

# Integrated Phrenic Activity in Hypercapnia and Hypoxia

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Phrenic nerve electrical activity was recorded and integrated in 14 dogs during hypercapnia without hypoxia and during hypoxia without hypercapnia. The dogs were anesthetized with intravenous pentobarbital or chloralose. In dogs anesthetized with either agent, hypercapnia primarily augmented the electrical activity of each phrenic nerve burst, while hypoxia increased burst frequency more than integrated phrenic nerve burst activity. Heart rate slowed during both hypoxia and hypercapnia. There was a small rise in systemic blood pressure with hypoxia but not with hypercapnia. When both arterial carbon dioxide and oxygen tension were kept constant, large, but not small, increases in systemic blood pressure decreased burst electrical activity. Hypoxia and hypercapnia may act on different subunits of the respiratory center.

SEVERAL STUDIES suggest that when ventilation is increased the mechanical properties of the lung and chest wall largely determine the relative changes in tidal volume and respiratory frequency needed to maintain the new level of ventilation.<sup>1-3</sup> However, the alterations in tidal volume and frequency observed may also be influenced by the nature of the chemical or neural stimulus driving ventilation. For example, Haldane suggested that hypoxia causes mainly an increased respiratory fre-

quency while hypercapnia primarily augments the tidal volume.<sup>4</sup>

The purpose of this study was to compare the effects of hypoxia and hypercapnia on respiratory frequency and tidal volume in dogs. In these animals spontaneous respiratory movements were abolished so that respiratory mechanics could not influence the observed ventilatory response. In addition, the dogs were studied during apnea so that mechanical ventilation could not affect respiratory frequency by vagal stimulation. In order to evaluate the output of the respiratory center in this situation phrenic nerve activity was recorded and integrated. Previous work has shown that the integrated phrenic nerve activity is linearly related to tidal volume over a wide range in spontaneously breathing subjects.<sup>5</sup> Thus, the integrated phrenic nerve activity in the paralyzed animals could be used to estimate potential tidal volume, *i.e.*, the tidal volume that would be generated by the phrenic nerve burst if the animal were spontaneously breathing. Also, the frequency of the phrenic nerve burst per minute is the same as respiratory frequency in spontaneously breathing subjects. Therefore, the integrated phrenic nerve activity multiplied by the frequency per minute of the phrenic nerve bursts is a measure of potential minute ventilation. The effect of hypercapnia without hypoxia was studied by denitrogenating dogs and connecting their lungs to a reservoir containing 100 per cent oxygen (apneic oxygenation experiments). By stopping artificial ventilation, exposing the same paralyzed dogs to room air and simultaneously infusing Tris buffer (tris(hydroxymethyl)aminomethane) intravenously, hypoxia without hypercapnia could be produced (hypoxia experiments). This use of Tris buffer to limit the rise in arterial CO<sub>2</sub> tension during asphyxia is an extension of the technique described by Nahas.<sup>6</sup> The animals were anesthetized with

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Received from the Department of Medicine, University of Illinois, 840 South Wood Street, Chicago, Illinois 60612. Accepted for publication June 20, 1967.

This work was supported in part by U.S.P.H.S. Grant HE-09617, National Heart Institute, National Institutes of Health, Bethesda, Maryland and by a grant from the Tuberculosis Institute of Chicago.

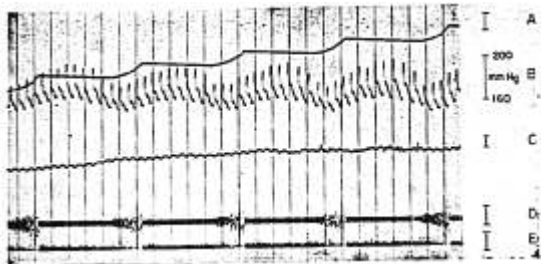


FIG. 1. A recording of phrenic nerve activity during apnea. A is the integrated phrenic nerve activity; B is the systemic blood pressure; C shows a pneumographic tracing; D shows the actual phrenic nerve bursts, while E shows the rectified phrenic nerve burst.

either chloralose or pentobarbital to determine whether these drugs affected phrenic nerve response differently.

