Noradrenergic modulation of XII motoneuron inspiratory activity does not involve \( \alpha_2 \)-receptor inhibition of the \( I_h \) current or presynaptic glutamate release

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Submitted 7 September 2004; accepted in final form 30 November 2004

Noradrenergic modulation of XII motoneuron inspiratory activity does not involve \( \alpha_2 \)-receptor inhibition of the \( I_h \) current or presynaptic glutamate release. **J Appl Physiol** 98: 1297–1308, 2005. First published December 3, 2004; doi:10.1152/japplphysiol.00977.2004. —Noradrenaline has powerful and diverse modulatory effects on hypoglossal (XII) motoneuron activity, which is important in maintaining airway patency. The objective was to test two hypotheses that \( \alpha_2 \)-adrenoceptor-mediated, presynaptic inhibition of glutamatergic inspiratory drive (Selvaratnam SR, Parkis MA, and Funk GD. *Brain Res* 805: 104–115, 1998) and postsynaptic inhibition of the hyperpolarization-activated inward current (Hille B. *J Neurosci* 17: 49–60, 1997) modulate XII inspiratory activity. Nerve and whole cell recordings were applied to rhythmic medullary slice preparations from neonatal rats (postnatal day 0–4) to monitor XII inspiratory burst amplitude and motoneuron properties. Application of an \( \alpha_2 \)-receptor agonist (clonidine, 1 mM) to the XII nucleus reduced inspiratory burst amplitude to 71 ± 3% of control but had no effect on inspiratory synaptic currents. It also reduced the \( I_h \) current by ~40%, but an \( I_h \) current blocker (ZD7288), at concentrations that blocked ~80% of \( I_h \), had no effect on inspiratory burst amplitude. The clonidine inhibition was unaffected by the GABAA antagonist (bicuculline), but attenuated by the \( \alpha_2 \)-antagonist rauwolscine and the imidazoline 1 (I1) antagonist efaxoaxan. The I1 agonist rilmenidine, but not the \( \alpha_2 \)-agonist UK14304, inhibited XII output. Clonidine also reduced action potential amplitude or impaired repetitive firing. Although a contribution from \( \alpha_3 \) and in particular I1 receptors remains possible, results demonstrate that 1) noradrenergic modulation of XII inspiratory activity is unlikely to involve \( \alpha_2 \)-receptor-mediated presynaptic inhibition of glutamate release or modulation of \( I_h \); 2) inhibition of repetitive firing is a major factor underlying the inhibition of XII output by clonidine; and 3) \( I_h \) is present in neonatal XII motoneurons but does not contribute to shaping their inspiratory activity.

**METHODS**

**Brain slice preparation.** Experiments were performed on rhythmically active transverse medullary slices from neonatal rats ranging in age from P0 to P4 (n = 83). Techniques for preparation of rhythmically active brain slices are described in detail elsewhere (21, 61). Briefly, rats were anesthetized with diethyl ether and decerebrated, and

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Noradrenergic signaling cascades, for example, exert powerful and diverse modulatory effects on respiratory activity of XII motoneurons (20, 49, 51, 58). The predominant effect in mice is an \( \alpha_1 \)-receptor-mediated potentiation of inspiratory output that increases approximately ninelfold between postnatal day 0 (P0) and postnatal day 14 (P14). This excitatory effect is complemented by a small, \( \beta \)-receptor-mediated component and attenuated by a putative \( \alpha_2 \)-receptor-mediated inhibition of inspiratory output (58). The \( \alpha_2 \)-receptor-mediated inhibition of inspiratory output is proposed to involve \( \alpha_2C \)-receptors because these, but not \( \alpha_2A \)-receptors, are expressed in the XII nucleus (54, 63). However, the underlying mechanisms are not known. One hypothesis is that postsynaptic \( \alpha_2 \)-receptors hyperpolarize and inhibit XII motoneurons by shifting the voltage dependence of activation of the \( I_h \) current (hyperpolarization-activated inward current) toward more negative potentials (50). An alternative hypothesis is that the inhibition is due to \( \alpha_2 \)-receptor-mediated presynaptic inhibition of glutamatergic synaptic drive to XII motoneurons. Indeed, inspiratory drive to XII motoneurons is glutamatergic (21), and \( \alpha_2 \)-receptor-mediated, presynaptic inhibition of glutamatergic transmission is well documented in the nervous system (6, 7, 30, 42, 46).

Thus the primary goal of this study was to determine, using rhythmically active medullary slice preparations from neonatal rats, whether the noradrenergic modulation of XII inspiratory activity involves \( \alpha_2 \)-receptor-mediated presynaptic inhibition of glutamate release or postsynaptic modulation of the \( I_h \) current. A secondary objective was to determine the role of the \( I_h \) current in shaping the inspiratory activity of XII motoneurons. In most rhythmic motor behaviors, block of \( I_h \) attenuates both frequency and output magnitude (24, 37, 39). However, the effects on respiratory-related behavior recorded from rhythmically active medullary slices of blocking \( I_h \) are controversial. At low concentrations the \( I_h \) blocker ZD7288 has no effect on rhythm or motor output (41), whereas at higher concentrations it potentiates both frequency and burst amplitude (64). Because ZD7288 was applied to the bath in these experiments where it would affect both rhythm generating and drive transmission elements, it remains to be determined whether block of \( I_h \) solely within the motoneuron pool has any impact on inspiratory output.

