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Effects of carbon dioxide on extracellular potassium accumulation and volume in isolated frog spinal cord

Eva Syková [1], R. K. Orkand [1,2], A. Chvátal [1], I. Hájek [1], and N. Kříž [1,3]

[1] Institute of Physiological Regulations, Czechoslovak Academy of Sciences, Bulovka, Pav. 11, CS-180 85, Prague 8, Czechoslovakia
[2] Institute of Neurobiology, University of Puerto Rico, Medical Sciences Campus, Old San Juan, PR 00901, USA
[3] Department of Physiology, Medical Faculty of Hygiene, Charles University, CS-120 00, Prague 2, Czechoslovakia

Abstract. A 6–10-fold increase in pCO_2 in the superfusing Ringer solution increased the volume of the extracellular space (ECS) and changed the spatial distribution and amplitude of the extracellular K^+ accumulation which resulted from dorsal root stimulation. Using the increase in tetraethylammonium concentration ($[TEA^+]$) resulting from iontophoretic injection of that ion in the extracellular fluid as an indication of the volume of the ECS, it was found that in high pCO_2 the ECS volume in spinal dorsal horn increased by more than 60%. In addition, in the presence of raised pCO_2 we also observed the following: (1) The rate of diffusion of TEA^+ into the dorsal horn increased. (2) The accumulation of K^+ evoked by single or tetanic stimulation of the dorsal root was less. (3) The clearance of K^+ was slowed down. (4) The regions where K^+ accumulated were more restricted. (5) The K^+ evoked depolarization of the primary afferent fibres decreased. (6) In contrast to TEA^+, the rate of diffusion of K^+ into the dorsal horn decreased. The effects of an increase in pCO_2 on K^+ accumulation and clearance appear to result from an increase in ECS volume and a possible decrease in glial electrical coupling which interferes with glial spatial buffering of K^+.

Key words: CO_2 – Frog – Glial cells – Potassium – Spinal cord – Size of extracellular space

Introduction

A number of studies of transient changes in extracellular potassium concentration ($[K^+]_o$) have been carried out in the mammalian (Krnjević and Morris 1972; Vyklický et al. 1972; Kříž et al. 1975) and amphibian spinal cord (Syková et al. 1976; Czéh et al. 1981). During tetanic electrical stimulation of an afferent input, $[K^+]_o$ increased up to three times normal, primarily in the deeper layers of the dorsal horn. In the upper dorsal horn and in the ventral horn the accumulation was much less. Various observations support the conclusion that fluctuations in $[K^+]_o$ in the spinal cord are compensated for by changes in the activity of the neuronal Na^+/K^+ ATPase (for review see Syková 1983). In addition the glial cell "spatial buffer mechanism" (Orkand et al. 1966; Coles and Orkand 1983; Gardner-Medwin 1983) might contribute to K^+ homeostasis in the spinal cord. Spatial buffering is a process which produces a passive flux of K^+ from regions of elevated $[K^+]_o$ to those of lower $[K^+]_o$ via electrically coupled glial cells due to regional differences in glial membrane potential. The differences in membrane potential arise from local variations in $[K^+]_o$ (Coles 1985).

We studied the effects of an increase in pCO_2 on the changes in $[K^+]_o$ evoked in the isolated frog spinal cord either by electrical stimulation of dorsal roots or by superfusing the cord with Ringer solution containing increased K^+. The exposure to CO_2, a treatment reducing intracellular pH, decreases coupling between cells in various tissues including neuroglia (Turin and Warner 1980; Spray et al. 1981; Tang et al. 1985). We wanted to disrupt the spatial buffering of K^+ in the glial syncitium and see how this procedure affected the movement of K^+ through the frog spinal cord.

Methods

Experiments were carried out on 36 isolated spinal cords of the frog (*Rana temporaria*). The dissection of the spinal cord and its maintenance have been described in detail elsewhere (Syková et al. 1976). The isolated cords were mounted in a chamber through which oxygenated bicarbonate-buffered Ringer solution (95% O_2 and 5% CO_2) of the following composition (in mM) was circulated at about 5 ml/min: NaCl, 112.0; KCl, 3.0; $CaCl_2$, 1.8; $NaHCO_3$, 10.0; glucose 1 g/l, pH 7.0 ± 0.1. In some experiments Ringer solution contained 30 mM $NaHCO_3$. During the experiment the fraction of CO_2 in the gas mixture was increased from 5% to 30–50%. In high pCO_2 the pH of Ringer solution was adjusted at room temperature (20–22°C) to 7.0 ± 0.1 or 7.3 ± 0.1 either with 30 mM $NaHCO_3$ and titration with NaOH, or by adding 70 mM $NaHCO_3$. Proportionally less NaCl was added in all solutions when $NaHCO_3$ was increased. No significant differences in our results were obtained using the different methods of pH maintenance or when the experiments were carried out in pH 7.0 or 7.3. Solutions were cooled to 17–19°C just before entering the experimental chamber. Dorsal root (DR) 8 or 9 was stimulated supramaximally (rectangular pulses of 5 V or less; duration 0.1 ms) with bipolar silver electrodes. DC recordings of the dorsal root potentials (DRPs) were made from the unstimulated dorsal root with bipolar platinum electrodes.

