Published in final edited form as:

Respir Physiol Neurobiol. 2013 April 1; 186(2): 214–220. doi:10.1016/j.resp.2013.02.015.

Serotonergic mechanisms are necessary for central respiratory chemoresponsiveness *in situ*

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Abstract

Evidence from *in vivo* and *in vitro* experiments conclude that serotonin (5-HT) neurons are involved in and play an important role in central respiratory CO₂/H⁺ chemosensitivity. This study was designed to assess the importance of 5-HT neurons and 5-HT receptor activation in the frequency and amplitude components of the hypercapnic response of the respiratory network in the unanesthetized perfused *in situ* juvenile rat brainstem preparation that exhibits patterns of phrenic nerve discharge similar to breathing *in vivo*. Exposure to a hypercapnic perfusate increased phrenic burst frequency and/or amplitude, the neural correlates of breathing frequency and tidal volume *in vivo*. Hypercapnic responses were also assessed during exposure to ketanserin (5-HT₂ receptor antagonist), and 8-OH-DPAT (inhibiting 5-HT neurons via 5-HT_{1A} autoreceptors). Neither of these drugs substantially altered baseline activity, however, both abolished hypercapnic responses of the respiratory network. These data illustrate that 5-HT neurons and 5-HT receptor activation are not required for respiratory rhythm generation *per se*, but are critical for CO₂ responses *in situ*, supporting the hypothesis that 5-HT neurons play an important role in central ventilatory chemosensitivity *in vivo*.

Keywords

hypercapnia; perfused *in situ* brainstem preparation; ketanserin

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1. Introduction

Brainstem serotonin-synthesizing neurons (5-HT neurons) project widely throughout the central nervous system, and comprise a complex and broad neuromodulatory system (Jacobs and Azmitia, 1992). 5-HT neurons have been implicated in the modulation of brainstem respiratory neurons, influencing breathing in a number of ways ranging from stimulation and/or stabilization of respiratory-related rhythmogenesis *in vitro* (Pena and Ramirez, 2002; Richter et al., 2003; Ptak et al., 2009) to the generation and/or stabilization of gasping (Tryba et al., 2006; Toppin et al., 2007). Increased activity of 5-HT neurons is also known to stimulate breathing *in vivo* (Hodges and Richerson, 2008; Depuy et al., 2011).

There is now strong evidence in favor of 5-HT neurons being involved in the brain's ability to detect changes in tissue PCO₂/pH and elicit ventilatory responses that return arterial blood gases to a normal level (Richerson, 1995; Corcoran et al., 2009a). The medullary raphe, which includes a major portion of the brain's 5-HT neurons, has been identified as one of the primary sites for central chemosensitivity *in vivo* (Veasey et al. 1995; Bernard et al., 1996; Nattie and Li, 2001; Messier et al., 2002; Hodges et al., 2004; Nattie et al., 2004; Taylor et al., 2005; Penatti et al., 2006; Dias et al., 2007). Hypercapnic acidosis increases the firing rate of 5-HT neurons in rat primary cell culture and acute brainstem slices (Richerson, 1995; Wang et al., 2001). 5-HT mechanisms are linked to central chemosensitivity, as treatment with selective 5-HT reuptake inhibitors augments ventilatory responses to hypercapnia in intact animals, and pharmacologic inhibition or neurotoxic lesions of specific subsets of 5-HT neurons decrease such responses (Nattie et al., 2004; Taylor et al., 2004, 2005). These data suggest that a subset of 5-HT neurons function as respiratory chemoreceptors and alter ventilation to maintain blood gas/pH homeostasis (reviewed by Richerson, 2004). This proposed role of 5-HT neurons, and the neurotransmitter and receptor mechanisms involved, however, remain controversial (Guyenet et al., 2005; Richerson et al., 2005).

Recent findings using genetically modified mice support the conclusion that 5-HT neurons contribute to hypercapnic ventilatory responses. Adult *Lmx1b*^{fl/p} mice and male Pet-1 knock-out mice, which lack the majority of central 5-HT neurons, both display a reduction in the CO₂ response (Hodges et al., 2008, 2011). A deficit in CO₂ chemoresponsiveness in animals lacking some or all 5-HT neurons implicates 5-HT neuron-mediated mechanisms in such chemosensitivity *in vivo*. Additionally, acute silencing of 5-HT neurons in adult mice *in vivo* using expression of inhibitory G protein coupled receptors on 5-HT neurons also leads to a 50% decrease in the ventilatory response to CO₂ *in vivo* (Ray et al., 2011).

