Original Articles

The Respiratory Effects of Carbon Dioxide in The Cat

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Although concentrations of CO_2 greater than 10 per cent have been characterized as depressant to respiration, in the experiments reported here concentrations from 5 to 40 per cent stimulated respiratory minute volume ($\dot{V}_E$) in the anesthetized and in the decerebrate cat. The stimulatory effect was maintained for at least four hours. Sixty per cent CO_2 depressed ventilation in some cats, but stimulated it in others. The mean $\dot{V}_E$ at this concentration was one-half of the control value. Unanesthetized cats showed a greater respiratory response to CO_2 than barbiturate-anesthetized or decerebrate animals, and $\dot{V}_E$ was not depressed by 60 per cent CO_2 in any of these animals. Recordings of phrenic nerve electrical activity disclosed central respiratory stimulation in all animals by all concentrations of CO_2.

STANDARD references state that CO_2 concentrations up to 10 per cent produce ventilatory stimulation but in higher concentrations produce ventilatory depression.[1-6] Surprisingly, only the effects of low CO_2 concentrations have been documented. The respiratory effects of high concentrations of CO_2 have received little attention. Moreover, the data available come from investigations primarily concerned with non-respiratory effects of CO_2, thus fragmentary and difficult to interpret. In a few instances concentrations of 12 and from 30 to 40 per cent CO_2 in air have been observed to produce respiratory depression in animals anesthetized with barbiturates[10-11] and a similar response was noted during severe hypoxia subsequent to the use of morphine.[12] However, in other instances, animals and man have been exposed to 15 to 75 per cent CO_2 without detriment.[13-17] Despite the potential importance of the resulting knowledge, no systematic analysis of the effects on respiration of high concentrations of CO_2 has been made. Therefore, we examined the respiratory response of cats exposed to 5 to 60 per cent CO_2 for periods of from ten minutes to four hours.

Methods

Fifty male and female cats averaging 3.4 kg. in weight were fasted for twelve to twenty-four hours. Forty were anesthetized by an intraperitoneal injection of 0.6 ml./kg. Dial-Urethane (Ciba). Six were narcotized with 20 per cent CO_2 and 80 per cent O_2, and prepared for tracheotomy by local infiltration with 1 per cent lidocaine. Four were anesthetized with cyclopropane and decerebrated at the midcollicular level. The trachea was cannulated in all. In addition, in all except those prepared with 20 per cent CO_2 and pretracheal lidocaine, the fifth cervical contribution to a phrenic nerve was divided and the proximal portion placed on a bipolar platinum electrode. The skin surrounding the recording site was retracted to retain a protective

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The experiments reported herein were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care" prepared by the NAS-NRC Committee on the Guide for Laboratory Animal Resources.

petroleum pool. In all animals rectal temperatures were maintained between 36 and 37.5° C. by infrared heating.

Gas mixtures were supplied to the tracheal cannula through an infant non-rebreathing valve (Sierra Engineering Co.) and a one-liter wet test gas meter (Albany Meter Co.) Gas concentrations were prepared with conventional rotameters calibrated by displacement and checked by gas chromatographic analysis of samples from the tracheal cannula. (Type K molecular sieve column and Model 154 L Vapor Fractometer, Perkin Elmer Corp.) Electrical activity recorded from the phrenic nerve was displayed on an oscilloscope (Type 502 A, Tektronix, Inc.) and recorded on magnetic tape for later examination and photography.

In the control period, 100 per cent O_2 was supplied to the decerebrate cats, and to thirty-one of the Dial-Urethane anesthetized cats. After thirty to sixty minutes, the gas was changed to 5 per cent CO_2 and 95 per cent O_2. The effects of this mixture on phrenic nerve and ventilatory activity and, in six animals, arterial P_{O_2}, P_{CO_2} and *p*H (Model 113, Severinghaus and Clark electrodes—Instrumentation Laboratories) were noted. After ten minutes, the CO_2 content of the inspired gas was increased to 10 per cent, and another ten-minute exposure begun. Measurements of phrenic nerve activity, ventilation and arterial gases were repeated. Subsequently, similar ten-minute exposures were made to successively higher concentrations of CO_2. For 16 randomly selected animals this sequence was terminated at a predetermined concentration of CO_2 and the exposure to that concentration prolonged for four hours.

