Carotid sinus nerve is involved in cardiorespiratory responses to intracarotid injection of capsaicin in the rat

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Wang, Rurong, Fadi Xu, Jianguo Zhuang, and Cancan Zhang. Carotid sinus nerve is involved in cardiorespiratory responses to intracarotid injection of capsaicin in the rat. J Appl Physiol 100: 60-66, 2006. First published September 8, 2005; doi:10.1152/japplphysiol.00302.2005.—The carotid sinus nerve (CSN), important in cardiorespiratory modulation, mainly contains C fibers (CSCFs). Previous studies have demonstrated that selective stimulation of bronchopulmonary C fibers (PCFs) via right atrial injection of capsaicin (Cap; ~0.25 µg) results in an apnea (~3 s) associated with hypotension and bradycardia. The present study was undertaken to determine the effects of activating CSCFs on cardiorespiratory activities. Intracarotid injection of Cap was performed before and after bilateral transection of the CSN in anesthetized and spontaneously breathing rats. Our results showed that 1) low doses of Cap (up to 2 ng) produced an increase in minute ventilation by elevating both tidal volume and respiratory frequency with the threshold dosage at 1.0 ng (P < 0.05); 2) high doses (4–64 ng) generated an apnea (prolongation of expiratory duration by ~8-fold) and hypertension (P < 0.05); 3) bilateral transection of the CSN reduced excitatory and inhibitory respiratory responses by 30 and 81%, respectively, and increased the hypertension by 88% (P < 0.05); and 4) the same doses of Cap delivered into the right atrium to stimulate PCFs failed to evoke detectable cardiorespiratory responses. Our results suggest that compared with PCFs, CSCFs are more sensitive to Cap stimulation and that activation of these fibers significantly modulates cardiorespiratory activity in anesthetized rats.

bronchopulmonary C fibers; myelinated and nonmyelinated fibers; chemoreceptor; baroreceptor

MORPHOLOGICAL STUDIES HAVE SHOWN that ~75% of the afferent fibers in the vagal branches innervating the respiratory tract are nonmyelinated (C) fibers (1). These bronchopulmonary C fibers (PCFs) are sensitive to various exogenous chemical substances and endogenously released mediators (17, 19, 22, 28). PCFs are believed to be involved in regulating cardiorespiratory function under pathophysiological conditions (7, 28). Small doses of phenylbiguanide (PBG) or capsaicin (Cap), a selective stimulant to C fibers, delivered into pulmonary circulation elicit rapid, shallow breathing coupled with bradycardia and hypotension in rats (4, 34) and rabbits (34). In sharp contrast, relatively large doses of PBG and Cap produced a brief apnea lasting for ~3 s, also associated with this cardiovascular inhibition (27, 48, 50). Further studies have demonstrated that these cardiorespiratory responses are PCFs mediated (7, 49).

The carotid sinus nerve (CSN) also contains C-afferent fibers (CSCFs), predominately arising from the carotid chemoreceptors and baroreceptors. It is well established that the majority of fibers in the CSN are C fibers (3, 11, 13, 26, 33, 36, 44, 45). With the use of single-unit recording techniques, C fibers were identified in cat CSN with a five-to-one ratio of C fibers vs. myelinated fibers (11). Investigators, employing a retrograde trace and electron microscopy, confirmed that ~85% fibers within the CSN were C fibers (33, 36). Interestingly, the C fibers activated by stimulation of the carotid chemoreceptors are not responsive to stimulation of the sinus baroreceptor, and vice versa (13), indicating the specificity of two types of C fibers in response to different stimulations. With respect to the functions of these C fibers, neonatal Cap treatment to inactivate C fibers significantly attenuated the pressor responses to bilateral carotid artery occlusion and the ventilatory response to hypoxia in anesthetized adult rats (3). Although the contributions of chemoreceptor and baroreceptor C fibers to cardiorespiratory functions have been investigated separately, the overall effects of activating CSCFs (both types of C fibers) on cardiorespiratory activity remain unknown. We hypothesized that stimulation of CSCFs would, similar to PCFs, alter cardiorespiratory activity.