Recordings from 5-HT neurons in primary cell culture and acute brainstem slices have demonstrated very large responses to mild acidosis (Richerson, 1995; Wang et al., 1998, 2001, 2002), suggesting that these neurons may play a particularly large role in the normal response to hypercapnia. However, it has recently been suggested that the magnitude of 5-HT neuron chemosensitivity *in vivo* is smaller than the degree of chemosensitivity seen *in vitro* (Mulkey et al., 2004; Depuy et al., 2011). This argument, however, was based on studies conducted in anesthetized *in vivo* preparations in which the ventilatory response to CO₂ was severely depressed, without consideration of the potential confounding influences of anesthesia or the specific location of 5-HT neurons under investigation (see Corcoran et al., 2009a for review). The experimental system used in our current study, the unanesthetized, decerebrate arterially perfused *in situ* juvenile rat brainstem preparation (St-John and Paton, 2000), retains the integrity of intact respiratory networks *in vivo*, while avoiding potentially confounding influences of anesthesia or emergent properties of genetically modified model organisms. This preparation retains responsiveness to CO₂-induced changes in pH, albeit with lower sensitivity than the normal *in vivo* situation,

possibly due to the decerebration that is used (Day and Wilson, 2005) or to impaired pH regulation due to a decrease in CO₂ transport (Richerson and Getting, 1990). In the present study, we used the perfused *in situ* brainstem preparation and pharmacological agents to assess the importance of 5-HT neuromodulation in altering respiratory drive in response to hypercapnia. 8-OH-DPAT is commonly used in respiratory studies to inhibit 5-HT neuron transmitter release via activation of hyperpolarizing 5-HT_{1A} autoreceptors (McCall and Clement, 1989; Sharp et al., 1989; Messier et al., 2002; St-John and Paton, 2000). Thus we assessed the phrenic nerve response of our preparation to a hypercapnic challenge before and during application of 8-OH-DPAT to isolate the role of 5-HT neurons. We also evaluated the change in chemoresponse produced by ketanserin, a 5-HT₂ receptor antagonist, to determine the role of post-synaptic 5-HT₂ receptors. Together these experiments are designed to illustrate the importance of 5-HT neuron mediated contributions to chemoresponsiveness.

2. Methods

2.1. Experimental preparations

Animal husbandry and experimental procedures conformed to guidelines set by the National Institutes of Health Office of Laboratory Animal Welfare and the United States Department of Agriculture Animal Welfare Act, and were approved by the Institutional Animal Care and Use Committee of the University of Alaska Fairbanks. Juvenile male albino rats (24–30 days old; 70–140 g; Sprague-Dawley strain; Simonson Laboratories) were used for decerebrate arterially perfused *in situ* brainstem preparations, as previously described (St-John and Paton, 2000; Toppin et al., 2007). Briefly, animals were pretreated with intraperitoneal heparin sodium injection (0.7 – 1.5 ml, 1000 USP; Baxter, Deerfield, IL) to prevent blood clotting during subsequent surgery. Procedures were preceded by deep anesthesia using isoflurane. Anesthesia was assessed by the absence of a withdrawal response to firm toe pinch and cessation of spontaneous breathing movements. Anesthesia was discontinued as animals were decerebrated rostral to the superior colliculi, and subsequent procedures were conducted in the absence of anesthesia. Preparations were transected below the diaphragm and immersed in ice-chilled perfusate. Preparations were then placed either supine or prone in a recording chamber. The descending aorta was cannulated retrogradely with a double-lumen catheter and preparations were perfused with solution at a temperature of 31°C. The perfusate contained the following: MgSO₄ (1.0 mM); KH₂PO₄ (0.25 mM); KCl (4.0 mM); NaHCO₃ (25 mM); NaCl (115 mM); CaCl₂ (2.0 mM); dextrose (10 mM) and Ficoll 70 (0.1785 mM). Under baseline conditions, the perfusate was equilibrated with 95% O₂-5% CO₂ (pH ~7.4). Perfusion pressure was maintained at approximately 40–60 mmHg by adjustments in flow rate achieved through regulating the speed of a peristaltic perfusion pump. The initial 50 ml of perfusate through each preparation was discarded to eliminate blood, and the remaining volume (approximately 300 ml) was recirculated. The neuro-muscular blocker gallamine triethiodide (60 mg/l) was added to the perfusate to eliminate movement. One phrenic nerve was isolated from the diaphragm and drawn into a glass suction electrode to record efferent nerve activity (phrenic neurogram). The signal was amplified (×10,000; DAM50, WPI) and filtered (band-pass 10 Hz – 1 kHz). Data were digitized at 2 kSamples/s with a computerized data acquisition system (AD Instruments, Powerlab) directly, and again on a second channel after being passed through a moving averager (CWE; 50 ms time constant).

