Focal CO₂ dialysis in raphe obscurus does not stimulate ventilation but enhances the response to focal CO₂ dialysis in the retrotrapezoid nucleus

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Submitted 1 February 2008; accepted in final form 29 April 2008

Dias MB, Li A, Nattie E. Focal CO₂ dialysis in raphe obscurus does not stimulate ventilation but enhances the response to focal CO₂ dialysis in the retrotrapezoid nucleus. J Appl Physiol 105: 83–90, 2008. First published May 1, 2008; doi:10.1152/japplphysiol.00120.2008.—Simultaneous inhibition of the retrotrapezoid nucleus (RTN) and raphe obscurus (ROb) decreased the systemic CO₂ response by 51%, an effect greater than inhibition of RTN (−24%) or ROb (0%) alone, suggesting that ROb modulates chemoreception by interaction with the RTN (19). We investigated this interaction further by simultaneous dialysis of artificial cerebrospinal fluid equilibrated with 25% CO₂ in two probes located in or adjacent to the RTN and ROb in conscious adult male rats. Ventilation was measured in a whole body plethysmograph at 30°C. There were four groups (n = 5): 1) probes correctly placed in both RTN and ROb (RTN-ROb); 2) one probe correctly placed in RTN and one incorrectly placed in areas adjacent to ROb (RTN-peri-ROb); 3) one probe correctly placed in ROb and one probe incorrectly placed in areas adjacent to RTN (peri-RTN-ROb); and 4) neither probe correctly placed (peri-RTN-peri-ROb). Focal simultaneous acidification of RTN-ROb significantly increased ventilation (Vₑ) up to 22% compared with baseline, with significant increases in both breathing frequency and tidal volume. Focal acidification of RTN-peri-ROb increased Vₑ significantly by up to 15% compared with baseline. Focal acidification of ROb and peri-RTN had no significant effect. The simultaneous acidification of regions just outside the RTN and ROb actually decreased Vₑ by up to 11%. These results support a modulatory role for the ROb with respect to central chemoreception at the RTN.

control of breathing; serotonin; brain stem

HYPERCAPNIA ELICITS A NUMBER of compensatory responses in a wide variety of animal species, such as an increased ventilatory response and a reduced body temperature (3, 23, 24). Central chemoreceptors that detect changes in CO₂/pH and regulate breathing are located in the brain stem close to the surface of the ventral medulla (9, 10, 16, 20, 21, 34) and in other locations widely distributed within the brain stem (6, 9, 10, 18, 23, 24, 26, 33, 34, 36), including the serotonergic neurons of the medullary raphe (MR; raphe magnus, pallidus, and obscurus) (2, 8, 11, 12, 25, 29, 33, 34, 42) and glutamatergic neurons of the retrotrapezoid nucleus (RTN) that express the transcription factor Phox2b (1, 7, 10, 17, 18, 22, 26, 30, 35, 39).

Some studies have demonstrated the effects in vivo of focal tissue acidification into the MR. Bernard et al. (2) have shown that the microinjection of acetazolamide (carbonic anhydrase inhibitor) into the MR of anesthetized rats increases the amplitude of the phrenic nerve discharge. In addition, CO₂ microdialysis into MR increases ventilation in conscious rats (25) and goats (11). However, these studies have investigated pre-dominantly the rostral MR (magnus and pallidus), and no data exist about the effects of focal tissue acidification into the raphe obscurus (ROb) in vivo.

Recently, the ROb and RTN were studied simultaneously to investigate the relative importance of the RTN and MR in the central chemoreception (19). To this end, simultaneous inhibition was performed using microdialysis of muscimol (GABA_A receptor agonist) into the RTN and the serotonin (5-HT_1A) receptor agonist (R)-(+-8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT) into the ROb. Inhibition of RTN neurons with muscimol caused hyperventilation and reduced the ventilatory response to CO₂. Inhibition of caudal MR serotonergic neurons with 8-OH-DPAT had no significant effect on the CO₂ response, but simultaneous inhibition of the ROb and the RTN produced an enhanced hyperventilation and a greater reduction of the CO₂ response (19). These data suggest that the caudal MR does not by itself contribute to chemoreception directly but may modulate breathing by interaction with RTN.

