Volume of activation of the Hering-Breuer inflation reflex in the newborn infant

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Polynomial regression analyses revealed a progressive increase (per ml/kg increase in lung volume at occlusion) of end-inspiratory volume [14.9% reduction in inspiratory time occurring rose from functional residual capacity (FRC) to end-inspiratory volume (14.9% reduction in inspiratory time (per ml/kg increase in lung volume at occlusion)]. All infants showed a significant increase in inspiratory time (17.1% increase (per ml/kg increase in lung volume at occlusion)). Polynomial regression analyses revealed a progressive increase in strength of HBIR from FRC to ~4 ml/kg above FRC. Eighteen infants showed no further shortening of inspiratory time and 10 infants no further lengthening of expiratory time with increasing occlusion volumes, indicating maximal stimulation of the reflex had been achieved. There was a significant relationship between strength of HBIR and respiratory rate, suggesting that HBIR modifies the breathing pattern in the neonatal period.

Hassan, A., J. Gossage, D. Ingram, S. Lee, and A. D. Milner. Volume of activation of the Hering-Breuer inflation reflex in the newborn infant. J Appl Physiol 90: 763–769, 2001.—Although the Hering-Breuer inflation reflex (HBIR) is active within tidal breathing range in the neonatal period, there is no information regarding whether a critical volume has to be exceeded before any effect can be observed. To explore this, effects of multiple airway occlusions on inspiratory and expiratory timing were measured throughout tidal breathing range using a face mask and shutter system. In 20 of the 22 healthy infants studied, there was significant shortening of inspiration because the volume at which occlusion occurred rose from functional residual capacity (FRC) to end-inspiratory volume [14.9% reduction in inspiratory time (per ml/kg increase in lung volume at occlusion)]. All infants showed a significant increase in expiratory time [17.1% increase (per ml/kg increase in lung volume at occlusion)].

The precise source of the HBIR remains incompletely understood; the effect of inflationary reflex has been extensively investigated and is largely attributable to inputs from slowly adapting pulmonary stretch receptors (1), the vagal afferents of which have conduction velocities of 14–59 m/s. The fibers from these receptors are also carried by the vagus nerves and have conduction velocities in the range of 3.6–25.8 m/s.

Several methods have been used to assess HBIR activity in infants. The first to be introduced, rapid lung inflation (11), is no longer recommended because it has been shown that the duration of the resultant apnea is related more to peripheral chemoreceptor input than to HBIR activity (29).

Alternatively, the strength of the HBIR has been assessed from the relative change in respiratory cycle time during brief airway occlusion. If this is carried out at end expiration, that is at functional residual capacity (FRC) (7), there will functional blockade of stretch-receptor activity producing a prolongation of inspiratory time, which is thought to be proportional to the strength of the HBIR (8). The reason for this is that during inspiration the increase in lung volume is accompanied by an increase in vagal afferent inputs from airway slowly adapting mechanoreceptors. This phasic tidal volume-related information, in combination with central mechanisms, inhibits further inspiratory activity (4). Thus, in the absence of lung volume expansion,
as during an occlusion at FRC, the inspiratory duration of the effort should depend only on the central threshold for inspiratory inhibition and so exceed unoccluded inspiration time (9). This has been shown to occur in newborn dogs (12) and human infants (20). The alternative approach has been to maintain an elevated lung volume by end-inspiratory occlusion, resulting in the persisting stimulation of stretch receptors, with subsequent inhibition of inspiratory effort and prolongation of expiratory time if the HBIR is active (8). It has been assumed that the effects of the HBIR on respiratory timing are totally eliminated by occlusion at FRC so that inspiratory time is then dependent on unmodified central drive and will be prolonged. What is not known is whether, as in anesthetized cats (17), there is a critical volume that has to be exceeded before the HBIR is stimulated or whether the reflex is activated increasingly throughout the tidal breathing range.

