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Inhibitory effects of repeated hyperoxia on breathing in newborn mice

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ABSTRACT: Brief oxygen therapy is commonly used for resuscitation at birth or prevention of hypoxaemia before procedures during the neonatal period. However, O_2 may severely depress breathing, especially when administered repeatedly. The aim of the present study was to test the effects of repeated hyperoxia on breathing control in newborn mice.

A total of 97 Swiss mouse pups were assigned to O_2 or air on post-natal day 0, 1 or 2. Each pup in the O_2 group was subjected to four hyperoxic tests (100% O_2 for 3 min followed by 12 min normoxia), whereas pups in the air group were maintained in normoxia. Breathing variables were measured using flow-through barometric plethysmography.

O_2 significantly decreased minute ventilation as seen in a decrease in respiratory rate. This decrease became significantly larger with repeated exposure and ranged -17– -26% for all ages combined. Furthermore, hyperoxia increased total apnoea duration, as compared with the baseline value.

In newborn mice, repeated hyperoxia increasingly depressed breathing. This finding further supports a need for stringent control of oxygen therapy, most notably repeated oxygen administration in the neonatal period for premature newborn infants and those carried to term.

KEYWORDS: Neonate, oxygen, respiration

Oxygen is the most commonly used treatment at birth and is an integral part of the respiratory support provided in neonatal units. Approximately 5–10% of neonates require resuscitation at birth [1], and most of these neonates are born prematurely [2]. Furthermore, short periods of O_2 therapy are used in critically ill neonates to prevent hypoxaemia when mechanical ventilation is not available, especially during transfer to the neonatal intensive care unit and before lumbar puncture [3], tracheal suction [4, 5], bronchoscopy [6, 7] and bottle feeding [5, 8].

The use of 100% O_2 instead of room air has been challenged [1] based on studies showing cerebral blood flow reduction [9], generation of oxygen free radicals that cause or worsen brain injury [2, 10–12], increased rates of bronchopulmonary dysplasia [13] and retinopathy [14]. In addition, the inhibitory effects of hyperoxia on breathing may compromise oxygenation after O_2 administration, particularly in pre-term humans, who are more susceptible to apnoeas [15]. Although this inhibitory effect of hyperoxia may be considered minor compared to other adverse effects of oxygen therapy, it may become worrisome when O_2 is administered repeatedly, for instance to nonventilated pre-term infants with recurrent cyanosis after O_2 withdrawal. The present authors reasoned that repeated hyperoxia, which alternately inhibits and stimulates chemoreceptors (upon return to normoxia), may induce potentiation, which is commonly observed with a variety of respiratory stimuli such as hypoxia [16, 17]. For ethical reasons, these effects are difficult to investigate in human neonates.

The aim of the present study was to assess the inhibitory effects of repeated hyperoxia (administration of 100% O_2) on ventilation in newborn mice. Breathing variables were measured non-invasively in freely moving pups to mimic human infants. The newborn mice were tested from birth (post-natal day 0 (P0)) to P2. The post-natal resetting of peripheral chemoreceptors occurs during this period in mice [18]. P2 is a period of high vulnerability for white matter injury, which is a major consequence of hypoxia in the newborn brain, and corresponds to the high-risk period from 23–32 weeks post-conceptional age in humans [19].

METHODS

Animals

Mouse pups from Swiss female mice (IFFA-CREDO, L'Arbresle, France) were housed at 24°C with a 12-h/12-h light/dark cycle and fed

For editorial comments see page 4.

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ad libitum. The experimental protocols complied with the animal research guidelines established by the Institut National de la Santé et de la Recherche Médicale (French National Institute for Health and Medical Research). Pups were examined on the first day of life (P0), with a 12-h uncertainty regarding time from birth to testing, on P1 or on P2. At each age, the pups were randomly assigned to O_2 or air with a 2:1 ratio. The weight differences between the O_2 and air groups were not significant at any of the three ages. Two complementary experiments in 2-day-old pups were run.

Whole-body flow plethysmography

Respiratory variables in unrestrained newborn mice were measured noninvasively using whole-body flow barometric plethysmography as previously described [20]. The mice were left unrestrained since restraint may affect baseline ventilation and ventilatory responses to chemical stimuli, as previously shown in adult mice [21].

