Acute hypoxia prolongs the apnea induced by right atrial injection of capsaicin

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The majority of afferent nerves arising from the lungs and airways are conducted in the vagus nerves and their branches, 75% of which are nonmyelinated (C) fibers. Right atrial bolus injection of capsaicin (Cap) or phenylbiguanide produces a brief expiratory apnea (several seconds) in different species (2, 17, 19, 25, 26, 29, 32, 40, 42). This apnea is often followed by rapid shallow breathing, bronchoconstriction, hypersecretion of mucus, hypotension, and bradycardia (7). This Cap-induced inspiratory inhibition is dependent on the integrity of vagus nerves because bilateral vagotomy eliminates this apnea (5, 7). Other studies further indicate that this apnea was achieved by selectively activating vagal pulmonary C fibers (PCFs). For example, with use of a single-fiber recording technique, it has been documented that the right atrial injection of Cap dramatically increased PCF activity (PCF_A) with little effect on the activities of other types of vagal afferent fibers (16, 23).

Cap-induced apnea is centrally mediated, and the nucleus tractus solitarius (NTS), especially the commissural subnucleus (cNTS), is the first site in the neural circuitry where afferent signals from primary PCFs are transmitted and susceptible to modulation. PCF afferents have been demonstrated to terminate in the cNTS by using the antidromic activation technique (3, 20). In agreement, studies utilizing Fos protein immunoreactivity have shown that stimulation of PCFs increases cNTS neural activity in ferrets (14). cNTS neurons can integrate vagal inputs with convergent signals from peripheral chemoreceptors, especially the input of carotid body chemoreceptors (29).

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In the present study, we addressed four major questions. First, is there an interaction between hypoxia and stimulation of PCFs? And if so, does it affect the control of breathing? Second, is this interaction species dependent? Third, how important are the vagal inputs to generate this interaction? Finally, does hypoxia alter the PCF response to Cap?

METHODS

General protocols. The experimental protocols described in this study were approved by the Institutional Animal Care and Use Committee in compliance with the Animal Welfare Act. They were in accordance with the Guide for the Care and Use of Laboratory Animals (DHHS Publication No. 85-23 (NIH), Revised 1985, Office of Science and Health Reports, 8750-7587/03 $5.00 Copyright © 2003 the American Physiological Society http://www.jap.org

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Bethesda, MD 20892). Two series of experiments were conducted in the present study. Series I experiments were performed in anesthetized (chloralose and urethane; 100 and 500 mg/kg ip, respectively), tracheotomized, and spontaneously breathing Sprague-Dawley rats (250–350 g; n = 13) and guinea pigs (450–600 g; n = 4). The left femoral artery was cannulated for monitoring arterial blood pressure (ABP) and heart rate (HR) and collecting blood samples. The right jugular vein was isolated, and a catheter (50-gauge tubing, ~10-cm length) was advanced close to the right atrium for injection of agents. The inserted depth of the catheter was determined by measuring the distance from the heart (felt from the heart beat) to the cannulating site before implantation. Supplemental anesthesia, as needed, was administered intravenously to suppress corneal and withdrawal reflexes. The trachea was cannulated below the larynx with a short cannula connected to a one-way breathing valve. Respiratory flow (V) was measured with a pneumotachograph and a differential pressure transducer (model MP-45, Validyne). The flow signal was integrated (PowerLab) to generate tidal volume (VT). The pneumotachograph was made of stainless steel and had a linear flow-pressure relationship in the range of 0–20 ml/s and a flow resistance of 0.046 cmH2O·ml−1·s with a dead space of ~0.2 ml. A three-way stopcock was attached to the inspiratory line of the one-way breathing valve and used to manipulate the inhaled gas mixture. The end-tidal PCO2 (PETCO2) level was monitored via an infrared CO2 analyzer (model 78356A, Hewlett Packard). Animals were placed into a rigid metal frame with the head fixed in a stereotaxic apparatus (Kopf). Bleeding was controlled with an absorbable hemostat (Surgicel and Gelfoam) and the use of a bipolar coagulator (model 440S, Radionics). The core temperature was monitored with a rectal probe and controlled with an absorbable hemostat (Surgicel and Gelfoam) and by a computer (PowerLab) for later analysis. In spontaneously breathing animals, baseline values (control) were collected and averaged for 1 min immediately after vehicle injection. The cardiorespiratory variables returned to the control level, after the establishment of the single-unit PCF response to a right atrial injection of Cap (0.5 µg) during a controlled experiment, the same Cap challenge was repeated during acute hypoxia (10% O2; ~1 min).

