Short-term modulation of the exercise ventilatory response in goats: effects of 8-OH-DPAT and MPPI

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Received 4 November 1999; accepted in final form 13 July 2000

Henderson, Daniel R., and Gordon S. Mitchell. Short-term modulation of the exercise ventilatory response in goats: effects of 8-OH-DPAT and MPPI. Am J Physiol Regulatory Integrative Comp Physiol 279: R1880–R1888, 2000.—Increased respiratory dead space increases the exercise ventilatory response, a response known as short-term modulation (STM). We hypothesized that STM results from a spinal, serotonin (5-HT)-dependent mechanism. Because 5-HT1A autoreceptors on caudal brain stem raphe neurons inhibit 5-HT release, we hypothesized that 5-HT1A-receptor agonists would inhibit, whereas 5-HT1A-receptor antagonists would enhance, STM. Ventilatory and arterial blood-gas measurements were made at rest and during exercise (4.0–4.5 km/h, 5% grade) in goats with the respiratory mask alone or with increased dead space (0.20–0.25 liter), before and after intravenous administration of the 5-HT1A-receptor agonist 8-hydroxy-2-(di-n-propylamino)tetrabenazine (8-OH-DPAT; 0.1 mg/kg) or the antagonist 4-iodo-2-pyridinylbenzamide (MPPI; 0.08 mg/kg). 8-OH-DPAT increased the slope of the arterial PCO2 vs. metabolic CO2 production relationship and decreased the ventilation vs. metabolic CO2 production relationship during exercise with increased dead space (not with the mask alone), indicating an impairment of STM. In contrast, MPPI had minimal effects on any measured variable. Although nonspecific effects of 8-OH-DPAT cannot be ruled out, impaired STM is consistent with the hypothesis that STM requires active raphe serotonergic neurons and 5-HT release.

The ventilatory response to moderate exercise is feed forward with respect to arterial blood-gas regulation such that arterial blood gases are maintained appropriately in the face of changing metabolic demands (4, 7). In goats, the exercise ventilatory response is characterized by modest hyperventilation and a slight decrease in arterial PCO2 (PaCO2) from rest to moderate exercise (5, 23). A unique aspect of this exercise ventilatory response is revealed with small increases in respiratory dead space in both goats (23) and humans (30). With increased dead space (e.g., bronchodilation), alveolar ventilation decreases and PaCO2 increases at rest, yet the same PaCO2 decrease from rest to exercise is maintained by an augmentation of the exercise ventilatory response known as short-term modulation (STM) (1, 22, 24). Activation of spinal serotonin (5-HT) receptors is necessary for STM, because spinal administration of methysergide (nonselective) or ketanserin (5-HT2 selective) impairs STM (26). On the other hand, spinally administered pindolol, a 5-HT1-receptor (and β-adrenergic-receptor) antagonist, has little effect on STM (D. R. Henderson, D. L. Turner, and G. S. Mitchell, unpublished observations). Thus activation of spinal 5-HT2 receptors is not necessary for full manifestation of STM, with no evidence for any role of spinal 5-HT1 receptors.

Spinal 5-HT-receptor activation requires 5-HT release from the descending projections of caudal medullary raphe nuclei (14). Rhythmic motor activity (e.g., walking or hypercapnia stimulated breathing) increases caudal raphe neuron activity in awake animals (15, 33), thereby increasing extracellular concentrations of spinal 5-HT during exercise (9). Thus one would hypothesize that, because STM requires spinal 5-HT release, a decrease in raphe neuron activity would impair STM. Similarly, one might hypothesize that increased raphe neuron activity would enhance STM.

