STIMULATION-EVOKED CHANGES IN EXTRACELLULAR pH, CALCIUM AND POTASSIUM ACTIVITY IN THE FROG SPINAL CORD*

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S u m m a r y

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Double-barrel ion-sensitive microelectrodes were used to measure activity-related changes in extracellular pH (pH_e), potassium and calcium concentration ($[K^+]_e$ and $[Ca^{2+}]_e$) in the spinal dorsal horns of frogs. Repetitive stimulation (30-100 Hz) of the dorsal root evoked transient acidification in the lower dorsal horn by 0.25 pH units, which was accompanied by an increase in $[K^+]_e$ by 4-5 mmol/l and a decrease in $[Ca^{2+}]_e$ by 0.5 mmol/l. The pH_e changes were found to have a typical depth profile and increased with the stimulation frequency, intensity and duration. The maximum of pH_e changes was reached in 25-30 s of stimulation, and when stimulation continued further no greater pH_e changes were achieved. Similarly as the K^+ and Ca^{2+} transients, the pH_e reached a ceiling level, which was 0.2-0.25 pH units more acid than the pH of the Ringer solution. The poststimulation K^+ undershoot below the resting K^+ level (3 mmol/l) was accompanied by an alkaline shift below the original pH base line. The rise time of the pH_e changes was slower than that of $[K^+]_e$ and $[Ca^{2+}]_e$ changes. However, the redistribution of all the ionic changes had a similar time course.

The clearance of changes in $[K^+]_e$ and pH_e was slowed by ouabain. The depression of the acid shift required higher concentrations of ouabain than the depression of the alkaline shifts. Acetazolamide, a carbonic anhydrase inhibitor, depressed the acid and enhanced the alkaline shift. Superfusion of the cord with elevated $[K^+]_e$ was accompanied by a prompt and progressive acid shift, the lowering of $[K^+]_e$ by an alkaline shift. The stimulus-evoked K^+ increase and acid shift were depressed during the elevated $[K^+]_e$, while the alkaline shift was enhanced. Spontaneous elevations of $[K^+]_e$ were accompanied by acid shifts of a similar time course.

The results are discussed in terms of stimulus-evoked changes in extracellular strong ion differences $[SID]_e$, and of their possible physiological significance.

Key words: Extracellular pH - Extracellular calcium - Extracellular potassium - Spinal cord - Frog

INTRODUCTION

Numerous studies on the mammalian as well as the amphibian CNS In which the ion-sensitive microelectrodes were used demonstrated the dynamic changes in ionic composition of the extracellular space (for review see Nicholson 1980, Somjen 1979, Syková 1983, Syková 1986). In the spinal cord of both mammals and amphibians, there are a number of studies presenting data about the amplitude, time course and possible physiological significance of K^+ changes ($[K^+]_e$) during the stimulation of various afferent inputs. Information about

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stimulation evoked transient changes in extracellular pH (pH_e) and Ca^{2+} concentration ($[Ca^{2+}]_e$) in spinal dorsal horns is, however, lacking.

In the present study, we used ion-sensitive double-barrel microelectrodes to follow transient changes in extracellular pH, calcium and potassium concentration in the spinal dorsal horn microenvironment of the frog spinal cord. We found that all the three variables have the similar depth profile In the isolated frog spinal cord and depend on the frequency, intensity and duration of stimulation. The time courses of extracellular pH, calcium and potassium changes were compared, with respect to their possible mechanisms.

METHODS

Experiments were performed on isolated spinal cords of the frog (*Rana temporaria*). The dissection of the spinal cord was described in detail elsewhere (Syková et al. 1976]. The isolated cords (n=31) were mounted in a chamber and superfused with oxygenated, bicarbonate-buffered Ringer solution (95 % O_2 and 5 % CO_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 ^{O}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. Ouabain (Sigma) or acetazolamide (Lachema) were dissolved in Ringer solution shortly before use.

Dorsal root (DR) eight or nine was stimulated supramaximally (rectangular pulses of 5 V or less and 0.1 ms) with bipolar silver electrodes. DC recordings of the dorsal root potentials (DRPs) were made from the adjacent dorsal root with bipolar platinum electrodes.

