Hering-Breuer reflex in conscious newborn rats: effects of changes in ambient temperature during hypoxia

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Merazzi, Daniele, and Jacopo P. Mortola. Hering-Breuer reflex in conscious newborn rats: effects of changes in ambient temperature during hypoxia. J. Appl. Physiol. 87(5): 1656–1661, 1999.—In a previous study in conscious normoxic newborn rats, we found that the strength of the Hering-Breuer reflex (HB reflex) was greater (188%) at high (36°C) than at low (24°C) ambient temperature (T_a). D. Merazzi and J. P. Mortola. Pediatr. Res. 45: 370–376, 1999). We now asked what the effect would be of changes in T_a during hypoxia. Rat pups at 3–4 days of age were studied in a double-chamber airflow plethysmograph. The HB reflex was induced by negative body surface pressures of 5 or 10 cmH_2O and quantified from the inhibition of breathing during maintained lung inflation. Rats were first studied at T_a = 32°C in normoxia, followed by hypoxia (10% O_2 breathing). During hypoxia, oxygen consumption (V_O_2) averaged 47%, and HB reflex increased by 115%, of the corresponding normoxic values, confirming that in the newborn, differently from the adult, hypoxia does not decrease the strength of the HB reflex. As hypoxia was maintained, lowering T_a to 24°C or increasing it to 36°C, on average, had no significant effects on V_O_2 and the HB reflex. However, with 5-cmH_2O inflations, the HB reflex during the combined hypoxia and hyperthermia was significantly stronger than in normoxia. We conclude that in conscious newborn rats during normoxia the T_a sensitivity of the HB reflex is largely mediated by the effects of T_a on thermogenesis and V_CO_2; in hypoxia, because thermogenesis is depressed and V_O_2 varies little with T_a, the HB reflex is T_a independent. The observation that the reflex response to lung inflations during hypoxic hyperthermia can be greater than in normoxia may be of importance in the pathophysiology of apneas during the neonatal period.

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METHODS

Experiments were performed in 85 newborn Sprague-Dawley rats from 20 litters, at 3 and 4 days of age (day 0 = day of birth), in either the morning or afternoon. Experiments were approved by the Animal Ethics Committee of McGill University.
University. The pregnant dams had free access to food and water and were maintained in individual cages at Ta between 20 and 25°C, relative humidity of 50–53%, and a 12:12-h dark-light cycle.

The main group of rats was used for measurements of the HB reflex. Separate groups of rats were used for measurements of body temperature (Tb) and gaseous metabolism. These measurements were performed at Ta of 32°C, which is slightly below thermoneutrality for rats of this age (14), first in normoxia for 20 min, followed by 20 min in hypoxia. Measurements were then continued for a third period of 20 min, with hypoxia maintained, with one-half of the rats at Ta = 24°C ("cold") and the other one-half at Ta = 36°C ("warm"). Numbers of animals, or sets of animals in the case of metabolic measurements, used for each experiment are given in Table 1 and in the pertinent sections of RESULTS.

HB reflex. The animal was placed in the rear chamber of a double-chamber plethysmograph, identical to that previously adopted (11, 12). The animal's head emerged into the front chamber by passing through multiple layers of paraffin sealing film (Parafilm), which provided complete separation of the rear from the front chamber. The front chamber (−30 ml) was for measuring the airflow (V˙) via a small pneumotachograph connected to a differential pressure transducer (Validyne, Northridge, CA). Tidal volume (VT) was obtained by electronic integration of the V signal. A steady flow of 80 ml/min, controlled by a flowmeter, was continuously delivered through the anterior chamber, to avoid any accumulation of expired air. This flow consisted of either air, for the normoxic runs, or hypoxia, obtained by connecting the anterior chamber to an anesthesia bag filled with a calibrated 10% O₂ gas mixture.

The rear chamber (−30-ml volume) was for the application of negative body surface pressures (P; i.e., positive trans-respiratory system pressures). To this end, one opening could be rapidly switched to a vacuum source by turning a stopcock. A second opening was connected to a polyethylene tube, the resistance of which was adjusted to obtain the desired P during application of the vacuum. P was continuously monitored by a Validyne pressure transducer. The three signals (V, Vt, P) were recorded on paper (Gould pen recorder) at a speed of 25 mm/s (Fig. 1). Temperature was measured in both the front and rear chambers by a small tungsten-constantan thermocouple (DP30, Omega, Stamford, CT).

