Effects on breathing of focal acidosis at multiple medullary raphe sites in awake goats

M. R. Hodges,¹ P. Martino,¹ S. Davis,¹ C. Opansky,¹ L. G. Pan,² and H. V. Forster¹,³

¹Department of Physiology, Medical College of Wisconsin, ²Department of Physical Therapy, Marquette University, and ³Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin 53226

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Hodges, M. R., P. Martino, S. Davis, C. Opansky, L. G. Pan, and H. V. Forster. Effects on breathing of focal acidosis at multiple medullary raphe sites in awake goats. J Appl Physiol 97: 2303–2309, 2004.—To gain insight into why there are chemoreceptors at widespread sites in the brain, microtubules were chronically implanted at two or three sites in the medullary raphe nuclei of adult goats (n = 7). After >2 wk, microdialysis (MD) probes were inserted into the microtubules to create focal acidosis (FA) in the awake state using mock cerebral spinal fluid (mCSF) equilibrated with 6.4% (pH = 7.3), 50% (pH = 6.5), or 80% CO₂ (pH = 6.3), where MD with 50 and 80% CO₂ reduces tissue pH by 0.1 and 0.18 pH unit, respectively. There were no changes in all measured variables with MD with 6.4% at single or multiple raphe sites (P > 0.05). During FA at single raphe sites, only 80% CO₂ elicited physiological changes as inspiratory flow was 16.9% above (P < 0.05) control. However, FA with 50 and 80% CO₂ at multiple sites increased (P < 0.05) inspiratory flow by 18.4 and 30.1%, respectively, where 80% CO₂ also increased (P < 0.05) tidal volume, heart rate, CO₂ production, and O₂ consumption. FA with 80% CO₂ at multiple raphe sites also led to hyperventilation (∼2 mmHg), indicating that FA had effects on breathing independent of an increased metabolic rate. We believe these findings suggest that the large ventilatory response to a global respiratory acidosis reflects the cumulative effect of stimulation at widespread chemoreceptor sites rather than a large stimulation at a single site. Additionally, focal acidification of raphe chemoreceptors appears to activate an established thermogenic response needed to offset the increased heat loss associated with the CO₂ hyperpnea.

central chemoreception; control of breathing

There is a great deal of evidence of CO₂/H⁺ chemoreceptors at widespread sites in the brain, including the nucleus of the solitary tract (NTS), retrotrapezoid nucleus and parapyramid al region (RTN/Ppy), medullary raphe nuclei (MRN), locus coeruleus, fastigial nucleus of the cerebellum, and pre-Bötzinger complex (2, 5, 11, 15–16, 18, 22–25, 27, 30–31, 34). Studies in reduced preparations provide insight into the cellular aspects of chemosensitivity, but other studies are necessary to confirm chemoreceptor function in the intact animal under physiological conditions.

One technique utilized to study chemoreceptor function in the intact, unanesthetized animal is creation of focal acidosis (FA) by microdialysis (MD) in putative chemoreceptor regions of mock cerebral spinal fluid (mCSF) equilibrated with high levels of CO₂ (11, 15, 16, 24–25). Indeed, Nattie et al. (16, 24–25) reported that FA in the NTS, RTN, or MRN in rats increase minute ventilation in a state-independent (NTS) or state-dependent (RTN, MRN) manner. In addition, our laboratory recently reported that FA in the MRN of goats also increases ventilation during wakefulness but found no effect of FA on breathing during sleep (11). However, in both rats and goats, the resulting increases in breathing ranged from 8 to 28% above control ventilation, which is small compared with a 250% increase during an equivalent systemic (brain) acidosis resulting from inhalation of 7% CO₂. The relatively small increases in breathing during FA may result because no single chemoreceptor site can account for the overall CO₂ sensitivity, but rather activation of multiple sites is required (23). To our knowledge, there are no published data on the ventilatory effects of FA at multiple brain sites during physiological conditions. Therefore, the major aim of this study was to test the hypothesis that FA at multiple chemoreceptor sites will have a greater effect on breathing than FA at one chemoreceptor site. Obtaining these data may provide insight into the question of why there are chemoreceptors at multiple sites in the brain.