K^+ activity was measured by means of double-barrel K^+-sensitive microelectrodes filled with a liquid ion-exchanger (Corning 477317) as described elsewhere (Kříž et al. 1975). Double-barrel pH electrodes had a tip diameter of 3 - 5 μm. The ion-sensitive barrel contained a pH-sensitive cocktail (Amman et al. 1981) which we obtained from Fluka, and the backfilling solution was composed of (mmol/l): KH_2PO_4 40.0, NaOH 23.0, NaCl 15.0 (pH 7.0). The reference barrel was filled with 1 mmol/l magnesium acetate solution. When the tip was broken to a size 3 μm or greater, the electrodes responded with about 58 mV/pH unit. 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 $CaCl_2$. The reference barrel was filled with 150 mmol/l NaCl. The response time of microelectrodes to changes in pH and Ca^{2+} activity was comparable to the potassium response of K^+-sensitive microelectrodes.

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

ACTIVITY-DEPENDENT CHANGES IN pH.

High-frequency repetitive electrical stimulation (10-100 Hz) of dorsal roots evoked transient extracellular acidification in the dorsal horn from the pH_e base line 7.2 - 7.3 to 7.0 - 7.1, i.e. by 0.20 - 0.25 pH units (Fig. 1). The acid phase reached a plateau (a so-called "ceiling" level) after 25-30 s of stimulation, and had a slower rise time than the simultaneously recorded increase in $[K^+]_e$ (Fig. 2 and Fig. 3). When stimulation continued for more than 30 s the pH_e began to recover, similarly as was the case with $[K^+]_e$ (Czéh et al. 1981, see also Figs. 2, 3 and 4).

A typical depth profile of the stimulation-evoked acid shifts was found in the frog spinal cord with the greatest changes in the lower dorsal horn at depths about 300 - 400 μm (Fig. 1).

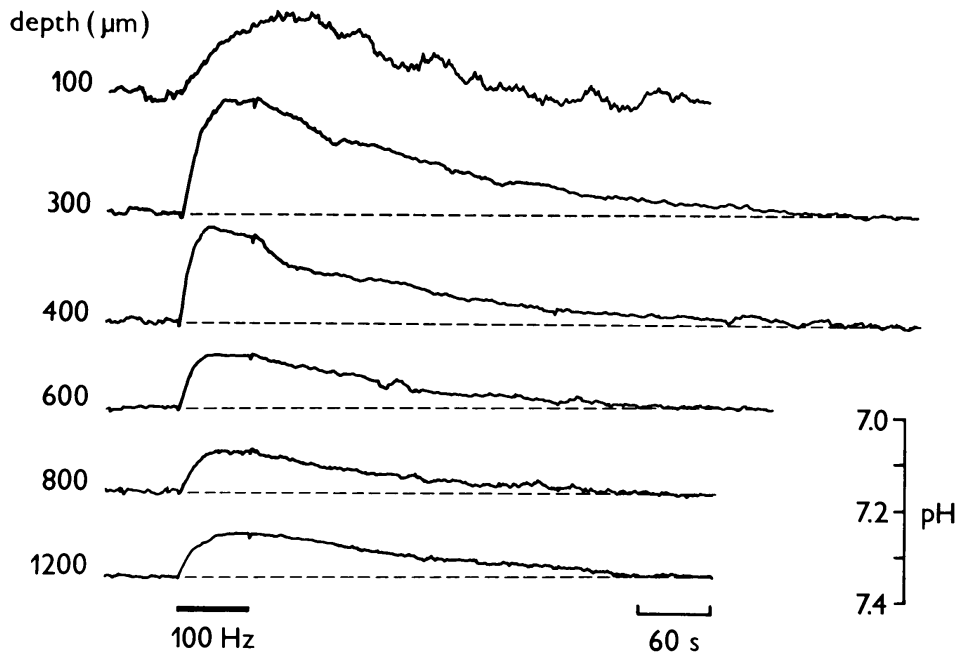

Fig. 1. The distribution of changes in pH_e in isolated frog spinal cord induced by tetanic stimulation of 8th dorsal root at 100 Hz. The greatest acid shift was found at a depth of about 400 μm from the dorsal spinal surface, i.e. at a depth corresponding to the lower dorsal horn.

