Apneic threshold for CO\textsubscript{2} in the anesthetized rat: fundamental properties under steady-state conditions

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Boden, A. G., M. C. Harris, and M. J. Parkes. Apneic threshold for CO\textsubscript{2} in the anesthetized rat: fundamental properties under steady-state conditions. J. Appl. Physiol. 85(3): 898–907, 1998.—Experiments were performed to measure the apneic threshold for CO\textsubscript{2} and its fundamental properties in anesthetized rats under steady-state conditions. Breathing was detected from diaphragmatic electromyogram activity. Mechanical hyperventilation resulted in apnea once arterial PCO\textsubscript{2} (P\textsubscript{ACO\textsubscript{2}}) had fallen far enough. Apnea was not a reflex response to lung inflation because it did not occur immediately, was not prevented by vagotomy, and was reversed by raising P\textsubscript{ACO\textsubscript{2}} without changing mechanical hyperventilation. The apneic threshold was measured by hyperventilating rats mechanically with O\textsubscript{2} until apnea had occurred and then raising P\textsubscript{ACO\textsubscript{2}} at constant hyperventilation until breathing reappeared. The mean P\textsubscript{ACO\textsubscript{2}} level of the apneic threshold in 42 rats was 32.8 ± 0.4 Torr. The level of the threshold did not depend on the volume at which the lungs were inflated. The level of the threshold, under steady-state conditions, was the same when approached from hypocapnia as from eupnea. The level of the threshold could be raised by 9 Torr by chronic elevation of the eupneic P\textsubscript{ACO\textsubscript{2}} level by 18 Torr.

Hypocapnia; breathing; carbon dioxide

There is considerable interest in the effects of raising body temperature on breathing, both under normal conditions, such as during exercise, and in clinical situations, such as fever. We wished to study in the rat the effects of stimulation of the thermoregulatory system on the properties of the respiratory control system. In the present paper we have therefore measured one of the fundamental properties of the respiratory control system, the minimum level of PCO\textsubscript{2} in arterial blood (P\textsubscript{ACO\textsubscript{2}}) that is necessary for breathing to occur under steady-state conditions.

At normal body temperature, breathing is primarily under chemical control, which is dominated by the level of CO\textsubscript{2}. The chemical-control system modulates breathing to the extent that the P\textsubscript{ACO\textsubscript{2}} is normally maintained around a set point of 40 Torr (22, 26, 33), and there exists a steep and linear relationship between breathing and P\textsubscript{ACO\textsubscript{2}}. Thus a fall in P\textsubscript{ACO\textsubscript{2}} leads to a reduction in breathing. Lowering P\textsubscript{ACO\textsubscript{2}} far enough causes apnea in a range of species, when studied under anesthesia or in non-rapid-eye-movement sleep when consciousness will not affect breathing (15, 33). An apneic threshold can then be defined as the P\textsubscript{ACO\textsubscript{2}} level at which breathing either just disappears or just reappears. In this study we have identified breathing from the reappearance of rhythmic activity in the diaphragm muscle. It is already known, however, that different stages in the generation of breathing have different threshold levels of CO\textsubscript{2} for their appearance. Thus the threshold P\textsubscript{ACO\textsubscript{2}} level for the reappearance of diaphragmatic activity is higher than the threshold P\textsubscript{ACO\textsubscript{2}} level for the reappearance of activity in neurons in the respiratory network (14).

When the Po\textsubscript{2} in arterial blood (P\textsubscript{O\textsubscript{2}}) is high (during, for instance, breathing of 100% O\textsubscript{2}), breathing is determined mainly by the P\textsubscript{CO\textsubscript{2}} within the central nervous system (CNS), which is detected by central chemoreceptors. The P\textsubscript{CO\textsubscript{2}} of the central chemoreceptors is closely related to P\textsubscript{ACO\textsubscript{2}} but, because of the properties of the blood-brain barrier, changes in P\textsubscript{ACO\textsubscript{2}} are followed by the central chemoreceptors with a finite delay (4). Consequently, to use P\textsubscript{ACO\textsubscript{2}} to estimate the P\textsubscript{CO\textsubscript{2}} of the central chemoreceptors, time must be allowed for P\textsubscript{CO\textsubscript{2}} in arterial blood and in the CNS to reach a steady-state equilibrium (9).

