Hypercapnia-induced long-term depression of respiratory activity requires $\alpha_2$-adrenergic receptors

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Bach, K. B., and G. S. Mitchell. Hypercapnia-induced long-term depression of respiratory activity requires $\alpha_2$-adrenergic receptors. J. Appl. Physiol. 84(6): 2099–2105, 1998.—We investigated the effects of repeated hypercapnic episodes (inspired $\text{CO}_2$ fraction $= 0.10$) on posthypercapnic respiratory nerve discharge. Anesthetized (urethan), vagotomized, and artificially ventilated rats were presented with three consecutive 5-min episodes of hypercapnic hypercapnia, separated by 5 min of hypoxemic normocapnia (inspired $\text{O}_2$ fraction $= 0.5$). Respiratory nerve discharge and blood gases were recorded before and 30 and 60 min after the final hypercapnic episode. Posthypercapnic, arterial $\text{PCO}_2$ was maintained within 1 Torr of initial baseline values. Integrated phrenic and hypoglossal burst amplitudes decreased posthypercapnia by up to $46 \pm 17$ and $55 \pm 13$% of baseline values, respectively, and remained reduced for at least 1 h (long-term depression [LTD]). The protocol was repeated in rats pretreated with the $\alpha_2$-adrenergic antagonists yohimbine HCl (0.5 mg/kg; n = 7) or 2-[2-(2-methoxy-1,4-benzodioxanyl)]imidazoline (RX-821002) HCl (0.25 mg/kg; n = 3). Both drugs attenuated LTD in the phrenic and hypoglossal neurograms. Results indicate that episodic hypercapnia elicits a yohimbine- and RX-821002-sensitive LTD of respiratory nerve activity in rats, suggesting that LTD requires $\alpha_2$-receptor activation.

respiratory control; catecholamines; yohimbine; RX-821002; phrenic nerve; hypoglossal nerve; norepinephrine

**EPISODIC CAROTID BODY chemoreceptor activation with hypoxia, or episodic electrical stimulation of carotid sinus nerve afferent fibers, results in a long-lasting enhancement of ventilatory activity, referred to as long-term facilitation (9, 14, 20). Long-term facilitation of phrenic and hypoglossal nerve activity is dependent on serotonin receptor activation and has been observed for at least 1 h after stimulus cessation (1, 26).

Increases in arterial $\text{PCO}_2$ ($\text{PaCO}_2$; or decreases in blood pH) also activate carotid chemosensitive neurons, although not to the same degree as does hypoxia (15). If carotid chemosensitive neuron activation per se is sufficient to elicit long-term facilitation, then hypercapnia may induce the underlying mechanism. Morris et al. (29) observed long-term facilitation of phrenic nerve activity after repeated intracarotid arterial injections of $\text{CO}_2$-saturated saline in anesthetized cats. However, when carotid body-denervated cats were exposed to a single (10-min) hypercapnic episode, long-term facilitation was not observed (26). The episodic nature of the stimulus, as well as the integrity of the carotid chemosensitive neurons, may be important for the induction of long-term facilitation.

We hypothesized that episodic exposure to severe, systemic hypercapnia would elicit long-term facilitation in anesthetized rats with intact carotid bodies. Instead, we found that episodic hypercapnia ($\text{PaCO}_2 =$ 80–95 Torr) resulted in a long-lasting depression of phrenic and hypoglossal nerve activity that persisted for at least 1 h after the final hypercapnic episode. We refer to this new neural mechanism as long-term depression. The purpose of this study is to characterize this phenomenon and certain aspects of its underlying mechanism.

Hypercapnia could activate an inhibitory mechanism to override the manifestation of long-term facilitation elicited by episodic carotid chemosensitive activation. For example, it is known that hypercapnia activates brain stem noradrenergic neurons (8, 17, 19). Norepinephrine released from these neurons can inhibit respiratory output by activating $\alpha_2$-adrenergic receptors (10). We hypothesized that hypercapnia-induced release of norepinephrine overrides long-term facilitation by acting on $\alpha_2$-adrenergic receptors to cause long-term depression.

