e$ ceiling level in rat spinal cord was attained in about 6–8 s, while the ceiling level of the

acid shift was reached in about 15–20 s. When stimulation lasted longer, a gradual decrease of both transients, $[K^+]_e$ and pH_e occurred immediately after the ceiling levels were reached. Recovery of the $[K^+]_e$ and pH_e changes to the original base lines took about 5 min and had similar time courses. The poststimulation alkaline undershoot below the original pH_e base line had a very similar time course to the poststimulation K^+-undershoot. These results suggest that the recovery of the activity-related K^+ and pH_e changes may have common underlying mechanisms, the most important being Na^+/K^+ pump activity (Kříž et al. 1975) and perhaps glial cell spatial buffering (Astion and Orkand 1988, Syková et al. 1988).

To examine further the relationship between $[K^+]_e$ and pH_e we superfused the isolated spinal cords of frogs with Ringer solution containing elevated $[K^+]_e$. After the spinal cord superfusion with 10 mmol/l K^+ a quasi-steady state was reached in the depth 300–400 μm within about 5 min. The $[K^+]_e$ during the superfusion was only the fraction of $[K^+]$ in the Ringer solution, i.e. the $[K^+]_e$ in the depth of 300–400 μm reached only 6–8 mmol/l K^+. The elevation in $[K^+]_e$ depolarized the neuronal elements, fibres and glial cells and was accompanied by a prompt and progressive acid shift of about 0.2 pH units (Fig. 2). The stimulation-evoked increase in $[K^+]_e$ as well as the acid shift both are depressed during the superfusion, while the poststimulation alkaline shift is enhanced. During the superfusion, the pH_e remained acid and returned to the base line only when $[K^+]_e$ returned to the standard bath concentration of 3 mmol/l K^+.

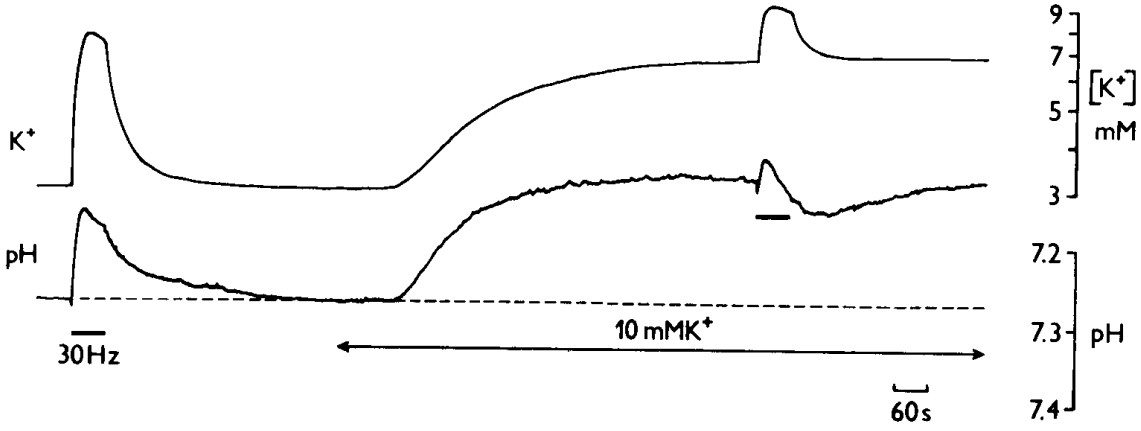

Fig. 2: Simultaneous pH_e and $[K^+]_e$ records during superfusion of the isolated frog spinal cord with solution containing elevated $[K^+]$ (10 mmol/l). The transient changes in pH_e and $[K^+]_e$ evoked by stimulation at 30 Hz prior and during the superfusion.

