Impact of changes in inspired oxygen and carbon dioxide on respiratory instability in the lamb

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Wilkinson, Malcolm H., Kah-Ling Sia, Elizabeth M. Skuza, Vojta Brodecky, and Philip J. Berger. Impact of changes in inspired oxygen and carbon dioxide on respiratory instability in the lamb. J Appl Physiol 98: 437–446, 2005. First published October 8, 2004; doi:10.1152/japplphysiol.00532.2004.—We examined the effect of hypoxia and hypercapnia administered during deliberately induced periodic breathing (PB) in seven lambs following posthyperventilation apnea. Based on our theoretical analysis, the sensitivity or loop gain (LG) of the respiratory control system of the lamb is directly proportional to the difference between alveolar PO2 and inspired PO2. This analysis indicates that during PB, when by necessity LG is >1, replacement of the inspired gas with one of reduced PO2 lowers LG; if we made inspired PO2 approximate alveolar PO2, we predict that LG would be approximately zero and breathing would promptly stabilize. In six lambs, we switched the inspired gas from an inspiratory oxygen fraction of 0.4 to one of 0.12 during an epoch of PB; PB was immediately suppressed, supporting the view that the peripheral chemoreceptors play a pivotal role in the genesis and control of unstable breathing in the lamb. In the six lambs in which we administered hypercapnic gas during PB, breathing instability was also suppressed, but only after a considerable time lag, indicating the CO2 effect is likely to have been mediated through the central chemoreceptors. When we simulated both interventions in a published model of the adult respiratory controller, PB was immediately suppressed by CO2 inhalation and exacerbated by inhalation of hypoxic gas. These fundamentally different responses in lambs and adult humans demonstrate that PB has differing underlying mechanisms in the two species.

Cheyne-Stokes respiration; hypoxia; hypercapnia; sleep apnea syndromes; control of breathing; periodic breathing

PERIODIC BREATHING (PB), or Cheyne-Stokes respiration, appears under a wide range of physiological and clinical conditions. It is particularly prevalent during sleep, occurring at sea level in apparently normal-term and preterm infants (22, 43), at high altitude in normal adults (27), and after hypoxic exposure in experimental animals (11, 21). It also occurs in patients with congestive heart failure (36), with idiopathic central sleep apnea (52), with bilateral brain stem lesions (40), and in periodic obstructive sleep apnea (41). A form of PB can also be found in hibernating animals (7, 34).

Although it is often assumed that the underlying mechanisms causing PB in infants and adults are similar, in each case involving instability of the chemical control system that regulates breathing, there are some marked differences in the pattern of PB and its responses to changes in inspired gas that remain largely unexplained. For example, in preterm infants, the waxing and waning pattern of PB that is characteristic of the adult is replaced by a more or less abrupt on-off pattern that shows little change in tidal volume during the breathing phase (42); this pattern resembles that seen in hibernating animals (34). Furthermore, although the response to hyperoxic inspired gas applied during PB is generally similar in adults and infants (17, 27, 51) in that the instability is transiently enhanced as evidenced by the immediate increase in the duration of the apneic pause, incidental observations show that the response to hypoxia applied during PB in preterm infants (44) is apparently opposite to that of adults (48). In the preterm infant, PB is suppressed by hypoxic inspired gas, whereas it is enhanced in adults. When a comparison is made between experimental animal models of PB and infants, further major discrepancies are revealed. Unlike in human infants (and adults), spontaneous PB has not been reported in intact experimental animals and cannot be induced during exposure to hypoxic inspired gas (11, 21), even though hypoxic stimulus of the carotid bodies, consistently implicated in PB (11, 25, 50, 51), is at a maximum during hypoxia; curiously, in animals, PB appears only after the return to room air after hypoxic exposure.

Although theoretical models of the respiratory control system have been proposed that support the view that PB involves the activity of central and peripheral chemoreceptors either individually or in concert (25, 31), it is not clear whether all of the conditions/discrepancies cited above can be accounted for by one theoretical model. Nor are there experimental tests currently available to determine which chemosensitive receptor systems are responsible for a particular type of instability. As a result, the treatment of these breathing disorders lacks a firm scientific foundation. This problem is particularly evident in the treatment of breathing irregularities in the infant where commonly used drug therapies are aimed at stimulating both central and peripheral chemoreceptor activity (8) with little recognition of the potential impact these interventions may have on the stability of the developing respiratory control system.

A profound effect of increasing the inspired gas concentration of O2 during PB is evident in our earlier work in the lamb and human infant. In both models, we demonstrated that the unstable breathing pattern of PB was transiently exacerbated by administration of hyperoxic inspired air in a setting in which the initial conditions included hypoxemia at the peripheral chemoreceptors. We interpreted this observation in terms of hyperoxic inspired gas increasing the loop gain (LG), or more specifically the plant gain (for oxygen), of the respiratory
control system (4, 50, 51), such that increments in ventilation cause larger changes in arterial PO$_2$ (PaO$_2$) than if air were the inspired gas. This observation suggests that if we were to adopt the opposite strategy and apply hypoxic inspired air during PB in the lamb, we would transiently reduce or even suppress the ventilatory instability of PB as a result of a reduction of the LG of the respiratory control system to <1. This prediction is supported by a theoretical analysis presented in the present work.

