Waters, Karen A., and Kellie D. Tinworth. Effect of stimulus cycle time on acute respiratory responses to intermittent hypercapnic hypoxia in unsedated piglets. J Appl Physiol 94: 2465–2474, 2003. First published February 7, 2003; 10.1152/japplphysiol.00421.2002—To determine whether stimulus frequency affects physiological compensation to an intermittent respiratory stimulus, we studied piglets (n = 43) aged 14.8 ± 2.4 days. A 24-min total hypercapnic hypoxia (HH) (10% O₂-6% CO₂-balance N₂ = HH) was delivered in 24-, 8-, 4-, or 2-min cycles alternating with air. Controls (n = 10) breathed air continuously. Minute ventilation and temperature were not different between the 2-min and 24-min groups, with neither different from controls during recovery. Piglets exposed to 8-min cycles had ventilatory stimulation, whereas those exposed to 4-min cycles had significant depression of ventilation. Despite this, piglets in these intermediate intermittent HH (IHH) groups (8- and 4-min cycles) showed more severe acidosis and attenuated temperature changes (P < 0.001 and P < 0.01 for pH and temperature vs. 24 min, respectively). Cycle time affected the ability of young piglets to tolerate IHH. More severe respiratory acidosis developed when IHH was delivered in intermediate (4 min or 8 min) cycles compared with the same total dose as a single episode or in short (2 min) cycles.

ventilatory responses; hypercapnia; cyclical

THE VENTILATORY RESPONSE OF a whole animal to hypoxia represents the integration of respiratory, metabolic, and circulatory adjustments (9, 13). Ventilatory responses, as well as the contributions of chemoreceptor, neurotransmitter, and metabolic responses to the depression, vary with age and species (22). During early development, the ventilatory response to a sustained hypoxic stimulus usually includes peripheral and central chemoreceptor responses, changes in central neurotransmitter levels, and decline in metabolic rate (2).

The piglet is an excellent model for cardiorespiratory control during early development, because brain and cardiorespiratory maturation is equivalent to that of human infants at the time of birth (29). In the early postnatal period, ventilatory responses of piglets show equivalent, but faster, postnatal maturation of these systems compared with humans (23). For example, in the early postnatal period, piglets show a typical decline in ventilation during sustained exposure to hypoxia (29, 35). At this age, they demonstrate the later fall in ventilation to levels that remain above baseline, a response known as short-term depression (26, 34). After the first week, when piglets are exposed to hypoxia they predominantly increase respiratory frequency, with a tendency for respiratory frequency to increase during the course of a sustained exposure (22, 34). By 2 wk of age, this ventilatory strategy means that piglets maintain ventilation above baseline throughout a sustained (30 min) hypoxic exposure (34). Piglets also show peak vulnerability to peripheral chemosensory denervation at 12–15 days, suggesting that development of peripheral chemoreceptor function at this critical period is essential for the development of other functions (4). Taken together, these studies show that piglets aged 12–15 days old are at an age of vulnerability for exposure to noxious cardiorespiratory stimuli compared with neonates or older animals (14, 24).

Clinical conditions more often cause cyclical or intermitent hypercapnic hypoxia (IHH) than sustained exposure to hypoxia or to hypercapnia. In the clinical situation, IHH may occur during brief apneic cycles with rapid recovery to baseline, or for sustained episodes during entire sleep times, most commonly recurring during rapid eye movement sleep. Conditions underlying such exposures during infancy include repetitive apnea, upper airway obstruction, and respiratory compromise during sleep in infants with chronic lung disease or prone sleeping leading to repeated entrapment in face-down positions (8, 28, 33).

When young piglets are exposed to intermittent hypoxia, their ventilatory responses show later decline compared with the same stimulus in naive animals, whether the intermittent hypoxia was acute (same day) or chronic (daily) (31, 34). This tendency for ventilatory responses to be depressed (or more immature) after repeated exposure to a respiratory stimulus may, in part, be explained by the greater role of peripheral chemoreceptors in the newborn compared with adults, or alternatively it may be due to changes in the pattern of central neurotransmitter release (12). These studies suggest that an intermittent stimulus has a predomi-
nantly depressant effect during early development. However, studies in adult animals and in young animals of other species have shown that an intermittent stimulus enhances the ventilatory response. In adult mammals, this can take the form of progressive augmentation of the amplitude of the acute response, or long-term facilitation of baseline ventilation, again possibly involving central and peripheral mechanisms (15, 17, 26). In young rats, ventilation may also be enhanced by exposure to intermittent hypoxia, although in this model enhancement means loss of the later decline (roll-off) in ventilation, and nitric oxide is implicated as the central nervous system neurotransmitter responsible for the change (10).

