Postnatal development of right atrial injection of capsaicin-induced apneic response in rats

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Wang, Rurong, and Fadi Xu. Postnatal development of right atrial injection of capsaicin-induced apneic response in rats. J Appl Physiol 101: 60–67, 2006. First published March 30, 2006; doi:10.1152/japplphysiol.00085.2006.—Apnea and respiratory failure often occur in infants with pulmonary disease. Bronchopulmonary C-fiber (PCF)-mediated apnea is an important component of respiratory dysfunction. This study was undertaken to define the postnatal development of PCF-mediated apnea. The experiments were conducted in five groups of anesthetized, tracheotomized, and spontaneously breathing rats with ages at postnatal days P1–3, P7–9, P14–16, P21–23, and P56–58. Right atrial bolus injection of three doses of capsaicin (Cap), equivalent to 2, 4, and 8 μg/kg used previously in 450-g rats, was applied to stimulate PCFs. We found that 1) Cap-induced apneic response [percent change from the baseline expiratory duration (TE%) values] and the sensitivity of this response (ΔTE%/μg⁻¹) were significantly greater in the rats <P10 than those >P10; 2) the Cap-induced apneas were vagally dependent in all rats tested; and 3) bivagotomy-induced prolongation of TE was much greater in the rats <P10 than those >P10. From these findings we concluded that, compared with the older rats (>P10), the newborn rats have a stronger PCF-mediated respiratory inhibition that may contribute to infants’ vulnerability to respiratory failure.

The majority of afferent nerves arising from the lungs and airways are conducted through the vagus nerves and their branches; of these, 75% are bronchopulmonary C fibers (PCFs). PCF endings are located in proximity to the pulmonary capillaries and the epithelium of conducting airways (11) and are sensitive to various exogenous chemical substances and endogenously released mediators (9, 33, 39, 40). Right atrial bolus injection of capsaicin (Cap), a pungent active ingredient of hot peppers (6), has been used predominantly to stimulate PCFs to produce a brief apnea (several seconds) in both anesthetized and awake animals (33). This apnea is defined as central apnea because a similar apnea is also denoted on the phrenic efferent nerve (27, 53). This apnea is excitable in rats (27), rabbits (26), and mice (41, 43), and achieved through the reflex involved in the central nervous system (14, 44, 58). The apnea is usually followed by rapid, shallow breathing, bronchoconstriction, increased mucous secretion, hypotension, and bradycardia (11).

Apnea is one of the common respiratory disorders in premature infants and infants with pulmonary diseases. It was demonstrated that 25% of infants whose birth weight was less than 2,500 g and 80% of those less than 1,000 g have apneic episodes during neonatal life (35). Respiratory syncytial virus (RSV) infections in infants and young children cause more than 120,000 hospitalizations in the United States every year, and some of these patients need mechanical ventilation because of apnea and respiratory failure. Infants younger than 3 mo and those who are premature are particularly vulnerable (1, 8, 28). Recent studies showed that right atrial injection of Cap (10 μg/kg) elicited a brief apnea in P14 weanling rats (where P represents postnatal age in days), but the same dose of Cap caused a long-lasting apnea leading to 66% mortality 2 days after RSV infection (48). More interesting is the subsequent observation that RSV infection significantly increased both Cap-induced apneic duration and the sensitivity of PCF response to Cap in P14 but not P35 anesthetized rats (W. Peng and F. Xu, unpublished observation). These results strongly suggest that the RSV facilitation of the PCF-mediated respiratory inhibition is age dependent in the rat.