In this study, we test the predicted effect of hypoxia applied during PB in a newborn lamb model of PB our laboratory has established in earlier studies (50, 51). In addition, because there is theoretical evidence that CO$_2$ administration should suppress PB in adult humans (31, 33) and because CO$_2$ administration has recently been used clinically in an attempt to control breathing instability in both adults (2, 32, 47) and infants (1), we also examined the effect of CO$_2$ administration on PB. Our immediate aim was to compare and contrast the effects of inspired hypoxic and hypercapnic gas on the unstable newborn respiratory control system. The results of the experimental study and our theoretical analysis clearly show that manipulations of inspired CO$_2$ and O$_2$ can be used to establish the dominant receptor system driving a particular breathing instability.

METHODS

Theoretical Model

Background. In the past, a number of mathematical models have been used to describe quantitatively the oscillations in breathing that appear as part of PB (25, 31, 33). Because these models incorporate nonlinear differential system equations, a solution is usually sought using either an analog or digital computer simulation. The effect of changing the system parameters must then be investigated by using repeated simulations.

An alternative approach to determining factors that are likely to destabilize (or stabilize) control systems is perturbation analysis (39), which is widely used by engineers as the means for evaluating the performance of nonlinear control systems (20, 39). Importantly, this technique has been successfully applied in studies of the human respiratory control system (25). In this method, the system equations are linearized around a particular equilibrium point, and the stability of the system under study is examined locally by assuming small perturbations from this point. The advantage of this method is that the impact of changes in multiple system parameters can often be expressed within a single equation so that the interaction between these parameters is easily deduced. Although the method is not strictly applicable to making precisely quantitative predictions in nonlinear systems undergoing limit-cycle oscillations, such as those that occur in PB, the local stability of the system can still be assessed by performing an analysis at a number of different equilibrium points. Typically, these points are chosen so as to be not too close to known nonlinearities such as an apneic threshold. The method is particularly useful in determining whether an instability is likely to develop (or be suppressed) for the particular equilibrium conditions under consideration. For the respiratory system, these equilibrium conditions may include the current values of Pa$_0$ and arterial PCO$_2$ (PaCO$_2$) at a receptor site. Ultimately, a full numerical simulation, supported by the insights gained from a perturbation analysis, can provide a detailed picture of the performance of the respiratory control system after a disturbance, as we demonstrate later.

The particular issue we seek to address is what effect changes in the inspired concentration of oxygen have on breathing stability. In particular, since the parameter that determines stability is the system LG, we seek to determine an analytical expression for LG in the lamb.

Lamb model. In the 10-day-old lamb, the magnitude of the rapid response to inspired hypercapnic gas, which is mediated by the peripheral chemoreceptors, is small (9, 14, 50). Calculations based on the data of Carroll et al. (14), and our own unpublished data, suggest that within the brief three- to four-breath time span of the ventilatory period of PB in the lamb, only an 8% increase in ventilation is predicted when a CO$_2$ stimulus similar to that expected during PB is applied (~7 Torr; Ref. 16). By contrast, during a ~10% step change in arterial oxygen saturation, approximating in magnitude that seen during PB in the lamb, we typically observe a 60% increase in ventilation over this same time period. It appears, therefore, that the 10-day-old lamb has a powerful response to hypoxia but lacks the multiplicative effect of hypoxia on the slope of the CO$_2$ response that is present in humans and is assumed to originate within the carotid bodies.

The central CO$_2$ controller contribution to LG, and hence the instability of PB, is also likely to be small (25). It is clear from Fig. 6 of Carroll et al. (14) that the response to hypercapnia in the peripheral chemoreceptor-denervated lambs is still not fully developed 60 s after exposure to hypercapnia. Therefore, if we assume a conservative time constant of 1 min for the central CO$_2$ response, the filtering effect of this time constant on the 10-s oscillation in PaCO$_2$ during PB is likely to be severe. Our calculations suggest that a 7-Torr oscillation in PaCO$_2$ would be reduced to 0.18 Torr in the brain tissue if we assume, as is usually the case, that the central receptors monitor cerebral tissue levels of CO$_2$. Thus the contribution of these receptors to the oscillatory drive of PB will be small. Furthermore, a more detailed calculation of LG for the central CO$_2$ control loop, using the equations given by Kho et al. (25) and using published values of brain weights and cerebral blood flow for the lamb (3), gives a central contribution to LG of only 0.03 during hypoxic challenge, confirming our view that the central receptors make a negligible contribution to oscillatory ventilatory drive during PB in the lamb and can reasonably be neglected in the formulation of the analytical expression for LG.

Accordingly, for the purpose of analysis, the major factor directly controlling breathing instability in the lamb is assumed to be the peripheral chemoreceptor sensitivity to hypoxia. By contrast, as we have outlined above, the peripheral CO$_2$ response has a negligible direct role in the genesis of respiratory instability in the 10-day-old lamb because the peripheral receptors are apparently unable to adequately follow the relatively “fast” changes of arterial CO$_2$ that occur during PB. Nonetheless, as discussed later, increased (nonoscillatory) CO$_2$ stimulus of the central and peripheral chemoreceptors can have a marked indirect effect on LG by increasing ventilation and consequently increasing the mean Pa$_0$ at the peripheral chemoreceptors. In addition, nonlinearities associated with the apneic threshold can also affect LG, as we discuss below.

