Published in final edited form as:

Respir Physiol Neurobiol. 2008 May 31; 161(3): 230–238.

SPINAL SEROTONIN RECEPTOR ACTIVATION MODULATES THE EXERCISE VENTILATORY RESPONSE WITH INCREASED DEAD SPACE IN GOATS

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Abstract

Small increases in respiratory dead space (V_D) augment the exercise ventilatory response by a serotonin-dependent mechanism known as *short-term modulation (STM)*. We tested the hypotheses that the relevant serotonin receptors for *STM* are in the spinal cord, and are of the 5-HT₂-receptor subtype. After preparing adult female goats with a mid-thoracic (T6–T8) subarachnoid catheter, ventilation and arterial blood gases were measured at rest and during treadmill exercise (4.8 km/h; 5% grade) with and without an increased V_D (0.2–0.3 L). Measurements were made before and after spinal or intravenous administration of a broad-spectrum serotonin receptor antagonist (methysergide, 1–2 mg total) and a selective 5-HT₂-receptor antagonist (ketanserin, 5–12 mg total). Although spinal methysergide had no effect on the exercise ventilatory response in control conditions, the augmented response with increased V_D was impaired, allowing P_{aCO_2} to increase from rest to exercise. Spinal methysergide diminished both mean inspiratory flow and frequency responses to exercise with increased V_D . Spinal ketanserin impaired P_{aCO_2} regulation with increased V_D , although its ventilatory effects were less clear. Intrathecal dye injections indicated CSF drug distribution was caudal to the upper cervical spinal cord and intravenous drugs at the same total dose did not affect *STM*. We conclude that spinal 5-HT₂ receptors modulate the exercise ventilatory response with increased V_D in goats.

Keywords

exercise hyperpnea; ventilatory control; serotonin; spinal cord; modulation; plasticity; hypercapnia; breathing

1. INTRODUCTION

Small increases in respiratory dead space increase the exercise ventilatory response in goats (Mitchell, 1990) and humans (Poon, 1992; Wood et al., 2007), maintaining P_{aCO_2} regulation

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with respect to its resting level. Modulation of the exercise ventilatory response with increased dead space cannot be accounted for by changes in chemoreceptor feedback from rest to exercise (Mitchell, 1990). Since the mechanism that augments exercise ventilatory responses with increased dead space is reversible within a single exercise trial, it has been referred to as short-term modulation (*STM*; Bach *et al.*, 1993; McCrimmon *et al.*, 1995; Mitchell and Babb, 2006).

Short-term modulation may involve changes in (spinal) respiratory motoneuron excitability, possibly due to afferent feedback from thoracic respiratory muscles (Mitchell *et al.*, 1984; Schaefer and Mitchell, 1989; Mitchell, 1990) or serotonergic modulation from descending brainstem-spinal cord projections of raphe neurons (Bach *et al.*, 1993; Mitchell *et al.*, 1993; McCrimmon *et al.*, 1995; Henderson and Mitchell, 2000). The latter hypothesis is based on the observations that the broad-spectrum serotonin receptor antagonist, methysergide, and whole-body serotonin depletion with p-chlorophenylalanine, impair *STM* with increased respiratory dead space in goats (Bach *et al.*, 1993). However, systemic drug administration prevented conclusions regarding the location or subtype of the serotonin receptors relevant to *STM*.

Spinal serotonergic projections may alter the “gain” of respiratory motoneurons, thereby accounting, at least in part, for *STM* (McCrimmon *et al.*, 1995). Although most of the necessary elements in this proposed mechanism have been established for phrenic motoneurons (Bach *et al.*, 1993; McCrimmon *et al.*, 1995; Reklung *et al.*, 2000), little is known concerning serotonergic modulation of other spinal respiratory motoneuron pools potentially involved in *STM* (McCrimmon *et al.*, 1995).

The objectives of the present study were to test the hypothesis that spinal serotonin receptors modulate the exercise ventilatory response with increased V_D (*ie.* *STM*), and to identify the relevant serotonin receptor subtype. Adult goats with chronic subarachnoid catheters in the thoracic spinal cord were studied to allow intra-spinal serotonin receptor antagonist administration. To control for possible systemic distribution of spinally administered drugs, experiments were repeated in the same goats on different days following intravenous administration of the same total dose. Drug distribution within spinal CSF was estimated by injections of Evans' Blue Dye. Portions of this work were published in preliminary form (Mitchell *et al.*, 1993; 1995).