2.2. Treatments: pharmacological agents and hypercapnia

We administered known agonists and antagonists of the 5-HT_{1A} and 5-HT_{2A} subclasses of receptors in two experimental series. In the first series, the 5-HT_{1A} receptor agonist (*R*)-(+)-8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) was administered at a

concentration of 1.5 μM . Populations of 5-HT_{1A} receptors are located on soma and dendrites of serotonin neurons. These function as inhibitory autoreceptors stimulated by extracellular 5-HT. Activation of 5-HT_{1A} autoreceptors by 8-OH-DPAT (or 5-HT) results in hyperpolarization, decreased 5-HT neuron firing, and reduced neurotransmitter release (McCall and Clement, 1989; Sharp et al., 1989). 8-OH-DPAT is commonly used in respiratory studies to inhibit 5-HT neuron transmitter release (Messier et al., 2002; St-John and Paton, 2000). Excitatory postsynaptic 5-HT_{1A} receptors are also present on some dendrites targeted by axonal projections from 5-HT neurons, and post-synaptic action of 8-OH-DPAT must be considered when results are interpreted (Corcoran et al., 2009b; Manzke et al., 2009; Richter et al., 2004). In the second series, we administered the high affinity 5-HT_{2A} receptor antagonist ketanserin tartrate (5 μM). Both drugs were obtained from Sigma Aldrich. Doses were chosen that were not expected to disrupt eupneic burst pattern in our preparation (Toppin et al., 2007; Ptak et al., 2009), leaving intact baseline activity so that the effect on the CO₂ response could still be evaluated.

The levels of O₂ and CO₂ in perfusate were controlled by equilibrating the perfusate reservoir with appropriate gas mixtures (verified with a CO₂ analyzer; CD-3A, Applied Electrochemistry). Baseline conditions approximate normocapnic plasma *in vivo*; perfusate equilibrated with 95% O₂ - 5% CO₂ enters the aorta at PCO₂ and pH of approximately 33 mmHg and 7.4, respectively. Lacking hemoglobin, solution hyperoxia (PO₂ $\approx$ 600 mmHg) is necessary to maintain O₂ content sufficient to meet tissue metabolic demands. This unavoidable hyperoxia was constant under all conditions. Hypercapnic challenges approximating a 4% change in inspired CO₂ (perfusate equilibrated with 91% O₂ - 9% CO₂; Pco₂ 60 mmHg; pH 7.2).

2.3. Experimental protocol

Phrenic burst frequency tended to increase over time in our preparations (also reported by Day and Wilson, 2005), but stabilized after approximately 60 minutes. Therefore, respiratory motor output was monitored for 60 – 75 minutes under control conditions to ensure a stable baseline. In pilot and control experiments, repeated CO₂ elevations produced consistent and repeatable changes in phrenic burst output with hypercapnia in a given preparation (Figure 1; n=14). As such, a repeated measures design was employed, with all preparations undergoing a hypercapnic challenge both before and during drug application. Preliminary experiments revealed that 5 minutes was sufficient to reach the maximal response to this level of hypercapnia, and confirmed that there was no degradation in the response to elevated CO₂ in repeated trials.

After baseline measurements, the preparation was exposed to mild hypercapnia via the perfusate (5 minutes) and then returned to normocapnia for an additional 5 minutes. A pharmacological agent was added to the perfusate (8-OH-DPAT, n=6; ketanserin, n=8) during normocapnia. After 5 minutes, the hypercapnic challenge was repeated in the presence of the drug. Phrenic nerve activity during the final minute of normocapnia, hypercapnia, recovery normocapnia, normocapnia with drug, and hypercapnia with drug were analyzed.