Brain stem raphe neurons express 5-HT1A autoreceptors that inhibit raphe neuron activity (8, 14, 15). Thus agonists to these receptors decrease the firing rate of dorsal and caudal raphe neurons in awake cats (2, 8, 33); 5-HT1A-receptor antagonists increase dorsal raphe neuron activity (2, 28), although effects on caudal raphe neuron activity have not been reported. Altering caudal raphe neuron activity would alter 5-HT release, including the spinal 5-HT release postulated to be necessary for STM. Thus we hypothesized that systemically administered 5-HT1A-receptor agonists would impair STM, whereas antagonists would enhance, STM with increased respiratory dead space. Although this approach is limited by potential nonspecific effects on 5-HT1A receptors located at other locations within the central nervous system, it nevertheless provides another means of testing our central hypothesis that STM requires serotonergic modulation at critical sites in the respiratory control system. Our objectives were to test the following specific hypotheses in goats: 1)
systemic administration of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a 5-HT1A-receptor agonist, would impair STM with increased dead space; and 2) systemic 4-iodo-N-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylbenzamide (MPPI), a 5-HT1A-receptor antagonist, would enhance STM.

METHODS

Animals. Experiments were conducted on two groups of female goats (each n = 7; 45 ± 4 and 55 ± 7 kg, respectively), familiarized with laboratory procedures such as wearing a tight-fitting respiratory mask and exercising on a motor-driven treadmill (model Q65, Quinton Instruments). At least 8 wk before experiments, the goats were surgically prepared with a translocated carotid artery under either halothane (MPPI experiments) or isoflurane (8-OH-DPAT experiments) anesthesia to allow repeated placement of arterial catheters. Before experiments, the goats were fasted overnight with water provided ad libitum.

Ventilatory and blood-gas measurements. At rest and during steady-state exercise on a treadmill (5–7 min), at least three 0.8-ml arterial blood samples were sequentially drawn into 1.0-ml heparinized syringes. Blood samples were capped, stored on ice, and later analyzed by using an automated blood-gas analyzer (model ABL-330, Radiometer, Copenhagen, Denmark) for pH, PCO2, and PO2. Each sample was corrected to the rectal temperature determined with a thermistor (model 401, Yellow Springs Instruments, Yellow Springs, OH). Throughout an experiment, arterial blood pressure was measured through the arterial catheter with a pressure transducer (model P23-ID, Gould Instruments, Cleveland, OH).

A tight-fitting face mask equipped with a Hans Rudolph valve (series 2600, Hans Rudolph, Kansas City, KS) was used to measure inspired ventilation (Vi) through a pneumotachograph (Fleisch no. 2, OEM Medical, Richmond, VA) attached to the inspiratory port. Pressure differences across the pneumotachograph were measured with a differential pressure transducer (±2 cmH2O; model MP-45, Validyne, Northridge, CA) and a carrier preamplifier (model CD-15, Validyne). The carrier preamplifier output of each breath was integrated by using a Gould resetting integrator (Gould Instruments) to produce a signal proportional to tidal volume (VT). The integrator signal was calibrated each day by using the same experimental sequence with the exception of no drug administration (i.e., step 4 omitted).

Data analysis. Differences between the slopes of the relationships between PaCO2 and VCO2 (ΔPaCO2/ΔVCO2) with the mask alone and with increased dead space (0.25 liter minus mask only) were compared pre- vs. postdrug by using a paired t-test. Resting values, as well as the slopes of the relationships between ventilatory and blood-gas variables with respect to VCO2, were compared pre- vs. postdrug, for mask only and with added dead space, by using a one-way repeated-measures ANOVA. Post hoc analysis was conducted by using t-tests with the Bonferroni correction for multiple comparisons (SigmaStat, Jandel, San Rafael, CA). All values are reported as means ± SE. P < 0.05 was considered statistically significant.

RESULTS

Effects of drugs on goat behavior. Immediately after 8-OH-DPAT administration (0.1 mg/kg), behavioral effects were observed, including head sway, hindlimb weakness, lower lip droop, increased chewing, a transient increase in blood pressure, postural instability, and agitation. These behavioral effects were generally transient, and some of them persisted for up to 1 h after drug administration. After 2 h, behavioral effects were no longer observed. Similar behaviors were observed in four goats administered 0.05 mg/kg 8-OH-DPAT.