Methods for lowering P\textsubscript{ACO\textsubscript{2}} in conscious rats are not yet in general use. The simplest method of lowering P\textsubscript{ACO\textsubscript{2}} in rats is to apply mechanical hyperventilation while rats continue to breathe, but this hyperventilation can only be applied under anesthesia. When mechanical hyperventilation is applied, it must be established first that apnea is caused by hypocapnia and not through any inhibitory effect of mechanical inflation of the lungs. Mechanical inflation can alter the pattern of spontaneous breathing. For instance, sustained rather than rhythmic mechanical inflation of the lungs slows the respiratory frequency and can induce apnea (13). When applied rhythmically, however, mechanical inflation can produce less inhibition of spontaneous breathing. Rhythmic mechanical inflation produces even less inhibition if the imposed inflation occurs at a particular point in the expiratory phase of spontaneous breathing (12). Furthermore, when connected to a ventilator, animals will adjust their breathing pattern (even under anesthesia) so that the applied inflation produces minimal disruption of spontaneous breathing. Animals make this adjustment by altering the timing of their breathing so that their own inspiration coincides with the gap between mechanical inflations. In the present paper, therefore, we have made a careful study of the apneic threshold in the rat, paying particular attention to the possible confounding effects of pulmonary stretch reflexes, and we have made the measurements under steady-state conditions where it is reasonable to assume that P\textsubscript{ACO\textsubscript{2}} reflects P\textsubscript{CO\textsubscript{2}} in the CNS.

Finally, in the course of the work, it became apparent that the P\textsubscript{ACO\textsubscript{2}} level of the apneic threshold might not be
an absolute level but rather one dependent on the eupneic PaCO2. We have therefore tested this hypothesis experimentally.

**METHODS**

All experiments were performed under appropriate authority from project and personal licenses from the United Kingdom Home Office.

**Surgical preparation.** Studies were performed on 49 adult male Sprague-Dawley rats (~300 g) under urethane anesthesia (1.25 g/kg ip); 42 rats were eucapnic with mean PaCO2 levels of 41.8 ± 0.5 Torr, and 7 rats were spontaneously hypercapnic with mean PaCO2 levels of 55.8 ± 5 Torr. A femoral vein and artery were cannulated to inject additional anesthetic, to monitor arterial blood pressure, and to sample blood. The depth of anesthesia was monitored carefully, and urethane (25-mg doses iv) was given if pinching the tail produced a change in blood pressure or heart rate. A tracheal cannula was inserted to measure pressure and airflow. During the experiments, end-tidal CO2 levels were monitored by using a Beckman LB-2 medical gas analyzer fitted with a heated sample inlet tube, although all gas and pH measurements were made on samples of arterial blood. To measure electrical activity (electromyogram [EMG]) of the diaphragm muscle, wire electrodes of stainless steel were sewn into the main body of the muscle. Electrode position was chosen to ensure that EMG activity continued throughout the duration of inspiratory airflow. Rectal temperature was measured and kept at 37°C by using a thermostatically controlled heating blanket. In three rats the cervical vagus nerves were sectioned bilaterally. Data were digitized by using aCED1401plus interface (Cambridge Electronic Designs, Cambridge, UK), displayed by using the CHART program, and stored on 35-megabyte optical disks. Electrocardiogram artifacts were digitally removed from the diaphragmatic EMG record.

After surgical preparation, we selected only rats that had PaCO2 levels of ≤45 Torr during spontaneous (eupneic) breathing in O2-enriched air, i.e., with PaCO2 levels no higher than those reported for unanesthetized rats (11, 22, 26, 29). We describe separately the results from a group of seven rats with abnormally high eupneic PaCO2 levels (see Influence of chronic changes in the eupneic PaCO2 level on the apneic threshold).

**Induction of apnea.** The frequency of spontaneous breathing in O2-enriched air was measured over a 30-min period in each rat after surgery. The tracheal cannula was then connected to a Harvard small-animal ventilator (model 50-1916, with an inspiratory-to-expiratory ratio of 1:1). The ventilator was fitted with a quick-release valve to enable rapid disconnection from the rat. The ventilator was supplied with humidified gas containing either 100% O2 or mixtures of CO2 in O2. The ventilator was set at the eupneic breathing frequency of the rat. Hypocapnia was induced by increasing inflation volume at constant ventilation frequency until diaphragmatic EMG activity disappeared. Blood samples were taken 4 min after apnea occurred to ensure equilibration of PCO2 between arterial blood and the CNS.

**Mechanism of apnea.** Once apnea was established, the ventilator was disconnected by opening the quick release valve. The time from disconnection to the onset of spontaneous breathing was measured. After the rats breathed spontaneously for 4 min, the valve was closed, and the time from reconnection of the ventilator to apnea was also measured.

Three rats were bilaterally vagotomized during spontaneous breathing. The ventilator was then reconnected to test whether mechanical ventilation would still induce apnea.