Our hypothesis was that there was a volume above FRC that had to be exceeded before the HBIR was activated in the neonatal period. The aim of this project was therefore to measure the relative strength of the HBIR throughout the respiratory cycle in the neonate by performing occlusions at various points during inspiration and to assess the effect of these on the timing of the subsequent expiration and inspiration.

METHODS

Technique. A manual shutter that was designed and provided by Dr. J. M. Stocks (Institute of Child Heath, London, UK) had a dead space of 5 ml. The shutter included openings for the attachment of a face mask and a heated pneumotachograph (Godart, size 0.00) and an additional port for a pressure transducer (±50 cmH₂O; model DP103, Validyne). The shutter was closed using a camera shutter release system that depressed a plunger, thereby sealing off the face mask.

The output from the pneumotachograph was electronically integrated against time to derive volume, and this signal along with the output of the pressure transducer were continuously displayed on a digital storage oscilloscope (series 400, Gould). These signals were also stored on magnetic tape using a four-channel magnetic tape recorder (Racal).

Procedure. The infants were first allowed to settle, and measurements were taken while they appeared to be in non-rapid-eye-movement sleep using the criteria of Prechtl (22); i.e., they were breathing regularly, were not making body movements, and were not observed to make rapid eye movements. Measurements were discontinued if the infants stirred and not recommenced until the above conditions were reestablished. A soft face mask with an inflated ring was placed on the infant’s face covering the infant’s mouth and nose, using a small amount of Vaseline to ensure an airtight seal. After a minimum of 20 regular breaths, an occlusion was performed by activating the manually operated shutter for a period of 3–4 s. The aim was to obtain occlusions throughout the inspiratory part of the tidal breathing cycle by observing the volumes at occlusion on the oscilloscope. From the pressure and volume traces, it was also possible to check whether there were any major leaks in the system. We aimed to perform 15–20 occlusions, spread evenly throughout the inspiratory cycle. We allowed at least 20 normal breaths between occlusions to minimize the potential influence of reflex habituation. Occlusions were rejected if there was evidence of a leak around the face mask, as demonstrated by a shift in the end-tidal baseline after the occlusion had been released or by any associated body movement indicating that infants’ sleep state had been disturbed.

Calibration of the equipment was performed before each study. The pneumotachograph was calibrated by injecting and withdrawing 20 ml of air from a syringe, and the output was stored on the Racal recorder. The pressure transducer was calibrated against a 20-cm column of water.

The signals were played back from the magnetic tape recorder onto chart paper, using a twin-channel direct-writing recorder (Gould Godart).

The following measurements were taken from each occlusion. 1) From the volume trace, tidal volume and inspiratory time and expiratory time before an occlusion (Fig. 1) and the volume above FRC at which the occlusion had occurred were measured. The FRC was determined from the end-expiratory point of the preocclusion breath. 2) From the mouth pressure trace, the subsequent expiratory time and inspiratory time during the occlusion were measured (Fig. 1). With the use of these data, graphs were constructed for each subject, plotting the effects of the occlusion on inspiratory time (the ratio of inspiratory time after occlusion to inspiratory time before occlusion) against the lung volume above FRC during the occlusion (Fig. 2). In addition, the ratio of expiratory time after occlusion to expiratory time before occlusion was also plotted against the volume above FRC measurements (Fig. 3). With the use of Microsoft Excel, a straight best-fit line was plotted and the gradient of this line was recorded. This slope represented the mean percent increase or decrease in inspiratory or expiratory time per milliliter per kilogram increase in volume at occlusion for each infant.

The gradients from all the infants studied were then used to derive mean values, SDs, and 95% confidence intervals (CIs) for the group as a whole, using a CI analysis program. Because the distribution of the data points suggested that the relationships between the volumes at occlusion and the strengths of the HBIR were not linear, second-order polynomial regression curves were constructed for the inspiratory and expiratory data.
for each infant individually and on the total group data. A P value of <0.05 was taken to be significant.

Using the inspiratory and expiratory times before each occlusion, an average respiratory rate was calculated for each subject. The respiratory rates were then plotted against the inspiratory and expiratory gradients to assess whether there was a significant relationship between respiratory rate and the strength of the HBIR.