K^+ activity was measured by means of double-barrelled K^+-sensitive microelectrodes (Corning 477317) as described elsewhere (Kříž et al. 1975; Czéh et al. 1981). The increase in pCO_2 did not affect the potential of K^+-sensitive microelectrode or its sensitivity to K^+. Changes in the extra-

Offprint requests to: E. Syková

cellular volume were measured using a method which permits determination of the time course of local changes in the extracellular space (Phillips and Nicholson 1978; Nicholson et al. 1979). In principal, the concentration of tetraethylammonium ($[TEA^+]$), which is essentially impermeable and therefore remains in the extracellular space, is measured by K^+-sensitive microelectrodes, which are 400–600 times more sensitive to these ions than to K^+ (Neher and Lux 1973; Kříž and Syková 1981). The increase in $[TEA^+]$, which is injected iontophoretically at regular intervals into extracellular space, is inversely proportional to the volume. Single, double or four channel iontophoresis pipettes were filled either with TEACl or TEABr (5 or 10 mM) or with 1 M KCl, and inserted into the cord at the same depth as K^+-sensitive microelectrodes but at a distance of 80–200 μm. Similar results were obtained in some experiments when TMA^+ or choline was used. The iontophoretic current was 10–40 nA; a small constant positive bias current was used to prevent dilution of the fluid in the tip of the pipette.

Results

A single electrical stimulus applied to the dorsal root evokes a dorsal root potential (DRP) and an increase in $[K^+]_o$ in the lower dorsal horn of the frog spinal cord (Czéh et al. 1981). As shown in Fig. 1, perfusion of the spinal cord with Ringer solution bubbled with 50% CO_2 decreased the amplitude of DRPs as well as the amplitude of transient increase in $[K^+]_o$. Similar effects of increased pCO_2 were found after block of synaptic transmission with 2 mM Mn^{2+} or 20 mM Mg^{2+} added to the normal Ringer solution.

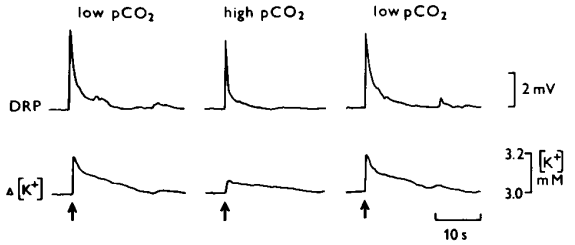

Fig. 1. The effect of raised pCO_2 on dorsal root potentials (DRP) and $[K^+]_o$ measured in lower dorsal horn and evoked by a single electrical stimulus (*arrow*) applied to the adjacent dorsal root. Low pCO_2: recordings in normal (5%) CO_2. High pCO_2: recordings after 15 min in raised (50%) CO_2

Tetanic stimulation of a dorsal root produced an increase of $[K^+]_o$ in the segment of the stimulated root, which had a typical depth profile, a sustained depolarization of the adjacent dorsal root (Syková et al. 1976; Czéh et al. 1981), (Fig. 2), and a decrease in ECS volume (Syková 1987). At sites of maximal increase in $[K^+]_o$ the active K^+ reabsorption was fastest due to substantial activation of Na^+/K^+ pump activity (Krnjević and Morris 1975; Kříž et al. 1975; Czéh et al. 1981). The DRPs have two components, the second, slower one is attributed primarily to a $[K^+]$ increase around the primary afferent terminals (Syková and Vyklický 1978; Czéh et al. 1981). Increasing pCO_2 in the superfusate decreased the stimulation evoked increase in $[K^+]_o$, slowed down the poststimulation K^+ clearance (Fig. 3) and depressed the slow K^+-component in DRPs (Fig. 2). Increasing the stimulation frequency increased the rise in $[K^+]$. Figure 3 shows that in high pCO_2 the K^+ redistribution, after approximately the same K^+ load, was significantly slowed down. In addition, in high pCO_2 the stimulation-evoked changes in $[K^+]_o$ were more restricted. Figure 2 shows that in normal pCO_2 the increase in $[K^+]_o$ in the upper dorsal horn (depth about 0.1 mm) and in upper ventral horn (depth about 0.8–1.0 mm) is still very high even when the main source of K^+ is the interneurones in the lower dorsal horn (depth 0.3–0.6 mm) (Czéh et al. 1981). However, the stimulation-evoked increase in $[K^+]_o$ in the remote areas was smaller in high pCO_2.