2. METHODS

2.1 Experimental animals

Experiments were conducted on 15 adult female goats familiarized with wearing a respiratory mask and standing/running on a treadmill. The goats were surgically prepared with a translocated carotid artery to allow placement of an arterial catheter and a subarachnoid catheter connected to a subdermal Ommaya Reservoir (Heyer-Schulte NeuroCare, Pleasant Prairie, WI, USA), thus allowing repeated sterile access to the cerebro-spinal fluid for drug administration. At least two weeks were allowed for recovery before experiments began. Goats were fasted the night before experiments. All procedures and protocols conformed with and were approved by the University of Wisconsin, School of Veterinary Medicine, Animal Care and Use Committee.

2.2 Surgical Preparation

Under halothane anaesthesia, a single segment laminectomy and limited durotomy were performed at T₁₀–T₁₂. A catheter was inserted into the subarachnoid space and advanced at least two centimetres rostrally (T₈–T₁₀). The catheter was secured to an Ommaya Reservoir

placed subdermally and sutured into position. The goats were recovered and carefully monitored for signs of spinal injury or inflammatory reactions. The reservoir/catheter was flushed daily with sterile saline to prolong patency. All drug or saline injections into the CSF were done using sterile techniques.

Preoperative treatments included: 1) Dexamethasone (0.2 mg/kg, i.m., 1 day before), a steroidal anti-inflammatory agent, to prevent swelling and spinal compression; 2) Antibiotics immediately pre-surgery (10mg/kg ampicillin, i.m.); 3) Prednisone (day of surgery; 2 mg/kg, i.v.). During surgery, treatments included: 1) Continuous infusion of lactated Ringer's solution (10 mg/kg/hr, i.v.); 2) Antibiotics (ampicillin 10 mg/kg, i.m.); and 3) Monitoring: blood gases, temperature, blood pressure and ECG. Postoperative care included: 1) Maintenance of body fluids with lactated Ringer's solution if necessary; 2) Prednisone (i.m.): 3 days @ 1 mg/kg, 3 days @ 0.5 mg/kg, 3 days @ 0.25 mg/kg. After the final steroid dose, at least one week was allowed before experiments.

2.3 Measurements

A tight-fitting facemask (dead space ~ 200 ml), equipped with a Hans-Rudolph valve (series 2600, Hans Rudolph, Kansas City, KS, USA), was used to measure inspired ventilation ($\dot{V}_I$) via a pneumotachograph (Fleisch no. 2, OEM Medical Inc., Richmond, VA, USA) connected to the inspiratory port. Pressure across the pneumotachograph was measured with a variable reluctance differential pressure transducer (± 2 cm H₂O; Validyne, MP-45, Cleveland, OH, USA) and a carrier preamplifier (Validyne, CD-15, Northridge, CA, USA). The carrier output was integrated with a resetting integrator (Gould Inst., Cleveland, OH, USA) to produce a signal proportional to tidal volume (V_T). The integrator signal was calibrated at the beginning and end of each experimental day with three known volumes from a calibration syringe. The inspired minute ventilation ($\dot{V}_I$) was calculated from V_T and breathing frequency. The mixed expired CO₂ fraction was determined with an infrared CO₂ analyzer (Beckman, LB-2, Sensor Medics Inc., Anaheim, CA, USA) after the expired gas passed through a mixing chamber and desiccant column. Metabolic CO₂ production ($\dot{V}_{CO_2}$) was calculated as described previously (Mitchell, 1990).

Measurements were made at rest and during exercise (4.8km/hr, 5% grade) without a mask, with the mask alone and with a small dead space (0.20–0.3 L) added to the mask. For the measurement of ventilatory responsiveness to CO₂ at rest, the goats breathed through the mask with increased dead space (0.6 L for 8 min). Ventilatory responsiveness to CO₂ at rest (S) was calculated as the slope of the ventilatory response to changes in the arterial partial pressure of carbon dioxide (P_{aCO_2}) elicited by increased dead space ($S = \Delta \dot{V}_I / \Delta P_{aCO_2}$). Measurements of S were used to estimate the exercise gain or make model predictions in each goat as described previously (Mitchell, 1990). All measurements were made with a background of slight hyperoxia (25–30% O₂).

2.4 Experimental Protocol

Baseline data consisted of blood gas measurements during a rest/run sequence (5 min rest followed by 5 min run @ 4.8 km/hr, 5% grade; blood samples were collected during the 6th min of rest and exercise) with the goat wearing no respiratory mask. This was followed by a slow injection of saline into the Ommaya Reservoir via a barbitage technique. After 10 min, hypercapnic responsiveness was measured by increasing dead space (0.6 L) at rest. Another rest/run sequence was then performed while the goat was wearing a respiratory mask. After the goat's body temperature and respiratory rate had returned to baseline levels (approximately 15–20 min post-exercise; see Mitchell, 1990), the rest/run sequence was repeated with 0.2 L dead space added to the mask. Methysergide or ketanserin was then administered to the spinal CSF (total dose of methysergide in 1 ml of saline; ketanserin in 3 ml due to limited solubility),

and 10 min were allowed for drug distribution. To emphasize the effects of drug administration during exercise with increased dead space, the protocol was repeated in a reverse sequence: rest and run with increased dead space followed by rest and run with the mask alone. The protocol ended with another measurement of $\dot{V}_E$.