Because MPPI administration produced no visible effects on goat behavior, the efficacy of the MPPI dose was tested in one goat to determine whether it prevented the blood pressure and behavioral effects of 8-OH-DPAT (0.05 mg/kg; Fig. 1). Twenty minutes after MPPI administration (0.08 mg/kg), 8-OH-DPAT (0.05 mg/kg) had no detectable blood pressure or behavioral effects, thus providing evidence for effective 5-HT1A-receptor antagonism at the MPPI dose used in these investigations (Fig. 1).

Resting ventilation and blood gases. After 8-OH-DPAT (0.1 mg/kg), PaCO2 decreased slightly (−1.7 mmHg) when goats breathed through the mask alone, but was increased (−1.7 mmHg) with dead space (both P < 0.05; Table 1). With the mask alone and with increased dead space, Vi increased (−366 and 220%,
respectively; both $P < 0.05$), largely due to an increase in respiratory frequency ($f$) ($-621$ and $208\%$, respectively; both $P < 0.05$). VT decreased with the mask alone ($-42\%$; $P < 0.05$) but was unaffected with dead space. After 8-OH-DPAT, resting $\dot{V}CO_2$ was elevated with increased dead space ($-85\%$; $P < 0.05$) but not with the mask alone (Table 1).

None of the measured ventilatory or blood-gas values were affected by MPPI, with the exception of a decrease in resting $\dot{V}I$ with dead space ($-26\%$; $P < 0.05$; Table 2). Apparent changes in $\text{PaCO}_2$, VT, and $f$ were not significant (Table 2).

During sham experiments, resting ventilation when goats breathed through the mask alone was elevated during the second trial ($-43\%$; $P < 0.05$; Table 3). This time-dependent increase in $\dot{V}I$ with dead space was mediated by a significant increase in $f$ ($-72\%$; $P < 0.05$), with an accompanying decrease in VT ($-20\%$; $P < 0.05$). $\text{PaCO}_2$ was not affected by these shifts in ventilatory pattern. No differences in resting ventilation or blood gases were detected in the first vs. the second trial with added dead space (Table 3).

**Exercise ventilatory response and STM.** After 8-OH-DPAT, $\Delta\text{PaCO}_2/\Delta\dot{V}CO_2$ during exercise with dead space was significantly elevated from $-2.9$ to $+2.6$ mmHg·min/l ($P < 0.05$; Figs. 2A and 3A), indicating an impairment of STM. Impaired arterial CO$_2$ regulation during exercise with added dead space was accompanied by a significant decrease in the exercise ventilatory response with added dead space [i.e., the slope of the relationship between $\dot{V}I$ and $\dot{V}CO_2$; Figs. 2D and 3D]. Thus the exercise ventilatory response with added dead space displayed less of an augmentation after 8-OH-DPAT ($-40\%$ predrug vs. $-5\%$ postdrug relative to mask only; Fig. 3D). A similar, although smaller, change in $\Delta\dot{V}I/\Delta\dot{V}CO_2$ was observed in sham (no drug) experiments ($-30\%$ first vs. $-8\%$ second trial; Fig. 3F; $P < 0.05$). However, $\Delta\text{PaCO}_2/\Delta\dot{V}CO_2$ exhibited no significant time- or trial-dependent effects (Fig. 3C).

Table 1. Resting measurements pre- and post-8-OH-DPAT with the mask alone or with added dead space (0.25 liter)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Predrug</th>
<th>8-OH-DPAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mask only</td>
<td>Dead space</td>
</tr>
<tr>
<td>$\text{PaCO}_2$, mmHg</td>
<td>39.4 ± 1.1</td>
<td>39.7 ± 1.1</td>
</tr>
<tr>
<td>$\dot{V}I$, l/min</td>
<td>10.2 ± 1.5</td>
<td>19.6 ± 4.3</td>
</tr>
<tr>
<td>$\dot{V}CO_2$, l/min</td>
<td>0.20 ± 0.02</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>VT, l/breath</td>
<td>0.59 ± 0.04</td>
<td>0.59 ± 0.04</td>
</tr>
<tr>
<td>Respiratory frequency, breaths/min</td>
<td>38.1 ± 11.0</td>
<td>38.1 ± 11.0</td>
</tr>
<tr>
<td>$T_I$, s</td>
<td>0.75 ± 0.14</td>
<td>0.75 ± 0.14</td>
</tr>
</tbody>
</table>

