Ciba Foundation Symposium 139

PROTON PASSAGE ACROSS CELL MEMBRANES

A Wiley – Interscience Publication

1988

JOHN WILEY & SONS

Chichester · New York · Brisbane · Toronto · Singapore

Extracellular pH and stimulated neurons

E. Syková, J. Svoboda, A. Chvátal and P. Jendelová

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

Abstract. The use of ion-sensitive microelectrodes enabled us to follow the dynamic changes in extracellular pH (pH_e) together with those in the extracellular concentration of some biologically important ions, particularly K^+ and Ca^{2+}. Activity-related changes in pH_e were studied in isolated spinal cords of frogs and in spinal cords of rats *in vivo*. Repetitive electrical stimulation of an afferent input led either to triphasic alkaline-acid-acid changes (90% of frogs) or to triphasic alkaline-acid-alkaline changes (10% of frogs and rats) with the greatest changes in the lower dorsal horns. The transient acid shift by as much as 0.15–0.25 pH units is dominant and builds up during the stimulation. The changes in pH_e were also found in response to various adequate stimuli applied to the skin on the hind limb. Using specific inhibitors of Na^+/H^+ exchange, K^+-Cl^- co-transport, Cl^-/HCO_3^- exchange, the Na^+/K^+ pump and carbonic anhydrase, we found pH_e homeostasis to be impaired and stimulation-induced changes in pH_e decreased. We conclude that the pH_e changes evoked by electrical or adequate stimulation of an afferent input are not determined by changes in extracellular strong ion concentration differences due to accumulation of lactate, since we found no effect of NaF, a metabolic blocker of lactate production. However, lactate accumulation has been demonstrated during seizures, spreading depression and anoxia. Recently, it has been recognized that the observed pH_e changes can affect permeability of membrane ionic channels, neuronal excitability and glial cell function.

1988 Proton passage across cell membranes. Wiley, Chichester (Ciba Foundation Symposium 139) p 220–235

The depolarization of neurons is accompanied by translocation of ions, particularly of H^+, Na^+, K^+, Cl^-, Ca^{2+}, HCO_3^-, and of other molecules across their membranes, which modifies the extracellular ionic composition and extracellular space (ECS) volume (for review see Nicholson 1980, Syková 1983, 1986, Thomas 1984). Recently, it has been recognized that changes in pH_e by only several tenths of pH units evoke marked effects on neuronal ionic channels (Krishtal & Pidoplichko 1980, Iljima et al 1986, Konnert et al 1987), affect neuronal excitability (Gruol et al 1980, Balestrino & Somjen 1988) and glial cell function (Deitmer & Schlue 1987, Walz & Hinks 1987).

The use of ion-sensitive microelectrodes enabled us to follow the dynamic ionic changes, including pH, in ECS of the central nervous system (CNS) as a result of neuronal activity. Until recently, the dynamic changes in the extracellular pH (pH_e) have been recorded under a variety of pathological experimental conditions. Transient changes in pH_e by several tenths of pH units with dominating acid shifts of 0.1–0.5 pH units were found in the rat cortex and cerebellum during local surface electrical stimulation (Urbanics et al 1978, Kreig et al 1983), seizures (Siesjö et al 1985), spreading depression, anoxia and ischaemia (Lehmenkühler et al 1981, Kreig et al 1983, Harris & Symon 1984, Mutch & Hansen 1984). Under more 'physiological' conditions, the changes in pH_e by as much as 0.25 pH units were found during repetitive electrical stimulation in the isolated rat optic nerve or vagus nerve (Ransom et al 1985, Endres et al 1986) and in the spinal cord of rats and frogs during stimulations of peripheral nerves (E. Syková and J. Svoboda, unpublished work 1987, Chvátal et al 1988).

The mechanism of activity-evoked transient changes in pH_e and the mechanisms by which pH_e is stabilized are far from clear and probably not the same under physiological and pathophysiological conditions. According to Stewart (1981), the pH_e in body tissues is determined by three independent variables: 1) extracellular strong ion difference ($[SID]_e$) (strong ions means completely dissociated ions); 2) extracellular total weak acid concentration ($[A_{tot}]_e$); and 3) partial pressure of CO_2 (p_{CO2}). These three independent variables completely define pH_e and other dependent variables such as $[H^+]$, $[HCO_3^-]$, $[OH^-]_e$ and $[CO_3^{2-}]_e$. Out of these three independent variables, changes in $[SID]_e$ represented by the value of strong base cations minus strong acid anions are likely to be the most important mechanism involved in the activity-related pH_e changes in the CNS, since substantial changes in $[A_{tot}]_e$ and p_{CO2} are unlikely (Stewart 1981, Kreig et al 1983). The extracellular changes in the concentration of strong ions, particularly Na^+, K^+, Ca^{2+}, Cl^- and possibly lactate, occur during neuronal activity and may therefore considerably affect pH_e. It may be useful to state here Stewart's (1981) important conclusions, so simple and so different from conventional thinking: . . . '1. Given the assumption that $[A_{tot}]$ stays constant and that p_{CO2} is not affected by membranes because they are so CO_2 permeable, it then follows that body fluids can only interact across membranes by exchanging strong ions so as to alter their [SID] values.

