Hyperventilation evoked by activation of the vicinity of the caudal inferior olivary nucleus depends on the fastigial nucleus in anesthetized rats

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Zhuang J, Xu F, Frazier DT. Hyperventilation evoked by activation of the vicinity of the caudal inferior olivary nucleus depends on the fastigial nucleus in anesthetized rats. J Appl Physiol 104: 1351–1358, 2008. First published March 20, 2008; doi:10.1152/japplphysiol.00824.2007.—Several studies have demonstrated that cerebellar deep nuclei, particularly the rostral fastigial nucleus (FNr), are involved in respiratory modulation. These nuclei receive inputs from the contralateral caudal inferior olivary nuclei of the medulla. The objectives of this study were to determine whether electrical and chemical activation of the vicinity of the caudal inferior olivary nuclei (vIOc) affected respiration and, if true, whether the FNr was involved in the vIOc stimulation-evoked ventilatory responses. Experiments were conducted in 30 anesthetized and spontaneously breathing rats. Our results showed that 1) electrical (25 or 100 μA at 10 or 20 Hz for 10 s) and chemical (1 or 100 mM, 25–50 nl N-methyl-D-aspartate) stimulation of the vIOc augmented ventilation predominantly via increasing tidal volume; 2) the responses to the electrical stimulation were almost eliminated by lesion of the contralateral FNr via microinjection of ibotenic acid; and 3) the respiratory responses to electrical stimulation in the vicinity of the rostral IO were 65–70% smaller compared with that evoked by vIOc stimulation. These findings strongly suggest that vIOc neurons play a significant role in modulation of respiratory activity, largely depending on their projections to the FNr.

Cerebellum; sudden infant death syndrome; inferior olive projections; hypertension

THE INFERIOR OLIVARY NUCLEUS (IO), a nuclear complex located ventromedially in the medulla oblongata, has been amply documented to play important roles in motor functions, e.g., motor coordination and motor learning (2, 14) and visceral renal functions (28, 40). Recent evidence from clinical observations has implied a possible IO involvement in cardiorespiratory dysfunction. First, victims of sudden infant death syndrome, a sleep apnea syndrome precipitated by defective control of involuntary respiration, often show neurotransmitter receptor deficiencies (10), developmental abnormalities (16), and prominent neuropathological changes in the IO (15). Second, a specific neuronal loss in the IO was found in infants with severe perinatal asphyxia (36). Third, a tremendous alteration of c-Fos immunoreactivity was found in the IO of asphyxiated rats as compared with nonasphyxia cases, with the effects more specific in any other region related to respiration (29). Expression of the fos gene in neuronal nuclei has been proposed to reflect second-messenger activation and, hence, serves as a sensitive indicator of cellular responses induced by various stimuli. In concert with the clinical observations, the possible role of the caudal IO (IOc) in cardiorespiratory modulation has also been reported in animals. For example, electrical stimulation to the IOc was reported to cause an apnea in anesthetized cats (20, 30), whereas lesion of the IOc by 3-acetylpyridine in anesthetized rats attenuated the ventilatory responses to acute hypoxia and hypercapnia by 50% (21). Because the IO resembles a crinkled sac with an opening directed toward the midline, the respiratory alterations induced by electrical stimulation or local chemical lesion of the IO are likely the result of affecting the vicinity of the IOc (vIOc). In support of the facilitatory effects of the vIOc on respiration, acute exposure to hypoxia caused activation of vIOc neurons in the anesthetized rats, as evidenced by the significant increase of c-fos immunoreactivity in the IOc (50). The inhibitory role of the vIOc revealed by electrical stimulation appears to be in direct opposition to the excitatory effects observed in the ventilatory responses to hypoxia and hypercapnia. However, these differences may be the result of using a high frequency of stimulation (>100 Hz).

