EXTRACELLULAR pH, [K^+], AND SYNAPTIC TRANSMISSION IN THE DORSAL HORN OF THE SPINAL CORD OF HYPERCAPNIC RATS

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More than a century ago it was shown in experiments on animals that inhalation of a gas mixture with high CO_2 concentration has a narcotic action [12]. It is now well known that hypercapnia inhibits synaptic transmission in the CNS [4, 7, 8, 11], blocking bulbar vasomotor and respiratory neurons [5]. Irrespective of how CO_2 acts on synaptic transmission in the CNS, the result of this action is a change in the extracellular and intracellular concentrations of K^+, Na^+, Ca^{2+}, and H^+ ions, a result widely discussed in the literature [2]. However, the experimental data in most cases were obtained on cold-blooded animals in vitro [3, 6, 9, 10, 14], which left the problem of the action of CO_2 and H^+ unresolved on account of species differences and the inadequate nature of the reactions of the isolated preparations in vitro. By means of ion-selective electrodes it became possible to record pH and [K^+] in nerve tissue together with its electrical response.

The aim of this investigation was to use K^+- and pH-ion-selective electrodes to determine quantitative correlations between the electrical response (in our case, the focal potential) and ion concentrations at 20% hypercapnia.

EXPERIMENTAL METHOD

Experiments were carried out on 12 Wistar rats weighing 250-300 g. The rats were anesthetized with CC-chloralose in a dose of 100 mg/kg. The trachea and carotid artery were cannulated, allowing artificial ventilation of the lungs and measurement of the blood pressure. Laminectomy was performed at the L_2-L_6 level. The animals were switched to artificial respiration after injection of the muscle relaxant Myo-Relaxin in a dose of 20 mg/kg intraperitoneally. The exposed spinal cord was moistened with Ringer's solution for mammals or with mineral oil at a temperature of 37°C. The focus of maximal activity was found by recording evoked potentials (EP) from the dorsal surface of the spinal cord. The body temperature was maintained at 37-38°C. Electrodermal stimulation was carried out through paired steel acupuncture needles, inserted subcutaneously into the left and right hind limbs of a rat in the region of the calf: with square positive pulses with a duration of 150 μsec and a strength of 10 mA, evoking a supramaximal response on the surface of the spinal cord. K^+-activity was recorded by means of two-channel K^+-sensitive glass microelectrodes with ion-exchange resin, as described previously [13]. The tip of one channel was siliconized and filled with K^+-ion exchanger (Corning 477317) at a distance of 200-300 μ from the end. The space thus left was filled with 0.5 M KCl. The second channel was neutral and filled with 150 mM NaCl. The potential arising on the K^+-sensitive electrode was measured differentially between two channels with high input resistance (10^{14} Ω) of the amplifier. The microelectrodes were calibrated in standard KCl solution containing 150 mM NaCl before each experiment. The procedure of preparing the pH-sensitive macroelectrodes was similar to that described previously. Two-channel glass micropipets with a tip 3-5 μ thick were made. The tip of the pH-sensitive channel was siliconized with 3-5% tri-N-butylchlorosilane in 1-chloronaphthalene and filled to a height of 300-500 μ with

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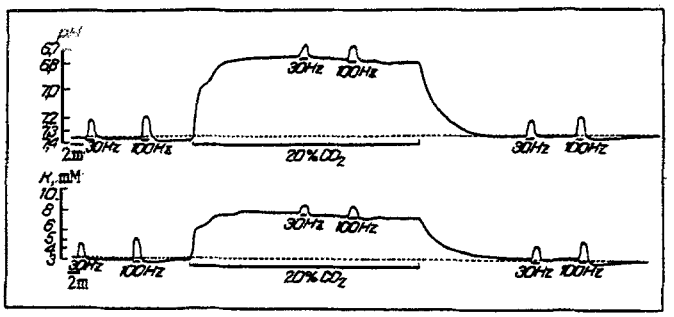

Fig. 1. Changes in extracellular K^+ concentration and pH_0 level in segment L_4 of the spinal cord at a depth of 600 μ before, during, and after addition of CO_2 to the inspired air.