The goal of the present study was to investigate the interaction between ROb and RTN by studying the responses to focal stimulation of these two sites in conscious rats during wakefulness by using reverse microdialysis of artificial cerebrospinal fluid (aCSF) equilibrated with 25% CO₂.

METHODS

General methods. All experimentation procedures and protocols were within the guidelines of the National Institutes of Health for animal use and care and were approved by the Dartmouth College Institutional Animal Use and Care Committee.

Surgery. Experiments were performed on adult male Sprague-Dawley rats weighing 280–350 g. Animals were submitted to general anesthesia by intramuscular administration of ketamine (100 mg/kg) and xylazine (20 mg/kg). The head and a portion of the abdomen were shaved, and the skin was sterilized with betadine solution and alcohol. Rats were fixed in a Kopf stereotaxic frame, and two dialysis guide cannulas (0.38-mm outer diameter) with a dummy were implanted into the medulla. One of them was placed into the caudal medullary raphe (ROb), and the other one was implanted into the RTN. The coordinates for probe placement into the ROb were 4.5 mm from the lambda, and 0.8–2.0 mm lateral from the lambda and 10.4–10.5 mm below the dorsal surface of the skull. The coordinates for RTN were 2.5 mm caudal and 1.8–2.0 mm lateral from the lambda and 10.6 mm below the dorsal surface (32). The guide cannula was secured with cranioplastic cement. Three electroencephalogram (EEG) electrodes were screwed into the right side of the skull, with the frontal electrode 2 mm anterior to bregma and 2 mm lateral to the midline, the parietal electrode 4 mm anterior to lambda and 2 mm lateral to the midline, and the ground electrode between the frontal and parietal electrodes. For the electromyogram...

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(EMG), a pair of wire electrodes was inserted deep into the neck muscle. The skull wound was sutured. The abdominal surface was shaved, the skin was sterilized, an incision was made through the linea alba, and a sterile telemetry temperature probe (TA-F20; Data Sciences, St. Paul, MN) was placed in the abdominal cavity. The incision was closed, and the animal was allowed to recover for 7 days.

**CO₂ dialysis solution.** The aCSF was equilibrated with 5 or 25% CO₂. The composition of the aCSF was (in mM) 152 sodium, 3.0 potassium, 2.1 magnesium, 2.2 calcium, 131 chloride, and 26 bicarbonate. The calcium was added after the aCSF was equilibrated with CO₂. The dialysis pump was run at a speed of 40 μl/min.

**Ventilation measurement.** The plethysmograph chamber used in these experiments is similar to the setup described by Jacky (14) and Pappenheimer (31). The volume of the plethysmograph was 7.6 liters, with a 3.5-liter top to protect the head pedestal. The analog output of the pressure transducer was converted to a digital signal and directly sampled at 150 Hz by a computer using the DataPac 2000 system. The animal chamber operates at atmospheric pressure, with the inflow and outflow of inspired gases balanced to prevent hyper- or hypobaric conditions in the box. The inflow gas was humidified, and the flow rate was controlled by a flowmeter (model 7491T; Matheson). The outflow port was connected to the in-house vacuum system via a flowmeter. A high-resistance “bleed” of the outflow line provided ~100 ml/min of outflow gas to the O₂ and CO₂ analyzers (Applied Electrochemistry). The flow rate through the plethysmograph was calibrated with 0.3-ml injections. Tidal volume (VT) (14, 24–26, 31) and breathing frequency (f) were calculated per breath to estimate ventilation (V̇E) per breath.

**Determination of body temperature.** The chamber temperature was measured by a thermometer inside the chamber. Rat body temperature was measured using the analog output via telemetry from the tempera-
ture probe. The rats were dialyzed with 5% CO₂ during equilibration aCSF began to exchange with the body. The dialysis pump was run at a speed of 40 μl/min.

