Role of the carotid bodies in chemosensory ventilatory responses in the anesthetized mouse

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Izumizaki, Masahiko, Mieczyslaw Pokorski, and Ikuo Homma. Role of the carotid bodies in chemosensory ventilatory responses in the anesthetized mouse. J Appl Physiol 97: 1401–1407, 2004.—We examined the effects of carotid body denervation on ventilatory responses to normoxia (21% O2 in N2 for 240 s), hypoxic hypoxia (10 and 15% O2 in N2 for 90 and 120 s, respectively), and hyperoxic hypercapnia (5% CO2 in O2 for 240 s) in the spontaneously breathing urethane-anesthetized mouse. Respiratory measurements were made with a whole body, single-chamber plethysmograph before and after cutting both carotid sinus nerves. Baseline measurements in air showed that carotid body denervation was accompanied by lower minute ventilation with a reduction in respiratory frequency. On the basis of measurements with an open-circuit system, no significant differences in O2 consumption or CO2 production before and after chemodenervation were found. During both levels of hypoxia, animals with intact sinus nerves had increased respiratory frequency, tidal volume, and minute ventilation; however, after chemodenervation, animals experienced a drop in respiratory frequency and ventilatory depression. Tidal volume responses during 15% hypoxia were similar before and after carotid body denervation; during 10% hypoxia in chemodenervated animals, there was a sudden increase in tidal volume with an increase in the rate of inspiration, suggesting that gasping occurred. During hyperoxic hypercapnia, ventilatory responses were lower with a smaller tidal volume after chemodenervation than before. We conclude that the carotid bodies are essential for maintaining ventilation during eupnea, hypoxia, and hypercapnia in the anesthetized mouse.

Keywords: carotid sinus nerve; chemodenervation; chemoreceptor; hypoxia; hypercapnia

Chemical respiratory control is subject to substantial variations among species. These variations are more common in respiratory responses to hypoxia, mediated mainly by the carotid body chemoreceptors, than in respiratory responses to hypercapnia, mediated by the central chemoreceptors. Considerable species differences have been described in the contribution of the carotid bodies to respiration. Several studies found that carotid body denervation abolishes hypoxic ventilatory responses in ponies, cats, rabbits, and rats, suggesting that the response arises solely from the carotid bodies (4, 6–8, 37). Other studies found that chemodenervation failed to abolish the responses to hypoxia in dogs and calves (5, 6). It has also been suggested that, in rats, other peripheral chemoreceptors or central neural mechanisms contribute to hypoxic responses (25). The extreme case occurs in guinea pigs, in which carotid body denervation has no effect on hypoxic ventilatory responses (10). The interpretation of these data has been complicated not only by species differences but also by confounding issues such as the use of anesthesia and possible adaptive changes in regulatory systems after chemodenervation (29, 34).

The mouse is a rodent species that is used increasingly for experimental respiratory studies. Use of these animals is motivated by the possibility of genetic engineering that would allow study of respiratory function after specific gene mutations. Biscoe and Pallot (3) undertook direct recording of carotid body activity in the mouse, and Donnelly and Rigual (13) did in vitro single-unit recording; both groups found that the mouse carotid body increased its discharge activity during hypoxia. Tankersley and colleagues (39–42) have identified strain differences in the control of breathing in this animal model. Yamaguchi et al. (44) observed morphological and functional differences of the carotid body between DBA/2J and A/J mouse strains and concluded that these differences might account for attenuated hypoxic ventilatory responses in the A/J mice. These results suggest the importance of the carotid body in the control of breathing in the mouse.

From a historical perspective, the role of the carotid body in physiological functions has usually been assessed by means of surgical carotid body denervation in other species. The aim of the present study was to determine the effect of carotid chemodenervation on hypoxic and hypercapnic ventilatory responses in the anesthetized mouse. We undertook the present study to characterize the steady-state short-term ventilatory responses to hypoxic hypoxia and hyperoxic hypercapnia in the urethane-anesthetized C57BL/6 mouse, which is widely used as a background strain of many genetically engineered mice. This investigation focused on extending the understanding of how carotid body denervation alters the interaction between components of the breathing pattern shaped by the chemosensory responses. We found that intact carotid body innervation is essential for the regulation of hypoxic ventilation and also has a role in normoxic ventilation and hypercapnic ventilatory stimulation in the mouse.