2. Only [SID] changes can bring about changes in $[H^+]$, $[HCO_3^-]$, or any other dependent variable, assuming constant p_{CO2} and $[A_{tot}]$.

3. Transport of weak ions, or dependent variable ions, such as H^+, HCO_3^-, and OH^-, across membranes separating body fluids cannot result in changes of $[H^+]$, $[HCO_3^-]$, etc, unless these ions are accompanied by strong ions, and then it is the effect of strong ions on [SID] that determines the $[H^+]$, $[HCO_3^-]$, etc, not the transport of the H^+ etc. Dependent ion transport is essentially

irrelevant to the determination of $[H^+]$, $[HCO_3^-]$, etc. . . .'

In our studies we examined transient changes in pH_e in the spinal dorsal horns of rats and in isolated spinal cords of frogs evoked by electrical as well as by adequate stimulation of the afferent input, i.e. by relatively 'physiological' stimulation. In all experiments *in vivo* as well as *in vitro*, triphasic changes in pH_e, alkaline-acid-alkaline, were found, and they exhibited a typical depth profile in the spinal dorsal horns which corresponded to stimulation-evoked neuronal excitation. We explored the origin of these changes and the mechanisms of post-stimulation pH_e homeostasis. We provide an interpretation of the observed activity-related pH_e changes and an insight into the pH_e regulatory mechanisms operating in the CNS.

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 a gas mixture of 5% CO_2/95% O_2. Arterial blood pressure was monitored and rectal temperature was maintained at 37 °C. Inhibitors of enzymic activity and of ionic transports were applied in an artificial cerebrospinal fluid which was pre-warmed to 37 °C and bubbled with 5% CO_2/95% O_2. Dissection of the isolated spinal cord was described in detail by Czéh et al (1981). Bicarbonate-buffered Ringer solution at 18–20 °C, pH 7.30–7.35 either of standard composition or with added inhibitors was used for spinal cord superfusion.

Double-barrelled ion-sensitive microelectrodes were used to measure dynamic changes in extracellular ionic activity. The ion-sensitive barrel contained either a pH-sensitive cocktail (Fluka) or K^+-sensitive Corning 477317 or a Ca^{2+}-sensitive cocktail (Fluka). The second barrel served as the reference electrode. For further details see Kříž et al (1975) and Chvátal et al (1988).

Results and discussion

Activity-dependent changes in pH_e

High-frequency repetitive electrical stimulation (10–100 Hz) of a peripheral nerve evoked transient extracellular acidification in the dorsal horns of rats and frogs by as much as 0.15–0.25 pH units. The acid phase reached a plateau (a so-called 'ceiling' level) after about 15–20 s of stimulation, i.e. it had a slower rise time than the simultaneously recorded increase in $[K^+]_e$ which reached a plateau after 6–9 s. The acid phase had a rise time almost as fast as the decrease in $[Ca^{2+}]_e$ (Fig. 1). When stimulation continued for more than 20–30 s, the pH_e began to return to the base line, as did the $[K^+]_e$ and $[Ca^{2+}]_e$ (Chvátal et al 1988). The acid shift was preceded by a very small initial

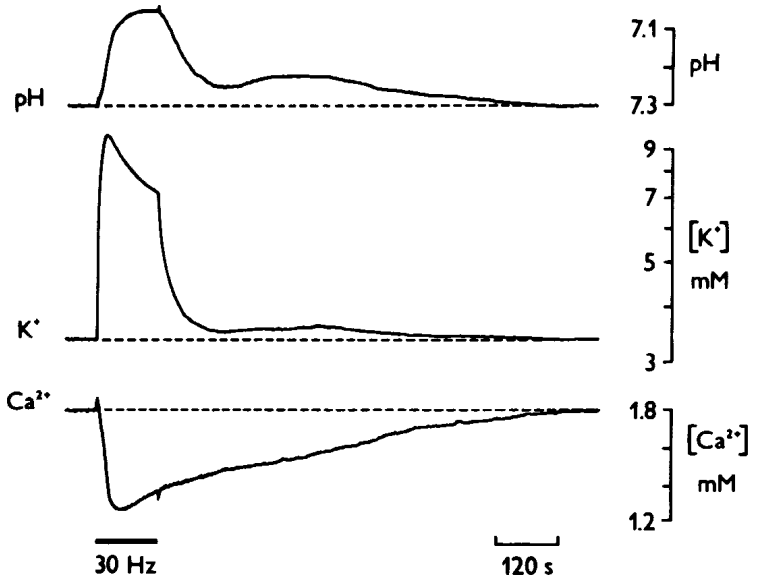

FIG. 1. Simultaneous records of changes in extracellular pH, $[K^+]$ and $[Ca^{2+}]$ in the 8th spinal cord segment of the isolated frog spinal cord. Changes produced by stimulation of the 8th dorsal root at a frequency of 30 Hz applied for 120 s were recorded in the lower dorsal horn, at a depth of 350 μm from the dorsal spinal surface. Tips of the three double-barrel ion-sensitive microelectrodes were about 100 μm apart.

alkaline shift (less than 0.01 pH units) lasting 1–2 s, clearly visible during only some experiments (Fig. 2). The initial alkaline shift was less pronounced in the dorsal horns than the previously described alkaline shift in the rat cortex during direct tissue stimulation (Kreig et al 1983). The latency of the initial alkaline shift corresponded to the rise in $[K^+]_e$.