Values are means ± SE. 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; $\text{PaCO}_2$, arterial $\text{PCO}_2$; $\dot{V}I$, inspired minute ventilation; $\dot{V}CO_2$, $\text{CO}_2$ production; VT, tidal volume; $T_I$, inspiratory time. *$P < 0.05$ compared with pre-8-OH-DPAT value.
Table 2. Resting measurements pre- and post-MPPI with the mask alone or with added dead space (0.20 liter)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Predrug Mask only</th>
<th>Predrug Dead space</th>
<th>MPPI Mask only</th>
<th>MPPI Dead space</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaCO₂, mmHg</td>
<td>41.8 ± 0.9</td>
<td>43.4 ± 0.9</td>
<td>42.1 ± 0.9</td>
<td>44.0 ± 1.0</td>
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<tr>
<td>V̇l, l/min</td>
<td>11.3 ± 1.4</td>
<td>17.3 ± 3.0</td>
<td>10.2 ± 2.0</td>
<td>12.8 ± 2.1*</td>
</tr>
<tr>
<td>VCO₂, l/min</td>
<td>0.24 ± 0.03</td>
<td>0.23 ± 0.03</td>
<td>0.20 ± 0.02</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>V̇r, l/breath</td>
<td>0.48 ± 0.04</td>
<td>0.58 ± 0.05</td>
<td>0.45 ± 0.05</td>
<td>0.57 ± 0.05</td>
</tr>
<tr>
<td>Respiratory frequency, breaths/min</td>
<td>25.7 ± 4.2</td>
<td>34.4 ± 8.5</td>
<td>27.6 ± 7.6</td>
<td>25.5 ± 4.8</td>
</tr>
<tr>
<td>Ti, s</td>
<td>0.95 ± 0.15</td>
<td>0.82 ± 0.16</td>
<td>1.03 ± 0.23</td>
<td>1.01 ± 0.19</td>
</tr>
</tbody>
</table>

Values are means ± SE. MPPI, 4-iodo-N-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylbenzamide. *P < 0.05 compared with pre-MPPI value.

MPPI had no significant effects on the relationships between ∆PaCO₂/∆VCO₂ or between ∆V̇l/∆VCO₂ with increased dead space (Fig. 2, B and E; Fig. 3, B and E, respectively). Although augmentation of the exercise ventilatory response with increased dead space compared with mask alone displayed a decreasing trend in most goats after MPPI, statistical significance was not attained because of a single goat that displayed a response opposite to the others (P = 0.27 with vs. P = 0.02 without the outlier; Fig. 3E).

Ventilatory pattern: V̇r and f. After 8-OH-DPAT administration, V̇r was significantly decreased during rest and exercise with the mask alone but not with increased dead space (Table 1, Fig. 4A). However, the slope of the V̇r vs. VCO₂ relationship (∆V̇r/∆VCO₂) was unchanged, either with the mask alone or with added dead space (Fig. 4A). At rest, f was elevated with the mask alone and with increased dead space (Table 1, Fig. 4D). However, the slope of the f vs. VCO₂ relationship (∆f/∆VCO₂) was not significantly altered by 8-OH-DPAT (Fig. 4D). Thus the relative decrease in the slope of the exercise ventilatory response with added dead space after 8-OH-DPAT could not be attributed specifically to changes in the regulation of either V̇r or f.

After MPPI administration, no differences were measured in V̇r or f at rest, either with the mask alone or with added dead space. Furthermore, ∆V̇r/∆VCO₂ and ∆f/∆VCO₂ during exercise were unaffected by MPPI, either with the mask alone or with increased dead space (Fig. 4, B and D, respectively).

During sham experiments, no significant differences were detected between the first and second trials in ∆V̇r/∆VCO₂ and ∆f/∆VCO₂, either with the mask alone or with increased dead space (Fig. 4, C and F, respectively). However, the f response showed a nonsignificant trend to increase similar to the 8-OH-DPAT and MPPI groups.