As a control for potential systemic drug distribution (e.g. across the blood-brain barrier), the entire protocol was repeated in the same goats on a separate day; during this control experiment, procedures were the same except that the goats were delivered intravenous injections of the same total dose of methysergide ($n = 7$) or ketanserin ($n = 5$) as administered to the spinal CSF in previous experiments. Systemic injections simulated maximum possible systemic drug concentrations following spinal injections, assuming that all drug had crossed the blood brain barrier and redistributed throughout the body *via* the circulation.

An additional concern regarding the spinal drug administration is the migration of drug through the CSF, possibly reaching brain sites. To examine the CSF distribution pattern of spinally administered drugs, Evans Blue dye was injected into the spinal CSF of six goats, using the same procedure as during drug injections. One goat was exercised for three minutes (4.0 km/hr, 5.0% grade) following dye injection. Approximately 60 min after dye injection, goats were euthanized with Beuthanasia solution and their spinal cords were exposed to observe dye distribution. No dye could be detected in the brainstem of any goat; dye remained localized to the spinal cord, spreading no further rostrally than the mid-cervical region. Radio-opaque dye was injected into the cervical spinal CSF of one goat to visualize cephalad flow under fluoroscopic examination; again, no dye was detected rostral to the mid-cervical region.

In each experimental condition, 3 or 4 samples (0.8 ml) of arterial blood were drawn into heparinized syringes from a catheter in an exteriorized carotid artery. The samples were stored anaerobically on ice until analyzed for pH, P_{CO_2} and P_{O_2} with a blood gas analyzer (model ABL 330, Radiometer, Copenhagen, Denmark). Each blood sample was analyzed at 37° C and then corrected to rectal temperature, recorded with a thermistor.

2.5 Drug Administration

Methysergide maleate (0.028 mg/kg in 1 ml) or ketanserin tartarate (0.25 mg/kg in 3 ml) were dissolved in saline and delivered to the Ommaya Reservoir using barbitage to gently mix the drug with the cerebral spinal fluid. To assure that most of the injected drug was delivered to the spinal CSF and not sequestered in the Reservoir, 1 ml of CSF was drawn out of the Ommaya Dome and stored in a sterile syringe prior to drug injection. Following drug injection, the 1ml of CSF was flushed back into the Ommaya Reservoir. Ketanserin was dissolved in a relatively large volume of saline due to its low solubility, particularly in artificial CSF. The solubility of ketanserin in CSF is sufficiently low that it is difficult to be certain of the effective ketanserin dose delivered to the thoracic spinal cord (ie. precipitation was possible when mixed with endogenous CSF).

2.6 Analysis

All ventilatory and metabolic data were collected with an on-line computer analysis system described previously (Warner and Mitchell, 1990; Bach *et al.*, 1993; Martin and Mitchell, 1993). Inspiratory peaks and timing were saved for each breath into a computer ASCII file and a second program was used for off-line editing and data summarization.

Ventilatory responses to exercise in each experimental condition are expressed as the change from rest to exercise or as the relationships between $\dot{V}_I$, $\dot{V}_T$ or f and $\dot{V}_{CO_2}$. Attention was focused on changes in Pa_{CO_2} from rest to exercise and the slope of the Pa_{CO_2} - $\dot{V}_{CO_2}$ relationship ($\Delta Pa_{CO_2}/\Delta \dot{V}_{CO_2}$) since these are in many respects the best indicators of short-term modulation

in goats (Mitchell, 1990). All values were averaged within and across animals and expressed as mean $\pm$ 1 SEM. Statistical comparisons among mean data groups were made *via* pairwise analyses utilizing T-tests with the Bonferroni correction for multiple comparisons (Wallenstein et al., 1980). $P < 0.05$ was considered significant.

3. RESULTS

3.1 Methysergide (non-selective antagonist)

Spinal methysergide administration had no effect on the slope of the relationship between PaCO_2 vs. $\dot{V}_{\text{CO}_2}$ when wearing the respiratory mask alone, indicating that exercise hyperpnea was not affected by spinal methysergide administration (Fig. 1A). However, with dead space added to the mask (Fig. 1B), the slope of the PaCO_2 vs. $\dot{V}_{\text{CO}_2}$ relationship was significantly increased compared to the pre-drug value, indicating that *STM* had been impaired. When the same low dose (0.028 mg/kg) of methysergide was administered systemically, there was no effect on the slope of the relationship between PaCO_2 and $\dot{V}_{\text{CO}_2}$ from rest to exercise, either with or without increased dead space (Figs. 1c and 1d). Thus, systemic drug spread could not account for loss of PaCO_2 regulation with increased V_D following intraspinal methysergide.