Several studies have investigated the immaturity of vagal afferent fibers. Using scanning electron microscopy, Hasan et al. (22) and Mortola (35) found that the number of vagal C fibers was higher in preterm lambs. Premature birth did not change the vagal Hering-Breuer reflex, and Arsenault et al. (2) showed that the PCF-mediated apneic response already existed immediately after birth in full-term lambs. In addition, Mortola suggested that vagal afferents played a more important role in control of breathing in early life because bivagotomy slowed breathing rate much more in the newborn than older animals (35). However, there has been no investigation into whether there is a postnatal development of the PCF-mediated apneic response, an important inhibitory component of the central control of breathing. Because of the high susceptibility to apnea and high population of vagal C fibers in early life, we tested the hypothesis that there was a postnatal development of the PCF-mediated respiratory response characterized by a stronger PCF-mediated apneic response in the newborn compared with the adult rats.

To examine our hypothesis, the apneic responses to right atrial injection of Cap were compared in five groups of anesthetized, tracheotomized, and spontaneously breathing rats with ages at P1–3, P7–9, P14–16, P21–23, and P56–58. The intensity of stimulation was equalized among the individual rats by adjusting the dose of Cap according to lung fluid volume. Our results showed that, compared with older rats, Cap-induced apnea and the sensitivity of this apnea as well as bivagotomy-induced prolongation of expiratory time (TE) were markedly greater in the rats <P10. In addition, the apnea was vagally dependent. These data, for the first time, demonstrate that the newborn rats (<P10), compared with older rats, have a stronger PCF-mediated respiratory inhibition that may contribute to infants’ vulnerability to respiratory failure.

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METHODS

General procedure. The experimental protocols described in this study were approved by the Institutional Animal Care and Use Committee in compliance with the Animal Welfare Act. All procedures were performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. This study includes two series of experiments conducted in anesthetized, tracheotomized, and spontaneously breathing male Sprague-Dawley rats of different ages. Isoflurane (3–4% for induction, ~1.0% for maintenance) was administered to maintain an adequate anesthetic level to suppress corneal and withdrawal reflexes. During conduction of the experimental protocols, the flow rate and anesthetic delivered from an anesthesia machine (SurgiVet, Phoenix, AZ) were similar in each group to maintain the anesthetic at approximately the same level throughout the experiment. The trachea below the larynx was exposed through a midline incision, cannulated, and connected to a pneumotachograph to record airflow. The flow signal was integrated by PowerLab/8SP (ADInstruments, Castle Hill, Australia) to generate expiratory duration (Te), tidal volume (Vt), respiratory frequency (f), and minute ventilation (Ve). The pneumotachograph (Frank’s Manufacturing, Albuquerque, NM) was made of stainless steel with a linear flow-pressure relationship in the range of 0–10 ml/s and a flow resistance equal to 0.046 cmH2O·ml⁻¹·s⁻¹. The dead space was varied for different diameters of the pneumotachograph: ~0.04, 0.08, and 0.2 ml for groups P1–3 and P7–9, P14–16 and P21–23, and P56–58, respectively. Core body temperature was monitored with a rectal probe and maintained at ~37.5°C by a heating pad and radiant heat lamp.

Adjustment of Cap doses injected in different-aged rats. Several previous studies have shown that right atrial injection of 1–2 µg/kg of Cap is essential to produce an apnea in anesthetized, spontaneously breathing adult rats with a body weight of ~450 g (33, 55, 57). This apnea was defined as having a Te at least four times longer than the control Te. To generate the equivalent intensity of PCF stimulation in different-aged rats, the dose of Cap injected in each rat was adjusted according to the individual lung fluid volume. Twenty-three rats ranging from P1 to P90 were used to determine the relationship between body weight and lung fluid volume, defined as the difference between the wet and dry weight of the lungs. The distribution of different-aged animals used is detailed in Fig. 1, bottom. After weighing and adequate anesthesia (pentobarbital sodium, 60 mg/kg, intraperitoneal), the animal’s chest was opened to expose the lungs and heart. The right and left pulmonary arteries and, subsequently, the pulmonary veins were sequentially ligated to separate the blood circulating in the lungs from that circulating systemically. The whole lung with the trapped blood was carefully removed and weighed, and the result was recorded as the wet weight of the lungs. The wet lung was dried in an isostemperature oven (Fisher Scientific, model no. 97-920-1, Pittsburgh, PA) at 60°C for 72 or 96 h. After drying in the oven for 48 h, the lungs were weighed once every day. If the final two weights of the lungs were the same, this weight was defined as dry weight of the lungs. Usually, baking for 48 h is enough to completely dry the lung.