To establish the required LG equation for the lamb, we have used the results of Kho et al. (25), modified to include the appropriate peripheral controller response for the lamb. To implement the analysis, we assume a small perturbation (~1) in alveolar ventilation (V$_A$) and proceed around the peripheral chemoreceptor control loop (see Fig. 1) calculating the resultant perturbations in Pa$_0$, at the heart and carotid bodies until we finally calculate the response of the controller (V$_P$). The ratio of the original disturbance, V$_A$ to the controller response V$_P$ gives the LG of the peripheral control loop (see APPENDIX), which is given by

\[
[LG] = \left(\frac{P_{a0} - P_{a0}^*}{ \left(kG_p e^{-\omega \tau_c} \right)} \right) \left|F(\omega, \tau_c)\right|
\]

where $F(\omega, \tau_c)$ is the magnitude of the frequency-dependent portion of the LG expression in Eq. A5; $\omega$ is radian frequency; $\tau_c$ is the collective effect of all the lung and tissue washout time constants and circulatory delays in Eq. A5; $P_{a0}$ and $P_{a0}^*$ are the mean Pa$_0$, at the lung and
Impact of the apneic threshold on LG.

The presence of an apneic threshold in the respiratory controller may have a significant effect on the magnitude of LG computed from linear theory. However, on the assumption that the oscillation in \( P_{\text{aO}_2} \) or \( P_{\text{aCO}_2} \) that drives ventilation is approximately sinusoidal, a method known as describing function analysis (20, 24) can be used to calculate an equivalent linear gain for any nonlinearity. Importantly, this linearization allows the powerful array of linear techniques, including the application of the Nyquist stability theorem that states that \( LG \geq 1 \) and \( \phi = 180^\circ \) for instability to occur. Unlike in linear systems, the equivalent linear gain may vary with the amplitude and the mean value of the driving oscillation, so this equivalent gain must be specified at a particular value of the driving function. Applying this method to the analysis of the well-known linear respiratory controller plus threshold, it can be shown that the equivalent gain of the controller is always less than or equal to the linear gain (24). On the other hand, if the respiratory controller gain is increased below eupnea compared with that above, as proposed in the lamb (4, 50, 51), the controller gain will be increased from its

### Experimental Protocols

**Control.** Lambs were hyperventilated with air for a period of 5 min, after which the ventilator was turned off. By adjusting the tidal volume and respiratory frequency during the hyperventilation period, it was possible to produce a posthyperventilation apnea (PHA) that was terminated at an \( SaO_2 \) of 40–50%. During PHA (i.e., before the commencement of breathing), the inspired gas was switched from an inspired oxygen fraction (\( F_{\text{IO}_2} \)) of 0.21 to an \( F_{\text{IO}_2} \) of 0.4. Previous experiments have shown that this procedure produces PB in 95% of lambs (51). The epoch of PB produced was allowed to terminate spontaneously.

**Hypoxic test.** The procedure outlined in control was repeated except that, after two cycles of PB had occurred and before the ventilatory period of the third cycle was initiated, the inspired gas was switched abruptly to an \( F_{\text{IO}_2} \) of 0.12. This level of hypoxia was sufficient to maintain \( SaO_2 \) close to 50% for the 1-min duration of the challenge. At the end of this period, the inspired gas was returned to sleep onset) before threshold crossings develop. In this case, linear theory, as exemplified by Eq. 1, is adequate to predict LG and the onset of instability.

**Animal preparation.** Surgical preparation of animals in this study was identical to that already described in detail (50, 51). We anesthetized seven lambs of 11–20 days of age with a mean weight of 8.6 ± 0.8 kg using intravenous \( \alpha \)-chloralose (80 mg/kg as a starting bolus followed by 20 mg·kg\(^{-1}\)·h\(^{-1}\) as a continuous infusion). The lambs were intubated and ventilated with a Bournes BP-200 infant ventilator. All animals were maintained under an effective but light level of anesthesia by continuously monitoring blood pressure and heart rate and by making regular tests for a response to stimulation of the inner canthus of the eye. The tracheal tube was attached to a ventilator circuit, through which gas flowed at 30 l/min, a very high flow that enabled us to change between hypoxic, normoxic, hyperoxic, or hypercapnic gas mixtures within 1 s. The circuit permitted us to ventilate the animal or to allow the animal to ventilate itself.

We inserted a catheter nonocclusively into the carotid artery for blood gas sampling and for measurement of blood pressure (HP 1280 blood pressure transducer connected to a Hewlett-Packard 8805B Carrier Amplifier). Heart rate was derived from the blood pressure signal (Neotrace NT 122). A second nonocclusive catheter in the jugular vein was used for blood gas sampling and infusion of glucose saline (5% glucose in 0.9% saline at a rate of 4.0 ml·kg\(^{-1}\)·h\(^{-1}\)). We maintained rectal temperature within the range of 39.8 ± 1°C using an overhead radiant heater.