The effects of intraspinal methysergide on PaCO_2 and $\dot{V}_I$ or its components of respiratory frequency (f), tidal volume (V_T) and the mean inspiratory flow (V_T/T_I) during exercise with increased dead space are shown in Figs. 2 and 3 respectively. During exercise with increased dead space; the value of PaCO_2 was significantly increased (42.3 ± 1.6 to 45.0 ± 1.5 ; $p < 0.05$) and $\dot{V}_I$ was decreased ($p < 0.05$; Fig. 2). In accordance, both f and V_T/T_I were significantly decreased ($p < 0.05$), with only a slight tendency for decreased V_T ($p > 0.05$; Fig. 3). Thus, both respiratory timing (f) and inspiratory drive (V_T/T_I) were affected by intraspinal methysergide, but only during exercise with increased dead space. Similar effects were not seen at rest or during exercise with the mask alone.

When data from the seven goats are plotted individually (Fig. 4) as changes in the slopes of the relationships between PaCO_2 or $\dot{V}_I$ and $\dot{V}_{\text{CO}_2}$ (*i.e.* $\Delta\text{PaCO}_2/\Delta\dot{V}_{\text{CO}_2}$ and $\Delta\dot{V}_I/\Delta\dot{V}_{\text{CO}_2}$, dead space compared to mask only) before and after drug administration, a pattern was observed that is consistent with an attenuation of *STM*. Figs. 4A and 4C express the change in the slope of the PaCO_2 vs. $\dot{V}_{\text{CO}_2}$ relationship caused by the addition of dead space relative to mask only, shown both before and after spinal methysergide. Expressed in this way, a value of zero would indicate precise *STM*. Prior to methysergide administration, the slopes of all seven goats were between 0 and 5. Any increase from those individual values following methysergide indicates a relative loss of *STM*. Indeed the change in the slope of the PaCO_2 vs. $\dot{V}_{\text{CO}_2}$ relationship increased following intraspinal methysergide in all 7 goats (Fig. 4A; $p < 0.05$). Conversely, augmentation of the exercise ventilatory response caused by increased dead space (Fig. 4B) decreased in 5 of 7 goats, consistent with a loss of *STM* ($p < 0.05$). As expected, no changes in the slopes of the PaCO_2 vs. $\dot{V}_{\text{CO}_2}$ relationship or the exercise ventilatory response were observed following systemic methysergide administration (Figs. 4C and 4D).

The V_T and f responses during exercise ($\Delta V_T/\Delta\dot{V}_{\text{CO}_2}$ and $\Delta f/\Delta\dot{V}_{\text{CO}_2}$) with and without dead space were unchanged by methysergide (Figs. 5A and 5B; both $p > 0.05$). The change in the mean inspiratory flow (V_T/T_I) in relation to $\dot{V}_{\text{CO}_2}$ (Fig. 5C) increased with the addition of dead space prior to, but not following spinal methysergide (pre-methysergide with mask only: 1.5 ± 0.1 vs. pre-methysergide with 0.25 L dead space: 2.1 ± 0.2 , $p < 0.05$; post-methysergide with mask only: 1.8 ± 0.1 vs. post-methysergide with 0.25 L dead space: 1.9 ± 0.2 , $p > 0.05$). Changes in the duty cycle (T_I/T_{TOT}) as a function of $\dot{V}_{\text{CO}_2}$ caused by the addition of dead space were unaffected by spinal methysergide (Fig. 5D). Collectively, these data confirm that the same trends are evident when data are normalized to changes in metabolic CO_2 production, indicating that methysergide directly impaired *STM* expression.

3.2 Ketanserin (5-HT₂ antagonist)

Following intraspinal ketanserin administration, the slope of the relationship between PaCO₂ and V̇CO₂ caused by the addition of dead space (Fig. 6A) increased in 6 of 6 goats from an initial value near zero ($p < 0.05$). In contrast to methysergide, the change in the exercise ventilatory response caused by the addition of dead space tended to increase following intrathecal ketanserin, although this effect was not significant (Fig. 6B; $p > 0.05$). There were no consistent effects on f , V_T or V_T/T_I responses following intraspinal ketanserin (data not shown). No significant effects on either PaCO₂ or V̇_I regulation were observed following intravenous ketanserin (Figs. 6C and 6D).