2.4. Data and statistical analyses

We monitored phrenic nerve activity as an index of central respiratory output. Samples were taken for analysis for each treatment as indicated above. The moving averages of phrenic nerve discharge were quantified as duration of burst (neural inspiration, T_I, s), time between bursts (neural expiration, T_E, s), burst frequency (f_R : 60/[T_I + T_E], min⁻¹), and burst peak amplitude (a neural index of tidal volume; nV_T; Eldridge, 1971). Neural minute ventilation was calculated as the product of f_R and nV_T. The maximum response to CO₂ was calculated

as the ratio of the values in hypercapnia and normocapnia. This was repeated with data obtained before and during drug exposure. To determine if hypercapnia had an effect on respiratory motor output, and whether or not a given pharmacological treatment affected the magnitude of this response, two-way repeated-measures ANOVA were performed on absolute values for all variables except nV_T (which was recorded in arbitrary units). To establish the statistical significance of the effect of CO_2 on nV_T , the non-parametric Wilcoxon signed rank test was applied to values normalized to initial baseline values. To evaluate the effect of pharmacological treatment on baseline activity, paired t-tests were used for all parameters. Data are expressed and shown graphically as mean $\pm$ standard error of the mean. The criterion level for statistical significance was $P < 0.05$.

3. Results

3.1. Eupnea

Under initial normocapnic conditions, phrenic bursts occurred at a mean rate of 26.9 ± 1.8 bursts/minute. Mean T_I was 0.76 ± 0.08 seconds and mean T_E was 1.76 ± 0.14 seconds. Bursts exhibited the typical incrementing pattern normally associated with eupneic integrated phrenic nerve discharge in this *in situ* preparation (Paton, 1996; St-John and Paton, 2000).

3.2. 8-OH-DPAT

Baseline hypercapnic response—In the 8-OH-DPAT series, hypercapnia prior to drug administration increased neural minute ventilation to $14 \pm 5\%$ above the control value (Fig. 1). Phrenic burst frequency increased by $12 \pm 6\%$; however, no significant changes were resolved in T_E nor T_I (Fig. 2). No consistent change in nV_T was observed with hypercapnia.

Effect of 8-OH-DPAT on eupnea and the hypercapnic response—Eupneic bursts continued following administration of $1.5 \mu M$ 8-OH-DPAT. While the frequency of phrenic bursts was not altered in response to 8-OH-DPAT, T_I was significantly increased (to $15 \pm 3\%$ above control) (Fig. 3). nV_T was also significantly increased (to $14 \pm 4\%$ above control). In the presence of 8-OH-DPAT, the frequency response to hypercapnia seen in untreated preparations was absent (Fig. 2). In addition, treatment with 8-OH-DPAT abolished the increase in neural minute ventilation seen with hypercapnia (Fig. 1).

3.3. Ketanserin

Baseline hypercapnic response—An increase in CO_2 resulted in a significant increase in phrenic burst frequency prior to drug administration in the ketanserin series (to $10 \pm 4\%$ above control) (Fig. 4). This was due to a decrease in T_E (to $89 \pm 4\%$ of control). The frequency response was accompanied by a significant increase in nV_T (to $8 \pm 2\%$ above control). As a result of the increase in both frequency and nV_T , there was an increase in neural minute ventilation (to $20 \pm 4\%$ above control) (Fig. 1).

Effect of ketanserin on eupnea and the hypercapnic response—Ketanserin ($5 \mu M$) did not alter baseline eupneic burst pattern in any of the eight preparations (Fig. 3). However, this dose was sufficient to abolish the neural response previously evoked by exposure to hypercapnia (Fig. 4). Following ketanserin treatment, hypercapnia did not produce any significant change in phrenic burst parameters.