The pup was placed in the setup, with the chambers preset at 32°C by means of a large-size heating lamp. After 5 min, negative pressures of 5 or 10 cmH₂O were applied in random order for the next 15 min, at −1-min intervals during quiet breathing. Each pressure was maintained for a few seconds, until the first inspiratory effort after lung inflation (Fig. 1). Hypoxia (10% O₂) was then initiated, and, after 5 min, the inflation maneuvers were repeated for the following 15 min. With hypoxia always maintained, Ta was then changed to either 36°C (warm; n = 20) or 24°C (cold; n = 19) by adjusting the distance of the heating source or cooling the animal chamber with ice-wet pads, respectively. In either case, Ta reached the desired new value within 5 min, at which time the inflation maneuvers were once again repeated for the following 15 min. Therefore, the total experiment lasted 60 min, a duration that, by itself, does not determine any significant change in the HB reflex (12). At the end of the experiment, Tb was measured and the pup was returned to the dam.

Inflations were performed during the inspiratory phase of the breathing cycle (23% of the cases) or during the first (15%), middle (25%), or last one-third (37%) of the expiratory phase, with no significant difference in this distribution among experimental conditions. As P was applied, lung volume increased and breathing temporarily stopped until the chemical drive was strong enough to overcome the vagal inhibition. When gross body movements occurred during the inflation they were readily recognizable, and these runs were discarded. On average, for each inflation pressure within each experimental condition, four runs were analyzed. The total time from the beginning of the expiration immediately before the application of P to the onset of the next inspiration during the maintained inflation (Tεinfl) was measured (Fig. 1), and the HB reflex was quantified as the "inhibitory ratio" (IR), i.e., the ratio between Tεinfl and the average expiratory time (Tε) of the five breaths preceding the inflation (12). This analysis was performed by use of a graphics tablet connected to a minicomputer.

Gaseous metabolism. VO₂ and VCO₂ were measured with an open-flow system, with a setup and a methodology similar to that previously adopted (12, 14). Rat pups were studied in sets of four animals, with separators to impede huddling, the magnitude of which influences metabolic rate and is affected by hypoxia (15, 22). The pups were in the metabolic chamber,

### Table 1. Number and body weight of animals for each experimental condition

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>Weight, g</th>
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<tbody>
<tr>
<td>Hering-Breuer reflex</td>
<td></td>
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<tr>
<td>Normoxia, hypoxia, hypoxia at Tₐ = 24°C</td>
<td>19</td>
<td>9.7 ± 0.3</td>
</tr>
<tr>
<td>Normoxia, hypoxia, hypoxia at Tₐ = 36°C</td>
<td>20</td>
<td>9.8 ± 0.2</td>
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<tr>
<td>Body temperature</td>
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<tr>
<td>Normoxia, hypoxia, hypoxia at Tₐ = 24°C</td>
<td>5</td>
<td>9.4 ± 0.3</td>
</tr>
<tr>
<td>Normoxia, hypoxia, hypoxia at Tₐ = 36°C</td>
<td>5</td>
<td>9.4 ± 0.3</td>
</tr>
<tr>
<td>Gaseous metabolism (no. of sets, 4 pups in each)</td>
<td>10</td>
<td>9.7 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of animals; Tₐ, ambient temperature. All animals were 3 or 4 days old.
which was preset at $T_a = 32^\circ C$; 20 min in hypoxia followed, and an additional 20 min of hypoxia in cold or warm conditions. Gases were passed through a drying column, sampled by appropriate gas analyzers for measuring the O$_2$ and CO$_2$ concentrations, and the values were continuously displayed on a computer monitor. VO$_2$ and VCO$_2$ were computed over several minutes as the product of the inflow-outflow gas concentration difference and the steady gas flow (235 ml/min) delivered through the chamber. Metabolic data are presented normalized by the weight (in kg), at STPD conditions. At the end, Tb was measured in each pup.