**HYPOGLOSSAL** (XII) motoneurons contribute to a number of rhythmic motor behaviors that are functional from the time of birth including swallowing, coughing, chewing, and vocalization (4, 53). They also play an important role in breathing whereby they help to maintain upper airway patency during inspiration by increasing genioglossus muscle tone. Their critical role in maintaining airway patency and the fact that airway dysfunction during sleep is implicated in obstructive sleep apnea and some cases of sudden infant death syndrome, combined with their utility for examining basic questions of signal processing, underlie the intense interest in understanding the neuromodulatory control of XII motoneuron excitability (18, 45, 52, 55, 62).

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the brain stem-spinal cord was isolated in artificial cerebrospinal fluid (aCSF) containing (in mM) 120 NaCl, 3.0 KCl, 1.0 CaCl$_2$, 2.0 MgSO$_4$, 26 NaHCO$_3$, 1.25 NaH$_2$PO$_4$, 20 d-glucose, equilibrated with 95% O$_2$-5% CO$_2$, at room temperature. The brain stem-cervical cord was pinned to a wax chuck and sectioned by using a vibratome (Pelco-101, Ted Pella). A series of 100 to 200-μm sections were cut and examined for landmarks until the compact division of nucleus ambiguous was observed in at least two sections, whereon a single 700- to 800-μm section was cut. This slice extended from the caudal margin of the compact nucleus ambiguous to the obex and contained the premotor complex, rostral ventral respiratory group, XII motor nuclei, and rostral XII nerve rootlets.

For studies examining changes in XII nerve inspiratory output, slices were pinned caudal surface up in a recording chamber (10-ml volume) and superfused with aCSF (27–29°C, chamber pH between 7.50 and 7.56) (36) at a flow rate of 20 ml/min. In whole cell recording experiments, slices were perfused at 2–3 ml/min in a 0.5-ml chamber. The pH of the solution in the recording chamber was between 7.50 and 7.56 and within the range necessary for maintaining relative alkalinity. This is a particularly important factor in these experiments because, in addition to the typical concern regarding maintaining the charge of proteins within the slice, clonidine contains an imidazoline ring. Thus its chemical activity might be altered if the pH of the aCSF deviated significantly from that required to maintain relative alkalinity. Further supporting that the efficacy of clonidine persists under in vitro conditions are the observations that application of clonidine to the ventrolateral medulla causes a reduction in frequency whether applied in vivo (Ref. 12; anesthetized rats) or in vitro (Ref. 27; rhythmically active medullary slices).

Thirty minutes before the start of data collection, extracellular K$^+$ concentration was raised from 3 to 9 mM. Elevated K$^+$ was not required to activate rhythm network activity but to maintain long-term respiratory network activity (21). Although the confounding influence of elevated K$^+$ must always be considered, the possibility that it affected our conclusions is remote. Our main objectives were to determine whether clonidine presynaptically inhibited glutamatergic inspiratory activity and whether its modulation of I$_{h}$ influenced inspiratory output. Elevated K$^+$ depolarizes XII motoneurons by ~10 mV (68). This will cause a mild reduction in the driving force for the K$^+$ component of the glutamatergic current [predominantly a taurine-3-hydroxy-5-methylisoxazolopyrionic acid (AMPA)-mediated Na$^+$ and K$^+$ current (21)]. However, the reversal potential of the glutamate current will remain significantly above resting potential. Thus glutamate will still produce a depolarizing input and any attenuation of glutamate release by clonidine will appear as a reduction in XII burst amplitude. In the context of I$_{h}$, extracellular K$^+$ has minimal effect on its reversal potential and increases the slope of its current-voltage relationship (47). This will enhance I$_{h}$ magnitude and should in fact enhance our ability to detect any effect of blocking I$_{h}$ on inspiratory activity. All experiments and procedures were approved by the local animal ethics committees and performed in accordance with New Zealand and Canadian guidelines for the care, handling, and treatment of experimental animals.

Drugs and drug application. Drugs used included the α$_2$-receptor agonists clonidine (Sigma, Oakville, Ontario, Canada; 0.1–1 mM) and UK14304 (Tocris, Northpoint, Bristol, UK; 0.5 mM), the α$_2$-receptor antagonists rauwolscine (RBI, Natick, MA; 10 μM) and idazoxan (RBI; 10 μM), the organic I$_{h}$ blocker ZD7288 [N-ethyl-1,6-dihydro-1,2,3-dimethyl-6-(methylamino)-N-phenyl-4-pyrimidinamine; Tocris; 0.1–0.5 mM], the imidazoline 1 (I$_1$) receptor agonist rilmenidine (RBI; 1 mM), the I$_{h}$ antagonist efaroxan (RBI; 10 μM), and the GABA$_A$ antagonist (+)-bicuculline (Sigma; 10 μM). Most of drugs were made up as concentrated stock in aCSF solution, then diluted with aCSF containing 9 mM K$^+$, aliquoted into 1-ml Eppendorf tubes, and frozen for future use. UK14304 was made up as 100 mM stock in DMSO. (+)-Bicuculline was dissolved in chloroform to make 20 mM stock solution. Effects of UK14304 and (+)-bicuculline were compared with effects of vehicle. Rauwolscine and (+)-bicuculline were made fresh before each experiment. The concentration of K$^+$ in injected solutions always matched the concentration of K$^+$ in the extracellular solution.