Right atrial injection of Cap. The first series of experiments were performed in five groups of rats with ages at P1–3, P7–9, P14–16, P21–23, and P56–58 to determine the postnatal development of the Cap-induced apnea. Eight rats were used in each group. The right jugular vein was isolated, and a catheter advanced close to the right atrium for injection of Cap. The inserted depth of the catheter was determined by measuring the distance from the heart (felt from the heartbeat) to the cannulation site before implantation. The position of the cannula was confirmed by autopsy after the experiments. Fifty-gauge tubing was used in the rats of P56–58, and the tip of the tubing was narrowed by heat pulling to allow for insertion in younger animals. Rather than just fully loading the Cap solution, the length of the catheter was slightly lengthened (~1.5 mm) to minimize immediate Cap diffusion-induced PCF stimulation. The lengths of the catheter were varied for each animal group to account for the different load volumes of Cap solution. A stock solution of Cap (400 µg/ml, Sigma-Aldrich, St. Louis, MO) was made in a vehicle of 10% Tween 80, 10% ethanol, and 80% isotonic saline. Cap solutions of the desired concentration were prepared with saline dilution. Because 0.1 ml of the loading Cap solution and 0.2 ml of the flushing volume were used previously in 450-g rats, both volumes were adjusted by the ratio of lung fluid volume in the tested rats vs. lung fluid volume in the 450-g rats. Therefore, the Cap concentration was the same for all rats, although the injection volumes and dosages were varied. Each animal received three different concentrations of Cap equivalent to 2.0, 4.0, and 8.0 µg/kg used in the 450-g rats, and these doses were defined as Cap-A, -B, and -C stimulations. A 15-min interval was required between two stimulations, with each injection performed after stabilizing the cardiorespiratory variables for at least 5 min. Routinely, in each animal vehicle injection equivalent to the Cap volume was conducted to serve as the sham control.

Right atrial injection of Cap in vagotomized rats. The second series of experiments tested whether the Cap-induced apnea was vagally mediated. The cervical vagal nerves were bilaterally isolated and looped with suture in 10 rats (four rats in P7–9, and two rats in P14–16, P21–23, and P56–58, respectively). Twenty minutes after bilateral transection of the vagal nerves, Cap doses equivalent to 4.0 and 8.0 µg/kg used in the 450-g rats were administered.

Data acquisition and analysis. The relationship between animals’ lung fluid volume and body weight was analyzed by plotting the lung fluid volume against body weight and fitting these numbers into an exponential equation: \( Y = a(1 - e^{-bX}) \), in which \( Y \) is lung fluid volume and \( X \) is body weight. The values of \( a \) and \( b \) were obtained by using the SYSTAT SigmaPlot software (Point Richmond, CA) and statistically examined by using ANOVA. Raw data of airflow were digitized, monitored, and recorded by using a PowerLab/8sp (ADInstruments) connected to a computer employing the PowerLab Chart 5 software (ADInstruments). Respiratory variables including Te, Vt, f, and Ve were derived by the online calculation functions of the
software. The baseline values (control) were collected and averaged for 30 s immediately before Cap stimulation and represented by absolute values. An initial apneic response that occurred immediately after each Cap administration was determined by measuring the prolonged \( \Delta T_E \) and represented by percent change from the baseline \( \Delta T_E \) values (\( \Delta T_E/\% \)).