Effects of changes of $T_a$ on Tb. The animals were placed in the double-chamber plethysmograph, preset at $32^\circ C$, used for the measurements of the HB reflex. Rectal temperature was measured with a very fine tungsten-constantan thermocouple inserted about 15 mm in the rectum, and its value was taken as representative of Tb. $T_a$ and Tb measurements were registered every 2 min for the entire 1-h experiment, i.e., 20 min in normoxia, 20 min in hypoxia, and an additional 20 min in hypoxia in either warm or cold conditions.

Statistical analysis. Data are presented as group means ± SE. The statistical significance of differences between two groups of data from the same animals was evaluated by two-tailed paired t-test. Comparisons between normoxia and hypoxia at each of the three temperatures ($32$, $24$, and $26^\circ C$) were performed, first, by ANOVA; post hoc Bonferroni limitations were then applied to assess the statistical significance for the four comparisons of interest, i.e., each of the three hypoxic conditions vs. normoxia, and hypoxia-cold vs. hypoxia-warm. In all cases, a significant difference was considered at $P < 0.05$.

RESULTS

HB reflex at $32^\circ C$: normoxia and hypoxia. As expected (28), the IR was greater at 10 than at 5 cmH$_2$O inflation pressures. On average, the IR value in hypoxia was 115 ± 5% of normoxia, a difference that was not statistically significant. When the two inflation pressures were individually considered, IR at 5 cmH$_2$O was significantly greater in hypoxia than in normoxia ($7.2$ vs. $6.1$, $P < 0.05$, Fig. 2), whereas at the higher pressure IR did not differ significantly ($14.7$ vs. $14.6$).

HB reflex in hypoxia: effect of changes in $T_a$. During hypoxia, the effect of changes in $T_a$ on the HB reflex varied depending on the inflation pressure (Fig. 2). At 5 cmH$_2$O inflation, the reflex was significantly stronger in the warm than in the cold condition. In addition, in the warm condition, the reflex was stronger than during the control normoxic phase. At 10 cmH$_2$O inflation, warming tended to have an opposite effect, i.e., that of reducing the strength of the reflex in comparison to cold, although it did not differ significantly from normoxia. On average, therefore, changes in $T_a$ during hypoxia had minimal and insignificant effects on IR, which at $36^\circ C$ averaged only 2% more than at $24^\circ C$.

In summary, IR during hypoxia was slightly greater than during normoxia at $32^\circ C$ (Fig. 3, solid symbols) and by approximately the same amount at various $T_a$ (+12% at $24^\circ C$, +15% at $32^\circ C$, +14% at $36^\circ C$). None of these changes, individually taken, reached statistical significance; only when all the results at the three $T_a$ were combined did the overall effect of hypoxia indicate a significant increase in IR, on average by $14 ± 3\%$ ($P < 0.01$). For comparison purposes, Fig. 3 also presents the data previously obtained in same-age rats during normoxia (12); with warming, IR increased, and in the cold condition it decreased, compared with the value at $32^\circ C$, and at $36^\circ C$ it averaged 188% of the value at $24^\circ C$.

Gaseous metabolism. At $32^\circ C$, VO$_2$ in normoxia averaged $55 ± 3$ ml·kg$^{-1}$·min$^{-1}$; during hypoxia it dropped to $26 ± 1$ ml·kg$^{-1}$·min$^{-1}$ ($P < 0.01$), or ~47% of normoxia. This hypoxic value was not significantly altered by changes in $T_a$. In fact, in cold and in warm conditions, VO$_2$ averaged $24 ± 2$ and $28 ± 2$ ml·kg$^{-1}$·min$^{-1}$, respectively, with no significant difference between the two (Fig. 4).
Effects of changes of $T_a$ on $T_b$. At $T_a = 32^\circ C$, $T_b$ was similar between normoxia and hypoxia ($33.5 \pm 0.3$ and $34.3 \pm 0.3^\circ C$, respectively). At the end of the warm and cold hypoxic phases, $T_b$ averaged $37.2 \pm 0.2$ and $29.7 \pm 0.6^\circ C$, respectively (Fig. 5, “basal measurements,” ○). Similar $T_b$ values were recorded after the measurements of metabolic rate and HB reflex (Fig. 5, filled symbols).