The depth profile of pH_e changes corresponded well to the depth profile of the stimulation evoked increase in $[K^+]_e$ (Czéh et al. 1981) and to the decrease in $[Ca^{2+}]_e$ (Fig. 5).

When stimulation was discontinued, the pH_e returned to the base line with an approximately similar time course as the simultaneously recorded K^+ transients. Frequently, the recovery was not smooth but the second spontaneous acid shift occurred during the post-stimulation recovery to the original pH_e base line, which was associated with a spontaneous increase in $[K^+]_e$ of a similar time course (Fig. 2). In two of our experiments a poststimulation K^+ undershoot was recorded, which was associated with an alkaline shift by 0.02 - 0.03 pH units (Fig. 3). The poststimulation K^+ undershoot is only rarely present in the isolated frog spinal cord, while it can be found regularly in the mammalian spinal cord (see Syková 1983). However, when present in our experiments, both the K^+ undershoot and alkaline shift had a similar time course and outlasted the stimulation for about 6 - 8 min. Both the stimulation-evoked acid shift and alkaline shift were found to increase with the stimulation frequency and intensity in a manner similar to the previously described K^+ transients (Syková et al. 1976, Czéh et al. 1981).

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 alkaline shift (see Fig. 3). It has already been demonstrated that the ceiling level of $[K^+]_e$ in the frog spinal cord is increased and that the poststimulation K^+ redistribution is slowed down by ouabain (Syková et al. 1985).

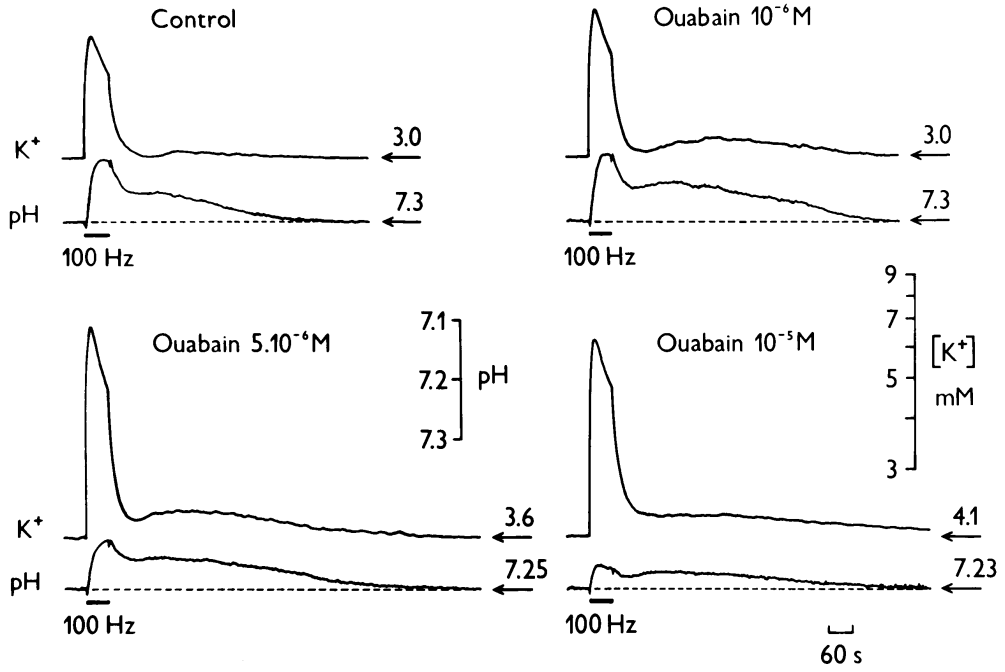

Fig. 2. Simultaneously recorded changes in pH_e and $[K^+]_e$ at a depth of 400 μm from the dorsal spinal surface in response to tetanic stimulation of 8th dorsal root. The effects of spinal superfusions with a solution containing ouabain at concentrations 10^{-6}, 5.10^{-6} and 10^{-5} mmol/l. The arrows and accompanying numbers indicate the base line of pH_e and $[K^+]_e$ and its actual values after 15 min of superfusion with ouabain.