METHODS

Animals. This study was approved by the Ethics Committee of Showa University. Experiments were conducted at room temperature (22 ± 1°C) on 21 male C57BL/6CrSlc mice [12–16 wk old for ventilatory measurements (n = 17 mice) and 20 wk old for metabolic measurements (n = 4 mice)].

Chemodenervation. Animals were anesthetized with an intraperitoneal injection of urethane (1.4 g/kg) and allowed to breathe spontaneously. Supplemental doses of anesthesia (15% of the initial dose)
were given if the mouse withdrew its limb in response to surgical incision or pinch during the course of the experiment. The mouse was tracheostomized through a midline incision in the anterior neck, and a cannula was introduced into the trachea. The rostral part of the trachea and the esophagus were ligated and transected in the midneck region. The rostral tracheoesophageal stump was retracted rostrally. The superior cerebral ganglion with surrounding adventitia, which covers the carotid artery bifurcation area in the mouse, was removed on each side to expose the common, external, and internal carotid arteries. After control measurements of ventilatory responses were obtained, the sinus nerve was identified and cut bilaterally at the point of branching off from the glosopharyngeal nerve. Because preliminary experiments showed that a successful denervation of the carotid bodies eliminated a hypoxic increase in respiratory frequency (f; breaths/min), the adequacy of chemodenervation was verified by the disappearance of the f increase in response to transient hypoxia (15% O2 in N2).

Measurement of ventilatory variables. Ventilation was measured with a whole body, single-chamber plethysmograph (model PLY3211, Buxco Electronics, Sharon, CT). The system comprised a Plexiglas experimental chamber equipped with two pneumotachographs. The chamber received a continuous airflow at 1 l/min via a flow pump-reservoir system (model PLY1020, Buxco Electronics). Pressure difference between the experimental and reference chambers was measured with a differential pressure transducer. The pressure signal was amplified and then integrated by data analysis software (Biosystem XA for Windows, Buxco Electronics). The flow signal generated by a breathing animal in the chamber arises from either expansion (decompression) of the alveolar gas as a consequence of Boyle’s law or from heating and humidification of inspired air (1, 27). The decompression component is related to airway resistance, and the heating and humidification component is related to tidal volume (VT). Onodera et al. (33) showed that VT measured with whole body plethysmography was similar to that measured with pneumotachography under normal conditions in urethane-anesthetized mice, which suggests that the decompression component is small under such conditions. Because no significant bronchocstriction was expected in the present study, we assumed that the respiratory signal from the chamber was created mainly by the heating and humidification component. A calibration volume of 0.5 ml of ambient air was introduced into the chamber with a syringe before recordings. Barometric pressure, body weight, and rectal temperature were measured routinely before and after experiments to enable calculation of VT (ml BTPS) per kilogram. Values of f, VT, minute ventilation (VE; ml/min BTPS), inspiratory time (TI), and expiratory time (TE) were computed breath by breath throughout all baseline and experimental periods and were stored in computer memory for offline analysis. A 10-s average was taken for each variable during hypercapnic or hypoxic exposure. With the exception of the ratio of TI to total respiratory time (TI/Ttot, Ttot = TI + TE), the results are presented as a percentage of the control value collected for 30 s before each trial.

Ventilatory responses. The total number of mice undergoing ventilatory response tests was 17. All 17 mice received the ventilatory tests with the carotid sinus nerves (CSNs) intact. Ventilatory responses after chemodenervation were studied in 11 of the 17 mice; 6 of the 17 mice were not tested after chemodenervation because chemodenervation was unsuccessful in 5 of the 6 mice because of accidental bleeding from carotid arteries, and the sixth mouse failed to recover from ventilatory depression elicited by a transient 15% hypoxic test trial. Each of the 17 mice was killed after the experiment by an intraperitoneal injection of an excessive dose of pentobarbital sodium.