Movements were detected based on changes in the baseline respiratory signal, using a previously validated criterion:

$$(V_i - V_e) - (V_i + V_e) \quad (1)$$

where V_i and V_e, respectively, were the magnitudes of the inspiratory and expiratory limbs of the volume signal [20].

The plethysmograph was composed of two Plexiglas cylinders serving as the animal (40 mL) and reference (70 mL) chambers, immersed in a thermoregulated water-bath that maintained the temperature at 32.8°C. A 100-mL·min^{-1} flow of dry air (Hi-Tec airflow stabiliser; Bronkhorst, Uurlo, The Netherlands) was divided into two 50-mL·min^{-1} flows through the chambers, thus avoiding CO_2 and water accumulation. The differential pressure between the two chambers (transducer; DRUCK-EFFA, Asnières, France; range ± 0.1 mb) was filtered (bandwidth 0.05–15 Hz at -3 dB), converted to a digital signal (Instrunet model 200 14-bits converter; GW-Instruments, Somerville, MA, USA) at a sample rate of 100 Hz, and processed by custom-written software (Software Superscope II; GW-Instruments). The time constant of the pressure decay within the system (0.35 s) was measured by injecting 2 μL into the measurement chamber. This allowed measurement of breathing frequencies within the 0.5 Hz–10 Hz range at -3 dB. Calibration was carried out before each session by injecting 2 μL of air into the animal chamber from a microsyringe (Ito Corporation, Tokyo, Japan). The pressure rise induced by this injection was of similar magnitude to that induced by a pup. Body temperature was not continuously recorded during ventilatory measurements but was measured immediately after the plethysmographic recordings. Considering the limitations of flow barometric plethysmography in newborn mice, the absolute values of tidal volume (V_T) and minute ventilation (V'_E) presented here should be considered with caution, whereas the absolute total respiratory time (t_{tot}) values are valid.

Design

Each pup was tested once on P0, P1 or P2. At each age, the pups were randomly assigned to oxygen or air with ratios of 3:1 (P0 and P1) or 2:1 (P2). Mean ± SD weights in the O_2 group on days P0, P1 and P2 were: 1.49 ± 0.26 g (n=22); 1.72 ± 0.11 g (n=11); and 1.95 ± 0.27 g (n=26), respectively. In the air group, mean weights on days P0, P1 and P2 were: 1.62 ± 0.11 g (n=17); 1.74 ± 0.17 g (n=8); and 2.01 ± 0.28 g (n=13), respectively. The weight differences between the O_2 and air groups were not significant at any of the three ages.

The experimental design is summarised in figure 1. After ~3-min waiting time to allow adaptation to the chamber, baseline V'_E was recorded for 3 min. In the O_2 group, the airflow through the plethysmograph was then switched to a 100% O_2 flow for 3 min and back to air for the next 12 min. This sequence was repeated three times (total duration 63 min). The normoxic control pups were constantly maintained in normoxia in the plethysmograph to look for possible effects of isolation on breathing pattern, and for drifts in breathing variables. In preliminary experiments, it was ensured that breathing variables were identical when pups were exposed to continuous airflow from the hospital compressed air system or to alternation of compressed air from the hospital system and an air bottle (to mimic O_2 administration). Simultaneous measurements of breathing variables and body temperature were carried out in 3 additional pups on P2 (mean weight 2.20 ± 0.16 g).

Furthermore, two complementary experiments were run in 2-day-old pups. First, in a follow-up experiment, an independent sample of 10 pups (weight 1.84 ± 0.09 g) were exposed to an additional O_2 test 1 h after completing the 100% O_2 protocol (fig. 1b). Secondly, to examine the effects of repeated exposures to lower O_2 concentrations, an independent sample of 16 pups (2.13 ± 0.21 g) were exposed to 30% O_2, instead of 100% O_2 (fig. 1c).