Data acquisition and analysis. Raw data of ABP and mean arterial blood pressure (MAP) were recorded in all animals. Inspiratory and expiratory duration (Ti and Te, respectively), VT, f, and inspiratory minute ventilation (Vt; product of VT and f) in spontaneously breathing preparation and PCF were recorded on a polygraph (model 7D, Grass) and/or by a computer (PowerLab) for later analysis. In spontaneously breathing animals, baseline values (control) were collected and averaged for 1 min immediately before chemical challenges. The cardiorespiratory responses to hypoxia were measured for 20 s just before administration of Cap. TE was determined by measuring the longest period during which VT signals were not detectable acutely hypoxic for ~1 min (Hypoxia + Cap). The hypoxic exposure was terminated ~15 s after completion of the Cap injection. The tracheal cannula was connected to a ventilator (model 55-0798, Harvard Apparatus) if the apneic duration lasted too long (>30 s in average). The animal was artificially ventilated with VT at 8–10 ml/kg and at a respiratory frequency (f) of 50–55 breaths/min until spontaneous breathing was restored. The tracheal cannula was then reconnected with the apparatus containing the pneumotachograph and one-way breathing valve. The same protocols (Cap and subsequent Hypoxia + Cap) were repeated twice in seven rats and three times in one rat. In addition, these protocols were performed in four guinea pigs, in which Cap dosage was increased to 1 or 2 µg to produce a detectable apnea. Arterial blood samples were taken before hypoxia and at the initial apneic period induced by Hypoxia + Cap (5–10 s after the onset of apnea). In 4 of these 11 rats and in 1 other rat, the vehicle instead of Cap was administered before and during acute hypoxia to determine whether hypoxia also affects the response to vehicle injection.

Reproducibility of Cap-induced apnea. These experiments (n = 6) were designed to determine whether the apneic duration in response to Cap changed over time. Bolus injection of the same dose of Cap (0.5 µg) during normoxia (room air) was repeated twice with an interval of 30 min.

Dependence of extremely long-lasting apnea on vagal integrity. The cervical vagus nerves were carefully isolated bilaterally and looped with suture in some rats. The ventilatory responses to Cap and Hypoxia + Cap were tested before and after bilateral vagotomy in 5 of the 11 rats and were tested only after bilateral vagotomy in 1 other rat. All chemical stimulations were applied at least 30 min after vagotomy to obtain stabilized baseline cardiorespiratory values.

PCF in response to Cap and Hypoxia + Cap. Series II experiments were conducted in seven anesthetized, paralyzed, and artificially ventilated rats with a midline thoracotomy. Single PCF was recorded to determine the effect of acute hypoxia on PCF responsiveness to Cap. The inspiratory outlet of the ventilator was placed under 3 cmH2O to maintain a near-normal functional residual capacity. The methods for single-PCF unit recording were the same as those described previously (17, 22). Briefly, the right cervical vagus nerve was carefully isolated and sectioned; under a microscope, a thin filament was teased away from the distal end of the cut vagus and placed on a platinum-iridium hook electrode. Action potentials were amplified and monitored by audio monitor and displayed on an oscilloscope-computer screen. The thin filament was further split until the afferent activity from a single unit was electrically isolated. A moistened Q-tip was used to probe the lung surface and to ensure that the PCF recorded arose from the lung rather than from the heart. After the establishment of the single-unit PCF response to a right atrial injection of Cap (0.5 µg) during the controlled experiment, the same Cap challenge was repeated during acute hypoxia (10% O2; ~1 min).

