Changes in Brain pH Response to CO_2 after Prolonged Hypoxic Hyperventilation

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PERSISTENT HYPERVENTILATION following prolonged overbreathing of hypoxic origin (1) and of mechanically induced origin (2) has been reported. The fall in alkali reserve of the blood during the time of the hyperventilation has been suggested as the mechanism for this continuing hyperventilation after the initial cause has been removed (3). In experiments in which the arterial blood pH and CO_2 content of the subject were determined at the same time that the respiratory minute volume response was recorded, it was found that reduction in alkali reserve of the blood would not account entirely for the increased respiratory responsiveness to CO_2 inhalation following 24 hours of passive hyperventilation (4).

It has been suggested that the respiratory center becomes more sensitive to CO_2 after prolonged acapnia (5) but no mechanism to explain such an increase in sensitivity has been proposed.

The present study was undertaken following the suggestion that a reduction in buffer capacity for CO_2 of the cells of the respiratory center themselves might play a part in this mechanism. On the assumption that the respiratory center cells would reflect changes in the brain as a whole, a method was devised for determining the CO_2 titration curve of brain homogenate.

Such titrations have been carried out on the brains of 10 pairs of guinea pigs, one animal of each pair having been exposed to hypoxic hyperventilation for 24 hours and the other animal of each pair having been kept under similar laboratory conditions but at normal oxygen tension.

METHODS

Hypoxic hyperventilation was produced by placing the guinea pigs in a metal chamber 6′ in length and 2.5′ in diameter and evacuating the chamber to a pressure of approximately 290 mm. Hg pressure. This low pressure was maintained with a continuous ventilation through the chamber for the 24-hour period. During this time both the experimental and the control animals were given water but no food. Immediately upon removing the guinea pig from the

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chamber it was decapitated along with its control mate and the heads were immediately frozen in liquid air.

The solidly frozen brain was then removed and ground to a fine powder in a cold mortar. Two grams of this powdered brain were weighed to the nearest 5 mg., and 20 ml. of cold distilled water were added. The brain and water mixture was kept frozen to a mushy ice and ground to a smooth homogenate in a mortar. Five-ml. aliquots of this homogenate were placed in 500-ml. tonometers and one of three CO_2/O_2 mixtures flushed through the tonometer. The CO_2 mixtures contained 1.80 per cent, 3.17 per cent, and 4.99 per cent CO_2 in oxygen. Gas analyses were made with the .5-ml. Scholander analyzer (6). Equilibration of the homogenate with the gas mixture was carried out by ro-

TABLE 1. HYDROGEN ION CONCENTRATIONS, EXPRESSED AS M/L × 10^8 OF BRAIN HOMOGENATES EXPOSED TO DIFFERENT CO_2 TENSIONS FOR NORMAL AND HYPOXIC HYPERVENTILATED GUINEA PIGS

GUINEA PIG PAIR NO.	1.80% CO_2		3.17% CO_2		4.99% CO_2	
	Normal	Hyperventilated	Normal	Hyperventilated	Normal	Hyperventilated
1	26.9	33.1	42.7	47.9	56.2	66.1
2	28.1	33.1	42.7	51.3	58.9	70.8
3	26.3	25.7	44.7	39.8	66.1	55.0
4	27.5	26.9	34.7	49.0	56.2	66.1
5	27.5	25.7	42.7	41.7	58.9	66.1
6	28.8	32.4	39.8	44.7	64.6	58.9
7	29.5	33.9	42.7	51.3	57.5	64.6
8	30.2	34.7	44.7	53.7	64.6	75.9
9	25.1	35.5	38.9	55.0	57.5	70.8
10	28.8	28.8	44.7	42.7	63.1	64.6
Ave.	27.87 ± 0.51	30.98 ± 1.26	41.83 ± 1.06	47.71 ± 1.75	60.36 ± 1.26	65.89 ± 1.96

tating the tonometer on an ice bath for 15 minutes. At the end of this time the homogenate was taken up without exposure to air into 5-cc. syringes and the pH of each sample was determined with a glass electrode pH meter maintained at 17°C. in a constant temperature room. Approximately 5 minutes were allowed for the homogenate to come up to the temperature of the glass electrode after removal from the ice bath and before the pH was determined. The glass electrode was checked against a standard buffer before and after each determination and duplicate determinations were made on each sample. Reproducibility of the pH value by this method was usually within .005 pH units. The entire procedure from decapitation to pH determination was carried out on one pair of guinea pigs in the same afternoon.