Thus the specific purposes of the present study were 1) to characterize long-term depression of hypoglossal and phrenic motor outputs after episodic hypercapnia in carotid body-intact rats and 2) to determine whether long-term depression requires activation of $\alpha_2$-adrenergic receptors. To address these questions, we studied responses to episodic hypercapnia in phrenic and hypoglossal motor activity, before and after systemic pretreatment with two $\alpha_2$-adrenergic-receptor antagonists: 17-hydroxyyohimban-16-carboxylic acid methyl ester hydrochloride (yohimbine HCl) and 2-[2-(2-methoxy-1,4-benzodioxanyl)]imidazoline hydrochloride (RX-821002 HCl).

**METHODS**

Experimental preparation. Experiments were conducted on 27 adult male rats (Harlan Sprague Dawley, Madison, WI) with weights ranging from 318 to 782 g. The animals were anesthetized initially with isoflurane (2.5–3.0% in 50% $\text{O}_2$–$\text{N}_2$) and slowly converted to urethan anesthesia (1.3–1.6 g/kg iv) over a period of ~30 min. The adequacy of anesthesia was assessed by testing corneal reflexes and blood pressure responses to toe pinch. Supplemental urethan was usually not necessary but could be administered as needed through a catheter implanted in a femoral vein. To maintain acid-base and fluid balance, a slow infusion of sodium bicarbonate (5.0%) and lactated Ringer solution (50:50, 1.7 ml·kg$^{-1}$·h$^{-1}$) was initiated 30 min after venous catheter placement.

All rats were prepared with a tracheostomy through which they were artificially ventilated (Harvard rodent respirator), and tracheal pressure was measured (Statham pressure transducer, P23-id). The lungs were hyperinflated approximately once per hour to prevent atelectasis. The rats were
paralyzed (pancuronium bromide, 2.5 mg/kg) and vagotomized bilaterally to prevent entrainment of respiratory motor outflow with the ventilator. End-tidal \( \text{PCO}_2 \) (\( \text{PETCO}_2 \)) was monitored with a flow-through capnograph (Novametrix, Wallingford, CT) with sufficient response time (<75 ms) to measure rat \( \text{PETCO}_2 \). \( \text{PETCO}_2 \) values obtained from this capnograph closely approximated \( \text{PaCO}_2 \) (usually within 1–2 Torr). Blood samples were drawn from a catheterized femoral artery to determine blood gases and pH (ABL 330; Radiometer, Copenhagen, Denmark), which were corrected to the rat’s rectal temperature. Blood pressure was also monitored at the femoral artery (Statham pressure transducer, P23-id). Rectal temperature was maintained between 37.3 and 38.3°C with a heated table.

Phrenic and hypoglossal nerves were isolated unilaterally by a dorsal approach, cut distally, and desheathed. The nerves were submerged in mineral oil and placed on bipolar silver recording electrodes. Nerve activity was amplified (>10,000; CWE BMA 831, Ardmore, PA), band-pass filtered (100 Hz-5 kHz), and integrated (Paynter filter CWE 821; time constant: 100 ms). The integrated signal was digitized (Scientific Solutions, Lab Master DMA, Solon, OH) and processed with computer software developed in “house.”

EExperimental protocol. After completion of the surgical preparation, 60 min were allowed for the nerve signal to stabilize in hyperoxia [inspired \( O_2 \) fraction (\( \text{FiO}_2 \)) = 0.50; arterial \( \text{PO}_2 \) (\( \text{Paa}_O_2 \) > 150 Torr) and normocapnia (−3 Torr above the apneic threshold; see Table 1). Baseline nerve activity was achieved by manipulating inspired \( CO_2 \) and respiratory pump rate and/or volume while \( \text{PETCO}_2 \) values were monitored until both phrenic and hypoglossal nerve activity attained low but stable levels of activity. The \( CO_2 \) thresholds for hypoglossal and phrenic nerve activity were nearly the same in these rats (−39 Torr for both nerves). The protocol began with a baseline control arterial blood sample (0.3 ml drawn into a 0.5-ml heparinized glass syringe; unused blood was injected into the animal). All subsequent blood samples were compared with this initial baseline value. Baseline nerve activity was recorded, followed by three 5-min episodes of hyperoxic hypercapnia [inspired \( CO_2 \) fraction (\( \text{FiCO}_2 \)) = 0.10; \( \text{PETCO}_2 \) = 80–95 Torr] separated by 5 min of hyperoxic normocapnia. The focus of our analysis was the poststimulus response; therefore, blood samples were not taken during hypercapnic episodes (to minimize overall blood volume removed from the animals). Nerve activities were recorded, and blood samples were drawn at 30 and 60 min posthypercapnia.