We first tested the hypothesis that respiratory and temperature compensations to sustained hypcapnic hypoxia (HH) would be similar to those previously documented during sustained hypoxia. Piglets were exposed to a sustained (24 min) HH stimulus and compared with a control group breathing fresh air in the same study environment. Our second hypothesis was that some stimulus cycle times of IHH would have a detrimental influence on the capacity of young piglets to compensate for second and subsequent exposures. To examine this, we studied respiratory responses to the same total “dose” of an HH stimulus delivered at different cycle times. The 24-min exposure was used as our standard, for comparison with the responses of young piglets exposed to 3 IHH cycles (8, 4, and 2 min) but the same total (24 min) duration of exposure.

METHODS

Mixed-breed miniature piglets were transported from a commercial piggery 3.0 ± 3.0 days (mean ± SD) after birth and then housed in an animal facility with light exposure between 12 noon and 12 midnight. Aseptic surgery was undertaken under general anesthetic on day 12.8 ± 2.4, when piglets weighed 2.6 ± 0.6 kg. Anesthesia was induced by using a face mask delivering 1–3% isoflurane with 30–50% nitrous oxide in O2 and continued throughout surgery via an endotracheal tube, adjusted according to the level of spontaneous respiratory efforts and heart rate. The piglets were ventilated throughout the anesthetic, and heart rate was monitored continuously by use of surface electrodes. An arterial catheter was placed in the descending aorta via the right femoral artery, tunneled subcutaneously to exit on the ipsilateral right flank, and protected in the pocket of jackets that were worn from the time of surgery. Analgesia commenced intraoperatively with paracetamol rectal suppository to a total dose of 100 mg/kg (27). Studies commenced a minimum of 48 h after surgery to permit full recovery from anesthetic. The piglets were unsedated at the time of study and had returned to normal feeding and playful activity. Average weight gain was 130 ± 30 g/day during the period of the study. After the final study, all animals were killed painlessly with an overdose of pentobarbitone. Ethical approval for the study was obtained from the Animal Ethics Committee of the University of Sydney.

Ventilatory Responses to HH

Ventilation was monitored for a 5-min baseline in air, followed immediately by a 48-min study period. All study animals had a total exposure of 24 min to HH (10% O2–6% CO2-balance N2), and a total of 24-min recovery time in air. Arterial blood samples were taken for gas analysis at baseline; at 8, 16, and 24 min into the stimulus time; and after 8, 16, and 24 min of recovery in air. Arterial gas tensions, pH, base excess (BE), and hemoglobin (Hb) were measured by an automated blood-gas analyzer (model 520, ABL, Radiometer, Copenhagen, Denmark). All values were corrected to the arterial temperature of the animal, which was recorded along with box (ambient) temperature at the time each blood sample was taken (ESO-1 and Thermalert TH-8, Physitemp Instruments, Clifton, Nj). Each piglet was randomly assigned to one stimulus pattern, and all studies were performed in a normally dark (sleep) time for the piglets.

The study environment comprised a sealed, temperature-regulated Perspex box. Box temperature was maintained by using a servo-controlled incubator that was modified to suit the experimental setup (RI 250, Thermoline, Smithfield, NSW, Australia). Piglets were placed in a vinyl hammock within the box to maintain their head position relative to the respiratory monitoring devices, while still permitting movement. Flow was recorded via a calibrated, heated-pneumotachograph (4500A, Hans-Rudolph, Kansas City, MO), stimulated to a full face mask. The mask was sealed against the snout by a layer of thixotropic gel under soft rubber (from a party balloon), inside the firm rubber seal of an anesthetic mask designed for animals (small 1582 or medium 1583, Lypppard, NSW, Australia). The inspiratory limb provided fresh gas flow and incorporated a gas-tight three-way tap to permit rapid switching between reservoir bags containing air or the premixed HH gas. The mean time for stabilization at the new gas level was 19.4 ± 9.0 s into hypoxia and 19.3 ± 4.6 s into recovery. A one-way valve was incorporated into the expiratory limb of the circuit to prevent side streaming of air into the gas mix, and O2 and CO2 concentrations were measured continuously by use of a gas analyzer (Daxet AS3 capnometer), sampling on the distal side of the pneumotachograph.