The average barometric pressure on the days of the experiment was 741 mm. Hg and this figure was used in computing the pCO_2 for the various mixtures.

RESULTS

The hydrogen ion concentrations as calculated from the pH's of the brain homogenate at the different CO_2 tensions are presented in table 1. The mean (H^+) at all CO_2 tensions was greater for the animals that had been exposed to 24 hours of hypoxic hyperventilation than it was for the controls. As might be expected, there is a greater variation among the animals of the hyperventilated group than there is among the control group. However, an examination of the data by the method of analysis of variance indicates that there is a probability of less than 0.01 that the differences between the hypoxic hyperventilated animals and the control animals could have occurred by chance alone.

When comparisons between control and hyperventilated animals are made by animal pairs, it can be seen that in 7 cases the change is in the direction of a given pCO_2 producing a lower pH, in 2 cases (*pairs 5* and *10*) there is very

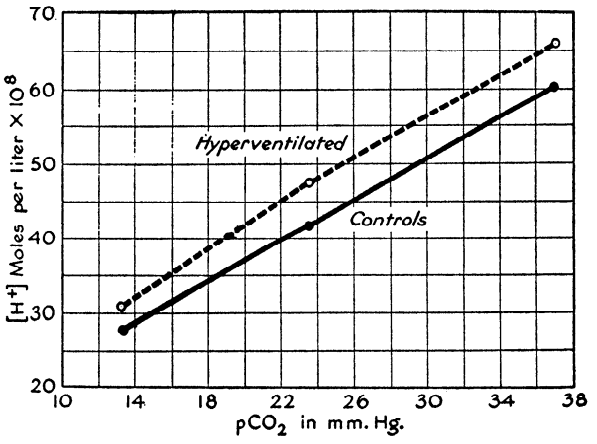

Fig. 1. EFFECT OF 24 HOURS of hypoxic hyperventilation on the CO_2 titration curves of guinea pig brain homogenate.

little difference between the members of the pair, and in one pair (*no. 3*) the change is in the opposite direction.

The curves of the average hydrogen ion concentration as a function of pCO_2 for the 2 groups are presented in figure 1. The difference in position of the two groups is apparent but any difference in slope of the 2 curves is not significant.

DISCUSSION

In previous work (7) the acid-base changes resulting from prolonged mechanically induced hyperventilation in the presence of normal oxygen tension have been found to be essentially the same as those resulting from hypoxic hyperventilation. For convenience in these experiments hypoxia was utilized as the means of producing hypocapnia.

It is recognized that the method of determining the CO_2 titration curve

of the brain included the extracellular as well as the intracellular components, and a reduction in the buffer content of the extracellular components would influence the position of the curves for the brain as a whole. However to account for the differences on this basis alone would require a fall in plasma alkali reserve several times as large as those which have been observed in humans subjected to 24 hours of hyperventilation.

Although CO_2 exerts a stimulating effect on the respiratory center apart from its effect on pH, it must also be considered in the effect it has on the hydrogen ion concentration. It is conceivable that the stimulating effect of a given pCO_2 in the arterial blood would be enhanced if that CO_2 tension produced a lower pH in the respiratory center cells. Such a reduction in buffer content of these cells might account for the increased sensitivity of the center to CO_2 following prolonged hyperventilation. In this connection it should be pointed out that a marked fall in the plasma level of inorganic phosphate has been found to take place with hyperventilation (7, 8). A shift of this anion from extracellular compartment into the chemosensitive cells of the respiratory center might be concerned in this mechanism.

SUMMARY AND CONCLUSIONS

The CO_2 titration curves of brain homogenate were determined for 10 guinea pigs which had been exposed to hypoxic hyperventilation for 24 hours and for 10 normal guinea pigs. A given pCO_2 produced a lower pH in the brain homogenate of the hyperventilated animals than it did in the controls. It is suggested that this reduction in the buffering ability of the brain for CO_2 following prolonged hyperventilation may be the mechanism by which the increased sensitivity of the respiratory center to CO_2 following such prolonged hypocapnia is brought about.

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