Similar effects of high pCO_2 on DRPs and on $[K^+]_o$ were found when synaptic transmission was blocked (Fig. 4). After the addition of 2 mM $MnCl_2$ the ventral root discharge and the focal potentials evoked by stimulation of the dorsal root disappear, while the DRPs are not eliminated (Syková and Vyklický 1977). The remaining DRPs result

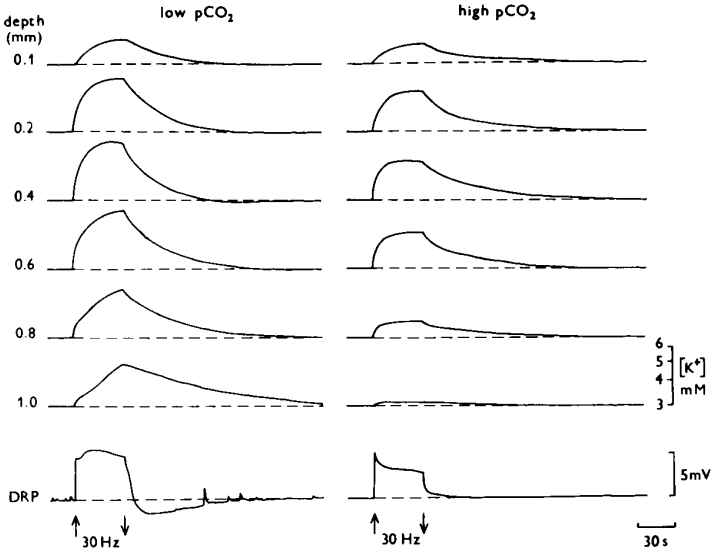

Fig. 2
Effects of raised pCO_2 on spatial distribution of changes in $[K^+]_o$ and depolarization of dorsal root (DRP, *bottom trace*) evoked by tetanic stimulation of adjacent dorsal root (supramaximal stimulation at 30 Hz for 40 s). The increased pCO_2 decreases the stimulation evoked increase in $[K^+]_o$ especially to 0.8 and 1.0 mm depth, i.e. in depth corresponding to the intermediate region and to the upper ventral horn, respectively. Note the absence of slow, K^+-component in DRP in raised pCO_2. Stimulation in high pCO_2 was repeated every 5 min, starting 10 min after its application

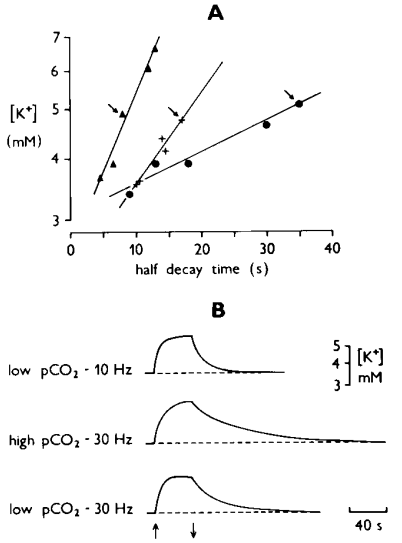

Fig. 3 A, B. Effects of raised pCO_2 (50%) on the stimulation evoked increase in $[K^+]_o$ and on poststimulation K^+ redistribution. **A** The increase in $[K^+]_o$ was produced by tetanic stimulation of a dorsal root (supramaximal stimulation for 40 s) at varying frequencies. The half-decay time of the increase in $[K^+]$ was measured from records obtained 400 µm below the dorsal surface of the cord. *Triangles:* recordings in normal (5%) CO_2 at 3, 10, 30, 100 and once more 3 Hz. *Circles:* recordings after 10–50 min in raised CO_2 at frequencies 3, 10, 30, 100 and once more 10 Hz. *Crosses:* partial recovery 10–50 min after normal CO_2 (5%) was returned at frequencies 3, once more 3, 10, 30, 100 Hz. For each line increasing the frequency of stimulation increased the final level of $[K^+]$ recorded. **B** Examples of the responses producing approximately the same K^+ load (2 mM) achieved by varying the frequency of stimulation in low and high CO_2 (the half-decay time measured from these curves is marked by the *arrows* in **A**