Fig. 2 shows that low doses of ouabain (from 10^{-7} to 10^{-6} mol/1) applied in Ringer solution enhanced both $[K^+]_e$ and pH_e ceiling levels. At concentrations from 5.10^{-6} mol/1 the pH_e ceiling level decreased. However, at all concentrations tested (from 10^{-7} to 10^{-5}) ouabain prolonged the poststimulation recovery of $[K^+]_e$ and pH_e to their base lines. Furthermore, superfusion of the cord with ouabain elevated the resting level of $[K^+]_e$ by as much as 1.1 mmol/1 and evoked an acid shift in pH_e base line by 0.07 pH units. These data demonstrate that poststimulation clearance processes of elevated pH_e and K^+ are both related to the Na^+-K^+ pump activity. The acid shift decreased only when relatively high concentrations of ouabain were used.

To examine the relationship between $[K^+]_e$ and pH_e we superfused the spinal cord with solutions in which the $[K^+]$ was increased or lowered. Fig. 4 shows the effect of 10 mmol/1 K^+ on $[K^+]_e$ and pH_e recorded in the lower dorsal horn. The superfusion elevated $[K^+]_e$ which was accompanied by a prompt and progressive acid shift of almost 0.2 pH units. The stimulation-evoked increase in $[K^+]_e$ as well as the acid shift both become depressed during the superfusion, while the post-stimulation alkaline shift becomes enhanced (Fig. 4). 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/1 K^+. Lowering the $[K^+]$ in the superfusing

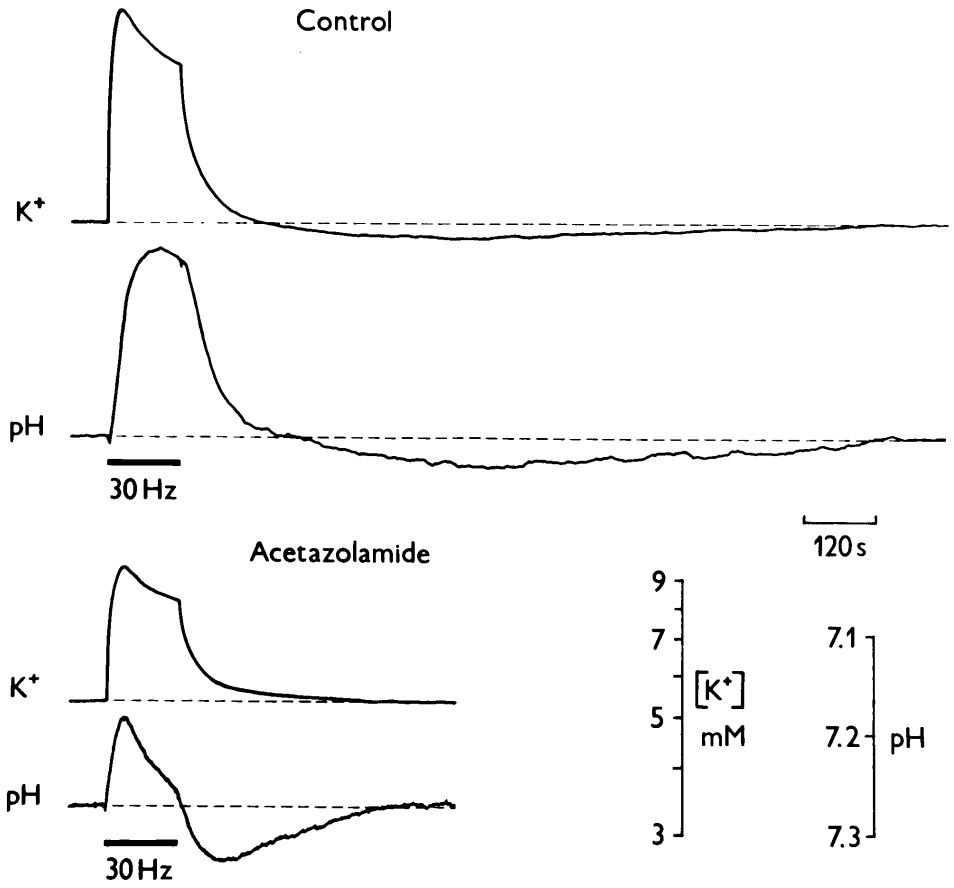

Fig. 3. Effect of acetazolamide (4 mmol/l) on simultaneously recorded changes in pH_e and $[K^+]_e$ in the lower dorsal horn as evoked by tetanic stimulation of dorsal root at 30 Hz. Note the poststimulation K^+ undershoot and alkaline shifts.