Ventilatory response to hyperoxia

Breathing variables and apnoeas were determined using a recently developed automatic classification method [20]. Briefly, apnoeas were defined as ventilatory pauses longer than twice the duration of the preceding breath, whereas t_{tot} in s, V_T in μL·g^{-1}, and V'_E calculated as $V_T \cdot t_{tot}^{-1}$ and expressed in μL·s^{-1}·g^{-1} were calculated on apnoea-free periods. Breathing

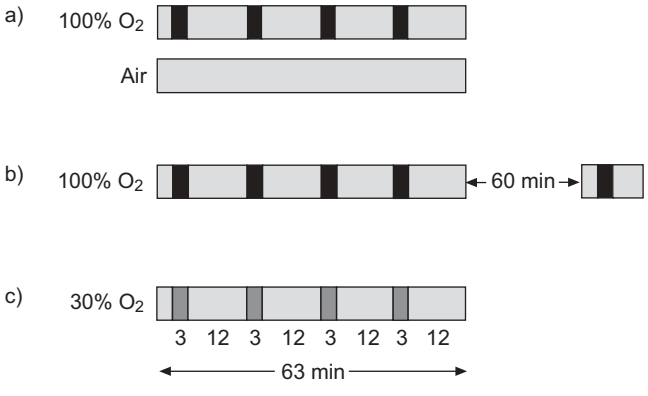

FIGURE 1. Study protocols. Breathing variables were recorded for 63 min. a) For the 100% O_2 group, after 3 min of normoxia (□), the gas in the plethysmograph was changed to 100% O_2 (■) for 3 min then returned to normoxia for 12 min. This cycle was repeated three more times (four cycles in all). In the air group, the pups were continuously maintained in normoxia. b) In an independent sample of pups, an additional 100% O_2 test was performed 1 h after completing the 100% O_2 protocol. c) An independent sample of pups was exposed to repeated 30% O_2 (■) instead of 100% O_2.

variables were averaged over consecutive 30-s periods. For each test, baseline values for these variables were calculated as the mean value over the 3 min of air exposure preceding the test (or the corresponding period in the air group).

The ventilatory response to hyperoxia was evaluated based on the maximal V'_E decline, *i.e.* the minimal V'_E value (min. V'_E) over the 3 min of O_2 exposure expressed as the percentage of the baseline level [22], according to the formula:

$$100 \times (\text{min. } V'_E\text{-baseline } V'_E)/\text{baseline } V'_E \quad (2)$$

This formula accounts for possible interindividual differences in the time course of the V'_E response to O_2. The V_T and the t_{tot} responses to O_2 were determined using the same formula with the values of V_T and t_{tot} corresponding to min. V'_E. The same calculations were calculated in the air group. This yielded a negative value for the V'_E response because min. V'_E was smaller than the sample mean.

Total apnoea duration was calculated as the total apnoeic time during each 3-min period, *i.e.*, before, during and after O_2 exposure in the O_2 group (or air in the air group). The apnoea response to O_2 was calculated as the difference between air and O_2; percentages could not be calculated, because some pups had no apnoeas during air breathing, *i.e.* had an apnoea duration of 0 s.

Statistics

Breathing variables and apnoeas were subject to ANOVAs with group (O_2 *versus* air) and age (P0, P1 and P2) as the between-subject factors and test (from 1–4) as the within-subject factor. To take into account the heterogeneous correlations among the repeated time measurements, the degrees of freedom were adjusted using the Greenhouse and Geisser factor, which is a conservative downward correction to the degrees of freedom [23]. Within-subject main effects and interactions are presented, together with p-values based on these adjusted degrees of freedom. When the interaction analysis was significant, contrast analyses were conducted to detect where the significant differences lay. Values are presented as mean±SD in the text and tables and mean±SEM in the figures.

RESULTS

Baseline breathing variables

Baseline V'_E levels were similar in the two groups (table 1). These values did not show any significant group or age effects. However, in both groups, a small but significant V'_E increase was found from the first to the fourth test ($p<0.0016$; the group differences were not significant). This drift was ascribable to a V_T increase (main effect for test: $p<0.0001$, not shown), whereas the corresponding change in t_{tot} was not significant (not shown).