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RESULTS

Comparison of the cardiorespiratory responses to Cap and Hypoxia + Cap. The reproducibility of Cap-induced apnea was tested in six rats. Experimental recordings illustrating the reproducibility of Cap-induced apnea in an anesthetized and spontaneously breathing rat are displayed in Fig. 1A, and group data are summarized in Fig. 1B. Before hypoxic exposure, right atrial injection of Cap (0.5 μg) produced an apnea that lasted for ~5 s and was accompanied by hypotension and bradycardia (Fig. 1, left). This protocol, repeated 30 min later, caused very similar cardiorespiratory responses (Fig. 1, right). Statistically, the apneic duration and associated hypotension induced by the first trial of Cap injection were not significantly different from those elicited by the second trial (4.42 ± 0.46 vs. 4.25 ± 0.26 s for Te, P > 0.05; 80.13 ± 11.40 vs. 88.02 ± 10.91 mmHg for MABP, P > 0.05). We tested the effect of acute hypoxia on Cap-induced apnea in 20 trials of 11 rats. Hypoxia produced a marked potentiation on the apneic response to Cap (>500%, 14 trials) in eight rats and slight prolongation (<100%, 5 trials) or little alteration (3 trials) in three rats. The Cap-induced apnea usually occurred within one or two breaths after Cap bolus injection. When Cap injection was repeated during acute hypoxia (~1 min), the rat immediately stopped breathing for an extremely long period, which was interrupted by a period of artificial ventilation. The ventilatory responses to Cap were compared before and during hypoxia (Fig. 2, top). The apnea (4.37 ± 0.53 s) induced by Cap injection was extremely prolonged (69.67 ± 11.97 s; P < 0.01) during acute hypoxia. Hypoxia itself has not significantly altered Te (P > 0.05). In addition, the apneic durations induced by the first and second trial of Hypoxia+Cap (76.36 ± 13.59 vs. 64.83 ± 18.91 s; P > 0.05) were not significantly different in seven rats. The cardiovascular responses to Cap were also compared before and during hypoxia (Fig. 2, bottom). Compared with the hypotension induced by Cap (control, 133.28 ± 3.94 mmHg; Cap, 85.48 ± 7.95 mmHg; P < 0.05) and hypoxia alone (control, 127.50 ± 3.20 mmHg; hypoxia, 91.75 ± 5.96 mmHg; P < 0.05), Hypoxia+Cap produced a lower MABP (66.75 ± 5.42 mmHg; P < 0.05).

In general, the cardiorespiratory responses to Cap and Hypoxia+Cap in guinea pigs (n = 4, 6 trials) were similar to those observed in the rats. As summarized in Fig. 2 (top), Cap injection generated an apnea (4.8 ± 1.05 s) that became extremely long (79.6 ± 3.93 s; P < 0.01) when Cap was administered during acute hypoxia. Hypoxia increased ventilation by ~43% with no significant change in Te. Hypoxia+Cap caused a lower MABP (48.13 ± 2.09 mmHg; P < 0.05) than that produced by Cap injection alone (67.20 ± 0.99 mmHg at control; 57.78 ± 2.80 mmHg after Cap; P < 0.05). Interestingly, hypoxia itself did not significantly alter MABP (64.63 ± 2.52 mmHg; P > 0.05), which is different from that observed in rats. Individually, hypoxia lowered ABP significantly in eight rats (e.g., Fig. 3A). In three others, hypoxia did not profoundly affect ABP (e.g., Fig. 3B). Three points should be noted. First, the long-lasting apnea occurred in the rat with (Fig. 3A) or without remarkable hypoxia-induced hypotension (Fig. 3B) and persisted even after ABP returned to the control level after the initial hypotension (Fig. 3, A and B). Second, most of guinea pigs tested did not show significant hypoxia-induced hypotension. Third, a shorter apneic duration was only observed in one of three rats with a much lower hypotensive response to hypoxia.

As a sham-control, saline instead of Cap was delivered during normoxia and acute hypoxia in five rats. Saline injection during normoxia did not cause a remarkable change in Te (Table 1). Acute hypoxia for ~1 min significantly increased ventilation by ~83% via elevating both VT and f. The latter is achieved by

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Fig. 1. Reproducibility of capsaicin (Cap)-induced apnea. A: experimental recordings of the responses to the first (left) and second trial of Cap injection (right) in an anesthetized and spontaneously breathing rat. Traces from top to bottom are arterial blood pressure (ABP), tidal volume (VT), and airflow (V). Horizontal bar under traces, time marker; arrows, point of injection. B: group data (6 trials in 6 rats) showing reproducibility of Cap-induced apnea [expiratory duration (Te)]. Values are means ± SE. *P < 0.05 between control and the responses.

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shortening Ti instead of Te. In addition, acute hypoxia decreased MABP with no significant changes in HR. Saline injection during acute hypoxia did not significantly change any cardiorespiratory variables.