The sensitivity of the apneic response to Cap was obtained by \( \Delta T_E/\% \) divided by Cap doses (\( \Delta T_E/\% \cdot \mu g^{-1} \)) (30, 57). The latency of the respiratory responses was determined by measuring the duration between the initiation of the Cap injection and the beginning of the apnea. Ventilatory responses of 5 s immediately after the initial apnea were measured and defined as the secondary respiratory responses. The duration required for recovery of these responses to the levels of preinjection was also measured. Group means were reported as means \( \pm SE \). Two-way ANOVA for repeated measures followed by Fisher’s least significant difference test was used to analyze the statistical significance of the differences in 1) the initial apneic response, 2) the PCF sensitivity, 3) the secondary respiratory response, and 4) these variables before and after bivagotomy among the groups. One-way ANOVA also was used for comparing the baseline respiratory activities and latency of and recovery duration from Cap stimulation among the five groups of rats. \( P \) values \(< 0.05 \) were considered significant.

RESULTS

Relationship between lung fluid volume and body weight. As illustrated in Fig. 1, lung fluid volume was nonlinearly related to body weight following the equation: \( Y = 1.671(1 - e^{-0.006X}) \), in which \( Y \) and \( X \) represent lung fluid volume and body weight, respectively. The values of \( a \) (1.67) and \( b \) (0.0063) are significant (\( P < 0.0001 \)). The coefficient of determination (\( R^2 \)) was 0.99 (\( P < 0.0001 \)), demonstrating an excellent fitting of this equation in reflecting the relationship between lung fluid volume and body weight. The Cap doses and concentrations of loading and flushing Cap administered in the 450-g rats were used as the reference for adjusting these values in the present study. Three steps were taken to make these adjustments. First, the lung fluid volume (\( Y_1 \) value) in the tested rats of this study and the lung fluid volume (\( Y_2 \) value) in the 450-g rats were calculated by using this equation. Second, according to the ratio of \( Y_1 \) vs. \( Y_2 \), the Cap dose used in the tested rats (\( D_1 \)) was calculated from the dose used in the 450-g rats (\( D_2 \)). Third, the volumes of loading and flushing Cap administered in the tested rats were adjusted based on the ratio of \( D_1 \) vs. \( D_2 \). In each animal, three concentrations of Cap, i.e., -A, -B, and -C equivalent to 2.0, 4.0, and 8.0 \( \mu g/kg \) Cap used in the 450-g rat, were administered.

Cap-induced apnea and the sensitivity in evoking this apnea. We compared the Cap-induced apneic duration in the five groups of rats. In general, the apneic durations observed in rat pups <P10 were profoundly longer than those noted in older rats. Typical experimental recordings of the ventilatory response to Cap in a P7 and a P14 pup are illustrated in Fig. 2. As shown, right atrial injections of three concentrations of Cap equivalent to 2.0, 4.0, and 8.0 \( \mu g/kg \) Cap used in the 450-g rats produced an immediate apnea in both rats, and these \( \Delta T_E \) responses appear to be Cap dose dependent, especially in the P7 rat. Compared with the P14 rat, the apneic durations induced by Cap stimulations were longer in the P7 rat. With respect to the apneic duration, our group data (Fig. 3A) showed that 1) compared with control Te, Cap-A, -B, and -C stimulation significantly prolonged Te by 5- to 63-fold in five groups of rats (\( P < 0.05 \)); 2) there were no significant differences of the apneic response elicited by Cap-A stimulation (equivalent to 2.0 \( \mu g/kg \) Cap used in the 450-g rats) in five groups of rats (\( P > 0.05 \)); 3) the apneic response to Cap seems to be dose dependent in P1–3 and P7–9 rats but was not so in other groups; and 4) Cap-B and -C stimulation-induced apneic responses were age dependent. In other words, the greatest apneic response was observed in P1–3 rats among all tested rats; this response was greater in P7–9 compared with the rats >P10, and no difference was observed among P14–16, P21–23, and P56–58 rats. Vehicle injection did not significantly alter ventilation in all five groups of rats. Te (control vs. vehicle injection) in P1–3, P7–9, P14–16, P21–23, and P56–58 rats was 0.440 ± 0.036 vs. 0.460 ± 0.038 s; 0.453 ± 0.029 vs. 0.466 ± 0.039 s; 0.542 ± 0.023 vs. 0.564 ± 0.028 s; 0.604 ± 0.023 vs. 0.610 ± 0.033 s; and 0.564 ± 0.038 vs. 0.589 ± 0.048 s, respectively (\( P > 0.05 \)). To determine whether the sensitivity of Cap-induced apnea is changed as a condition of development, we compared the sensitivity of PCF-mediated apnea, defined as \( \Delta T_E/\% \cdot \mu g^{-1} \), in differently aged rats. As illustrated in Fig. 3B, the sensitivity was age dependent; it was greatest in P1–3 among all tested rats and greater in P7–9 rats compared with the rats >P10. Interestingly, the sensitivities were not significantly different among P14–16, P21–23, and P56–58 rats. The respiratory patterns immediately after the initial apnea were also compared. As exhibited in Fig. 2, an initial apnea followed by two to three secondary apneas was observed in the P7 rat when higher Cap concentrations were administered, but these responses did not