Ventilatory response to hyperoxia

Large proportions of the 63-min recordings were free of movement artefacts in both the O_2 (mean±SD P0: 90±4; P1: 86±7; and P2: 92±3%) and the air group (P0: 92±4; P1: 90±5; and P2: 92±3%).

At all ages, on average over the four tests, hyperoxia compared to air caused a significant decline in V'_E (table 2). The V'_E decline in the O_2 group was chiefly ascribable to a t_{tot} increase, whereas changes in V_T were small (table 2). Because of the time needed to flush the chamber with O_2, the min. V'_E was achieved during the second 30-s period of O_2 exposure. The V'_E decline was reversed within a 3-min period upon return to normoxia: the analysis of post-hyperoxic values (calculated over successive 3-min periods from the return to normoxia) did not indicate significant differences between the O_2 group and the air group.

The comparison between the O_2 and air groups (~10% difference between groups on P0 and P1 and 20% on P2) showed a significant increase in the O_2-induced V'_E decline on P1 and P2. This effect was supported by a significant group-by-age interaction and by the between-group and within-group pairwise comparisons described in detail in table 2. The group differences were not accounted for by changes in baseline levels, which were small and similar in the two groups, as previously mentioned (table 1). Thus, a significant response to hyperoxia, consisting mainly of t_{tot} changes, was present on the day of birth and increased over the next 2 days. This effect was abolished by a return to normoxia at all ages and for all test numbers (data not shown). As for V'_E, reversal of the apnoea effect occurred within 3 min after the return to normoxia. Post-hypoxic values of apnoeas calculated over successive 3-min periods did not show significant differences between the O_2 group and the air group.

Finally, it was determined whether V_T and V'_E values were influenced by body temperature changes during hyperoxia. Body temperatures (measured in a separate sample of three pups subjected to the same protocol as the O_2 group) showed remarkable stability throughout the experiment. Individual ranges of temperature changes were 0.7, 0.5 and 0.7°C, respectively, and variation coefficients were 0.5, 0.3 and 0.4%, respectively.

Apnoeas

In normoxia, total apnoea duration was significantly longer on P1, compared to P0 and P2, with no significant difference between the O_2 and air groups (table 3). Hyperoxia significantly increased mean total apnoea duration at all ages (fig. 2), as compared to the pre-oxygen period (group-by-period interaction, $p<0.0001$; fig. 3). The increase in mean apnoea duration was not significantly affected by age (group-by-period interaction nonsignificant). When the combined mean values for all four tests were considered, the mean±SD increase in apnoea duration from air to O_2 was 8.7±10.8 s , 8.4±16.4 s and 5.0±8.9 s on P0, P1 and P2, respectively (nonsignificant differences). In the air group, there were virtually no changes at any of the three ages. The apnoea increase was reversed by the return to normoxia, at all ages and for all test numbers (data not shown).

Finally, neither the linear correlation coefficient nor the Spearman rank correlation between the total apnoea duration and the mean V'_E decline was significant overall or in any of the age groups.

Effect of repeated exposure to oxygen

The V'_E decline caused by O_2 (expressed as % of baseline) increased significantly with repeated exposure in the O_2 group

TABLE 1 Baseline minute ventilation (V'_E) in air before each 3-min exposure to 100% O_2

Age days	Baseline V'_E $\mu L \cdot g^{-1} \cdot s^{-1}$									
	O_2 group					Air group				
	Test 1	Test 2	Test 3	Test 4	Mean over tests	Test 1	Test 2	Test 3	Test 4	Mean over tests
0	9.7±3.4	10.4±3.6	10.6±3.6	10.5±3.4	10.3±3.5	10.3±2.6	10.0±2.5	10.4±2.7	10.5±3.4	10.3±2.8
1	11.1±3.7	11.1±2.5	12.3±2.7	13.1±3.3	11.9±3.1	9.2±4.4	9.5±3.3	9.8±3.3	10.0±3.2	9.6±3.5
2	10.1±2.6	10.6±2.6	10.7±3.0	10.6±2.8	10.5±2.7	9.9±3 .2	10.8±2.8	10.9±3.1	11.1±3.2	10.7±3.1
Mean	10.1±3.1	10.6±3.0	11.0±3.2	11.0±3.2	10.3±3.0	10.0±3.2	10.2±2.8	10.6±3.0	10.6±3.2	10.3±3.1

Data are presented as mean±SD. Baseline V'_E increased significantly from test 1–4 ($p<0.0009$), by ~9% in the O_2 group and 6% in the air group (nonsignificant difference). Group and age had no significant effects on V'_E, neither as the main effect or as an interaction with the remaining factors.