**Anatomical analysis.** At the end of the experiment, the rats were killed by injection of barbiturate, and the brain stems were frozen and sectioned. Removal of the microdialysis probe tips for the RTN-ROb group (A), with both probe tips correctly placed, and for the RTN-peri-ROb group (B), with probe tips correctly placed in the RTN but incorrectly placed for the ROb. Figure 1A, left, shows schematically that all five probe tips were within the RTN region. The mean distance from bregma for these five probe tips was −10.90 ± 0.19 (SE) mm with a range of −10.52 to −11.5 mm. Figure 1A, right, shows schematically the locations of the probe tips in the ROb. The mean distance from bregma was −12.72 ± 0.30 mm with a range of −12.0 to −13.8 mm. Figure 1B, left, shows schematically that all five probe tips were within the RTN region. The mean distance from bregma for these five probe tips was −10.96 ± 0.20 mm with a range of −10.40 to −11.6 mm. Figure 1B, right (each panel is split to show 4 levels of probe tip locations), shows schematically the locations of the probe tips incorrectly placed outside of the ROb. The mean distance from bregma was −12.66 ± 0.37 mm with a range of −11.3 to −13.8 mm. These five probe tips were lateral to the midline and dorsal or ventral to the ROb.

**RESULTS**

**Anatomy.** There were 20 (of 49) successful experiments in which all data were obtained and both probe tips were located close to the areas of interest. Figure 1 summarizes the locations of the microdialysis probe tips for the RTN-ROb group (A), with both probe tips correctly placed, and for the RTN-peri-ROb group (B), with probe tips correctly placed in the RTN but incorrectly placed for the ROb. Figure 1A, left, shows schematically that all five probe tips were within the RTN region. The mean distance from bregma for these five probe tips was −10.90 ± 0.19 (SE) mm with a range of −10.52 to −11.5 mm. Figure 1A, right, shows schematically that all five probe tips were within the RTN region. The mean distance from bregma for these five probe tips was −10.96 ± 0.20 mm with a range of −10.40 to −11.6 mm. Figure 1B, right (each panel is split to show 4 levels of probe tip locations), shows schematically the locations of the probe tips incorrectly placed outside of the ROb. The mean distance from bregma was −12.66 ± 0.37 mm with a range of −11.3 to −13.8 mm. These five probe tips were lateral to the midline and dorsal or ventral to the ROb.
was $-11.27 \pm 0.14$ mm with a range of $-10.86$ to $-11.6$ mm. Figure 2B, right, shows schematically the locations of the probe tips outside of the ROb. Three were outside of the midline, and two were dorsal. The mean distance from bregma was $-13.5 \pm 0.1$ mm with a range of $-13.24$ to $-13.7$ mm.

Responses to dialysis of the RTN and ROb, simultaneously, with 25% CO$_2$. This group ($n = 5$) had an average body weight of $256 \pm 7$ g and a body temperature of $37.7 \pm 0.1^\circ$C. The average values during air breathing were $81 \pm 5$ min$^{-1}$ for $f$, $1.11 \pm 0.04$ ml/100 g for $V_t$, and $81 \pm 5$ ml·min$^{-1}$·100 g$^{-1}$ for $V_E$. Oxygen consumption was $2.0 \pm 0.17$ ml·min$^{-1}$·100 g$^{-1}$. Figure 3 shows the percent change relative to baseline of $V_E$ (A), $V_t$ (B), and $f$ (C) resulting from simultaneous 25% CO$_2$ dialysis in the RTN and ROb. $V_E$ was increased significantly ($P < 0.01$, repeated-measures ANOVA) with values greater than baseline at 5 and 10 min after onset of dialysis ($P < 0.05$, post hoc analysis).

