Development of Ventilatory Response to Transient Hypercapnia and Hypercapnic Hypoxia in Term Infants

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ABSTRACT

Whereas peripheral chemoreceptor oxygen sensitivity increases markedly after birth, previous studies of ventilatory responses to CO₂ in term infants have shown no postnatal development. However, the hypercapnic challenges applied have usually been long-term, which meant that the effect of central chemoreceptors dominated. Oscillatory breathing, apneas, and sighs cause transient Pco₂ changes, probably primarily stimulating peripheral chemoreceptors. We wanted to assess whether the immediate ventilatory responses to step changes in inspired CO₂ and O₂ in term infants undergo postnatal developmental changes. Twenty-six healthy term infants were studied during natural sleep 2 d and 8 wk postnatally. Ventilatory responses to a randomized sequence of 15 s hypercapnia (3% CO₂), hypoxia (15% O₂), and hypercapnic hypoxia (3% CO₂ + 15% O₂) were recorded breath-by-breath using a pneumotachometer. Response rate, stimulus-response time, and response magnitude were analyzed with ANOVA after coherent averaging. Response rate increased with age by 30% (hypercapnia), 318% (hypoxia), and 302% (hypercapnic hypoxia). Response rate during hypercapnic hypoxia exceeded rate during hypercapnia plus rate during hypoxia in wk 8, but not on d 2. Time to half-maximum response decreased by 3.4 s with age for the two hypercapnic stimuli but

was unchanged for hypoxia. Response magnitude was unchanged for hypercapnia, but increased for the two hypoxic stimuli. In conclusion, an interaction between the effects of hypercapnia and hypoxia on ventilatory response rate emerged between postnatal d 2 and wk 8 in term infants. Concomitantly, stimulus-response time to hypercapnic stimuli declined markedly. The development of a prompt response to transient hypercapnia may be important for infant respiratory stability. (*Pediatr Res* 55: 302–309, 2004)

Abbreviations

Fico₂, fraction of inspired carbon dioxide
f_R, respiratory rate
Paco₂, partial pressure of arterial carbon dioxide
V_T, tidal volume
Ṡ_E, minute ventilation
Ṡ_E 45, integrated ventilatory response for 45 s after the onset of a 15-s stimulus
Ṡ_E median, median value of ventilation in the period ± 5 s from the end of a 15-s stimulus
Ṡ_E max, maximum value of ventilation during a response, determined after signal filtering

Peripheral and central chemoreceptors are crucial for the precise control of respiration. The carotid, aortic, and central chemoreceptors are functional even in fetal life (1, 2), but the transition to continuous breathing calls for rapid adjustments. The marked increase in arterial Po₂ at birth brings about a corresponding resetting of carotid and aortic chemoreceptor O₂ sensitivity, which takes place during the first postnatal days and weeks (1, 3–5). In contrast, studies of postnatal CO₂ chemosensitivity show divergent results. Steady-state CO₂ sensitivity increased after birth in some (6–8) but not all species

(9). Preterm infants displayed an age-related increase in steady-state CO₂ sensitivity (10, 11), whereas term infants showed no such postnatal development (12, 13). Dynamic CO₂ sensitivity was unchanged during the first month in neonatal lambs (8, 14), whereas in piglets the relative contribution of peripheral chemoreceptors to CO₂ stimulation increased postnatally (15).

Ultimately, the combined effects of Pco₂, arterial Po₂, and pH determine the level of ventilation. Nonlinear interaction between Pco₂ and Po₂, *i.e.* increased CO₂ chemosensitivity at increasing levels of hypoxia, has been described in carotid and aortic nerve recordings (7, 8, 16) and in ventilatory responses in animals (17, 18) and adult humans (19). Whether interaction between Pco₂ and Po₂ is present at birth and whether it increases postnatally differs from one species to another (7, 16, 20).

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There have been few studies of infant respiratory control during transient hypercapnia, and to our knowledge, the hypercapnic-hypoxic ventilatory interaction has not been assessed in term infants. These are areas of interest because a fast-feedback system to maintain P_{aCO_2} level is crucial for respiratory stability. Infants display a variety of oscillatory breathing patterns, sighs, and apneas, which produce marked but transient changes in P_{aCO_2} . Observed stimulus–response times indicate that the effect of peripheral chemoreceptors must dominate over that of central chemoreceptors in these situations (17, 21). The maturation of infant respiratory oscillations after a spontaneous sigh has been linked to peripheral chemoreceptor development (22), and it has been proposed that the emerging CO₂–O₂ interaction at the peripheral chemoreceptor level underlies the postnatal increase in hypoxic sensitivity (16).

We hypothesized that the peripheral chemoreceptors in healthy term infants undergo a postnatal increase in CO₂ sensitivity, resulting in changes in the time course of ventilatory responses to step increases in P_{CO_2} . Secondly, we hypothesized that during simultaneous exposure, effects of hypercapnia and hypoxia on ventilation would interact, and that such interaction could develop postnatally.