**DISCUSSION**

The results of this study confirmed that, in conscious newborn rats, hypoxia does not reduce the strength of the HB reflex. They also indicated that during hypoxia temperature has very little effect on the intensity of the reflex. This latter result is in sharp contrast to the temperature sensitivity of the HB reflex during normoxia (Fig. 3).

During the apnea of the maintained lung inflation, oxygen is consumed and carbon dioxide is produced at a rate that depends on the metabolic processes. The animal's chemosensitivity eventually dictates the level of arterial hypoxia and hypercapnia at which the chemical drive overcomes the inhibitory inputs from the pulmonary stretch receptors. Hence, the HB reflex, quantified as the apnea during maintained lung inflation, reflects the balance between the stretch receptors' inhibition on breathing and the stimulation of breathing by the increasing chemical drive. The former depends on the magnitude of lung inflation and the degree of adaptation of the stretch receptors. The latter depends on chemosensitivity and metabolic rate. The interplay of these factors has been studied in conscious and anesthetized adult animals. A depression of chemosensitivity or a reduction in metabolic rate, as during anesthesia, increases the strength of vagal reflexes (1, 7, 19, 20). On the other hand, stimulation by hypoxia or hypercapnia reduces the HB reflex (1). In a previous study in conscious newborn rats at $T_a = 32–34^\circ C$, the strength of the HB reflex was reduced by hypoxia at postnatal day 8, whereas it was not affected or even slightly increased in younger rats (11). Similarly, in the 3- and 4-day-old rats in the present study, hypoxia did not lessen, but, in fact, slightly increased, the strength of the HB reflex. The insensitivity of the HB reflex to hypoxia in the early neonatal period probably reflects the combination of the low ventilatory chemosensitivity and the hypometabolic response to hypoxia (4, 9, 13).

During hypoxia, increasing $T_a$ to 36°C or lowering it to 24°C had insignificant effects on the HB reflex, which, at either of the above $T_a$, averaged approximately those at 32°C (Fig. 3, solid symbols). This lack of sensitivity to changes in $T_a$ during hypoxia differs markedly from what has been previously observed in rats of the same age during normoxia (12), where the HB reflex significantly decreased in cold and increased in warm conditions (Fig. 3, open symbols). In fact, during normoxia at 36°C, IR averaged 188% of the value at 24°C, whereas during hypoxia at 36°C it was only 102% of, and not significantly different from, the value at 24°C. Hence, in the newborn rat, the temperature sensitivity of the HB reflex depends on the level of oxygenation, being very pronounced in normoxia and absent in hypoxia. Several factors, and their interplay, are likely to be responsible for this difference.

The $T_b$ values presently observed in the hypoxic rats (Fig. 5) in cold and warm conditions are very similar to those previously measured in warm and cold conditions during normoxia (12). The changes in $T_b$ with $T_a$ and their similarities between normoxia and hypoxia are not surprising. In fact, in small newborn mammals, including the rat, $T_b$ is of overwhelming importance in determining $T_b$, and the presence (i.e., in normoxia) or

**Fig. 4.** Oxygen consumption and carbon dioxide production at 32°C in normoxia (open symbols) and during hypoxia (solid symbols) at 32, 36 (warm), and 24°C (cold). In each condition, $n = 10$ sets of 4 pups each. Bars, SE. *Significantly different from hypoxia, $P < 0.05$.