During spontaneous breathing, the spinal cord of one intact rat was transected just caudal to the phrenic outflow. The ensuing hypotension was corrected by bolus injections of the α1-agonist phenylephrine (15 µg iv). The ventilator was then reconnected to test whether mechanical ventilation would still produce apnea.

**Measurement of the apneic threshold.** After 4 min of hypocapnic apnea, the PICO2 in the inspired gas (PICO2) was increased stepwise, by increasing the flow of CO2 to the ventilator, to raise PaCO2 in a controlled manner. With each increase in the flow of CO2, a corresponding decrease was made in the flow of O2 so that inflation volume remained constant. The frequency of mechanical ventilation also remained constant. The first step increase of PICO2 was with a 2% mixture, and thereafter PICO2 was increased by one 0.05% step every 2 min until rhythmic breathing was detected.

To detect the reappearance of rhythmic breathing as PICO2 was raised, the diaphragmatic EMG signal was simultaneously played through a loudspeaker and displayed on the computer monitor. It was striking that the return of rhythmic EMG activity above background noise could usually be detected by ear before rhythmic EMG activity could be seen above background electrical activity. For this reason the apneic threshold was always taken as the PaCO2 at which rhythmic EMG activity was just audible. Once audible, rhythmic EMG activity was monitored for 2 min, and PICO2 was adjusted if the amplitude of EMG activity changed from its perceived threshold level. Once it was established that the amplitude of EMG activity was stable, PICO2 was kept at this composition for a further 4 min to ensure steady-state equilibration. Only then was a blood sample taken to measure the PaCO2 level of the apneic threshold.

**Inflation volume and the apneic threshold.** The influence of the changes in inflation volume used to raise or lower PaCO2 on the apneic threshold PaCO2 level was measured in two ways.

First, the apneic threshold was approached from hypocapnic apnea, by using reductions in inflation volume to raise PaCO2. This was a two-stage procedure during which inflation volume was first increased to produce a 4-min period of apnea and was then reduced by one 0.5-ml step every 2 min until rhythmic diaphragmatic EMG activity was just audible. Over the next 2 min, inflation volume was adjusted if the audible amplitude of EMG activity changed from the perceived threshold level. Inflation volume was then kept at this volume for a further 4 min, after which a blood sample was taken to measure the PaCO2 level of the apneic threshold.

Second, the apneic threshold was approached from eupnea, by using increases in inflation volume to lower PaCO2. After the rats spontaneously breathed for 20 min in O2-enriched air, the ventilator was connected and the inflation volume was increased by one 0.5-ml step every 2 min until rhythmic diaphragmatic EMG activity just disappeared. Over the next 2 min, inflation volume was adjusted if audible EMG activity reappeared. Inflation was then kept at this volume for 4 min, after which a blood sample was taken to measure the PaCO2 level of the apneic threshold. For each rat, both measurements of the apneic threshold after changes in inflation volume were compared with the threshold measurement made when PICO2 was changed while inflation volume was kept constant.

**Effect of vagotomy and the prevailing PaCO2 on the apneic threshold.** The apneic threshold was measured at constant inflation by increasing PICO2 both before and after vagotomy in three rats.
In the group of normal rats, we determined whether chronic hypercapnia could raise the level of the apneic threshold. This was achieved by measuring their apneic threshold before and after 20 min of hypercapnia, induced by using a two-stage procedure. First, the inflation volume of mechanical ventilation was increased until apnea occurred. Then, while rhythmic inflation continued at this inflation volume, $P_{l CO_2}$ was increased to raise $P_{A CO_2}$ to 10–20 Torr above eupneic levels. Hypercapnia was maintained for 20 min, after which $P_{l CO_2}$ was lowered, at constant inflation volume, to reestablish the apneic threshold. Measurement of the $P_{A CO_2}$ at which rhythmic diaphragmatic EMG activity just disappeared was made under steady-state conditions as described in Measurement of the apneic threshold.

Once the experiments were completed, the animals were killed with an overdose of anesthetic.

Statistical analysis was performed on normally distributed data by using the Student's t-test. Values are means ± SE.

RESULTS

After surgery, and during spontaneous breathing in $O_2$-enriched air, the mean blood-gas and pH values for all 42 rats were $P_{O_2}$ of 207 ± 21 Torr, $P_{CO_2}$ of 41.8 ± 0.5 Torr, and pH of 7.353 ± 0.005. Their eupneic frequency (to which the ventilator was set) was 120 breaths/min, and tidal volume was 2 ml.