Changes of arterial blood gases during long-lasting apnea. The corresponding changes in blood gases and pH at the initial period of this long-lasting apnea are depicted in Fig. 4 (n = 4, 6 trials). Acute hypoxia significantly decreased arterial Po2, arterial PCO2, and arterial O2 saturation but increased arterial pH. At the beginning of artificial ventilation (~30 s after the apnea was started), PETCO2 was 55 ± 7.3 Torr.

Importance of vagal integrity on long-lasting apnea. We compared the ventilatory response to Cap before and during acute hypoxia in the intact and vagotomized preparations. Typical recordings are depicted in Fig. 5, A–C. As shown, Cap injection into the right atrium brought about a brief apnea (Fig. 5A), and this response disappeared after bilateral vagotomy (Fig. 5B). Subsequently, Cap injection was repeated during hypoxia, in which no apnea was observed although hypoxia still induced pronounced hypotension (Fig. 5C). The absence of an apneic response to Cap after bilateral vagotomy was confirmed in three rats (Fig. 5D). It is noteworthy that after vagotomy, Cap did not significantly reduce MABP, but hypoxia-induced hypotension persisted. In two rats, as shown in Fig. 5B, Cap still produced a hypotension.

PCF A responses to Cap and Hypoxia + Cap. Hypoxic sensitization of PCF responsiveness to Cap was observed in seven rats. Figure 6A presents typical experimental recordings from an anesthetized and artifi-

![Image](attachment://image.png)

Fig. 2. Group data comparing the cardiorespiratory responses to Cap before and during acute hypoxia (10% O2 for ~1 min; Hypoxia + Cap). Top: apneic responses (Te) to Cap before and during hypoxia in 11 rats (A; 20 trials) and 4 guinea pigs (B; 6 trials). Bottom: mean ABP (MABP). Values are means ± SE. *P < 0.05 between control and the responses. †P < 0.05 between responses to Hypoxia + Cap and Cap or hypoxia alone.

![Image](attachment://image.png)

Fig. 3. Effect of ABP responses to acute hypoxia on the occurrence of the long-lasting apnea. Presence (A) and absence (B) of significant hypoxia-induced hypotension obtained from 2 anesthetized and spontaneously breathing rats are shown. In each panel, traces from top to bottom are ABP, VT, and V. Horizontal bar under traces, time marker; arrows, point of injection or hypoxia. Note that the long-lasting apnea persisted when ABP returned to the control level after the initial hypotension (A) and occurred without remarkable hypoxia-induced hypotension (B).
cially ventilated rat. During room air, PCFs were generally quiescent, and Cap injection evoked an abrupt burst of action potentials within 2 s after the injection (Fig. 6A, top). Hypoxia did not alter baseline PCF \( A \) but it significantly increased PCF \( A \) in response to Cap injection (middle). Ten minutes later, the response returned to control (bottom). The group data (Fig. 6B) indicated no significant difference of baseline PCF \( A \) (averaged over 10 s) between room air and hypoxia (normoxia, 0.16 \( \pm \) 0.06 impulses/s; hypoxia, 0.24 \( \pm \) 0.04 impulses/s; \( n = 10; P > 0.05 \)). However, in sharp contrast, the PCF \( A \) responses to Cap were significantly increased by hypoxia. PCF \( A \) firing rate (Fig. 6B, a) in response to the same dose of Cap during hypoxia was elevated from 3.34 \( \pm \) 0.76 to 7.65 \( \pm \) 1.32 impulses/s \( P < 0.05 \), and burst duration (Fig. 6B, b) was prolonged from 1.12 \( \pm \) 0.18 to 2.32 \( \pm \) 0.31 s \( P < 0.05 \).

**DISCUSSION**

**Role of interaction between acute hypoxia and stimulation of PCFs is important in control of breathing.** The present study found that acute hypoxia amplifies PCF-mediated inspiratory inhibition to such an intense degree that ventilation is terminated for 60–70 s in both anesthetized rats and guinea pigs. The similarity of hypoxic prolongation of Cap-mediated apneic duration in both animals suggests that the same mechanism might be also present in other species. In the present study, bolus Cap injection produced a brief apnea that lasted for \( \sim 4 \) s, similar to data reported in the cat, rat, mouse, guinea pig, rabbit, dog, and monkey (2, 7, 17, 19, 25, 29, 32, 40, 42). The PCF-mediated inhibitory effect on inspiratory drive is elicited by activating the second-order neurons residing in the CNS, which in turn alters the activities of respiratory-related neurons in the brain stem (40). Acute hypoxia stimulates ventilation that is characterized by an immediate excitatory phase (\( \sim 1 \) min; termed as early response) followed by a decline ("roll-off") that remains above the prehypoxic level (1, 12, 31). The former is the result of stimulating peripheral chemoreceptors, predominantly carotid chemoreceptors, whereas the latter is related to central nervous system (CNS) hypoxic depression (1, 12). In the present study, acute hypoxia significantly increased ventilation before Cap challenge in both rats and guinea pigs. Because Cap was injected after exposure to hypoxia for \( \sim 1 \) min, activation of carotid chemoreceptors mainly generated this ventilatory augmentation.