Responses to dialysis of the RTN region with 25% CO$_2$. This group ($n = 5$) had an average body weight of $356 \pm 28$ g and body temperature of $37.6 \pm 0.1^\circ$C. The average values during air breathing were $71 \pm 4$ min$^{-1}$ for $f$, $0.89 \pm 0.06$ ml/100 g for $V_t$, and $59 \pm 4$ ml·min$^{-1}$·100 g$^{-1}$ for $V_E$. Oxygen consumption was $1.9 \pm 0.17$ ml·min$^{-1}$·100 g$^{-1}$. Figure 4 shows the percent change relative to baseline of $V_E$, $V_t$, and $f$ resulting from simultaneous 25% CO$_2$ dialysis in the RTN and peri-ROb. $V_E$ was increased significantly ($P < 0.02$, repeated-measures ANOVA) with values greater than baseline at 15 (11%) and 20 min (15%) after onset of dialysis ($P < 0.05$, post hoc analysis). There was no significant effect on $V_t$ or $f$.

Responses to dialysis of the ROb region with 25% CO$_2$. This group ($n = 5$) had an average body weight of $291 \pm 19$ g and body temperature of $37.7 \pm 0.1^\circ$C. The average values during air breathing were $71 \pm 5$ min$^{-1}$ for $f$, $1.21 \pm 0.09$ ml/100 g for $V_t$, and $83 \pm 5$ ml·min$^{-1}$·100 g$^{-1}$ for $V_E$. Oxygen consumption was $2.22 \pm 0.17$ ml·min$^{-1}$·100 g$^{-1}$. Figure 5 shows the percent change relative to baseline of $V_E$, $V_t$, and $f$. 

Fig. 1. Schematized anatomical cross sections show location of dialysis probe tips (solid ovals) for animals with both tips correctly located in the retrotrapezoid nucleus (RTN; A, left; $n = 5$) and raphe obscurus (ROb; A, right; $n = 5$) and for animals with the RTN probe tip correctly located (B, left; $n = 5$) and the ROb probe tip located incorrectly (peri-ROb; B, right; $n = 5$). The shaded areas delineate schematically the RTN (below the facial nucleus; left) and the ROb (midline; right). The numbers below the cross sections refer to millimeters caudal to bregma. Scale bar, 1 mm. VII, facial nucleus; Sol, nucleus of the solitary tract; Amb, nucleus ambiguous. The drawings are from the atlas of Paxinos and Watson (32). The actual RTN probe tip locations for A were, relative to bregma: $-10.48$, $-10.52$, $-10.70$, $-11.5$, for ROb: $-12.0$, $-12.3$, $-12.5$, $-13.2$, $-13.8$; and the actual RTN probe tip locations for B were, relative to bregma: $-10.40$, $-10.52$, $-11.00$, $-11.3$, $-11.6$; for ROb: $-11.3$, $-12.4$, $-12.8$, $-13.00$, $-13.8$. 

Fig. 2. Right, shows schematically the locations of the probe tips outside of the ROb. Three were outside of the midline, and two were dorsal. The mean distance from bregma was $-13.5 \pm 0.1$ mm with a range of $-13.24$ to $-13.7$ mm.

This group ($n = 5$) had an average body weight of $256 \pm 7$ g and a body temperature of $37.7 \pm 0.1^\circ$C. The average values during air breathing were $81 \pm 5$ min$^{-1}$ for $f$, $1.11 \pm 0.04$ ml/100 g for $V_t$, and $81 \pm 5$ ml·min$^{-1}$·100 g$^{-1}$ for $V_E$. Oxygen consumption was $2.0 \pm 0.17$ ml·min$^{-1}$·100 g$^{-1}$.

Figure 3 shows the percent change relative to baseline of $V_E$ (A), $V_t$ (B), and $f$ (C) resulting from simultaneous 25% CO$_2$ dialysis in the RTN and ROb. $V_E$ was increased significantly ($P < 0.01$, repeated-measures ANOVA) with values greater than baseline at 5 and 10 min after onset of dialysis ($P < 0.05$, post hoc analysis).

Responses to dialysis of the RTN region with 25% CO$_2$. This group ($n = 5$) had an average body weight of $356 \pm 28$ g and body temperature of $37.6 \pm 0.1^\circ$C. The average values during air breathing were $71 \pm 4$ min$^{-1}$ for $f$, $0.89 \pm 0.06$ ml/100 g for $V_t$, and $59 \pm 4$ ml·min$^{-1}$·100 g$^{-1}$ for $V_E$. Oxygen consumption was $1.9 \pm 0.17$ ml·min$^{-1}$·100 g$^{-1}$.