Changes in the breathing pattern caused by mechanical inflation. When each rat was first connected to the mechanical ventilator, spontaneous breathing continued, but, as would be expected, its pattern was altered. Each rat established a relationship between its breathing pattern and the inflation rhythm of the ventilator. This new relationship was characterized by the peak of the breathing pattern and the inflation rhythm of the ventilator. Each rat established a relationship between its breathing pattern and the inflation rhythm of the ventilator. This new relationship was characterized by the peak of the breathing pattern and the inflation rhythm of the ventilator.

Induction of apnea and its dependency on $P_{A CO_2}$. After an appropriate increase in the volume at which the chest was rhythmically inflated, apnea occurred in all rats after a sufficient period of time. We chose first an inflation volume (mean of 11 ± 1 ml as measured in 22 rats) that brought $P_{A CO_2}$ to 21 ± 1 Torr, i.e., to a $P_{A CO_2}$ level well below that which we subsequently found to be the threshold level. The minimum inflation volume necessary to achieve apnea was not measured at this stage.

The presence of apnea depended on the maintenance of an adequate level of hypercapnia rather than on the degree of pulmonary stretch that the rhythmic inflation caused. Thus during apnea, while inflation volume was kept constant, rhythmic diaphragmatic EMG activity reappeared if $CO_2$ was added to the inspired gas. Moreover, apnea could still be induced after all pulmonary stretch afferents were removed by vagotomy in three rats, and, in one rat, apnea could still be induced after chest afferents originating below the phrenic outflow were removed by cord transection at C6. Furthermore, if rhythmic inflation at high volume was suddenly applied, the time taken for apnea to appear would be too long to be due to the sudden onset of an inhibitory reflex from pulmonary stretch receptors. Thus, in six intact rats, the sudden imposition of rhythmic inflation at high volume induced apnea only after a latency of 26 ± 7 s. Figure 2A shows this latency in one rat. This latency corresponded to the time necessary for the gradual decline in $P_{A CO_2}$ as seen by monitoring end-tidal $P_{CO_2}$. Similarly, if rhythmic inflation at high volumes was suddenly removed, the time taken for breathing to reappear would be too long to be due to the sudden removal of an inhibitory reflex from pulmonary stretch receptors. Thus, in the same six rats, when the quick-release valve was opened during hypocapnic apnea, spontaneous breathing reappeared only after a mean latency of 20 ± 4 s. Figure 2B shows this latency in one rat. This latency accords with the time necessary for the rise in arterial blood of $CO_2$ produced by metabolism. Finally, the disappearance or reappearance of breathing with these step changes in $P_{A CO_2}$ did not require the presence of pulmonary afferents, because they still occurred at similar latencies after vagotomy in two rats (see Fig. 2, C and D).

Measurement of the $P_{A CO_2}$ level of the apneic threshold at constant inflation volume. The $P_{A CO_2}$ level of the apneic threshold was measured from hypocapnic apnea by increasing $P_{l CO_2}$ while inflation volume and frequency were kept constant. Figure 3 shows examples of diaphragmatic EMG activity in one rat at eupnea, at the apneic threshold. The mean $P_{A CO_2}$ level of the apneic threshold for all 42 rats was 32.8 ± 0.4 Torr, the mean $P_{A O_2}$ at threshold was 207 ± 21 Torr, and the mean pH was 7.353 ± 0.005. The mean difference between the $P_{A CO_2}$ level at eupnea and at the apneic threshold was 9.0 ± 0.4 Torr (P < 0.05 by paired t-test).

The level of the apneic threshold in three rats was 6 ± 1 Torr lower after bilateral vagotomy.

Inflation volume and the apneic threshold. Although the mean $P_{A CO_2}$ level of the apneic threshold was lower after vagotomy, we found that the apneic threshold $P_{A CO_2}$ level under steady-state conditions did not depend on the inflation volume used. This was shown in eight intact rats by comparing the level of the apneic threshold measured by adding $CO_2$ to the inspired gas (at a constant inflation volume of 11 ml) with the level measured by raising (or lowering) $P_{A CO_2}$ by changing inflation volume (at a constant $P_{l CO_2}$, i.e., 0 Torr). When inflation volume was increased or decreased (at constant ventilation frequency), the inflation volume at which the threshold was found was one-half (5.8 ± 1 ml or 5.6 ± 0.8 ml, respectively) of the mean volume (11 ± 1 ml) normally used to measure the threshold. The mean $P_{A CO_2}$ levels of the apneic threshold measured by raising or lowering the inflation volume were not significantly different (35.0 ± 3 or 34.0 ± 3 Torr, respectively; P > 0.05 by paired t-test), as shown in Fig. 4.
More importantly, these levels were not significantly different from the mean \( P_{\text{a}CO_2} \) level of the apneic threshold measured at 11-ml inflation either in the same 8 rats (34.5 ± 0.2 Torr; \( P > 0.05 \) by paired t-test) or in all 42 rats (32.8 ± 0.4 Torr; \( P > 0.05 \) by unpaired t-test). Changing the inflation volume increased the variance of the mean apneic threshold (see Fig. 4) because of a methodological difference between the two procedures. The minimum change in inflation volume that we could make (≈13% of inflation volume used at the apneic threshold) was greater than the minimum change in \( P_{\text{ICO}_2} \) we could make (≈1% of \( P_{\text{ICO}_2} \) at the apneic threshold). Hence there was less precision in approaching the apneic threshold by changing inflation volume than by changing \( P_{\text{ICO}_2} \).