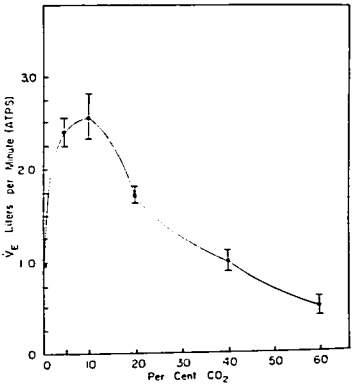

FIG. 1. The response of Dial-Urethane anesthetized cats to CO_2 in O_2. Mean respiratory minute volume ($\dot{V}_E$) is plotted, plus and minus standard error. $\dot{V}_E$ is corrected to ambient temperature and pressure, saturated (ATPS).

To study the effect of oxygen on the respiratory response to CO_2, nine cats anesthetized with Dial-Urethane were given 20 per cent O_2 and 80 per cent N_2 during the control period. They were exposed to increasing concentrations of CO_2, as described above, except that the O_2 content of the inspired gas was maintained at 20 per cent and the N_2 content reduced as the CO_2 concentration was increased.

Results

The ventilatory response reported here is the respiratory minute volume during the tenth minute of exposure ($\dot{V}_{E_{CO_2}}$). These data are plotted for the thirty-one Dial-Ure-

TABLE 1. Control $\dot{V}_E$ in Anesthetized Cats

Anesthetic	Gas Mixture ($\%O_2$)	Gas Mixture ($\%N_2$)	Animals	Weight (kg.)	$\dot{V}_{E_C}$ l./min. mean±S. E.	*P**
Dial-Urethane	100	—	31	3.3	0.99±0.07	0.01
Dial-Urethane	20	80	9	3.9	1.36±0.12	0.01
Decerebrate	100	—	4	3.4	0.82±0.05	
20% CO_2+local lidocaine	100	—	6	3.3	— — —	

* Difference in $\dot{V}_{E_C}$ between decerebrate and Dial-Urethane anesthetized cats breathing 100 percent O_2 is not significant ($P>0.15$).

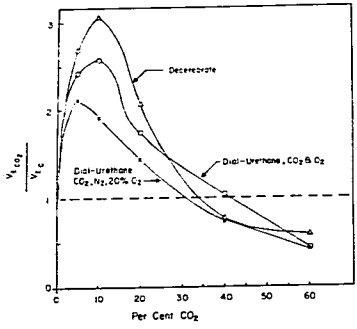

FIG. 2. The respiratory effects of CO_2 on: decerebrate cats, Dial-Urethane anesthetized cats breathing CO_2 and O_2, and Dial-Urethane anesthetized cats breathing CO_2, 20% O_2 and N_2. Ordinate is the ratio of $\dot{V}E_{CO_2}$ in $\dot{V}E_C$. The apparent differences among the three groups are not statistically significant at any CO_2 concentration ($P > 0.06$).

thane anesthetized cats given mixtures of CO_2 and O_2 (fig. 1). The figure shows that $\dot{V}E_{CO_2}$ was greater than the control value at all CO_2 concentrations below 40 per cent. Forty per cent CO_2 resulted in ventilatory activity approximately equivalent to that observed with 100 per cent O_2, and 60 per cent CO_2 resulted in a minute ventilation about one-half of the control value.

Figure 2 presents these data and the data obtained from the decerebrate animals and the Dial-Urethane anesthetized cats limited to 20 per cent O_2. Since there were differences among the three groups in the control values of minute ventilation ($\dot{V}E_C$) (table 1), the ventilatory response to CO_2 is expressed with relation to $\dot{V}E_C$. That is, each animal is used as its own control and the ratio $\dot{V}E_{CO_2}/\dot{V}E_C$ presented. The respiratory response to CO_2 of the three experimental groups was the same. That of the decerebrate animals did not differ significantly from that of either group of Dial-Urethane anesthetized cats at any CO_2 concentration ($P > 0.10$), nor were there any significant differences among anesthetized cats breathing CO_2 in O_2 and those breathing the same CO_2 concentration in N_2 and 20 per cent O_2.