**Fig. 5.** Average body temperature of rat pups at 3–4 days of age, during hypoxia at ambient temperature of 32°C ($n = 10$), and in hypoxia at 24 (cold; $n = 5$) or 36°C (warm; $n = 5$). For both cold and warm conditions, mean body temperature values immediately after end of measurements of metabolic rate ($n = 15$) and Hering-Breuer reflex ($n = 19–20$) are also indicated. Oblique line, identity line; bars, SE. *Significantly different from 32°C, $P < 0.05$. ‡Significantly different from 24°C, $P < 0.05$. Downloaded from http://ajp.physiology.org/ by 10.220.22.247 on April 20, 2017
absence (i.e. in hypoxia) of the metabolic response to cold does not make any appreciable difference to the effect of $T_a$ on $T_b$ (14). The fact that, at the various $T_a$, $T_b$ was approximately the same between normoxia and hypoxia, whereas the HB reflex was very different, should imply that $T_b$ is not an important parameter in determining the strength of the HB reflex. This is surprising. In fact, temperature influences neuronal activity and could affect the HB reflex at multiple sites of the reflex loop, including the stretch receptors’ sensitivity to lung inflation (2, 25) and the central effectiveness of vagal inputs (3, 5, 8, 29). Changes in temperature modify the breathing pattern and its effect on the vagal reflexes (5, 6). One might postulate that $T_b$ in the normoxic newborn, as in the adult, also has a direct effect on the intensity of the HB reflex and that this effect is shadowed by the large changes in metabolic level, which was very high in the cold and low in the warm condition. This interpretation, however, would not hold during hypoxia; in fact, hypoxia, the metabolic level and the strength of the HB reflex were essentially constant and independent of temperature, despite the large changes in $T_a$. Hence, it seems reasonable to conclude that in the conscious newborn rat the effects of cooling and warming temperatures on the strength of the HB reflex cannot be attributable to the changes in $T_b$ per se, but, rather, to the corresponding changes in metabolic rate. In normoxic conditions cooling stimulates and warming lowers metabolic rate, and the reflex becomes weaker and stronger, respectively. With hypoxia, the chemical stimuli from the peripheral chemoreceptors should reduce the strength of the HB reflex compared with normoxia, as it happens in adults (1, 20), but the drop in metabolic level offsets this effect. Finally, during hypoxia, because the metabolic responses to changes in temperature are suppressed, ambient cooling and warming have negligible effects on the intensity of the reflex.

The combined analysis of the results at the two inflation pressures revealed a small trend for the HB reflex to increase in hypoxia, independently of temperature. However, when the effects of raising $T_a$ were separately analyzed at each of the two inflation pressures, a significant trend emerged indicating an increase in the reflex response to small inflations, and a slight drop with large inflations (Fig. 2). Airway tension is the stimulus activating the stretch receptors (23), and changes in the mechanical properties of the airways with temperature may have altered the pressure-tension relationship, but it is difficult to see how this could have resulted in opposite effects at the two inflation pressures. It is more likely that the higher pressures also stimulated the rapidly adapting irritable receptors, which are facilitatory in breathing (28), hence reducing the inhibition from the stretch receptors.

The observation that in the newborn rat hypoxia not only did not decrease but also could actually strengthen the intensity of the HB reflex when the temperature increased is of considerable interest. In fact, it implies that the vagal inhibition on ventilation in the early neonatal period can be increased when hypoxia is combined with hyperthermia, an association that has been often suspected in the pathophysiology of sudden infant death (24, 26). This could be important in the pathophysiology of neonatal apneas, particularly if one considers that hypoxia lowers the set point of thermoregulation; hence, even values of temperatures considered normal in normoxia can act as hyperthermic conditions during hypoxia (18, 21).

In conclusion, the present results in newborn rats have indicated that hypoxia did not decrease the intensity of the HB reflex and that changes in $T_a$ between 24 and 36°C had no significant effects, contrary to what was previously observed during normoxia. The most likely interpretation is that, in addition to chemosensitivity, changes in metabolic rate play a most important role in determining the magnitude of the reflex and its $T_a$ sensitivity. Finally, the observation that the reflex response to small lung inflations during hypoxic hyperthermia can actually be greater than in normoxia may be of importance in the pathophysiology of apneas during the neonatal period.

The authors thank Lina Naso for technical assistance and Teresa Trippenbach for critical reading of the manuscript.

This study was supported by funds from the Medical Research Council of Canada. D. Merazzi was financially supported at McGill University by a grant from the Fondi, Miglierina, Varese, Italy, and was on leave from Hospital S. Anna, Como, Italy. Present address of D. Merazzi: Div. of Neonatology, Neonatal Intensive Care Unit, S. Anna Hospital, via Napoleon 60, 22100 Como, Italy.

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Received 30 December 1998; accepted in final form 29 January 1999.

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