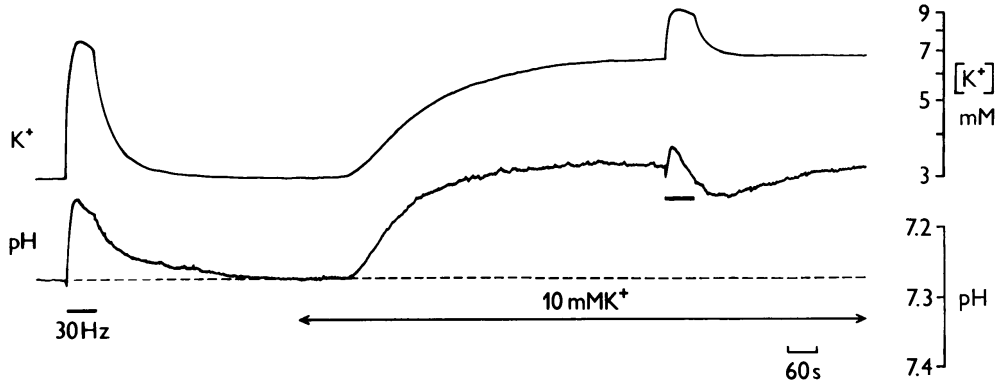

Fig. 4. Simultaneous pH_e and $[K^+]_e$ records during superfusion of the cord with a solution containing elevated $[K^+]$ (10 mmol/l). The transient changes in pH_e and $[K^+]_e$ evoked by stimulation at 30 Hz prior to and during superfusion.

solution below the standard bath concentration of 3 mmol/1 resulted in a concomitant alkaline shift (not shown).

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 elevations in $[K^+]_e$ were always accompanied by acid shifts with a similar time course.

The application of acetazolamide (4.0 or 8.0 mmol/l), a carbonic anhydrase inhibitor, resulted in an alkaline shift of the pH_e baseline by 0.05 pH units. The stimulation-evoked acidification was depressed and the post-stimulation alkaline shift was significantly larger (Fig. 3). Superfusion with acetazolamide also elevated the K^+ base line by 0.5 - 0.8 mmol/1, and this elevation was accompanied by a decrease in stimulation-evoked K^+ transients.