METHODS

Subjects. Twenty-six randomly selected infants (16 girls) were studied at 2 d and 8 wk postnatally. Inclusion criteria were healthy infant, birth weight ≥ 2500 g, Apgar scores ≥ 8 at 1 and 5 min, spontaneous vaginal delivery to term, and uncomplicated pregnancy. Exclusion criteria were any chronic maternal illness and alcohol or illicit drug abuse. Median birth weight was 3482 g (range, 2940–4390), gestational age was 39 wk 6 d (range, 38 wk 0 d–41 wk 2 d), and maternal age was 30.2 y (range, 22.3–41.3 y). Fourteen women were nonsmokers, seven women smoked ≤ 5 cigarettes/d, and five women smoked 10–20 cigarettes/d. Regional Ethics Committee approval and written informed consent from parents were obtained, and mothers were often present during experiments.

Procedures. Full details of this method of assessing chemoreceptor reflexes in infants have been published previously (23). The infants were studied during a daytime nap in a crib, sleeping in the supine position. Inspiratory gases were supplied through a lightweight, handheld face mask. Instantaneous switching between breathing gas mixtures of different composition was made possible by using computer-controlled electromagnetic valves. Gases were delivered dry at room temperature; the flow was 6.25 L/min. The 21-mL face mask would thus be filled 0.2 s after a switch in delivered gas, whereas the functional dead space of the system, estimated at 1.5 mL (23), would be filled almost instantly.

Repeated chemoreceptor challenges were presented in a block randomized sequence during a full sleep period. The test gases were hypercapnia (3% CO₂ in 21% O₂), hypoxia (15% O₂), and hypercapnic hypoxia (3% CO₂ and 15% O₂). Each test gas period lasted 15 s and was followed by a 75-s period during which medical air was delivered in the mask.

f_R , V_T , and $\dot{V}_E$ were calculated for each breath cycle from a pneumotachograph connected to the mask (23). Beat-by-beat heart rate was determined online from a three-lead ECG. Continuous measurements of CO₂ and O₂ immediately in front of the infants' nostrils were obtained by infrared spectroscopy and paramagnetic method, respectively (Artema Multigas Monitor MM201, Sundbyberg, Sweden; sampling rate 150 mL/min), and end-tidal values were estimated for each breath. Timed gas valve status was stored together with the physiologic data.

Data selection. Epochs of quiet sleep were selected on the basis of behavioral criteria. We discarded stimulation periods in which pneumotachograph recordings indicated that mask leakage had occurred, or in which a sigh occurred during the 20 s preceding stimulation.

Gas analysis measurements were inspected to ensure that inspired oxygen during hypoxic stimulation did not exceed 15% and that F_{iCO_2} during hypercapnic stimulation did not fall below 3%. This would happen if peak inspiratory flow exceeded the flow of test gas, resulting in dilution of the stimulus with room air. Some accepted responses included a single breath cycle (an exaggerated breath or a sigh) in which such dilution did occur, but only test gas was inhaled in the ensuing breath cycles.

Ventilatory response categories. For each experiment and test gas type, each ventilatory response was categorized as being 1) smooth, with a gradual rise and fall, 2) spiked, *i.e.* a smooth response with one or two exaggerated breaths at its maximum phase, or 3) sighlike, in which the breathing pattern seemed unaffected until a sudden sigh was elicited.

Quantitative analysis. Ventilatory and end-tidal gas data were converted to 5-Hz time series, and recordings in a time window from 50 s before to 150 s after the onset of each accepted stimulation period were aligned so that the onset of stimulus coincided before averaging. Time 0 was taken as the beginning of the first inspiration after a switch to test gas.

Coherently averaged $\dot{V}_E$, V_T , and f_R responses and the course of end-tidal CO₂ and O₂ values were calculated for each infant, age, and type of test gas. The averaged ventilatory responses were analyzed in a virtual instrument programmed in LabVIEW 5.0 (National Instruments Corp., Austin, TX, U.S.A.), as described previously (24). For illustration purposes, the coherently averaged $\dot{V}_E$, V_T , and f_R response of the entire group was also calculated for each stimulus–age combination (Figs. 1 and 2).

Quantification of stimulus. The delivery of test gas from mask to lung was considered to be shown by the change in end-tidal CO₂ and O₂ values from baseline to 2 s after stimulus start, which is too early for any chemoresponse to have occurred. The ensuing rate of change in end-tidal CO₂ and O₂ (in kilopascals per second) was computed using linear regression for the interval 2–6 s after stimulus start. Maximum stimulus strength was taken as the change in end-tidal values from baseline to plateau level, *i.e.* the median value in the period 10–15 s after stimulus start.