A Fleisch no. 0 pneumotachograph, in conjunction with a Gaertec MP-15 differential pressure transducer, was used to measure respiratory airflow, and this signal was electronically integrated to provide tidal volume (HP-8815A electronic integrator). End-expired \( P_{\text{CO}_2} \) and \( P_{\text{O}_2} \) were measured using a Morgan 901 MK2 CO\(_2\) analyzer and an Amatek S-3A oxygen analyzer, respectively. The probe of a Nellcor pulse oximeter (model N-200) was placed across the lower jaw, but with the tongue reflected out of the way so that the light beam passed through the soft tissues of the jaw.

**Pulse oximeter calibration.** We calibrated the Nellcor N-200 pulse oximeter over the arterial oxygen saturation (\( SaO_2 \)) range from 40 to 100% using the \( SaO_2 \) (Radiometer OSM-2 hemoximeter) of samples of carotid artery blood taken while the animal breathed progressively more hypoxic gas mixtures. All saturation values reported in this paper were corrected by using a curve relating the real (Radiometer) saturation to the indicated (Nellcor) saturation. At the end of the protocols, the lambs were killed with an overdose of anesthetic (Letharb, 150 mg/kg; Virbac, Sydney, Australia). All surgical and experimental procedures conformed with the guidelines established by the National Health and Medical Research Council of Australia and had the approval of the Standing Committee in Ethics in Animal Experimentation of Monash University.
RESULTS

Hypoxia During PB

There was no significant difference in the duration of PHA between control 1, hypoxia, and control 2 (22.5 ± 2.9 vs. 25.0 ± 2.1 vs. 23.2 ± 2.6 s, respectively), in nadir SaO2 during PHA (42.3 ± 3.3 vs. 40.7 ± 3.2 vs. 42.6 ± 3.6%, respectively), or in the end-tidal CO2 before PHA (3.1 ± 0.2 vs. 3.2 ± 0.3 vs. 3.2 ± 0.3%, respectively). Thus conditions before the induction of PB were similar in the three tests. In all six lambs challenged with hypoxia during PB, the instability was immediately terminated after the switch of \( F_{1O2} \) from 0.4 (hypoxia) to 0.12 (hypoxia) as illustrated in Figs. 2 and 3. Significantly, the mean \( SaO2 \) immediately before the challenge (56.5 ± 4.1%) was not different to that immediately after the challenge (53.7 ± 4.4%), suggesting that \( PaO2 \), the mean \( PaO2 \) at the peripheral chemoreceptors, was similar during PB and during the regular breathing that resulted from switching inspired gas to a hypoxic mixture. A second estimate of the prechallenge \( SaO2 \), deduced using interpolation of the saturation data to account for poor oximeter tracking (see Fig. 2) was similarly not significantly different from that immediately after the challenge. In this case, the prechallenge \( SaO2 \) was 57.3 ± 4.1 vs. 53.7 ± 4.4% immediately after the challenge.

All lambs once again exhibited PB (mean epoch duration of 44.5 ± 6.8 s) when the inspired gas was returned to an \( F_{1O2} \) of 0.4 after 1 min of hypoxia (Figs. 2 and 3). The mean epoch duration of PB for control 1 was 41.1 ± 7.8 s and for control 2 was 45.3 ± 12.0 s (P = not significant).

Hypercapnia During PB

As in protocol 1, there was no significant difference in the duration of PHA between control 1, hypercapnia, or control 2 (20.8 ± 3.4 vs. 21.7 ± 2.1 vs. 23.5 ± 2.6 s, respectively) in nadir \( SaO2 \) during PHA (41.3 ± 3.8 vs. 43.8 ± 3.9 vs. 41.4 ± 2.0%, respectively) or in the end-tidal CO2 before PHA (2.8 ± 0.2 vs. 3.0 ± 0.3 vs. 3.0 ± 0.3%, respectively). In the six lambs challenged with hypercapnic gas during PB, there was a significant reduction in the poststimulus epoch

an \( F_{1O2} \) of 0.4 until after the resumption of stable breathing. At the end of the hypoxic test, the protocol described in control was repeated, with PB again allowed to terminate spontaneously. As a result, each hypoxic challenge was bracketed by a control procedure. The study sequence comprised control 1, hypoxia, and control 2.

Hypercapnic test. This protocol was similar to that described in the hypoxic test, except that the inspired gas during PB was switched after one cycle to an inspired CO2 fraction of 0.05 in a gas mixture containing an \( F_{1O2} \) of 0.4. To quantify the central CO2 stimulus and the peripheral O2 stimulus at the resolution of PB, we took a jugular venous blood sample to determine the jugular venous \( P_{CO2} \) (\( P_{iCO2} \)), and we also noted the corrected value of \( SaO2 \) at the onset of regular breathing. Each hypercapnic challenge was bracketed by an identical control challenge, during which the inspired gas remained at an \( F_{1O2} \) of 0.4 throughout. The study sequence was made up of control 1, hypercapnia, and control 2.