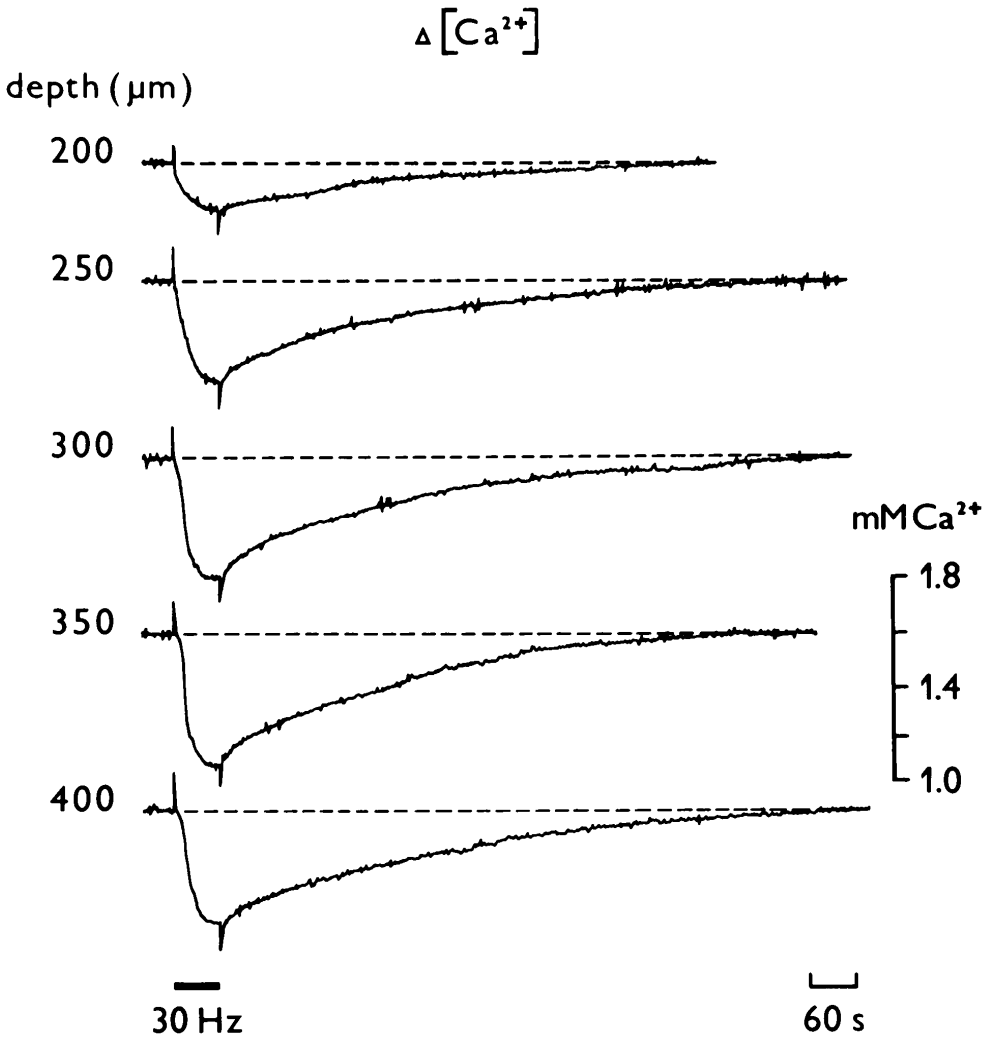

Fig. 5. The distribution of stimulation-evoked changes in $[Ca^{2+}]_e$ in spinal dorsal horn. The maximal decrease in $[Ca^{2+}]_e$ evoked by stimulation of 8th dorsal root at 30 Hz was found at a depth of about 350 μm.

ACTIVITY-DEPENDENT CHANGES IN $[Ca^{2+}]_e$.

Fig. 5 shows the typical time course and depth profile of the decrease in $[Ca^{2+}]_e$ evoked by repetitive electrical stimulation of the dorsal root. The decrease in $[Ca^{2+}]_e$ was found to increase with stimulation intensity and frequency. The largest decrease in $[Ca^{2+}]_e$ by 0.5 mmol/1 was found at stimulus frequency 30 - 100 Hz in the lower dorsal horn, at depth 300 - 400 μm, i.e. in the region where the maximal changes in $[K^+]_e$ and pH_e were found. The decrease in $[Ca^{2+}]_e$ reached a plateau after 20 - 25 s of stimulation, it had a slower rise time than the simultaneously recorded increase in $[K^+]_e$, and a faster time course compared to the stimulation-evoked acid shift (Fig. 6). There was partial recovery to the standard bath $[Ca^{2+}]$ during the stimulation. When stimulation was discontinued, the recovery to bath $[Ca^{2+}]_e$ (1.8 mmol/l) took about 8 min.