Blood pressure was maintained within 10–20 mmHg of control at all times poststimulation with intravenous fluid infusion as described previously (1). Sustained changes in blood pressure of 20 mmHg or less from control values cause little consistent change in respiratory activity in rats (1, 36).

The entire protocol was repeated in two separate groups of animals, pretreated with either the \( \alpha_2 \)-receptor antagonist yohimbine HCl (0.5 mg/kg; \( n \) = 9; 2 of the animals were studied with background normoxia instead of hypoxia) or with the more specific \( CO_2 \)-receptor antagonist RX-821002 HCl (0.25 mg/kg; \( n \) = 3). Both drugs were given intravenously (in saline vehicle) 20 min before the first hypercapnic episode. Another group of rats (\( n = 5 \)) was given systemic norepinephrine injections (0.1–10.0 µg/kg iv) to determine the ventilatory effects of circulating norepinephrine.

The protocol was repeated in a group of untreated rats exposed to more moderate episodic hypercapnia (\( \text{FiCO}_2 = 0.05; \text{PETCO}_2 = 60 \) Torr; \( n = 3 \)). Sham experiments, in which the protocol was repeated in rats that were prepared and treated identically to the other groups but were never exposed to episodic hypercapnia, were conducted previously by using this preparation (1) and showed no ventilatory effects because of time-dependent factors (e.g., changes in blood pressure or anesthetic depth).

Data analysis. Peak amplitude and frequency (bursts/min) of phrenic and hypoglossal nerve activity were averaged over 50 bursts for each recorded data point. Averaged amplitude data were then normalized as a percent change from baseline activity, and as a change expressed as a percentage of the (\( CO_2 \)-stimulated) maximum nerve activity. The latter form of normalization obviates concerns about expressing data in terms of the percent increase above an arbitrary (low) baseline value (14). Statistical analysis was conducted by using paired t-tests with the Bonferroni correction for multiple comparisons. Differences were considered significant if \( P < 0.05 \); values are described as means ± SE.

RESULTS

Episodic hypercapnia decreased poststimulus phrenic and hypoglossal nerve activities for at least 1 h after the final hypercapnic episode. As shown in Fig. 1, baseline activities were initially recorded from the phrenic and hypoglossal nerves, followed by three episodes of hypercapnia (only the first is shown). In Fig. 1A, the amplitudes of both integrated phrenic and hypoglossal nerve bursts were depressed below baseline levels at all posthypercapnic time points. This depression persisted for the 60 min observed. It is unknown how long this effect lasts or whether it is reversible because we only followed the response for 1 h. Over longer periods, we were concerned that deterioration of the preparation or of our neural recordings would obscure the behavior. Data were not included in our analyses if \( \text{PaCO}_2 \) was >1 Torr from the baseline value (Table 1). Therefore, changes in \( \text{PaCO}_2 \) are unlikely to be responsible for changes in phrenic or hypoglossal nerve activity observed after episodic hypercapnia. It is necessary to adhere to such rigid criteria because rats (as well as other mammals) respond briskly to small (1.5- to 2.0-Torr) fluctuations in \( \text{PaCO}_2 \) near the apneic threshold, which could augment or obscure the degree of long-term depression observed.

In the rat represented in Fig. 1A, detectable neural activity was in fact completely absent at 30 min posthypercapnia. Respiratory nerve activity was restored by 1 h, although at a reduced burst frequency and amplitude. A slight yet persistent decrease in blood pressure
is evident after episodic hypercapnia in this rat, although it remained within 20 mmHg of baseline at all times. Blood pressure tended to increase during the hypercapnic exposures and then return to prestimulus levels several minutes later. We did not attempt to control blood pressure during hypercapnic stimulations.