**Significance of this interaction in control of breathing.** It is well established that pulmonary inflammation, edema, and congestion are potent stimulants of PCFs (7, 23). For example, Undem et al. (38) discovered PCF hypersensitivity in guinea pigs with bronchial hyperreactivity induced by ovalbumin sensitization. Discharges of sensory neurons in the nodose ganglion of sensitized, compared with nonsensitized, animals were enhanced, as indicated by a significant depolar-

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**Table 1. Effects of saline injection into the right atrium during normoxia and hypoxia on cardiorespiratory variables in the rat**

<table>
<thead>
<tr>
<th></th>
<th>( V_t ), ml</th>
<th>( f ), breaths/min</th>
<th>( V_i ), ml/min</th>
<th>( T_i ), s</th>
<th>( T_e ), s</th>
<th>MABP, mmHg</th>
<th>HR, beats/min</th>
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<tbody>
<tr>
<td><strong>Normoxia</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ctrl</td>
<td>4.25 ( \pm ) 0.33</td>
<td>77.89 ( \pm ) 3.07</td>
<td>328.98 ( \pm ) 26.35</td>
<td>0.31 ( \pm ) 0.01</td>
<td>0.47 ( \pm ) 0.03</td>
<td>120.43 ( \pm ) 3.01</td>
<td>332.16 ( \pm ) 11.8</td>
</tr>
<tr>
<td>Saline</td>
<td>4.06 ( \pm ) 0.14</td>
<td>78.54 ( \pm ) 2.93</td>
<td>353.41 ( \pm ) 34.2</td>
<td>0.31 ( \pm ) 0.01</td>
<td>0.46 ( \pm ) 0.02</td>
<td>117.27 ( \pm ) 3.26</td>
<td>324.34 ( \pm ) 8.80</td>
</tr>
<tr>
<td><strong>Hypoxia</strong></td>
<td></td>
<td></td>
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<tr>
<td>Ctrl</td>
<td>3.90 ( \pm ) 0.24</td>
<td>78.83 ( \pm ) 4.99</td>
<td>291.04 ( \pm ) 22.59</td>
<td>0.31 ( \pm ) 0.02</td>
<td>0.45 ( \pm ) 0.04</td>
<td>105.90 ( \pm ) 4.29</td>
<td>315.25 ( \pm ) 17.59</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>5.72 ( \pm ) 0.57*</td>
<td>90.00 ( \pm ) 7.33*</td>
<td>532.87 ( \pm ) 80.37*</td>
<td>0.25 ( \pm ) 0.01*</td>
<td>0.44 ( \pm ) 0.05</td>
<td>76.44 ( \pm ) 7.75*</td>
<td>313.82 ( \pm ) 18.45</td>
</tr>
<tr>
<td>Saline</td>
<td>5.85 ( \pm ) 0.57*</td>
<td>91.01 ( \pm ) 7.23*</td>
<td>596.51 ( \pm ) 78.44*</td>
<td>0.25 ( \pm ) 0.02*</td>
<td>0.43 ( \pm ) 0.05</td>
<td>74.55 ( \pm ) 5.52*</td>
<td>320.83 ( \pm ) 19.08</td>
</tr>
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</table>