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Responses to dialysis of the ROb region with 25% CO$_2$. This group ($n = 5$) had an average body weight of $291 \pm 19$ g and body temperature of $37.7 \pm 0.1^\circ$C. The average values during air breathing were $71 \pm 5$ min$^{-1}$ for $f$, $1.21 \pm 0.09$ ml/100 g for $V_t$, and $83 \pm 5$ ml·min$^{-1}$·100 g$^{-1}$ for $V_E$. Oxygen consumption was $2.22 \pm 0.17$ ml·min$^{-1}$·100 g$^{-1}$.
resulting from simultaneous 25% CO₂ dialysis in the ROb and peri-RTN. There was no significant effect.

Responses to dialysis with 25% CO₂ with both probes incorrectly placed: peri-RTN-peri-ROb. This group (n = 5) had an average body weight of 269 ± 5 g and body temperature of 37.8 ± 0.1°C. The average values during air breathing were 83 ± 9 min⁻¹ for f, 1.09 ± 0.04 ml/100 g for VT, and 88 ± 7 ml·min⁻¹·100 g⁻¹ for V˙E. Oxygen consumption was 2.27 ± 0.17 ml·min⁻¹·100 g⁻¹. Figure 6 shows the percent change relative to baseline of V˙E, VT, and f resulting from simultaneous 25% CO₂ dialysis in the peri-RTN and peri-ROb. V˙E and VT were decreased significantly (P < 0.01, repeated-measures ANOVA). There was no significant effect on f.

DISCUSSION

Experimental design. In this study we used a chronic, unanesthetized rat model with two preimplanted guide tubes that allowed insertion of dialysis cannulas for subsequent reverse microdialysis in the RTN and ROb using aCSF equilibrated with 25%. The microdialysis cannula (probe) tip has dimensions of 1 mm in length and 0.24 mm in diameter. We perfused the dialysis probe at 40 μl/min, a flow rate necessary to produce the focal acidification as the CO₂ that enters the tissue is rapidly cleared by diffusion and blood flow (17). An earlier study evaluated the focal stimulus intensity caused by CO₂ dialysis, with a pH electrode adjacent to the dialysis probe located into the RTN. The pH change was relatively small, approximately equivalent to that observed with a 6.6-Torr increase in arterial PCO₂ (17).

Focal acidification has been used in individual sites such as the RTN and the medullary raphe to study the role of each in central chemoreception (11, 17, 18, 25, 26). Acidification of the RTN in its rostral portion (18) and the medullary raphe in its rostral portion (raphe magnus) (11, 25) in vivo stimulates respiratory output, indicating that both sites participate in the CO₂ response and that each site contains chemosensitivity. It is difficult to determine the functional importance of chemoreceptor sites by using this approach, since in the intact conscious animal, the control system is functional and responsive such that focal stimulation increases ventilation and the resultant hypocapnia inhibits other chemoreceptor sites, including the carotid body, and minimizes the overall increase in V˙E (17).
The time course when focal acidification does produce increased \( \dot{V} \dot{E} \) commonly is like that shown in Fig. 5 with an initial increase, followed by a decrease to a level that is still above baseline or control. This time course could be due to an initial excitation of the chemoreceptors at the site of focal acidification, followed by a slower inhibition as other chemoreceptor sites respond to the hypocapnia.

We limited each rat to a single dialysis test with both probes perfused with aCSF equilibrated with 25% CO\(_2\) to minimize the tissue disruption or damage caused by the insertion of the dialysis probe. Because of this constraint, the relatively low success rate of placing both probe tips in the desired locations (5 of 20 rats that successfully completed the entire protocol had both correctly placed), and the unexpected observation of an inhibition of \( \dot{V} \dot{E} \) if both probes were placed just outside the regions of interest (Fig. 2B) as well as the incorrectly placed probes in ROb (Fig. 1B) and RTN (Fig. 2A) were clearly not within the two regions of interest but were within 1–2 mm of them.