Influence of chronic changes in the eupneic \( P_{\text{a}CO_2} \) level on the apneic threshold. In a separate group of seven rats with chronic and abnormally high eupneic \( P_{\text{a}CO_2} \) levels of 55 ± 5 Torr (mean \( P_{\text{a}O_2} \) 224 ± 28 Torr, pH 7.32 ± 0.03), the mean \( P_{\text{a}CO_2} \) level of their apneic threshold (40 ± 3 Torr) was higher than the mean threshold level in the rats with normal blood-gas levels. This suggested that the level of the apneic threshold was not an absolute level but was related to the eupneic...
This hypothesis was tested by measuring the apneic threshold in four normal rats before and 20 min after their mean eupneic PaCO₂ levels were artificially raised from 41.0 ± 1.2 to 58.7 ± 3.3 Torr. Figure 5 shows that this significantly raised the mean level of their apneic threshold from 34.6 ± 1.4 to 43.2 ± 2.7 (P < 0.05 by paired t-test).

Finally, the observation that chronic hypercapnia alters the level of the apneic threshold raises the question of whether the level of the apneic threshold as normally measured in our experiments might have been artificially lowered by the short time the animals were held at hypocapnic apnea. Figure 4 shows that this was not the case because, in the same animal, the measured PaCO₂ level of the apneic threshold was the same whether the animal started from eucapnia or from the 4-min period of hypocapnia. Thus only if the eupneic PaCO₂ is chronically changed (for at least 20 min) does the PaCO₂ level of the apneic threshold also change.
DISCUSSION

These results show that sufficient rhythmic inflation of the chest can lead to apnea in the anesthetized rat by causing arterial hypocapnia. The results define the 

PaCO₂ level of the apneic threshold in the anesthetized rat under steady-state conditions. They demonstrate that the PaCO₂ level of the apneic threshold does not depend on whether the apneic threshold is approached by raising or by lowering PaCO₂. The PaCO₂ level of the apneic threshold is also not measurably influenced by the volume of rhythmic inflation. The threshold PaCO₂ level is not absolute, however, in that it can be raised by chronic elevation of the eupneic PaCO₂ level.

Rhythmic inflation changes the pattern of breathing and can cause hypocapnic apnea. The tidal volume and frequency of spontaneous breathing in the anesthetized rat are sensitive to maintained chest inflation (13, 22). This sensitivity is believed to be mediated predominantly by pulmonary stretch receptors, which have afferents that run in the pulmonary branches of the vagus nerves (13). We show here (see Fig. 1A) that the patterning of breathing is altered by rhythmic inflation, even when inflation is applied at the eupneic frequency and tidal volume. Rhythmic inflation at eupneic levels will have little effect on PaCO₂ levels so the mechanism would be expected to involve feedback from pulmonary stretch receptors. This expectation is confirmed by the demonstration that this alteration in breathing pattern is abolished by vagotomy (see Fig. 1B). We show also that rhythmic inflation alters the amplitude of breathing (see, for example, in Fig. 2A the decrease in the amplitude of diaphragmatic EMG activity when rhythmic chest inflation is suddenly applied but before hypocapnia had time to develop). This alteration involves pulmonary stretch receptors because it also is abolished by vagotomy (see Fig. 2D).

Despite these measurable effects of rhythmic inflation on spontaneous breathing, the occurrence of apnea during rhythmic ventilation was dependent only on 

Fig. 3. Diaphragmatic EMG activity measured in arbitrary units in the same rat as in Fig. 1. This rat was chosen because an EMC rhythm was partially visible at audible apneic threshold (C). For comparison, absence of an EMG rhythm during hypocapnic apnea is shown in B and presence of an EMG rhythm during eupnea is shown in A.