The rate of $\dot{V}_E$ increase was estimated using linear regression during the interval 2–10 s after start of stimulation, a period when the response should result almost solely from stimulation

Figure 1. Coherently averaged ventilatory responses from 26 term infants. Hypercapnia (black circles), hypoxia (line only), and hypercapnic hypoxia (open circles) lasted 15 s (gray box). Ventilatory response delay was approximately 2 s. Response rates increased with age, most markedly for hypoxia and hypercapnic hypoxia. Hypercapnic responses were slowest to reach half-maximum value and remained longest above baseline, especially on d 2. For all stimulus-age combinations, ventilation returned to prestimulus values well before the 20-s baseline period of the next stimulation.

of the peripheral chemoreceptors (21, 25). Data from the initial 2 s of stimulation was omitted because lung-to-chemoreceptor transportation time makes it unlikely that chemoreceptor responses would occur in this time interval. Linear regression on data from a fixed time interval does not differentiate between an age-related increase in the steepness of the response slope and a reduction in response delay with an unaltered response slope. We nevertheless chose this method because the low number of breaths in the time interval of study would introduce considerable uncertainty if, for example, we used a polynomial curve fit.

Interaction of CO_2 and O_2 effects was assessed by subtracting the rates of ventilatory increases in response to hypercapnia and to hypoxia from the rate of response to hypercapnic hypoxia. If the effects are purely additive, this calculation will return a value close to zero. Interaction of effects will result in a positive value.

Ventilatory response magnitude was quantified by three measures: 1) $\dot{V}_{E45}$, 2) $\dot{V}_{E\text{median}}$, and 3) $\dot{V}_{E\text{max}}$. The measures

Figure 2. Coherently averaged tidal volume and respiratory rate responses from 26 term infants. Hypercapnia (black circles), hypoxia (line only), and hypercapnic hypoxia (open circles) lasted 15 s (gray box). Most of the $\dot{V}_E$ response was caused by an increase in V_T . For hypercapnia and hypercapnic hypoxia, a reduction in stimulus-response time with increasing age was apparent. V_T returned to baseline shortly after the end of stimulation. The f_R response was more prolonged and contributed to the $\dot{V}_E$ response mainly on d 2, when it was identical during hypercapnia and hypercapnic hypoxia. A drop in f_R at the time of maximum response signifies the frequent occurrence of sighs. The f_R response to hypoxia was very small and is omitted for clarity.

of response magnitude were normalized with respect to the baseline value, *i.e.* the median value in the 20 s preceding stimulation.

Stimulus-response time was determined as the time from the onset of the first breath receiving test gas to the half-maximum $\dot{V}_E$ value. This point occurred on a steep part of the curve, making it a more robust measure than the onset of ventilatory increase or the time of maximum $\dot{V}_E$ response.

Before the maximum value and time-course of responses were assessed, high-frequency variation was removed from the signal with a 20-order low-pass Butterworth filter with a cutoff frequency of 0.20 Hz. Time distortion of the signal was avoided by running the data forward and then backward again through the filter. The maximum values and the time at which they occurred in the resulting filtered responses were determined automatically, the search being limited to the first 30 s after the start of stimulation. The value halfway between the baseline and the maximum value was computed, and its time of occurrence on the filtered curve was found. Plots of the unfil-

tered data and the filtered version with calculated time-points indicated were always inspected to ensure a sensible fit.

Statistical analysis. Values are medians with nonparametric near 95% confidence intervals, unless otherwise stated. For multiway analysis, we used a general mixed model of variance (BMDP 3V, release 7.0, 1993; BMDP Statistical Software Inc., Cork, Ireland), which uses maximum likelihood methods and allows repeated measures ANOVA to be applied to unbalanced data sets with missing observations. The magnitude and rate of change in end-tidal O₂ and CO₂ values for different test gas types and age groups were compared. The distribution of ventilatory response categories (smooth, spiked, or sighlike) was analyzed for effects of test gas type, postnatal age, and response magnitude expressed as $\dot{V}_{E\text{median}}$. The response rate and magnitude of ventilatory response (expressed as $\dot{V}_{E\ 45}$, $\dot{V}_{E\text{median}}$, and $\dot{V}_{E\text{max}}$) were analyzed for effects of test gas type and postnatal age. Stimulus–response time was analyzed for effects of test gas type, postnatal age, and $\dot{V}_E$ response magnitude.

RESULTS

Successful recordings during quiet sleep were obtained in all 26 infants on d 2 and in 20 of 26 infants in wk 8 (Table 1). The number of repeated responses obtained per experiment was similar for the three test gas types and was unrelated to age (Table 1). Mean $\dot{V}_E$, V_T , and heart rate during air breathing increased with age ($p < 0.01$), whereas f_R decreased ($p < 0.02$). Both V_T and f_R increased while test gases were being supplied ($p < 0.03$ for all comparisons; Fig. 2). V_T increased more than f_R when CO₂-containing test gases were supplied but not during hypoxia ($p < 0.01$ at both ages).