**Inhibition of the CO\(_2\) response with both probes incorrectly placed.** An unexpected result was that with acidification of two sites with probes placed just outside the regions of interest, we observed a decrease in \( \dot{V} \dot{E} \) of up to 11%, on average, compared

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**Probe placement.** We studied four groups of rats based on post hoc identification of probe tip location: RTN-ROb, with both probes correctly placed; RTN-peri-ROb, with the RTN probe correctly placed and the ROb probe placed outside of the ROb; peri-RTN-ROb, with the RTN probe placed outside of the RTN and the ROb probe correctly placed; and peri-RTN-peri-ROb, with both probes placed incorrectly. For both groups with correct RTN probe placement, the locations in RTN were almost identical (Fig. 1). For both groups with correct probe placement in ROb, the placements in ROb were almost identical (Figs. 1A and 2A). The probes of the group in which were probes were placed outside the regions of interest (Fig. 2B) as well as the incorrectly placed probes in ROb (Fig. 1B) and RTN (Fig. 2A) were clearly not within the two regions of interest but were within 1–2 mm of them.

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We limited each rat to a single dialysis test with both probes perfused with aCSF equilibrated with 25% CO\(_2\) to minimize the tissue disruption or damage caused by the insertion of the dialysis probe. Because of this constraint, the relatively low success rate of placing both probe tips in the desired locations (5 of 20 rats that successfully completed the entire protocol had both correctly placed), and the unexpected observation of an inhibition of \( \dot{V} \dot{E} \) if both probes were incorrectly placed, we decided to use only aCSF equilibrated with 25% CO\(_2\) in both probes in all experiments. We found that simultaneous acidification in two probes interfered with the normal sleep cycling that we observe during this type of experiment, so we have reported only data obtained in wakefulness.

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**Inhibition of the CO\(_2\) response with both probes incorrectly placed.** An unexpected result was that with acidification of two sites with probes placed just outside the regions of interest, we observed a decrease in \( \dot{V} \dot{E} \) of up to 11%, on average, compared
with the initial baseline value. We are uncertain of the source of this inhibition of $V_\text{E}$, but its presence supports the specificity of the stimulatory responses observed with microdialysis of aCSF equilibrated with 25% CO$_2$ in probes that were correctly placed within the confines of putative central chemoreceptor locations.

*High-CO$_2$ dialysis in ROb did not stimulate breathing*. Prior work that used this approach (CO$_2$ microdialysis) in the MR focused on the more rostral part of MR and did show stimulation of $V_\text{E}$ (25). In the present study, with the focal acidification produced more caudally in ROb, there was no stimulation of $V_\text{E}$ relative to baseline. We conclude that there is a heterogeneity of chemoreceptor function within the medullary raphe. Studies of the effects of focal inhibition or lesions within the medullary raphe on the CO$_2$ response support this concept. Lesions of the raphe magnus have large inhibitory effects on the CO$_2$ response (8). Inhibition of raphe 5-HT neurons by dialysis of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT rostrally inhibits the CO$_2$ response (41), whereas more caudally there is no inhibition (19, 38). Focal stimulation of the

**Fig. 5. **Ventilatory response to acidification of the ROb with simultaneous acidification at sites lying outside the RTN, expressed as a percentage of baseline. There were no significant effects.

**Fig. 6. **Ventilatory response to simultaneous acidification at sites lying outside the RTN and the ROb, expressed as a percentage of baseline. Asterisk at far right indicates a significant ($P < 0.01$) increase as determined by repeated-measures ANOVA on the absolute values for $V_\text{E}$ and $V_\text{T}$. There was no significant effect on $f$.  