Methods

Data were obtained on adult goats (6 female and 1 male) weighing 50.6 ± 5.4 kg. The goats were housed and studied in an environmental chamber with a fixed ambient temperature and photoperiod. All goats were allowed free access to hay and water, except for periods of study. The goats were trained to stand comfortably in a stanchion during periods of study. All aspects of the study were reviewed and approved by the Medical College of Wisconsin Animal Care Committee before the studies were initiated.

Surgical Procedures

Instrumentation surgery. An initial surgery was performed to elevate a 5-cm segment of the carotid arteries. In this and subsequent surgeries, the goats were anesthetized initially with a combination of ketamine and xylazine, intubated, and mechanically ventilated. Throughout surgery, anesthesia was maintained with 1–1.5% halothane in O₂. Under sterile conditions, the carotid arteries were isolated from the vagni, they were elevated superficial to the muscle, and the skin was sutured. After surgery, the goats received cefitfur sodium (2 mg/kg) daily as an antibiotic for 1 wk.

Microtubule implantation surgery. After ≥3 wk, a second surgery was performed to chronically implant two or three microtubules (MTs) into the medullary raphe (n = 7). An occipital craniotomy was created, and dura mater was excised to expose the posterior cerebel lum and dorsal aspect of the medulla for visualization of the obex. The dorsal surfaces of the medulla, the obex, and the midline were all used as reference points for stereotaxic coordinates in the dorsoventral, rostrocaudal, and mediolateral planes. The implantation sites were

Address for reprint requests and other correspondence: M. R. Hodges, 706 LCI, Dept. of Neurology, Yale Univ., 333 Cedar St., New Haven, CT 06520 (E-mail: hodges@yale.edu).

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along the caudal (raphe obscurus) and rostral (raphe pallidus) midline. The MTs used were 18-gauge stainless steel tubes 70 mm in length. After placement, the MTs were secured with screws in the bone and dental acrylic. Arterial blood pressure (BP) and rectal temperature were continuously monitored throughout the duration of surgery. Laboratory personnel monitored the goats continuously for a minimum of 24 h after the MT implantation surgery. Most goats were unable to maintain normal sternal recumbent posture and/or stand for 3–6 h postsurgery. Two goats were unable to stand unassisted for ≤3 days after the implant. However, after full recovery, all animals were studied. Food and water intake were monitored closely in all goats daily after the implantation surgery. Brain edema was minimized with dexamethasone injections (0.4 mg·kg⁻¹·day⁻¹ iv for 2 days, then decreasing by 0.05 mg·kg⁻¹·day⁻¹) three times a day for 1 wk. Infection was minimized with chloramphenicol injections (20 mg/kg iv) for 3 days and with daily injections thereafter of cefitufur sodium (2 mg/kg) and gentamycin (3 mg/kg). Buprenorphine was administered 3–12 h after implantation to minimize pain.

Physiological Measurements

For all studies, a fitted mask was taped firmly to the snout, and a two-way breathing valve was attached to the mask to measure inspiratory flow (VI) with a pneumotachograph and for collection of expired air analyzed for O₂ and CO₂ concentrations required for O₂ uptake (V̇O₂) and CO₂ production (V̇CO₂) calculations. A chronically placed catheter in the elevated carotid artery was used to measure arterial BP and heart rate (HR) and for arterial blood sampling to obtain pH, arteriole PO₂, and arteriole PCO₂ (PacO₂) values (model 278, Ciba-Corning). Rectal temperature of the animal was measured at regular intervals.

Whole body ventilatory CO₂ sensitivity was assessed on multiple days before and on days of FA studies. Room air (RA) breathing, BP, and HR were measured for 30 min before exposure to three levels of elevated inspired CO₂ (2.5, 5.0, and 7.5% CO₂ in RA). Arterial blood samples were drawn during the control period and during the fourth and fifth minute of each CO₂ exposure level. The changes in expired ventilation (Ve) and PacO₂ from RA breathing to all levels of CO₂ were used to determine the slope of the relationship between VI and PacO₂, and they were used as an index of CO₂ sensitivity.