Quantification of stimulus. The initial (2 s) decrease in end-tidal Po₂ during hypoxia and increase in end-tidal PCO₂ during hypercapnia were slightly larger on d 2 than in wk 8 (Table 2; Mann-Whitney *U* test; $p < 0.05$ for both comparisons). A similar trend during hypercapnic hypoxia was not statistically significant.

The rate of fall in end-tidal Po₂ during the second through sixth seconds of stimulation increased from d 2 to wk 8, and the increase was similar for hypoxic and hypercapnic-hypoxic test gas [0.27 kPa/s (0.25–0.32) on d2, and 0.34 kPa/s

(0.32–0.37) in wk 8; Mann-Whitney *U* test, $p < 0.02$]. The rate of rise of end-tidal PCO₂ in the same time interval did not change with age and was similar for hypercapnic and hypercapnic-hypoxic test gas [0.06 kPa/s (range, 0.05–0.07 kPa/s)].

The baseline to plateau change in end-tidal values (Table 2) did not differ between age groups for any test gas. Hypoxia and hypercapnic hypoxia induced similar drops in end-tidal Po₂, whereas end-tidal PCO₂ increased slightly more during hypercapnia than during hypercapnic hypoxia (median difference, 0.13 kPa; Wilcoxon signed rank test $p < 0.01$).

Ventilatory response type distribution. Ventilatory response type distribution differed according to test gas type and postnatal age at the time of study ($p = 0.02$), but was unaffected by the magnitude of the ventilatory response ($\dot{V}_{E\text{median}}$). Age dependency was strong only for hypercapnic responses, during which a high frequency of spiked responses (30%) declined by two thirds from d 2 to wk 8 ($p = 0.078$), and sighlike responses declined from 5 to 1% ($p = 0.021$), whereas smooth responses became more frequent ($p = 0.007$). Hypoxia caused spiked or sighlike responses least often (12%) ($p < 0.035$), whereas hypercapnic hypoxia frequently produced spiked responses (24%) and more sighlike responses than the other stimuli ($p < 0.05$ for both comparisons). A positive correlation was found between the fractions of spiked responses during hypercapnic hypoxia and those during hypercapnia ($p < 0.001$).

Rate of ventilatory increase—interaction of hypercapnia and hypoxia. Overall, the rate of $\dot{V}_E$ response increased with age ($p < 0.001$). The magnitude of the age effect differed among test gas types ($p < 0.001$), the response rates to hypercapnia, hypoxia, and hypercapnic hypoxia increasing by 30%, 318%, and 302%, respectively (Table 3). The increase in the hypercapnic response rate was smaller because the rate was already relatively high on postnatal d 2, probably reflecting the high frequency of spiked responses.

The combined effect of hypercapnia and hypoxia on the rate of $\dot{V}_E$ increase was purely additive on d 2 (Fig. 3), the calculation rate during hypercapnic hypoxia minus the sum of rates during hypercapnia and during hypoxia returning a value that did not differ from zero [0.18 L/min² (range, –0.30 to 0.9 L/min²; Wilcoxon signed rank sum test $p > 0.20$]. In contrast, in wk 8, the rate during hypercapnic hypoxia exceeded the sum of the rates during single stimuli by 2.22 L/min² (range, 1.08 to 5.94 L/min²; $p < 0.01$). Thus, a more-than-additive interaction between the effects of hypercapnia and hypoxia on the initial rate of $\dot{V}_E$ increase had emerged.

Time course of ventilatory responses. Stimulus-response time, *i.e.* the time from onset of test gas to half-maximum $\dot{V}_E$ response, depended on both test gas type and postnatal age (Fig. 4). On postnatal d 2, the stimulus-response time during hypercapnia was 2.3 s longer than during either hypercapnic hypoxia ($p = 0.046$) or hypoxia ($p = 0.011$). However, by postnatal wk 8, the stimulus-response time during the two hypercapnic test gases had declined by 3.4 s, whereas the response time during hypoxia was unchanged ($p = 0.002$). The difference in age effect between test gas types was significant regardless of whether sighlike responses were included or excluded from the analysis. In a multiple regression analysis of

Table 1. Study age, baseline physiologic data, and responses

	Session 1	Session 2
Postnatal age (d)	2.1 (1.5–3.3)	57.3 (51.6–79.1)
Time since meal (min)	60 (10–150)	90 (20–170)
$\dot{V}_{E\text{baseline}}$ (L/min)*	0.88 (0.58–1.14)	1.04 (0.76–1.55)
V_T baseline (mL)*	19.9 (14.0–29.5)	30.9 (19.0–42.7)
f_R baseline (breaths/min)†	44.9 (28.8–63.2)	37.0 (29.9–51.5)
HR _{baseline} (beats/min)*	110.3 (83.4–141.0)	123.0 (106.1–148.7)
No. of responses§ to		
3% CO ₂	4 (1–9)	4 (1–7)
15% O ₂	4 (2–10)	5 (0–8)
3% CO ₂ + 15% O ₂	4 (2–9)	4.5 (1–8)

Values are medians and ranges. Difference between age groups: * $p < 0.001$; † $p < 0.02$, according to Mann-Whitney *U* test. § Accepted responses to repeated test gas periods in individual experiments, *i.e.* the number of single responses underlying each infant's coherently averaged response.