The effect of a block of synaptic transmission on pH_e changes

Repetitive electrical stimulation of peripheral input leads to accumulation of K^+ and to other dynamic ionic changes in the extracellular space, which are not only result of postsynaptic neuronal activity, but are also largely the outcome of activity in primary afferent fibres (for review see Syková, 1983). It can therefore be assumed that dynamic pH_e changes are partly of postsynaptic (i.e. neuronal) origin and partly of presynaptic origin (i.e. due to depolarization of primary afferent fibres). We blocked synaptic activity in the isolated frog spinal cord by superfusing the spinal cord with Ringer solution containing only 0.5 mmol/l $CaCl_2$ and either 2–4 mmol/l $MnCl_2$ or 20 mmol/l $MgCl_2$. The pH_e changes evoked by repetitive stimulation were markedly reduced; spontaneous activity, the initial alkaline shift as well as the second poststimulation acid shift always disappeared completely and the amplitude of the acid shifts during stimulation fell to 25–35% (Fig. 3) (Syková, Chvátal and Jendelová unpublished results). These results demonstrate that depolarized postsynaptic elements are responsible for most of the changes in the pH_e. Like other dynamic extracellular ionic changes, however, some of the pH_e changes are the result of depolarization of the primary afferent fibres themselves.

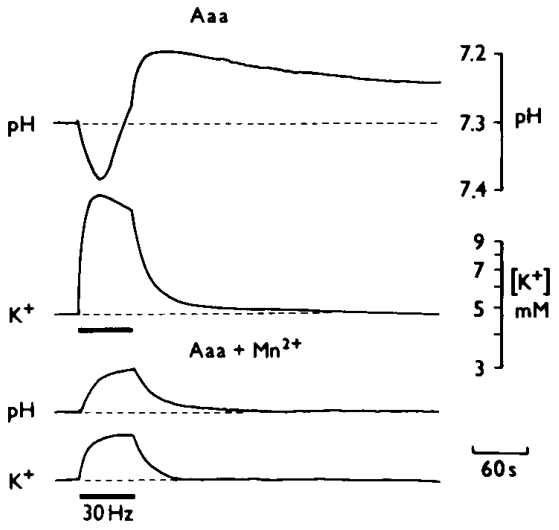

Fig. 3: Effect of acetazolamide (10^{-4} mmol/l) on simultaneously recorded changes in pH_e and $[K^+]_e$ in the isolated frog spinal cord (superfused with Ringer solution buffered with HEPES) as evoked by tetanic stimulation of dorsal root at 30 Hz. Note the enhanced initial alkaline shift and the onset of acid shift before the end of stimulus train. Lower records: Effect of Mn^{2+} (2 mmol/l) applied in Ringer solution with acetazolamide on the pH_e and $[K^+]_e$ changes. Note the complete block of the initial alkaline shift, and the decrease in the stimulation-evoked acid shift and $[K^+]_e$.

Application of inhibitors

To clarify the mechanisms of the observed changes in pH_e we studied the effects of various inhibitors of enzymatic activity and of ion transport mechanisms across cell membranes. In particular, we concentrated on the role of carbonic anhydrase, the enzyme which catalyzes the hydratation of CO_2 and is noncompetitively inhibited by acetazolamide, on the role of Na^+/K^+ ATPase inhibited by ouabain and on lactate production blocked by NaF. Furthermore we investigated the role of the Na^+/H^+ exchange, Cl^-/HCO_3^- exchange, $Na^+/Cl^-/HCO_3^-/H^+$ antiport, Na^+-HCO_3^- cotransport, K^+-Cl^- cotransport, and voltage sensitive H^+ channels.

pH_e changes after acetazolamide

The *in vivo* control of pH_e is ensured by an extremely effective HCO_3^-/CO_2 buffer system. The high buffering capacity of the extracellular fluid may hamper the resolution of acid-base perturbations. The pH_e changes were therefore studied after application of acetazolamide. The pH_e base line following application of acetazolamide in concentrations of 1–5 mmol/l was either not significantly changed or was about 0.01–0.05 pH units more alkaline. However, stimulation-evoked alkaline-acid shifts were greatly accelerated and enhanced both in spinal cord of rats *in vivo* as well as in isolated spinal cord of frogs superfused by Ringer solution buffered with bicarbonate. The stimulation-evoked changes in $[K^+]_e$ were not altered. In Ringer solution buffered with HEPES or TRIS, the enhancement of the acid-base changes was even larger the initial alkaline shift being more than ten times larger after application of acetazolamide (Fig. 3). The rise time of the initial alkaline shift after acetazolamide treatment corresponded to the time course of the stimulation-evoked increase of $[K^+]_e$.