Previous studies have shown that low-frequency (<50 Hz) stimulation of the cerebellar fastigial nucleus (FN) and vestibular nucleus elicits the excitatory respiratory responses, but high-frequency stimulation leads to an apnea (12, 42). In addition to the possible role in respiratory modulation, the IOc has also been suggested to participate in cardiovascular regulation. Waldrop and Iwamoto chemically stimulated the vIOc by microinjection of glutamate and elicited an increase in arterial blood pressure (ABP) in anesthetized cats (40). Other investigators found a profound functional magnetic resonance imaging signal decline in the IO during pharmacological depressor challenges in anesthetized adult cats, supporting an involvement in blood pressure regulation (11). To date, it remains unclear whether electrical stimulation of the vIOc at lower stimulating frequencies (<50 Hz) would produce hyperventilation and hypertension in anesthetized rats and, if so, whether the responses are uniquely evoked by stimulation of the vIOc rather than the vicinity of the rostral IO (vIOr).

IO neurons receive inputs from a variety of areas of the central nervous system, e.g., the spinal cord (8), trigeminal nuclei (38), vestibular nuclei (34), deep cerebellar nuclei (33, 34), raphe nuclei (35), and medullary reticular formation (35), and are innervated extensively via glutamatergic connections (5, 6, 31). Activation of N-methyl-D-aspartate (NMDA) and non-NMDA receptors enhances the tone of excitatory activities of IO neurons (5, 19, 31). Of particular interest is that activation of NMDA receptors is necessary for the generation of spontaneous oscillations and the production of physiological responses to electrical and chemical activation of the vIOc.
rhythmicity of IO neurons (31). Because our preliminary data have shown that electrical stimulation of the vIOc at lower frequencies (<50 Hz) evokes excitatory effects on respiration, further study is needed to clarify whether NMDA receptors in the vIOc play a role in the cardiorespiratory modulation.

Both electrophysiological (1, 8, 17) and morphological (7, 8, 17, 34) studies have demonstrated that IOc neurons project to the contralateral cerebellar deep nuclei, especially the FN, directly via the collaterals of climbing fibers or indirectly through the cerebellar Purkinje cells. The rostral FN (FNr) has been well documented to play an important role in cardiorespiratory modulation. Bassal and Bianchi (3) first reported an altered respiratory response to electrical stimulation of the FNr in cats. Subsequent studies confirmed that low-frequency electrical (3, 22, 43) or chemical activation (44) of FNr neurons predominantly elevated ventilation, often associated with a pressor response in both anesthetized cats and rats. Ablation of this region attenuated the respiratory response to severe hypercapnia and hypoxia (44, 45). Guraut and Delgado-Garcia (9) have shown in conscious cats that some respiratory-modulated neurons in the FN are synchronically activated by stimulation of the IOc, which is in agreement with the morphological observation that the FNr predominantly receives afferents from the IOc. Therefore, it begs the question as to whether the vIOc-mediated cardiorespiratory responses are dependent on the FNr. Accordingly, the objectives of this study were to test the three hypotheses that 1) electrical (at low frequencies) and chemical stimulation of the vIOc would lead to hyperventilation and hypertension in the anesthetized rat; 2) these responses are largely dependent on the integrity of the FNr; and 3) the vIOc is, compared with the vIOr, uniquely involved in cardiorespiratory modulation.

METHODS

All procedures described in this study were conducted under protocols approved by the Institutional Animal Care and Use Committee at Lovelace Respiratory Research Institute. The latter is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Animal preparations. Experiments were conducted in 30 anesthetized, tracheotomized, and spontaneously breathing male Sprague-Dawley rats (body weight: 350–450 g). Anesthesia was induced using a mixture of chloralose and urethane (100 and 500 mg/kg ip) and supplemented as needed via a left femoral vein cannula to suppress corneal and withdrawal reflexes. The right femoral artery was cannulated for monitoring and recording the ABP and heart rate (HR) through a blood pressure transducer (AD Instruments ML.T0380, Castle Hill, Australia). The catheter was filled with heparinized saline and connected to a bridge preamplifier (AD Instruments ML224). The trachea was cannulated below the larynx with a short metal cannula and connected to a pneumotachograph. Respiratory flow was measured with the pneumotachograph via a differential pressure transducer (AD Instruments ML141). The pneumotachograph was made of stainless steel and had a linear flow-pressure relationship in the range of 0–20 ml/s with a flow resistance of 0.046 cmH2O·l·s−1 and a dead space of ~0.2 ml. End-tidal pressure of CO2 was monitored via an infrared CO2 analyzer (MicroCapStar, model 15-10000, Ardmore, PA) and maintained at the appropriate level throughout the experiments. The core temperatures of the animals were monitored with a rectal probe and maintained at ~37.5°C by a heating pad and radiant heat.