Signals were amplified on a Grass model 8 polygraph and then digitized by using a commercially available eight-channel data-acquisition program (Labdat, V 5.2, RHT-Infodat, Montreal, PQ, Canada). The sampling frequency was 100 Hz. Calibrated recordings included concentrations of O2 and CO2 just distal to the pneumotachograph and calibrated flow from the pneumotachograph. Minute ventilation (VE), tidal volume (Vt), and respiratory frequency (f) were derived from these raw signals using a commercially available digital data analysis system (Abreath, RHT-Infodat). Ventilation is expressed in milliliters corrected for weight (ml/kg) and time (ml·kg−1·min−1). The f is expressed as breaths per minute.

Study Groups, Including IHH

To evaluate the effects of varying stimulus cycle time, inspired gas concentrations, total exposure time, and total recovery times were equivalent in all groups. Recovery cycles had the same duration as the stimulus cycle in all cases. The study groups included controls and piglets exposed to a total of 24-min HH comprising 10% O2–6% CO2–balance N2, and 24-min recovery time, the total study time for all animals being 48 min. The following are the group profiles: control piglets placed in the study environment, breathing fresh air continuously for 48 min; A (24 min), single stimulus and recovery cycle, each of 24-min duration; B (8 min) stimulus and recovery cycle durations of 8 min; C (4 min), stimulus and recovery cycle durations of 4 min; and D (2 min), stimulus and recovery cycle durations of 2 min. The stimulus was, therefore, intermittent (IHH) for groups B (8 min), C (4 min), and D (2 min).
Analysis

Outcome measurements included VE (ml·kg⁻¹·min⁻¹), VT (ml/kg), f, arterial gases, piglet rectal temperature, pH, and BE. Use of sampled means, such as the last minute before each blood sample, did not alter the results for changes in ventilation, so values are presented as mean during the stimulus, except where otherwise specified.

Physiological data were reviewed, artifact was excluded, and ventilation was averaged over consecutive 15-s intervals by using the analysis software associated with the data-acquisition software (Anadat and Abreath, RHT-InfoDat). The Abreath program calculates ventilatory parameters, including VE, VT, and f, from the calibrated flow signal. Mean values for each piglet were collated for comparisons among groups and used to compare ventilatory parameters among piglets exposed to an intermittent stimulus. For comparison of arterial gases, pH, BE, and body temperature, data were normalized to the mean of the 5-min baseline recording before group comparisons were made. Data are presented as normalized to the mean of the 5-min baseline recording of arterial gases, pH, BE, and body temperature, data were acquired in 2-min blocks. The Abreath program calculates ventilatory parameters, including VE, VT, and f, from the calibrated flow signal. Mean values for each piglet were collated for comparisons among groups and used to compare ventilatory parameters among piglets exposed to an intermittent stimulus. For comparison of arterial gases, pH, BE, and body temperature, data were normalized to the mean of the 5-min baseline recording before group comparisons were made. Data are presented as means ± SD in the text and tables and means ± SE in the figures, unless otherwise stated. The time base over which results were averaged is detailed in the relevant section of the results. Comparisons for data among groups at baseline or single-point data were performed by one- or two-way ANOVA. Comparisons among groups and across time were performed by using general linear modeling for repeated measures, in SPSS for Windows (version 10.0, Chicago; IL). A P value of ≤0.05 was considered statistically significant. Bonferroni correction for multiple comparisons was used in post hoc analyses.

RESULTS

Piglets were aged 14.8 ± 2.4 days at the time of their study and weighed 2.6 ± 0.6 kg. Details of the piglet physical and physiological characteristics are provided in Tables 1 and 2.