Our experiments were carried out in anesthetized and spontaneously breathing rats. The role of CSCFs was determined by comparing the cardiorespiratory responses to intracarotid injection of Cap before and after bilateral transection of the CSN. We found that a low-dose (1 and 2 ng) intracarotid injection of Cap evoked an excitatory respiratory response, whereas high doses (4–64 ng) produced a long apnea associated with hypertension. After bilateral transection of the CSN, both types of respiratory responses were significantly decreased, and hypertension was increased. These results suggest that activation of CSCFs is able to modulate cardiorespiratory activity in anesthetized rats.

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. Twenty-seven Sprague-Dawley male rats (400–500 g) were anesthetized with Nembutal (40–50 mg/kg ip). The anesthesia was maintained by supplemental urethane (200–300 mg·kg⁻¹·h⁻¹ iv) to suppress corneal and withdrawal reflexes. The trachea below the larynx was exposed through a midline incision, cannulated, and connected to a one-way breathing valve via a pneumotachograph to record airflow. The flow signal was integrated by PowerLab/8SP (ADInstruments, Castle Hill, Australia) to generate tidal volume (VT). The pneumotachograph was made of stainless steel with a linear flow-pressure relationship in the range of 0–20 ml/s, a flow resistance of 0.046 cmH2O·ml⁻¹·s, and a dead space of ~0.2 ml. A three-way switch
was attached to the inspiratory inlet of the one-way breathing valve and used to manipulate the inhaled gas mixture to maintain end-tidal \( \text{PCO}_2 \) (\( \text{P}_{\text{ETCO}}_2 \)). The latter was monitored by an infrared CO\(_2\) analyzer (model 78356A, Hewlett-Packard, Louisville, KY). Rich oxygen was applied throughout the experiment as a baseline. The left femoral artery and vein were cannulated for monitoring cardiovascular activity and administering the anesthetic, respectively. The tracheal pressure (\( \text{Ptr} \)) was recorded by a pressure transducer that was connected to a side port of the tracheal cannula. Animals were placed into a rigid metal frame with the head fixed in a stereotaxic apparatus (model 1404, Kopf, Tujunga, CA). Core body temperature was monitored with a rectal probe and maintained at \( \sim 37.5^\circ\text{C} \) by a heating pad and radiant heat lamp.

**Intracarotid injection of small and large doses of Cap before and after transection of the CSN.** The right common carotid artery was isolated, and a catheter (PE 50-gauge tubing) was advanced until its tip was placed \( \sim 3 \) mm caudal to the carotid bifurcation in 14 rats. A stock solution of Cap (400 \( \mu\text{g/ml}, \text{Sigma-Aldrich, St. Louis, MO} \)) was made in a vehicle of 10% \( \text{Tween 80}, 10\% \text{ ethanol}, \) and 80% isotonic saline. During each injection, solutions of the desired concentration were prepared with saline dilution, loaded in the catheter (0.1 ml), and then flushed into the common carotid artery by injection of saline (0.2 ml). Our preliminary data showed that 1 ng Cap seemed to be the threshold to evoke detectable excitatory respiratory responses, whereas doses > 4 ng generated apnea. Therefore, to test the cardiorespiratory responses to low doses of Cap, 0.5, 1.0, and 2.0 ng of Cap were sequentially injected into six rats, whereas eight other rats only received a 2.0 ng injection. Large-dose injections were initiated at 4.0 ng in 12 of 14 rats. If this large dose failed to generate a distinct apnea, a greater dose(s), i.e., 16.0 ng (\( n = 5 \)) and/or 64.0 ng (\( n = 3 \)), was applied. Once the apnea was generated, this threshold dose of Cap for apnea was used for the subsequent protocols. To prevent pressure stimulation of carotid baroreceptors, the injection speed was controlled at \( \sim 15 \) s. A 20-min interval was required between two trials with each injection performed after stabilization of cardiorespiratory variables for at least 10 min. Routinely, vehicle injection with the same volume was conducted to serve as the sham control. Before Cap administration, the CSN was carefully isolated bilaterally or unilaterally (right side) and looped with sutures for later transection. After the Cap test described above, the CSN was bilaterally transected in seven rats that subsequently were exposed to 10% \( \text{O}_2 \) (balance with \( \text{N}_2 \)) for 1 min to confirm the successful bilateral CSN transection. Intracarotid injection of Cap (2 ng and 4 – 64 ng) was then applied again. The same Cap applications were also conducted in another three rats after unilateral transection of the CSN to test the possible difference between bilateral and unilateral section.