DISCUSSION

These experiments demonstrate that a 5-HT₁A receptor agonist (8-OH-DPAT) impairs STM of the exercise ventilatory response with increased respiratory dead space in goats. In contrast, a 5-HT₁A receptor antagonist (MPPI) had no discernable effects. The results of this study are consistent with the hypothesis that STM requires 5-HT release from raphe neurons and subsequent activation of a 5-HT-receptor subtype distinct from 5-HT₂ receptors (presumably 5-HT₂ receptors; Ref. 26). However, the present results also suggest that enhanced serotonergic function is unable to augment STM, consistent with a previous report using serotonin reuptake inhibitors (11).

Behavioral effects of 8-OH-DPAT. The experimental doses of 8-OH-DPAT and MPPI were determined empirically, by monitoring blood pressure and behavioral effects of 8-OH-DPAT. Many of the behavioral effects reported to result from 8-OH-DPAT administration in monkeys (e.g., head sway, hindlimb extension; Ref. 27) were present in these goats at doses of 0.05 and 0.1 mg/kg. Administration of MPPI (0.08 mg/kg) was sufficient to block all observable behavioral and blood pressure effects of 8-OH-DPAT (Fig. 1), thus providing evidence that this was an effective dose of the antagonist. One concern is that behavioral effects (e.g., postural changes) resulting from 8-OH-DPAT administration may have nonspecifically impaired STM.

Table 3. Resting measurements of sham STM experiments with the mask alone or with added dead space (0.25 liter)

<table>
<thead>
<tr>
<th>Variable</th>
<th>First STM Trial</th>
<th>Second STM Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaCO₂, mmHg</td>
<td>41.6 ± 0.9</td>
<td>41.1 ± 0.8</td>
</tr>
<tr>
<td>V̇l, l/min</td>
<td>11.0 ± 1.1</td>
<td>15.7 ± 2.4*</td>
</tr>
<tr>
<td>VCO₂, l/min</td>
<td>0.25 ± 0.02</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>V̇r, l/breath</td>
<td>0.60 ± 0.04</td>
<td>0.48 ± 0.03*</td>
</tr>
<tr>
<td>Respiratory frequency, breaths/min</td>
<td>20.9 ± 2.7</td>
<td>36.0 ± 6.8*</td>
</tr>
<tr>
<td>Ti, s</td>
<td>1.05 ± 0.12</td>
<td>0.67 ± 0.13</td>
</tr>
</tbody>
</table>

Values are means ± SE. STM, short-term modulation. *P < 0.05 compared with first STM trial value.
several arguments will be put forth that this was not a major factor in these studies.

The experimental dose of 8-OH-DPAT (0.1 mg/kg) is in excess of doses observed to decrease caudal raphe neuron activity in cats. In cats, an 8-OH-DPAT dose of 0.01–0.02 mg/kg (iv) is sufficient to decrease caudal raphe neuron activity, with little further affect at higher doses (33). Behavioral effects, consistent with those observed in goats, become apparent in cats at drug doses higher than 0.02 mg/kg (S. Veasey, personal communication). Although a lower dose of 8-OH-DPAT may have been sufficient to achieve raphe neuron inhibition while avoiding behavioral effects in these studies, we were concerned with the duration of drug action. Because our intent was to obtain rest and exercise measurements with the mask alone and with increased dead space, it was necessary to use a dose sufficient to stably inhibit raphe neurons for a period of at least 1 h. Nevertheless, behavioral effects from 8-OH-DPAT were transient, did not prevent the goats from performing any exercise protocol, and did not greatly impair the exercise ventilatory response with a mask alone (however, see Loss of STM with mask alone).