Occipital craniotomy. After completing the above procedures, the animals were placed into a rigid metal frame with their heads fixed in a stereotaxic apparatus (David Kopf, Tujunga, CA). Stereotactic coordinates were determined according to the rat brain atlas (29a). A hole (~6-mm diameter) was drilled at the midline of the skull (with the center positioned at 12.0 mm posterior to the bregma) for stereotaxically inserting the stimulating electrode or a microneedle into the given region of the IO and/or the FNr. Bleeding was controlled with bone wax, an absorbable hemostat (Surgicel and Gelfoam, Ethicon, Johnson & Johnson, Somerville, NJ), and the use of a bipolar coagulator (Radiomics, model 440S, Burlington, MA). The underlying tissue was covered by a 2 × 2 gauze sponge saturated with mineral oil to prevent drying.

Experimental protocols. Four series of experiments were performed in the present study. The cardiorespiratory responses to 100-μA electrical stimulation of the right vIOc at lower frequencies (10, 20, and 50 Hz) were tested in the first series (n = 14), since these stimulations could evoke excitatory ventilatory responses in our preliminary studies. High-frequency stimulations (75 and 100 Hz) were also utilized to confirm the presence of an apneic response as observed in cats (20, 30). To test the specificity of the vIOc in modulating respiration, electrical stimulations were also applied to the vIOr (bregma = 11.8 mm) in 6 of the 14 rats. The second series was designed to determine the threshold of electrical stimulating intensity required to evoke the vIOc-mediated excitatory ventilatory response (n = 5). We tested whether 10 or 25 μA at 10 and 20 Hz was able to initiate a detectable excitatory ventilatory response. The other 8 of the 14 rats used in the first series were employed to evaluate the dependence of the FNr in the vIOc-mediated responses in the third series. The cardiorespiratory responses to electrical stimulation of the vIOc were compared before and after microinjection of ibotenic acid (IA) into the left side of the FNr. The fourth series was conducted in 11 rats to reveal the effects of unilateral microinjection of 100 mM NMDA (50 nl, n = 6; and 100 nl, n = 2) and 1 mM (25 nl, n = 3) into the vIOc on cardiorespiratory activity. Stabilization of the baseline cardiorespiratory variables for at least 10 min was allowed before conducting the experimental protocols, with a 5-min interval allowed between the two electrical stimulations.

Electrical stimulation of the vIOc. Stereotaxic coordinates were used to unilaterally position the tip of a stainless steel, concentric bipolar electrode (Rhodes Medical Instruments, model NE-100, Woodland Hills, CA) into the right IOc or IOr. Stimulation pulses were delivered from a stimulator (Grass, model S88, Quincy, MA) at the beginning of either the inspiratory or expiratory phase. The stimulating intensity was 0.2-ms duration pulses at 10, 25, or 100 μA, whereas the stimulating frequencies were varied (5, 10, 20, 50, 75, 100 Hz with a 10-s duration) and randomly ordered.

Microinjection of IA in the FNr. A microinjection needle (25 gauge, 0.5 μl, Hamilton, Reno, NV) prefilled with 100 mM of IA (Sigma Chemical, St. Louis, MO) solution mixed with 2% Chicago Sky Blue in 150 mM saline was inserted into the left FNr. IA (200 nl) driven by a microinjection unit (model 5002, David Kopf Instruments) was delivered from the microneedle into the left FNr unilaterally over a 30-s period. The same electrical stimulation series were repeated 45 min after the microinjection of IA into the FNr.

Unilateral microinjection of NMDA into the vIOc. In these experiments, the microneedle was prefilled with NMDA solution (mixed with 2% Chicago Sky Blue dissolved in 150 mM saline; Sigma Chemical) and inserted into the right IOc. Delivery of NMDA (100 mM/50 or 100 nl; or 1 mM/25 nl) was driven by the microinjection unit from the microneedle into the vIOc over a 10-s period.