Figure 1B shows a representative recording in an animal after yohimbine pretreatment. In this rat, the amplitude and frequency of phrenic and hypoglossal bursts posthypercapnia were no longer depressed from baseline values.

These observations from individual rats were consistent with mean responses. When phrenic and hypoglossal nerve amplitudes are expressed as percent changes from baseline values (Fig. 2, A and B), the amplitudes of both nerves remained significantly depressed for at least 60 min after the final hypercapnic episode. Phrenic burst amplitude decreased by 46 ± 17 and 34 ± 15% at 30 and 60 min posthypercapnia, respectively; hypoglossal burst amplitude decreased by 55 ± 13 and 37 ± 12% at 30 and 60 min posthypercapnia, respectively (all P < 0.05). To minimize normalization artifacts caused by variable baseline nerve activities, data were also expressed as a change from baseline, normalized as a percentage of the maximal CO2-stimulated nerve burst amplitude (see Ref. 14 for discussion). Analyzed in this way, the results were qualitatively similar (data not shown). After pretreatment with yohimbine, phrenic and hypoglossal nerve amplitudes were no longer depressed after episodic hypercapnia when normalized in either manner. In fact, hypoglossal burst amplitude was significantly elevated at 1 h posthypercapnia (15 ± 6%, P < 0.01), a possible expression of long-term facilitation (1). Decreases in nerve burst frequency also played a significant role in long-term depression (Fig. 2).
Phrenic (and hypoglossal) burst frequency decreased by 21 ± 6 bursts/min at 30 min posthypercapnia (P < 0.05); at 60 min, the apparent depression was no longer significant. Rats pretreated with yohimbine showed no tendency to decrease nerve burst frequency after episodic hypercapnia. Pretreatment with another, more specific α2-antagonist (RX-821002 HCl) also attenuated long-term depression of burst amplitude (Fig. 3, A and B) and frequency (data not shown). Apparent increases in phrenic and hypoglossal burst amplitude posthypercapnia were not significant. One hour after the third hypercapnic episode, the response to a final hypercapnic episode was unchanged from values observed during episodic hypercapnia.

In one study conducted in normoxia (FIO2 < 0.21), phrenic burst amplitude and frequency still decreased 30 and 60 min after episodic hypercapnia, similar to experiments conducted in hyperoxia (data not shown). In two additional rats, yohimbine pretreatment before episodic normoxic hypercapnia revealed long-term facilitation (vs. depression) of phrenic and hypoglossal nerve activities after hypercapnia.

In experiments that consisted of episodic exposure to 3.0–5.0% inspired CO2, none of these animals (n = 3) displayed long-term depression after episodic hypercapnia (Fig. 4). Thus severe hypercapnia is necessary to elicit long-term depression.

To test the peripheral effects of norepinephrine on phrenic nerve activity in rats, we treated five animals with bolus injections of norepinephrine. The response of one rat to norepinephrine (2 µg/kg iv) is shown in Fig. 5. End-tidal CO2 was monitored and held as constant as possible. This response was qualitatively similar in all rats, regardless of the dose administered. Systemic norepinephrine resulted in a transient (~30-s) depression of phrenic and hypoglossal nerve activity coincident with an increase in blood pressure (perhaps reflecting the baroreceptor reflex). This depression was followed by a gradual rise in nerve amplitude over the next 5 min, reaching a value above baseline that lasted for at least 30 min. Blood pressure had returned to baseline values within 5 min of norepinephrine injection.