Abbreviation: HR, heart rate.

Table 2. Induced changes in end-tidal P_{O_2} and P_{CO_2} during stimulation

Gas	Age	Change in end-tidal P_{O_2} (kPa)		Change in end-tidal P_{CO_2} (kPa)	
		After 2 s	Plateau†	After 2 s	Plateau†
Hypercapnic hypoxia	d 2	-2.37	-4.29	0.85	1.24
	wk 8	-2.30	-4.20	0.75	1.17
Hypoxia	d 2	-2.41*	-4.41	-0.02	0.01
	wk 8	-2.08	-4.34	-0.01	0.08
Hypercapnia	d 2	-0.12	0.42	1.03*	1.34
	wk 8	-0.02	0.60	0.85	1.21

Values are medians calculated from coherently averaged time courses of end-tidal CO_2 and O_2 in 26 infants.

* Difference between age groups by Mann-Whitney U test ($p < 0.05$).

† Change from baseline to median value in the interval 10–15 s after the start of a 15-s test period.

Table 3. Ventilatory response rate and total ventilatory response to hypercapnic and hypoxic stimuli

Study age	Ventilatory response rate (L/min ²)		Increase in ventilation, integrated during 45 s (%)	
	d 2	wk 8	d 2	wk 8
Hypercapnia	1.50 (0.48–1.74)	1.95 (1.32–2.46)	13.5 (11.1–14.7)	13.4 (9.3–17.0)
Hypoxia	0.33 (0.24–0.60)	1.38 (0.66–1.80)	3.6 (1.7–8.9)	7.6 (6.4–9.9)*
Hypercapnic hypoxia	1.23 (0.72–1.92)	4.95 (3.18–6.54)	15.6 (14.9–20.8)	20.7 (17.4–24.9)†

Data from 26 term infants. Values are group medians and nonparametric, near-95% confidence intervals. Difference between age groups * $p = 0.09$, † $p = 0.036$. Response rate was calculated in the interval 2–10 s after the start of the 15-s stimulus.

Figure 3. Emergence of more-than-additive interaction between effects of hypercapnia and hypoxia on rate of ventilatory increase in 26 term infants studied at two ages. Boxes comprise 25th to 75th percentiles; 50th percentiles (median values) are marked. Bars show 10th and 90th percentiles. The points represent individual values of response rates during hypercapnia and during hypoxia subtracted from the response rate during hypercapnic hypoxia. In contrast to findings on postnatal d 2, the response rate during hypercapnic hypoxia clearly exceeded the sum of rates during hypercapnia and hypoxia in postnatal wk 8 ($p = 0.005$).

the effects of age, test gas type, $\dot{V}_E$ response rate, and $\dot{V}_{E\max}$ on stimulus-response time, the magnitude of the regression coefficients indicated that the age–test gas type interaction was independently responsible for approximately two thirds of the decline in stimulus–response time.

Magnitude of ventilatory responses. The increase in median ventilation after 15 s of hypercapnic hypoxia ($\dot{V}_{E\text{median}}$) was larger than the sum of $\dot{V}_{E\text{median}}$ during hypercapnia and $\dot{V}_{E\text{median}}$ during hypoxia, at both ages studied (Wilcoxon

Figure 4. Time from stimulus start to half-maximum (half-max) $\dot{V}_E$ response to hypercapnia, hypoxia, and hypercapnic hypoxia in 26 infants on postnatal d 2 (light gray) and in wk 8 (dark gray). Values were calculated in low-pass filtered coherently averaged responses from individual infants. Time to half-maximum response was longest during hypercapnia on d 2. By wk 8 it had decreased by 3.4 s for hypercapnia and hypercapnic hypoxia alike, whereas hypoxic response timing remained unchanged.

signed rank sum test; $p < 0.01$; Fig. 5). Thus, an interaction of effects of hypercapnia and hypoxia on early response magnitude was present. In contrast, the total ventilatory increase ($\dot{V}_E$ 45) in response to hypercapnic hypoxia equaled the sum of the total ventilatory increases to hypercapnia and to hypoxia (Table 3). With increasing age, the total response to hypercapnic hypoxia increased ($p = 0.036$), the total response to hypoxia tended to increase ($p = 0.09$), and the total response to hypercapnia was unchanged (Table 3). Neither $\dot{V}_{E\text{median}}$ nor $\dot{V}_{E\max}$ during hypercapnic hypoxia showed significant