(group-by-test interaction, $p<0.0005$; fig. 3a). In the O_2 group, the V'_E drop was about 70% larger during the fourth test than during the first test, with no significant effect of age. The small changes in baseline normoxic V'_E did not account for the V'_E decline (table 2).

The increase in apnoea duration caused by O_2 exposure was significantly greater during the second than the first test in the O_2 group and remained elevated thereafter (group-by-test interaction, $p<0.03$, with no significant effect of age; fig. 3b). This increase was not ascribable to differences in baseline levels, which were similar for the first and second tests (12.5±6.9 s and 11.7±16.9 s, respectively; fig. 3b).

Follow-up

The protocol of the main experiment (*i.e.* four successive 100% O_2 tests) was replicated in a smaller, independent sample of 10 P2 pups, which were re-exposed to a follow-up test 1 h after completing the last O_2 test. This experiment confirmed the repetition effects on V'_E ($p<0.030$, effects on apnoeas were not significant). After 1 h, the V'_E decline was partially reversed, and its value was not significantly different from the baseline value on test 1 (fig. 4).

30% O_2 effects

To determine whether repeated exposure to lower concentrations of O_2 also magnified the V'_E decline, an independent sample of 16 P2 pups were exposed to the same protocol after replacing the 100% O_2 stimulus by 30% O_2. The V'_E declines were -22.1±14.1, -23.9±13.2, -24.2±7.1, and -22.2±13.3% from test 1–4, respectively. In contrast to the 100% O_2 tests, repeated exposure had no significant effect on the V'_E decline, which remained the same over the four tests. As with the 100% O_2 test, the V'_E decline was chiefly ascribable to an increase in t_{tot} (27.0±14.4% on average over the four tests), whereas changes in V_T were small -2.1±4.5%. The V'_E decline was slightly

TABLE 2 Peak changes in breathing variables in newborn mice exposed to 100% O_2 or air for 3 min

Age days	Test	V'_E			t_{tot}			V_T		
		100% O_2	Air	p-value	100% O_2	Air	p-value	100% O_2	Air	p-value
0	1	-13.0±8.6	-7.3±8.7	NS	13.9±11.8	9.2±8.1	NS	-0.9±11.3	2.4±8.6	NS
	2	-18.8±12.8	-7.9±4.8	0.0002	17.1±15.5	3.4±8.2	0.0064	-5.6±10.2	-3.1±8.0	NS
	3	-20.2±13.4	-13.0±8.6	0.0430	22.3±17.0	10.4±13.0	0.0134	-2.0±9.8	-3.8±6.6	NS
	4	-22.7±13.5	-11.0±7.2	0.0002	23.3±14.1	9.5±10.2	0.0110	-4.8±12.9	-1.6±7.7	NS
1	1	-23.9±17.2	-19.7±7.6	NS	16.5±22.1	12.7±10.9	NS	-6.1±19.8	7.2±2.5	NS
	2	-25.5±9.2	-16.4±5.7	0.0275	18.8±17.6	10.3±10.0	NS	-10.5±11.2	-5.0±9.7	NS
	3	-27.7±13.5	-15.8±10.0	0.0206	23.0±17.6	8.7±14.7	0.0396	-12.0±10.4	-5.6±6.9	NS
	4	-36.3±11.9	-14.6±10.2	0.0001	24.8±21.3	-9±15.1	0.0011	-20.1±7.5	-5.3±8.1	0.0010
2	1	-20.1±13.0	-7.2±8.3	0.0009	23.0±16.4	2.3±8.9	NS	-0.9±7.4	1.3±5;1	NS
	2	-27.3±6.5	-8.9±9.5	0.0001	31.4±20.3	0.1±10.7	0.0001	-4.1±9.4	5.0±16.7	0.0148
	3	-25.7±10.5	-4.9±7.1	0.0001	24.7±14.4	4.7±9.7	0.0001	-8.1±10.2	10.1±21.0	0.0001
	4	-29.2 ±8.8	-5.3±4.1	0.0001	31.4±22.0	-4.0±6.3	0.0001	-7.9±8.8	0.2±6.9	0.0124