4. DISCUSSION

Our results indicate that *spinal* serotonin receptor activation is necessary for short-term modulation of the exercise ventilatory response with increased respiratory dead space in goats, and that 5HT₂-receptors are involved. Thus, *STM* represents a novel model of spinal, serotonergic modulation of a critical homeostatic control system in unanesthetized, freely behaving animals.

4.1 Short-term modulation

STM is a feature of the exercise ventilatory response revealed when resting ventilation and blood gases are altered experimentally. For example, small increases in respiratory dead space increase the exercise ventilatory response, maintaining PaCO₂ regulation with respect to its new resting level in goats (Mitchell, 1990) and humans (Poon, 1992; Wood et al., 2007). *STM* with increased dead space cannot be accounted for by changes in chemoreceptor feedback from rest to exercise (Mitchell, 1990), and is similar to the modulation observed with other experimental treatments that increase resting ventilation, including acid-base changes, hormonal alterations and manipulation of certain neurotransmitter systems (Dempsey *et al.*, 1984; Mitchell *et al.*, 1984). Although *STM* was previously shown to be serotonin-dependent (Bach *et al.*, 1993), the present study is the first demonstration that its major effect is *via spinal* serotonin receptor activation.

4.2 Drug distribution and selectivity

Attenuation of *STM* with intrathecal methysergide and ketanserin localizes the effect, but does not provide detailed information concerning the precise role played by serotonin receptor activation in the underlying mechanism. Serotonin likely affects multiple types of spinal neurons *via* actions on different receptors (Jacobs and Azmitia, 1992; McCrimmon *et al.*, 1995). Methysergide is a broad-spectrum serotonin receptor ligand, affecting most known serotonin receptor subtypes. However, the consistent results between methysergide and ketanserin suggest the probable involvement of 5-HT_{2A} receptors since ketanserin is highly selective for this serotonin receptor subtype.

Although drugs used in this study may have diffused along the spinal cord or crossed the blood brain barrier to a limited extent, effective drug concentrations were most likely restricted to the cervical/thoracic spinal cord. Intrathecal drug administration has been highly beneficial in numerous studies concerning spinal neuropharmacology (*e.g.* Yaksh and Rudy, 1976; Proudfit and Hammond, 1981; Baker-Herman and Mitchell, 2002). In such studies, hydrophilic drugs like methysergide (and morphine) remain concentrated in the CSF for long periods (Cousins and Mather, 1984). Further, cephalad flow of CSF from the spinal cord is limited; only 20–30% of spinally injected albumin reaches the brain in 12 hours in humans (Cousins and Mather, 1984). Although exercise and the quadrupedal stance of goats may exaggerate cephalad flow of CSF, our observation that Evan's Blue dye never reached the brain provides reassurance that we effectively restricted drug distribution to the intended sites via intrathecal injection.

4.3 Serotonin receptor activation is necessary for *STM*

In a previous study designed to test the hypothesis that *STM* is serotonin-dependent, methysergide was administered intravenously (1 mg/kg) prior to taking measurements during exercise with and without increased dead space (0.25 L). Ventilation and PaCO_2 regulation during normal exercise were unchanged, suggesting that serotonin receptor activation is not necessary for a normal exercise ventilatory response. The unique appearance of hypercapnia and graded ventilatory failure during exercise with increased dead space indicates that serotonergic influences are unique to *STM* (Bach *et al.*, 1993). Although these results demonstrate that serotonin receptor activation is necessary for *STM*, certain findings were not in complete agreement with predictions. For example, methysergide disrupted PaCO_2 regulation during exercise with increased dead space more than predicted based on *STM* attenuation alone. Furthermore, loss of PaCO_2 regulation resulted from a blunted exercise f response, with increased V_T and V_T/T_I responses. Both observations are contrary to predictions of the hypothesis that ventral, spinal serotonin receptor activation mediates increased respiratory drive with only (possible) secondary changes in f due to sensory receptor feedback. However, systemic methysergide administration undoubtedly affects serotonin receptor function at multiple CNS sites, many not directly involved in *STM*.