Baseline respiratory variables in air were measured for 30 s before the start of ventilatory response tests for both intact and chemodenervated conditions. Animals were challenged with three levels of inspired O2 (21, 15, and 10% O2 in N2) and hypercapnia (5% CO2 in O2) before (n = 17 mice, 27.9 ± 0.6 g) and after (n = 11 mice, 27.3 ± 0.6 g) chemodenervation. Normoxic and hypercapnic gas challenges lasted 4 min, and 10 and 15% hypoxia lasted 90 and 120 s, respectively. The order of applied gas conditions was chosen randomly for each experiment, and each gas challenge was followed by a 5-min interval in room air. The same order of conditions was maintained after chemodenervation with the exception of 10% hypoxia, which was always applied last; 10% hypoxia was profoundly depressing for the chemodenervated mouse, which made recovery prolonged or unattainable.

Measurement of metabolism. O2 consumption (VO2; ml/min STPD) and CO2 production (VCO2; ml/min STPD) were measured during normoxia (21% O2 in N2) before and after chemodenervation (n = 4 mice, 32.7 ± 1.2 g) with an open-circuit system (model ARCO-1000, ARCO System, Kashiwa, Japan). Each mouse was placed in a chamber where a steady flow of air was delivered by a vacuum pump. The ARCO system measured the fractions of O2, CO2, and N2 at the inflow and outflow of the chamber with a mass spectrometer; it also measured the flow rate with a pneumotachograph. After equilibration was achieved, the metabolic factors in the normoxic condition were measured for 5 min. VO2 and VCO2 were normalized by body weight (kg).

Statistical analysis. Results are expressed as means ± SE. We used a commercial software package (SPSS, SPSS Japan, Tokyo, Japan). Comparisons of ventilatory variables and responses were made between the 17- and 11-mouse groups, taken as independent groups. Data were analyzed by the unpaired t-test for baseline measurements in air and by two-way-repeated-measures ANOVA for ventilatory responses to test for a between-factor (chemodenervation) effect, a within-factor (time) effect, and any interaction between these two effects. Metabolic variables were analyzed by the paired t-test. Statistical significance was accepted at P < 0.05.

RESULTS

Adequacy of chemodenervation. For ventilatory response tests, 12 of 17 mice underwent transient hypoxia. In the first hypoxic trial, f did not increase in 9 of the 12 mice, and 1 of these 9 mice had sustained ventilatory depression after interruption of the trial and failed to recover. The data collected on this latter mouse were not included in the final calculations of the chemodenervated mouse group. The other 3 of the 12 mice responded to the first hypoxic trial with an obvious increase in f that disappeared after a successful sinus nerve section. For metabolic analysis, another group of four mice was used. The f increase was not seen in all four mice in the first hypoxic trial. In summary, the transient hypoxic test identified 11 of the 12 mice for use in ventilatory response tests with the CSN cut and another group of 4 mice for metabolic analysis.

Table 1. Respiratory variables during baseline breathing

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<th>Before CSN Section</th>
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<tr>
<td>f, breaths/min</td>
<td>188.8 ± 8.3</td>
<td>161.6 ± 7.0*</td>
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<tr>
<td>VT, ml/kg</td>
<td>1.46 ± 0.11</td>
<td>1.20 ± 0.05</td>
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<td>VT, ml/kg · 1·min−1</td>
<td>271.6 ± 19.3</td>
<td>192.8 ± 10.9*</td>
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<td>T1, s</td>
<td>0.106 ± 0.004</td>
<td>0.106 ± 0.004</td>
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<td>T1, s</td>
<td>0.230 ± 0.015</td>
<td>0.281 ± 0.019*</td>
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<td>VT/Ttot, ml/kg · s−1</td>
<td>14.0 ± 0.9</td>
<td>11.5 ± 0.5</td>
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<td>VT/Ttot</td>
<td>0.321 ± 0.006</td>
<td>0.280 ± 0.012*</td>
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<tr>
<td>VO2, ml/kg · 1·min−1</td>
<td>19.9 ± 0.9</td>
<td>18.8 ± 1.9</td>
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<td>VCO2, ml/kg · 1·min−1</td>
<td>15.9 ± 0.8</td>
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Values are means ± SE. CSN, carotid sinus nerve; f, respiratory frequency; VT, tidal volume; VE, minute ventilation; T1, inspiratory time; TE, expiratory time; Ttot, total respiratory time; VO2, O2 consumption; VCO2, CO2 production.
*Significant difference before and after CSN section, P < 0.05 (unpaired t-test).
Ventilatory and metabolic variables during air breathing. Baseline ventilatory and metabolic measurements in air before and after chemodenervation are given in Table 1. There were significant differences between the before and after values of f and V\text{E} but not of V\text{T}. A prolonged T\text{E}, observed after chemodenervation, accounted for reductions in f and T\text{I}/T\text{TOT}. Metabolic variables during normoxia were compared before and after chemodenervation in another group of four mice. There were no significant differences in V\text{O}_2 or V\text{CO}_2 values.