Figure 5. Magnitude of ventilatory response to hypercapnia (gray), hypoxia (light scatter), and hypercapnic hypoxia (heavy scatter) at two ages. Columns show means, error bars show 95% confidence intervals. Responses to hypercapnia and to hypoxia are stacked to facilitate comparison with response to hypercapnic hypoxia. The $\dot{V}_{E\text{median}}$ value around the end of the 15-s stimulation increased with age for hypoxia and remained unchanged for hypercapnia. A positive interaction of effects of hypoxia and hypercapnia was present at both ages; $\dot{V}_{E\text{median}}$ for hypercapnic hypoxia exceeded the sum of responses to hypercapnia and to hypoxia by 4.1% (range, 1.0–10%; $p < 0.01$). $\dot{V}_{E\text{max}}$ value increased with age for hypoxia and tended to decrease for hypercapnia, but there was no significant change for hypercapnic hypoxia and no detectable interaction.

changes with age (Fig. 5). Underlying this finding were age-related increases in both $\dot{V}_{E\text{median}}$ ($p = 0.005$) and $\dot{V}_{E\text{max}}$ ($p = 0.043$) for hypoxic responses, but no change in $\dot{V}_{E\text{median}}$ and a downward trend in $\dot{V}_{E\text{max}}$ for hypercapnic responses.

DISCUSSION

The main findings in this study of ventilatory responses in healthy term infants were 1) a more-than-additive interaction of hypercapnic-hypoxic effects on ventilatory response rate emerging during the first two postnatal months, and 2) a marked age-related decrease in stimulus-response time during hypercapnic stimuli but not during hypoxia. Taken together, these could suggest a maturation of peripheral CO₂ chemoreception.

Methodological considerations. This study was designed to assess the combined respiratory effects of hypercapnia and hypoxia. Stimulus duration and analytical approach were selected to differentiate as clearly as possible between the effects of peripheral and central chemoreceptor stimulation. After carotid chemoreceptor denervation, neonatal lambs exposed to a step increase in F_{ICO_2} showed no ventilatory response during the first 10 s of stimulation (21, 25), and the (central) ventilatory response was markedly reduced for approximately 25 s (21). In our study, the measures of response magnitude and the time to half-maximum response reflected both peripheral and

central chemoreceptor effects. In contrast, ventilatory response rate was calculated from the initial 10 s of the test period, when it should be determined almost exclusively by peripheral chemoreceptors.

Age-related changes in infant respiratory pattern and mechanics may confound the interpretation when chemoreceptor characteristics are inferred from respiratory responses to changes in fraction of inspired oxygen and carbon dioxide. Evaluating the strength and time profile of stimuli through end-tidal gas values, we found the initial (2 s) hypoxia and hypercapnia to be slightly greater on postnatal d 2 than in wk 8, although plateau stimulation strength was similar. Alveolar ventilation per body weight was probably similar at the two ages, but the younger infants may have had lower functional residual lung capacity, giving a shorter equilibration time after a switch of gas type. The early rate of change in end-tidal values increased with age for P_{O_2} but was unchanged for P_{CO_2} , and neither depended on whether stimuli were given alone or in combination. Overall, it could not be demonstrated that the delivery of test gas to the pulmonary circulation was faster or more complete in older infants or during steeper or larger ventilatory responses.

Upper and lower airway reflexes probably modified the $\dot{V}_E$ responses. The spiked responses might represent what is called Head's paradoxical inflation-augmenting reflex, in which a V_T increase itself triggers an unusually large breath cycle. Sighlike responses, most frequent during hypercapnia on postnatal d 2, could be the result of stimulation of laryngeal CO₂ receptors initiating the first step of arousal (26).

Development of ventilatory chemoreceptor responses. For hypercapnic responses, both the median value after 15 s of stimulation and the total integrated response were identical on d 2 and in wk 8 (Fig. 5 and Table 3). Thus, as early as the immediate neonatal period, the combined action of peripheral and central chemoreceptors closely controls P_{aCO_2} and thus pH during transient mild hypercapnia. However, in 8-wk-old infants, the time needed for $\dot{V}_E$ to return to baseline level after stimulation was shorter (Fig. 1), possibly reflecting a higher degree of peripheral handling of the CO₂ challenge. As expected, the magnitude of response to hypoxia increased with age, and the magnitude of hypercapnic-hypoxic responses increased accordingly.

Interaction of the effects of hypercapnia and hypoxia on the magnitude of response ($\dot{V}_{E\text{median}}$) was detectable by postnatal d 2 (Fig. 5). For the $\dot{V}_E$ response rate, CO₂-O₂ interaction emerged between d 2 and wk 8 (Figs. 1 and 3 and Table 3). Although a noninvasive study does not make it possible to rule out central nervous contribution, the developing more-than-additive effect of CO₂ and O₂ was demonstrated for a variable for which it is likely that peripheral chemoreceptors were mainly responsible. A positive interaction between CO₂ and O₂ has been described at the peripheral chemoreceptor level in several species (7, 8, 16, 27). Moreover, selective perfusion studies in awake goats (28) and studies in piglets (17) and adult humans (29) indicate that the hypercapnic-hypoxic ventilatory interaction is mediated largely by the peripheral chemoreceptors, with only an additive effect of central CO₂ chemoreception.