**Intracarotid injection of only large doses of Cap.** To determine whether the apnea induced by large doses of Cap was related to the accumulative effects of low doses of Cap previously received, the large doses of Cap were administered in 11 other rats without a previous Cap test in the same manner as described above. In these cases, large dose(s) of Cap (4, 16, and 64 ng) were applied to generate the apnea.

**Right atrial injection of Cap.** The right jugular vein was isolated, and a catheter was advanced close to the right atrium in 4 of the 11 rats that only received the large doses of intracarotid administration of Cap and two other rats without previous tests. The inserted depth of the catheter was determined by measuring the distance from the heart (felt from heartbeat) to the cannulating site before implantation. The position of the cannula was confirmed by autopsy after the experiments. The excitatory dose (2.0 ng) and largest apneic dose (64 ng) were injected into the jugular vein at the same rate as for the intracarotid Cap injection (\( \sim 15 \) s). This series of experiments was designed to test whether the cardiorespiratory responses to intracarotid administration could also be elicited by right atrial injection of identical doses of Cap.

**RESULTS**

Small doses of Cap increased \( \dot{V}_\text{E} \). Intracarotid administration of 1 and 2 ng, rather than 0.5 ng, of Cap produced an increase in \( \dot{V}_\text{E} \) by elevating \( f \) and \( \text{VT} \). As shown in Fig. 1, intracarotid injection of vehicle or 0.5 ng Cap did not significantly affect ventilation. However, injection of 1 and 2 ng of Cap brought about a significant increase in \( \dot{V}_\text{E} \) by elevating \( f \) and \( \text{VT} \). Statistically, the ventilatory responses to injection of 2 ng Cap were significantly greater than those to 1 ng-injection of Cap (Fig. 2). Therefore, 1 ng seems to be the threshold for the low-dose Cap-induced respiratory responses. The latency for the evoked respiratory responses and the duration required for recovery were not significantly different between the responses to 1 and 2 ng Cap (7.8 \( \pm \) 0.8 vs. 8.1 \( \pm \) 0.6 s; and 42.0 \( \pm \) 3.8 vs. 53.0 \( \pm \) 7.7 s; \( P > 0.05 \)). With respect to the cardiovascular responses, no significant changes were found when either vehicle or low-dose Cap was administered (Figs. 1 and 3).

Large doses of Cap produced an apnea and pressor response. An apneic response associated with hypertension was elicited by 4 ng Cap in four rats, 16 ng in five rats, and 64 ng in three rats. Although 4 or 16 ng failed to cause the apnea in
the latter, these injections induced immediate hypoventilation. Clearly, the threshold of Cap required to generate the apnea was varied among the rats. As shown in Fig. 4, vehicle did not affect the cardiorespiratory variables (left), whereas 16 ng of Cap profoundly depressed V̇E coupled with an increase in ABP (middle). In sharp contrast, administration of 64 ng of Cap generated a long apnea and a greater increase in ABP (right).