Clonidine, ZD7288, UK14304, and rilmenidine were pressure injected via triple-barreled pipettes (6–7 μm per barrel, outside-tip diameter) pulled from borosilicate glass capillaries (Clark Electromedical Instruments, Pangbourne, UK). Drugs were delivered at 10 psi, and injections were controlled via a programmable stimulator (Master-8, A.M.P.L., Jerusalem, Israel) linked to a solenoid. Injection pipettes were positioned over the ventromedial aspect of the XII nucleus by use of a motorized microdrive (PCM100, Newport, Irvine, CA) attached to a three-axis manual micromanipulator (M443 series, Newport).

Note that the concentrations of drugs used in the present study should not be directly compared with those in experiments in which similar agents are bath applied or applied to isolated cells. First, the concentration of drug decreases exponentially with distance from the pipette tip (43). Second, previous experiments with this preparation indicate that drug concentration must be at least 10-fold greater than the bath-applied concentration to produce similar effects (34).

Whole cell recording. Whole cell patch-clamp recordings from XII motoneurons were made under voltage conditioning by using infrared differential interference contrast microscopy. Patch electrodes (resistance 3.0–4.0 MΩ) were pulled on a horizontal puller (Sutter model P-97) from 1.2-mm outer diameter filamented borosilicate glass (Clark/WPI) and filled with potassium gluconate solution containing (in mM) 122.5 potassium gluconate, 17.5 KCl, 1 NaCl, 1 MgCl$_2$, 10 HEPES, 0.2 EGTA, 3 ATP-Mg, 0.3 GTP-Tris. Intracellular solution pH was adjusted to 7.2–7.3 with 5 M KOH. Intracellular signals were amplified and filtered (2–5 kHz low-pass Bessel filter) with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA), acquired at 8–20 kHz by using a Digidata 1200B analog-to-digital board and pClamp 6.0 software, and stored via pulse code modulation on VCR cassette (Vetter 400A).

Data analysis. Effects of drugs on XII nerve inspiratory burst amplitude were assessed with custom-written LabVIEW acquisition and analysis protocols and Excel software. The maximum inhibition induced by clonidine alone or in the presence of various antagonists was determined from a moving average of XII inspiratory burst amplitude (on the basis of 5 consecutive inspiratory events) during the 2-min control period immediately before drug onset, the 2-min period of drug application, and 5 min of drug washout. The maximum inhibition was then calculated by comparing the smallest value of the moving average obtained during drug application to the smallest value of the moving average obtained during control period. To determine whether a given effect was significant, the maximum inhibition induced by a given drug was compared with the inhibition produced by injection of vehicle into the same location.

To address the possibility that clonidine was acting on premotoneuron pools outside the XII nucleus, we verified that injections in the lateral segmental field within 300 μm of the lateral margin of the ventral half of the XII nucleus (n = 3), and in the dorsal portion of the...
XII nucleus \((n = 5)\), were without effect on inspiratory output. Moreover, clonidine-mediated decreases in frequency, indicative of clonidine diffusion to rhythm-generating regions in the ventrolateral medulla \((14, 15)\) were never observed.

Whole cell data were analyzed offline by using AxoGraph 4.4, Clampfit 8.0, and Excel software. Data are presented as means ± SE. All statistical analyses were performed on raw data, and differences in means were tested with Student’s or paired \(t\)-tests. Values of \(P < 0.05\) were assumed significant. Because of the slow onset of the clonidine response and evidence in the literature that the effects are slow to reverse \((50)\), most slices or motoneurons were exposed to clonidine only once. Thus the effects of rauwolscine, efaroxan, and rilmenidine were not explored with paired protocols, but by comparing the effects of clonidine in a control group with its effects in a different group. In only two sets of experiments were paired protocols used. The first compared the actions of clonidine with those of UK14304 and the order of treatment was varied. The second compared the actions of clonidine first in the presence and then the absence of (+) bicuculline.

**RESULTS**

**Clonidine inhibits XII nerve inspiratory activity in rats.** Clonidine significantly inhibits XII inspiratory burst amplitude in mice. The fact that this is mediated, at least in part, by \(\alpha_2\)-receptors was established by demonstrating that norepinephrine (NE), when applied in the presence of \(\beta\)-adrenoceptor antagonists, produces an inhibition of XII nerve inspiratory burst amplitude that is similar to the clonidine effect \((58)\). Our first objective in this study was to establish that this same inhibitory mechanism operates in rats. Clonidine was used at a high concentration \((1 \text{mM})\) to ensure maximal activation of \(\alpha_2\)-receptors throughout the XII nucleus, so that in subsequent experiments we could exclude the possibility that a negative effect on glutamatergic inputs or \(I_h\) reflected insufficient receptor activation. As shown for a single preparation in Fig. 1A, local application of clonidine \((120 \text{ s})\) over the XII nucleus produced a significant inhibition of inspiratory burst amplitude that reached a nadir during the second minute of application before returning to control \(7.5 ± 0.5 \text{ min} \,(n = 7)\) after clonidine application was stopped. Burst amplitude was reduced on average to \(71 ± 3\% \,(n = 10)\) of control. Injections of vehicle did not affect burst amplitude (Fig. 1B).