Potential ventilatory limitation. Ventilation during the postdrug exercise ventilatory response with dead space was substantially elevated compared with the same predrug trial (57 l/min predrug vs. 74 l/min postdrug; P < 0.05; Fig. 2D). At the higher level of ventilation, it is possible that mechanical constraints or mechanical feedback inhibition may have limited the exercise ventilatory response with dead space, thus preventing STM (23). However, we argue against a purely mechanical limitation because, after scaling for body mass (0.75 power), higher ventilatory outputs have been reported during exercise in goats (e.g., 87 l/min, Ref. 23; 95 l/min, Ref. 19). Sensory feedback inhibition remains a possibility for limiting STM in a nonspecific way after 8-OH-DPAT. However, we do not believe that this is the case because 8-OH-DPAT increased \( V_i \) largely by increases in \( f \) vs. \( V_T \) (which decreased slightly). Although not completely clear, we
suggest that elevated VT is a more potent stimulus to feedback inhibition (32). The decrease in VT with dead space after 8-OH-DPAT was, in turn, accompanied by a decreased VT/inspiratory time (VT/TI) response during exercise. Decreased VT and VT/TI responses during exercise with dead space after 8-OH-DPAT are consistent with decreased serotonergic augmentation of spinal motor output to respiratory muscles, rather than a ventilatory limitation caused by feedback inhibition.

**Loss of STM with mask alone.** Resting PaCO$_2$ with the mask alone was decreased after 8-OH-DPAT, reflecting increased resting ventilatory drive. This increased resting ventilatory drive alone should elicit STM, thereby achieving an exercise ventilatory response sufficient to achieve similar PaCO$_2$ regulation from rest to exercise pre- to postdrug (25). After 8-OH-DPAT there appeared to be a slight, although not significant, loss of PaCO$_2$ regulation during exercise with the mask alone ($\Delta$PaCO$_2$ = −2.1 mmHg pre- vs. −1.1 mmHg postdrug; $P > 0.05$; Fig. 2A). When expressed as $\Delta$PaCO$_2$/$\Delta$VCO$_2$ during exercise, PaCO$_2$ regulation was impaired (~3.5 pre- vs. −1.6 mmHg·min/l postdrug; $P < 0.05$ when compared with a paired t-test). A previously described model (23) was used to predict the decrease in PaCO$_2$ expected with increased dead space but without STM (i.e., no increase in exercise gain). The model predicted a PaCO$_2$ decrease of −1.7 mmHg postdrug (vs. −2.1 mmHg predrug). Thus there is suggestive evidence that the resting hyperventilation caused by 8-OH-DPAT did not elicit STM with the mask alone, possibly due to diminished serotonergic function.

**Possible sites of action.** Intravenous administration of both 8-OH-DPAT and MPPI precludes certainty regarding the predominant site of action. Indeed, 5-HT$_{1A}$ receptors act at other neuroanatomic locations relevant to ventilatory control (16, 17, 18, 31). In addition, 8-OH-DPAT has an affinity for other 5-HT receptors (e.g., 5-HT7; Ref. 13), such that some drug effects may have been mediated by actions at non-5-HT$_{1A}$ receptors. Once released, serotonin may interact with other neurotransmitter systems. Although other neurochemicals (e.g., catecholamines, peptides) are likely to modulate spinal pathways that contribute to the exercise ventilatory response and STM (12, 29), our laboratory found preliminary evidence that spinal dopamine or $\beta$-adrenergic receptors are not necessary in the underlying mechanism of STM (Ref. 10; unpublished...
observations). The potential involvement of other neurotransmitters and neuromodulators awaits further investigation.

8-OH-DPAT administration may exert unintended cardiovascular effects by acting at raphe neurons or other sites, such as central sympathetic neurons (6, 20, 21). Thus it is uncertain whether blood pressure or behavioral effects of 8-OH-DPAT are attributable to the actions of 5-HT1A autoreceptors on raphe neurons or to the activation of 5-HT1A receptors located at other sites in the central nervous system.

Actions of 8-OH-DPAT and MPPI on raphe neurons. Systemically administered 8-OH-DPAT and MPPI alter dorsal raphe neuron activity in awake cats (2, 8). More importantly, 8-OH-DPAT inhibits activity of raphe pallidus neurons (33), a more relevant group to respiratory control. By inhibiting caudal (obscurus/pallidus) raphe activity, 8-OH-DPAT is expected to diminish spinal serotonergic modulation during exercise with increased dead space, thereby impairing STM.