### Methods

Twenty-six mongrel dogs weighing 12 to 14 kilograms were studied. In the first series of experiments 20 dogs were examined. Of this twenty, twelve dogs were anesthetized with chloralose, 60–80 mg./kg. intravenously, while the remaining eight dogs were anesthetized intravenously with pentobarbital 30 mg./kg. In 14 dogs (9 chloralose anesthetized and 5 under pentobarbital anesthesia) the following procedures were performed: Each dog was intubated with a tracheal tube; catheters were inserted in both femoral arteries to permit the continuous recording of systemic blood pressure and to allow the arterial blood to be sampled; a pneumograph was placed about each animal's abdomen so that any voluntary respiratory movements could be detected; the femoral veins were cannulated so that Tris buffer could be infused as required.

The electrical activity of the phrenic nerve was measured and integrated as previously described.<sup>5</sup> In brief, this method consists of placing bipolar platinum electrodes on the surface of the phrenic nerve which was immersed in mineral oil. The electrode leads were connected to electronic amplifiers (Electronics for Medicine, White Plains, New York). In order to obtain a quantitative estimate of the electrical activity of these bursts, the output of the phrenic nerve was rectified, *i.e.*, the electrical signals were made positive and fed into an integrator with infinite delay time. The integrator allowed the integral to be ob-

tained with respect to any arbitrary base line, therefore background noise could be cancelled. Continuous and simultaneous records were obtained of phrenic nerve output, rectified phrenic nerve output, and integrated phrenic nerve output. Figure 1 shows the method of recording this output.

The animals were then paralyzed with succinylcholine and for the first experiment (apneic oxygenation) were artificially ventilated with 100 per cent oxygen at a constant rate for twenty minutes in a non-rebreathing circuit. Arterial blood samples were then obtained. In these samples pH was measured with a glass electrode (Radiometer, Copenhagen), arterial carbon dioxide tension with a Severinghaus electrode (Beckman), and arterial oxygen tension with a modified Clark electrode (Beckman). Artificial ventilation was then stopped and the animal connected via the endotracheal tube to a spirometer containing 100 per cent oxygen. Apneic oxygenation maintained 100 per cent arterial oxygen saturation throughout the six minutes during which arterial blood was periodically sampled. At the conclusion of six minutes, artificial ventilation at the same rate as previously described, was reinstated but room air was substituted for 100 per cent oxygen as the inspired gas.

At the end of 20 minutes, after arterial blood was sampled, artificial ventilation was stopped and 0.3 M Tris buffer was infused intravenously at a constant rate of 1.2 ml./Kg./minute with an electrically powered syringe (Harvard Apparatus). This infusion kept  $P_{aCO_2}$  tension constant while  $P_{aO_2}$  declined. Again, blood samples were obtained periodi-

cally and phrenic nerve activity and blood pressure continuously recorded throughout the three minutes of the experiment.

Hypoxia had a somewhat different effect on systemic blood pressure than did hypercapnia. Therefore, in six additional dogs (3 anesthetized with pentobarbital and 3 with chloralose) the effects of blood pressure changes on phrenic nerve activity were studied during apneic oxygenation with Tris infusion. The blood pressure changes were produced by the infusion of 0.04–0.08 mg./minute of levarterenol.

### Results

Figure 2 shows the changing values of  $P_{aCO_2}$  obtained in the 14 dogs during apneic oxygenation; while figure 3 illustrates how  $P_{aO_2}$  changed in the same animals in the studies on hypoxia. The initial  $P_{CO_2}$  in both experiments were identical ( $P_{aCO_2}$  37 mm. of mercury). At the end of six minutes of apneic oxygenation  $P_{aCO_2}$  was 80 mm. of mercury (standard error  $\pm 3$  mm.) while  $P_{aO_2}$  exceeded 200 mm. of mercury. Thus, a rising  $P_{aCO_2}$  with no hypoxia was achieved.  $P_{aO_2}$  at the beginning of hypoxia averaged 89 mm. of mercury and fell to 16 mm. of mercury at the end of three minutes. No significant change in  $P_{aCO_2}$  occurred since  $P_{aCO_2}$  tension at 1, 2, and 3 minutes of