FA studies. Studies began at least 2 wk after implantation (30). Throughout the protocol, all goats were in good health, with stable baseline breathing and PacO₂ values. During the first experiments performed in some animals, shivering occurred. These data were excluded from the final analysis of all physiological data.

The CMA (CMA Microdialysis, Solna, Sweden) 12 MD probes (20-kDa molecular mass cutoff) had a 70-μm shaft length, a 2-mm membrane length, and a 0.5-mm membrane diameter. Identical MD probes were used in our laboratory’s initial sets of experiments, when we established that MD with 50 and 80% CO₂ generated an extracellular fluid acidosis slightly greater than, or three times greater than, that observed with 7.5% inspired CO₂ in room air (11). The contents of the dialysate (mCSP) have been previously described (11).

VI, HR, BP, V̇O₂, and V̇CO₂ were measured continuously or at regular intervals during a 15-min control period, during 45 min of microdialysis, and for 15 min after termination of the dialysis flow. Arterial blood was drawn during the final 5-min period of the control, microdialysis, and recovery periods. Three different dialysate pH and Pco₂ conditions were tested: J) 6.4% CO₂ (pH = 7.31–7.36, Pco₂ = 41–47 Torr), 2) 50% CO₂ (pH = 6.5–6.6, Pco₂ > 250 Torr), and J) 80% CO₂ (pH = 6.3–6.4, Pco₂ > 250 Torr). These studies were performed in individual and multiple MTs with a flow rate (50 μl/min) identical to that previously described (11).

Histological studies. After completion of these protocols, the animals were euthanized (Beuthanasia), and the brain was perfused with PBS solution (pH = 7.35–7.4) and 4% paraformaldehyde fixative in PBS. The medulla was then removed, postfixed in 4% paraformalde-
FOCAL ACIDOSIS AT MULTIPLE SITES IN THE MEDULLARY RAPHE

Table 1. Resting breathing variables and CO2 sensitivity

<table>
<thead>
<tr>
<th>Goat</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vt, ml/min</td>
<td>8.3±0.3</td>
<td>8.2±0.7</td>
<td>10.7±0.9</td>
<td>8.5±1.0</td>
<td>6.1±0.1</td>
<td>6.6±0.5</td>
<td>7.5±0.6</td>
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<td>f, breaths/min</td>
<td>21.3±0.5</td>
<td>19.9±1.2</td>
<td>21.7±1.8</td>
<td>19.6±0.4</td>
<td>21.2±0.9</td>
<td>18.1±1.7</td>
<td>15.0±0.7</td>
<td>19.5±0.9</td>
</tr>
<tr>
<td>Vt, ml/breath</td>
<td>350±28</td>
<td>408±20</td>
<td>477±10</td>
<td>427±41</td>
<td>286±14</td>
<td>368±30</td>
<td>481±19</td>
<td>400±27</td>
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<tr>
<td>Vt, ml/breath</td>
<td>149±14</td>
<td>141±10</td>
<td>184±23</td>
<td>187±13</td>
<td>96±5</td>
<td>116±12</td>
<td>178±13</td>
<td>150±13</td>
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<tr>
<td>VCO2, ml/min</td>
<td>289±7</td>
<td>248±16</td>
<td>387±37</td>
<td>297±30</td>
<td>204±4</td>
<td>232±12</td>
<td>262±10</td>
<td>274±22</td>
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<tr>
<td>VCO2, ml/min</td>
<td>242.1±1</td>
<td>215±7</td>
<td>307±31</td>
<td>206±28</td>
<td>160±7</td>
<td>170±11</td>
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<td>RQ</td>
<td>0.83±0.02</td>
<td>0.88±0.04</td>
<td>0.78±0.02</td>
<td>0.79±0.02</td>
<td>0.8±0.01</td>
<td>0.73±0.01</td>
<td>0.88±0.02</td>
<td>0.8±0.03</td>
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<tr>
<td>PacO2, Torr</td>
<td>41.2±1.1</td>
<td>36.7±1.8</td>
<td>41.9±1.1</td>
<td>37.9±1.0</td>
<td>35.4±1.9</td>
<td>34.1±0.8</td>
<td>40.0±0.9</td>
<td>38.1±1.1</td>
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<tr>
<td>PacO2, Torr</td>
<td>96.0±2.4</td>
<td>108.5±2.9</td>
<td>99.1±3.0</td>
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<td>101.7±2.5</td>
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<td>105.4±1.5</td>
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<td>7.470±0.008</td>
<td>7.443±0.013</td>
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<td>7.481±0.006</td>
<td>7.451±0.003</td>
<td>7.473±0.012</td>
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<td>CO2 slope</td>
<td>1.5±0.4</td>
<td>2.0±0.3</td>
<td>1.5±0.6</td>
<td>2.4±0.4</td>
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<td>2.5±0.1</td>
<td>1.6±0.2</td>
<td>2.0±0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE for individual goats (goats A–G) and for the goats as a group at the time of focal acidosis studies. Vt, resting expired ventilation; f, breathing frequency; Vt, tidal volume; Vd, dead space volume; VCO2, O2 consumption; VCO2, CO2 production; RQ, respiratory quotient; PaCO2, arterial PCO2; PaO2, arterial PO2; CO2 slope, sensitivity. Note that all values for individual goats are within the normal range after implantation of microtubules (33) and on days before and during focal acidosis studies.