Changes Over Time

Control piglets showed no change in ventilatory or arterial blood-gas parameters over the same time period in the study environment (Fig. 1). To assess changes across time, data were analyzed in 2-min blocks. The most marked changes occurred within 6–8 min, and differences among groups were apparent by this time in both the HH and recovery periods. Sustained (24 min) HH led to stimulation of VE, VT, and f (Figs. 1, 2, and 3). None of the IHH groups showed any change in VT across successive HH cycles (Fig. 2), but there was a progressive increase in f for the sustained (24 min) and all IHH (8, 4, and 2 min) groups (Fig. 3). The absolute increase in f was smallest in groups B and D (8 and 6 breaths/min for 8- and 4-min groups, respectively), but the increase in group A (24 min) was 24 breaths/min (62 to 86) and in group D (2 min) was 19 breaths/min (65 to 84) (P < 0.001 for both). During recovery, group A (24 min) showed progressive decrease for all ventilatory parameters (P ≤ 0.001 for VE, VT, and f). For the IHH groups, minimum VT always reached baseline, and across cycles the changes in VT were only significant for group D (2 min) and group A (24 min), in which it decreased (P = 0.001 for both) (Fig. 3). Note that f increased across successive recovery periods for groups B (8 min, from 62 to 70 breaths/min, P = 0.02) and C (4 min, from 70 to 81 breaths/min, P < 0.001) (Fig. 3).

Ventilatory Responses

HH. During HH, VE and f were not different between groups A and D (24 and 2 min). Mean VE during HH was significantly lower for group C (4 min) than all other groups (Fig. 4A, P < 0.001 for all). VE was lowest in group A (24 min) compared with all other groups except group D (2 min) (Fig. 4A). Mean VE and VT during HH were significantly higher in group B (8 min) than all other groups (Fig. 4, A and B). Mean f during HH was significantly lower for group C (4 min) than all other groups, and lower in group B (8 min) than for groups A or D (24 or 2 min) (Fig. 4C).

Recovery. During recovery, mean VE was not different between group A (24 min) and controls, but for all IHH groups (2, 4, or 8 min) VE remained above baseline and there was no difference among them (Fig. 4A). Mean VT during recovery was significantly lower in group A (24 min) than all other groups including controls (Fig. 4B), highest in group D (2 min), and not different between group C (4 min) and controls. The f did not return to baseline for any of the IHH groups during recovery, with mean f between groups B and C (8 and 4 min) not different and both higher than the other two HH groups (groups A and D, 24 and 2 min). In a contrasting pattern to all others, f for group C (4 min) was higher in recovery than it was during HH (75.2 ± 6.0 vs. 63.9 ± 5.5 breaths/min, HH vs. recovery, respectively, P < 0.001) (Fig. 4C).

Respiratory drive (T I/TE). To assess respiratory drive, inspiratory time (T I) and the ratio of T I to total respiratory time were evaluated. During HH, T I in group A (24 min) decreased from 0.46 to 0.39 and in

Table 1. Physical characteristics of the piglets

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control, n = 10</th>
<th>Group A (24 min), n = 11</th>
<th>Group B (8 min), n = 9</th>
<th>Group C (4 min), n = 12</th>
<th>Group D (2 min), n = 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, days</td>
<td>13.2 ± 1.62</td>
<td>14.0 ± 3.2</td>
<td>12.0 ± 2.1</td>
<td>13.0 ± 2.3</td>
<td>11.5 ± 1.8</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>2.49 ± 0.59</td>
<td>2.98 ± 0.67</td>
<td>2.42 ± 0.67</td>
<td>2.57 ± 0.47</td>
<td>2.32 ± 0.36</td>
</tr>
<tr>
<td>Daily weight gain, g</td>
<td>90 ± 50</td>
<td>110 ± 80</td>
<td>110 ± 50</td>
<td>120 ± 30</td>
<td>100 ± 40</td>
</tr>
<tr>
<td>Gender, male:female</td>
<td>8:2</td>
<td>6:5</td>
<td>6:3</td>
<td>7:2</td>
<td>6:5</td>
</tr>
<tr>
<td>Rectal temperature, °C</td>
<td>40.0 ± 0.4</td>
<td>39.8 ± 0.7</td>
<td>40.1 ± 0.4</td>
<td>39.9 ± 0.3</td>
<td>39.6 ± 0.5</td>
</tr>
<tr>
<td>Box temperature, °C</td>
<td>27.5 ± 1.4</td>
<td>27.2 ± 1.6</td>
<td>27.1 ± 1.3</td>
<td>27.2 ± 1.0</td>
<td>28.7 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of piglets. See text for explanation of study groups.