Similar to previous results with high, systemic doses of methysergide (1 mg/kg), restricted intra-spinal doses (1–2 mg total) had no significant effects on the control exercise ventilatory response, but decreased the exercise ventilatory response (with associated hypercapnia) uniquely during exercise with increased dead space (Figs. 1, 2, 4). Thus, spinal serotonin receptor activation affects the exercise ventilatory response only with increased dead space, consistent with the postulated role of these receptors in *STM*. Diminished V_T/T_I responses following spinal methysergide (Fig. 5) are consistent with the hypothesis that serotonin receptors modulate respiratory motoneuron excitability, thereby causing *STM*. By decreasing motor neuron excitability, motor output at a given descending drive associated with exercise will be reduced. On the other hand, diminished exercise f responses are not easily explained (Fig. 3). Since we believe that methysergide was restricted to spinal CSF in this study, and did not reach the brainstem at an effective concentration within the relevant time frame (*i.e.* < 30 min), we hypothesize that methysergide had unanticipated effects on spino-medullary transmission of sensory afferent inputs to the thoracic and/or cervical spinal cord. For example, antagonism of serotonergic receptors that inhibit sensory inputs to the spinal dorsal horn may indirectly influence rhythm generation (*via* subsequent projections to brainstem respiratory neurons). The relevant serotonin receptors in this hypothesis are unclear since methysergide affects many 5-HT receptors (Hoyer *et al.*, 1994; Zifa and Fillion, 1992); it is unlikely to involve 5-HT₂ receptors since similar results were not observed with ketanserin. Thus, ketanserin may antagonize 5-HT₂ receptors on spinal respiratory motoneurons, specifically, whereas methysergide may alter respiratory frequency by additional actions on different serotonin receptor subtypes in the spinal dorsal horn (*e.g.* Marlier *et al.*, 1991; Zifa and Fillion, 1992).

4.4 Did intraspinal methysergide block all *STM*?

We previously addressed concerns that *STM* results from small and difficult to detect changes in PaCO_2 regulation from rest to exercise (acting *via* chemoreceptors) by: 1) repeating measurements with a large sample size to minimize errors due to experimental variability and 2) implementing a mathematical model to represent the expected behavior of individual goats without *STM* (Mitchell, 1990). The model was used to contrast with experimental data, and to provide quantitative alternate hypotheses as suggested by Grodins (1950). The combination of experimental data and model estimates of the feedforward exercise stimulus provide compelling evidence that changes in chemoreceptor feedback from rest to exercise cannot account for *STM* (Mitchell, 1990).

This same mathematical model (Bennett and Fordyce, 1985; Mitchell, 1990) can be used to predict system behavior if intra-spinal drug administration blocks *STM* during exercise with increased dead space. Thus, data collected at rest and during exercise from individual goats were used to estimate the magnitude of the feedforward exercise stimulus during control conditions in each goat without dead space or intraspinal drug administration. When this same exercise stimulus gain is used in a subsequent simulation to predict respiratory behavior of each goat with increased dead space, but without *STM*, the predicted change in PaCO_2 from rest to exercise increases from -2.8 mmHg (without dead space) to $+1.6$ (with dead space). These predictions compare favorably with observed behavior following spinal methysergide administration with ($+2.3$ mmHg) and without (-2.8 mmHg) increased dead space. The consonance between experimental results and model predictions suggest that intraspinal methysergide completely blocked *STM* elicited by increased dead space.

4.5 Proposed Mechanism of *STM*

Our working hypothesis is that *STM* results from serotonergic modulation of respiratory motor neuron excitability. However, modulation of spinal motor neurons must result from complex respiratory network interactions. For example, we propose that elevated PaCO_2 caused by increased respiratory dead space at rest stimulates chemoreceptors and augments the activity of brainstem respiratory neurons that, in turn, drive thoracic and phrenic motor neurons and increase breathing (see Fig. 7). Simultaneously, brainstem respiratory neurons (indirectly) or hypercapnia (directly) augment serotonergic raphe neuron activity with increased dead space (Morris *et al.*, 1996; Veasey *et al.*, 1995; Wang *et al.* 1998). Caudal raphe neurons project to the spinal cord and release serotonin near respiratory motor neurons, thereby activating post-synaptic 5-HT₂ receptors and increasing motor neuron excitability (Mitchell and Babb, 2006). In this scenario, the same descending drive from respiratory pre-motor neurons associated with exercise activates more excitable motor neurons, thereby enhancing respiratory motor output and mean inspiratory flow (*ie.* V_T/T_I).

Spinal methysergide decreased breathing frequency during exercise with increased dead space in a manner not predictable based on modulation of motor neuron excitability alone. Serotonin receptor activation may exert indirect effects on respiratory rhythm generation, for example via modulation of sensory synaptic inputs in the spinal dorsal horn (Feldman *et al.*, 2008; McCrimmon *et al.*, 2008). Our present findings support this hypothesis since effective methysergide doses were most likely restricted to the spinal cord (see above). Therefore, we suggest that at least some effects of intrathecal methysergide on respiratory frequency during exercise with increased dead space arise from changes in the modulation of primary afferent synaptic pathways in the spinal dorsal horn. The net result of intrathecal methysergide administration would be disinhibition of synaptic transmission along this ascending pathway to brainstem rhythm generating neurons, reducing breathing frequency during exercise with increased dead space, when serotonergic neurons are activated. This model suggests that multiple spinal, serotonergic mechanisms are operational during *STM*.