![Fig. 3. Phr (A) and XII (B) nerve amplitudes after episodic hypercapnia in RX-821002-treated rats (Δ; 0.25 mg/kg; n = 3). Change in amplitude is expressed as %baseline (prehypercapnic) amplitude. No depression was seen after hypercapnia in RX-821002-treated rats. Apparent increases in Phr and XII nerve amplitude posthypercapnia were not significant. Untreated (○; n = 7) and yohimbine-treated (●; n = 6) data. Iso-capnia was maintained within 1 Torr at all recorded data points (see Table 1).](image)

![Fig. 4. Phr (△) nerve amplitude after moderate episodic hypercapnia (inspired CO2 fraction = 0.03–0.05) in 3 rats. Change in amplitude is expressed as %baseline (prehypercapnic) amplitude. No depression was seen after episodic exposure to 3.0–5.0% CO2. Apparent increase in Phr nerve amplitude 60 min posthypercapnia is not significant. Rats exposed to 10.0% CO2 (●; n = 7) are shown for comparison. Iso-capnia was maintained within 1 Torr at all recorded data points (see Table 1).](image)

![Fig. 5. Integrated Phr neurogram from 1 rat showing response to a single intravenous bolus injection of norepinephrine (2 µg/kg; arrow). Figure illustrates that long-term response to peripherally administered norepinephrine is a gradual rise in nerve activity (after brief inhibition), making it unlikely that circulating norepinephrine is responsible for long-term depression after episodic hypercapnia.](image)
HYPERCAPNIA-INDUCED LONG-TERM DEPRESSION

DISCUSSION

This study demonstrates the existence of a unique mechanism, elicited by repeated exposures to high levels of inspired CO₂ and resulting in a long-term depression of respiratory nerve activity. This mechanism requires the activation of α₂-adrenergic receptors, although probably not acting at the peripheral chemoreceptors. The results are consistent with the hypothesis that noradrenaline is involved in the neuromodulation of upper airway (hypoglossal) as well as spinal (phrenic) respiratory motor output in rats, although the site of these effects remains uncertain.

Episodic hypercapnia depresses phrenic and hypoglossal nerve activity. Our original hypothesis was that episodic hypercapnia would elicit long-term facilitation of respiratory motor output similar to that observed after episodic hypoxia (1, 20). Carotid chemoafferent activation is sufficient to elicit long-term facilitation under most circumstances, and, because both hypercapnia and hypoxia increase the firing frequency of carotid chemoafferent neurons (2, 13), we predicted that severe hypercapnia would elicit long-term facilitation as long as the carotid body chemoreceptors were intact. Surprisingly, rats exhibited a prolonged decrease in phrenic and hypoglossal burst amplitude and frequency after episodic hypercapnia.

Our findings contrast with the results of Morris et al. (29), who observed that episodic intracarotid hypercapnia causes long-term facilitation of phrenic nerve activity in anesthetized cats. However, this apparent difference can be explained by differences in protocol, because they injected CO₂-saturated saline directly into the carotid artery, thereby activating the carotid chemoreceptors with minimal, direct effects on the central nervous system. In this way, they may have activated long-term facilitation while avoiding the potential inhibitory influence of central hypercapnia. The present study exposed rats to systemic hypercapnia, which stimulates both central and peripheral chemoreceptors, as well as exerting effects on other central nervous system structures. Thus systemic hypercapnia may activate mechanisms that underlie both long-term facilitation and long-term depression. Severe CO₂ (i.e., \( F_1_{CO_2} = 0.10 \)) may preferentially activate inhibitory mechanisms (long-term depression), creating an imbalance between excitation and inhibition that masks long-term facilitation.

The majority of our experiments were conducted in hyperoxia (\( F_1_{O_2} = 0.50 \)) to avoid exposing the rats to hypoxia during and between hypercapnic episodes. However, high background levels of O₂ may also affect the relative expression of long-term facilitation and long-term depression. Hyperoxia may have blunted the effect of hypercapnia, because increased inspired O₂ levels inhibit the action of CO₂ at the peripheral chemoreceptors (13). Nevertheless, in one animal studied during normoxia, long-term depression was still evident and indistinguishable from long-term depression produced in hyperoxia. Yohimbine blocked long-term depression in two normoxic rats exposed to episodic hypercapnia as expected but also revealed long-term facilitation of hypoglossal and phrenic nerve activity. Thus hypercapnia may be capable of eliciting both long-term facilitation and long-term depression of respiratory nerve activity: long-term depression via a central noradrenergic mechanism and long-term facilitation via peripheral chemoreceptor activation (i.e., activating raphe serotonergic neurons). Long-term depression may mask the expression of long-term facilitation unless α₂-adrenergic receptors are blocked, allowing hypercapnic activation of the peripheral chemoreceptors to be expressed (particularly during normoxic hypercapnia).