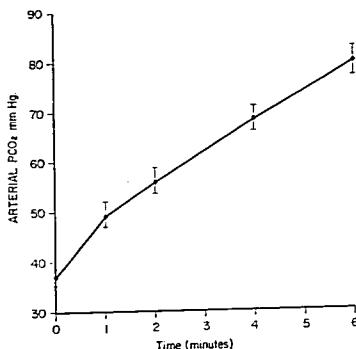


FIG. 2. The change in arterial carbon dioxide tension during apneic oxygenation. Values shown represent the mean  $\pm$  standard error.

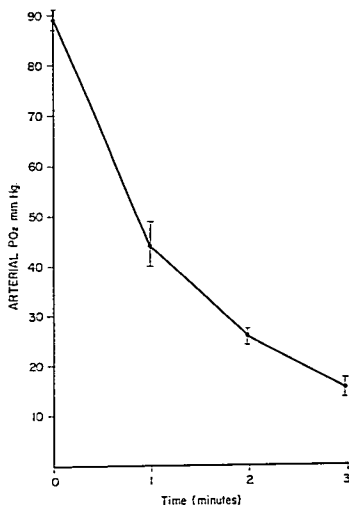


FIG. 3. The change in arterial oxygen tension during asphyxia and Tris infusion (hypoxia experiments). Values shown represent the mean  $\pm$  the standard error.

hypoxia averaged 37, 37, and 35 mm. of mercury, respectively.

Figures 3 and 4 show the experimental results in two different dogs. In both figures, the changes in the integrated electrical activity of each phrenic nerve burst and the number of bursts per minute are compared in two conditions: hypercapnia without hypoxia and hypoxia without hypercapnia. The changes in frequency and electrical activity are expressed as percentage of the initial values (the values obtained during the first 20 seconds of apnea). Figures 4a and 4b show the response during hypercapnia alone, while figures 5a and 5b show the response during hypoxia alone. In both dogs, when the  $P_{aCO_2}$  increased without hypoxia, the electrical activity per burst steadily increased, while the frequency of the bursts was unchanged or only slightly greater. In contrast, when the  $P_{aO_2}$  alone decreased, the burst frequency became much greater, while the electrical activity per burst was relatively unchanged.

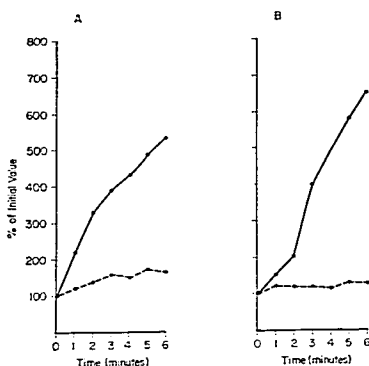


FIG. 4. The change in electrical activity of each phrenic nerve burst and in burst frequency/minute expressed as a percentage of the initial value in two dogs (A and B) during hypercapnia without hypoxia. The solid line shows the change in burst electrical activity, while the dotted line shows the change in frequency. In the dog shown in A, the  $P_{CO_2}$  was 41 mm. of mercury at the beginning of the experiment and 73 mm. at 6 minutes. In the dog shown in B, the initial  $P_{CO_2}$  tension was 34 mm. and it had increased to 65 mm. by 6 minutes.  $P_{aO_2}$  in both dogs exceeded 200 mm. of mercury throughout the experiments.

In the other dogs either hypoxia or hypercapnia increased both the frequency and the electrical activity of each phrenic nerve burst. However, in all dogs but one, for the same levels of potential ventilation the increase in frequency was relatively greater during hypoxia. Hypercapnia, on the other hand, caused a relatively greater increase in the electrical activity of each burst. This is shown in table 1 which compares the relative increase of electrical activity per burst and frequency in each of the 14 dogs when the potential ventilation (integrated electrical activity multiplied by frequency) is approximately twice its original level.