Values are means \( \pm \) SE of 7 trials in 5 rats. Ctrl, control; saline, vehicle injection; \( V_t \), tidal volume; \( f \), respiratory frequency; \( V_i \), inspiratory minute ventilation; \( T_i \), inspiratory duration; \( T_e \), expiratory duration; MABP, mean arterial blood pressure; HR, heart rate. *\( P < 0.05 \) between the control and the responses.
tzation of the resting membrane potential. In fact, the inflammatory mediators, such as histamine, bradykinin, and prostaglandins, can sensitize PCF endings and increase their excitability (6, 15, 21, 23, 38). PCF endings are also consistently activated by pulmonary congestion and edema. Paintal (27) named these afferents initially as “juxtapulmonary receptors” because of their sensitivity to increased interstitial fluid volume or lung pressure (28). Clinically, these pulmonary pathological conditions are usually associated with hypoxemia in patients with pulmonary obstructive diseases (asthma), sudden infant death syndrome (SIDS), and acute mountain sickness. Respiratory disorders, such as hypoventilation and apnea, are often observed in these patients whose conditions worsen and are even fatal when transient nocturnal hypoxemia occurs in sleep. For example, some patients with asthma or chronic bronchitis became cyanotic (hypoxemia) without increased ventilation, indicating a depressed ventilatory response to hypoxia (36). This depressed response has also been confirmed in an animal model of asthma (43), in which pulmonary inflammation was clearly documented (41). Patients with acute mountain sickness have hypoxia-induced pulmonary edema and pulmonary arterial hypertension, in which periodic breathing (episodic apnea) is a common symptom, especially at night (39). In addition, the pulmonary infection was reported in SIDS, and these patients are particularly vulnerable to transient nocturnal hypoxemia (33, 34). Our studies provide, for the first time, experimental evidence to show a functional interaction of acute hypoxia and stimulation of PCFs in control of breathing. In other words, acute hypoxia amplifies PCF-mediated inspiratory inhibition to cause a long-
lasting apnea. It seems possible that this interaction is involved in the pathophysiology of these respiratory disorders.

**Long-lasting apnea is partially due to hypoxic sensitization of PCF response to Cap.** A large body of evidence shows that Cap-induced apnea is dependent on pulmonary vagal inputs (reviewed in Refs. 5, 7, and 23). We found that the Cap-induced long-lasting apnea during hypoxia was eliminated after bilateral transection of cervical vagi, clearly indicating that vagal input is essential for the long-lasting apnea. Because of the potent inhibitory role of the vagus nerve (PCFs) in eliciting apnea, we attempted to determine whether hypoxia could amplify the PCF response to Cap and lead to this long-lasting apnea. In the study of single-unit PCF\(_A\), we observed that acute hypoxia did not significantly change baseline PCF\(_A\), but doubled PCF\(_A\) responses to Cap, indicating a peripheral interaction at the level of PCF endings. In other words, acute hypoxia can sensitize the PCF response to Cap. The absence of an hypoxic effect on baseline PCF\(_A\) has been reported (8); in anesthetized cats, activity of nonmyelinated vagal afferent neurons was recorded in the nodose ganglion, and their baseline activity was not altered by hypoxia (10 and 8% O\(_2\) exposure for 1 min). Hypoxic sensitization of a PCF response to Cap has not been documented but is supported by several previous reports. Hypoxemia-induced products, such as adenosine and lactic acid, have sensitized PCF endings (17, 23). The plasticity of PCF responsiveness to other types of chemical challenges has also been observed. For example, an increased excitability of PCF or sensory fiber somata in the nodose ganglia after exposure to cigarette smoke, ozone, sulfur dioxide, and ammonia has been documented (2, 18, 23). Chronic exposure to environmental tobacco smoke increased the PCF\(_A\) (2) and prolonged Cap-induced apneic duration (28). In addition, the inflammatory mediators, mentioned above, can also sensitize PCF endings and increase their excitability (6, 15, 21, 23, 38).