Fig. 4. Mean arterial P CO₂ (PaCO₂) level (44.1 ± 0.4 Torr) in the 8 rats during eupnea (A), at apneic threshold measured from hypocapnic apnea by raising Pco₂ in inspired gas at constant inflation volume (B), at apneic threshold measured from eupnea by increasing inflation volume (C), and at apneic threshold measured from hypocapnic apnea by decreasing inflation volume (D). None of the threshold levels are significantly different from each other, although variances are greater when inflation volume was changed than when inflation volume was kept constant.

Fig. 5. Mean PaCO₂ levels in 4 rats during eupnea (A), at their normal apneic threshold level (B), after 20 min of hypercapnia caused by raising Pco₂ in inspired gas from eupnea (C), and at their new apneic threshold after 20 min of hypercapnia (D). *P < 0.05, before vs. after 20 min of hypercapnia (paired t-test).
\( \text{PaCO}_2 \), falling sufficiently far. Breathing disappeared slowly rather than immediately (after the gradual decrease in \( \text{PaCO}_2 \), rather than after the sudden increase in inflation volume), and breathing reappeared when \( \text{CO}_2 \) was added to the inspired gas without a change being made in inflation volume or frequency. The occurrence of apnea did not depend on the enhanced feedback from pulmonary stretch receptors because, when rhythmic inflation was applied at volumes high enough to cause hypocapnia, breathing still disappeared after vagotomy.

We induced apnea initially at a \( \text{PaCO}_2 \) level well below the level of apneic threshold, by applying rhythmic inflation at a mean volume of 11 ml. This volume is within the volumes that have been published of vital capacity for rats of this weight (17). Prolonged and rhythmic chest inflation with volumes at or above this 11-ml volume may induce small but measurable changes in lung compliance and pulmonary edema (6). Pulmonary function, however, was not obviously compromised in our experiments. Thus we were able to change repeatedly from eucapnia to hypocapnia in the same animals throughout each experiment, and we did not observe the progressive development of hypoxia.

\( \text{PaCO}_2 \) level of the apneic threshold in different species and the effects of anesthesia. In these experiments the mean \( \text{PaCO}_2 \) level of the apneic threshold in anesthetized rats was 32.8 \( \pm \) 0.4 Torr (i.e., 9 Torr lower than the mean \( \text{PaCO}_2 \) level during eupnea). Although it is known that sufficient hypoxia lowers the \( \text{PaCO}_2 \) level of the apneic threshold (19, 28, 37), in our experiments all measurements of the apneic threshold were made in hyperoxia. Thus any slight variation around such a high \( \text{PaCO}_2 \) would not have influenced the \( \text{PaCO}_2 \) level of our apneic threshold.

We measured the threshold by recording diaphragmatic EMG activity under steady-state conditions. For comparison, however, there are no other reports of the \( \text{PaCO}_2 \) level of the apneic threshold measured under steady-state conditions in the anesthetized rat. Threshold levels are nevertheless similar in anesthetized humans (31 Torr), when breathing is measured from diaphragmatic EMG activity and with depths of anesthesia under which \( \text{PaCO}_2 \) is maintained at \( \sim \) 37 Torr (15), and in anesthetized dogs and cats (28–33 Torr) when breathing is measured from motoneurons or from airflow (9, 18, 19, 21).

We deliberately selected animals with minimal respiratory depression, by choosing only anesthetized rats with \( \text{PaCO}_2 \) levels during spontaneous breathing that were no higher that those described for unanesthetized rats (11, 22, 26, 29). Increases in the depth of anesthesia depress breathing and cause a rise in both the eupneic \( \text{PaCO}_2 \) level and the \( \text{PaCO}_2 \) level of the apneic threshold (15, 32). The \( \text{PaCO}_2 \) level of the apneic threshold should be lower in the absence of anesthesia because all anesthetics depress breathing to some extent. We know of no experiments in which \( \text{PaCO}_2 \) was lowered in unanesthetized rats, but, surprisingly, the apneic threshold measured without anesthesia in other species is at the level we found, or even higher. Thus the apneic threshold is 33–34 Torr in awake dogs (28, 30). In unanesthetized humans, the whole issue of direct measurement of the apneic threshold is more complex. Many additional problems include the following: 1) whether ventilation is voluntary or mechanical, 2) whether mechanical ventilation ceases or is maintained during hypocapnic apnea, 3) whether a steady-state equilibration of CNS \( \text{PCO}_2 \) and \( \text{PaCO}_2 \) has been established, and 4) whether the inflation frequency and volume influence the threshold level. With these problems in mind, during non-rapid-eye-movement sleep a threshold is reported at various levels from 4 Torr above to 6 Torr below eupneic \( \text{PaCO}_2 \) levels (8, 16, 34, 37). In some studies [reviewed by Meah and Gardner (27)], it was impossible to measure an apneic threshold in awake humans. In other studies, estimates of the mean \( \text{PaCO}_2 \) level of the apneic or recruitment threshold in awake humans vary upward from 34 to 42 Torr (1, 2, 20, 31, 35). Thus the apparently higher level of the apneic threshold in the absence of anesthesia suggests that a number of other factors, including in some cases conscious influences (33), may raise the level of the threshold.