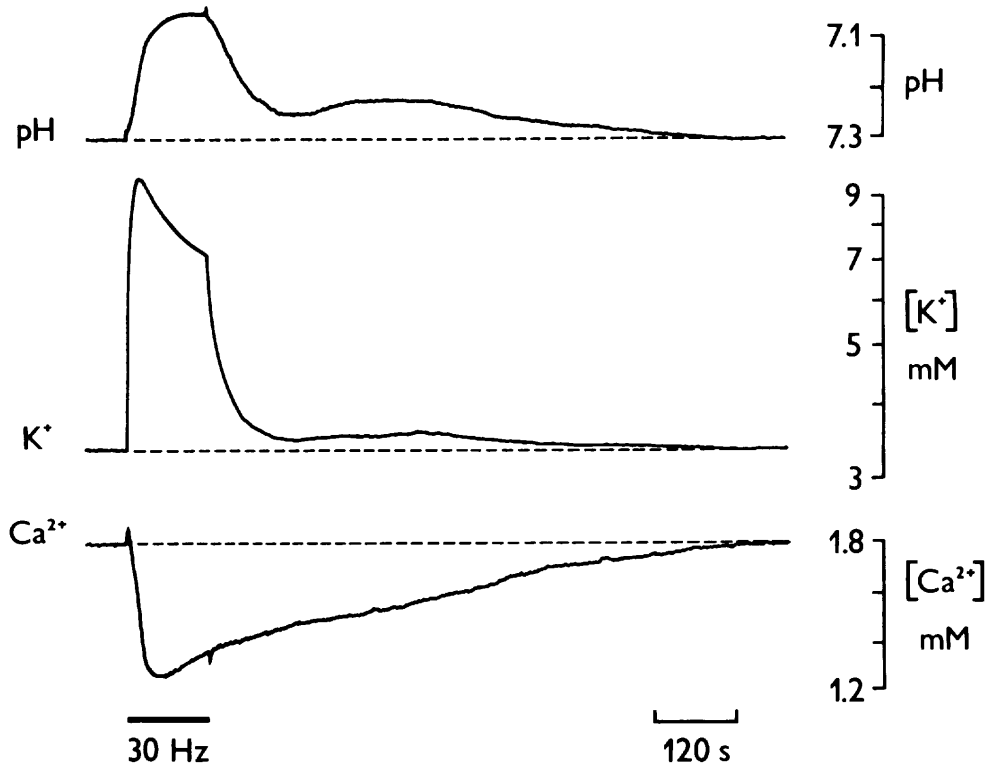

Fig. 6. Simultaneous records of pH_e, $[K^+]_e$ and $[Ca^{2+}]_e$ changes at a depth of 350 μm from the dorsal spinal surface. Stimulation at 30 Hz for 2 min. Tips of the three double-barrel ion-sensitive microelectrodes were separated by about 100 μm. Note the differences in rise time of the changes, and the similar time course of their post-stimulation recovery to original base lines.

DISCUSSION

Our study presents the first direct measurements of dynamic changes in pH_e in the spinal dorsal horn produced by stimulation of an afferent input and compares them with the previous findings in the brain (Kraig et al. 1983; Urbanics et al. 1978) and in peripheral nerves (Endres

et al. 1986; Ransom et al. 1985). It is apparent that stimulus-evoked neural activity leads to rather complicated extracellular pH changes in the CNS. In the spinal cord, similarly as in the brain and in peripheral nerves, the acidification dominates over the alkaline shifts. As has been shown in the cerebellum (Kraig et al. 1983) and in the isolated rat vagus nerve (Endres et al. 1986), the acid shifts were preceded by small initial alkaline shifts. When present in the isolated frog spinal cord, this initial alkaline shift was negligible (below 0.01 pH units, 3 s, see Figs. 1, 2, 3) and we can not exclude that it is not a stimulation artifact. In fact, a similar deflection was observed when the stimulation was switched off. Similarly, there is no initial alkaline shift in the rat optic nerve (Ransom et al. 1985). We therefore conclude that, in the frog spinal cord, the acid shift begins more rapidly than in the cerebellum, its rise time has a shorter delay after the onset of rise in $[K^+]_e$, it has a convex time course as the K^+ and Ca^{2+} transient changes (not the almost linear rise time observed in the cerebellum, Kraig et al. 1983), and when stimulation lasted for more than 30 s the pH_e attained its ceiling level, which was 0.2 - 0.25 pH more acid than the original pH_e base line. The rise time of the changes in $[Ca^{2+}]$ had almost the same time course as the increase in $[K^+]_e$ and they were therefore also faster than the pH_e changes (see Fig. 6).