– Fig. 2. Experimental recordings of the respiratory responses to right atrial injections of Cap in a P7 (A) and a P14 (B) rat. The 3 doses of Cap-A, -B, and -C solution are equivalent to 2 (left), 4 (middle), and 8 \( \mu g/kg \) (right) doses used in 450-g rats and are the same for Figs. 3 and 4. Traces from top to bottom are tidal volume (\( V_T \)), respiratory frequency (\( f \)), and minute ventilation (\( V_E \)). Arrows indicate onset of Cap injections. bpm, Breaths/min.
Exist in the P14 rat. Statistical results indicated that immediately after the initial apnea there was a significant elevation of VT in all animals (P < 0.05). In sharp contrast, as shown in Fig. 4, a significant reduction of f associated with remarkable TE prolongation (secondary apnea) was only observed in P1–3 and P7–9 rats.

Latency of and respiratory recovery from Cap stimulation. The latency of Cap-induced apnea and the recovery duration were compared in different-aged rats and are summarized in Fig. 5. The injection durations were 1.5 s in all five groups. The latency for the evoked apnea and the recovery durations from the apnea were not significantly different in five groups (P > 0.05).

Ventilation and ventilatory responses to Cap after bivagotomy. We tested the effects of bivagotomy on the ventilation and Cap-induced apneic responses in 10 rats (four rats in P7–9 and two rats in P14–16, P21–23, and P56–58, respectively). As illustrated in Fig. 6, bilateral transection of cervical vagus nerves significantly increased VR and decreased f predominantly via prolonging TE without significant VE changes in either group of rats. However, TE was prolonged by 576.2 ± 151.8% after bivagotomy in P7–9 rats but was 94.1 ± 10.4% in P14–58 rats (P < 0.001). It should be noted that the TE prolongations observed in the P14–58 rats were similar (96.1, 88.9, and 97.3% for P14–16, P21–23, and P56–58, respectively). After bivagotomy, right atrial injection of Cap did not significantly prolong the TE in all tested rats (P7–9 and P14–58) as depicted and summarized in Fig. 6 and Table 1, respectively.

Baseline respiratory variables in different-aged rats. The averaged age, body weight, and respiratory variables in each group of animals are summarized in Table 2. All of the listed variables changed in an age-dependent manner. As rats developed, VE and VR gradually and significantly increased. Most importantly, f was fastest in P1–3 rats in five groups and faster in P7–9 rats compared with the rats > P10; and there were no differences among the groups of P14–16, P21–23, and P56–58 rats. The normalized VE (ml·min⁻¹·kg⁻¹) was ~ 574, 809, 719, 735, and 487 in P1–3, P7–9, P14–16, P21–23, and P56–58 rats, respectively. Previous studies showed that VE values were 1,120, 1,480, and 1,317 ml·min⁻¹·kg⁻¹ in awake P3, P8, and P14 rats, respectively (3, 16), and barbiturate anesthesia reduced these VE values by ~48% (49). Compared with the former studies, our corresponding VE values (574, 809, and 719 ml·min⁻¹·kg⁻¹) using the anesthetized preparation were decreased by ~50%, which is consistent with the results reported by other investigators (3, 16).