**Clonidine effects do not involve presynaptic inhibition of glutamate release.** Because inspiratory drive to XII motoneurons is mediated by glutamate acting primarily at postsynaptic AMPA receptors \((21)\), we tested the hypothesis that the clonidine-mediated inhibition of XII nerve inspiratory burst amplitude is due to presynaptic inhibition of glutamate release from XII inspiratory premotoneurons. Whole cell voltage-clamp recordings were made from inspiratory XII motoneurons. Clonidine was then locally applied, and its effects on inspiratory synaptic currents were quantified by averaging the peak synaptic current and charge transferred per inspiratory cycle for five consecutive inspiratory cycles in control, in the presence of clonidine, and during drug washout. Inspiratory synaptic currents ranged from \(-30\) to \(-500\) \text{pA} in amplitude and from 300 to 450 ms in duration and were characterized by a rapidly incrementing and slowly decrementing envelope. Clonidine \((1 \text{ mM})\) consistently produced a small outward current that averaged \(39 ± 7 \text{pA} \,(n = 6)\), as indicated in the slow time scale trace in Fig. 2A. However, comparison of the peak inspiratory current (Fig. 2, B and C, \(n = 10\)) or the charge transfer per cycle (data not shown) relative to control revealed that clonidine was without significant effect on either parameter.

**Clonidine inhibits the hyperpolarization-activated inward current \(I_h\).** To elucidate the basis of the clonidine-induced outward current (Fig. 2A), voltage ramps from \(-90\) to \(+30\) \text{mV} were delivered before, during, and after clonidine application. The control \(I-V\) relationship was subtracted from the clonidine \(I-V\) relationship to produce the \(I-V\) relationship of the clonidine-induced current. The clonidine current reversed at \(-47 ± 3 \text{ mV} \,(n = 5)\) (Fig. 3A) and was associated with a significant \(24 ± 6\% \,(n = 4)\) and \(36 ± 9\% \,(n = 8)\) increase in input resistance in response to 0.1 mM and 1.0 mM clonidine, respectively, suggesting a dose dependence to this clonidine-mediated effect. This, plus the negative slope of the clonidine \(I-V\) relationship, suggested that the clonidine current resulted from modulation of \(I_h\), which in juvenile XII motoneurons is due to a decrease in the peak amplitude of \(I_h\) as well as a hyperpolarizing shift in its voltage dependence of activation \((50)\). To confirm that clonidine also affected \(I_h\) in neonatal motoneurons, membrane potential was stepped from a holding potential of \(-50 \text{ mV} \) to \(-120 \text{ mV} \) (5 steps, first step was \(-10 \text{ mV} \), remaining steps were \(-15 \text{ mV} \)); 800-ms step duration, 20 s between pulses) to activate \(I_h\) before, during, and after local application of clonidine. Currents activated by this series of voltage steps are shown in Fig. 3B. The instantaneous current jump \((I_{\text{inst}})\) was measured immediately after the capacitive transient (at the open circle in Fig. 3B). The steady-state current \((I_{\text{steady}})\) measured at the end of the 800 ms hyperpolarizing pulse (indicated by the closed circle in Fig. 3B) revealed an inwardly rectifying conductance. As described elsewhere \((41)\), this difference between \(I_{\text{steady}}\) and \(I_{\text{inst}}\) was defined as the \(I_h\) current on the basis of its voltage dependence and sensitivity to the organic blocker, ZD7288 (see Fig. 4B).
The \( I_h \) current first became apparent with steps to \(-75\) or \(-90\) mV, and it increased in magnitude with further hyperpolarization (Fig. 3B). This is shown for pooled data in Fig. 3C (\( n = 6 \)) where the magnitude of \( I_h \) is plotted vs. command potential. The average amplitude of \( I_h \) measured at \(-120\) mV was \(-375 \pm 50\) pA (\( n = 10 \)). As shown for a single XII motoneuron in Fig. 3B, and for pooled data in Fig. 3C (0.1 mM clonidine) and 3D, \( I_h \) (measured at \(-120\) mV) was reduced to \( 0.66 \pm 0.07 \) (\( n = 6 \)) and \( 0.59 \pm 0.13 \) (\( n = 4 \)) of control by local application of 0.1 and 1.0 mM clonidine, respectively. The effects of clonidine on \( I_h \) took more than 15 min to wash out.

Modulation of \( I_h \) by clonidine does not contribute to the inhibition of XII inspiratory output. To test the hypothesis that inhibition of \( I_h \) contributes to the clonidine-mediated inhibition of XII nerve inspiratory output, we examined the effects on XII

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**Fig. 2.** Clonidine-mediated inhibition of XII burst amplitude does not involve inhibition of glutamatergic inspiratory synaptic currents. 
A: long time scale recording of membrane current \( (I_m) \) in an inspiratory XII motoneuron showing that local application of clonidine over the XII nucleus induced a small outward current but had no effect on inspiratory synaptic currents. B: expanded trace of inspiratory synaptic current envelopes averaged from 5 consecutive inspiratory cycles in control (i), in clonidine (ii), and during recovery (iii). C: group data (\( n = 10 \)) showing effects of clonidine on peak inspiratory current. \( \Psi \), current-voltage (I-V) ramp or series resistance check.