Data are expressed as n or mean±SD and are a percentage of baseline values calculated over the 3 min of normoxia before each test. p-Values correspond to pairwise comparisons between the oxygen and air groups at a given age. All pairwise comparisons were conducted after checking that age-by-group interactions were significant for minute ventilation (V'_E; $p<0.0053$), total time of the respiratory cycle (t_{tot}) ($p<0.0004$), and tidal volume (V_T; $p<0.0248$).NS: nonsignificant.

TABLE 3 Mean baseline total apnoea duration before 100% O_2 or air

Age days	Baseline apnoea duration s									
	100% O_2					Air				
	Test 1	Test 2	Test 3	Test 4	Mean over tests	Test 1	Test 2	Test 3	Test 4	Mean over tests
0	14.4±21.1	13.1±16.3	12.2±18.3	13.1±18.0	13.2±18.2	4.0±4.6	9.8±7.7	10.2±10.2	10.6±9.4	8.6±8.5
1	21.9±14.0	20.0±16.0	17.0±12.3	20.1±16.4	19.8±14.3	17.8±19.3	28±22.4	22.3±26.9	25.7±25.2	23.4±23.2
2	7.0±11.8	7.1±16.9	7.9±17.0	9.5±19.9	7.8±16.4	3.0±4.0	2.9±4.7	1.1±1.5	3.2±5.4	2.6±4.1
Mean	12.5±16.9	11.7±17	11.2±16.9	12.8±18.7	12.1±17.3	6.6±11.0	11.3±14.6	9.6±15.6	11.3±16.0	9.7±12.1

Data are presented as mean±SD. Group differences were not significant. Values were significantly higher on P1 than on P0 ($p<0.0098$) and P2 ($p<0.0002$), in both groups.

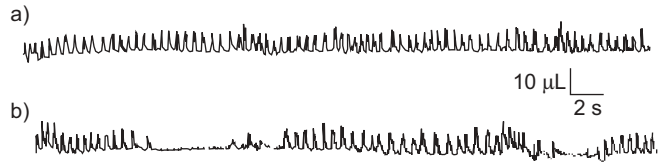

FIGURE 2. Representative respiratory traces in a 2-day-old mouse during a) air exposure and b) 100% O_2 exposure. At 30 s after the switch to O_2, apnoeas and a slight increase in breath duration occurred.

smaller than that caused by 100% O_2, but the difference was not significant.

The 30% O_2 stimulus slightly, but significantly, increased mean total apnoea duration at all ages, compared with the pre-oxygen period ($p<0.008$). The mean±SD increase in apnoea duration from air to O_2 was 1.9±4.9 s, 2.6±4.9 s, 1.5±4.0 s and 2.5±5.8 s from test 1–4, respectively. The repeated-exposure effect was not significant.

DISCUSSION

Repeated exposure to 100% O_2 was associated with increasing inhibitory effects of hyperoxia on V'_E, as a result of increases in t_{tot} and total apnoea duration, which is consistent with potentiating effects.

Methodological considerations

Whole-body plethysmography is the only method for measuring breathing variables in unrestrained newborn mice. This method has been validated against pneumotachography in larger animals but not in newborn mice, due to the lack of pneumotachographs designed for small animals. Furthermore, body temperature, which is inherent to equations for calculating V_T and V'_E, was not continuously measured during plethysmographic recordings, as this would have required restraining the pups. Thus, the absolute V_T and V'_E values in the present study should be considered with caution. However, these limitations do not invalidate the results, because the ventilatory depression caused by hyperoxia was explained by increases in t_{tot} and apnoea duration, two variables known to be reliably measured by plethysmography