These results suggest that the HCO_3^-/CO_2 extracellular buffer system slows and abolishes the activity-related pH_e changes. Decrease in the extracellular fluid buffering capacity probably unmasks the time course of activity-related transient pH_e changes in the close vicinity of active neurons.

Effects of ouabain, NaF and hypoxia

Ouabain has been shown to significantly slow the stimulation-evoked changes in $[K^+]_e$, slow their recovery, and block poststimulation K^+-undershoot. Similar effects were observed during partial hypoxia with an accompanying decrease in blood pressure to 70–80 mm Hg (for review see Syková 1983).

In spinal cords of rats as well as frogs, the application of ouabain (10^{-6}–10^{-5} mol/l) decreased the pH_e base line by about 0.05 pH units (Chvátal et al. 1988). The rise time of the stimulation-evoked acid shift was prolonged, as was the poststimulation pH_e recovery to the original base line. The poststimulation alkaline undershoot was diminished. The ceiling pH_e level, i.e. the maximal acid shift in rats *in vivo*, however, was not much smaller – it decreased by 15.3 ± 8.2% (Syková and Svoboda, in press). The depression by 20–50% was observed in isolated frog spinal cord when applied in concentrations up to 10^{-5} mol/l. The higher concentrations of ouabain depressed $[K^+]_e$ transient changes. Mild hypoxia which decreased blood pressure to 60–80 mm Hg, had similar effects on pH_e transient changes as ouabain.

NaF, which blocks the glycolytic part of metabolic processes and lactate production, did not have a significant effect on pH_e changes induced in the isolated spinal cord by tetanic stimulation of peripheral input (Syková et al. 1988b). Our results show that the lactate production demonstrated under the extreme conditions as anoxia, ischemia and epileptic seizures, did not play an important role in the origin of pH_e changes evoked by electrical and adequate stimulation.

Changes in pH_e after amiloride

In concentrations blocking Na^+/H^+ exchange (10^{-4}–10^{-3} mmol/l) amiloride decreased the pH_e baseline by about 0.01–0.05 pH units. While initial and poststimulation alkaline shifts were not affected, amiloride depressed the acid shift evoked by stimulation, the maximal depression being 26.8 ± 2.7% of the control in spinal cords of rats, and by 10–25% in the isolated spinal cords of frogs. We could not record the effect of amiloride on stimulation-evoked $[K^+]_e$ changes, since we found that K^+-sensitive microelectrodes were also sensitive to amiloride. However, concomitantly recorded field potentials were not decreased.

We conclude that amiloride does not block more than 10–25% of acid changes occuring during repetitive electrical stimulation and, therefore, Na^+/H^+ exchange is obviously neither the only nor the most important mechanism of the pH_e changes.

Effects of SITS and DIDS

The blockade of Cl^-/HCO_3^- exchange, and/or $Na^+/Cl^-/HCO_3^-/H^+$ antiport, and/or Na^+-HCO_3^- cotransport by the stilbene derivatives SITS or DIDS resulted in an acid shift in pH_e base line of 0.01–0.05 pH units and had a pronounced effects on alkaline-acid changes evoked by repetitive electrical stimulation. In the rat spinal cord the stimulation-evoked acid shifts were depressed by 27.8 ± 1.1% after application of SITS (1–3 mmol/l) onto the spinal cord surface and by 26.0 ± 6.0% after application of DIDS (1–2 mmol/l). Moreover, the rise time of the acid shift was slowed and the initial alkaline shift enhanced. In the isolated spinal cord of the frog SITS blocked pH_e changes by about 30%. The application of SITS or DIDS in these experiments affected neither the $[K^+]_e$ base line nor the concomitantly recorded stimulation-evoked transient increase in $[K^+]_e$. Applications of SITS or DIDS in higher concentrations or for longer periods than 30 min further depressed pH_e, but it also depressed $[K^+]_e$ transients, suggesting that nonspecific effects were involved (Syková et al. 1988, Syková and Svoboda, in press). Our findings show that membrane transports which can be blocked by stilbene derivates play an important part in the pH_i and the pH_e regulation.