It can be seen that in 5 dogs during hypercapnia an increase in electrical burst activity was associated with a decrease in the frequency of nerve bursts. In contrast, during hypoxia in two dogs electrical activity per burst actually decreased while frequency increased, and in three other dogs there was

virtually no change in electrical burst activity. In only one dog, frequency decreased both during hypoxia and during hypercapnia. During hypoxia, potential ventilation was approximately doubled by a 166 per cent increase in frequency and a 130 per cent increase in electrical activity per burst. On the other hand, there was a 204 per cent increase in electrical activity per burst during hypercapnia, with only a 105 per cent increase in frequency. The differences shown in frequency and electrical burst activity in hypoxia as compared to hypercapnia were highly significant. By the paired *t* test, the probability of this difference occurring by chance alone was less than 1 in 1,000. The table also shows that similar results were obtained with animals anesthetized with chloralose and with pentobarbital. These results suggest that hypoxia and hypercapnia incite a ventilatory change in divergent ways regardless of the anesthetic employed.

Since the infusion of Tris buffer in the experiment on hypoxia produced an average reduction in arterial hydrogen ion concentration of 12.0 nanamoles/liter (standard deviation =  $\pm 4.6$  nanamoles/liter), an additional study was conducted in six dogs. In these animals, following another period of artificial ventilation with oxygen, the animals were reconnected to the spirometer containing oxygen but Tris buffer was infused at a rate of 1.6

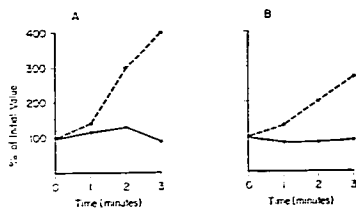


FIG. 5. The change in electrical activity of each phrenic nerve burst and in burst frequency/minute during hypoxia with constant  $P_{CO_2}$  tensions in the same two dogs (A and B) as in figure 4. The solid line shows the change in burst electrical activity, while the dotted line shows the change in burst frequency. In the dog shown in A,  $P_{aO_2}$  decreased from 75 to 6 mm. of mercury over the 3-minute period.  $P_{CO_2}$  was constant at 41 mm. In B,  $P_{aO_2}$  declined from 74 to 18 mm., while  $P_{CO_2}$  was 34 mm. of mercury initially and 33 mm. at the end of 3 minutes.

TABLE 1. The Relative Increase in the Electrical Activity of Each Phrenic Nerve Burst and Burst Frequency when Potential Ventilation is Doubled. Comparison of the Effect of Hypercapnia Without Hypoxia to Hypoxia Without Hypercapnia

Dog	Hypercapnia		Hypoxia	
	Burst Electrical Activity % of Initial Value	Burst Frequency % of Initial Value	Burst Electrical Activity % of Initial Value	Burst Frequency % of Initial Value
9 Dogs Anesthetized with Chloralose				
1	161	132	101	200
2	168	126	108	200
3	144	143	103	200
4	249	78	200	100
5	241	79	120	165
6	276	80	123	170
7	207	93	210	92
8	196	105	94	217
9	188	110	93	220
Average	203	105	128	174
5 Dogs Anesthetized with Pentobarbital				
10	328	63	156	139
11	200	100	118	167
12	143	137	123	156
13	178	100	113	158
14	190	115	152	143
Average	208	103	132	153

ml./kg./minute (apneic oxygenation and Tris infusion). This allowed hydrogen ion concentration to decrease without causing any change in arterial oxygen saturation or  $P_{aCO_2}$ . This greater infusion rate was necessary since the  $P_{aCO_2}$  increased more rapidly when the  $P_{aO_2}$  was kept constant than when the  $P_{aO_2}$  was allowed to fall. In these dogs, the initial  $P_{aCO_2}$  again averaged 37 mm. of mercury while the  $P_{aO_2}$  tension again exceeded 200 mm. of mercury in all 8 dogs. The average change in hydrogen ion concentration over three minutes was 12.2 nanomoles/liter (standard deviation  $\pm 4.3$  nanomoles per liter). In no experiment did the  $P_{aCO_2}$  change by more than 2 mm. of mercury. Also, in none of the six experiments did the integrated activity of each phrenic nerve burst or the frequency of each phrenic nerve burst per minute change by