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<td>5% CO\text{2}</td>
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Hypoxic ventilatory response. Ventilatory responses to hypoxia (10 and 15% O\text{2} in N\text{2}) and normoxia (21% O\text{2} in N\text{2}) were tested before and after chemodenervation. Switching to normoxia from air did not affect any of the variables before and after chemodenervation (data not shown). Representative tracings of the chamber flow signal during hypoxia in one mouse are given in Fig. 1 (top and middle traces). Both levels of hypoxia increased V\text{E} due to an increase in f rather than V\text{T} before chemodenervation (Fig. 1).

Fig. 1. Representative tracings of flow signals from the chamber of the whole body plethysmograph during exposure of a mouse to 15% O\text{2} (top traces), 10% O\text{2} (middle traces), and 5% CO\text{2} (bottom traces) before (left) and after (right) chemodenervation. Before chemodenervation, respiratory frequency (f) increased in response to both levels of hypoxia. After chemodenervation, f decreased during both levels of hypoxia, and a gasp-like pattern occurred in the course of the 10% O\text{2} exposure.
The significant interaction in \( f \) and \( V_e \) during both levels of hypoxia (all \( P < 0.05 \)) suggests that, after CSN section, \( V_e \) responses to hypoxia were lower than before section because \( f \) did not increase. After CSN section, \( f \) declined in response to hypoxia with decreased \( T_i/T_{tot} \) (Fig. 2 and see Fig. 4). A significant time effect in \( V_T \) with no chemodenervation effect or interaction during 15% hypoxia showed that increases in \( V_T \) in response to 15% hypoxia before and after chemodenervation were similar. However, during 10% hypoxia, significant interactions in \( V_T \), \( V_T/T_i \), and \( T_i/T_{tot} \) (all \( P < 0.05 \)) showed that the larger \( V_T \) response after chemodenervation was accompanied by a sudden increase in \( V_T/T_i \) and a drop in \( T_i/T_{tot} \). The chamber flow signal shows that a gasp-like pattern occurred during the course of 10% hypoxia after chemodenervation in this mouse (Fig. 1).

**Hypercapnic ventilatory response.** Representative tracings of the flow signal during hypercapnia in the mouse are given in Fig. 1 (bottom traces). \( V_e \) increased mainly because of increased \( V_T \) in response to hyperoxic hypercapnia both before and after chemodenervation (Figs. 3 and 4). The significant interactions between time and chemodenervation effects in \( V_T \) and \( V_e \) (both \( P < 0.05 \)) showed that the \( V_e \) response with time was significantly lower in chemodenervated mice and that this weaker response was due primarily to a smaller \( V_T \) response. Chemodenervation did not modify the \( f \) response to hypercapnia. The \( V_T/T_i \) response was significantly lower in the absence of sinus innervation (\( P < 0.05 \)).

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**Fig. 2.** Comparison of \( f \), tidal volume (\( V_T \)), minute ventilation (\( V_e \)), ratio of \( V_T \) to inspiratory time (\( T_i \)) (\( V_T/T_i \)), and ratio of \( T_i \) to total respiratory time (\( T_{tot} \)) (\( T_i/T_{tot} \)) during exposure to 15% \( O_2 \) (A) and 10% \( O_2 \) (B) before and after carotid body denervation. Values are means ± SE. Data are presented as a percentage of the control value before each trial, with the exception of \( T_i/T_{tot} \). Before the sinus nerves were cut, \( f \), \( V_T \), and \( V_e \) increased in response to both levels of hypoxia, whereas after chemodenervation there was a decline in \( f \) and thus \( V_e \) during hypoxia. There is a significant time effect \((* P < 0.05)\), a chemodenervation effect \((† P < 0.05)\), and interaction between the 2 effects \((‡ P < 0.05)\).