The effect of furosemide

Furosemide, a K^+-Cl^- cotransport inhibitor, causes the resting pH_e level in the isolated frog spinal cord to shift to the alkaline side by 0.1–0.15 ph units, but rises the resting K^+ level by several tenths mmol/l. It blocks acid shifts in the pH_e evoked by stimulation by 20–30% (Syková et al. 1988), but also the stimulation-induced $[K^+]_e$ changes by 10–20%.

In a number of tissues, K^+ shifts into the cells are accompanied by cotransport of Cl^-. Since K^+-Cl^- cotransport is associated with H_2O transport, the neuronal and glial cells may swell. Although the extent to which the K^+-Cl^- cotransport participates in the stimulation-induced changes in the size of the extracellular space (Syková 1987) is not yet clear, K^+ and Cl^- transport across the neuronal and glial cell membranes may lead to changes in pH_e homeostasis.

Block of H^+ channels

Various divalent and trivalent cations were used to test the possibility that H^+ or its equivalents leave or enter cells during repetitive electrical stimulation through voltage sensitive H^+ channels. The application of Mn^{2+} or Mg^{2+} onto the spinal cord surface depressed pH_e as well as $[K^+]_e$ transients, due to the blockade of synaptic transmission (see also Fig. 3). Blocking of synaptic transmission was also observed in our experiments after application of Cd^{2+} or Co^{2+}, but not of La^{3+}. Moreover, we found that the sensitivity of pH-sensitive microelectrodes was dramatically decreased by Cd^{2+}, Co^{2+} or Zn^{2+} in concentrations from 10^{-6}–10^{-3} mol/l (Chvátal and Syková, unpublished results), but not by similar concentrations of La^{3+}. All these ions were demonstrated to block voltage-sensitive H^+ channels in snail neurones (Meech and Thomas 1987, Thomas 1988b); however, we could only test the effect of La^{3+} on stimulation-evoked pH_e transient changes.

Application of $LaCl_3$ in a concentration of 1 mmol/l on the spinal cord surface in rats or superfusion of the isolated frog spinal cord with Ringer solution containing $LaCl_3$ decreased pH_e resting level by 0.05–0.3 pH units. In rats La^{3+} depressed the stimulation-evoked acid shift by 23.6 ± 5.2%, but the time course of the acid shift was not changed. The application of La^{3+} depressed the stimulation-evoked initial alkaline shift by more than 50%. The poststimulation alkaline undershoot was not altered by application of La^{3+}, and La^{3+} had no effect on stimulation-evoked K^+ transients (Syková and Svoboda, submitted). In the isolated frog spinal cord, superfused with Ringer solution buffered with HEPES and containing acetazolamide, La^{3+} depressed the enhanced initial alkaline shift by about 60–70% and the acid transients by 40–50%.

These results suggest that the activity-related initial alkaline shifts as well as acid shifts may result to great extent from the influx as well as from the efflux of H^+ through voltage sensitive H^+ channels.

Discussion and Conclusions

Our observations show that the pH_e in the unstimulated spinal cord is more acid than that in the CSF. Moreover, the pH_e is not the same at various depths in the spinal dorsal horns; it is most acid in regions of elevated $[K^+]_e$ due to spontaneous activity of interneurons in the lower dorsal horn (Syková et al. 1983, Svoboda et al. 1988). This finding suggests that H^+ are efficiently extruded from spontaneously active cells and this results in a more acid pH_e in lower dorsal horns than in areas with low levels of spontaneous activity or in the CSF.