A typical depth profile of the stimulation-evoked acid shifts was found in the spinal cord of rats and frogs, with the greatest changes in the lower dorsal horn (Fig. 3). The depth profile of pH_e changes corresponded well to the maximum of focal potentials elicited by single electrical stimuli, to the depth profiles of stimulation-evoked increases in $[K^+]_e$ (Czéh et al 1981) and to decreases in $[Ca^{2+}]_e$ (Chvátal et al 1988).

When stimulation was discontinued, the pH_e returned to the base line with an approximately similar time course as the simultaneously recorded K^+ transients. The post-stimulation K^+ undershoot recorded in the rat spinal cord *in vivo* was associated with an alkaline shift below the original pH_e base line by 0.02–0.03 pH units (Fig. 2). While the post-stimulation K^+ undershoot is present regularly in the mammalian spinal cord, it is present only rarely in the frog spinal cord (Syková 1983). The recovery in the isolated frog spinal cord was frequently not smooth but a second spontaneous acid shift occurred

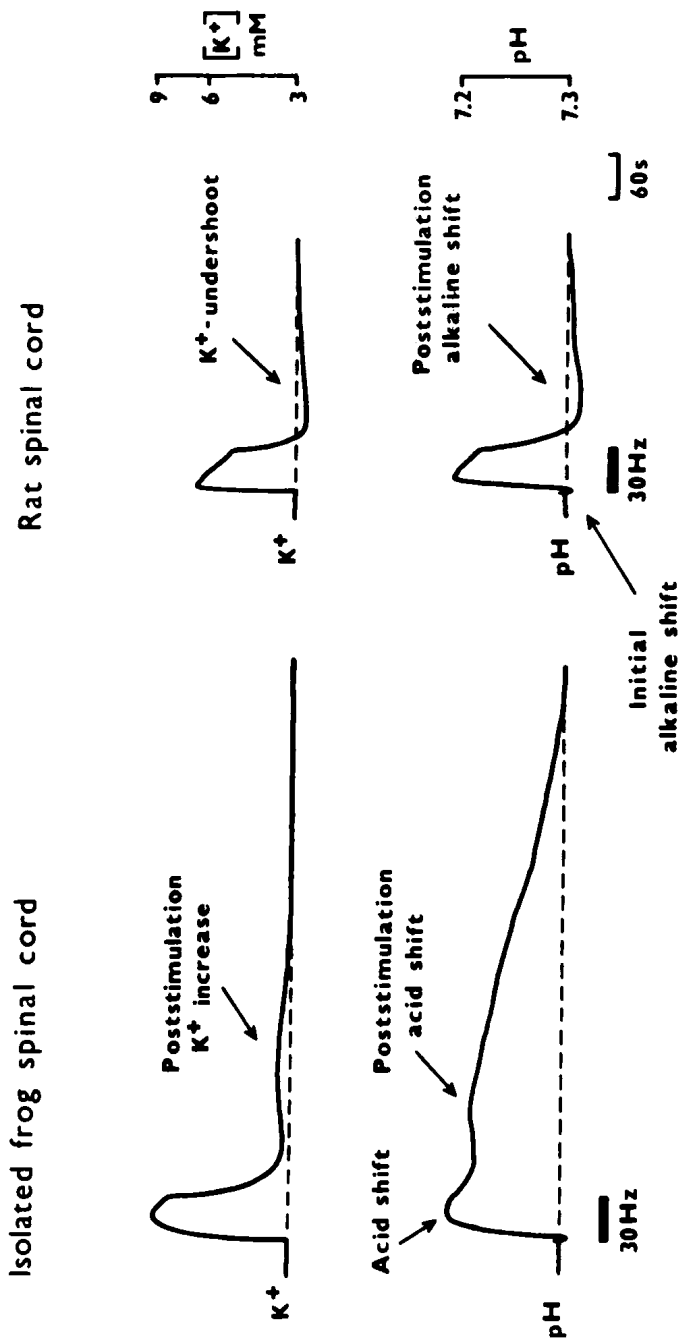

FIG. 2. Typical records of activity-related changes in extracellular pH and $[K^+]$ in the isolated frog spinal cord and in the rat spinal cord *in vivo*. Changes in pH_e and $[K^+]_e$ were produced in dorsal horns by electrical nerve stimulation at a frequency of 30 Hz applied for 60 s. Note the characteristic triphasic, alkaline-acid-acid changes in pH_e 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 $[K^+]_e$.