**Data Analysis**

In both the hypoxic and hypercapnic protocols, we measured the epoch duration of PB from the point at which the challenge was given until regular breathing was restored; this method of analysis was carried out for the control and the hypoxic and hypercapnic gas challenges alike. To test whether PB was induced under uniform initial conditions for each procedure, we measured the duration of PHA, the nadir value of \( SaO2 \), reached during PHA, and the end-tidal CO2 of the last ventilated breath before PHA. To assess whether the mean hypoxic stimulus at the carotid chemoreceptors immediately before and after the hypoxic challenge had changed, we estimated the mean \( SaO2 \) for one complete cycle of PB (including both the ventilatory and apneic periods) before the challenge and for an equal time period starting at the beginning of the hypoxic challenge; this was done by averaging the corrected \( SaO2 \) values taken every 2 s from the oximeter recording after allowing for the processing delay of the oximeter. If the oximeter failed to track the rapid changes in oxygen saturation for short periods as evidenced by a flat section on the \( SaO2 \) oximeter trace (see Fig. 2), either the data for that section were discarded or the data were interpolated over that period to obtain an estimate of the likely saturation (see Fig. 2). Both estimates are recorded in **RESULTS**.

**Statistics.** All data are means ± SE. Differences between means were tested using a one-way repeated-measures analysis of variance, with \( P < 0.05 \) being taken as the critical level and using Tukey's post hoc test for multiple comparisons.

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Fig. 2. Hypoxia during periodic breathing (PB) (data recording). Typical data recording showing the time course of respiratory flow rate (V), tracheal gas concentrations of oxygen and CO2 (ETO2 and ETCO2, respectively), pulse oximeter saturation (SpO2), and carotid artery pressure (Pca) during a typical hypoxic challenge during PB. The record commences during hyperventilation with a mechanical ventilator, and this is maintained until the ventilator is turned off at \( t1 \), after which the inspired gas is immediately switched to an inspired oxygen fraction (\( F_{1O2} \)) of 0.4. After a period of posthyperventilation apnea, during which \( SpO2 \) falls progressively, spontaneous PB is generated at \( t2 \), and after 2 cycles of PB the inspired gas was switched to an \( F_{1O2} \) of 0.12 at \( t3 \) for 1 min. Breathing was immediately stabilized and remained so until the inspired gas was switched back to an \( F_{1O2} \) of 0.4 at \( t4 \), after which PB reappeared. Notice that \( SpO2 \) just before the hypoxic challenge approximates that immediately after the challenge. The dotted sections of the \( SpO2 \) trace marked with an x are estimates of the time course of oxygen saturation during periods where pulse oximeter tracking was lost (see METHODS for further details).
duration of PB with respect to control (Figs. 4 and 5A). Epoch durations in the three experimental periods were 46.1 ± 8.2 s during control 1; 23.3 ± 8.0 s during hypercapnia (inspired CO$_2$ fraction = 0.05), and 51.2 ± 9.9 s during control 2. There was no significant difference in epoch duration between control 1 and 2.

The $\mathrm{P_{\text{VCO}_2}}$ that coincided with the commencement of regular breathing after PB was 46.0 ± 1.9 Torr in control 1, 45.8 ± 2.4 Torr during hypercapnia, and 45.5 ± 2.0 Torr during control 2 (Fig. 5B). There were no significant differences in these $\mathrm{P_{\text{VCO}_2}}$ values. The corrected value of $\mathrm{SaO_2}$ at the commencement of regular breathing was 90.3 ± 3.9 in control 1, 83.0 ± 5.9 during hypercapnia, and 90.1 ± 2.9 during control 2 (Fig. 5D). There was no significant differences in these $\mathrm{SaO_2}$ values.

To assess the impact of hypercapnia on PB; the minute ventilation during PB was compared between control 1, hypercapnia, and control 2. As shown in Fig. 4C, there was a significant increase in minute ventilation during hypercapnia (140.8 ± 15.0 ml·min$^{-1}$·kg$^{-1}$) compared with control 1 (116.5 ± 11.0 ml·min$^{-1}$·kg$^{-1}$) and control 2 (116.4 ± 9.8 ml·min$^{-1}$·kg$^{-1}$). There was no significant difference in minute ventilation between control 1 and control 2.

**DISCUSSION**

We report two principal findings in this study. First, inspiration of hypoxic gas during PB at a level chosen not to lead to further hypoxemia at the peripheral chemoreceptors immediately stabilizes breathing in the lamb. We interpret this effect of hypoxic gas in terms of a reduction in LG (plant gain for oxygen), making ventilation less effective at changing blood gases, reducing the overshoot in $\mathrm{PaO_2}$, and effectively braking the oscillation that causes PB. Second, hypercapnia also stabilizes breathing, but it does so only after a considerable delay; our findings also show that when continuous breathing resumes after an epoch of PB there is a similar level of jugular venous Pco$_2$ in the control and in the hypercapnia phases of the protocol, suggesting that breathing stabilizes when central Pco$_2$ reaches a threshold level. These findings strongly suggest that the stabilization of breathing brought about by hypoxic inspired gas during PB in the lamb is mediated via the rapidly responding, O$_2$-sensitive carotid bodies, whereas the stabilizing effect of inspired CO$_2$ is mediated via the CO$_2$-sensitive, slowly responding medullary chemoreceptors.