DISCUSSION

In the present study, we found that the amplitude of the apneic response to stimulation of PCFs is age dependent, i.e., the prolonged TE response to Cap and the sensitivity of Cap-induced apnea are significantly different as rats develop. The differences can be summarized as three major aspects. First, the apneic response and the sensitivity are greatest in P1–3 rats in five groups, and greater in P7–9 rats compared with rats P10, with little difference among the P14–58 rats.
The equivalent Cap stimulation administered in the P7–9 and P1–3 rats elicited an apnea two- to threefold longer than that observed in P14–58 rats, and the same was true for the sensitivity of the PCF-mediated apneic response. These data provide, for the first time, experimental evidence to convincingly show a stronger apneic response to right atrial injection of Cap in the rats.

Second, the apneic response appears to be Cap dose dependent in P1–3 and P7–9 rats but not in the older rats, further supporting the higher sensitivity in the rats than P10 as described above. Third, although an increase of VT was observed immediately after the initial apnea in all rats, the secondary apneas occurred only in the pups P1–3. The presence of the secondary apneas coupled with the greater initial apneic response clearly demonstrates that Cap generates stronger apneic responses in these newborn pups (<P10) compared with the older rats (P14–58). There are some similarities in the Cap-evoked apnea between the newborn and older rats. The latency (~1.5 s) and recovery time (~3 min) were similar in all tested animals, consistent with those previously reported in adult rats (33, 55, 57). In addition, deeper breaths (increased VT) were found immediately after the initial apnea in our tested rats. Absence of rapid, shallow breathing following the reflex apnea has been previously observed in anesthetized mice and rats (7, 42, 51).

The specificity of PCF involvement in the right atrial injection of Cap-induced apnea has been well established. For example, bilateral vagotomy (10, 11, 56) and inactivation of all C fibers or vagal C fibers, including PCFs, eliminated this apnea (25, 29, 31, 32). Cap administered into isolated perfused dog pulmonary circulation to prevent subsequent stimulation of extrapulmonary afferents also produced the expected phrenic neural apnea (10, 18). Moreover, when a single-fiber recording technique was used, right atrial bolus injection of Cap evoked a remarkable increase in the activity of PCFs, with little or no effect on the discharges of rapidly adapting stretch receptors and slowly adapting stretch receptors in the lung (23, 33). We tested the effects of bivagotomy on the Cap-induced apneic responses in rats aged from P7 to P58. We found right atrial