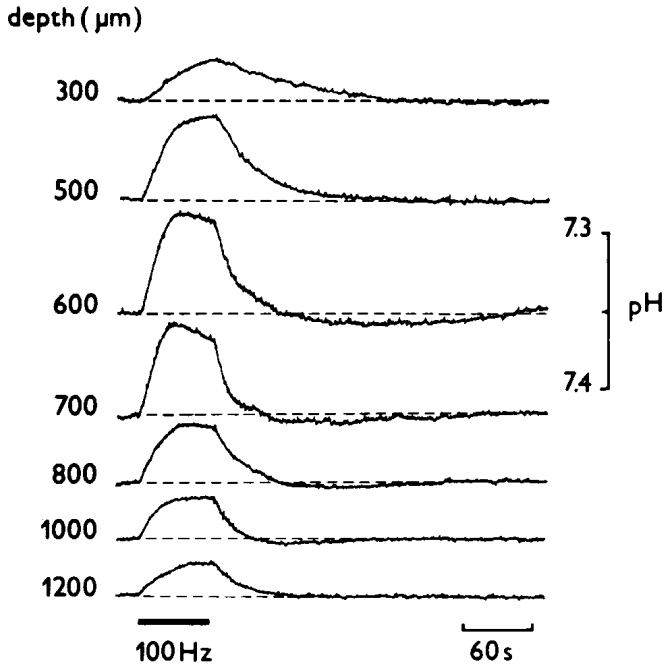

FIG. 3. Distribution of changes in extracellular pH in the L_5 spinal cord segment of a rat. Changes in pH_e in response to repetitive electrical stimulation at a frequency of 100 Hz applied for 60 s were recorded at various depths in the spinal cord, as measured from the dorsal spinal surface. Stimulation was delivered through a pair of needles inserted intramuscularly into the planta.

during post-stimulation recovery to the original pH_e base line, which was associated with a spontaneous increase in $[K^+]_e$ with a similar time course (Figs. 1 and 2). All four stimulation-evoked pH_e changes, 1) the initial alkaline shift, 2) the stimulation-related acid shift, 3) the post-stimulation alkaline shift ('pH_e undershoot') and 4) the post-stimulation acid shift, were found to increase with the stimulation frequency and intensity in a manner similar to that previously described for K^+ and Ca^{2+} transients (Czéh et al 1981, Chvátal et al 1988).

Transient acid shifts by 0.02–0.06 pH units were also found in the corresponding lumbar spinal segments when adequate stimuli (pinching of the toes with a pair of forceps, pinching of the skin, heat application) were applied to the skin of the ipsilateral hind limb (E. Syková & J. Svoboda, unpublished work 1987).

Spontaneous elevations in $[K^+]_e$ due to spontaneous neuronal firing were often seen in the frog spinal cord, especially when the cord was superfused with elevated $[K^+]$ or ouabain. The spontaneous elevation in $[K^+]_e$ was always accompanied by acid shifts of a similar time course.

TABLE 1 Effects of inhibitors on activity-related pH_e changes

	NaF	*Acetazolamide*	*Amiloride*		*Ouabain*	*Furosemide*	*SITS*	*Elevated* $[K]_e$
Effect of inhibitor	lactate production	carbonic anhydrase	high affinity Na^+ channel	low affinity Na^+/H^+ exchange	Na^+/K^+ pump	K^+-Cl^- co-transport	Cl^-/HCO_3^- exchange	Na^+/K^+ pump stimulation, membrane depolarization
Shift in resting level of pH_e (pH units)	alkaline (0.05)	alkaline (0.05)	0	alkaline (0.05–0.1)	acid (0.05)	alkaline (0.1–0.15)	alkaline (0.1)	acid (0.2)
Initial alkaline shift	0	0	↓	↑	0	↓ ↑	↓ ↓	↑ ↑
Activity-related acid shift	0	↓ ↓	↑	↓ ↓	↓ ↓ ↓ (slower)	↓ ↓ ↓	↓ ↓ ↓	↓ ↓
Post-stimulation alkaline shift	0	↑ ↑	0	0	↓ ↓ ↓	↓ ↓ ↓	↓	↑ ↑
Post-stimulation acid shift	↑ ↑/↓	↓ ↓	↑	↓ ↓	↓ ↓ (slower)	0	↓ ↓ ↓	↓ ↓

0, no effect; ↓–↓ ↓ ↓, decrease in amplitude; ↑–↑ ↑ ↑, increase in amplitude.