The results demonstrate that transient changes in pH_e occur in the spinal dorsal horn during neuronal activity evoked by repetitive electrical stimulation of the peripheral nerve, as well as during the application of adequate stimuli. The experiments further show that there is a high correlation between pH_e and $[K^+]_e$, i.e. with neural activity. The dominating change *in vivo* (or in bicarbonate buffered solution) evoked by all types of stimuli is the acid shift (Chvátal et

al. 1988, Syková et al. 1988b) demonstrated also in the rat cerebellum (Kraig et al. 1983). The acid shift evoked by repetitive electrical stimulation is preceded by an at least 20 times smaller initial alkaline shift the resolution of which is hampered by acid shift and by a bicarbonate buffering system. The new finding in spinal cord of the rat concerns the poststimulation alkaline shift – alkaline undershoot – which corresponds to the poststimulation K^+ undershoot.

The pH_e changes in the spinal cord may differ from those in the brain. In the rat brain, pH_e changes are biphasic alkaline-acid shifts (Kraig et al. 1983), while in the rat spinal cord they are triphasic, alkaline-acid-alkaline or alkaline-acid-acid (Syková et al. 1988b). The initial alkaline shift is less pronounced than that in the skate and turtle cerebellum *in vitro* (Rice and Nicholson 1988, Chesler and Chan 1988) and is even smaller than in the rat cerebellum *in vivo* (Kraig et al. 1983). This is apparently attributed to the higher buffering capacity of the rat ECF than that of the saline buffered with 5–20 mmol/l HCO_3^- or with HEPES. In fact, a transition from bicarbonate-buffered Ringer to HEPES-buffered Ringer has been observed to increase the amplitude of the initial alkaline shift in the turtle cerebellum (Chesler and Chan 1988). In the isolated frog spinal cord the amplitude of the initial alkaline shift was enhanced after the superfusion with Ringer solution buffered with HEPES and containing acetazolamide (Fig. 3). The recovery of pH_e changes and poststimulation alkaline undershoot were not affected by acetazolamide. The buffering capacity of the ECF, therefore, affects predominantly the initial alkaline pH_e transients and the rise time of the activity-related acid shift.

Although we observed triphasic pH_e changes, it is possible that the pH_e transients are preceded during neuronal activation by a fast initial extracellular acidosis, related to synaptic activity. The rapid pH_e fall evoked within the first 10 ms by single electrical stimuli, have been shown to be followed by an alkaline-acid shift in hippocampal slices of the rat (Krishtal et al. 1987). This acid transient was suggested to result from the released acid content of synaptic vesicles into the synaptic cleft, but this cannot be measured with pH-sensitive microelectrodes, the tips of which have a diameter of 2–5 μm. Moreover, changes faster than 0.1 s cannot be detected by microelectrodes with a response time of about 0.6 s (90% of the change). The absolute value of a change in pH_e depends, among other factors, on the buffering power of the extracellular medium. It is, therefore, obvious that activity-related changes in pH_e in the spinal dorsal horn as measured with pH-sensitive microelectrodes are likely to be much slower and smaller than those taking place close to the postsynaptic membranes, i.e. in the synaptic clefts. The upper limit for the pH_e change in the synaptic cleft after application of GABA has been estimated to be 7–10 times higher than that sensed by the extracellular pH-sensitive electrode (Kaila and Voipio in press).

Activity-stimulated cell metabolism is associated with the production of acids or CO_2. It is clear that large numbers of protons pass across cell membranes on a carrier of some sort (for review see Thomas 1988a). This carrier may be a special protein in the cell membrane, a weak acid, CO_2, or a weak base; extrusion of excess of H^+ or its equivalents from brain cells and the regulation of pH_i in vertebrate central neurons may involve Na^+/H^+ exchange, HCO_3^- dependent mechanisms and voltage activated H^+ channels.