Locations of the stimulating electrode and microneedle. The electrode coordinates utilized to stimulate the vIOc/vIOr were 11.6 mm caudal to the bregma, 1.0 mm lateral to the midline, and 8.8/9.0 mm from the surface of the cerebellar cortex, respectively. The microinjection site of the FNr was 11.6 mm caudal to the bregma, 1.0 mm lateral to the midline, and 5.5 mm from the surface of the cerebellar cortex. After completion of the above protocols, the animals were deeply anesthetized with an overdose of anesthetics and transcardially perfused.
and fixed with 4% paraformaldehyde for 10 min. The brain was then removed and cryostatically transected into 50-μm-thick sections. The sites where the stimulating electrode tip was placed and the centers of the areas marked by the Chicago Sky Blue were mapped on a grid.

Data acquisition and analysis. The baseline cardiorespiratory variables and their responses were monitored and recorded continuously throughout the experiment. Raw signals of respiratory flow and ABP were digitized and recorded by using a PowerLab/8sp (AD Instruments) connected to a computer employing the PowerLab Chart 5 software (AD Instruments). Mean ABP (MABP), HR, tidal volume (VT), respiratory frequency (fR), and minute ventilation (VE) were derived by the online calculation functions of the software. Baseline values (control) were collected and obtained from averaged values of 1-min data immediately before electrical stimulation of the vIOc and vIOr or microinjection of NMDA into the vIOc. The cardiorespiratory responses were normalized as the percent change from the baseline values. The responses were collected and measured for 1) last 5 s of the responses during electrical stimulation; 2) the longest apneic duration (TE) evoked by a given stimulation; and 3) 30 s of the peak VE response to the 100 mM and 5 s to the 1 mM NMDA injection. An apnea was defined as a TE threefold longer than the control. The latency for VT and ABP in response to electrical stimulations was determined by the time between the onset of stimulation and the initiation of a detectable response. All data in the text and figures are presented as means ± SE. One-way ANOVA with repeated tests followed by Tukey's multiple comparisons were used to test the cardiorespiratory responses to the electrical stimulation at low or high frequencies. Two-way ANOVA followed by Tukey's tests were used for comparisons between the responses elicited by vIOc and vIOr electrical stimulation, or before and after the FNr lesions at different frequencies. Paired t-tests were employed for comparing the differences of cardiorespiratory activities before and after NMDA injection. P values of <0.05 were considered significant.

RESULTS

Cardiorespiratory responses to 100-μA electric stimulation of the vIOc. Figure 1 shows typical experimental recordings of the cardiorespiratory responses to unilateral vIOc stimulation at different frequencies (10, 20, 50, 75, 100 Hz) in an anesthetized rat. Both the ventilatory and cardiovascular responses evoked by vIOc stimulation were frequency dependent. At low frequencies (10 and 20 Hz), electrical stimulation caused significant excitatory Ve responses, attributed to increases in both VT and fR. The ventilatory responses to 50-Hz stimulation were varied. An apneic response was evoked by 50 Hz in 7 of the 14 rats tested as illustrated in Fig. 1C (Te = 2.3 ± 0.6 s for the evoked response vs. 0.3 ± 0.02 s for baseline; P < 0.05), whereas an augmented ventilatory response (62% increase from 173 ± 16 ml to 276 ± 36 ml; P < 0.01) was observed in the other 5 rats, which was mainly due to an increase in VT (65 ± 11%; P < 0.05) but not fR (−2 ± 10%; P > 0.05). It appears that 50 Hz is near the threshold for inhibitory effects. When the stimulating frequencies were 75 and 100 Hz, inhibitory effects on Ve emerged, leading to an apnea (Fig. 1, D and E). Statistically, 10- and 20-Hz stimulation increased ventilation with a greater contribution from an elevation of the VT response, and the augmentation in ventilation was significantly greater with 20-Hz than with 10-Hz electrical stimulation (Fig. 2A). In sharp contrast, 75- and 100-Hz stimulation always produced a long-lasting apneic response, and the prolongation of Te induced by 100-Hz stimulation (8.3 ± 0.8 s) was similar to that evoked by 75-Hz stimulation (7.3 ± 0.9 s) (Fig. 2B). The averaged latency for the ventilatory responses to electrical stimulation was 0.08 ± 0.002 s, which was not significantly affected by the stimulating frequencies (Table 1). MABP responses to the vIOc electrical stimulations (10–100 Hz) were always excitatory, whereas the ventilatory responses were excitatory during low-frequency stimulation but inhibitory during high-frequency stimulation (Fig. 1). As shown in the group data (Fig. 2C), the pressor responses to stimulation of the vIOc were related to the stimulating frequency. Ten hertz failed to evoke significant pressor response, whereas in the stimulating range from 10 to 50 Hz the higher stimulating frequency elicited a greater elevation in MABP. However, the pressor response reached a plateau when the stimulation was >50 Hz. HR responses to vIOc electrical stimulation were excitatory at low frequencies (10 and 20 Hz) but inhibitory when 75- and 100-Hz stimulations were applied. The averaged latency (10–100 Hz) for the cardiovascular responses to electrical stimulation was 0.85 ± 0.05 s, ~10 times longer than that for the Ve responses (P < 0.01), with no differences among the stimulating frequencies (Table 1).