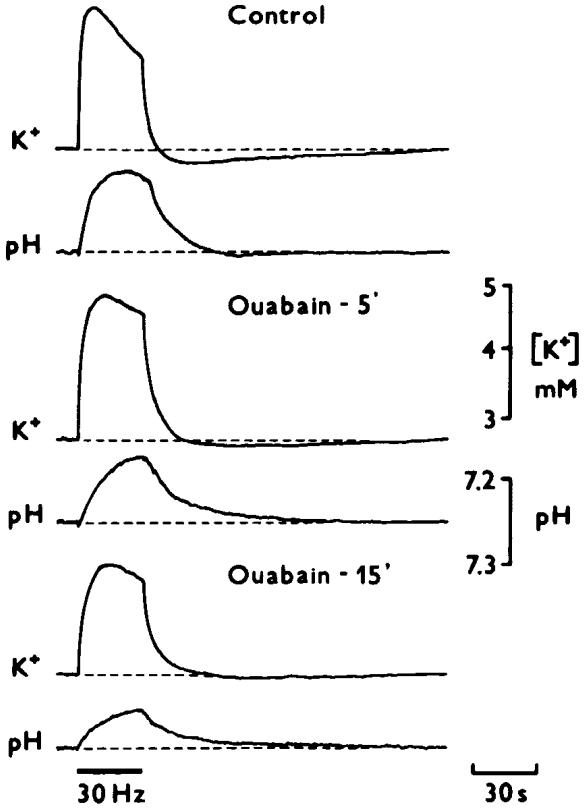

FIG. 4. Effects of ouabain applied to the spinal surface of the rat spinal cord. Simultaneous records of changes in pH_e and $[K^+]_e$ in the L_5 spinal cord segment at a depth of about 700 μm were evoked by repetitive electrical stimulation at a frequency of 30 Hz applied for 30 s through a pair of needles inserted into the planta. Note that the rise time of acid shift and its recovery to the base line are slowed down and the post-stimulation alkaline shift disappeared 5 and 15 minutes after application of 10^{-5} M ouabain.

To summarize, stimulation-evoked neuronal activity leads to rather complicated pH_e changes, triphasic alkaline-acid-alkaline shifts. The most prominent is the transient acidification which builds up during the stimulation. The overall activity-related change of acid-base balance does not last longer than the changes in $[K^+]_e$ and $[Ca^{2+}]_e$ and presumably $[Cl^-]_e$ and $[Na^+]_e$, i.e. the changes in $[SID]_e$.

pH_e homeostasis: application of inhibitors

Various inhibitors of enzymic activity and of ion transport were used to study pH_e homeostasis. We studied their effects on base line pH_e, initial alkaline

shift, acid shift which developed during the stimulation, and post-stimulation alkaline or acid shifts. The results are summarized in Table 1.

Alkaline shifts in pH_e. The origin of the initial alkaline shift remains obscure. One of the reasons is that the alkaline shift is very small, inconstant and short-lasting, nevertheless apparently not an artifact. In fact, an electrode artifact imitating an initial acid shift and resulting from measurement of pH as a potential difference between the very high resistance barrel containing the ion-exchanger and the low-resistance reference barrel, was seen in several of our experiments (see Fig. 2). We offer several hypotheses concerning the origin of the initial alkaline shifts and post-stimulation alkaline shifts which are based on our data as well as those of others, being aware of the fact that all the described alkaline shifts do not necessarily have the same mechanism.

Since it has been recognized that changes in pH_e are the consequence of alterations in $[SID]_e$, we can hypothesize about the initial and post-stimulation increase in $[SID]_e$. An increase of only 3 mM in $[SID]_e$ would account for a 0.05 pH unit alkaline change. The source of an increase in $[SID]_e$ might be due to K^+ which accumulate in ECS, the movement of Cl^- into glial cells (K^+-Cl^- co-transport), etcetera. The post-stimulation alkaline shift was found to be effectively blocked by furosemide, suggesting that K^+-Cl^- co-transport may play an important role in pH_e homeostasis. Another interesting possibility is that the increase in $[SID]_e$ results from the reduction of the volume of ECS (Syková 1987), without a comparable reduction in impermeable $[HCO_3^-]_e$. However, the $[HCO_3^-]_e$ could double as a result of extracellular space shrinkage. We cannot exclude the possibility that the initial alkaline shift is due to flow of $[HCO_3^-]$ down its electrochemical gradient, which in neurons results in efflux.

The uptake process, represented by the K^+ undershoot and almost certainly involving the Na^+/K^+ pump, seemed to have the same time course as the post-stimulation alkaline shift. Both were found to be selectively blocked by ouabain. At all concentrations tested (from 10^{-7} M to 10^{-4} M), ouabain prolonged the post-stimulation recovery of $[K^+]_e$ and pH_e to their base lines (Fig. 4). Furthermore, the superfusion of the spinal cord with ouabain increased the resting level of $[K^+]_e$ by as much as 1.1 mM and evoked an acid shift in pH_e base line by 0.07 pH units. These data demonstrate that both post-stimulation pH_e and K^+ homeostasis are related to the Na^+/K^+ pump activity.

Acid shifts in pH_e related to stimulation. With respect to Stewart's ideas, it is unlikely that the observed acid shifts result from a direct movement of H^+ through the neuronal membrane, even when changes in surface $[H^+]$ may occur through Na^+/H^+ exchange or the H^+ channel (Meech & Thomas 1987, Thomas 1984). The observed acid shifts of 0.15–0.25 pH units could be either