4. Discussion

4.1. Chemosensitiveness of the *in situ* preparation

Our primary objective was to determine the relative importance of 5-HT neurons and 5-HT receptor activation for central chemosensitiveness in an intact respiratory network. A first step was to confirm that our experimental system exhibited biologically relevant responses to hypercapnia, as have been identified in previous studies (St-John and Paton, 2000; Day and Wilson, 2005, 2007). We documented that the *in situ* preparation exhibited central hypercapnic responses expected of a decerebrate, vagotomized and mildly hypothermic rodent *in vivo*, without peripheral chemosensitivity (peripheral chemosensors are reported to be deactivated by hyperoxia in this preparation; Day and Wilson, 2007) (Martin-Body and Sinclair, 1987; Maskrey, 1990; Nattie et al., 1991). In our experiments, hypercapnia elicited a response by increasing respiratory frequency and neural minute ventilation. The response of this preparation to hypercapnia is much smaller than that seen *in vivo*. This is not due to the use of perfusion, since a large response to hypercapnia is preserved in the perfused guinea pig brain *in situ* (Richerson and Getting, 1990). The reasons for this are likely multifactorial, but may include a decrease in CO₂ transport by perfusate that does not contain hemoglobin or substitute (Richerson and Getting, 1990). Nonetheless, the preservation of a hypercapnic response in this preparation allows the direct study of central chemoreceptor responses in the absence of anesthesia.

4.2. 8-OH-DPAT facilitates ventilatory output

8-OH-DPAT is a commonly used 5-HT_{1A} receptor agonist that binds to somatodendritic 5-HT_{1A} autoreceptors on 5-HT neurons. 5-HT_{1A} autoreceptor activation causes hyperpolarization, resulting in reduced 5-HT neuron firing and neurotransmitter release (McCall and Clement, 1989; Sharp et al., 1989; Veasey et al., 1995). The effect of 8-OH-DPAT on baseline normocapnic activity in our preparation was relatively small. The augmentation of phrenic nerve discharge by increases in T_I and nV_T with 8-OH-DPAT treatment could be interpreted as showing that 5-HT neurons limit these variables. Alternatively, these results could be due to activation of post-synaptic 5-HT_{1A} receptors, which are expressed in several respiratory-related nuclei, including the hypoglossal motor nucleus, the retrotrapezoid nucleus and the pre-Bötzinger complex (pre-BötC; Okabe et al., 1997; Liu and Wong-Riley, 2010). 8-OH-DPAT is also a moderate affinity agonist for 5-HT₇ receptors (Bernard et al., 1993; Eriksson et al., 2008), suggested to exist in the pre-BötC (Richter et al., 2003), and activation of 5-HT₇ receptors could account for the increase in phrenic burst parameters that we observed. We have reported preliminary results showing that activation of post-synaptic 5-HT_{1A} receptors with 8-OH-DPAT in mice lacking central 5-HT neurons (thus absent 5-HT_{1A} autoreceptors) facilitates breathing (Corcoran et al., 2009b), perhaps through disinhibition of interneurons. We speculate that the mild stimulation of baseline respiratory output resulting from 8-OH-DPAT in the current study may reflect the actions of this agonist on post-synaptic 5-HT_{1A} autoreceptors and/or 5-HT₇ receptors. Regardless, this small change in baseline activity does not confound our subsequent interpretations of the effect of 8-OH-DPAT treatment on hypercapnic responsiveness.

4.3. The respiratory motor response to hypercapnia is absent in the presence of 8-OH-DPAT

Phrenic burst frequency no longer changed with hypercapnia during systemic application of 8-OH-DPAT, indicating that central chemosensitiveness is dependent on a process suppressed by 8-OH-DPAT. Potential impacts of 8-OH-DPAT on post-synaptic 5-HT_{1A} and 5-HT₇ receptors appear to facilitate ventilation (Corcoran et al., 2009b; Manzke et al., 2009; Richter et al., 2003) and would not be expected to dampen hypercapnic responsiveness.

Thus, our results are consistent with 8-OH-DPAT inhibiting 5-HT neurons (McCall and Clement, 1989; Veasey et al., 1995) and preventing their response to hypercapnia in the *in situ* perfused brain preparation. The mechanism of this effect is likely to be similar to that recently reported (Ray et al., 2011), where activation of DREADD (Designer Receptors Exclusively Activated by Designer Drugs) receptors expressed selectively in 5-HT neurons causes hyperpolarization and inhibition of 5-HT neurons by activation of Kir3, since 5-HT_{1A} receptors are also coupled to Kir3 in 5-HT neurons (Bayliss et al., 1997; Yasufuku-Takano et al., 2008). Populations of excitatory post-synaptic 5-HT_{1A} receptors are also located on neurons receiving axonal projections from 5-HT neurons (Corcoran et al., 2009b; Manzke et al., 2009; Richter et al., 2004). Post-synaptic action of 8-OH-DPAT activation of these receptors would not be expected to saturate synaptic activation of these cells and would not explain the loss of chemoresponsiveness we observe. These results, and their similarity to the influence of ketanserin (which does not activate post-synaptic 5-HT_{1A} receptors), suggest that 8-OH-DPAT treatment is acting as a pre-synaptic autoreceptor agonist. This result supports the hypothesis that 5-HT neurons are important for central chemoreception.