Measurements made under steady-state and non-steady-state conditions. The time taken for equilibration of \( \text{PaCO}_2 \) and central \( \text{PCO}_2 \) has not been measured in the rat, but the 4 min we allowed for equilibration is consistent with that used in other species (e.g., Ref. 4) and is double that used previously to ensure steady-state conditions in rats (7). Under non-steady-state conditions, e.g., rapidly raising \( \text{PaCO}_2 \) during hypocapnic apnea or rapidly lowering \( \text{PaCO}_2 \) during eupnea, a disequilibrium will exist between \( \text{PaCO}_2 \) and central \( \text{PCO}_2 \). The precise difference this disequilibrium causes between \( \text{PaCO}_2 \) and central \( \text{PCO}_2 \) will depend on the relative rates of change in \( \text{CO}_2 \) production and removal on each side of the blood-brain barrier.

One of the consequences of this disequilibrium (15, 38) is that the \( \text{PaCO}_2 \) level at the apneic threshold will be different when approached from hypocapnia than when approached from eupnea. Our results support this interpretation by demonstrating that, in the anesthetized rat when sufficient time is allowed to reach steady-state equilibration, there is no measurable difference in the mean \( \text{PaCO}_2 \) level of the apneic threshold when approached from hypocapnia or eupnea.

A further problem arises in experiments in which mechanical ventilation is stopped during hypocapnic apnea and attempts are made to sample blood at the same time that the first breath appears (32). Under these conditions, not only does the disequilibrium exist but also the variance in estimating the apneic threshold will be larger. This is because it is impossible always to sample blood simultaneously with the first breath.
This problem is clear when comparing the variance (±0.4 Torr) of our mean apneic threshold (32.8 Torr; n = 42), measured under steady-state conditions, with the variance (±0.55 kPa, i.e., ±4 Torr) of the mean (4.25 kPa, i.e., 32 Torr; n = 19), measured under non-steady-state conditions and when attempting to sample blood simultaneously with the first breath (32).

Inflation volume, hysteresis, and the apneic threshold. Although sufficient and maintained inflation of the chest can have marked inhibitory effects on breathing, it is quite possible that rhythmic inflation will have much weaker effects and may have no measurable effect on the level of the apneic threshold. We found that halving the volume used for rhythmic inflation had no effect on the PaCO2 level of the apneic threshold. We were unable to measure the PaCO2 level of the apneic threshold with rhythmic inflation at the eupneic tidal volume (i.e., when inflation volume was reduced further by one-half). This is because, with the ventilator we used, raising the frequency alone could not induce adequate hypocapnia. Nevertheless, in the one published measurement of the apneic threshold in the anesthetized rat with no mechanical inflation applied (albeit under non-steady-state conditions), Schwieger et al. (32) found the mean PaCO2 level of the apneic threshold (32 ± 4 Torr; n = 19) was almost the same as ours (32.8 ± 0.4 Torr; n = 42). In the one animal examined we also found no evidence for any effect on the apneic threshold of removal of afferents from the chest that would be carried in the spinal cord and originate below the level of C6. The conclusion that the volume of rhythmic chest inflation has little effect on the PaCO2 level of the apneic threshold is further supported by the observation that the level of the threshold was unchanged by a manipulation that increases the potency of the pulmonary stretch reflex. We found that the apneic threshold was the same when approached from hypocapnia as from eupnea (see Fig. 4, C and D), yet hypocapnia increases both the discharge of slowly adapting pulmonary stretch receptors and the ability of lung inflation to inhibit inspiration (3). Our experiments do not definitively exclude the possibility that there exists an effect of inflation volume on the PaCO2 level of the apneic threshold that is too small for us to measure. If such an effect exists, it might be measurable in rats during spontaneous breathing by manipulating PaCO2 levels by using extracorporeal gas exchange (39).