The question remains about the mechanisms of extracellular acidification. According to Stewart's ideas (1981), the pH_e in body tissues is determined by three independent variables: 1. Extracellular strong ion difference ($[SID]_e$), 2. total weak acid extracellular concentration ($[A_{tot}]_e$), and 3. the partial pressure of CO_2 (pCO_2). These three independent variables completely define pH_e and other dependent variables: $[HCO_3^-]_e$, $[OH^-]_e$ and $[CO_3^{2-}]_e$. Of these, changes in $[SID]_e$ represented by the value of the strong base cations minus strong acid anions are likely to be the most important mechanisms involved in the stimulation-evoked pH_e changes, since substantial changes in $[A_{tot}]_e$ and pCO_2 are unlikely (Kraig et al. 1983). The extracellular changes in the concentration of strong ions, particularly Na^+, K^+, Ca^{2+}, $C1^-$ and possibly lactate, occur during neuronal activity and may therefore considerably effect pH_e. From various direct measurements of extracellular strong ion activities and the subsequent calculations of $[SID]_e$ (Kraig et al. 1983) it has been shown that a decrease in $[SID]_e$ should be accompanied by acidification, while an increase in $[SID]_e$ by an alkaline shift.

Since the K^+, pH_e and Ca^{2+}-sensitive microelectrodes have almost the same response time, the difference in rise time of K^+ and extracellular acidification observed in our experiments during the stimulation points to the different mechanisms involved. It is unlikely that the acid shift is exclusively the result of a decrease in $[SID]_e$, which results from the changes in $[K^+]$, $[Na^+]$, $[Ca^{2+}]$ and $[Cl^-]$. At first, calculations which were made on the basis of data obtained by direct measurements of $[K^+]_e$, $[Na^+]_e$, $[Ca^{2+}]_e$ and $[Cl^-]_e$ by ion-sensitive electrodes (Kraig et al. 1983), suggest that during the stimulation we can expect an increase in $[SID]_e$ and not the decrease required for acidification. Kraig et al. (1983) points out that a decrease in $[SID]_e$, can be due to extrusion of lactate, a strong organic anion, but direct evidence for its production during the stimulation-evoked neuronal activity is missing. Secondly, both the K^+ ceiling level as well as acidification were enhanced by low doses of ouabain; however, ouabain in higher concentrations increased the K^+ ceiling level but decreased the acidification (Fig. 2). Stimulation-evoked acidification is therefore not directly related to the extracellular K^+ increase, and may be of metabolic origin. The fast clearance of pH_e and K^+ transients during stimulation and immediately after was also not affected by ouabain (Syková et al. 1985, Fig. 3). It was proposed that this recovery does not include active reabsorption by neuronal elements (Kříž et al. 1975) but is due to passive potassium clearance which involves the glial spatial buffering mechanism (Gardner-Medwin 1983).

One of the likely candidates for maintaining the H^+ homeostasis are glial cells, which actively control their intracellular pH (Deitmer and Schlue 1987). Deitmer and Schlue

suggested the presence of a Na^+-H^+ exchanger in the glial cell membrane. It has been shown that the exogenously applied K^+ produces a significant acid shift in the pure glial, enucleated, rat optic nerve (Ransom et al. 1985). Moreover, carbonic anhydrase is widely assumed to play an important role in pH_e regulation (see Kraig et al. 1983) and this is found in the CNS predominantly in the glia and myelin (Cammer et al. 1985; Rousel et al. 1979). Our findings that acetazolamide reduced the stimulation-evoked acidification are in accordance with the idea of the important role of glia in activity-related pH_e transients in the spinal cord.

The origin of the post-stimulation alkaline shift is also not clear. It was closely related to the K^+ undershoot which has been shown to result from enhanced Na^+-K^+ pump activity (see Syková 1983, Fig. 3). It is therefore possible that the alkaline shift is related either to an active Na^+-K^+ exchange or to Na^+-H^+ exchange, which is mediated by ATPase (Thomas 1984, Kaila and Voughan-Jones 1987). A further possibility is that the alkaline shift arises from an increase in $[SID]_e$, due to a reduction in extracellular space volume. The poststimulation shrinkage of the extracellular space in the frog spinal dorsal horn has been described recently (Syková 1987).

We may conclude that out data provide further evidence that neuronal activity in the CNS is accompanied by significant changes in pH of the neuronal microenvironment. In the light of recent findings, we can assume that the observed changes in spinal cord pH_e are functionally important. Changes in pH_e by only several tenths of pH units have been shown to evoke marked effects on neuronal ionic channels (Krishtal and Pidoplichko 1980, Iljima et al. 1986, Konnert et al. 1987), to affect neuronal excitability (Gruol et al. 1980) and glial cell function (Deitmer and Schlue 1987, Walz and Hinks 1987).

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