more than 10 per cent of the initial value. The average electrical activity of each phrenic nerve burst at the end of three minutes was 99 per cent of the initial value (standard deviation  $\pm 3$  per cent), while the minute frequency of each phrenic nerve burst averaged 98 per cent of the initial value (standard deviation  $\pm 4$  per cent). This suggested that a decrease in hydrogen ion of the same magnitude as noted in the experiments in hypoxia does not effect phrenic nerve activity. A slow rate of change of hydrogen ion intracellularly might explain this observation.

The effect of increasing hypoxia and hypercapnia on systemic blood pressure and heart rate in 14 dogs is shown in table 2. In both conditions there was a progressive slowing of heart rate more pronounced in the animals anesthetized with chloralose. As in Holmdahl's study,<sup>7</sup> systemic blood pressure remained essentially the same during apneic oxygenation. However, during hypoxia there was a small but progressive rise in both systolic and diastolic pressures. This was observed in the dogs regardless of the type of anesthesia.

TABLE 2. Changes in Systemic Blood Pressure and Heart Rate Observed in 14 Dogs in Hypercapnia Without Hypoxia and in Hypoxia Without Hypercapnia

Time in minutes from beginning of experiment	Average Systolic Pressure in mm Hg $\pm$ Standard Error	Average Diastolic Pressure in mm Hg $\pm$ Standard Error	Average Heart Rate/minute $\pm$ Standard Error
Effect of Hypercapnia			
0	178 $\pm$ 7	120 $\pm$ 7	157 $\pm$ 10
1	175 $\pm$ 9	129 $\pm$ 8	130 $\pm$ 12
2	171 $\pm$ 8	120 $\pm$ 9	119 $\pm$ 13
3	168 $\pm$ 8	114 $\pm$ 9	108 $\pm$ 8
4	170 $\pm$ 8	118 $\pm$ 7	98 $\pm$ 10
5	173 $\pm$ 8	119 $\pm$ 7	94 $\pm$ 11
6	172 $\pm$ 8	113 $\pm$ 7	86 $\pm$ 10
Effect of Hypoxia			
0	173 $\pm$ 9	124 $\pm$ 8	151 $\pm$ 18
1	177 $\pm$ 10	125 $\pm$ 9	115 $\pm$ 16
2	192 $\pm$ 11	128 $\pm$ 9	92 $\pm$ 12
3	210 $\pm$ 12	131 $\pm$ 9	85 $\pm$ 11

TABLE 3. Change in Integrated Phrenic Activity and Burst Frequency After the Intravenous Infusion of Norepinephrine

Dog	Increase in Systemic BP (mm. Hg)	Integrated Phrenic Nerve Activity % of Initial Value	Burst Frequency % of Initial Value
15	120/80	80	86
16	160/110	79	89
17	170/90	57	111
18	60/50	80	107
19	130/100	88	120
20	90/60	54	71
Mean	122/98	73	97

It seemed possible that the dissimilar effects of hypoxia and hypercapnia on blood pressure might account for the different effects of these two respiratory stimulants on phrenic nerve activity. In this case one would expect that a rising blood pressure would produce an increase in respiratory frequency and a decrease in the electrical activity of each phrenic nerve burst. Therefore, in 6 paralyzed dogs maintained at a constant  $P_{aCO_2}$ , arterial oxygen saturation by apneic oxygenation, and Tris infusion, increases in systemic blood pressure were produced by infusion of norepinephrine, and the effect on phrenic nerve activity observed. Small increases in blood pressure averaging 23/16 mm. of mercury produced no change in respiratory frequency and decreased the electrical activity per burst by 9 per cent. The effect of large alterations in systemic blood pressure are listed in table 3 where increases in systemic blood pressure averaging 122/98 mm. of mercury produced a variable effect on respiratory frequency but regularly reduced the integrated electrical activity of the phrenic nerve burst. This observation agrees with those made in spontaneously breathing animals where significant increases in systemic blood pressure (presumably by an effect on baroreceptors) have produced a decrease in ventilation.<sup>8</sup> However, since the increase in blood pressure in hypoxia was small and never exceeded 80/25 mm. of mercury, it seems unlikely that differences in systemic blood pressure alone can explain the dissimilar effects of

hypoxia and hypercapnia on phrenic nerve activity.