Our study with specific inhibitors shows that, in spinal cord, the excess of H^+ can be removed into the interstitial space by voltage activated H^+ channels, by Na^+/H^+ antiport, and by Cl^-/HCO_3^- exchange, and/or $Na^+/Cl^-/HCO_3^-/H^+$ antiport, and/or by Na^+-HCO_3^- cotransport. The acid shifts in spinal cord are of complex origin; each of the mechanisms studied contributes to the acid extrusion by cca 25–30%. Furthermore, the acid shift and especially its recovery are directly coupled to Na^+/K^+ pump activity. It is the K^+ uptake process, almost certainly the Na^+/K^+ pump (Syková 1983) or $Na^+/K^+/H^+$ pump, which has a time course similar to the H^+ uptake process.

Lactate production has been demonstrated in the rat cortex. Lactate was found to be released after epileptic activity. Its release had a slow time course. It occurred as a second acid peak after the first peak, lasting about 60 s, and then persisted after seizure activity for many minutes (Siesjö et al. 1985). The hypothesis that the acid shift is produced by extrusion of a

strong organic anion, and the most likely candidate is lactate which is a product of metabolic activity, was not confirmed in the isolated frog spinal cord during stimulation of the dorsal roots. The present findings also do not support the postulate that extracellular lactate accumulates in the rat spinal cord during electrical and adequate stimulation of peripheral nerves.

To conclude: It is evident from our results that the initial alkaline shift has a mechanism different from that of the acid shift and the poststimulation alkaline undershoot. The initial alkaline shift was blocked significantly by La^{3+} and enhanced by SITS and DIDS. Its fast time course and the effective blockade by Mn^{2+} and La^{3+} suggest that the influx of H^+ through the voltage sensitive H^+ channels could be the dominating mechanism. In fact, the initial alkaline shift in the isolated frog spinal cord has been shown to be enhanced by depolarization with 10 mmol/l K^+ applied into the perfusion solution (Chvátal et al. 1988). The initial alkaline shift is directly associated with Ca^{2+}-mediated electrical activity of neurons, since it was abolished by Mn^{2+} (Ca^{2+} channel blocker) (Fig. 3; Kraig et al. 1983). The increase of amplitude under the action of SITS or DIDS can either be due to the slowing of the acid shift rise time, which unmasks the initial alkaline shift, or to the fluxes mediated by membrane carriers such as Cl^-/HCO_3^- or $Cl^-/Na^+/H^+/HCO_3^-$ or Na^+-HCO_3^-. It is also an open question as to whether some of the observed relatively slow alkaline changes can be attributed to fast, GABA-activated bicarbonate fluxes through inhibitory postsynaptic channels (Kaila and Voipio 1987). The acid shift developing during stimulation has three main mechanisms, namely: 1. Na^+/H^+ exchange, 2. Cl^-/HCO_3^- exchange, and/or $Na^+/Cl^-/H^+/HCO_3^-$ antiport, and/or Na^+-HCO_3^- cotransport, and 3. H^+ efflux through the voltage sensitive H^+ channels. Finally, the poststimulation alkaline undershoot reflects a coupled clearance of K^+ and H^+ by active transport processes involving the Na/K pump.

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, Konnert et al. 1987), to affect neuronal excitability (Gruol et al. 1980, Balestrino and Somjen 1988), and cell-to-cell coupling (Roos and Boron 1981). It is, therefore, of great interest to study the dynamic changes in pH_e which accompany neuronal excitation. The role of glial cells in pH_e homeostasis remains to be elucidated, owing to their high permeability ot HCO_3^- and the nature of the membrane transport processes which regulate their pH_i (Astion et al. 1987, Deitmer and Schlue 1987, Kettenmann and Schlue 1988). On the other hand, changes in pH_e which result from neuronal activity may function as a signal between neurons and glial cells.