In awake, behaving cats, 0.01 mg/kg of 8-OH-DPAT is sufficient to virtually eliminate dorsal raphe neuron activity within 1 min of injection. Dorsal raphe neuron activity remains significantly decreased at 1 h, although it recovers substantially during that time (~65% of baseline; Ref. 2). Raphe obscurus/pallidus neurons displayed somewhat lower sensitivity to intravenous 8-OH-DPAT, responding at doses ranging from 0.01 to 0.02 mg/kg (32). Because the primary dose of 8-OH-DPAT used in these studies was 0.1 mg/kg, and postdrug data were collected within 1 h of administration, it is likely that raphe neuron activity remained decreased throughout the experimental protocol. Nevertheless, the pharmacodynamics of 8-OH-DPAT are unknown in goats and, therefore, may have influenced the outcome of these experiments to some extent.

In awake cats, 0.1 mg/kg of MPPI is sufficient to increase dorsal raphe neuron firing rate above baseline activity (~50%), with no further increase at higher doses. At this dose, firing rates of raphe neurons remained elevated 45 min after drug administration (2). Because the dose of MPPI used was sufficient to block the behavioral and cardiovascular actions of 8-OH-DPAT, it is unlikely that an inadequate MPPI dose could account the lack of STM enhancement in the

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Fig. 4. Tidal volume (VT; A, B, and C) and respiratory frequency (D, E, and F) expressed as functions of metabolic VCO2 for both mask only and with added dead space, before and after systemic administration of 8-OH-DPAT (A and D), MPPI (B and E), or no drug (sham) (C and F). Values are means ± SE. Missing error bars are small enough to be contained within the point. 8-OH-DPAT administration resulted in tachypnea; however, the slopes of and frequency relative to VCO2 were unchanged for either mask only or with added dead space in any experiment.
present study, although there may be some concerns about the duration of its effects. Furthermore, the effects of 5-HT<sub>1A</sub>-receptor antagonists on caudal raphe neurons have not been examined. Thus it has not been demonstrated that raphe neurons in goats have significant 5-HT<sub>1A</sub>-autoreceptor inhibition during the conditions of these experiments.

Thus the lack of enhancement of STM after MPPI indicates either the lack of ability to enhance spinal serotonergic modulation during hypercapnic exercise, a paucity of 5-HT<sub>1A</sub>-receptor autoinhibition in the caudal raphe nuclei of goats, or confounding influences via different populations of 5-HT<sub>1A</sub> receptors in the central nervous system or periphery. The possibility that STM is a saturable mechanism and that a maximal influence of 5-HT had already been attained during hypercapnic exercise, is supported by observations that goats are unable to regulate PaCO<sub>2</sub> with larger dead space volumes (i.e., no further STM; Ref. 23) and that STM is not enhanced after chronic 5-HT reuptake inhibition with fluoxetine (11). Thus there may be an inherent upper limit to 5-HT-dependent STM.

**Perspectives**

Serotonergic modulation, resulting from raphe neuron activity, is involved in a variety of motor behaviors (3, 14, 15, 25, 32, 34). The results of this study are consistent with the necessity of raphe neuron activity in response to ventilatory challenge in the form of added dead space during the exercise ventilatory response (i.e., STM), expanding on previous studies demonstrating that spinal 5-HT receptors are necessary for STM (25). Although the present study is consistent with our hypothesis that disruption of serotonergic modulation during ventilatory challenges interferes with STM, future studies addressing the effects of more specific methods of caudal raphe neuron suppression would be of interest.

We thank B. A. Hodgeman for assistance in preparation of Fig. 1 and S. M. Johnson for helpful critique of the manuscript. S. Veasey and C. Fornal provided critical discussion regarding raphe neuron effects of drugs used in these studies. This work was supported by National Heart, Lung, and Blood Institute Grants HL-36780 and HL-09905.

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