The apnea was followed by a hypoventilation that gradually recovered to baseline within ~3 min (not shown). Similar results were obtained from group data (Fig. 5). Compared with the control, Ṫ was not significantly altered by vehicle injection (-3.0 ± 1.0%; P > 0.05), but it was prolonged significantly by Cap (821.9 ± 105.1%; P < 0.05) with the latency of 7.4 ± 0.8 s. These large doses of Cap also significantly increased MABP from 136.2 ± 5.0 to 158.2 ± 6.7 mmHg (16.0 ± 5.1%; P < 0.05) with little effect on HR (375.3 ± 13.1 vs. 384.6 ± 15.2 beats/min, 2.4 ± 2.2%; P > 0.05). The recovery duration required for V̇E (170.4 ± 12.8 s) was significantly longer than that needed for hypertension (55.9 ± 11.0 s; P < 0.05).

To test whether the apnea induced by the large doses of Cap was related to the accumulative effects of low doses of Cap previously received, we compared the cardiorespiratory responses to injection of large dose of Cap in the rats with and without previous Cap tests. We found that the evoked changes in the apneic duration, MABP, and HR in the rats that previously received low doses of Cap were not significantly different from those in the rats without previous Cap tests (Ṫ %Δ = 821.0 ± 105.0 vs. 870.1 ± 236.6%; MABP %Δ = 16.3 ± 5.0 vs. 17.3 ± 6.2%; HR %Δ = 2.4 ± 2.2 vs. 2.7 ± 2.0%; P > 0.05). These data demonstrate that the apnea induced by the large dose of Cap was not due to the accumulative effects of low doses of Cap previously received.

Transection of the CSN attenuated the respiratory, but enhanced hypertensive, responses to Cap. The effects of a small dose (2 ng) and large dose (4–64 ng) of Cap on the cardiorespiratory responses were compared before and after bilateral or unilateral (right) transection of the CSN. Bilateral transection did not significantly change the baseline cardiorespiratory variables (Table 1), which is consistent with previous studies conducted in cats (43) and rats (15). The absence of the effects of carotid body denervation on baseline breathing may be due to anesthesia and hyperoxia used in our study that certainly blunted the reflex cardiorespiratory responses induced by denervation. Transection of the CSN reduced the ventilatory responses to hypoxia by 85% (P < 0.01; n = 7), indicating the success of the transection. The transection significantly decreased the ventilatory responses to 2 ng of Cap by 30% with little effect on cardiovascular activity (Fig. 6A). Interestingly, the transection almost eliminated the apneic response to large doses of Cap (81% reduction) and profoundly increased the hypertensive response by 88% compared with those responses observed in the intact rats (Fig. 6B). The effects of unilateral transection of the right CSN on cardiorespiratory responses were very close to those denoted after bilateral transection. The reductions of the augmented V̇E and the apneic response, and increases of hypertension were 33, 76, and 83%, respectively. These similarities suggest that the involvement of the contralateral CSN (to the injecting side) in the Cap-induced cardiorespiratory responses is limited. However, we cannot rule out the possibility that these cardiorespiratory responses may be somewhat buffered by aortic baroreceptors.

Right atrial injection of the same doses of Cap failed to evoke cardiorespiratory responses. We compared the cardiorespiratory responses to injection of vehicle and Cap (2 ng and
64 ng) into pulmonary circulation via the right atrium in 6 rats. As shown in Fig. 7, no detectible changes were observed in 

**DISCUSSION**

**CSN is involved in respiratory responses to intracarotid injection of Cap.** The major finding of this study is that intracarotid injection of Cap provokes significant respiratory responses. An excitatory respiratory response was produced by an intracarotid injection of 1–2 ng Cap, whereas a long apnea was induced by injection of 4–64 ng Cap. Most importantly, after bilateral transection of the CSN, both types of respiratory responses were substantially reduced; in particular, the apneic response was almost eliminated (81% reduction of apneic duration). Previous morphological (3, 13, 33, 36, 44, 45) and electrophysiological (8, 11) studies have demonstrated that the majority of the fibers in the CSN are C fibers that are activated by systematic administration of Cap. Because Cap has been used extensively as a selective stimulant to C fibers, our results provide experimental evidence that selective activation of CSCFs significantly alters respiration, especially producing an apnea. The apnea is central because a similar apnea denoted on the phrenic efferent nerve activity in response to an intracarotid injection of 64 ng Cap was also observed in paralyzed and ventilated rats (Wang R and Xu F, unpublished observation).