Cardiorespiratory responses to 100-μA electric stimulation of the vIOr. To test the specificity of the vIOc stimulation-evoked ventilatory responses, electrical stimulations were also delivered into the vIOr in 6 of the 14 rats. The results showed that ventilation was also increased by electrical stimulation of the vIOr at low frequencies (10 and 20 Hz) with a much smaller response amplitude compared with that evoked by vIOc stimulation. Numerically, compared with the vIOc-induced responses, the amplitude of the ventilatory response to

![Fig. 1. Typical experimental recordings of cardiorespiratory responses to electrical stimulation of the vicinity of the caudal inferior olivary nucleus (vIOc; 100 μA) at different frequencies in a rat. Traces from top to bottom are arterial blood pressure (ABP), airflow rate (Flow), tidal volume (VT), respiratory frequency (fR in breaths/min), minute ventilation (VE), heart rate (HR in beats/min), and the electrical stimulation mark (EST Mark; duration = 10 s).](http://jap.physiology.org)
stimulation of the vIOr was ~65% less at 10 Hz and ~70% less at 20 Hz, with VT being the primary variable (Fig. 3A).

With respect to the cardiovascular responses (Fig. 3B), the vIOc stimulation elicited hypertension that was similar to that evoked by vIOc stimulation. Different from vIOc stimulation, vIOr stimulation failed to evoke significant HR response.

Cardiorespiratory responses to 10- and 25-μA electric stimulation of the vIOc. Because the focus of this study was on the excitatory respiratory responses, in five rats we tested whether 10 or 25 μA was the threshold to initiate the excitatory cardiorespiratory responses to activate the vIOc. It was found that 25-μA stimulation of the vIOc at 10 and 20 Hz, rather than at 5 Hz, significantly increased ventilatory response via elevating both VT and/or fR with little effect on cardiovascular activity (Fig. 4, A and B). In two rats, the sites of stimulation were subsequently replaced at 1.0–1.2 mm dorsal and lateral to the vIOc to test the location specificity. We found that the same stimulations applied in these regions did not evict detectable excitatory responses (sites are marked in Fig. 4C). In addition, 10-μA stimulation at 20 Hz failed to significantly affect VE (1.8 ± 0.8%), VT (1.2 ± 0.7%), fR (0.4 ± 0.7%), MABP (0.2 ± 0.2%), or HR (0.2 ± 0.1%) (P > 0.05).