### Discussion

The results of this study show that in dogs with paralyzed respiratory muscles, the respiratory center (as reflected in phrenic nerve activity) responds differently when stimulated by hypercapnia as compared to hypoxia. This was true whether the dogs were anesthetized with chloralose or with pentobarbital. Hypercapnia produced primarily an increase in the integrated electrical activity of each phrenic nerve burst, while hypoxia mainly increased the frequency of such bursts, changing the integrated activity of each burst to a lesser extent. Since integrated electrical activity is proportional to tidal volume, the results support Haldane's observations on the dissimilar effects of hypoxia and hypercapnia on tidal volume and frequency.<sup>4</sup> They also support the thesis that in part the nature of the agent which provokes an augmented ventilation determines how augmented ventilation is attained.

Previous studies in a variety of spontaneously breathing animals and in man suggest that the mechanical properties of the lung and chest wall help determine the tidal volume and frequency at a given level of ventilation. While the investigations of Otis<sup>1</sup> and Agostoni<sup>2</sup> suggest that tidal volume and frequency are set so that respiratory work is minimal, Mead<sup>3</sup> believes that the frequency of breathing is selected so that average force exerted by the respiratory muscles is at a minimum. Mead also believes that the receptors which determine this are located in the lung rather than in the chest wall.

The relative importance of the nature of the respiratory stimulus and respiratory mechanics in setting the tidal volume and frequency in spontaneously breathing subjects cannot be determined from the results of the present study. In this study the respiratory muscles were deliberately paralyzed to eliminate the effects of mechanical properties of the lung and chest wall on response of the respiratory center. Thus, the results undoubtedly exaggerate the opposite effects of hypercapnia and hypoxia on phrenic nerve activity.

In addition, it is not clear how hypoxia and hypercapnia produce their dissimilar effects. The effect may be exerted directly on neural centers or indirectly caused by changes in systemic pressure or regional perfusion. Our results indicate that the difference in systemic blood pressure in hypoxia and hypercapnia alone cannot explain the observed difference in respiratory frequency.

During apneic oxygenation  $\text{CO}_2$  rises not only in arterial blood but also in the central nervous system.<sup>9</sup> Lambertsen's experiments indicate that changes in  $\text{P}_{\text{CO}_2}$  at both sites influence ventilation.<sup>10</sup> In the experiments on hypoxia, a hypoxia-induced rise in cerebral blood flow may have actually decreased central  $\text{P}_{\text{CO}_2}$ .<sup>11</sup> The opposite changes in central  $\text{P}_{\text{CO}_2}$  which may have been produced in this study might have influenced the relation between frequency of phrenic nerve burst and electrical activity.

Another explanation has been offered by Hugelin.<sup>12</sup> He showed in experiments in cats that the amplitude of phrenic nerve bursts and their frequency can be affected separately by a variety of maneuvers, and proposed that the respiratory center was composed of subunits which controlled either tidal volume, the length of inspiration, or the length of expiration. It is an intriguing possibility that hypoxia acts mainly on one such subunit while hypercapnia acts on another. Different kinds of chemoreceptors may affect one subunit more than another. For example, afferent nerve impulses from the peripheral carotid and aortic bodies stimulated by hypoxia may influence one such subunit, while hypercapnia acting, in addition, on other receptors may affect a different subunit.

### Summary

In dogs under pentobarbital or chloralose anesthesia, hypercapnia primarily increased the electrical activity of each phrenic nerve burst while hypoxia increased burst frequency more than the electrical activity of each burst.

The divergent effect of these two respiratory stimulants were not explained by changes in systemic blood pressure. It is possible that hypoxia and hypercapnia affect different subunits of the respiratory center.

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