**Fig. 3.** Clonidine reduces the magnitude of the hyperpolarization-activated inward (\( I_h \)) current. 
A: whole cell current-voltage relationship obtained in control and in the presence of clonidine were subtracted to give the I-V relationship of the clonidine current. B: membrane current responses to a series of hyperpolarizing pulses (800 ms) from \(-60\) to \(-120\) mV delivered in control and in the presence of 1.0 mM (top traces) or 0.1 mM (bottom traces, separate motoneuron from 1.0 mM) clonidine. \( I_h \) was measured as the difference between steady-state current measured at the end of the pulse (●) and instantaneous current jump measured immediately after the capacitive transient at the beginning of the pulse (○). C: average I-V relationship for \( I_h \) under control conditions and in the presence of 0.1 mM clonidine (\( n = 6 \)). D: histogram showing the magnitude of the \( I_h \) current evoked in 0.1 (\( n = 6 \)) and 1.0 mM (\( n = 4 \)) clonidine by a step from \(-60\) to \(-120\) mV, relative to that evoked by the same step in control. Note that different neurons were exposed to 0.1 or 1.0 mM clonidine *Significantly different from control, \( P < 0.05 \).
burst amplitude and $I_h$ amplitude of locally applying the $I_h$ channel blocker, ZD7288, to the XII nucleus. At 0.1 mM, ZD7288 blocked 80 ± 8% of $I_h$, reducing it from −377 ± 70 pA ($n = 13$) under control conditions to −40 ± 33 pA (Fig. 4, B and C). In spite of this, 0.1 mM ZD7288 had no effect on XII burst amplitude (Fig. 4, A and C). Inhibition of XII burst amplitude was only observed when the concentration of ZD7288 was increased to 0.5 mM, at which point $I_h$ was no longer detectable in individual XII motoneurons ($n = 2$) and XII burst amplitude fell significantly to 85 ± 5% of control ($n = 5$). This inhibition of XII burst amplitude peaked within 2–3 min, and recovery occurred gradually over 8–10 min.

Fig. 4. Block of the $I_h$ current by ZD7288 does not affect inspiratory burst amplitude. A: integrated recording of XII nerve inspiratory activity during local application of 0.1 mM (top trace) and 0.5 mM ZD7288 (bottom trace). B: membrane current responses to a series of hyperpolarizing pulses (800 ms) from −60 to −120 mV delivered to activate the $I_h$ current in control and in the presence of 0.1 mM ZD7288. C: graph showing the relationship between the concentration of ZD7288, XII nerve inspiratory burst amplitude (●, relative to control, $n = 5$) and the magnitude of the $I_h$ current (●; as measured in Fig. 3 and presented relative to the magnitude of $I_h$ evoked in control with a step from −60 to −120 mV). D: repetitive firing responses of a XII motoneuron to a suprathreshold current pulse (600 ms), and firing frequency vs. current relationships generated in control and after local application of 0.1 and 0.5 mM ZD7288. *Significant difference from control, $P < 0.05$. 
Reports in supraoptic neurons in superfused explants of rat hypothalamus suggest that high concentrations (30–60 \( \mu \)M in the bath) of ZD7288 caused a significant decrease in spike amplitude, and this effect was readily reversed with a wash period lasting 20–40 min, suggesting that ZD7288 has non-specific inhibitory actions on voltage-gated Na\(^+\) channels (23). We therefore examined the effects of ZD7288 on the repetitive firing properties of inspiratory XII motoneurons to help determine whether the inhibitory actions of ZD7288 at 0.5 \( \mu \)M were due to indirect actions or inhibition of the \( I_h \) current. Under current clamp conditions, motoneurons were held at \(-60\) mV and a series of 5 incrementing, depolarizing current pulses (600 ms, 20 pA increments) were delivered starting with an initial step of +200 pA. This protocol was repeated before, during, and after local application of ZD7288 to establish its effects on action potential amplitude, and the relationship between firing frequency and current (the \( f/I \) relationship). From the voltage responses to the same single current step before, during, and after ZD7288 shown in Fig. 4D, action potential amplitude and the \( f/I \) relationship were unaffected at the lower dose of 0.1 mM ZD7288 where control and ZD7288 curves overlap completely. However, at the higher dose of 0.5 mM, where effects on XII burst amplitude were apparent, ZD7288 significantly, and reversibly, reduced action potential amplitude. The effect on repetitive firing was variable. In some cells, the \( f/I \) relationship was minimally affected (Fig. 4D). However, in others the ability to fire continuously in response to a 600-ms depolarizing pulse was compromised. In these motoneurons, firing could not be sustained throughout the pulse while consecutive action potentials became progressively smaller in amplitude and longer in duration (data not shown). In summary, concentrations of ZD7228 (0.1 mM) that blocked 80 ± 8% of \( I_h \) were without effect on XII burst amplitude. Effects on XII burst amplitude were only observed at 0.5 mM where action potential amplitude or repetitive firing was inhibited.