Table 1. LVₜₐᵢ and Lₐᵢᵩᵩ response to electrical stimulations at 100 μA

<table>
<thead>
<tr>
<th></th>
<th>10 Hz</th>
<th>20 Hz</th>
<th>75 Hz</th>
<th>100 Hz</th>
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<tr>
<td>LVₜₐᵢ</td>
<td>0.078±0.006</td>
<td>0.082±0.003</td>
<td>0.071±0.004</td>
<td>0.074±0.005</td>
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<tr>
<td>Lₐᵢᵩᵩ</td>
<td>0.955±0.129*</td>
<td>1.002±0.123*</td>
<td>0.750±0.060*</td>
<td>0.830±0.040*</td>
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Data are means ± SE (n = 14). LVₜₐᵢ, latencies for minute ventilation; Lₐᵢᵩᵩ, latencies for arterial blood pressure. P > 0.05, among different stimulating frequencies. *P < 0.01, Lₐᵢᵩᵩ vs. LVₜₐᵢ.

Effects of FNr lesion on the cardiorespiratory responses to electrical stimulation of the vIOc. Figure 5 compares the typical excitatory respiratory responses to vIOc electrical stimulation before and 45 min after microinjection of IA into the FNr. As shown, the excitatory ventilatory responses to low-frequency stimulation at 100 μA in the FNr intact state were almost eliminated after FNr IA microinjection. Statistically, compared with the intact state, the FNr lesion almost eliminated the excitatory ventilatory responses to 100 μA at 10 and 20 Hz (>75%: Fig. 6A) and completely abolished the responses to 25 μA at the same stimulating frequencies (Fig. 7A). This FNr lesion-induced diminution was achieved by lowering both the VT and fR responses with a greater effect on the VT. MABP and HR responses to 100 or 25 μA at 10- and 20-Hz stimulation were not affected significantly by microinjecting IA into the contralateral FNr (Figs. 6B and 7B).

Cardiorespiratory responses to unilateral microinjection of NMDA. Unilateral microinjection of NMDA (100 mM, 50 nl) into the vIOc caused excitatory effects on ventilation in eight rats (Fig. 8, A and B). Although the fR was decreased after an initial transient increase compared with the baseline, the VT was greatly increased in response to the NMDA injection that
The significant increase in ventilation usually occurred within 1 min after the NMDA injection and reached a peak or plateau in 2–5 min. The recovery usually started 5–10 min after injection, with the variables usually returning to the control values 1–1.5 h after the NMDA injection. The excitatory nature of the cardiorespiratory responses to the NMDA microinjection was not changed by increasing or decreasing the NMDA dosage. Similar excitatory cardiorespiratory responses were evoked if a higher dose (100 mM, 100 nl) was microinjected into the vIOc. On the other hand, the lower dose and volume of NMDA (1 mM, 25 nl) microinjection induced a 45% increase in V˙E, mainly via elevating the VT response with no effect on cardiovascular activities (Fig. 8C). In addition to the response amplitude, the duration of the excitatory ventilatory response to low-dose NMDA was much shorter than that to high-dose. The peak response occurred at 1.8 ± 0.8 s postadministration, and the response was usually recovered within 2 min after the NMDA injection.

**DISCUSSION**

The vIOc is involved in respiratory modulation. In the present study, we found that low-frequency stimulation of the vIOc at either 25 or 100 µA significantly increases ventilation via elevating Vt and/or fR in the anesthetized rats. In contrast, although the same 100-µA stimulation applied in the vIOR also elicited excitatory ventilatory responses, the amplitude of the response was ~65–70% smaller. Interestingly, when high frequencies were applied (75 and 100 Hz), the vIOc stimulations produced an apnea. The mechanisms underlying the excitatory and inhibitory respiratory responses remain unknown, but it is plausible that a pool of neurons with different activation thresholds is recruited by the higher frequency stimulation. Indeed, the low-frequency stimulation-induced excitatory respiratory responses and high-frequency stimulation-induced apneic response have been reported previously in activation of other respiratory-related nuclei, such as the FNr (12), the vestibular nucleus (12), and the dorsolateral portion of the medulla in anesthetized rats and cats (27). Considering the
physical spread of current induced by electrical stimulation, we do not rule out a partial involvement from the structures near the vIOc in the electrical stimulation-induced respiratory responses presented in this study. However, there are several lines of evidence that do not fully support this notion. First, electrical stimulation (20 V, 240 Hz) previously used to induce the respiratory responses in other respiratory-related nuclei has shown the physical spread of current within a sphere of tissue <0.5 mm in radius (20, 30). A recent review (37) has specifically discussed the current spread in the microelectrical stimulation studies and quantitatively defined the current spread distance. Based on the calculations in this review, our electrical stimulation at 25 \( \mu \text{A} \) (10–20 Hz) should have an effective current spread distance of 0.2 mm. Second, if current spreads to nearby structures primarily contributing to the evoked responses, respiratory-related nuclei, such as the medullar ventral surface and raphe nuclei, would be mostly suspect. Previous studies have demonstrated that electrical stimulation of the medullary ventral surface only evokes excitatory respiratory responses that require a much greater stimulating frequency and intensity (>40 Hz, 300 \( \mu \text{A} \)) (24) compared with the stimulation used in the present study (<20 Hz, 25 \( \mu \text{A} \)). Electrical stimulation (20 Hz) of the caudal or rostral raphe of the medulla in rats and cats produced inhibitory rather than excitatory effects on respiration (4, 18). Third, the placement of the electrode 1.0–1.2 mm away from the vIOc failed to elicit the excitatory ventilatory responses in this study. Taken together, our data suggest that the vIOc, especially the vIOc, plays a role in modulating respiratory activities.