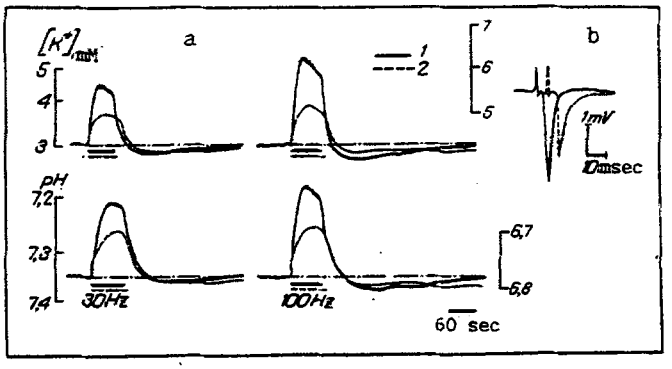

Fig. 2. Effects of CO_2 on post-stimulation shifts of pH and K^+ (a) and focal potential (b). a) Post-stimulation changes in $[K^+]_0$ and pH_0 in segment L_4 of the spinal cord at a depth of 600-700 μ, evoked by electrical stimulation of hind limbs at frequencies of 30 Hz and 100 Hz, through steel subcutaneous electrodes; b) averaged focal potential recorded through reference channel of pH-sensitive microelectrode. Continuous line – values of post-stimulation shifts of pH, K^+, and focal potential; broken line indicates post-stimulation values of pH, K^+, and focal potential after addition of 20% CO_2 to inspired air.

Hydrogen Ion Ionophore II-Cocktail A ("Fluka"), developed by E. Ammann and co-workers [1]. The channel with the ion-exchange resin was filled with the following solution (in mM): KH_2PO_4 40.0, NaOH 23.0, NaCl 15.0 (pH 7.0). The second channel consisted of the reference electrode, and it was filled with 0.15 M NaCl. The electrodes had the following characteristics: 58 mV/pH unit, resistance 700-1800 MΩ. The microelectrodes were calibrated in a standard pH solution with 0.15 M NaCl, 0.003 M $CaCl_2$. Both electrodes were inserted into the spinal cord perpendicularly: one to the left, the other to the right of the central artery. The focal potential was recorded between the reference channel of the pH-sensitive electrode and ground. The signal was analyzed on an ATAK-350 analyzer. Every 100 presentations were averaged. In the control state the rats breathed room air. Hypercapnic normoxia was created by replacing 20% of the air by 20% CO_2, i.e., the mixture inhaled consisted of 80% (21% O_2 + 79% N_2) and 20% CO_2. Blood samples measuring 60-80 μl were taken through the cannulated carotid artery four times during an experiment: once before, once immediately after, and again 20 and 40 min after addition of the CO_2. The samples were analyzed on an ABL 330 instrument (Radiometer).

EXPERIMENTAL RESULTS

The basic level pH_0 in the dorsal horn of the spinal cord changed in the course of hypercapnia as shown in Fig. 1. In the first 5 min after elevation of the CO_2 concentration in the inspired air to 20% the basic pH_0 level fell from 7.35 ±

TABLE 1. Partial Pressure of Blood Gases before and during Hypercapnic Normoxia

Background				Normoxia (40 min)			
pH	pCO_2	pO_2	SaO_2	pH	pCO_2	pO_2	SaO_2
7,37±0,03	33,5±1,8	103,9±7,6	97,2±0,4	6,71±0,02 $p<0,001$	193,4±4,3 $p<0,001$	135,0±6,5 $p<0,01$	91,0±1,4 $p<0,001$

0.01 pH units to 6.78 ± 0.09 and remained at that level during the next 35 min of CO_2 inhalation. Together with the observed rise of pH_0, changes took place also in $[K^+]_0$ (Fig. 1). Changes in $[K^+]_0$ to the maximal level took place in the course of 8-10 min, and its values rose from 3 mM to 5.14 ± 0.8 mM. After replacement of the CO_2 in the inspired air, the pH_0 and $[K^+]_0$ levels returned to their basic values observed before inhalation in the course of 10-15 min.

Besides the increase in $[K^+]_0$ and decrease in pH_0 immediately after the beginning of addition of CO_2, the diastolic and systolic blood pressure rose in the course of 0.3-0.5 min by 28.25 ± 1.25% and 33.25 ± 14.7% respectively. After removal of CO_2 the arterial pressure reverted to its initial level in the course of 0.3-0.5 min.

Electrodermal stimulation with a frequency of 30-100 Hz evoked a biphasic (acidosis – alkalosis) or triphasic (alkalosis – acidosis – alkalosis) shift of pH_0. The amplitude and duration of the changes in pH_0 increased with an increase in the intensity, duration, and frequency of stimulation (Fig. 2a). During stimulation pH_0 fell by 0.15-0.2 unit. After the end of stimulation pH_0 reverted to its basal level, after which the next phase of alkalosis after acidosis was formed, and amounted to 0.01-0.03 unit. The changes in pH_0 were closely linked with the change in $[K^+]_0$. Any shifts from the basal $[K^+]_0$ level evoked by stimulation were associated with pH_0 changes.