Are the inhibitory effects of clonidine mediated by \( \alpha_2 \)-receptors? Involvement of an \( \alpha_2 \)-receptor mechanism was established previously by the observation in mice that NE inhibits XII nerve inspiratory burst amplitude in the presence of propranolol (\( \beta \)-adrenoceptor antagonist) and prazosin (\( \alpha_1 \)-adrenoceptor antagonist). We tested the involvement of \( \alpha_2 \)-receptor signaling in the inhibition in rat by determining whether the inhibition was sensitive to \( \alpha_2 \)-receptor antagonism. The \( \alpha_2 \)-receptor antagonists, rauwolscine or idazoxan (10 \( \mu \)M), were applied to the bath 30 min before the local application of clonidine (1 mM) to ensure sufficient time for diffusion into the tissue. Rauwolscine (10 \( \mu \)M), which unlike idazoxan does not bind to I\(_1\) receptors (16), blocked the inhibitory actions of clonidine (n = 6; Fig. 5A). Idazoxan (10 \( \mu \)M) did not completely block the clonidine-mediated inhibition of inspiratory output, but reduced it to 83 ± 7% (n = 5) from 71 ± 3% (n = 10; Fig. 5B) in control.

In addition to their attenuation of the clonidine inhibition, the \( \alpha_2 \)-antagonists also reduced XII inspiratory burst amplitude. Rauwolscine reduced XII inspiratory burst amplitude to 83 ± 4% of control (Fig. 5C). The effects of idazoxan alone were variable. It reduced burst amplitude in some but not all preparations, such that average values (90 ± 6% of control) were not different from control. Yohimbine, another \( \alpha_2 \)-antagonist, completely and irreversibly blocked XII nerve output (data not shown). A consequence of the fact that the \( \alpha_2 \)-

![Fig. 5](http://jap.physiology.org/Downloadedfrom)
antagonists alone had an effect on inspiratory output was that the effects of clonidine tested in the absence and presence of antagonist are not directly comparable because they were performed on different baselines of respiratory activity.

Therefore, to further test whether \( \alpha_2 \)-receptor activation inhibits XII output in rats as seen in mice (58), we compared in eight preparations the effects on XII output of clonidine and a saturating concentration of the \( \alpha_2 \)-agonist, UK14304 (0.5 mM) (2, 48). Responses of a single rhythmic slice to local application of both agonists are shown in Fig. 6A. UK14304 had no effect when applied to the same site where clonidine potently inhibited XII output. This was the case whether UK14304 preceded or followed clonidine. Pooled data similarly indicate that burst amplitude in UK14304 (94 ± 5% of control, \( n = 8 \)) was no different than in control (Fig. 6B).

**Clonidine-mediated inhibition of XII output: alternative mechanisms.** Given that inspiratory activity was not affected by either mechanism through which \( \alpha_2 \)-receptors were hypothesized to act in the XII nucleus, and that the UK14304 data directly question a role for \( \alpha_2 \)-receptors in the clonidine-mediated inhibition in rats, the mechanism(s) underlying the inhibition remains unknown. We tested three additional possibilities.

**Imidazoline receptors.** UK14303 does not inhibit XII output and is selective for \( \alpha_2 \)-receptors, whereas clonidine inhibits XII output and activates both \( \alpha_2 \)-receptors and imidazoline (I1) receptors. We therefore tested the effects of the I1 receptor antagonist, efaroxan (10 \( \mu \)M), on the clonidine inhibition. Efaroxan did not completely block the clonidine-mediated inhibition, but significantly reduced its magnitude from 71 ± 3% to 91 ± 5% of control (\( n = 6 \); Fig. 7). Local application of the I1 agonist rilmenidine over the XII nucleus also produced a significant inhibition of XII inspiratory burst amplitude (85 ± 5%) that was similar in time course but smaller in amplitude relative to that evoked by clonidine.

**Clonidine-mediated potentiation of GABAergic input?** A subpopulation of inspiratory XII motoneurons receive GABA\(_A\) receptor-mediated inhibition during inspiration (56). To test the hypothesis that potentiation of this GABAergic input (8, 56) contributes to the clonidine-mediated inhibition of XII output, we examined the effects on the clonidine-mediated inhibition of bath applying the GABA\(_A\) antagonist (+)bicuculline (10 \( \mu \)M). Bicuculline alone increased burst frequency to 160 ± 19%, and decreased burst amplitude to 77 ± 9% of control (\( n = 5 \)). However, as shown for an individual preparation and pooled data (Fig. 8), the relative inhibitory actions of clonidine on XII inspiratory burst amplitude were virtually identical in the presence (72 ± 8%, \( n = 6 \)) or absence of (+)bicuculline (75 ± 8%, \( n = 6 \)).