Stimulus–response time was estimated by the time taken to reach the half-maximum $\dot{V}_E$ value. It would therefore increase with mask-to-lung-to-receptor transportation time, receptor and central nervous processing time, and value of maximum $\dot{V}_E$ response. A high $\dot{V}_E$ response rate would decrease stimulus–response time.

We found a 3.4 s decline in stimulus–response time with postnatal age for the two hypercapnic stimuli, but none for hypoxia. Regression coefficients indicated that approximately two thirds of this decline was independent of changes in $\dot{V}_{E\max}$ and $\dot{V}_E$ response rate; reduced central nervous processing time is unlikely to produce a change of this magnitude. No age-related reduction in mask-to-lung transportation time could be demonstrated, and in any case, this, like an age-related increase in cardiac output, would also have affected hypoxic responses. Thus, the isolated decline in stimulus–response time for hypercapnic responses is more readily explained by an increase in the contribution of peripheral CO_2 chemoreceptors relative to the central CO_2 chemoreceptors.

In neonatal piglets exposed to a square-wave increase in end-tidal CO_2 , the ratio of peripheral to total CO_2 chemosensitivity increased postnatally (15, 30). The estimated difference between peripheral and central chemoreceptors in lung-to-receptor transportation times was 2 s, and the peripheral $\dot{V}_E$ chemoresponse was extremely steep compared with the central response (estimated time constants were 1–3 s *versus* 60–70 s). Thus, an age-related increase in peripheral CO_2 chemosensitivity could have produced a reduction in the time taken to achieve half-maximum hypercapnic response of the magnitude observed in our study.

The timing of development cannot be determined exactly in a study with only two points of observation. However, in a previous study of 14 term infants studied 2 d and 4 wk postnatally (24), we found a decline in stimulus–response time to hypercapnia identical to that found in the present study (3.3 s *versus* 3.4 s; unpublished data). Thus, infant CO_2 chemosensitivity appears to undergo development during the first postnatal month. In various animal studies, the maturation of peripheral CO_2 chemosensitivity and CO_2 – O_2 interaction occurred from a few days to several months postnatally (7, 14–16, 20, 30).

It has been suggested that CO_2 – O_2 interaction at the level of the peripheral chemoreceptor or its innervation underlies the postnatal increase in O_2 sensitivity (16). In the present study, interaction between CO_2 and O_2 effects on the magnitude of the ventilatory response was present 36–79 h after birth, at a time when hyperoxic responses are clearly detectable (4). Moreover, interaction between CO_2 and O_2 effects on the $\dot{V}_E$ response rate emerged over the following weeks, a long-term development comparable to that of the hyperoxic responses observed in a previous study (4). However, several mechanisms may be responsible for the resetting of O_2 sensitivity. Further developmental studies, functional and biochemical and with a high “time resolution,” are warranted to clarify this matter.

CONCLUSIONS

In quietly sleeping healthy term infants, we found that the overall ventilatory handling of a hypercapnic challenge was fully functional on postnatal d 2, and CO_2 and O_2 interacted to increase the magnitude of the ventilatory response. However, during the following 8 wk, the ventilatory stimulus–response time to hypercapnic stimuli declined markedly, whereas a more-than-additive interaction between CO_2 and O_2 effects on ventilatory response rate emerged. We suggest that a developmental increase in the contribution of peripheral CO_2 chemoreceptors relative to the central CO_2 chemoreceptors may underlie our findings. Improved ability to detect and respond to transient changes in P_{CO_2} is important for infant respiratory stability, and may contribute to the age-related changes in breathing pattern seen in developing infants.