### Table 1. Ventilatory responses to capsaicin in the intact and vagotomized rats

<table>
<thead>
<tr>
<th>Cap Group</th>
<th>VT, Δ%</th>
<th>f, Δ%</th>
<th>VT, Δ%</th>
<th>TE, Δ%</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P7–9</td>
<td>1.12±1.72</td>
<td>1.27±1.05</td>
<td>1.59±2.42</td>
<td>−2.51±0.83</td>
</tr>
<tr>
<td>P14–58</td>
<td>1.29±3.93</td>
<td>5.57±2.44</td>
<td>2.03±3.14</td>
<td>−4.33±2.22</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P7–9</td>
<td>0.44±1.66</td>
<td>1.09±1.17</td>
<td>−1.13±1.16</td>
<td>−1.13±1.17</td>
</tr>
<tr>
<td>P14–58</td>
<td>0.51±3.02</td>
<td>−0.73±5.01</td>
<td>2.74±1.98</td>
<td>−1.26±4.19</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4 in P7–9 and 6 in P14–58 rats and 2 in P14–16, P21–23, and P56–58, respectively. Responses of VT, f, VT, and TE were depicted in A–D, respectively. Values are means ± SE. *P < 0.05 compared before and after bivagotomy; †P < 0.01 compared between the vagotomized P7–9 and P14–58 rats.
Table 2. Baseline respiratory variables in the 5 groups of rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, days</th>
<th>Weight, g</th>
<th>V̇E, ml/min</th>
<th>f, breaths/min</th>
<th>Vt, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1–3</td>
<td>1.63±0.32</td>
<td>8.63±0.46</td>
<td>4.93±0.60</td>
<td>105±5</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>P7–9</td>
<td>7.78±0.28</td>
<td>19.04±1.13</td>
<td>15.80±1.33</td>
<td>93±4</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td>P14–16</td>
<td>14.57±0.30</td>
<td>35.44±1.18</td>
<td>25.85±1.71</td>
<td>75±3</td>
<td>0.35±0.03</td>
</tr>
<tr>
<td>P21–23</td>
<td>21.63±0.26</td>
<td>64.01±2.54</td>
<td>46.08±3.37</td>
<td>68±2</td>
<td>0.68±0.06</td>
</tr>
<tr>
<td>P56–58</td>
<td>56.75±0.31</td>
<td>238.25±6.39</td>
<td>115.66±7.27</td>
<td>72±3</td>
<td>1.62±0.10</td>
</tr>
</tbody>
</table>

Values are means ± SE. P < 0.05 in V̇E and Vt among 5 groups; and f: P1–3 rats vs. others; and P7–9 rats vs. others.

injection of Cap failed to evoke not only the initial apneic response in all tested rats, but also the secondary apneas observed in pups <P10 after vagotomy. These findings suggest that, similar to adult rats, the Cap-induced strong apneic responses observed in newborn rats are mediated by vagal afferents, likely by PCFs. Moreover, both the initial and secondary apneas denoted in rats <P10 are mediated by PCFs.

There are several lines of evidence to support the immaturity of vagal C fibers. First, morphologically, the number of vagal C fibers was reported to be higher in preterm lambs (22, 35). Second, premature birth did not significantly alter the vagal Hering-Breuer reflex in lambs (2), and the PCF-mediated respiratory responses already existed in newborn lambs (2) and rabbits (15). Third, breathing movements are episodic in fetal life and abruptly change to be continuous after delivery, but continuous breathing is critically dependent on fetal maturity and intact vagal nerve function (21, 54). For example, the consequences of vagotomy were more dramatic in the newborn anesthetized and decerebrated cats, dogs, and kittens compared with the older ones, including a greater prolongation of Tr, possibly leading to death within a few hours (12, 50). In agreement with the latter, we also found that Vt increases and f reductions were greater in P7–9 rats than in older rats. All of these results demonstrate that vagal afferents play a more important role in control of breathing and that the PCF-mediated apneic reflex is much stronger in the rat pups <P10 compared with P14–58 rats. The mechanism underlying the overexpression of PCF-mediated apnea in younger rats remains unknown. It is possible that this high sensitivity is related to immaturity of nerve development characterized as containing more C fibers as mentioned above. In addition, accumulated evidence has demonstrated that PCFs can be chemically stimulated by local tissue heat (46), acidification (20, 24), hypercapnia (19, 34), and reactive oxygen species (47). PCFs are also mechanically sensitive to increased interstitial fluid volume or pressure in the lungs and airway mucosa (39, 45). It has been demonstrated that, compared with adults, the infants have a relatively higher temperature and metabolism (CO2 production) (36), especially in the premature (13), and a greater pulmonary circulation (4) that implies a higher lung fluid volume as observed in this study. These factors may also be involved in the genesis of high PCFs sensitivity and account for infants’, particularly the premature, vulnerability to respiratory failure. It is generally accepted that right atrial injection of Cap produces the apneic response via binding the transient receptor potential channel vanilloid subfamily member 1 receptor (52). The PCF afferents terminated at the nucleus tractus solitarius and local substance P is critical for prolonging the PCF-mediated apnea (37, 57). Importantly, respiratory failure observed in RSV-infected P14 rats elicited by right atrial injection of Cap disappears after intravenous injection of the antagonists of neurokinin 1 receptors and GABA receptor (48). Therefore, further studies are needed to determine whether the density of PCFs and transient receptor potential channel vanilloid subfamily member 1 of PCFs, and substance P and GABA levels in the nucleus tractus solitarius are higher in rats <P10 than those in older rats.