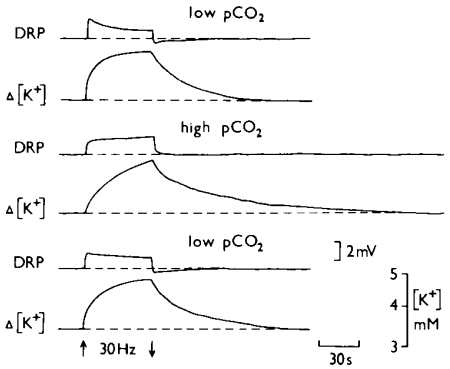

Fig. 4. The effect of high pCO_2 (50%) on the increase in $[K^+]_o$ ($\Delta[K^+]$) and depolarization of the dorsal root (DRP) evoked by tetanic stimulation of an adjacent dorsal rool after block of synaptic transmission with 2 mM Mg^{2+} and its recovery in low pCO_2 (5%). The rise in $[K^+]_o$ and its clearance are slowed after 15 min in high pCO_2. The K^+-sensitive microelectrode was 0.35 mm below the dorsal surface of the cord

from depolarization produced by K^+ released from stimulated primary afferents. Figure 4 shows that in high pCO_2 the poststimulation K^+ redistribution as well as the rise time of both DRPs and K^+ accumulation evoked by repeti-

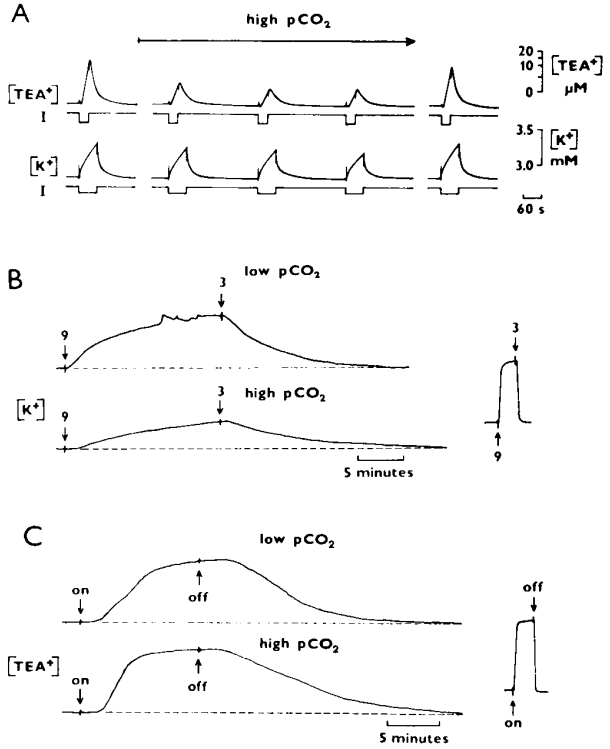

Fig. 5 A–C. The effect of high pCO_2 on extracellular space volume in spinal cord (**A**) and on diffusion of K^+ and TEA^+ into the dorsal horn (**B** and **C**). **A**: Changes in $[TEA^+]$ and $[K^+]$ as measured by K^+-sensitive microelectrodes were elicited by identical current pulses applied to iontophoretic pipette filled either with TEACl (four channel pipette, total iontophoretic current 40 nA) or with KCl (single channel pipette, iontophoretic current 10 nA). Records are from two different experiments. Recordings from depths of 0.4 mm and 0.2 mm, respectively. Separation of K^+-sensitive microelectrode from iontophoretic pipette was about 100–150 µm in both experiments. **B**: The $[K^+]$ in the superfusing Ringer solution was raised (from 3 to 9 mM and back to 3 mM) and the increase in $[K^+]_o$ was measured with a K^+-sensitive microelectrode located 0.6 mm below the dorsal spinal surface. Note that after 15 min in high pCO_2 the diffusion of K^+ was slowed. The CO_2 did not affect the microelectrode calibration shown on the right side. **C**: TEACl (2.5 mM) was added to the superfusing Ringer solution and the increase in $[TEA^+]$ measured with a K^+-sensitive microelectrode 0.5 mm below the dorsal spinal surface. After 15 min in high pCO_2 the diffusion of TEACl was faster. The calibration of the electrode in 2.5 mM TEACl prior to the experiment is on the *right side*

tive stimulation of the dorsal root are slowed. These effects of raised pCO_2 developed during 2–3 min and were reversible following the return of normal Ringer solution (5% CO_2).