Although our laboratory (4, 51) and others (17, 44) have already shown that hyperoxia applied during PB can transiently destabilize breathing further, this present work represents the first systematic demonstration that hypoxia applied in similar circumstances stabilizes breathing, at least in the lamb. LG is an effective unifying concept to explain the effects of increased or decreased oxygen level on PB. By reference to Eq. 1, it is clear that oxygen enters the LG equation that defines the respiratory controller of the lamb in two places. The first is via the difference between $\mathrm{P_{O_2}}$ and $\mathrm{PaO_2}$. The second is via $G_e^{-1}P_{\text{delt}}$, the peripheral chemoreceptor contribution to LG, which increases exponentially as $\mathrm{PaO_2}$ falls.

The first bracketed term in the numerator of Eq. 1 predicts that the introduction of hypoxic inspired gas during PB will decrease LG and therefore will tend to suppress breathing instability. Conversely, hyperoxia will increase the difference between $\mathrm{P_{O_2}}$ and $\mathrm{PaO_2}$, augment LG, and promote breathing instability. This effect can also be understood qualitatively without reference to the LG equation in that the breathing and apneic phases of PB result from cyclic increases and decreases in carotid body discharge (50, 51). During the apneic phase,
arterial O2 level falls, with the result that there is an increase of carotid body discharge that eventually exceeds the threshold needed to trigger breathing. Once breathing begins, PaO2 rises, carotid body discharge falls below the apneic threshold, and an apnea ensues. The inhalation of hypoxic gas during PB decreases the tendency of PaO2 to rise and to overshoot when breathing returns after the apneic pause. Without an overshoot in PaO2, the carotid bodies continue to discharge, and hence breathing continues. Hypoxia has the reverse effect, exacerbating the ventilatory overshoot and thereby enhancing the respiratory instability (51).

The second bracketed term in the numerator of the LG equation represents the contribution of the carotid bodies through their sensitivity to hypoxia. When PIO2 is suddenly reduced during the hypoxic challenge, any fall in LG through the effect of a decline in PIO2-PaO2 could be counteracted by a rise in the contribution of the carotid bodies via their sensitivity to the imposed hypoxia. For such a carotid body effect to occur in our study, there would have to be a fall in PaO2 during the hypoxic challenge compared with the control period of PB. That no immediate change in carotid body contribution occurs in our hypoxic challenge experiments is illustrated in Fig. 2 and is clear from our analysis showing that the mean SaO2 just before the challenge (56.5 ± 4.1%) is not significantly different from that occurring just after the challenge (53.5 ± 4.4%) (see also Fig. 2). Thus the switch to hypoxic inspired gas leads to a fall in LG as a result of the fall in PIO2-PaO2, and plant gain, thereby providing an explanation for the stabilization of breathing we observed.

Are there other possible explanations for our observations? Recently, a good deal of attention has been focused on the role of proximity of the eupneic point to the CO2 apneic threshold (10, 35, 45). The concept of a CO2 reserve, defined as the difference between the end-tidal CO2 at eupnea and the end-tidal CO2 at the apneic threshold, has developed (46), and its magnitude has been used as an explanation for the occurrence of apnea and PB. The CO2 reserve is reduced in such conditions as acute hypoxia (53) in patients with congestive heart failure (54) or when ventilatory drive is very low; importantly, these are conditions in which ventilatory instability occurs. In contrast, the CO2 reserve is increased when ventilatory drive is increased by nonhypoxic means, such as during treatment with theophylline and acetazolamide, and these drugs suppress PB (10, 23). Although we did not measure CO2 reserve, increased hypoxemia in the lamb could conceivably cause an increased CO2 reserve by shifting the CO2 response curve to the left without change in slope. However, this did not occur in our experiments because the mean level of hypoxemia did not change during the prehypoxic cycle of PB and the immediate post-PB period (see Fig. 2). Accordingly, an increase in CO2 reserve by this mechanism does not explain our findings. We conclude, therefore, that a decrease in LG, specifically plant gain (for oxygen), is the key mechanism stabilizing breathing in the lamb after hypoxic exposure during PB. The important role of plant and controller gain in controlling ventilatory instability has been stressed recently (18).

Our experimental results for challenge with inspired CO2 during PB are in marked contrast to our results with hypoxia, because although the epoch duration of PB was reduced after an increase in inspired CO2, this occurred only after a considerable delay and not instantaneously as in the hypoxic chal-

Fig. 5. Influence of hypercapnia on PB. A: epoch duration of PB after administration of hypercapnic gas was significantly reduced compared with control; *P < 0.05, n = 6. B: at the point where regular breathing resumed after PB, the jugular venous PCO2 (PrPCO2) was not significantly different between the control and hypercapnic challenge, suggesting that stimulation of the central CO2-sensitive chemoreceptors mediates the conversion to regular breathing after PB. C: hypercapnic gas applied during PB significantly increased minute ventilation. D: there was no significant difference in the arterial oxygen saturation (SaO2) at the start of regular breathing between the control period and the hypercapnic challenge with 5% CO2. The dotted lines show the mean nadir of SaO2 reached at the start of PB.
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leng. In addition, even though the epoch duration was shortened during the CO₂ challenge, the PB reverted to a regular pattern at the same value of ṖVO₂ as in the control run when no CO₂ was applied. Accepting that this measure accurately portrays central tissue CO₂ levels, as we have argued previously (51), this finding strongly suggests that PB is resolved when the central respiratory drive reaches a threshold value.