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Table 2. Physiological parameters of the piglets at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{E} ), ml·kg(^{-1})·min(^{-1} )</td>
<td>528.5 ± 213.2</td>
<td>545.8 ± 193.0</td>
<td>742.6 ± 155.9</td>
<td>716.8 ± 282.7</td>
<td>625.1 ± 133.9</td>
</tr>
<tr>
<td>( V_{T} ), ml/kg</td>
<td>11.6 ± 3.8</td>
<td>9.3 ± 2.0</td>
<td>14.3 ± 2.4</td>
<td>13.5 ± 3.0</td>
<td>13.2 ± 2.1</td>
</tr>
<tr>
<td>( f ), breaths/min</td>
<td>47.4 ± 10.2</td>
<td>63.6 ± 27.6</td>
<td>55.8 ± 9.3</td>
<td>54.0 ± 11.4</td>
<td>49.8 ± 7.8</td>
</tr>
<tr>
<td>( T_{E} ), s</td>
<td>0.55 ± 0.18</td>
<td>0.52 ± 0.27</td>
<td>0.50 ± 0.17</td>
<td>0.45 ± 0.14</td>
<td>0.48 ± 0.09</td>
</tr>
<tr>
<td>( T_{I} ), s</td>
<td>0.92 ± 0.32</td>
<td>0.75 ± 0.43</td>
<td>0.79 ± 0.19</td>
<td>0.88 ± 0.22</td>
<td>0.92 ± 0.20</td>
</tr>
<tr>
<td>( T_{tot} ), s</td>
<td>1.48 ± 0.39</td>
<td>1.27 ± 0.66</td>
<td>1.28 ± 0.30</td>
<td>1.32 ± 0.30</td>
<td>1.41 ± 0.24</td>
</tr>
</tbody>
</table>

Values are means ± SD. \( V_{E} \), minute ventilation; \( V_{T} \), tidal volume; \( f \), respiratory frequency; \( T_{I} \), inspiratory time; \( T_{E} \), expiratory time; \( T_{tot} \), duration of respiratory cycle.

Fig. 1. Raw data for minute ventilation (\( V_{E} \)) for all groups and control animals for comparison. Gray shading reflects periods of hypercapnic hypoxia (HH; 10% \( O_{2} \)-6% \( CO_{2} \)-balance \( N_{2} \)) for the relevant study group. □, Control (air only). A: ■, group A (24 min). B: ●, group B (8 min). C: ▲, group C (4 min). D: ■, group D (2 min). Minutes (min) refers to the exposure and recovery cycle times. Each data point is the average value for the preceding 15 s. The first data point is the average of the 5-min baseline recording for that group. For clarity, error bars have been removed.

In group B (8 min) from 0.46 to 0.37. In group C (4 min), \( T_{I} \) increased over time in HH from 0.35 to 0.41 (\( P < 0.0001 \) in all cases). During recovery, \( T_{I} \) in group A (24 min) increased from 0.40 to 0.51 and in group D from 0.42 to 0.49 (peaking at 18 min, \( P < 0.0001 \) in both cases). The ratio of \( T_{I} \) to expiratory time (\( T_{E} \)) was low in group C (4 min) during HH, when it was not different from controls (0.36 ± 0.07 vs. 0.36 ± 0.03), but in all other groups the ratio increased (\( T_{E} \) decreased) significantly during HH (0.43 ± 0.01, 0.47 ± 0.01, 0.38 ± 0.04, for groups A, B, and D, respectively). In other groups, the ratio increased during HH as expected (\( T_{E} \) shortened as \( f \) increased).

### Arterial Gases and Core Temperature

Control piglets had no change in arterial gas parameters over time, confirming that differences observed among the treatment groups were due to the pattern of HH. Raw and mean values for all HH-exposed piglets are shown in Table 3. Statistical comparisons in the table are for HH groups against the sustained exposure, or group A (24 min).

During HH, all arterial gas parameters differed from controls, and apart from \( P_{O_{2}} \) the differences persisted between recovery and baseline. During HH exposure, \( P_{O_{2}} \) was not different among groups, and \( P_{CO_{2}} \) and BE were also equivalent among groups A, B, and C (24, 8, and 4 min). Group D (2 min) had lower \( P_{CO_{2}} \) than these other groups (\( P = 0.04 \)). The fall in pH was greatest for group B (8 min) and lowest for group D (2 min). During HH, rectal temperature was not different among HH groups. The increase in Hb during HH for all groups (to 9.6 g/dl) was significantly different compared with the values of 9.1 g/dl at baseline and recovery (\( P = 0.03 \) and 0.002, respectively), with baseline and recovery values not different from each other. The HH levels and duration of exposure in this study meant that, over time, animals tended to show deterioration in pH and BE (Fig. 5). Note that group D (2 min) was the only exception and showed a trend to improved pH and BE over time.