4.4. A ketanserin-sensitive mechanism is involved in central chemoresponsiveness

We used ketanserin, a 5-HT_{2A/C} antagonist, to identify the specific receptor subtype involved in the CO₂ response *in situ*. This receptor subclass increases neuronal excitability, and plays a critical role in respiratory-related rhythm generation and modulation *in vitro* (Pena and Ramirez, 2002; Tryba et al., 2006; Ptak et al., 2009).

Application of ketanserin to the *in situ* preparation had little effect on eupneic phrenic nerve discharge, indicating a lack of reliance of spontaneous eupneic output on 5-HT₂ receptor activation. Similarly, other studies do not report a significant effect of 5-HT₂ receptor inhibition on breathing *in vivo* (de Souza Morena et al., 2010) or on eupneic respiratory output *in situ* (Toppin et al., 2007). In contrast, 5-HT has been shown to be essential to the maintenance of stable respiratory output in brain slices *in vitro* (Pena and Ramirez, 2002; Richter et al., 2003; Ptak et al., 2009) and *in situ* (Ptak et al., 2009). The reason for this discrepancy is not clear. It has been ascribed to a greater reliance on neuromodulation through 5-HT₂ receptors in the generation of stable respiratory-related output in brain slices, where many endogenous neuromodulatory factors are absent (Toppin et al., 2007).

However, that may not explain why others have shown a requirement for 5-HT_{2A} receptor activation *in situ* (Ptak et al., 2009). It is possible that the *in situ* preparation used by Ptak et al (2009) lacks some endogenous neuromodulators that are present in our preparations. The two studies both used *in situ* preparations. However, the cerebellum is removed in the preparations described by Ptak et al (2009). It may be that cerebellar influences contribute to the maintenance of burst discharge in the absence of 5-HT_{2A} receptor activation in our hands (Xu and Frazier, 2000), while this input is not present following cerebellectomy. Similarly, the level of decerebration used by Ptak et al (2009) may be more caudal than ours, eliminating potential non-5-HT-mediated neuromodulatory projections from more rostral areas. Finally, in the present studies the doses of the pharmacological agents were selected as ones that did not greatly alter baseline burst discharge (Toppin et al., 2007), so as not to confound interpretations of chemoresponsiveness. The higher doses used by Ptak et al (2009) did clearly demonstrate that 5-HT_{2A} receptor activation is required for baseline eupneic activity *in situ*. Regardless, our use of treatments that do not greatly alter baseline eupneic activity *in situ* facilitated our assessment of the involvement of 5-HT_{2A} receptor activation in chemoresponsiveness.

Ketanserin had a significant effect on the response to elevated CO₂. Addition of ketanserin to the perfusate abolished both the frequency and amplitude responses to increased CO₂ in the *in situ* preparation. This result illustrates that central chemoresponsiveness is dependent

on a ketanserin-sensitive process. As the primary action of ketanserin is to block post-synaptic 5-HT_{2A/C} receptors, we conclude that central chemoresponsiveness is dependent on 5-HT_{2A/C} receptor activation in our preparation. Again, this result lends further support to the hypothesis that the 5-HT system is necessary for central chemoreception.