**Fig. 3.** Comparison of \( f \), \( V_T \), \( V_e \), \( V_T/T_i \), and \( T_i/T_{tot} \) during hyperoxic hypercapnia (5% \( CO_2 \) in \( O_2 \)). Values are means ± SE. Data are presented as a percentage of the control value before each trial with the exception of \( T_i/T_{tot} \). \( V_e \) response is significantly lower after chemodenervation than before because of a smaller response in \( V_T \). Time effect \((* P < 0.05)\), chemodenervation effect \((† P < 0.05)\), and interaction between the 2 effects \((‡ P < 0.05)\) are significant.
N2 caused no ventilatory response. A lack of ventilatory denervation of anesthetized rats that few-breath administration of cats, hypoxic ventilatory responses were abolished acutely by Smith and Mills (37), who observed that, in anesthetized lack of increase in f. These results are similar to data presented were eliminated after carotid body denervation because of the significant role in the regulation of respiration in the anesthetized mouse.

We observed a small Ve in air, without metabolic changes, caused by a low f after acute carotid body denervation. In anesthetized intact wild-type mice, hypoxia decreases minute neural respiration relative to the normoxic control by 35%, with reductions in both the rate component and integrated phrenic activity (22, 23); this suggests that the carotid bodies sustain ventilation during normoxia in anesthetized mice. The contribution of the carotid body to eupneic breathing has been established in other animals. Smith and Mills (37) showed in anesthetized cats that carotid body denervation was followed by decreased ventilation with a reduction in f. Bouvet et al. (6) showed in awake rabbits that chemodenervation decreases ventilation and that this effect is related mainly to a decrease in f without changes in metabolism under normoxic conditions. Hypercapnia occurs commonly in other species during eupneic breathing after carotid body denervation (6, 32, 34, 35, 37). Such consistency among many experiments supports the view that carotid chemoreceptors play a significant role in the regulation of basal breathing in anesthetized mice.

We found that Ve responses to hypoxia in anesthetized mice were eliminated after carotid body denervation because of the lack of increase in f. These results are similar to data presented by Smith and Mills (37), who observed that, in anesthetized cats, hypoxic ventilatory responses were abolished acutely after resection of the carotid bodies with a reduction in the f response. Chiocchio et al. (8) showed in chronically chemo-denervated anesthetized rats that few-breath administration of N2 caused no ventilatory response. A lack of ventilatory response to acute hypoxia has also been observed in awake subjects after carotid body denervation in rabbits, ponies, and humans (4, 6, 7, 18). However, carotid body denervation does not always abolish ventilatory responses to acute hypoxia. In awake dogs with chronic carotid body denervation, acute hypoxia caused small ventilatory responses (6). Such residual hypoxic ventilatory responses were also observed in rats, goats, and calves (5, 25, 32, 34). It is likely that aortic chemoreceptors mediate these residual responses (4, 34). There may be remaining peripheral chemoreceptive tissues in rats (25). Because the anesthetized mice did not have residual hypoxic Ve responses after carotid body denervation, mice may have few other functional receptors that increase ventilation in response to hypoxia.

Carotid body denervation not only abolished Ve responses to 10 and 15% hypoxia in anesthetized mice but also caused obvious ventilatory depression during hypoxic gas exposure as a result of a sudden drop in f. A biphasic ventilatory response to sustained hypoxia is observed in mice and cats (17, 19, 20, 43); hypoxic exposure results in an abrupt increase in ventilation followed by a slow decline in ventilation, referred to as a roll-off (31). Because carotid sinus activity remains unchanged during the roll-off, the mechanism responsible for the ventilatory decline most likely involves the central nervous system (CNS) (43). Because the depressant effect of brain hypoxia is best shown in peripherally chemodenervated cats (30, 31), the elimination of carotid sinus activity during hypoxia probably uncovered the depressant effect of the CNS on ventilation. The CNS effect could account for the sudden ventilatory depression in chemodenervated mice.

Because the Ve increase in response to 15% hypoxia after section of the carotid sinus nerves was similar to that before chemodenervation, we believe that carotid body activity affects mainly the f response during hypoxia. A large increase in Ve was observed after carotid body denervation during 10% hypoxia, but this did not seem to be due to the direct effect of carotid body denervation. When hypoxia progresses, a gasping pattern, characterized as a high-amplitude phrenic burst with a short duration of inspiration and fast rate of rise (38), can develop (16, 31). When a large Ve occurred during the course of 10% hypoxia, increased Ve/Ti and decreased Ti/Ttot were observed, suggesting that this large Ve was caused by a compensatory mechanism such as gasping. A gasp-like pattern can be seen in the representative tracing.