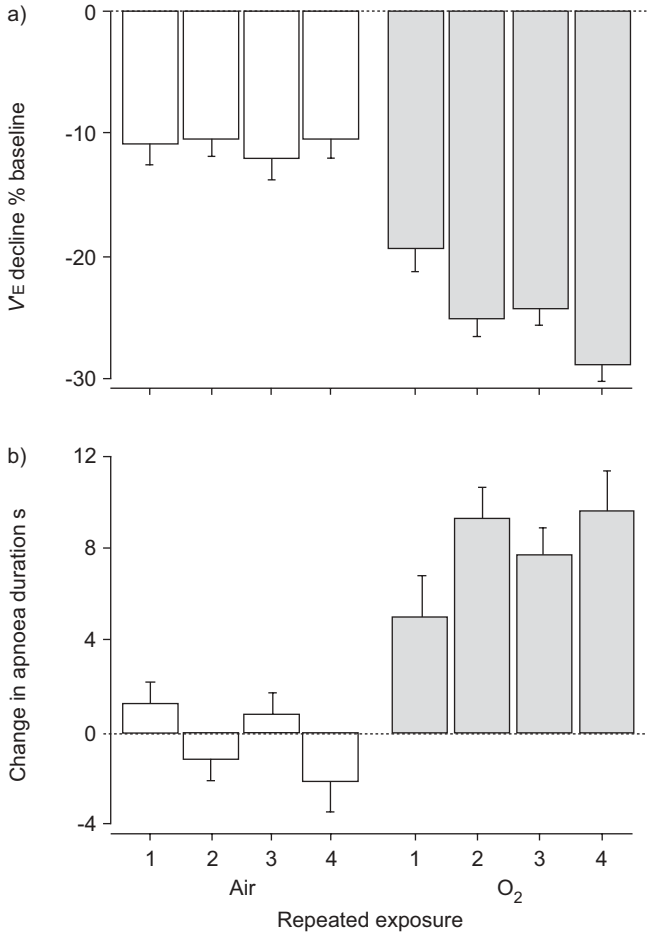

FIGURE 3. Effects of repeated 3-min O_2 exposures on minute ventilation (V'_E) and apnoea duration. Data are presented as mean±SEM and were averaged over the age groups. a) V'_E drop expressed as a percentage of baseline calculated over the 3-min period of normoxia preceding O_2 exposure. The V'_E drop increased significantly from one test to the next in the O_2 group (test 1 *versus* test 2, $p<0.0093$; test 3 *versus* test 4, $p<0.0112$). b) Apnoea duration increase from baseline calculated over the 3-min period of normoxia preceding O_2 exposure. The increase in total apnoea duration was significantly more marked after the first test in the O_2 group (test 1 *versus* test 2, $p<0.0397$). Repeated-exposure effects on V'_E and apnoeas were not influenced by age.

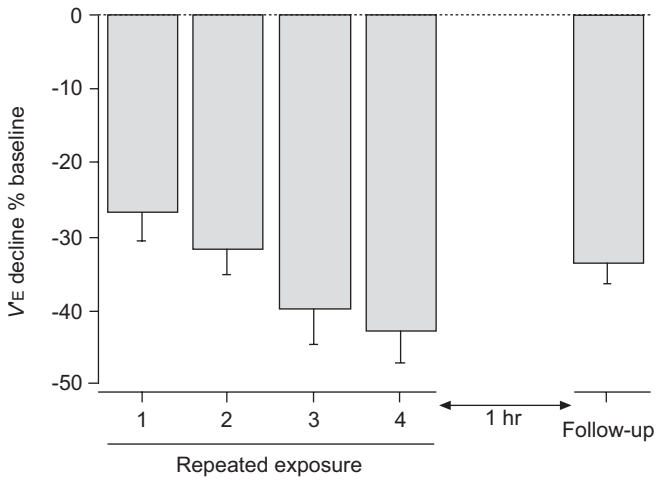

FIGURE 4. Resolution of repeated-exposure effects. Two-day-old pups were exposed to a follow-up test 1 h after completing the final 100% O_2 test. The minute ventilation (V'_E) decline induced by this test was not significantly different from baseline values on test 1.

[20]. Furthermore, the body temperatures measured in three animals during plethysmography showed very small variations.