### Recovery

During recovery periods in air, piglets exposed to HH remained different in all arterial gas parameters compared with controls. During recovery, groups A and D (24 and 2 min) had equivalent \( P_{O_{2}}, P_{CO_{2}}, \) pH, BE, and temperature, and they had largely recovered to baseline (not different to controls). Groups B and C (8 and 4 min) had least recovery and reached control values only for \( P_{O_{2}} \) and Hb. Notably, the fall in
rectal temperature was attenuated for groups B (8 min) and C (4 min) compared with groups A and D (24 and 2 min) \( (P < 0.01 \text{ in all cases}) \), and, associated with this, there was failure of recovery for PCO2, pH, and BE. None of these parameters were different between groups B and C (8 and 4 min). All animals did show subsequent complete recovery and returned to normal behavior and feeding, with no ill effects apparent after their acute studies.

**Summary of Effects of Stimulus Cycle**

In multiple linear regression analysis, cycle time was a significant contributor to the levels of V\(\dot{E}\) achieved. After adjustment, parameters that remained in the model for V\(\dot{E}\) and f included cycle time, arterial O\(\text{2}\) saturation, and Hb (for V\(\dot{E}\), \(R^2 = 0.88\), \(F = 27.4\), \(P < 0.001\)). Animals exposed to the short (2 min) cycle time had comparatively smaller changes in their pH and BE, no attenuation of the temperature drop compared with controls or 24 min exposure, and no progressive acidosis. Thus, despite having slightly lower respiratory stimulus as evaluated by arterial gas changes, group D (2 min) achieved equivalent ventilation (V\(\dot{E}\)) to group A (24 min) \( (2.1 \pm 0.22 \text{ vs. } 2.0 \pm 1.6 \text{ lkg}^{-1}\text{min}^{-1}, \text{respectively, not significant}) \). Although groups B and C (8 and 4 min) had largely equivalent blood-gas changes, along with attenuation of temperature, group C (4 min) had depressed ventilation, with lower V\(\dot{E}\) \( (1.8 \pm 1.3 \text{ lkg}^{-1}\text{min}^{-1}) \), and group C re-

![Fig. 2. Mean values for tidal volume (VT, ml/kg) for each 2 min. Gray shading reflects the HH exposure, with no shading during recovery periods. A: Control (air only). B: group A (24 min). C: group A (2 min). D: group D (2 min). min refers to the exposure and cycle time. Each data point is the average value for the preceding 2 min. Error bars show SE.](http://jap.physiology.org/)

![Fig. 3. Mean values for respiratory frequency (f) for each 2 min. Gray shading reflects the HH exposure, with no shading during recovery periods. A: Control (air only). B: group A (24 min). C: group B (8 min). D: group D (2 min). min refers to the exposure and cycle time. Each data point is the average value for the preceding 2 min. Error bars show SE.](http://jap.physiology.org/)
sponded with stimulation of ventilation, largely through VT (Fig. 4).

**DISCUSSION**

The main purpose of this study was to determine how IHH affects ventilatory responses during early development. To achieve this, the same total dose (inspired gas concentration and total duration of exposure) was delivered, but different groups experienced different patterns of delivery. We first confirmed that adaptive responses, usually seen in piglets during hypoxia, were elicited during a sustained exposure to HH. We then demonstrated that when the same stimulus was delivered intermittently, the duration of the intermittent cycles had a significant influence on the ventilatory responses, with some patterns of IHH having a negative effect on physiological outcomes.

In this study, we showed that exposure to sustained (24 min) HH invoked the same patterns of ventilatory and temperature compensation as those previously described for piglets in sustained hypoxia with hypocapnia (31). When the same total HH stimulus was delivered intermittently (IHH) and compared with the 24-min (sustained) HH stimulus, 4-min IHH was associated with depressed ventilatory responses, attenuated temperature changes, and more severe acidosis. In contrast, 8-min exposures showed severe acidosis but stimulated ventilation. The 2-min exposures were short enough to invoke equivalent $V_{E}$, $f$, and temperature responses such that adaptation was generally not different from that observed during the sustained (24 min) stimulus.