Resting Breathing

Ventilatory variables while breathing RA and whole body CO2 sensitivity were stable and within the normal physiological range for goats over 4–5 days before and during the days of FA studies (Table 1). Both BP (8.4 ± 3.3%) and HR (16.9 ± 6.0%) tended to increase with increasing inspired CO2, but these changes were not significantly different from control (P ≥ 0.083; n = 5).

Physiological Responses to FA at Single Raphe Sites

Microdialysis of mCSF equilibrated with 6.4% CO2 (control) or 50% CO2 had no effect on all calculated variables during the dialysis or recovery period (P ≥ 0.101). However, FA with 80% CO2 at a single caudal or rostral raphe site increased Vt and VT (P ≤ 0.006), but it had no effects on all other variables during the dialysis or recovery periods (P ≥ 0.209). Two-way ANOVA analyses comparing 6.4 and 80% CO2 dialysis effects indicated significant increases in Vt, Vt, and breathing frequency during FA at single raphe sites (P < 0.05; Fig. 2).

FA at Multiple Raphe Sites

Microdialysis with 6.4% CO2 had no effect on all measured variables (P > 0.153). Vt increased (P = 0.015) with FA at multiple raphe sites with 50% CO2, with no effects on all other measured variables (P > 0.05). Microdialysis with 80% CO2 increased Vt, VT, VT/Ti, VdO2, VdCO2, and HR (P < 0.05) and it led to a small but significant hyperventilation (~1.6 Torr; P < 0.005) with no effect on breathing frequency, RQ, Vd, Vd/Vt, or BP (P > 0.05). CO2 dialysis at multiple raphe sites with both 50 and 80% CO2 increased Vt compared with 6.4% CO2, where the effect of 80% CO2 was greater than that of 50% CO2 at two time points (P > 0.05; Fig. 3). Dialysis with 80% CO2 at multiple sites had greater effects on Vt and VdO2 (P < 0.05) than dialysis with 80% CO2 at single raphe sites, but VT and HR were not different (P > 0.05; Fig. 4).

DISCUSSION

The major findings of these studies were that FA at two or three medullary raphe sites has greater physiological effects than the equivalent FA at one site and that focal acidosis alters physiological functions other than breathing.