Changes in pH_0 and $[K^+]_0$ evoked by electrodermal stimulation changed significantly after the addition of 20% CO_2 to the inspired air (Fig. 2a). After establishment of the basal level of pH_0 and $[K^+]_0$ at the 5th-10th minute after addition of CO_2, the acidotic shift evoked by stimulation was reduced by 36.9 ± 8.5% during stimulation with a frequency of 30 Hz and by 41.9 ± 6.1% during stimulation with a frequency of 100 Hz (Fig. 2a). This change of the pH_0 shift was accompanied by reduction of the $[K^+]_0$ shift during stimulation with a frequency of 30 Hz by 11.5 ± 1.33% and during stimulation with a frequency of 100 Hz by 17.3 ± 1.52%. Reduction of the post-stimulation shifts of pH_0 and $[K^+]_0$ on the addition of CO_2 did not change significantly throughout the period of inhalation. After removal of the CO_2, the poststimulation shifts were restored after 10-15 min to the preinhalation level.

The averaged recorded potential recorded through the reference channel of the pH-sensitive electrode enabled changes in electrical activity of the dorsal horn of the spinal cord in the region of Rexed's laminae IV-VI at a depth of 500-700 μ to be compared with ion-exchange during hypercapnia and normoxia. Changes in the focal potential in one experiment after addition of 20% CO_2 to the inspired air are shown in Fig. 2b. On average the amplitude of the focal potential fell by 16.8 ± 4.18% after addition of CO_2.

Reduction of the amplitude of the focal potential in response to single electrodermal stimulation took place as the pH_0 and $[K^+]_0$ levels changed, and it was established after the basal level of pH_0 and $[K^+]_0$ had flattened out on a plateau. After removal of CO_2, with restoration of the basal level of pH_0 and $[K^+]_0$ the amplitude of the focal potential assumed the preinhalation values.

Aggregated changes in blood gas composition before and after addition of 20% CO_2 to the inspired air (n = 8) are given in Table 1. The measurements were made in vitro four times in the course of 60 min: once before, once after, and twice during inhalation of CO_2. As Table 1 shows, the pH of the blood, pCO_2, and SaO_2 differed significantly after the beginning of CO_2 addition, and after its removal they returned to their original level. It must also be noted that the tissue pH_0 and the blood pH did not differ significantly throughout the experiment.

On the basis of these results it can be concluded that 20% hypercapnia increases the proton concentration in the extracellular space of the dorsal horns of the rat spinal cord, and this may perhaps be the cause of the increase in the extracellular K^+ concentration. An increase in proton and K^+ ion concentrations reduces their response to peripheral nerve stimulation. However, the main results of this redistribution of ions is general inhibition of synaptic transmission and also, evidently, the narcotic action which, in our experiments, was demonstrated as lowering of the focal potential of the dorsal horn of the spinal cord.

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EFFECT OF DEPRIVATION OF THE PARADOXICAL PHASE OF SLEEP ON ACTIVITY OF OPIATE RECEPTORS ISOLATED FROM RAT BRAIN SYNAPTIC MEMBRANES

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Paradoxical sleep deprivation (PSD) in mammals is a convenient model with which to study the effect of extremal factors on metabolic and mediator processes in the brain [1]. It has been found that during PSD not only is the sensitivity of the rat brain to dopamine reduced [8], but the total concentration of dopamine receptors and their affinity for ligands also are reduced [14]. Similar changes in the number of binding sites and the affinity of β-adrenoreceptors during PSD have been observed by Mogilnicka [11]. PSD in rats also is reflected in the state of their opiatergic system: the concentration of Met-enkephalin is increased whereas that of Leu-enkephalin is reduced in the hypothalamic region and hippocampus [3], although the β-endorphin concentration is unaffected [3, 12]. Workers in our laboratory also have shown that 24-hourly PSD in rats causes a change in the state of the opiate receptors located in synaptic membranes, and leads to a decrease in the concentration of binding sites of the antagonist, namely ^{3}H-naloxone [5]. In this case a tendency was observed for affinity of the receptors for this ligand to be increased. Meanwhile a change in the molecular organization of the synaptic membranes was found after PSD: a decrease in their lipoperoxide content, an increase in their degree of hydrophobicity, and a shift of the cationic—anionic balance [6]. In this connection the following problem arises: are the changes in functional properties of the receptor apparatus the result of changes in the molecular organization of the synaptic membranes, or are they due to a change in the properties of the opiate receptors themselves and, in particular, of opiate-binding proteins.

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