To evaluate the ventilatory response to hyperoxia, a min. V'_E over the 3-min O_2 exposure period was used instead of the mean decrease over the total O_2 exposure period [22]. Min. V'_E during hyperoxia is thought to depend only on suppression of the peripheral chemoreceptor drive. However, the increase in metabolism caused by hyperoxia [24] may elicit excitatory inputs to breathing that may partially counteract the ventilatory fall caused by peripheral chemoreceptor silencing. Thus, the mean V'_E value calculated over the entire hyperoxic period may confound excitatory and inhibitory inputs to breathing. Furthermore, the current method for assessing the ventilatory response to hyperoxia accounted for possible interindividual differences in the timecourse of the V'_E response to O_2.

The study period (P1–P3) encompassed major post-natal changes in respiratory sensitivity to hypoxia. Previous studies showed that significant changes in ventilatory responses to chemical stimuli take place around 12 h after birth in mice [18]. In human infants, post-natal resetting of peripheral chemoreceptors occurs within days to weeks after birth [25]. The effects of hyperoxia beyond this critical period were not examined in the present study. Oxygen causes ventilatory depression in mature mammals [26], and may yield similar potentiation effects to those found here. However, the present study focused on the early post-natal period during which ventilatory depression may be aggravated by other sources of respiratory instability, in particular immaturity of the central chemoreceptors [27].

Development of the hyperoxic response

Hyperoxia induced depression of ventilation in the present study, as previously reported in mouse pups 1–2 days after birth [28], as well as in other newborn mammals including humans [29]. The present results establish that the V'_E decline is present on P0 and is accompanied by apnoeas. A previous study has suggested that the post-natal resetting of peripheral chemoreceptors occurs within 6–12 h after birth in mice [18]. This maturation effect may account for the increase in the V'_E decline after P0 in the present study.

The V'_E decline in the O_2 group was chiefly ascribable to a t_{tot} increase, whereas V_T changes were small. In a study of human pre-term infants, the V'_E decline after 3 min of O_2 exposure was due to a significant decrease in respiratory rate with little or no change in V_T on P2, whereas the opposite breathing pattern changes were found on P6 [15] and in term infants during P2–P6 [29]. Taken together, these results suggest that the breathing strategy during hyperoxia undergoes rapid developmental changes and that, in this respect, newborn mice resemble pre-term infants shortly after birth. This similarity further supports the validity of newborn mice as a model for studying early breathing disorders in pre-term human infants and at a lesser stage in neonatal period in term.

Effects of repeated O_2 exposure

The V'_E decline and total apnoea duration tended to increase with repeated 100% O_2 exposure, whereas both V'_E and apnoea duration returned to baseline levels between 100% O_2 exposures. These effects were transient; after 1 h, re-exposure to 100% O_2 did not induce a significantly larger decline in V'_E compared to the first test.

Finally, the increase in ventilatory depression observed with repeated 100% O_2 was not found with a lower O_2 concentration (30%). Exposure to 30% O_2 significantly inhibited ventilation, but this effect did not become stronger with repeated exposure.

Repeated hyperoxia was associated with alternating inhibition (during hyperoxia) and stimulation of peripheral chemoreceptors (upon return to normoxia). However, the effects shown in the current study departed from potentiation effects induced by repeated hypoxia in that the V'_E changes induced by repeated hyperoxia did not outlast the exposure to the stimulus [16]. Sustained changes in metabolic rate from one test to the other may also contribute to the increase in ventilatory depression with repeated hyperoxia. Hyperoxia has previously been found to increase O_2 consumption and CO_2 production in newborn mammals [24, 30]. However, the rapid return of V'_E and apnoea duration to pre-O_2 baseline levels upon return to normoxia after each O_2 exposure in the current experiments militates against a sustained increase in metabolic rate.

Conclusion

Repeated O_2 administration exacerbated O_2-induced ventilatory depression by increasing the minute ventilation decline and apnoea duration in newborn mice. These findings support that systematic preventive oxygenotherapy should be avoided. They may indicate a need to revise current resuscitation strategies for premature infants. Other studies are warranted to determine the age at which this detrimental effect disappears.

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