Limitations of Study

In our model, the capability to generate FA in the medullary raphe under physiological conditions requires chronic implantation of stainless steel MTs. Previously, implantation of two MTs into other respiratory-related nuclei was accomplished with minimal surgical and/or recovery complications, largely due to avoiding passing the microtubules through the cerebellum. Caudal raphe regions lie caudal to the posterior aspect of the cerebellum, and therefore they can be reached without damaging the cerebellum. However, to reach more rostral raphe sites required passing MTs through the medial cerebellum. In initial goats, we only implanted one MT in each of the caudal and more rostral raphe, and the goats recovered well; thus, in subsequent goats, we implanted two MTs caudally and one more rostrally, or two rostrally. The latter goats in particular had a longer than normal recovery period because it took 4 days before they were able to stand unassisted. Therefore, we limited the number of raphe sites tested in a given animal because of the limitations of recovery from the surgical approach.

Additionally, the interpretations of these data are in the context of one nucleus: the medullary raphe. We did not test the effects of focal acidification at more than one chemosensitive nucleus but rather the effects of multiple acid foci within one chemoreceptive nucleus. That is to say that these data do not specifically address multiple chemoreceptor nuclei function per se, nor the previously noted state-dependent function of the raphe nucleus.

In previous investigations, pHi measurements were made within ~200 μm of the site of FA to determine the degree of acidification created with various CO2/H+ levels (11). In the present study, we did not measure pHi changes created with FA, and therefore we cannot directly confirm that we have indeed generated a greater degree of acidosis with FA at multiple sites or whether there were regional differences in the degree of acidification. However, there are lines of evidence that address these issues. First, we previously found that the pHi change at the MD site was greater when two MD probes (CMA 11) were
simultaneously used in parallel compared with the pH change using one probe (unpublished observations). Second, local pH regulatory mechanisms do not appear to differ greatly between medullary regions because we found no differences in the change in the extracellular fluid pH with increasing inspired CO₂ fraction (0.07) when measurements were made in the caudal or rostral raphe or in the retrotrapezoid, facial, or gigantocellularis reticularis nuclei (unpublished observations). These findings plus the finding that breathing and metabolic rate increase more with multiple vs. single sites of microdialysis lead us to conclude that indeed a FA was created at multiple sites.

A final consideration is the potential trauma or tissue scarring that could conceivably occur with multiple insertions of the MD probe. Indeed, the probe is repeatedly passed into the medulla 2 mm beyond the ventral-most aspect of the MTs to test the effects of single vs. multiple sites. Thus the probes are inserted a minimum of two times at a given site, potentially

Fig. 2. Ventilatory response to focal acidosis with 6.4 and 80% CO₂ at single midline raphe sites. Values are means ± SE expressed as a percentage of control for 6 goats. Inspiratory flow (V̇i), tidal volume (Vₜ), and breathing frequency (f) during control, dialysis (dashed line), and recovery periods are shown. Dialysis of 80% (●) but not 6.4% (○) CO₂ increased V̇i and Vₜ (P < 0.05, 1-way repeated-measures ANOVA). Significant effects of treatment (6.4 vs. 80% CO₂) by 2-way ANOVA were detected for V̇i, Vₜ, and f, where pairwise comparison differences are noted by asterisks (P < 0.05).

Fig. 3. Ventilatory response to focal acidosis with 6.4, 50, and 80% CO₂ at multiple midline raphe sites. Values are means ± SE expressed as a percentage of control. V̇i during control, dialysis (dashed line), and recovery periods is shown. Dialysis of 50% CO₂ (5 goats) and 80% CO₂ (6 goats) but not 6.4% CO₂ (n = 6 goats) increased V̇i (P < 0.05, 1-way repeated-measures ANOVA). Significant effects for treatment by 2-way ANOVA were detected between all conditions, where differences between 50 and 80% CO₂ are noted by asterisks (P < 0.05).
creating a physical barrier for diffusion by scar tissue generation with multiple insults. Although scarring may be a factor, we believe that this is unlikely because of the dramatic increase in $V_t$ ($+163\%$) observed after ibotenic acid injection performed after completion of these studies that indicates minimal or no diffusion barrier (unpublished observations).