Finally, when the occlusion was carried out within 10% of the onset of inspiration, the time for inspiration of that breath as well as the subsequent breath was measured to assess whether increasing chemoreceptor drive during the period of occlusion could be influencing the results.

The study was considered and approved by the Ethics Committee of St. Thomas’ Hospital, and informed and written consent was obtained from at least one of the parents. Subjects. A total of 22 neonates (9 male and 13 female subjects) were recruited from the postnatal ward at St. Thomas’ Hospital. Their mean gestational age was 39 ± 1 (SD) wk (range 36–41 wk), and their mean birth weight was 3.32 ± 0.51 kg (range 2.54–4.42 kg). At time of testing the mean age was 3 ± 1.6 days (range 1–5 days). None of the infants tested had any known congenital abnormalities or any evidence of respiratory, neuromuscular, or cardiac disease, nor were any of the infants on any drugs or assisted ventilation. The studies were usually performed after the infant had been fed and was in quiet sleep. Parents were often present during measurements.

RESULTS

Satisfactory data were obtained on all 22 infants, although, in ~25% of the studies, the infants roused at some time in response to shutter closure. Further measurements were only made once the infant had returned to quiet sleep as determined on behavioral criteria (22). A total of 428 satisfactory inspiratory occlusions were performed on the 22 infants. Twenty of the 22 infants showed a significant relationship between volume above FRC during occlusion and shortening of inspiratory time.

The mean percent change in inspiratory time was −14.9% (per ml/kg increase in lung volume over the entire breath) (95% CI −11.9 to −17.9; Table 1). All 22 infants showed a significant relationship between occlusion volume and lengthening of expiratory time. The effect of the volume at occlusion on expiratory timing was similar in magnitude with a mean increase of 17.0% (95% CI 14.2–19.9%).

When the effects of volume at occlusion on changes in inspiratory timing were compared with the effects on expiratory timing in the 22 infants, there was little correlation (Fig. 4; P < 0.2).

Analysis of the slopes from the babies comparing respiratory rate and the strength of the Hering-Breuer inflationary reflex (HBIR) showed that was a significant correlation between the two variables, the stronger the reflex the higher the respiratory rate [Fig. 5 (P < 0.01), Fig. 6 (P < 0.02)].

The polynomial regression analyses showed that, in 18 of the 22 infants, there was a progressive effect on inspiratory time from the FRC up to ~4 ml/kg (Fig. 7) but that increasing the occlusion volume above that tended to have no additional effect. Ten of the 22 also

Table 1. Overall results of the strength of the HBIR in all subjects

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>%Change per ml/kg increase in volume</th>
<th>P value</th>
<th>%Change per ml/kg increase in lung volume</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−11.88</td>
<td>&lt;0.001</td>
<td>9.7</td>
<td>&lt;0.01</td>
</tr>
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<td>−24.09</td>
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</tr>
<tr>
<td>4</td>
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<td>&lt;0.01</td>
<td>9.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>32.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
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<tr>
<td>10</td>
<td>−06.43</td>
<td>NS</td>
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</tr>
<tr>
<td>11</td>
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<tr>
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<tr>
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<td>25.25</td>
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Mean ± SD −14.94 ± 7.12 17.05 ± 6.92

95% CI −12.0 14.2 to 19.94

Strength of Hering-Breuer inflationary reflex (HBIR) is indicated by % change in inspiratory and expiratory timing. CI, confidence interval. NS, not significant.
showed a progressive increase in expiratory time, up to \(4\) ml/kg. This effect was still apparent when the data points from all 420 occlusions in the 22 infants were analyzed together (Figs. 7 and 8).

In 87 occlusions in the 22 infants, occlusion occurred within 10% of the onset of inspiration. The mean time for inspiratory activity of the initial occluded breath was \(0.824 \pm 0.314\) s and for the subsequent inspiratory effort was \(0.880 \pm 0.277\) s \((P = 0.0001,\) paired \(t\)-test). On 62 occasions there was no measurable difference, on 24 occasions the second inspiration was longer, and in 1 occasion it was shorter.