On the other hand, the respiratory response of the six animals initially narcotized with 20 per cent CO_2 was different from the Dial-Urethane and decerebrate animals (fig. 3). Since there was no control period free of CO_2 for these animals, the results are expressed directly as $\dot{V}E_{CO_2}$. The mean response of this group was greater at each CO_2 concentration than the mean responses of the Dial-Urethane anesthetized and the decerebrate cats ($P < 0.05$). The differences are not ascribable to differences in body mass, since the mean weights of the three groups of animals are the same within 0.1 kg. (table 1).

The results of prolonged exposure of cats anesthetized with Dial-Urethane to CO_2 are presented in figure 4. Because of differences in $\dot{V}E_C$ among animals exposed to the several CO_2 concentrations, results are again expressed by the ratio $\dot{V}E_{CO_2}/\dot{V}E_C$. With 20 and 40 per cent CO_2, the ratio reached a steady state within about 20 minutes, and remained there for the rest of the 4-hour exposure. All six of the animals exposed to 20 per cent CO_2 and

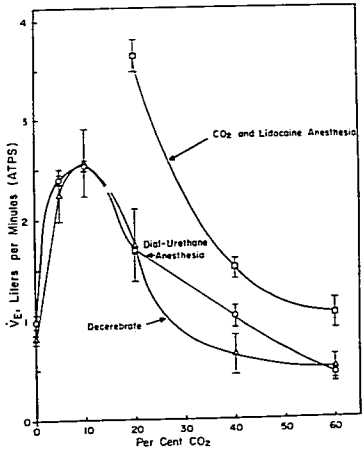

FIG. 3. Effects of CO_2 on $\dot{V}E$ of: Cats prepared under pretracheal lidocaine infiltration and 20 per cent CO_2, decerebrate cats, and Dial-Urethane anesthetized cats breathing CO_2 and O_2. Mean $\dot{V}E$ is plotted, plus and minus standard error.

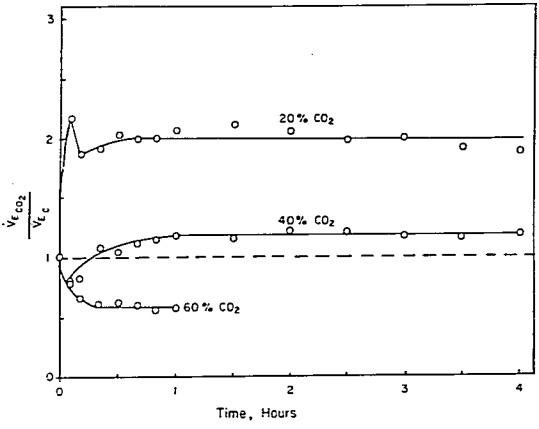

FIG. 4. Respiratory response of Dial-Urethane anesthetized cats to continuous exposure to CO_2. Ordinate represents the ratio of $\dot{V}E_{CO_2}$ to $\dot{V}E_C$.

the four exposed to 40 per cent CO_2 survived. In contrast, two of six animals exposed to 60 per cent CO_2 died after 60 minutes, a third died after 90 minutes, and a fourth after 210 minutes. For this reason, figure 4 only contains data from the first hour. The 2 animals surviving four hours on 60 per cent CO_2 had final $\dot{V}E$ values greater than their controls ($\dot{V}E_{CO_2}/\dot{V}E_C = 1.33$ and 1.65).

A typical recording of the efferent electrical activity in the phrenic nerve is presented in figure 5. Each rhythmic "burst" of activity represents an inspiratory effort which was usually proportional to the duration and amplitude of the burst. It is evident that increasing the CO_2 concentration of the inspired gas to 5, 10 and 20 per cent produced an increase in the frequency and amplitude of the rhythmic bursts, while the duration of individual bursts was progressively shortened. This change paralleled the increase in ventilation shown in figure 1. At CO_2 concentrations of 40 and 60 per cent, the rhythmic pattern of activity was interrupted by protracted periods of continuous electrical discharge. This prolonged activity, associated with inspiratory spasm of the muscles of respiration, persisted for up to 30 seconds. During this time there was no effective ventilation. Spasm terminated with a final intensification of electrical activity and a gasping inspiratory effort (fig. 5, 60 per cent CO_2, 90 minutes). This was followed by slow rhythmic respiration, which, after a variable period gave way to another burst of tonic inspiratory activity. The periods of prolonged discharge could be terminated at any time by momentary compression of the animal's thorax, a maneuver that initiated expiration and a return to slow rhythmic breathing. Inspiratory spasm was never observed under control conditions, nor at CO_2 concentrations less than 40 per cent.