Physiological Effects of FA at Single and Multiple Raphe Sites

FA with microdialysis of high CO$_2$ has previously been utilized to test for intracranial chemoreceptor function (11, 16, 24–25). The changes in breathing observed from FA in regions such as the caudal and rostral NTS, RTN/Ppy, and caudal midline raphe range from 15 to 28\% in rats and from 8 to 13\% in goats. The changes in breathing observed with FA at single raphe sites in our laboratory’s previous report (11) and in the present study were similar in magnitude and time course. In the present study, FA with 80\% CO$_2$ at single rostral raphe sites transiently increased $V_t$ (16.3 ± 4.9\%), $V_t$ (11.1 ± 3.8\%), and breathing frequency (5.5 ± 1.5\%), similar to our laboratory’s previous report where dialysis with 80\% CO$_2$ increased $V_t$ (12.0 ± 2.0\%), $V_t$ (13.3 ± 1.9\%), and breathing frequency (5.9 ± 1.6\%). Additionally, we detected no differences in response to FA in caudal vs. more rostral raphe regions in both studies despite a greater number of total, neurokinin-1 receptor-expressing, and serotonergic (chemosensitive) neurons in the rostral midline raphe (12).

FA at multiple raphe sites had a greater effect than FA at single raphe sites with an equivalent CO$_2$ level, where dialysis of 80\% CO$_2$ had a greater effect on average than did 50\% CO$_2$. 

![Graph showing physiological effects of focal acidosis with 80\% CO$_2$ at single or multiple raphe sites. Values are means ± SE expressed as a percentage of control. $V_t$, $V_t$, metabolic rate [O$_2$ consumption (V_{O2})], and heart rate (HR) during control, dialysis (dashed line), and recovery periods are shown. Focal acidosis with 80\% CO$_2$ at single (6 goats) and multiple (6 goats) sites increased $V_t$ and $V_t$ ($P < 0.05$, 1-way repeated-measures ANOVA with Bonferroni post hoc). Focal acidosis with 80\% CO$_2$ at multiple but not single sites increased $V_{O2}$ and HR ($P < 0.05$, 1-way repeated-measures ANOVA with Bonferroni post hoc). Significant differences from a 2-way ANOVA comparison of focal acidosis with 80\% CO$_2$ at single and multiple sites are noted by asterisks ($P < 0.05$).]
Breathing maximally increased 30% from control, which represents a fraction of the overall ventilatory response to 7.5% inspired CO₂ (~250%). Increases in ventilation with FA in the medullary raphe correlate well with the idea of a "dose response" to acidification, by increasing the CO₂/H⁺ content or number of acidic foci (Fig. 5). In light of the evidence for widespread central chemoreceptor sites, it therefore seems likely that the ventilatory response to inspired CO₂ reflects a cumulative effect of stimulation of multiple chemoreceptor sites. This postulate does not preclude the possibility that certain chemoreceptor sites may be more or less sensitive to pH or CO₂ or that they may function in a state-dependent manner (21–23). Additionally, the functional contribution of each chemoreceptor site may differ in terms of VT and/or breathing frequency. Breathing effects from FA with microdialysis of high CO₂ in the RTN were due solely to increases in VT (rats), whereas FA in the raphe (rats and goats), NTS (rats), or pre-Bötzinger complex (cats) had both VT and breathing frequency effects (11, 16, 24, 25, 30). Finally, it is likely that there are differences in the capabilities of central chemoreceptor sites to locally regulate pH as indicated by the findings of heterogeneous distribution and pH regulation of glia in the RTN and NTS in neonatal rat brain slices (8). In other words, the role of breathing in correction for a FA may not be the same at all sites in the brain.