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REFERENCES

1. Blanco CE, Dawes GS, Hanson MA, McCooke HB 1984 The response to hypoxia of arterial chemoreceptors in fetal sheep and newborn lambs. *J Physiol (Lond)* 351:25–37
2. Hilaire G, Duron B 1999 Maturation of the mammalian respiratory system. *Physiol Rev* 79:325–360
3. Kumar P, Hanson MA 1989 Re-setting of the hypoxic sensitivity of aortic chemoreceptors in the newborn lamb. *J Dev Physiol* 11:199–206
4. Søvik S, Lossius K, Eriksen M, Grøgaard J, Walløe L 1999 Development of oxygen sensitivity in infants of smoking and non-smoking mothers. *Early Hum Dev* 56:217–232
5. Gauda EB, Lawson EE 2000 Developmental influences on carotid body responses to hypoxia. *Respir Physiol* 121:199–208
6. Guthrie RD, Standaert TA, Hodson WA, Woodrum DE 1981 Development of CO_2 sensitivity: effects of gestational age, postnatal age, and sleep state. *J Appl Physiol* 50:956–961
7. Carroll JL, Bamford OS, Fitzgerald RS 1993 Postnatal maturation of carotid chemoreceptor responses to O_2 and CO_2 in the cat. *J Appl Physiol* 75:2383–2391
8. Calder NA, Kumar P, Hanson MA 1997 Development of carotid chemoreceptor dynamic and steady-state sensitivity to CO_2 in the newborn lamb. *J Physiol (Lond)* 503:187–194
9. Haxhiu-Poskurica B, Carlo WA, Miller MJ, DiFiore JM, Haxhiu MA, Martin RJ 1991 Maturation of respiratory reflex responses in the piglet. *J Appl Physiol* 70:608–616
10. Rigatto H, Brady JP, de la Torre Verdusco R 1975 Chemoreceptor reflexes in preterm infants: II. The effect of gestational and postnatal age on the ventilatory response to inhaled carbon dioxide. *Pediatrics* 55:614–620
11. Frantz ID, Adler SM, Thach BT, Taeusch Jr HW 1976 Maturation effects on respiratory responses to carbon dioxide in premature infants. *J Appl Physiol* 41:41–45
12. Haddad GG, Leistner HL, Epstein RA, Epstein MA, Grodin WK, Mellins RB 1980 CO_2 -induced changes in ventilation and ventilatory pattern in normal sleeping infants. *J Appl Physiol* 48:684–688
13. Schafer T, Schafer D, Schlafke ME 1993 Breathing, transcutaneous blood gases, and CO_2 response in SIDS siblings and control infants during sleep. *J Appl Physiol* 74:88–102
14. Canet E, Kianicka I, Praud JP 1996 Postnatal maturation of peripheral chemoreceptor ventilatory response to O_2 and CO_2 in newborn lambs. *J Appl Physiol* 80:1928–1933
15. Wolsink JG, Berkenbosch A, DeGoede J, Olivier CN 1993 Maturation of the ventilatory response to CO_2 in the newborn piglet. *Pediatr Res* 34:485–489
16. Pepper DR, Landauer RC, Kumar P 1995 Postnatal development of CO_2 – O_2 interaction in the rat carotid body *in vitro*. *J Physiol (Lond)* 485:531–541
17. Wolsink JG, Berkenbosch A, DeGoede J, Olivier CN 1992 The effects of hypoxia on the ventilatory response to sudden changes in CO_2 in newborn piglets. *J Physiol (Lond)* 456:39–48
18. Daristotle L, Berssenbrugge AD, Bisgard GE 1987 Hypoxic-hypercapnic ventilatory interaction at the carotid body of awake goats. *Respir Physiol* 70:63–72
19. Masuda A, Ohyabu Y, Kobayashi T, Yoshino C, Sakakibara Y, Komatsu T, Honda Y 2001 Lack of positive interaction between CO_2 and hypoxic stimulation for $P(\text{CO}_2)$ -VAS response slope in humans. *Respir Physiol* 126:173–181
20. Wolsink JG, Berkenbosch A, DeGoede J, Olivier CN 1994 Ventilatory interaction between hypoxia and hypercapnia in piglets shortly after birth. *Respir Physiol* 96:25–35

21. Carroll JL, Canet E, Bureau MA 1991 Dynamic ventilatory responses to CO₂ in the awake lamb: role of the carotid chemoreceptors. *J Appl Physiol* 71:2198–2205
22. Fleming PJ, Goncalves AL, Levine MR, Woollard S 1984 The development of stability of respiration in human infants: changes in ventilatory responses to spontaneous sighs. *J Physiol (Lond)* 347:1–16
23. Søvik S, Eriksen M, Lossius K, Grøgaard J, Walløe L 1999 A method of assessing ventilatory responses to chemoreceptor stimulation in infants. *Acta Paediatr* 88:563–570
24. Søvik S, Lossius K, Walløe L 2001 Heart rate response to transient chemoreceptor stimulation in term infants is modified by exposure to maternal smoking. *Pediatr Res* 49:558–565
25. Carroll JL, Bureau MA 1988 Peripheral chemoreceptor CO₂ response during hyperoxia in the 14-day-old awake lamb. *Respir Physiol* 73:339–349
26. Lijowska AS, Reed NW, Chiodini BA, Thach BT 1997 Sequential arousal and airway-defensive behavior of infants in asphyxial sleep environments. *J Appl Physiol* 83:219–228
27. Lahiri S, Mulligan E, Nishino T, Mokashi A 1979 Aortic body chemoreceptor responses to changes in Pco₂ and Po₂ in the cat. *J Appl Physiol* 47:858–866
28. Daristotle L, Bisgard GE 1989 Central-peripheral chemoreceptor ventilatory interaction in awake goats. *Respir Physiol* 76:383–391
29. Yang F, Khoo MC 1994 Ventilatory response to randomly modulated hypercapnia and hypoxia in humans. *J Appl Physiol* 76:2216–2223
30. Wolsink JG, Berkenbosch A, DeGoede J, Olivier CN 1991 Ventilatory sensitivities of peripheral and central chemoreceptors of young piglets to inhalation of CO₂ in air. *Pediatr Res* 30:491–495