TABLE 2. Arterial Blood Changes in Acute Hypercapnia

Inspired Gas		P_{O_2} (mm.Hg)	P_{CO_2} (mm.Hg)	pH
(%O_2)	(%CO_2)			
		568	28	7.32
100	0	(500–622)	(21–35)	(7.26–7.37)
		602	44	7.21
95	5	(547–675)	(42–47)	(7.18–7.27)
		497	72	7.04
90	10	(350–645)	(57–82)	(7.00–7.12)
		448	124	6.85
80	20	(320–525)	(90–161)	(6.82–6.91)
		334	252	6.67
60	40	(254–410)	(207–287)	(6.64–6.71)
		186	381	6.55
40	60	(95–242)	(284–411)	(6.52–6.58)

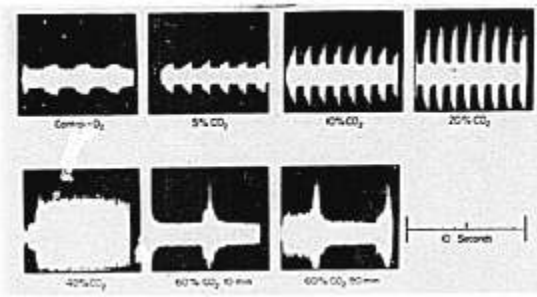

FIG. 5. Effects of CO_2 on phrenic nerve electrical activity in Dial-Urethane anesthetized cat. Each burst of electrical discharges is associated with an inspiratory effort. Increased frequency and amplitude of bursts occur. At CO_2 concentrations of 40 and 60 per cent, the cyclic pattern of respiration is broken up by periods of continuous inspiratory activity during which ventilation drops to zero.

Arterial blood gas tensions and *p*H values from cats anesthetized with Dial-Urethane are given in table 2. This table presents data from 6 animals whose mean ventilatory response to CO_2 was the same as that shown in figure 1 at all CO_2 concentrations. These data document the intensity of the acidemia produced by the CO_2 and indicate that hypoxemia did not develop even when 60 per cent CO_2 and 40 per cent O_2 were given and $\dot{V}E_{CO_2}$ averaged one-half of the control value.

No generalized seizures occurred in any of the animals. The only adventitious movements observed consisted of peripheral myoclonus, generally seen in animals breathing 20 per cent CO_2.

Discussion

In the present study we attempted to focus on the respiratory action of CO_2 by minimizing hypoxia and by employing several anesthetic techniques. We found that CO_2 concentrations of 40 per cent or less produced no depression of ventilation in anesthetized or decerebrate cats. Cats given no drugs except CO_2 and local lidocaine tolerated 60 per cent CO_2 without respiratory depression.

These findings are complemented by the results of phrenic nerve recordings. Although $\dot{V}E$ and other respiratory parameters are often used to assess response to CO_2, they provide indirect and limited information about the respiratory center.[18] Lourenco *et al.* have demonstrated the usefulness of phrenic nerve activity as a measure of the central respiratory response.[19] The records of the present study suggest that the reduced ventilation which occurred at the highest CO_2 concentration may not have been due to respiratory depression. Phrenic nerve discharge increased with increasing CO_2 in animals breathing 5 to 20 per cent CO_2 and in this range, the normal pattern of bursts of activity alternating with periods of electrical silence was maintained. Consequently, ventilation increased *pari passu* with CO_2 concentration. Under the influence of 40 and 60 per cent CO_2, however, the inspiratory activity became so intense that the expiratory phase was compromised, and tidal gas exchange ceased. The source of this intense inspiratory excitation is unknown, but since phrenic motoneurones are not directly excited by CO_2,[20] it must derive from higher center, perhaps because of a depressant action of CO_2 on inspiratory-inhibiting peripheral or cortical influences.[21-22] But whatever the mechanism, the observations suggest that high CO_2 concentrations act more to disorganize respiration than to depress it.