**Adaptive Responses to a Sustained Stimulus**

This study describes the responses of young animals to a combined stimulus of hypercapnia and hypoxia. We are not aware of any systematic studies of the responses to HH during early development, when hypoxic and hypercapnic ventilatory responses are most commonly studied independently of one another. The ventilatory responses to hypercapnia and hypoxia are likely to be additive at this age, rather than multiplicative, although it is possible these levels of PCO$_2$ and PO$_2$ have a depressant interaction on the slope of the ventilatory response (25). With regard to the ventilatory and temperature responses to a sustained stimulus, we found similar patterns to those documented previously in slightly older piglets during hypoxia (27). That is, $V_{E}$ was maintained above baseline, predominantly through a sustained increase in $f$. Piglets at this age also show a sustained increase in VT. Although this may be attributed to the lack of decline in $f$ during sustained exposure, this maintenance or increase in $f$ is a consistent feature of the response of young animals to hypoxia, in piglets as well as other species (22, 31, 34). It is important to note that temperature fell in our control animals in the same environment. Thus the fall in temperature during sustained HH was unchanged, rather than being a physiological response to the stimulus. It was not the purpose of this study to evaluate effects of age on the responses to HH, and so it is not clear whether the response is fully mature or still plastic at this stage of life in piglets (1, 26, 31).

**Influences of Stimulus Cycle Time on Responses to HH**

Previous studies of intermittent hypoxia with hypocapnia in piglets showed depression of the ventilatory responses, compared with a sustained stimulus of equivalent duration, whether the studies were acute or chronic (31, 34). In this study, we found depression of ventilation and attenuation of temperature responses in group C (4 min), in which we also observed more severe acidosis. By using the model summarized by Powell et al. in 1998 (26), the effect of an intermittent stimulus would be dictated by the point at which it interrupted the various responses to hypoxia. Slower
components of the ventilatory responses may be more readily disrupted because they are more susceptible to the timing of the changes (5, 20, 21).

A new feature in this study was that our short stimulus (2 min) could effectively elicit the same response as a continuous exposure. We interpret this to mean that 2-min exposures were short enough to be “equivalent” to a sustained stimulus of the same inspired gases for most of the ventilatory and physiological responses we studied. Despite the marked differences in stimulus cycle duration between sustained (24 min) and short (2 min) cycles, respiratory parameters showed the same pattern of change over “time in HH” (Fig. 1), and temperature changes were also largely equivalent.

Body temperature was used as a surrogate measure of metabolic rate because the two change in a similar pattern over time in hypoxia (31). Our laboratory’s previous study also demonstrated that metabolic (temperature and $O_2$ uptake) responses were attenuated during cyclical compared with sustained hypoxia (31). Significant attenuation of body temperature changes was observed only in the intermediate stimulus cycle groups (8 and 4 min). The relevance of the fall of body temperature over time in our control group is not clear, but it was also observed in groups A and D (24 and 2 min). The most likely explanation for the attenuation is that unseeded animals increased activity during the excitatory stimulus (31). It may also relate to the mechanisms relevant to studies by Côty et al. (3), who found no effect on the metabolism of piglets after repeated, albeit much slower exposures to hypoxia. We are not aware of other data relating to temperature changes under control conditions in young animals.

Studies showing how slow the recovery of ventilatory responses can be or how long the effects of a single exposure may last provide insights into the phenomena we observed. Easton and colleagues (5) have shown that hypoxic responses in adult humans take as long as 60 min to recovery. Another study (16) illustrated that, in newborn rats, very short (16 breaths) alternations in inspired gases are equivalent to a sustained stimulus of the same duration. It has also been proposed that short courses of intermittent hypoxia will enhance ventilatory drive, but recurrence over a longer period of time or an overall larger cumulative number of cycles would decrease the hypoxic ventilatory response (11).