4.6 Possible Significance of *STM*

The capacity for *STM* indicates an ability to adapt the exercise ventilatory response so that it remains appropriate when faced with different physiological or experimental circumstances. For example, *STM* may be significant in compensating for the onset of disease, helping to maintain an appropriate exercise ventilatory response despite progressively deteriorating mechanics and gas exchange. As an example, one goat prepared for study (but not included in mean data) developed a respiratory infection characterized by a productive cough and mucous drainage from the nostrils prior to experimentation. We were unable to culture the microorganism responsible for this pathology. The goat displayed only limited *STM* when dead space was added to the mask prior to drug administration (Fig. 8). Following spinal

methysergide, PaCO_2 regulation during exercise with increased dead space deteriorated, indicating a striking loss of *STM*. One interpretation of these limited data is that the sick goat was already using *STM* to offset spontaneous lung pathology. When dead space was added to the mask, regulation of PaCO_2 became difficult and was less effective than in healthy goats. Upon spinal methysergide administration, regulation was profoundly impaired because the natural, compensatory mechanism had been eliminated.

Following a single exercise trial with increased dead space, subsequent exercise ventilatory responses return to normal (Mitchell, 1990). Thus, *STM* is readily reversible and represents modulation *versus* (experience-dependent) plasticity (Mitchell and Johnson, 2003). In contrast, when goats are subjected to repeated hypercapnic exercise trials, subsequent exercise trials without the dead space cause an exaggerated exercise ventilatory response and greater hypocapnia than normal--a form of respiratory plasticity originally referred to as long-term modulation (Martin and Mitchell, 1993). Long-term modulation is serotonin-dependent (Johnson and Mitchell, 2001), suggesting that cellular/synaptic mechanisms of *STM* may initiate long-term modulation (Mitchell and Babb, 2006), much as short-term memory formation is necessary for consolidation into long-lasting memories. We predict that long-term modulation requires the same spinal serotonin receptor activation as *STM*, but that repeated activation is necessary to elicit its underlying mechanism. This hypothesis awaits experimental verification.

Acknowledgements

The National Institutes of Health (HL36780 and HL69064) supported this research; D.T. supported in part by the Wellcome Trust. We thank B. Hodgeman and K.B. Bach for excellent assistance during manuscript preparation.

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Figure 1.

Arterial PCO₂ *versus* CO₂ production at rest and during exercise before (open circles) and after methysergide administration (closed circles) (means $\pm$ SEM). Administration of methysergide to the thoracic spinal fluid (n = 7) had no significant effects on the exercise ventilatory response when wearing the mask alone (A), but decreased the exercise ventilatory response with additional dead space, thereby causing mild hypercapnia relative to the same conditions following control vehicle injection (B). Systemic methysergide at a total dose of 1–2mg (*ca.* 0.028 mg/kg) had no detectable effects on PaCO₂ with or without increased dead space (C and D). Thus, although systemic injection of methysergide at this low dose was without effect, spinal application effectively blocked short-term modulation, indicating that spinal serotonin receptors modulate ventilation during exercise with increased dead space. *Indicates that mean value post-drug is significantly different from pre-drug value ($p < 0.05$).

Figure 2.

Arterial PCO₂ (PaCO₂) and ventilation (V_I) during exercise with increased dead space before (open bars) and after (solid bars) methysergide was administered to the thoracic spinal fluid (means ± SEM). As shown in Fig. 1, PaCO₂ increased significantly during exercise with increased dead space following methysergide (A), an effect caused by decreased ventilation (B). * p < 0.05 relative to pre-drug value.

Figure 3.

Respiratory frequency (f), tidal volume (V_T) and mean inspiratory flow (V_T/T_I) during exercise with increased dead space before (open bars) and after (solid bars) spinal methysergide administration (means $\pm$ SEM). Frequency decreased significantly during exercise with increased dead space following methysergide (A) while tidal volume remained unchanged (B). Mean inspiratory flow, a measure of inspiratory drive, decreased following methysergide (C).

* $p < 0.05$ relative to pre-drug value.

Figure 4.

Spinal methysergide diminishes ventilatory compensation for increased dead space (0.2–0.3 L) in 7 goats (solid circles). Changes in the slope of the PaCO_2 versus CO_2 -production relationship caused by the addition of dead space are presented before (Pre-) and after (Post-) intraspinal (A) and intravenous (B) methysergide. Zero change indicates that short-term modulation precisely maintained PaCO_2 regulation from rest to exercise; increases from zero indicate a loss of short-term modulation. Spinal methysergide impaired short-term modulation in each goat (A, $p < 0.01$), but not systemic methysergide (B). Corresponding decreases in the change in the exercise ventilatory response elicited by increased dead space were observed following intraspinal (C, 5/7 goats; $p < 0.05$) but not systemic methysergide (D). *Indicates that the mean value post-drug is significantly different from pre-drug value ($p < 0.05$).