In addition to VT, VT, and breathing frequency effects, we noted significant increases in metabolic rate, BP, and HR with dialysis of high CO₂ in the raphe obscurus and pallidus in these and previous investigations (11). The increase in metabolic rate with FA at multiple raphe sites likely contributes to the ventilatory response. However, coincident with the substantial increased metabolic rate was a small but significant 2-Torr decrease in PaCO₂, indicating that there is an effect on ventilation independent of the increased metabolic rate. This finding is consistent with FA in the RTN of awake rats, which increased Ve 24% and led to a 5-Torr reduction in PaCO₂ (15). Additionally, the resulting systemic hypoxemia may also inhibit other chemoreceptors (both central and peripheral), which may contribute to the relatively small increases in breathing observed with FA.

The increased metabolic rate and the cardiovascular changes with FA at multiple raphe sites likely reflect the postulated influence of the medullary raphe on sympathetic drive, affecting thermogenesis [brown adipose tissue (BAT) metabolism], peripheral vascular tone (3), BP, and HR. Retrograde tracer injections into interscapular BAT indicate that, among others, the raphe pallidus may play a role in premotor regulation of sympathetic nerve activity (1, 4). Activation (via disinhibition) of raphe pallidus neurons with bicuculline or intravenous leptin administration both increased BAT sympathetic nerve activity, BAT temperature, expired CO₂ (19, 20), as well as HR and BP (19). Microinjection of the 5-HT type 1A receptor agonist 8-hydroxy-2(di-n-propylamino)tetralin into the raphe pallidus eliminated the leptin-evoked response, further implicating a role for the raphe in thermogenesis (19).

Although some previous reports have shown that the increase in VO₂ observed during CO₂ inhalation is due to the increased O₂ cost (work) of breathing (9, 17, 29, 32), others have shown VO₂ increased to a greater degree than predicted by O₂ cost of breathing alone with increasing inspiratory PCO₂ (14). Additionally, Pappenheimer (28) found that the hypercapnia-induced hyperpnea increased VO₂ by 31%, whereas a similar hypoxia-induced hyperpnea decreased VO₂ by 18% in rats. Similarly, in ponies and oxen, VO₂/V̇E was greater during a CO₂-induced hyperpnea compared with a thermal-induced hyperpnea (10, 13). In addition, in ponies, the increased VO₂ during CO₂ inhalation was accentuated or attenuated during exposures to cold or hot environmental temperatures, respectively (13). These data led to the conclusion that the increase in VO₂ during hypercapnia represents in part a response to offset the respiratory heat loss, thereby contributing to the maintenance of homeoothermy.

The observation that a large fraction of the ventilatory response may be related to increased metabolic rate indicates that the traditional concept of intracranial chemoreception is too narrow. Indeed, one of the postulates for why chemoreceptors are located at several brain sites is that they do not all affect the same physiological functions or serve the same purpose, i.e., cardiovascular function, thermogenesis, and arousal. Intracranial chemoreceptors at some sites might be primarily respiratory (RTN), whereas other sites might be primarily thermogenic or cardiovascular (medullary raphe) or may serve to increase arousal (pontine raphe, locus coeruleus). In other words, hypercapnia places multiple demands on the
body, which are met by activation of multiple systems and/or functions, each under control by chemoreceptors at different sites in the brain.

Finally, other studies in piglets, goats, and rats have previously shown that medullary raphe lesions attenuate whole body CO₂ sensitivity (7, 12, 26). Because the present study further establishes that chemoreceptors in the raphe stimulate breathing during physiological conditions, the lesion-induced attenuation of CO₂ sensitivity may reflect a specific effect on chemoreceptors rather than an effect on a nonchemoreceptor neuromodulation of breathing by the raphe.

CONCLUSIONS

We conclude that J) the magnitude of the increase in breathing observed with FA at single or multiple raphe sites is dependent on the degree of acidification, by increasing either the CO₂/H⁺ dialysate concentrations or the number of sites of FA and 2) FA in the medullary raphe affects multiple physiological variables, in addition to ventilation.

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Present address of M. R. Hodges: 706 LCI, Dept. of Neurology, Yale University, 333 Cedar St., New Haven, CT 06520.

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