Ketanserin not only blocks 5-HT_{2A/C} receptors but also has affinity for alpha-1 adrenergic receptors (Hoyer et al., 1987) as well as limited affinity for 5-HT₇ and histamine H1 receptors (Wouters et al., 1985; Shen et al., 1993; Jasper et al., 1997). The disruption of central chemoresponsiveness in our preparation, therefore, may have resulted from a dependence of chemoresponsiveness on alpha-1 adrenergic, 5-HT₇ or histamine H1 receptor activation. However, as the primary outcome of the ketanserin treatment was similar to that resulting from 8-OH-DPAT, and that we have observed following application of the mixed 5-HT_{1,2} receptor antagonist methysergide (Harris et al., 2003), the most parsimonious conclusion is that elimination of the hypercapnic ventilatory response by ketanserin was due to inhibition of 5-HT_{2A} receptors. This has been observed regarding ketanserin's hypotensive effect, where alpha-adrenergic blockade makes a minimal contribution compared to the serotonergic system (Naslund et al., 1988).

4.5. Conclusions

Our data demonstrate that in an unanesthetized, decerebrate, and arterially perfused *in situ* preparation, administration of 8-OH-DPAT or ketanserin blocked the ventilatory response to hypercapnia. The feature unifying the pharmacological action of the two agents tested is a decrease in serotonergic neurotransmission. 8-OH-DPAT activates 5-HT_{1A} autoreceptors on 5-HT neurons, attenuating 5-HT neuron firing, and ketanserin blocks post-synaptic 5-HT₂ receptors. Taken together, our data suggest that activation of 5-HT neurons, release of 5-HT and activation of post-synaptic 5-HT₂ receptors are critical for normal chemoresponsiveness. Our results are consistent with the growing pool of data indicating that serotonin neurons, particularly those of the medullary raphe, play an important role in central respiratory chemoreception (Richerson, 2004; Dias et al., 2007; Hodges et al., 2008; Corcoran et al., 2009a; Ptak et al., 2009).

Acknowledgments

The authors would like to thank Megan Hoffman for her contribution to the reported experiments. This study was supported by the NINDS (Specialized Neuroscience Research Programs; U54 NS041069-06A1), the NICHD (P01HD36379 & R01HD052772), and the VAMC.

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Highlights

We assess the role of 5-HT neurons and receptors in the hypercapnic response of the *in situ* rat brainstem preparation.

Exposure to hypercapnia increased phrenic burst frequency and/or amplitude.

Hypercapnic responses were abolished when exposed to ketanserin (5-HT₂ receptor antagonist).

Hypercapnic responses were abolished when exposed to 8-OH-DPAT (5-HT_{1a} agonist, inhibits firing of serotonergic neurons).

Figure 1.

The effect of hypercapnia on the neural correlate of minute ventilation is blocked by 8-OH-DPAT and ketanserin. A) Data are means $\pm$ SE of hypercapnic values expressed as a percentage of normocapnic control values. Significant differences from baseline are denoted by an asterisk (*) and significant differences between untreated and treated groups are denoted by a number sign (#) (two-way RM ANOVA, $P < 0.05$). The control preparations did not receive a drug in their second hypercapnic exposure; both differed from baseline but were no different from each other. B) Raw and integrated traces of phrenic nerve activity during normocapnia (5% CO_2) and hypercapnia (9% CO_2) in a control preparation (ie. untreated).

Figure 2. 8-OH-DPAT (1.5 μM) blocks the phrenic nerve response to hypercapnia. A) Data are means $\pm$ SE of hypercapnic values expressed as a percentage of normocapnic control values. Significant difference from baseline are denoted by an asterisk (*) ($n = 6$; two-way RM ANOVA, $P < 0.05$). B) Traces of integrated phrenic nerve activity during normocapnia (5% CO_2) and hypercapnia (9% CO_2), before and during application of 1.5 μM 8-OH-DPAT (DPAT).

Figure 3. 8-OH-DPAT and ketanserin did not affect baseline phrenic burst parameters in the *in situ* preparation. Data are means $\pm$ SE of burst parameter values during pharmacological administration expressed as a percentage of baseline values. Significant differences from baseline are denoted by an asterisk (*) (paired t-test, $P < 0.05$).

Figure 4.

Ketanserin (5 μ M) blocks the phrenic nerve response to hypercapnia. A) Data are means $\pm$ SE of hypercapnic values expressed as a percentage of normocapnic control values.

Significant differences from baseline are denoted by an asterisk (*) and a number sign (#) indicates a significant difference between untreated and treated groups ($n = 8$; two-way RM ANOVA, $P < 0.05$). B) Traces of integrated phrenic nerve activity during normocapnia (5% CO_2) and hypercapnia (9% CO_2), before and during application of 5 μ M ketanserin (KET).