There are two additional and important conclusions to be drawn from the fact that in our anesthetized rats the apneic threshold was the same when approached from hypocapnia as from eupnea. First, this fact indicates that any cerebrovasoconstriction caused by hypocapnia did not influence the PaCO2 level of the apneic threshold. Second, this fact contrasts with the effects on the threshold PaCO2 level of approaching it from hypocapnia or eupnea in unanesthetized humans. In unanesthetized humans, the level is apparently lower when approached from eupnea than when approached from hypocapnic apnea (31). Such hysteresis has resulted in the distinction being made between an “apneic threshold” (when lowering PaCO2 from eupnea) and a “recruitment threshold” (when raising PaCO2 from hypocapnic apnea). The causes of this hysteresis are not clear. Our results show that in the anesthetized rat there is no significant difference between the apneic threshold and the recruitment threshold.

The lack of effect of changing inflation volume on the apneic threshold in anesthetized rats contrasts with the increase in the PaCO2 level of the recruitment threshold when the inflation volume is increased in unanesthetized humans (23, 35). Furthermore, increasing the frequency of rhythmic inflation has similar effects in humans (25). In humans these effects may be mediated not by afferents carried in the pulmonary branches of the vagus nerve but by afferents entering the spinal cord at the level of C1–C5, although this point is still under debate (25, 35, 36).

Additional effects of vagotomy. While the volume of chest inflation has no measurable effect on the PaCO2 level of the apneic threshold, we have shown in three rats that the PaCO2 level of the apneic threshold was lowered by vagotomy. This lowering of the PaCO2 level of the apneic threshold under steady-state conditions is similar to that already described in anesthetized and vagotomized rats under non-steady-state conditions (24). Vagotomy also lowers the apneic threshold in anesthetized dogs (18). Because vagotomy lowers the PaCO2 level of the apneic threshold under anesthesia, but the volume of chest inflation has no measurable effect on the threshold level, it must be concluded that this effect of vagotomy on the apneic threshold in our experiments is not a result of removing the inputs from pulmonary stretch receptors. This conclusion also raises the question of the extent to which the profound changes in the frequency and depth of spontaneous breathing in the rat after vagotomy are caused solely by removal of pulmonary stretch afferents. Our experiments do not answer this question, but it is apparent that, under anesthesia, the apneic threshold is sensitive to afferents carried in the vagus nerves that do not relay information on rhythmic lung inflation. Again, these results contrast with those obtained without the use of anesthesia. Vagal cooling does not lower the apneic threshold in awake dogs (30), although the effect of lung transplantation on the level of the apneic threshold is less clear in humans (25, 35).

Apneic threshold is a relative and not an absolute PaCO2 level. Although increasing the depth of anesthesia raises the eupneic PaCO2 level and the apneic threshold PaCO2 level (15, 32), all rats were initially given the same dose of anesthetic per kilogram body weight. It is not clear why spontaneous breathing was depressed in seven rats (their mean eupneic PaCO2 was 14 Torr above normal levels), but the fact that their
mean apneic threshold was 7 Torr above normal suggested that the apneic threshold might be related to the eupneic $P_{ACO_2}$, in a manner independent of the depth of anesthesia. We tested this hypothesis in four normal rats by comparing the apneic threshold before and after raising the $P_{ACO_2}$ by 18 Torr during spontaneous breathing for 20 min. We found that chronic hypercapnia raised the mean $P_{ACO_2}$ level of the apneic threshold by 9 Torr. Anesthetic depression would not have caused this rise because no additional anesthetic was applied either just before or during this period. This rise also is unlikely to be due to the mechanism of respiratory afterdischarge or short-term potentiation (10). Afterdischarge should temporarily sustain breathing when $P_{ACO_2}$ is suddenly lowered and therefore should result in breathing disappearing at a lower than normal $P_{ACO_2}$. We found, however, that the opposite effect occurred; i.e., breathing disappeared at a higher than normal $P_{ACO_2}$. Although we do not yet know either the minimum time required to reset the apneic threshold or the central mechanisms involved, this is the first demonstration that the apneic threshold for CO₂ must be relative to the prevailing $P_{ACO_2}$ level rather than being at an absolute level of CO₂.

We have described for the anesthetized rat a technique to measure the $P_{ACO_2}$ level of the apneic threshold, one fundamental property of the respiratory control system. We have measured what this level is and have identified the principle experimental variables that do, or do not, influence this level. Throughout these experiments, body temperature was kept at 37°C. It is now appropriate to investigate the effects of changing body temperature on the $P_{ACO_2}$ level of apneic threshold.

The authors are grateful to Dr. Prem Kumar for help and advice and for the loan of some of the equipment used in these experiments. Some of these results have been previously published in abstract form (5).

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Received 15 August 1997; accepted in final form 21 April 1998.

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