Spinal Methysergide (1-2mg/kg)

Figure 5.

Spinal methysergide diminishes short-term modulation *via* a decreased mean inspiratory flow response ($\Delta(V_T/TI)/\Delta \dot{V}_{CO_2}$) caused by the addition of respiratory dead space (C; $p < 0.05$). Values are represented for mask only (open bars) and with increased dead space (solid bars) before (Pre-) and after (Post-) intraspinal methysergide administration. Although spinal methysergide decreased frequency during exercise with increased dead space (Fig. 3), the apparent decrease in the slope of the frequency *versus* $\dot{V}_{CO_2}$ relationship caused by methysergide did not attain significance (B). Methysergide also had no significant effects on changes in the slopes of the relationships between V_T (A) or $TI/TTOT$ (D) and $\dot{V}_{CO_2}$ caused by the addition of dead space. *Indicates a significant change caused by the addition of dead space ($p < 0.05$). # Indicates that the change caused by dead space is different pre- *versus* post-drug administration ($p < 0.05$).

Figure 6.

Spinal ketanserin (0.25 mg/kg) diminishes compensation for dead space ($n = 6$). Mean changes in the slope of the PaCO_2 versus CO_2 production relationship caused by the addition of dead space are plotted before (Pre) and after (Post) intrathecal ketanserin. Zero change indicates that short-term modulation precisely maintained PaCO_2 regulation from rest to exercise; increases from zero indicate a loss of short-term modulation. Whereas spinal ketanserin impaired PaCO_2 regulation (panel A; $p < 0.05$), corresponding changes in the slope of the ventilation versus CO_2 production relationship showed an insignificant tendency to increase (panel B; $p > 0.05$). Systemic application of the same total dose of ketanserin had no significant effect on changes in the slopes of the PaCO_2 or VI versus CO_2 production relationship during exercise (Panels C, D). Mean values $\pm$ SEM. *Indicates a significant difference Pre- versus Post-ketanserin ($p < 0.05$).

Figure 7.

Hypothetical mechanisms modulating the exercise ventilatory response when respiratory dead space is increased. Inputs that increase resting ventilatory drive elicit short-term modulation by activating serotonergic brainstem neurons directly, or indirectly *via* integrating premotoneurons (integral sign). Descending serotonergic projections from caudal raphe (obscurus and/or pallidus) are postulated to alter the excitability of spinal respiratory motoneurons to synaptic inputs from the descending feedforward exercise stimulus *via* effects on 5-HT_2 receptors (1). A decreased VT/TI response to exercise following spinal methysergide is consistent with the hypothesis that spinal methysergide antagonized the effects of serotonin on respiratory motoneuron excitability, thereby reducing respiratory motor outflow at a given descending drive associated with exercise (Fig. 5). However, the diminished frequency during

exercise with dead space (Fig. 3) implies at least some involvement of supraspinal mechanisms since it must at some point involve the respiratory rhythm generator (signified by oscillating symbol). Since methysergide was restricted to the spinal CSF in this study, and did not reach the brainstem at an effective concentration within the relevant time frame (*i.e.* < 30 min), we suggest that methysergide affected spinomedullary transmission of sensory afferent input, possibly *via* serotonergic receptors in the spinal dorsal horn. Thus, we postulate that descending serotonergic projections (5-HT; from raphe magnus) inhibit sensory integration in the spinal dorsal horn *via* non-5-HT₂ receptors (2). Such an effect from changes in the integration of spinal sensory inputs would indirectly alter rhythm generation, thereby accounting for changes in the frequency response to exercise with increased dead space when spinal methysergide is applied.

Figure 8.

Spinal serotonin receptor antagonism with methysergide diminishes compensation for exogenous dead space to a greater extent in one goat with a spontaneous respiratory infection of unknown etiology (open circles) *versus* 7 normal goats (solid circles; same goats as in Fig. 4). Pre-drug, short-term modulation was already compromised in response to exogenous dead space in the sick goat, and spinal methysergide caused the greatest compromise of any goat similarly tested (total $n = 8$). This observation may suggest that respiratory infection, with the subsequent impairment of pulmonary gas exchange and mechanics, had already elicited short-term modulation, thus preserving an adequate exercise ventilatory response; therefore, it was most severely affected by the loss of short-term modulation post-methysergide. This goat provides suggestive evidence that short-term modulation may play an important role in compensating for progressive deterioration of mechanics and/or gas exchange during lung disease.