**vIOc-mediated respiratory modulation is related to the FN.** Another important finding in the present study is that contralateral lesion of the FNr eliminates the vIOc-mediated excitatory response by 25-\( \mu \text{A} \) stimulation, clearly demonstrating the critical role of the integrity of the FNr in vIOc-mediated respiratory responses. Consistent with this finding, it has been reported that some respiratory-modulated neurons in the FN were synaptically activated by stimulation of the IOc in conscious cats (9). Morphologically, IOc neurons project to cerebellar Purkinje cells and deep nuclei, particularly to the contralateral FNr (33). Several studies have demonstrated that, among the cerebellar deep nuclei, the FNr may play the most important role in respiratory modulation because it contains respiratory-modulated and chemosensitive neurons (9, 42, 47) and is the most involved in respiratory and cardiovascular modulation (9, 22, 23). Stimulation of FNr neurons modulates neuronal activity of the medullary respiratory group (42), medial vestibular nucleus (12, 49), and the medullary gigantocellular nucleus (48). These results combined with our data suggest that the vIOc-mediated respiratory responses greatly relay through the FNr and its direct or indirect connections to the respiratory central network.

**vIOc neurons are responsible for respiratory modulation.** The vIOc-mediated respiratory responses could be the result of activation of local neurons, or fibers of passage, or both.

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**Fig. 7.** Effects of contralateral FNr chemical lesion on the ventilatory (A) and cardiovascular responses (B) to the vIOc electrical stimulations at 25 \( \mu \text{A} \). *\( P < 0.05 \) and **\( P < 0.01 \), comparison between the responses and the baseline values. †\( P < 0.05 \), comparison between before and after contralateral FNr chemical lesion. The locations of microinjection of ibotenic acid into the FNr are illustrated in C (n = 5).