CO₂ has long been thought to stimulate central chemoreceptors via changes in extracellular fluid pH (11). Hypercapnia-induced increases in extracellular HCO₃⁻ concentration ([HCO₃⁻]), sampled from cerebrospinal fluid and homogenized brain tissue, have been associated with decreases in ventilation in awake rats (30). In principle, increased [HCO₃⁻] could persist after normocapnic conditions had been restored, thereby accounting for the decreased ventilatory activity observed in the present study. However, Nattie (30) observed that reduced ventilation occurred only after 3 h of continuous 11.0% CO₂. Ventilation did not decrease in rats after 15 min of hypercapnia, although some increase in extracellular fluid [HCO₃⁻] had already occurred by this time ([HCO₃⁻] levels were measured during the hypercapnic exposure and not after return to normocapnia). Because the hypercapnic stimuli used in the present study were short (5-min duration) and long-term depression occurred up to 1 hour after the hypercapnic stimulus was removed, it is unlikely that brain pH regulation is responsible for long-term depression. Furthermore, arterial pH was constant at all recorded data points.

Yohimbine and RX-821002 block hypercapnia-induced long-term depression. Systemic pretreatment with yohimbine blocked long-term depression of phrenic and hypoglossal nerve activity, thereby suggesting that α₂-adrenergic receptors are necessary in the underlying mechanism. Yohimbine primarily antagonizes α₂-adrenergic receptors, but it is also a suspected antagonist at serotonergic and dopaminergic receptors (34), making it difficult to conclude with certainty that the α₂-receptor is necessary for long-term depression. However, because a more specific drug, RX-821002 HCl (25), also blocked long-term depression, it seems that α₂-receptors are necessary in the underlying mechanism.

Systemic yohimbine, but not RX-821002, consistently increased baseline nerve activity. This increase was largely transient, but nerve activity usually did not completely return to baseline by the start of the hypercapnic exposures. The sustained increase in baseline nerve activity after yohimbine administration in anesthetized rats may be due to the suppression of an α₂-receptor-mediated tonic inhibitory effect, although the lack of a similar effect after RX-821002 casts doubt on this possibility. Hilaire et al. (22) hypothesized that a tonic noradrenergic depression of respiratory rhythm originates in the pons, because α₂-receptor antagonists
increase respiratory activity in in vitro neonatal rat preparations that include the pons and medulla but not in medullary preparations.

Episodic 5.0% inspired CO2 does not result in long-term depression. Because episodic exposure to 5.0% inspired CO2 did not result in long-term depression of phrenic or hypoglossal nerve activity, it appears that long-term depression requires severe episodic hypercapnia. Thus the role of long-term depression in awake, active animals is doubtful. Still, animals are often exposed to high levels of CO2 during experimental protocols, assuming, perhaps incorrectly, that there are no long-lasting effects from this treatment.

Other investigators have shown that episodes of 5.0% inspired CO2 have little effect on interepisode ventilation. For example, Gozal et al. (16) exposed normal children to episodic hypercapnia (5.0%) and saw no difference in normocapnic ventilation between episodes. However, they did not record ventilation for >5 min poststimulation, which is not long enough to observe long-term depression in rats (data not shown). Because CO2 stores had not yet reached steady state within 5 min after a hypercapnic episode, it was not possible to determine whether the mechanism of long-term depression had been elicited between hypercapnic episodes in the present study. Millhorn et al. (26) did not observe long-term depression in anesthetized cats after a single 10-min exposure to 5.0% inspired CO2. Their protocol, however, precluded making direct comparisons with the present study: 1) they used cats, a species for which there is (as yet) no evidence of long-term depression; 2) the cats used in their study were exposed to a single episode of moderate (vs. episodes of severe) hypercapnia; and 3) their animals were carotid denervated.