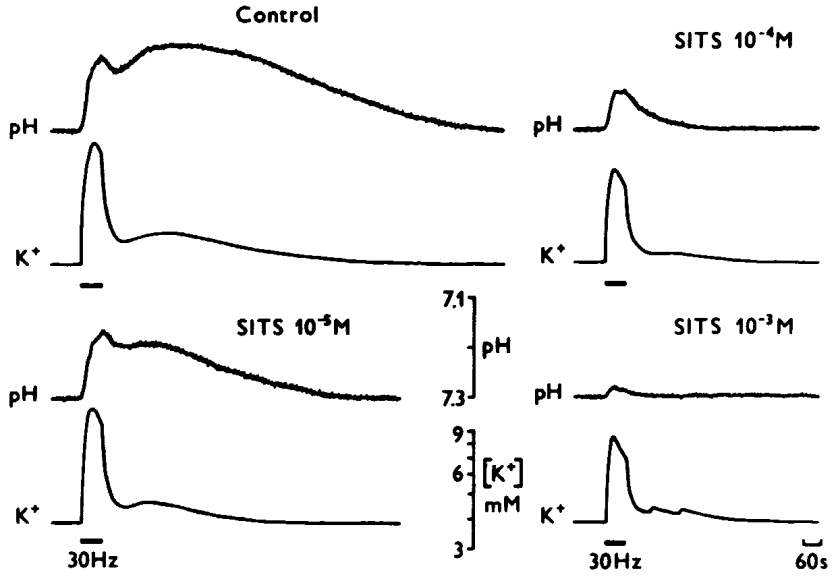

FIG. 5. Depression of simultaneously recorded changes in pH_e and $[K^+]_e$ in the lower dorsal horn of the isolated frog spinal cord by superfusion of the cord with SITS (10^{-5}, 10^{-4} and 10^{-3} M). Changes were evoked by tetanic stimulation of the 8th dorsal root at a frequency of 30 Hz applied for 60 s.

a consequence of ion movements leading to alterations in $[SID]_e$ of about 8–12 mM (for calculations see Kreig et al 1983) or due to the metabolic production of acid.

In our experiments, the observed attenuation of acid shifts due to amiloride was about 20–40%, to furosemide 60–80% and to SITS 80–90% (Fig. 5). The fact that NaF, which blocks the glycolytic portion of metabolic processes and lactate production, is almost without effect suggests that under the relatively 'physiological' conditions in our experiments the dominating mechanism is not alteration in $[SID]_e$ due to lactate production. Ion extrusion mechanisms across cell membranes (Na^+/H^+ or Cl^-/HCO_3^- exchange or K^+-Cl^- co-transport) play an important role in pH_e homeostasis. A decrease in $[SID]_e$ is probably due to extrusion of Cl^- or HCO_3^-, since production of strong acid anions is unlikely. Lactate may be produced under extreme conditions, e.g. seizure, spreading depression or anoxia. Siesjö et al (1985) found that lactic acid values during seizures were not significantly altered during the first 30–60 s. They suggest that the Na^+/H^+ exchange across cell membranes is enhanced due to increased Na^+ permeability. The resulting influx of Na^+ and efflux of H^+ would lead to extracellular acidification. Another argument against the role of lactate production in stimulation-evoked acid shifts is the fact that normalization of extracellular lactate content is slow, lasting about 15 minutes after the seizures, while the pH recovery

observed in our experiments (especially *in vivo*) was almost as fast as the build up and never longer than the recorded changes in extracellular concentration of K^+ and Ca^{2+}. There is other evidence against extracellular lactate accumulation. When the peripheral nerve was stimulated for five or more minutes the pH_e changes (similarly, the changes in $[K^+]_e$ and $[Ca^{2+}]_e$) were not enhanced; on the contrary, once they had attained a plateau, the acidification (and the elevated $[K^+]_e$ and $[Ca^{2+}]_e$ gradually decreased to the original base line (E. Syková et al, unpublished work 1987). The situation in the spinal cord may, however, be different from that in the cerebellum and cortex where long-term direct tissue stimulation may lead to accumulation of lactate (Kreig et al 1983).

Carbonic anhydrase in the brain is found predominantly in the glia and myelin (see Kreig et al 1983). Since the fluid in the extracellular space is buffered by an HCO_3^--CO_2 system, inhibition of carbonic anhydrase by application of acetazolamide (4.0 or 8.0 mM) could result in changes of pH_e. Indeed, acetazolamide evoked an alkaline shift of the pH_e base line by about 0.05 pH units. The stimulation-evoked acidification was depressed and the post-stimulation alkaline shift was enhanced. Our findings, as well as those of Kreig et al (1983), provide evidence that carbonic anhydrase and glia play a significant role in pH_e homeostasis.

Post-stimulation or delayed acid shifts were always accompanied by a spontaneous rise in $[K^+]_e$ and were selectively blocked by 4 mM Mn^{2+} while the acid shifts which build up during the stimulation were depressed by only 60–80% (E. Syková et al, unpublished work 1987). This suggests that post-stimulation acid shift is postsynaptic in nature, while the pH_e changes during the stimulation arise from activity in neurons, as well as in pre-synaptic afferent fibres themselves (see Syková 1983).

Conclusions

We conclude that acid-base changes in neuronal microenvironment occur under physiological as well as pathological conditions and may significantly affect CNS function.

It is apparent that the Na^+/H^+ exchange, K^+-Cl^- co-transport, Na^+/K^+ pump activity, Cl^-/HCO_3^- exchange (or Na^+-H^+-HCO_3^-/Cl^- exchange), carbonic anhydrase and, presumably, the extracellular space shrinkage due to neuronal and/or glial cells swelling play an important role in pH_e homeostasis. One of the pertinent questions at the present time is to what extent the changes in pH_e are due to ionic changes in neurons or in glial cells or due to changes in extracellular space volume. We need to study and clarify the role of glia in activity-related pH_e homeostasis. Recently described HCO_3^- conductance in the glial cell membrane (Astion et al 1987) points to the mechanism that will tend to buffer temporary changes in pH_e at the expense of glial

pH_i. To elucidate further the mechanisms of acid-base changes in the extracellular space may require the use of methods other than those available at present. However, it is evident that the described pH_e changes are of physiological significance and as such support the concept of the extracellular space as a dynamic structure maintaining the brain cell microenvironment and communication channel between cells.