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**Fig. 8.** Cardiorespiratory responses to unilateral NMDA microinjection into the vIOc. Experimental recordings in an anesthetized rat are depicted in A (see Fig. 1 for traces). Group data for V\( _{\text{t}} \), tidal volume VT, f\( _{\text{R}} \), MABP, and HR responses to high- and low-dose of NMDA microinjected into the vIOc are exhibited in B (n = 8) and C (n = 3). The right side of B and C shows the locations of microinjection (see Fig. 2 for abbreviations), and in B, one site completely overlapped with another. *\( P < 0.05 \) and **\( P < 0.01 \), the responses vs. baseline. No statistical analysis was made for the data presented in C.
NMDA receptors extensively exist on (5, 25, 31) and are excitatory to IO neurons (31). Thus we tested whether micro-injection of NMDA into the vIOc could alter respiration. Our results showed that NMDA administered into the vIOc elicited an increase in Vr, leading to hyperventilation. Because the Chicago sky blue-marked spread area of microinjection was mainly restricted to the IO, the evoked excitatory effect on ventilation is most likely achieved by activation of vIOc NMDA receptors. As mentioned above, the vIO-mediated excitatory or inhibitory respiratory responses were stimulation frequency-dependent. Contrasted with electrical stimulation in this study, the respiratory responses evoked by low (1 mM, 25 nl) and high dose (100 mM, 100 nl) of NMDA microinjection into the vIOr were always hyperventilatory due to an elevation in Vr. Because the excitatory respiratory responses to NMDA persisted during delivery of greatly different doses of NMDA, we believe that the nature of the response is largely independent of the limited diffusion. An interesting question arises as to why such greatly varied NMDA doses always increase the ventilation, different from the excitatory and inhibitory ventilatory responses to high- and low-electrical stimulating frequency, respectively. These differences may result from 1) the intensity of stimulation not being equivalent between chemical and electrical stimulation of the vIOc and 2) electrical stimulation that presumably activated both neurons and fibers of passage, whereas chemical stimulation of the vIOc involved only the nuclear neurons. Nevertheless, our results confirmed that vIOc neuronal activation modulated the respiratory activities. Previous studies have indicated that the IO contains neither respiratory-modulated neurons (9) nor chemosensitive neurons in the rat (32). However, the latter has been challenged by a recent finding that the immunoreactivity of carbonic anhydrase IV, an abundant marker in central chemoreceptor neurons (26), was prominently present within the IO (41).

Stimulation of vIOc evokes cardiovascular responses. Waldrop and Iwamoto (40) have demonstrated that chemical stimulation of the vIOc elicits a pressor response in anesthetized cats. In another study, investigators found a dramatic fMRI signal decline in the IO during pharmacological depressor challenges in anesthetized adult cats, supporting an IO involvement in blood pressure manipulation (11). Similarly, our data showed that electrical and chemical stimulation of the vIOc produced hypertension associated with bradycardia. The respiratory responses are not secondary to the cardiovascular responses for two reasons. First, the latency for evoking the respiratory responses is significantly shorter than that required for the cardiovascular responses (Table 1). Second, electrical stimulation elicited both the excitatory and inhibitory respiratory responses, but always pressor responses. Third, the recovery time for the respiratory responses was much shorter than for the cardiovascular responses (<20 vs. 35 s). These vIOc-mediated cardiovascular responses were not significantly altered by FNr lesions, suggesting that they are not dependent on the integrity of the FNr. In other words, the IO neurons responsible for modulating the respiratory and cardiovascular responses belong to different subsets. This finding suggests that the FNr is within the efferent pathway of the vIOc-mediated respiratory but not cardiovascular responses. The cardiovascular responses to stimulation of the IO are unlikely evoked by stimulating the nearby raphe neurons because stimulation of raphe produced inconsistent cardiovascular responses: a small depressor (39) or a small pressor response (13).

Limitation of experimental methods. The IO projects to Purkinje cells that inhibit the FNr. Although the vIOc-mediated respiratory responses are largely dependent on the integrity of the FNr, we cannot, based on the data obtained from this study, distinguish whether these respiratory responses are mediated by IO projections directly to FNr and/or through the Purkinje cells. In this study, we focused on the excitatory respiratory responses to electrical stimulation of the vIOc. Thus we only tested the relevant mechanisms underlying the excitatory responses, such as NMDA and FNr involvement. Further studies are needed to clarify whether GABA in the vIOc is responsible for the vIOc-mediated respiratory inhibition and whether vIOc-mediated cardiorespiratory responses are also dependent on the FNr.

In summary, our major findings in the present study can be summarized as follows. First, low-frequency electrical or chemical stimulation of the vIOc altered respiratory responses characterized by an increase in ventilation predominantly via elevating Vt dependent on the integrity of the contralateral FNr. Second, electrical or chemical stimulation of the vIOc also elicited hypertension concurrent with bradycardia that is independent of the FNr. Third, low-frequency electrical stimulation of the vIOc evoked a hyperventilation that is 65–70% smaller than that observed in vIOc stimulation. We conclude that vIOc neurons play a significant role in the modulation of cardiorespiratory activity. Moreover, the vIOc effects on facilitating respiration rather than modulating cardiovascular activity is largely achieved by projection to the FNr.

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GRANTS

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