Although there are no directly comparable studies of the respiratory effects of CO_2 under other conditions or in other species, several investigators have noted failure of CO_2 to depress respiration. Barbour and Seevers[13] were able to keep rats in an atmosphere from 15 to 25 per cent CO_2 up to 30 days. Schaeffer and Niemoeller[14] had a similar experience with guinea pigs and 15 per cent CO_2. Concentrations of 30 and 40 per cent CO_2 were tolerated by anesthetized dogs for two-hour periods in the blood volume studies of Billings and Brown.[15] In preliminary studies we have found no differences in the responses to CO_2 between dogs and cats anesthetized with Dial-

Urethane.[22] Lambertsen exposed man briefly to 30 per cent CO_2 without noting respiratory depression.[16] In the most complete studies previously reported, Graham *et al.*[17] observed spontaneous respiration in dogs breathing 75 per cent CO_2 for one hour. Although they noted that an apneic phase sometimes occurred in animals breathing CO_2-rich gas mixtures, they also suggested that high CO_2 concentrations may be free of respiratory depressant actions.

It is noteworthy that ventilatory depression by CO_2 has been documented only in animals whose respiratory response was reduced by drugs or hypoxia. Barbiturates are well known for their ability to depress respiration,[10-11] and the severe hypoxia resulting from large doses of morphine[12] has been shown to change stimulatory CO_2 concentrations into respiratory depressants.[24] Suggestion of a similar effect was found in our study. The animals anesthetized with Dial-Urethane were less tolerant of CO_2 than those treated solely with CO_2. Furthermore, although the animals limited to 20 per cent O_2 did not differ from the others in the initial ventilatory response, they were less able to tolerate prolonged exposures to CO_2. All six cats given 60 per cent CO_2 and 40 per cent O_2 survived for at least one hour, but two of the four cats given 60 per cent CO_2, 20 per cent O_2 and 20 per cent N_2 died within 45 minutes.

Carbon dioxide is a potent agent which in high concentrations can produce general anesthesia,[25-26] convulsions,[27-28] cardiac arrhythmias,[29] electrolyte shifts,[20-21] and pulmonary parenchymal damage.[14] It is properly viewed with caution in clinical medicine. However, our results suggest that one possible deleterious effect, ventilatory depression, may occur only with CO_2 concentrations far higher than is generally supposed.

Summary

Anesthetized and decerebrate cats exposed to CO_2 showed no respiratory depression over a range of concentrations from 5 to 40 per cent CO_2. The administration of 60 per cent CO_2 caused V_E to fall to one half of the control value, and though some of these animals died after one hour, others continued to show respiratory stimulation after four hours. We used V_E as the major criterion of response to CO_2. This criterion sometimes masked stimulatory effects of CO_2 on the respiratory center, effects made evident by recordings of phrenic nerve activity.

The V_E response to CO_2 of locally-anesthetized animals was greater than that of decerebrate or Dial-Urethane anesthetized cats, and their V_E was not depressed by 60 per cent CO_2.

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Nervous System

ANOXIA ON NERVE FIBER Single nerve fibers with Ranvier node isolated from the sciatic nerve of the rat were subjected to oxygen-free (nitrogen substituted) solutions with the following results: (1) The action potential declined slowly at first (for 15 minutes), then more rapidly to a graded response by 20 min. The effect was reversible when the preparation was re-exposed to oxygen again. The effect was also reversible by application of a hyperpolarizing current, (2) the resting membrane potential and resistance declined slightly during the first 15 min., significantly during the period of time of spike extinction, and (3) the critical firing level remained relatively constant throughout anoxia and recovery. The electrical effects of anoxia could be duplicated by reducing the calcium concentration of the medium. The effects of metabolic inhibition by sodium cyanide (2 m*M*) are identical to the effect of oxygen replacement by nitrogen. An hypothesies was suggested that membrane calcium-binding property is ATP-dependent. In the absence of oxidative metabolism, energy calcium is released from the membrane resulting in decline and extinction of the action potential and reduction of membrane potential and resistance. (*Maruhashi, J., and Wright, E. B.: Effect of Oxygen Lack on the Single Isolated Mammalian (Rat) Nerve Fiber, J. Neurophysiol. 30: 434 (May) 1967.*)