**Clonidine-mediated inhibition of voltage-gated Na\(^+\) channels.** Analysis of compound action potential amplitude recorded from nonmyelinated nerve fibers in rabbit suggests that high concentrations of clonidine can have anesthetic effects via block of voltage-gated Na\(^+\) channels (22). To test the potential contribution of this mechanism to the clonidine-mediated inhibition of XII inspiratory output, we repeated the repetitive firing protocol described above for ZD7288 and examined the effects of clonidine on the repetitive firing properties of inspiratory XII motoneurons. From the voltage responses to the same single current step before, during, and after clonidine shown in Fig. 9A, it is apparent that 0.1 mM clonidine is without effect on action potential amplitude or discharge frequency. At 1.0 mM, however, clonidine reduced action potential amplitude. Effects of 1.0 mM clonidine on firing frequency were variable. The slope of the \( f/I \) relationship was unaffected in some motoneurons (2/6; Fig. 9A), but the ability to fire repetitively throughout the 600-ms pulse was compromised in others (4/6; Fig. 9B). Repeating the firing protocol at 5 and 10 min after the end of the clonidine application revealed that the effects of clonidine on action potential amplitude and repetitive firing and XII inspiratory burst amplitude washed out with a similar time course. Firing responses were back to control after 10, but not 5, min of drug washout.

**DISCUSSION**

The predominant effect of NE on motoneurons is an \( \alpha_1 \)-receptor-mediated excitation (20, 49, 51, 58). However, considerable evidence supports \( \alpha_2 \)-receptor-mediated inhibition of motoneuron output. For example, \( \alpha_2 \)-receptor mechanisms contribute to the long-term depression of phrenic and XII output induced by episodic hypercapnia (3) and suppress the responses of trigeminal motoneurons to oral stimulation (29).

In addition, clonidine inhibits EMG activity and the flexor responses of spinal rats to afferent nerve volleys (65). Finally, the inspiratory output of XII motoneurons is inhibited by clonidine alone and by NE when coapplied with \( \alpha_1 \)- and \( \beta \)-adrenoceptor antagonists (58). Whether these inhibitory ac-
The space-clamp problem. However, the disadvantage of this approach, which is common to the analysis of mEPSCs, is that it is impossible to determine whether the synapses assessed with these methods are inspiratory in nature. The purpose of this study was to test whether an α2-receptor mechanism presynaptically inhibits glutamatergic inspiratory inputs. This should not imply that mEPSC analysis and evoked potentials are not of enormous value. For example, in the context of the inspiratory synapse examined here, if α2-receptor activation decreased mEPSC frequency without affecting glutamate inspiratory drive, it would suggest the differential modulation of inspiratory and noninspiratory glutamatergic inputs. Evoked potentials are also of considerable value in that, whereas the functional relevance of an evoked input (from the lateral tegmental field, for example) may be unknown, the input will be relatively stable and involve a reasonably constant pool of synapses. Evoked potential are also important in developmental studies because of the difficulty in older animals of maintaining spontaneously active networks in vitro. In summary, our analysis does not exclude the possibility that a presynaptic α2-receptor mechanism operates at noninspiratory glutamatergic synapses. It does, however, suggest that this mechanism does not modulate inspiratory inputs to XII motoneurons.

![Graph](image)

**Fig. 7.** Imidazoline receptor mechanisms may contribute to the clonidine-mediated inhibition. Group data showing the magnitude of the inhibition evoked by clonidine when applied alone and when applied (in different preparations) 30 min after bath application of the imidazoline 1 (I1) receptor antagonist efaxoxan (10 μM, n = 6). Effects of locally applying rilmenidine (1.0 mM, 120 s, n = 3) are also shown. Responses to rilmenidine and clonidine were also from separate slices. *Significant difference from sham; †significant difference from clonidine; P < 0.05.

Noradrenergic modulation of XII inspiratory activity does not involve α2-receptor-mediated presynaptic inhibition of glutamatergic drive. Presynaptic, α2-receptor-mediated inhibition of glutamatergic transmission is well documented at multiple levels of the nervous system (6, 7, 30, 42, 46). For example, glutamatergic excitatory postsynaptic potentials (EPSCs) in dorsal motor vagal neurons (6), sympathetic preganglionic neurons (42), dorsal horn neurons (46), and cultured hippocampal neurons are inhibited by activation of presynaptic α2-receptors. Clonidine also reduces the amount of glutamate that is released spontaneously from spinal synaptosomes (28) and from slices in response to capsaicin (66).

In spite of this database, our observation that clonidine, at concentrations that should completely saturate α2-receptors, has no effect on glutamatergic inspiratory currents in XII motoneurons strongly suggests that a presynaptic α2-receptor-mediated inhibition of glutamate drive does not operate at the XII preinspiratory motoneuron-to-XII motoneuron synapse. Because the inspiratory synaptic current is composed of multiple events distributed over the somatodendritic tree, we cannot exclude the possibility that an α2 inhibitory effect was masked by a postsynaptic effect of clonidine which, through increases in motoneuron Rm, effectively “boosted” synaptic currents arriving on unclamped regions of the dendritic tree and counterbalanced an α2 inhibition. We consider this possibility unlikely. Individual synapses contributing to the inspiratory current will be widely distributed over the tree, with distal synapses less effectively “space-clamped” than proximal synapses. Thus the potential boosting effect will vary between individual synapses as a function of their distance from the soma. We consider it a remote possibility that this variable amplification of distal inputs would exactly match a presynaptic inhibition of glutamate drive.