Systemic norepinephrine injections do not result in long-term depression. In principle, increased circulating norepinephrine could be responsible for long-term depression by acting at the peripheral chemoreceptors via inhibitory α2-receptors (24). Previous studies have shown that increases in circulating norepinephrine have different effects on ventilation depending on species. For example, norepinephrine stimulates ventilation in primates (5), but intracarotid infusions of norepinephrine depress ventilation via α-receptor activation in goats (31). The effect of systemic norepinephrine injections in anesthetized rats has not been previously investigated to our knowledge. In this study, systemic norepinephrine caused a brief depression (in s) of respiratory nerve activity, followed by a long-lasting increase, suggesting that hypercapnic stimulation of systemic norepinephrine release is not a likely explanation for long-term depression.

Possible mechanisms of long-term depression. Our working hypothesis is that episodic hypercapnia, at least in part, elicits long-term depression via activation of noradrenergic neurons with inhibitory projections to medullary sites involved in ventilatory control. Norepinephrine is produced and released from several discrete regions in the brainstem. At least two of these, the A5 nucleus (in the caudal ventrolateral pons) and the locus coeruleus (A6), increase their firing rates when exposed to hypercapnia (8, 17). Furthermore, there is evidence that the A5 region and the locus coeruleus send projections to relevant respiratory centers. Dobbins and Feldman (7) have shown that A5 and locus coeruleus neurons send direct projections to brainstem respiratory neurons, thus indicating the necessary neural pathways for an involvement in long-term depression.

Phrenic nerve activity increases when acetazolamide (a carbonic anhydrase inhibitor) is injected locally near the locus coeruleus (4), suggesting that activation of locus coeruleus noradrenergic cells by local increases in H+/CO2 facilitates (vs. depresses) respiratory motor activity. In seeming contrast to this study (4), our results suggest that increased brain CO2 or H+ levels activate a noradrenergic mechanism that ultimately inhibits phrenic and hypoglossal motor activity. Either species differences or activation of distinct noradrenergic cell groups (e.g., locus coeruleus vs. A5) may account for inhibitory vs. excitatory effects on respiratory motor output, thus accounting for these apparent differences in results.

Previous studies have shown that norepinephrine, and the α2-adrenergic receptor specifically, is involved in inhibition of respiratory nerve activity. Hilaire et al. (22) and Homma et al. (23) observed that norepinephrine tonically inhibits activity of the medullary respiratory rhythm generator via α2-receptors. Hilaire et al. (22) also hypothesized that a tonic noradrenergic depression of respiratory rhythm originates in the pons of rats because α2-receptor antagonists increase respiratory activity in in vitro neonatal rat preparations that include the pons and medulla. Bulbar respiratory neurons treated directly with norepinephrine and clonidine, an α2-receptor agonist, decrease their firing frequency (3). Inhibition of neural activity by norepinephrine may be mediated by 1) α2-autoreceptors, which regulate further norepinephrine release; 2) α2 heteroreceptors, which have been found on serotonergic raphe neurons and inhibit serotonin release (18); and/or 3) α2-receptors on a variety of postsynaptic sites (6, 28). Norepinephrine could be acting through any or all of the above α2-adrenergic receptor sites to cause a long-lasting reduction in phrenic and hypoglossal nerve activity.

Hypercapnia-induced release of norepinephrine could block long-term facilitation by acting on α2-adrenergic receptors located on raphe serotonergic neurons. Long-term facilitation is serotonin dependent in rats (1), and norepinephrine can act on raphe cell bodies and terminals to inhibit raphe excitability and serotonin release (33). Noradrenergic synaptic contacts have been identified on serotonergic neurons in the caudal raphe nuclei of the cat and rat by using immunocytochemistry (32, 35). It is possible that α2-receptor antagonists block long-term depression by antagonizing inhibitory receptors on serotonin terminals, increasing serotonin release, and enhancing long-term facilitation. In other words, α2-receptor antagonism may tip the scales from long-term depression toward long-term facilitation. This “push-pull” system makes sense from a control perspec-
tive, because long-term modulatory phenomena such as long-term facilitation and long-term depression may require a high degree of regulation to prevent wide, unchecked, and inappropriate swings in ventilatory drive.

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