ROLE OF pH IN THE MECHANISM OF ACTION OF CO_2 ON SMOOTH MUSCLE OF THE CEREBRAL ARTERIES

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In the cerebral circulatory system carbon dioxide acts as a vasodilator [2, 4, 5, 7-9, 11]. Thanks to its physicochemical properties CO_2 passes easily through the tissue barriers and gives rise to considerable pH changes in the intercellular fluid [2, 4, 5, 7-9, 11]. Accordingly, to explain the mechanism of action of carbon dioxide, a "pH hypothesis" has been suggested, according to which the ultimate factor responsible for the vasomotor activity of CO_2 is a change in the H^+ concentration in the medium surrounding the smooth muscle, and even in the cytoplasm of the smooth muscle cells (SMC) themselves [1, 2, 8]. An unsolved problem in this hypothesis is whether CO_2 acts directly on SMC of the cerebral arteries and what is the role of pH in this mechanism. This paper is devoted to an analysis of this problem.

EXPERIMENTAL METHOD

Changes in smooth muscle tone of the isolated cerebral arteries were recorded during changes in pCO_2 and pH in the Krebs' salt solution in which the preparations were kept. All experiments were conducted on strips of the intracranial portion of the human internal carotid artery by means of a mechanotron dynamometer, under pH and pCO_2 control and at constant temperature (37 ± 0.5°C). The preparations (15 × 2 mm) were obtained from blood vessels of clinically healthy subjects, male and female, dying suddenly from trauma or asphyxia, and aged from 18 to 46 years. Strips in which the muscle preserved its functional capacity, i.e., could contract spontaneously and exhibited high reactivity to the depolarizing action of potassium ions (80 mM KCl), were used for the investigation. Altogether 34 preparations were studied; death had occurred between 2.5 and 17 h previously. Tests were carried out with hypercapnic solution (pCO_2 50-60 and 70-80 mm Hg or 6.66-7.00 and 9.33-10.66 kPa), prepared by saturating Krebs' solution with gas mixtures of the appropriate composition. Before each procedure pH was corrected with hydroxymethylaminomethane. In some experiments acetazolamide

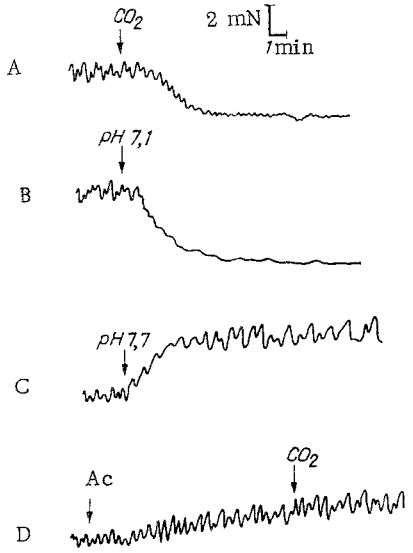

Fig. 1. Changes in smooth muscle tone of arteries. A) Under the influence of CO_2 (pCO_2 60 mm Hg); B) with decrease in pH; C) with increase in pH of medium; D) under the influence of acetazolamide (10^{-5} M).

KEY WORDS: cerebral arteries; smooth muscles; hypercapnea; pH; acetazolamide

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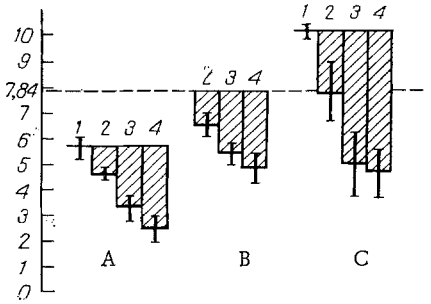

Fig. 2. Effect of pCO_2 on tone of SMC of internal carotid artery. 1, 2, 3) pCO_2 30-40, 50-60, and 70-80 mm Hg, respectively. A, B, C) pH 7.0-7.2, 7.3-7.4, and 7.5-7.8, respectively. Ordinate, strength of smooth-muscle tone (in mN). Broken lines shows level of tone of strips under initial conditions.

(Diacarb from Polfa, Poland), dissolved beforehand in 0.2 N NaOH solution in a concentration of 10^{-3} M and added to the Krebs' solution in a concentration of up to 10^{-5} M, was used as the pharmacologic agent.

EXPERIMENTAL RESULTS

Under the original conditions (pCO_2 1-5 mm Hg, or 0.13-0.67 kPa, pH 7.3-7.4) spontaneous contractions were observed with a frequency of 2 to 5 waves/min. Under the influence of hypercapnic solutions, and with pH constant (7.3-7.4) a decrease was observed in the initial tone of the preparation, together with weakening of the spontaneous contractions (Fig. 1A).