References

Ammann D, Lanter F, Steiner R A, Schulthess P, Shijo Y, Simon W (1981) Neutral carrier based hydrogen ion selective microelectrode for extra- and intracellular studies. Anal Chem 53: 2267–2269

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

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

Balestrino M, 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

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

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

Deitmer J W, 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

Endres W, Grafe P, Bostock H, Bruggencate G Ten (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, Smith T G Jr (1980) Hydrogen ions have multiple effects on the excitability of cultured mammalian neurons. Brain Research 183: 247–252

Harris R J, Symon L (1984) Extracellular pH, potassium, and calcium activities in progressive ischemia of rat cortex. J Cerebr Blood Flow Metabol 4: 178–186

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

Kaila K, Voipio J (1987) Postsynaptic fall in intracellular pH induced by GABA-activated bicarbonate conductance. Nature 330: 163–165
Kaila K, Voipio J (in press) Gaba-activated bicarbonate conductance: Influence on E_{GABA} and on postsynaptic pH regulation. In: Alvarez-Leefmans F J, Russell J M (eds) Chloride Channels and Carriers in Nerve, Muscle and Glia
Kettenmann H, Schlue-W-R (1988) Intracellular pH regulation in cultured mouse oligodendrocytes. J Physiol (Lond) 406: 147–162
Konnerth A, Lux H D, Morad M (1987) Proton induced transformation of calcium channel in chicks dorsal root ganglion cells. J Physiol (Lond) 386: 603–633
Kraig R P, Ferreira-Filho C R, Nicholson C (1983) Alkaline and acid transients in cerebellar microenvironment. J Neurophysiol 49: 831–850
Krishtal O A, Osipchuk Yu V, Shelest T N, Smirnoff S V (1987) Rapid extracellular pH transients related to synaptic transmission in rat hippocampal slices. Brain Research 436: 352–356
Kříž N, Syková E, Ujec E, 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, 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, Zidek W, Staschen M, Caspers H (1981) Cortical pH and pCa in relation to DC potential shifts during spreading depression and asphyxiation. Pages 225–229 in: Syková E. Hník P, Vyklický L (eds) Ion-Selective Microelectrodes and Their Use in Excitable Tissues. Plenum Press, New York, London
Meech R W, Thomas R C (1987) Voltage-dependent intracellular pH in *Helix aspersa* neurones. J Physiol (Lond) 390: 433–452
Mutch W A C, Hansen A J (1984) Extracellular pH changes during spreading depression and cerebral ischemia: Mechanisms of brain pH regulation. J Cerebr Blood Flow Metabol 4: 17–27
Ransom B R, Carlini W G, Connors B (1985) Brain extracellular space: Developmental studies in rat optic nerve. Ann New York Acad Sci 481: 87–105
Rice M E, Nicholson C (1988) Behavior of extracellular K^+ and pH in skate *(Raja erinacea)* cerebellum. Brain Research 461: 328–334
Roos A, Boron W F (1981) Intracellular pH. Physiol Rev 61: 296–434
Siesjö B K, von Harewehr R, Nergelius G, Nevander G, Ingvar M (1985) Extra- and intracellular pH in the brain during seizures and in the recovery period following the arrest of seizure activity. J Cerebr Blood Flow Metabol 5: 47–57
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, Motin V, Hájek I, 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 (1987) Modulation of spinal cord transmission by changes in extracellular K^+ activity and extracellular volume. Can J Physiol Pharmacol 65: 1058–1066
Syková E, Kříž N, 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, Kříž N (1988a) Effects of carbon dioxide on extracellular potassium accumulation and volume in isolated frog spinal cord. Pflügers Arch 421: 183–187
Syková E, Shirayev B, Kříž N, Vyklický L (1976) Accumulation of extracellular potassium in the spinal cord of frog. Brain Res 106: 413–417
Syková E, Svoboda J, Chvátal A, Jendelová P (1988b) Extracellular pH and stimulated neurons. Pages 220–232 in: Thomas R C (ed) Proton Passage Across Cell Membranes. John Wiley & Sons, Ltd., Chichester
Syková E, Svoboda J (in press) Extracellular alkaline-acid-alkaline transients in the rat spinal cord evoked by peripheral stimulation. Brain Res
Thomas R C (ed) (1988a) Proton Passage Across Cell Membranes. John Wiley & Sons, Chichester
Thomas R C (1988b) 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, Lübbers D W (1978) Time course of changes of extracellular H^+ and K^+ activities during and after direct electrical stimulation of the brain cortex. Pflügers Arch 378: 43–53