A significant difference in the Ve time courses before and after carotid body denervation was also observed during hyperoxic hypercapnia, indicating that the carotid bodies contribute to hypercapnic ventilatory responses in the mouse. The relative importance of central vs. peripheral chemoreceptors in the ventilatory response to hypercapnia has long been debated. The contribution of the carotid bodies to hypercapnic ventilatory responses was observed after carotid body denervation in awake goats and dogs (34, 35). Pan et al. (34) found that in awake goats, ventilatory sensitivity to CO2 was reduced from control by 60% after carotid body denervation, suggesting a large role for the carotid bodies in hypercapnic ventilatory responses. However, peripheral chemoreceptors do not contribute to hypercapnic ventilatory responses in some species. In awake and anesthetized cats, CO2 sensitivity can be abolished by lesions on the ventral lateral medullary surface (28, 36); in the anesthetized rabbit, acute carotid body denervation does not change hypercapnic ventilatory responses (21).

Several studies of humans have shown that hyperoxia eliminates peripheral CO2 responsiveness (2, 11, 26). Our results, however, suggest that peripheral chemosensitivity to CO2 is not abolished with a hyperoxic background in the anesthetized

![Fig. 4. Comparison of f, VT, and VE at the end of 15% hypoxia (left) and 5% hypercapnia (right) before and after section of the carotid sinus nerves. Values are means ± SE. Data are presented as percentage of the control value before each trial. After section, there is ventilatory depression as a result of reduced f during hypoxia. Ventilatory response to CO2 is lower after chemodenervation than before, because of a smaller VT.](http://jap.physiology.org/)
mouse. Rodman et al. (35) attributed 40% of the whole body ventilatory response to CO₂ during hypoxia directly to the carotid bodies. Measurements of carotid sinus nerve activity also show that hypoxia cannot remove sensitivity of the carotid body to CO₂ (14, 15, 24). These results support the notion that the carotid bodies can respond to hypercapnia under hyperoxic conditions in the anesthetized mouse.

Unlike hypoxic responses, lower Vₑ responses to hypercapnia in chemodenervated mice were due primarily to smaller V_T responses. In conscious rats, Coles et al. (9) showed that CSN section eliminated f responses to hypoxia but did not affect f responses to hypercapnia. In contrast, Rodman et al. (35) observed in awake dogs reduced ventilatory responses to hyperoxic hypercapnia with reductions in both f and V_T after carotid body denervation. Whether the differences in the effect of chemodenervation on breathing pattern during hypercapnia are due to species differences needs to be considered.

It is important to consider whether limitations in our methodology influenced our results. In the present study, we did not use independent sham-operated mice. The surgical trauma to the mouse was similar before and after chemodenervation because control data were collected after the carotid bifurcations were exposed and the trachea was cannulated. Additionally, in three mice, there was an obvious ventilatory response to a trial hypoxic test conducted immediately before the sinus nerve section. This observation suggests that the surgical procedure itself, without the sinus nerve cutting, does not abolish hypoxic ventilatory responses. We did not measure blood pressure or arterial blood gases in the present study. There is the same sort of inconsistency in cardiovascular responses to carotid body denervation as exists for respiration. Chemodenervation affects cardiovascular status in anesthetized cats (37) and in unanesthetized old piglets but not young piglets (12). The interruption of afferent pathways for carotid sinus baroreceptors in the carotid sinus nerves may have influenced our results. Measurement of blood pressure or blood gases would have given us a clue as to what was happening in breathing pattern generation, especially during hypoxia in the mouse. Because there are major differences in ventilatory response and in morphology and function of carotid bodies among various mouse strains, strain differences in the chemodenervation effect on respiration may also exist.

The present study found that section of the carotid sinus nerves resulted in reduced ventilation under normoxic conditions, a lack of hyperpnea with subsequent ventilatory depression in response to hypoxia, and decreased ventilatory responses to hyperoxic hypercapnia. The carotid bodies are essential for maintaining ventilation during eupnea, hypoxia, and hypercapnia in the anesthetized mouse.

GRANTS

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