After PHA in the lamb, transient PB develops as a direct result of the increased LG provided principally by the hypoxic stimulation of the peripheral chemoreceptors (50, 51). The fact that respiratory instability is present implies that LG exceeds 1 during the epoch of PB and falls below 1 on return to regular breathing. During this period, both Pao₂ and cerebral CO₂ rise as they return to their prehyperventilation values. Accordingly, central CO₂ chemoreceptor drive is being progressively augmented and peripheral oxygen drive decremented. Thus LG, which we have argued is determined principally by peripheral oxygen drive in the lamb, falls progressively with time throughout the PB epoch. Eventually, at some specific value of Pao₂, which we refer to as Pao₂, LG falls to 1, and soon after regular breathing returns.

The result of adding CO₂ to the inspired gas during PB is relatively easy to visualize since the resulting extra CO₂-sensitive central drive increases ventilation during PB (see Fig. 5C) so that both Pao₂ and cerebral CO₂ rise more rapidly and Pao₂ is reached earlier, shortening the PB epoch. Because the sum of the central neural drive and the peripheral hypoxic drive determined by Pao₂ will be the same as previously (unless the apneic threshold changes), it follows that ṖVCO₂ must be the same also. Clearly, the experimental finding that ṖVO₂ sampled at the commencement of regular breathing after an epoch of PB is the same during both the control protocol and the hypercapnia protocol indicates an important role for central CO₂ chemosensitivity in terminating respiratory instability in the lamb.

The issue of how the properties of the respiratory controller of the lamb relate to the adult human case is an interesting one. What is immediately evident from perturbation analysis is that the adult human controller has, in addition to the peripheral O₂ component present in the lamb, a peripheral CO₂ component (25). These two components, each with separate LG, which we will refer to as LGO₂, analogous to our Eq. 1, and LGCO₂, respectively (this component of LG is not present in the lamb), arise because of the multiplicative effect of hypoxia on CO₂ sensitivity in the adult human. The system LG is then equal to the vector summation of LGO₂ and LGCO₂, with the contribution to LG of the central CO₂ controller during PB being neglected (25). A further important difference is that the human controller apparently has a relatively fixed apneic threshold for CO₂ (19, 30), whereas the lamb and several other species do not (12, 28). It seems likely that these fundamental differences are the reason hypoxia normally precipitates PB in sleeping humans (6, 27), whereas it has never been reported to do so in the lamb.

A full discussion of the impact these controller differences would have on the genesis of PB is beyond the scope of this paper, but several differences might be expected. First, the
traditional view of PB being an oscillation about a fixed apneic threshold for CO₂ (38) is not applicable to the lamb since the oscillation in PaO₂ apparently dominates the generation of PB in this species. Second, the effect of inspired CO₂ on the resolution of PB might be accelerated in the adult human because of the brisk carotid body response to CO₂, a feature not present in the lamb. In effect, the inspiration of CO₂ in humans reduces LGCO₂ in much the same way that hypoxia reduces LGO₂ in the lamb. We note in passing that the absence of a brisk response to inspired CO₂ during PB in the lamb and the failure to suppress PB immediately on exposure to CO₂ suggests that LGCO₂ is small and supports our view that PB is driven primarily by oscillations in PaO₂. Third, although the effect of hypoxia on the peripheral oxygen controller in the adult human would be expected to be similar with LGO₂ falling after hypoxia, as in the lamb, hypoxia could augment the human LGCO₂ so that PB may be unchanged or even enhanced on switching to a hypoxic inspired gas during PB.

We can take the first steps toward testing these important predictions by performing a numerical simulation using an established model of the adult human respiratory control system (25, 49). The results of this simulation, illustrated in Fig. 6, show that challenge with hypoxia during the transient PB that follows hyperventilation has a radically different effect than in the lamb: it clearly enhances PB. Furthermore, it is clear from the LG calculations included in Fig. 6 that, although LGO₂ falls after hypoxic challenge as in the lamb, the LGCO₂ rises and the overall system LG is increased, leading to an enhancement of the instability. Challenge with CO₂ suppresses PB as in the lamb, but, as expected, it does so more rapidly because of the brisk carotid body response to hypercapnia in the human. This result is supported by the experimental results of Cherniack et al. (15) that clearly demonstrate the rapid suppression of servorespirator-induced PB that follows the addition of CO₂ to the inspired gas in adult cats, a model that has been shown to exhibit a brisk response to changes in end-tidal CO₂ (5).

Further support for our predictions comes from the experimental observation (48) that hypoxia applied during PB in snorers does not suppress PB. This observation and our modeling results raise the interesting possibility that a hypoxic challenge during PB could differentiate between the form of respiratory controller present in the lamb, in which the carotid bodies are rapidly responsive only to oxygen vs. the multiplicative respiratory controller of the adult human in which the peripheral chemoreceptors are rapidly responsive to both oxygen and CO₂.