An interesting finding in this experiment is that the doses of Cap required for activating CSCFs are very low compared with the dosage utilized in stimulating PCFs. A large number of experiments have shown that right atrial bolus injection of Cap, ranging from 0.25 to 1 µg, produced an apnea for ~3 s in anesthetized spontaneously breathing rats (28, 50). In sharp contrast, the doses we used were 4–64 ng that were delivered for ~15 s, which results in a significant reduction of Cap concentration administered. Surprisingly, the intracarotid injection of such low dose of Cap produced a longer apnea (~6 s). The fact that such low doses of Cap can evoke the remarkable respiratory responses, especially long apnea predominantly induced by activation of CSCFs, leads us to believe that the CSCFs are very sensitive to Cap stimulation. The significance of our study is twofold. First, our data, for the first time,

| Table 1. Baseline cardiorespiratory variables before and after transection of carotid sinus nerve |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Before          | After           |                 |                 |                 |                 |                 |
| Vt ml/min, breaths/min, Vt ml, Tt s, Tt s, MABP, mmHg, HR, beats/min | 148±26, 73±5, 2.0±0.2, 0.33±0.02, 0.52±0.04, 135±8, 378±12 | 137±21, 71±6, 1.9±0.2, 0.32±0.02, 0.57±0.06, 134±8, 381±10 |

Values are means ± SE; n = 7 rats. Bilateral transection of carotid sinus nerve did not affect euepic breathing, including minute ventilation (Vt), respiratory frequency (f), tidal volume (Vt), inspiratory duration (Ti) and expiratory duration (Te) as well as mean arterial blood pressure (MABP) and heart rate (HR).
reveal the overall effects of activating both types of C fibers on cardiorespiratory activities, although a number of previous studies have demonstrated the separate roles of baroreceptor and chemoreceptor C fibers (3, 5, 13, 29, 32). Second, activation of CSCFs may play a role in pathogenesis of respiratory disorders during sleep. Recent epidemiological studies have revealed that 40% of patients with systemic hypertension and nocturnal hypoxemia have sleep apnea (23, 30, 38). However, the mechanisms underlying this apnea remain unclear. Our data that intracarotid injection of large doses of Cap generates hypopnea and apnea raise a possibility that simultaneously stimulating baroreceptor (hypertension) and chemoreceptor (hypoxemia) C fibers may be responsible for the genesis of the sleep breathing disorders in these patients. Additionally, there is evidence to show that intravenous injection of interleukin-1β, a prototype of the proinflammatory cytokines involved in acute and chronic inflammatory responses (9), alters ventilation. Low doses of interleukin-1β stimulate ventilation (16), whereas high doses depress respiratory and anoxic survival in rats (37). Moreover, interleukin-1β has been suggested to be involved in sudden infant death syndrome (18, 40). Because a strong expression of interleukin-1 receptor has been reported in rat carotid body (47), it is possible that the respiratory roles interleukin-1β plays are through CSCFs.