References

Astion ML, Coles JA, Orkand RK 1987 Effects of bicarbonate on glial cell membrane potential in Necturus optic nerve. Neurosci Lett 76:47–52

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

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

Czéh G, Kříž N, Syková E 1981 Extracellular potassium accumulation in the frog spinal cord induced by stimulation of the skin and ventrolateral columns. J Physiol 320:57–72

Deitmer JW, 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 388:261–283

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

Gruol DL, Barker JL, Huang LM, McDonald JF, Smith Jr TG 1980 Hydrogen ions have multiple effects on the excitability of cultured mammalian neurons. Brain Res 183:247–252

Harris RH, Symon L 1984 Extracellular pH, potassium and calcium activities in progressive ischaemia of rat cortex. J Cereb Blood Flow Metab 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 Natl Acad Sci USA 83:654–658

Konnert A, Lux HD, Morad M 1987 Proton-induced transformation of calcium channel in chick dorsal root ganglion cells. J Physiol (Lond) 386:603–633

Kreig RP, Ferreira-Filho CR, Nicholson C 1983 Alkaline and acid transients in cerebellar microenvironment. J Neurophysiol 49:831–850

Krishtal OA, Pidoplichko VI 1980 A receptor for protons in the nerve cell membrane. Neuroscience 5:2325–2327

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. In: Syková E et al (eds) Ion-selective microelectrodes and their use in excitable tissues. Plenum Press, New York, p 225–229

Meech RW, Thomas RC 1987 Voltage-dependent intracellular pH in Helix aspersa neurones. J Physiol (Lond) 390:433–452

Mutch WAC, Hansen AJ 1984 Extracellular pH changes during spreading depression and cerebral ischemia: Mechanisms of brain pH regulation. Cereb Blood Flow Metab 4:17–27

Nicholson C 1980 Dynamics of the brain cell microenvironment. Neuroscience Res Prog Bull 18:177–322
Ransom BR, Carlini WG, Connors BW 1985 Brain extracellular space: Developmental studies in rat optical nerve. Ann New York Acad Sci 481:87–105
Siesjö BK, von Hanwehr 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 the seizure activity. J Cereb Blood Flow Metab 5:47–57
Stewart PA 1981 How to understand acid-base. Elsevier, New York
Syková E 1983 Extracellular K^+ accumulation in the central nervous system. Prog Biophys Mol Biol 42:135–189
Syková E 1986 Activity-related fluctuations in extracellular ion concentrations in the central nervous system. NIPS 1:57–61
Syková E 1987 Modulation of spinal cord transmission by changes in extracellular K^+ activity and extracellular volume. Can J Physiol Pharmacol 65:1058–1066
Thomas RC 1984 Experimental displacement of intracellular pH and the mechanism of its subsequent recovery. J Physiol 354:3–22
Urbanics R, Leniger-Follert E, Lübbers DW 1978 Time course of changes of extracellular H^+ and K^+ activities during and after direct electrical stimulation of the brain cortex. Pflügers Arch Eur J Physiol 378:47–53
Walz W, Hinks EC 1987 Extracellular hydrogen ions influence channel-mediated and carrier-mediated K^+ fluxes in cultured mouse astrocytes. Neuroscience 20:341–346

DISCUSSION

Frömter: I should like to add to your list of possible mechanisms that might explain these observations: rheogenic Na^+-HCO_3^- co-transport. When the cell depolarizes on stimulation, HCO_3^- could enter the cell, leaving the extracellular fluid more acidic. The blocking effect of SITS supports this interpretation.

Oberleithner: This is indicated by your work with furosemide and SITS. Rheogenic Na^+-HCO_3^- co-transport depends on the cell membrane potential. We have done similar experiments in frog distal cells, where this system is present. HCO_3^- is moved across the basolateral cell membrane because of changes in membrane potential. I think that you could explain elegantly most of the your findings, if you postulate rheogenic Na^+-HCO_3^- co-transport.

Syková: I agree, that is possible.

Schlue: Is the pH in the extracellular spaces of the spinal cord different from the pH in the bathing medium?

Syková: The resting pH level *in vitro* was essentially the same as that in Ringer solution, i.e. pH 7.3-7.35. In rat spinal cord the extracellular pH (pH_e) is slightly more acidic (7.1–7.25) than the pH of blood. We also measured the extracellular p_{CO2} and p_{O2}; these were within the physiological range.

We measured pH_e within the spinal cord *in vivo* and found that it varies at different depths from 7.1 to 7.25. In dorsal horn we described the various K^+ concentrations which are related to the spontaneous activity of the inter-

neurons (Syková et al 1983). Many neurons in the dorsal horn fire spontaneously. This spontaneous firing is accompanied by permanent elevation of the extracellular K^+ concentration in the lower dorsal horn, and may also be accompanied by a decrease in pH_e.