It is important to note that different components of the ventilatory response were affected differently. As discussed, slower responses may be more readily disrupted by an intermittent stimulus. The significant variability of $f$ and body temperature among the stimulus cycles that we studied suggests that our study design was particularly relevant to these components of the response to HH. The time course of the $f$ response in young piglets is >2 min, compared with $V_t$, for which responses were faster and were not apparently disrupted during our intermittent exposures. Because the cumulative exposure in all our groups was equivalent, we conclude that the impact of an intermittent stimulus does depend on where in the “response cycle” the stimulus was interrupted.
Influences of Stimulus Cycle Time on the Ability of Piglets to Compensate for HH

Apart from the 2-min IHH group, piglets had progressive acidosis during the HH exposure used in the study. This study cannot determine whether poorer ventilatory responses or greater arterial gas disturbances were the primary determinant of this phenomenon. Similarly, we did not study the effects on central neurotransmitters that may combine to produce an overall depressant effect on the ventilatory response, and it is beyond the scope of this discussion to review all factors contributing to respiratory depression in the presence of hypoxia (see Ref. 11). On the other hand, it seems likely that the animals were developing some form of metabolic (lactic) as well as respiratory acidosis toward the end of the exposure. A previous study specifically examining metabolic responses, for which piglets in this age group had persistent sympathetic and lactic acid responses when other age groups showed lessening of these responses (3), suggests that this may be an age-specific phenomenon. It is also possible that chronic exposure would alleviate this depression to some extent, given the results in other age groups from the study of Côté et al., and evidence that athletes can train to stop developing lactic acidosis while maintaining their ventilatory threshold (3, 7).

Limitations of the Study

The same inspired gases were delivered to all animals in this study, but the stimulus at the level of the arterial gases was not equivalent for the short (2 min) stimulus cycles. This is an important constituent of our results, in that the greater ventilatory response observed in this group effectively constitutes stimulation of the ventilatory responses to HH, relative to the other IHH groups. The hypoxic stimulus, and therefore carotid body stimulation, is fast and would be expected to have equilibrated in all study groups. On the other hand, the central CO₂ chemoreceptor stimulus may have varied among the study groups. The switch between gases was manual, and the changeover times were shortest in the 2-min group (13.5 ± 2.2 and 16.2 ± 1.8 s into and out of HH, respectively) and longest in the 24-min group (28.6 ± 12.8 and 22.6 ± 5.3 s into and out of HH, respectively). Continuous rather than intermittent monitoring of Po₂ and Pco₂ would help to elucidate how this component affected the results. Alternatively, such continuous monitoring or arterial gases would permit studies with the same arterial stimulus to all groups.

Sleep state was not analyzed or controlled for in this study. Although piglets were studied during a normal “sleep” time and settled to sleep at baseline, they consistently aroused at the onset of the stimulus. This is to be anticipated, because animals arouse if the new gas mix is not introduced slowly (4). Hypercapnia is a particularly potent arousal stimulus, especially if delivered rapidly (6). An additional fact is that EEG becomes very hard to interpret after rapid gas switches to hypoxia during early development, and well-defined EEG criteria for sleep staging are lost (32). Our priority was to determine the ventilatory and metabolic responses to the stimulus, but more detailed analysis of arousal frequency, for example, may reveal additional group differences (18). Similarly, cardiac responses may to some extent explain the severity of acidosis seen in the intermediate (4 min and 8 min) groups because very high CO₂ levels can cause reduced cardiac output and bradycardia in carotid body-intact animals (30). These previous studies do not indicate whether the differences in Pco₂ between our groups would be sufficient to cause significant differences in cardiovascular effects. Our laboratory’s previous study of intermittent hypoxia in the presence of hypocapnia did not find that heart rate responses of piglets ex-
plained or followed the ventilatory changes to an inter-
mittent stimulus (31).

In conclusion, in this study we examined how the pattern of delivery affected acute responses to an IHH stimulus. Young piglets experiencing very short (2 min) but repeated events (e.g., apnea) appear to tolerate the stimulus better than a single, sustained event of equivalent total duration, although both stimuli elicited equivalent ventilatory and temperature responses. Animals experiencing intermediate (4 and 8 min) stimulus cycles showed depression of \( V_{E} \), attenuated metabolic responses, and more severe arterial gas disturbances. Thus by altering stimulus delivery pat-
terns, we elicited different ventilatory and physiological responses in young piglets that translated into differences in the animals’ overall ability to tolerate acute episodes of HH. We conclude that stimulus cycle time has an independent and signifi-
cant effect on responses to the total cumulative dose of IHH. This may determine how a young animal compensates for a HH exposure so that, in clinical situations, the progressive respiratory acidosis that we observed during “interme-
diate” cycles may be life threatening, whereas the same total exposure given in very brief cycles could be rela-
tively well tolerated.

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