**DISCUSSION**

The results of this study show that there is a progressive stimulation of the HBIR from the FRC, presumably due to progressive recruitment of stretch receptors as lung volume increases but that the maximum effect on inspiratory time occurs at \(~4\) ml/kg above the end-expiratory volume in most infants.

To investigate this reflex, we used the occlusion technique (8, 23). However, we also performed multiple occlusions throughout inspiration. We then calculated the percent change in expiratory and inspiratory time against the volume above FRC at which the lung was occluded. This provided us with a measure of reflex activity throughout the tidal breathing range.

Unlike previous investigators, we did not base our measurements on the inspiration in which the occlusion was carried out but rather on the next inspiration, i.e., the one immediately after the first occlusion expiration. This was essential to avoid artifacts produced by attempting to measuring the HBIR effect when variable inspiratory volume change had already occurred. This would have been a particular problem for measurements as the end-inspiratory point was approached.

This raised the potential problem that chemoreceptor drive might be increasing during the relatively long period of the occlusion and have a significant effect on the results. For this reason, we measured inspiratory time of both the inspiration during which occlusion was carried out as well as the subsequent inspiration in 87 occlusions in which the occlusion occurred within 10% of the onset of inspiration. Although the second inspiratory breath tended to be longer than the first, this effect was only seen in 24 of the 87 occlusions, and the mean effect was only 6.8%, making it unlikely that the results were affected by changing chemoreceptor drive.

We used volumes above FRC rather than absolute lung volume because there is a wide scatter of FRC measurements in healthy newborn infants with a value of \(35 \pm 1\) (SD) ml in a recent study on 289 infants born to smoking and nonsmoking women (19). It would also have been impractical to carry out lung volume
measurements between each occlusion. It is possible that absolute lung volume changed during studies. However, our laboratory has previously shown that, over 30-min periods, the mean change in lung volume during quiet sleep is $<6$ ml (26). If the volume had changed by much more than that, it is unlikely that we would have been able to show the highly significant correlations between lung volume above FRC and strength of the HBIR.

As in other studies into the HBIR in the neonatal period, sleep state was defined on behavioral characteristics (22). This has obvious limitations, but data were only collected when the infants were breathing quietly and without nonrespiratory body movements. There are no published studies on the effects of sleep state on the reflex in the neonatal period so that it is not possible to state whether sleep has a significant effect. The dramatic falls of lung volume on transfer from quiet to rapid-eye-movement sleep reported by Henderson-Smart and Read (15) are now thought to be artifactual and have not been confirmed by other studies (26). In one study, the difference in FRC between sleep states was less than the changes in FRC in any given sleep state (26).

The system also had a dead space of $\sim$1.2 ml/kg. In a previous study (27), our laboratory has shown that an added dead space of 1.1 ml/kg will increase minute ventilation by 37%. Approximately two-thirds of this is due to increase in tidal volume and the remaining on-third to increase in respiratory rate. Both this and stimulation from the use of a face mask will increase tidal volume so that measurements at and near to end inspiration were probably above the normal tidal breathing range.

However, using this technique, we were able to demonstrate a significant shortening of inspiration in each of 20 of the 22 infants and lengthening of expiration because volume of occlusion was increased in all 22 infants. The mean effect was for inspiratory time to be increased by $\sim$200% when occlusion was carried out at the onset of inspiration and an $\sim$200% increase in expiration when occlusion occurred at end inspiration. These results are essentially the same as those recently published by Rabbette and Stocks (24), whose figures were 199 and 197%, respectively. Although the mean effects on inspiratory and expiratory timing were similar, there was poor correlation when these results were compared in individual infants. Although the methods of analysis are different, this is in agreement with the finding of Brown et al. (5), who examined the effect of occlusion at both FRC and end inspiration in the same infants and also found a poor correlation.