Morad: In the experiments where you increase the K^+ concentration in the bath, H^+ was secreted into the extracellular space. In those experiments, did you add SITS to see whether you could dissociate the efflux of K^+ from depolarization?

Syková: Not yet.

Morad: It's curious that K^+ always seems to be coming out at the same time that H^+ is coming out.

Syková: In the spinal cord there are spontaneous changes of K^+. Sometimes there is a burst of firing, especially in isolated spinal cord, which is associated with the K^+ increase, and with a spontaneous acid shift that follows a similar time course.

Morad: But when you added SITS, did the K^+ efflux decrease?

Syková: Yes, but the depression of the K^+ transients with SITS was only about 20–30%, while there was nearly complete inhibition of the pH change.

Grinstein: You showed that *in vivo* there was a much greater inhibition of the K^+ flux than *in vitro*.

Syková: The effect of SITS is generally the same in the spinal cord of the rat *in vivo* and the spinal cord of the frog *in vitro*. I showed the experiment *in vivo*, in which the inhibition of K^+ accumulation 15 minutes after the application of 3mM SITS was greater, but less than the inhibition of the acid shift. *In vivo* we applied SITS to the surface of the spinal cord, and therefore used a higher concentration than that used *in vitro* (see Fig.5).

Grinstein: You don't have any explanation for why the K^+ flux is depressed by SITS?

Morad: The issue is whether the neurons are firing. In the presence of SITS, you can say that you are blocking some excitatory process so the neurons aren't all firing, resulting in accumulation or depletion of ions. To investigate this, I would simply increase the K^+ concentration, as you did. You can then examine whether H^+ accumulates and how it is affected by SITS. In this way, you can decide whether this is a secondary or primary phenomenon.

Syková: Yes, SITS can partially block excitation. However, when we applied SITS, we observed an almost immediate, rapid effect on pH, while the K^+ accumulation was not substantially depressed. The effect on K^+ transients was slower, i.e. it required a longer application of SITS.

Thomas: You are expecting SITS to diffuse a long way. This rat presumably still has an intact blood supply.

Syková: That is completely intact. You have to apply SITS in higher concentrations. *In vivo* we recorded pH and K^+ changes at a depth of 700 μm, applied 3 mM SITS to the spinal surface, then recorded five and fifteen minutes later.

Thomas: Whereas the frog spinal cord doesn't have a blood supply and so it is more accessible.

Schlue: You stated that part of the changes in external pH are due to shrinkage of the extracellular space. Is it possible to exclude this contribution in the same experiment by blocking KCl uptake, for example, with furosemide?

Syková: The inhibition of transient extracellular pH changes with furosemide varies from 40–80%. One question is what is the source of that shrinkage of the extracellular space? We don't know whether only the neurons or the glial cells or both are swelling. A possible relationship between swelling of the cells and pH_e changes is under investigation in our laboratory.

Kaila: I wonder whether KCl co-transport has to be implied in the increase in cell volume. If the product of the extracellular concentrations of K^+ and Cl^- increases, that would account for cell swelling caused by an influx of ions through plasmalemmal channels.

Syková: Several mechanisms have been proposed for the activity-related shrinkage of the extracellular space, including transport of KCl. We can't tell at the moment what is the main mechanism of that shrinkage, or to what extent the glial cells, neurons and/or fibres are swelling.

Thomas: The HCO_3^- efflux that you would predict, Kai, to occur through GABA-activated channels would cause an extracellular alkalinization rather than an acidification.

Kaila: Yes, a GABA-activated bicarbonate conductance should produce an initial alkalosis at the extracellular surface.

Syková: There is an initial alkalinization.

Kaila: It looks very fast. Do you think it could be truncated by the slow response time of the H^+-selective microelectrode?

Thomas: It might, in addition, be swamped by the subsequent acidification.

Syková: That's exactly what happens. If you block the acidification or slow it down, the initial alkaline shift becomes larger. These are two antagonistic processes. It was shown in cerebellum by Kraig et al (1983) that the size of the initial alkaline shift depends on the frequency of stimulation. The alkaline shift seems to be related to synaptic activity.

Thomas: Eva, you are not sure where the pH changes are, presumably your recording site is chosen simply as an area of maximal changes. You don't know how close you are to the main front. We are all interested in H^+ fluxes over some distance. In snail neurons the effect was magnified by reducing the buffering power. Have you looked at extracellular buffering power?

Syková: We haven't, but I assume that the buffering power is about 30–34 mM.

Thomas: Your solutions contain HCO_3^- but that was a lousy buffer for the space outside the snail neuron. The buffering power could be much lower than you think. Would it be possible to add 20 mM Hepes to your solution?

Syková: It would be possible in experiments on isolated frog spinal cord, but essentially we obtained the same results *in vivo* and *in vitro*.

Thomas: In the frog spinal cord it might be very interesting if you were to do the experiment with a higher concentration of Hepes.

References

Kraig RP, Ferreira-Filho CR, Nicholson C 1983 Alkaline and acid transients in cerebellar microenvironment. J Neurophysiol 49:831–850

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