An obvious inference from our results in the lamb and our simulation for the adult human is that the presence of a brisk CO₂ response at the carotid bodies has a major impact on the effect of hypoxia on PB. Interestingly, there is evidence that the respiratory controller of the newborn human infant, and indeed the infants of some other species, resembles the lamb rather than the adult human. In a series of experiments aimed at characterizing the respiratory response to hypoxia in preterm infants, Rigatto et al. (44) observed that when hypoxia was applied during PB in one infant it immediately restored regular breathing, a response that is clearly consistent with the human newborn having a controller similar to the lamb. Other evidence that the peripheral chemoreceptors of the newborn predominantly have an oxygen sensitivity comes from studies in both in vivo and in vitro preparations of the immature cat (13) and rat (37). In these preparations, over a period of weeks, there is a shift toward a controller that manifests a multiplicative type CO₂-oxygen interaction characteristic of the human adult. We suggest that the application of hypoxia or CO₂ during PB may provide a simple means for determining whether the respiratory controller has a simple oxygen-sensitive character in early development before converting to a multiplicative controller with postnatal development.

We are currently exploring this possibility using a numerical model of the lamb respiratory control system that incorporates a central CO₂ control loop and an additive peripheral oxygen feedback loop representing an early life model that then matures progressively toward a multiplicative interaction, where both peripheral O₂ and CO₂ responses are present as in the adult controller. This more accurate model should also allow us to determine whether the peripheral O₂ feedback loop is in fact dominant in the lamb as we contend in this present work. The model should also allow us to examine the impact of the apneic threshold on breathing instability and LG in the lamb.

In conclusion, there is considerable evidence from our work and that of others that during PB the newborn respiratory controller may be dominated by O₂ sensitivity before converting to a controller in which O₂ modulates the response to CO₂. The methods developed in this paper, which essentially manipulate LG during PB by changing the inspired gas, represent a powerful tool for discriminating the stage of development of the infantile respiratory control system. This information may in turn allow more appropriate use of drugs to control LG of specific receptor systems and to suppress breathing instabilities.

**APPENDIX**

Using a perturbation analysis following that used by Khoo et al. (25) and applying it to the peripheral chemoreceptor control loop of the lamb (see Fig. 1), a disturbance in V˙A (V̇ΔA) will cause a disturbance in PaO₂ given by

$$\dot{\Delta}P_{aO_2}(s) = \dot{V}_A(s) \frac{(P_{aO_2} - \dot{P}_{aO_2})}{V_O} \frac{(1 + \tau_T s)}{\tau_T(s + 1/\tau_E)}$$  \hspace{1cm} (A1)

where \(\tau_T\) is tissue washout time for oxygen; \(\tau_E\) is overall washout time for oxygen, including the effect of tissue washout by perfusion and lung washout by perfusion and ventilation; and \(s\) is complex frequency (rad/s).

This disturbance (\(\Delta P_{aO_2}\)) is filtered as it passes through the heart and associated vasculature and is further delayed by \(\tau_n\) seconds in transit to the carotid bodies. Denoting the filtered and delayed stimulus that arrives at the carotid bodies as \(\hat{P}_{aO_2}\), we can write (29)

$$\hat{P}_{aO_2}(s) = \frac{e^{-\tau_1 s}}{(1 + \tau_1 s)(1 + \tau_2 s)}$$  \hspace{1cm} (A2)

where \(\tau_1\) and \(\tau_2\) are filtering time constants and \(\tau_p\) is circulation delay (lung to carotid body).

This disturbance in partial pressure stimulates the carotid bodies to produce a change in ventilation \(V_P\). In previous work, our laboratory has shown that the lamb, unlike the human, has a negligible brisk response to hypercapnia (50), suggesting that the usual multiplicative effect of hypoxia on the carotid body response to \(\hat{P}_{aCO_2}\) is absent under the conditions of our experiments, and as a result the exponential response to hypoxia (the peripheral controller response) can be adequately modeled as
where \( k \) is a constant, \( \overline{PaO_2} \) is mean \( PaO_2 \), at the peripheral chemoreceptor. Differentiating Eq. A3 with respect to \( \overline{PaO_2} \), and expressing the differential as a small perturbation yields

\[
V_p = G_k e^{-\frac{\overline{PaO_2}}{k}}
\]

(A3)

And finally, if we assume as did Khoo et al. (25) that the dead-space controller as

\[
V_p(s) = -kG_k e^{-\frac{\overline{PaO_2}}{k}} \overline{PaO_2}(s)
\]

(A4)

This expression can be used to derive the magnitude and phase of LG. However, for our purposes, it is sufficient to recognize that the magnitude of LG can be written as

\[
|LG| = \left| \frac{\overline{PaO_2} - \overline{PaO_2}}{(kG_k e^{-\frac{\overline{PaO_2}}{k}})} \right| |F(\omega, \tau_s)|
\]

(A6)

where \( |F(\omega, \tau_s)| \) is the magnitude of the frequency-dependent portion of the LG expression in Eq. A5, \( \omega \) is radian frequency, and \( \tau_s \) is the collective effect of all the time constants in Eq. A5.