PCFs are not involved in respiratory responses to intracarotid injection of Cap. Another one of our interesting findings is that right atrial injection of 2 or 64 ng Cap produced no discernable respiratory responses. A number of studies have shown that a relatively small dose of PBG or low levels of Cap administered into pulmonary circulation elicited shallow, rapid breathing, whereas administration of large doses of PBG or high levels of Cap using the same approach caused apnea (4, 17, 34, 41). These respiratory responses were dependent on the integrity of vagal nerves (6, 7, 49, 50) and were mediated by PCFs (14, 20). One might conjecture that intracarotid injection of Cap alters respiration partially by activating PCFs. Our results ruled out this possibility because the same doses of Cap that generated respiratory responses via intracarotid injection failed to elicit detectable respiratory response when they were delivered into the right atrium. Indeed, the characteristics of the cardiorespiratory responses to intracarotid and right atrial injection of Cap previously reported (28, 50) are distinctly different. First, right atrial injection of 0.1 g Cap brought about rapid and shallow breathing, but intracarotid injection of 1–2 ng Cap led to rapid and deep breathing. Second, as discussed above, intracarotid injection of 4–64 ng Cap produced an apnea (~6 s) that is markedly longer than the apnea (~3 s) induced by right atrial injection of 0.25–1.0 μg Cap. Third, immediately after the apnea, a hypventilation was observed with intracarotid injection, but shallow and rapid breathing was often denoted with right atrial injection of Cap. Fourth, intracarotid injection produced hypertension without affecting cardiac activity, but right atrial injection generated hypotension and bradycardia. These results lead us to believe that CSCFs instead of PCFs are heavily involved in the respiratory responses to intracarotid injection of Cap.

Fig. 6. Comparison of the cardiorespiratory responses to intracarotid injection of small and large doses of Cap before and after transection of the carotid sinus nerve (CSN). Responses of V̇E, MABP, and HR to small dose (2 ng) Cap (A) and responses of V̇E, MABP, and HR to large doses of Cap (B) are presented. Values are means ± SE; n = 7 rats. *P < 0.05 between control (zero) and the Cap-induced responses. †P < 0.05 before and after transection of CSN.

Fig. 7. Cardiorespiratory responses to right atrial injection of small (2 ng) and largest (64 ng) apneic dose of Cap. Responses of V̇E (A), Ti (B), MABP (C), and HR (D) are presented. Values are means ± SE; n = 6 rats.
Depressor induced by stimulation of CSCFs was masked by hypertensive response to intracarotid injection of large dose of Cap. Our results showed that cardiovascular activity was not significantly altered by intracarotid injection of low doses of Cap, but a hypertension was observed when the doses were >2 ng. Surprisingly, we subsequently found that the hypertensive response was strengthened after bilateral transection of the CSN. These data demonstrated that CSCFs stimulated by Cap, likely the baroreceptor C fibers, had a depressor effect that was masked by a pressor response elicited by a large dose of Cap. The central nervous system may be the source responsible for the hypertension. Investigators have reported that intracarotid injection of Cap causes a hypertension in the rats with CSN transection, implying a possible central nervous system effect of Cap on hypertension (51). Indeed, administration of Cap into the fourth ventricle or cisterna magna (39), the rostral ventrolateral medulla (42), or ventral medullary surface (24) has been demonstrated to increase ABP. In the present study, neither low nor large doses of Cap altered HR. Because baroreceptor stimulation can cause both sympathetically activating and parasympathetically withdrawing, the lack of HR responses might be due to parasympathetic effects of anesthetic. The respiratory responses to intracarotid injection of Cap are not secondary to its effect on cardiovascular activity. Low doses of Cap produced ventilatory stimulation without effect on cardiovascular variables. Although large doses of Cap initiated an increase in ABP and an apnea at the same time, the recovery duration required for apneic response was much longer than the associated hypertensive response, implying that the corresponding pathways responsible for these respiratory and cardiovascular responses are different.

In conclusion, our results can be summarized as follow. First, compared with right atrial injection previously reported (6, 17, 19, 27, 34, 41), the doses required for generating the respiratory responses to intracarotid injection were much smaller, yet the evoked apnea lasted much longer. Furthermore, the elicited hypopnea or apnea associated with hypertension was dependent on Cap doses. Second, both excitatory and inhibitory respiratory responses were substantially reduced, and the hypertension was increased after bilateral transection of the CSN. Third, the same dose of Cap delivered into the right atrium failed to evoke detectable cardiorespiratory responses. Our results suggest that CSCFs are sensitive to Cap stimulation, and activation of these fibers significantly modulates cardiorespiratory activity in anesthetized rats.

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