The scatter of results around the regression line suggested that there was not a progressive activation of the HBIR throughout the tidal breathing range. For this reason, we constructed polynomial regression curves. These revealed that the progressive recruitment of the inspiratory effects of the HBIR was occurring predominantly over the lower two-thirds of the tidal volume, i.e., up to $\sim$4 ml/kg above the FRC, but that occlusion at higher volumes had no greater effect,
indicating that maximal stimulation of the HBIR had already been achieved.

To our knowledge, this is the first time it has been shown not only that the HBIR is active from FRC in term infants but also that maximal stimulation is achieved within the tidal volume range. A similar effect was seen on expiratory timing but significant in only 10 of the 22 infants. These findings were contrary to our expectations. We had hypothesized that the newborn infant would show a pattern of activation similar to that described by Knox (17) in the anesthetized cat. It is possible that some of the differences were due to technique because Knox attempted to obtain the reflex by brief inflations during expiration rather than by repeated inspiratory occlusion.

These findings are apparently in conflict with those of Rabbette and Stocks (24), who carried out multiple occlusions throughout respiration in four neonates and three 1-yr-old infants. They claimed that, in the neonates, no HBIR effect could be found until a volume of $\sim4$ ml/kg was exceeded and that at high lung volumes, achieved by occluding infants during sighs, the effect was increased by up to sevenfold. However, their multiple occlusions were carried out during expiration rather than inspiration, which our own studies have shown produces a weaker stimulus to the HBIR than occlusion during inspiration (unpublished observations), and they assume that the very striking prolongation of expiration after occlusion during a sigh is due to the HBIR, rather than a reduction in peripheral chemoreceptor drive as occurs during imposed lung inflation (29). Their claim would suggest that the brief apnea, which so constantly follows sighs (29) and is presumably responsible for their very prolonged expiratory times, is due to the HBIR and not to transient changes in arterial carbon dioxide concentrations.

If their measurements during sighs are disregarded, then their data also show a progressive increase in the HBIR from FRC. Part of the reason for disregarding this part of their data may be that they defined a minimal HBIR as a 25% change in inspiratory timing (24) so that subtle changes may have been overlooked.

One defect of this study was that we did not assess the degree of dynamic lung inflation (6) that was occurring in each infant, which according to Kosch and Stark (18), may be as much as 14.4 ml in the supine position. If this volume is taken into account, then the HBIR would show a progressive increase throughout the tidal breathing range. However, our aim was to study the functional effect of the reflex over the tidal range adopted by each infant. The dynamic lung inflation may partially explain the differences in responses of term and 1-yr-old infants because this progressively disappears over the first 12 mo of life (10).

The claim that there is a strong association between respiratory rate and the strength of the HBR in the infant has recently been challenged by Gagliardi and Rusconi (13) on the grounds that the calculation of the respiratory rate and the strength of the reflex are both based, in part, on the same data, i.e., preocclusion expiratory or inspiratory time. The effect of this is that for a constant shortening in inspiratory time, the percent change will be greater when the respiratory rate is higher and the preocclusion inspiration shorter. If this were the sole reason for the reported relationship, then there should be a direct relationship between respiratory rate and strength of the HBR. However, in this study there is an $\sim7$-fold increase in the strength of the HBR associated with a 2.5-fold increase in respiratory rate, a far greater effect than can be explained by Gagliardi and Rusconi’s arguments. These data therefore reconfirm that the HBR does modify the pattern of breathing in the neonatal period, although the association was not particularly strong ($r = 0.51$, $P < 0.02$).

In conclusion, the results show that there is activation of the HBIR throughout the respiratory cycle and that there is a progressive increase in strength of the reflex from the FRC. Maximal stimulation of the reflex is reached at $\sim4$ ml/kg above the FRC. It is possible that, even at FRC, the HBIR has an effect. The strength of the HBR does have a significant correlation with respiratory rate, supporting the claim that the HBIR modifies the breathing pattern in the neonatal period.

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