*High-CO$_2$ dialysis in RTN did stimulate breathing*. The RTN probe location provides access to the neurons within the RTN that lie near the surface in the rostral ventrolateral medulla, just beneath the facial nucleus and extending from the rostral part of the facial nucleus to a few hundred micrometers caudally (7, 10, 22, 35). Many studies have demonstrated the importance of the RTN in the control of breathing. In anesthetized animals, focal inhibition or lesions of the RTN causes apnea and severe reductions in CO$_2$ sensitivity (1, 23, 30, 40). In conscious animals, lesions cause decreased breathing and CO$_2$ sensitivity (1, 24), whereas focal acidification stimulates breathing (18). One type of RTN chemoreceptor neuron has been identified as expressing the transcription factor Phox2b as well as being glutamatergic (39).
In prior work, focal 25% CO₂ dialysis in the RTN increased \( V_{\text{E}} \) by 24% of baseline during wakefulness (18). This response was bigger than that obtained in the present study, a 15% increase in \( V_{\text{E}} \) compared with baseline. Three factors may account for this difference. First, the microdialysis probes were located in the more rostral region of RTN in the animals of the previous study compared with the location of the probes in the present study. Second, the present experiments were performed at an ambient temperature within the thermoneutral zone of the rats, whereas the earlier study used room temperature, which is below the normal rat thermoneutral zone. The ambient temperature can affect the CO₂ response (41). Third, the excitatory response of focal RTN acidification could have been attenuated by any inhibition arising from the simultaneous acidification in the peri-ROb region.

**Simultaneous high-CO₂ dialysis in RTN and ROb did produce an enhanced breathing response.** Focal acidosis at both the RTN and ROb increased \( V_{\text{E}} \) by 22% compared with baseline, an effect that included increases in both \( V_T \) and \( f \). This was greater than observed with focal acidification of either RTN or ROb alone. There are anatomical data that support the interaction between RTN and ROb. Retrograde tracing with neurobiotin injected into the RTN has shown that neurons of ROb were labeled, indicating that RTN has afferent input from ROb (7, 35). Recent data show that the Phox2b-positive putative chemoreceptor neurons in the RTN possess 5-HT receptors and can be excited by local 5-HT application (21). Thus connectivity and receptor presence indicate that ROb 5-HT neurons can affect RTN neurons.

In a previous study, inhibition of RTN neurons with muscimol, with simultaneous dialysis of aCSF (control) in or near the caudal MR (ROb), caused hypoventilation and reduced the \( V_{\text{E}} \) response to 7% CO₂ by 24% (19). Inhibition of caudal MR (ROb) serotonergic neurons with the 5-HT1A receptor agonist 8-OH-DPAT, with simultaneous dialysis of aCSF in or near the RTN, caused hypoventilation but had no significant effect on the CO₂ response. Inhibition of both the RTN and the caudal MR (ROb) simultaneously produced enhanced hypoventilation and a substantial 51% decrease in the CO₂ response (19). These results suggest a modulatory role of caudal medullary raphe, and our present results corroborate this hypothesis.

We conclude that simultaneous focal acidification of both RTN and ROb potentiates the effects of focal acidification of either RTN or ROb alone. This conclusion is apparent despite the unexpected inhibition of the CO₂ response observed if both probes were incorrectly located just outside the regions of interest. The mechanism for the potentiation of the response is not known. It possibly occurs as a result of 5-HT-mediated excitation of RTN Phox2b-chemosensitive neurons. As shown by the in vitro data of Mulkey et al. (21), increased 5-HT input to these RTN neurons could enhance their CO₂-stimulated output. Such a mechanism could help to explain our results. Corelease of substance P by these 5-HT neurons could also stimulate RTN chemoreceptor neurons as well as other respiratory neurons that possess neurokinin-1 receptors (NK1Rs). Lesions of NK1R-immunoreactive cells in the ventral medulla substantially decreased the CO₂ response, indicating their importance in this function (27). Lesions of both NK1R-expressing neurons and processes and 5-HT neurons together in the medullary raphe inhibit the systemic CO₂ response to a greater extent than either lesion alone, also suggesting an interaction of 5-HT, substance P, and NK1Rs in central chemoreception (29).

In addition, 5-HT neurons in ROb could directly affect respiratory neurons in the pre-Bötzinger complex and the ventral respiratory group via 5-HT and or substance P.

**GRANTS**

This work was supported by National Heart, Lung, and Blood Institute Grant R37 HL-28066 (to E. Nattie). M. B. Dias was supported by a grant from the Fundação de Amparo a Pesquisa do Estado de São Paulo.

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