![Figure 6. Pulmonary C-fiber activity (PCF\(_A\)) responses to Cap challenges.](image)
Importance of the CNS in this long-lasting apnea. Hypoxia+Cap leads to a long-lasting apnea that is 16-fold longer than that induced by Cap alone. In comparison, acute hypoxia increased PCF response to Cap by approximately twofold. These data cast doubt on whether hypoxic sensitization of PCFs alone is adequate to explain the full expression of this long-lasting apnea. Indeed, we recently observed that after bilateral transection of carotid sinus nerves, right atrial injection of Cap still caused brief apnea but that Hypoxia+Cap did not generate the extremely long-lasting apnea (F. Xu, Q.-h. Gu, T. Zhou, and L.-Y. Lee, unpublished data). These observations strongly support, besides hypoxic sensitization of PCFs, a central interaction of inputs from peripheral chemoreceptors and PCFs in the expression of this long-lasting apnea. Actually, the assumption is supported by the accepted concept that the ventilatory responses to acute hypoxia and right atrial injection of Cap are centrally mediated and elicited predominantly by stimulating carotid chemoreceptors and PCF endings, respectively. The mechanism underlying this long-lasting apnea is unknown. Recently, Mutoh et al. (25) found that, in guinea pigs, Cap stimulation of PCFs produced a brief apnea that became 10-fold longer when substance P was preinjected into the vicinity of the cNTS. Their subsequent studies convincingly showed that endogenous substance P induced by tobacco smoke exposure in young guinea pigs could increase NTS expiratory neuronal activity concomitant to a prolonged apnea denoted by the cessation of the phrenic discharge (2). It has been established that hypoxia causes an increase of substance P release in the NTS as measured by microdialysis (24). This enhanced release of substance P is probably due to activation of carotid body chemoreceptors because the release was blocked by denervation of the carotid sinus nerves (37). Therefore, it seems reasonable to postulate that hypoxia stimulates carotid chemoreceptors and causes the release of substance P in the cNTS, which in turn amplifies PCF-mediated inspiratory inhibition and causes this long-lasting apnea.

Criticisms of experimental methods. Ventilatory responses to hypoxia involve more complex mechanisms during a roll-off period (1, 12, 31). This prolonged hypoxia not only stimulates carotid chemoreceptors but also inhibits the CNS, resulting in hypoxic ventilatory depression. In the present study, no attempt was made to evaluate the effect of Cap injection during later hypoxia (the roll-off period) on the breathing because our goal was to elucidate the interaction between stimulation of peripheral carotid chemoreceptor and PCFs. Absence of rapid shallow breathing after the reflex apnea is consistent with previous studies conducted in anesthetized mice or rats (see discussion in Ref. 30). Because the level of PETCO2 was not constant, the arterial PCO2 was progressively elevated during the apneic period (without artificial ventilation). It has been reported that hypercapnia can modulate PCF sensitivity. In anesthetized cats, 80% of PCFs increased their discharge frequency when the end-tidal CO2 concentration was elevated from 0.02 to 0.10 impulses/s (8). Furthermore, in anesthetized rats, PCF response to Cap was also increased by transient alveolar hypercapnia (11). Is the long-lasting apnea dependent on this apnea-induced hypercapnia? We believe that hypercapnia has a limited role in the expression of this long-lasting apnea because it persisted for a long period even after the rat was artificially ventilated (see Fig. 3). Because hypoxia significantly decreases PETCO2 as the result of hyperventilation, we can rule out the partial contribution of this hypoxia-induced hypocapnia to the long-lasting apnea. In addition, this long-lasting apnea is not secondary to the hypotension after acute hypoxia. As shown in Fig. 3A, the long-lasting apnea persisted when ABP returned to the control level immediately after application of artificial ventilation. Moreover, in three rats (see Fig. 3B) and all guinea pigs (Fig. 2) tested, hypoxia did not significantly produce hypotension, but Cap injection during hypoxia still led to long-lasting apnea. Furthermore, several lines of evidence indicate that this long-lasting apnea is not due to time. First, the apneic durations induced by the first and second Cap injection (0.5 μg) with an interval similar to that between Cap and Hypoxia+Cap were not significantly different. Second, saline injection during hypoxia did not lead to apnea. Finally, this long-lasting apnea consistently appeared in the rats in which the Hypoxia+Cap was repeated two or three times. Vagal afferent C-fiber endings are also located in the myocardium and coronary arteries, largely in the left ventricle (13). Our data cannot completely rule out the possibility that these C fibers contribute to the long-lasting apnea.

Summary of results and conclusion. In the present study, we found that the brief expiratory apnea induced by right atrial injection of Cap was intensely potentiated (16-fold) by acute hypoxia in both rats and guinea pigs. Bilateral vagotomy abrogated the apneic responses to both Cap and Hypoxia+Cap. Acute hypoxia did not significantly affect baseline PCF, but it increased the PCF response to Cap by twofold. In conclusion, these data, in opposition to our original hypothesis, demonstrate that acute hypoxia can amplify rather than attenuate the pulmonary C fiber-mediated inhibitory effect on inspiration and produce a long-lasting apnea. This interaction relies on the vagal inputs and is partially due to hypoxic sensitization of PCF response to Cap. This interaction may be involved in the etiology of certain respiratory disorders observed in patients with pulmonary obstructive diseases (asthma), SIDS, and acute mountain sickness.

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