In other experiments ($n = 6$), high pCO_2 produced an increase of extracellular volume. The same amount of TEA^+, TMA^+, choline or K^+ iontophoresed into the ECS of the spinal cord produced a smaller concentration increase in high pCO_2 (30–50%) than in normal pCO_2 (5%) (Fig. 5A). In spinal cords superfused with Ringer solution and bubbled with 50% pCO_2 the calculated increase in extracellular volume measured by the decrease in $[TEA^+]$ produced by iontophoresis (Phillips and Nicholson 1978; Nicholson and Phillips 1981) was 60.5% ± 10.0 (mean ±

SE, $n = 4$). The same effect was observed if the pH of the superfusate was kept at 7.3 ($n = 2$).

The diffusion of substances and ions in central nervous tissue extracellular space is subject to the constraints of an extracellular volume or pore space fraction and a tortuosity factor which increases the path-length of diffusion particles (Nicholson and Phillips 1981). In our experiments the raised pCO_2, which led to an increase in ECS volume, slowed down K^+ movement from superfusing solution into the spinal cord. Figure 5B shows that increase of $[K^+]$ in Ringer solution from 3 to 9 mM increased $[K^+]_o$ in deeper layers of the spinal dorsal horn with half-time of less than 5 min. In high pCO_2 the K^+ diffusion into the cord was slowed down, the half-time was more than 15 min (Fig. 5B). In contrast, the penetration of TEA^+ (2.5 or 5.0 mM) into the spinal dorsal horns was more rapid in high pCO_2 (Fig. 5C). These findings suggest that the diffusion of K^+ into the frog spinal cord was slowed down even when the extracellular volume fraction increased. This could result from uncoupling of the glial cells by high pCO_2 (Tang et al. 1985), i.e. due to a disruption in the glial cells spatial buffer mechanism (Gardner-Medwin 1983).

Discussion

Our experiments show that an increase in pCO_2 in extracellular milieu may: (1) slow down poststimulation K^+ redistribution due to glial cell uncoupling and (2) increase the size of the extracellular space. In contrast to our observations, (Figs. 1–3) one would expect that glial cells uncoupling should slow the clearance of K^+ and thereby lead to greater increases in $[K^+]_o$ in dorsal horn. The increase of extracellular volume may explain the observed decrease in transient poststimulation changes in $[K^+]_o$. Alternatively, neurones may release less K^+ due to a reduced K^+ conductance. Moreover, elevated pCO_2 depresses the excitability of neuronal preparations by a direct effect either on intracellular pH or on neuronal membrane, i.e. not due to action of changes on extracellular pH (Carpenter et al. 1974; Caspers and Speckman 1974; Balestrino and Somjen 1988).

It has been suggested that spatial buffer mechanism in the mammalian brain implements K^+ redistribution much more efficiently than K^+ diffusion in the extracellular space (Gardner-Medwin 1983). Recently, evidence for the spatial buffer mechanism has been found in the retina of the honeybee drone (Coles and Orkand 1983). In the spinal cord the glial cells are well shaped for spatial buffering; they form syncytium and form processes from the surface of the cord to intermediate region (Sasaki and Mannen 1981). Our experiments on isolated spinal cord do suggest that the glial cell spatial buffer mechanism contributes to the clearance of stimulation-evoked increases in $[K^+]_o$, since clearance was slowed down in high pCO_2 (Fig. 3). The glial cell spatial buffer mechanism may be rather important for the diffusion of K^+ from its source to the more remote areas. This can explain the smaller changes in $[K^+]_o$ in the upper dorsal horn and in the ventral horn and the diminished K^+ component of DRPs (see Figs. 1 and 2).

The observed effects of high pCO_2 are probably not due to CO_2 evoked depression of synaptic transmission resulting from hypoxia. At first, high pCO_2 did not substantially affect the first synaptic component of DRPs (see Fig. 1). Furthermore, all effects of high pCO_2 were found during block of synaptic transmission. Secondly, the frog spinal cord is not as sensitive to hypoxia as the mammalian cord. We did not find a depolarization of DR or an increase in resting level of $[K^+]_o$ in high pCO_2, as is the case during hypoxia (see Syková 1983). Moreover, a decrease and not an increase of the extracellular volume was found during hypoxia (Hansen and Olsen 1980) and during the extracellular acidification (Syková et al. 1987).

Our data support the hypothesis that in the case of local increases in $[K^+]$, efficient K^+ clearance through the spinal cord involves K^+ fluxes through glial cells driven by K^+ concentration gradients (spatial buffer mechanism). However, the relative importance of K^+ clearance by this process as compared to the role of the Na^+/K^+ pump in neurons and glia remains unknown.

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