Meanwhile, when pH shifted towards the acid side and pCO_2 remained constant (30-40 mm Hg, 4-5.33 kPa) relaxationof the smooth muscle of the strips also was observed (Fig. 1B). In these cases the spontaneous contraction disappeared. A change in pH of the solution to the alkaline side led to an increase in tone of the preparations and strengthening of spontaneous contractions (Fig. 1C).

The results of the combined influence of CO_2 and pH are shown in Fig. 2. Clearly against a background of low pH values (7.0-7.2) the magnitude of the hypercapnic effects diminished, whereas when pH values were high (7.5-7.8) the relaxing effect of CO_2 was stronger than originally. However, a statistically significant change in the values studied was found only when pH values were high ($P < 0.05$).

To study the role of intracellular pH in the mechanism of the effects of CO_2, acetazolamide, an inhibitor of intracellular carbonic anhydrase was used. It was added to the solution with pH 7.3-7.4. After 5-10 min, in 3 of 7 cases it caused a very small increase in the initial tone and in the strength of the spontaneous contractions (Fig. 1B). It is interesting to note that the action of the hypercapnic solution (pCO_2 50-70 mm Hg, or 6.66-9.33 kPa) on all seven preparations kept under these conditions was greatly weakened or was not exhibited at all.

It can be concluded from these results that even if pH is stabilized, CO_2 retains its ability to affect smooth muscle tone of the cerebral arteries. At the same time, the reactivity of the muscle to hypercapnic stimulation increased in a more alkaline medium and was slightly reduced when pH in the solution was lowered. It will also be evident that pH has an independent effect on the smooth-muscle tone of the internal carotid arteries. Consequently, the possibility that CO_2 exerts a direct action on SMC in the blood vessels of the human brain must be recognized, and at the same time, it must be assumed that the extracellular H^+ concentration can modulate the effects of hypercapnea and can also play an independent role in the regulation of tone of arterial SMC.

The extracellular pH factor in the direct action of CO_2 appears to be secondary, the role of intracellular pH in this effect must be regarded as more important. Experiments with blocking of intracellular carbonic anhydrase by acetazolamide shows that marked depression of activity of this enzyme significantly weakened the hypercapnic responses. Considering that intracellular carbonic anhydrase hydrates CO_2, which is followed by dissociation of carbonic acid into H^+ and HCO_3^- [6], it can be tentatively suggested that the action of "exogenous" CO_2, appearing in the cytoplasm under hypercapnic conditions, must inevitably be connected with a change in the intracellular H^+ concentration.

The results are thus evidence of relative independence of the effects of pH and pCO_2, acting from outside on SMC of the cerebral arteries, and at the same time, they suggest the close interaction between these two factors inside the cell.

These observations can explain the disappearance of hypercapnic responses in the cerebral circulation observed previously in response to administration of acetazolamide to animals [2, 9].

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ROLE OF SEROTONIN OF NEURONS AND NEUROPIL IN THE VISUAL SYSTEM

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There have been many studies of the principles governing serotonin (5-HT) metabolism [8]. However, problems concerned with the 5-HT content and its turnover in different types of cells of the CNS have not hitherto been studied, despite the fact that methods of isolating fractions enriched with neurons and neuroglial cells from brain tissue have recently been developed [14].

One of us (M.G.U.) suggested previously that 5-HT plays a specific role in the activity of the visual system [3]. Accordingly, in the present investigation the 5-HT content was studied in fractions enriched with neurons and neuropil (neuroglial cells and axodendritic fragments taken together) of the visual and motor areas of the rat cortex, using early visual deprivation as the model.

EXPERIMENTAL METHOD

Male Wistar rats were used. The animals of group 1 served as the control, the rats of group 2 were visually deprived (kept for 50 days after birth in darkness), and group 3 consisted of rats exposed to light for 3 h after 50 days of visual deprivation. Altogether six experiments were carried out on each group. The animals of all three groups were decapitated, the brain was removed, and the visual and motor areas of the cortex were isolated. All operations of the brain were performed at 0°C. The tissue of the corresponding regions from 5-7 rats in each group was pooled and used to obtain the cell fractions. Fractions enriched with neurons and neuropil (neuroglial cells + axodendritic fragments) were isolated by the method in [13, 14]. The 5-HT content in these fractions was determined by a spectrofluorometric method [12], in the modification of [4, 5], and the protein content by the method in [11].

KEY WORDS: serotonin; visual system; deprivation; visual cortex.

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