The augmented PCF-mediated apnea may contribute to respiratory failure observed in infants with pulmonary disease. Apnea and respiratory failure in infants with RSV infection is a typical example. RSV has been suggested to be a precipitating factor in sudden infant death syndrome (17, 38), and RSV infection was found in 22% of sudden infant death syndrome cases. A number of studies have reported that RSV-induced apnea and respiratory failure often present in infants and young children, especially those younger than 3 mo and those who are premature (1, 5, 8, 28). Interestingly, a high vulnerability of respiratory failure in RSV-infected postnatal rats has been observed. Right atrial injection of Cap (10 μg/kg) elicited a brief apnea in awake 2-wk-old weanling rats, but the same dose of Cap caused a long-lasting apnea leading to 66% mortality in age-matched rats 2 days after RSV infection (48). This convincingly demonstrates that RSV infection amplifies PCF-mediated apnea in early life of rats.

It would be ideal to adjust Cap doses injected in different-aged rats based on the pulmonary circulation volumes. However, so far as we know, data on pulmonary circulation volumes in newborn and postnatal rats is lacking. We are aware of the limitation of adjusting Cap doses on the basis of the lung fluid volume because the lung fluid volume contains not only the fluid of pulmonary circulation but also the intracellular fluid and interstitial fluid of the lungs. It is generally accepted that cardiac output (ml·kg⁻¹·min⁻¹) is highest in the newborn and gradually falls as the child develops. The cardiac output in newborn infants at rest is ~350 ml and falls over the first 2 mo of life to ~150 ml; it then gradually decreases to ~75 ml and is maintained at this level in the normal adult (4). This concept is somewhat consistent with the relationship between the rats’ lung fluid volume and body weight obtained in this study, indicating that lung fluid volume is relatively greater in younger rats compared with older rats. Previous studies showed that stimulation of PCFs produced the apnea associated with hypotension and bradycardia in adult animals (33, 55, 57). It would be interesting to know whether these cardiovascular responses are altered by development. However, the wall of carotid artery was very fragile in newborn rats, making it nearly impossible to insert a catheter into the carotid artery. In our pilot experiments, we found that the apneic responses elicited by right atrial injection of a Cap-A dose (equivalent to 2.0 μg used in the 450-g rats) were not significantly different among the different-aged rats. Thus we infer that the differences of the threshold of Cap-induced cardiovascular response, if present, would be not significant. Several investigators have pointed out that right atrial injection of a relatively lower dose of Cap leads to rapid, shallow breathing in adult animals (33). No attempt was made in this study to address the issue.

In summary, apnea and respiratory failure often occur in infants with pulmonary disease. PCF-mediated apnea is an important component of respiratory dysfunction. The present
study investigated the postnatal development of PCF-mediated apnea. We found that 1) Cap-induced apneic response and the sensitivity of the apneic response were the greatest in P1–3 rats among all rats tested, greater in the rats <P10 than >P10; 2) the Cap-induced apneas were vagally dependent; and 3) bivagotomized-induced prolongation of Ti was much greater in rats <P10 than in those >P10. These findings lead to a conclusion that, compared with adults, the rat during early life has a stronger PCF-mediated respiratory inhibition that may contribute to infants’ vulnerability to respiratory failure.

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GRANTS

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REFERENCES


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