Analysis of miniature EPSC (mEPSC) amplitude and frequency distributions would not resolve this problem. Like the inspiratory current, these distributions are constructed from analysis of widely distributed inputs. Direct stimulation of synaptic inputs to the XII motoneurons and comparison of paired-pulse responses in control and clonidine would address the space-clamp problem. However, the disadvantage of this approach, which is common to the analysis of mEPSCs, is that it is impossible to determine whether the synapses assessed with these methods are inspiratory in nature. The purpose of this study was to test whether an α2-receptor mechanism presynaptically inhibits glutamatergic inspiratory inputs. This should not imply that mEPSC analysis and evoked potentials are not of enormous value. For example, in the context of the inspiratory synapse examined here, if α2-receptor activation decreased mEPSC frequency without affecting glutamate inspiratory drive, it would suggest the differential modulation of inspiratory and noninspiratory glutamatergic inputs. Evoked potentials are also of considerable value in that, whereas the functional relevance of an evoked input (from the lateral tegmental field, for example) may be unknown, the input will be relatively stable and involve a reasonably constant pool of synapses. Evoked potential are also important in developmental studies because of the difficulty in older animals of maintaining spontaneously active networks in vitro. In summary, our analysis does not exclude the possibility that a presynaptic α2-receptor mechanism operates at noninspiratory glutamatergic synapses. It does, however, suggest that this mechanism does not modulate inspiratory inputs to XII motoneurons.

![Graph](image)

**Fig. 8.** Potentiation of GABAergic transmission does not contribute to the clonidine-mediated inhibition. Integrated recording of XII nerve inspiratory activity from a rhythmic medullary slice (A) and pooled data (B) showing that the effects of locally applying clonidine (1 mM, 120 s) over the XII nucleus were the same during and after bath application of (+) bicuculline (10 μM, n = 6). Responses to clonidine in bicuculline and control were from the same slices.
MODULATION OF XII MOTONEURON ACTIVITY BY CLONIDINE

Noradrenergic modulation of XII inspiratory activity does not involve α2-receptor-mediated inhibition of the Ih current. Inwardly rectifying currents such as Ih have been characterized in diverse groups of cells (47). The unique property of Ih to increase its activation on hyperpolarization is of particular interest in rhythm-generating systems for its ability to produce pacemaker-like depolarizations between consecutive oscillations. However, the depolarizing sag that follows membrane hyperpolarization and results from activation of Ih and the postinhibitory rebound that occurs with removal of the hyperpolarizing influence are important determinants of the integrative and firing properties of many nonpacemaking neurons, including motoneurons (47, 51). In XII motoneurons, Ih expression increases developmentally (5). However, a significant current is present in neonatal motoneurons through which α2-receptors, like many other receptor systems (51), could act to modulate inspiratory activity. Indeed, the observations in juvenile XII motoneurons (49) that clonidine modulates Ih in an idazoxan-sensitive manner was the basis of the hypothesis tested here, that α2-receptor-mediated inhibition of Ih in XII motoneurons inhibits inspiratory-related activity. In juveniles, clonidine has multiple effects on Ih, reducing the peak amplitude, shifting the voltage dependence of activation in the hyperpolarizing direction, and slowing the time course of activation. Clonidine also inhibited Ih in neonatal XII motoneurons. Surprisingly, however, concentrations of clonidine or ZD7288 that inhibited Ih in individual XII motoneurons were without effect on XII burst amplitude, indicating that α2-receptor-induced inhibition of Ih does not contribute to the modulation of XII inspiratory output.

Whether the effects of clonidine on Ih in neonates are similar to those described in juveniles remains unknown. It was not examined in this study because our objective was to determine whether modulation of Ih by clonidine affected inspiratory activity. With the observation that complete block of Ih did not influence inspiratory output, defining the mechanisms underlying the 40% attenuation of Ih by clonidine was not a priority.

Modulation of Ih itself does not contribute to the baseline activity of neonatal XII motoneurons. The role of Ih in modulating the inspiratory activity of XII motoneurons is controversial. As outlined by Bayliss et al. (5), Ih is normally active at rest and therefore contributes to setting the resting membrane potential (5, 67). They also proposed that Ih contributes to the subthreshold and repetitive firing behavior of motoneurons, as well as the integration of synaptic inputs. Because resting membrane potential sits on the lower portion of the Ih activation curve, Ih will be activated with only minor membrane hyperpolarization. In contrast, depolarizations will have a smaller influence on Ih magnitude. In other words, Ih may prevent prolonged hyperpolarizations in response to inhibitory inputs without compromising depolarizing responses to excitatory inputs (5). On the basis of this hypothesis, one would not predict a major influence of Ih or its modulation on excitatory inspiratory motoneuron activity in neonates in vitro. Only two studies have examined the role of Ih in modulating rhythmic inspiratory activity (41, 64). However, these studies yielded conflicting results. Moreover, they were not designed to focus specifically on Ih modulation of XII inspiratory motoneuron activity. The Ih antagonist ZD7288 was applied to the solution bathing the entire rhythmically active medullary slice preparation. Thus it was not possible to determine whether the effect on XII output was due to an action at the XII premotor-to-inspiratory motoneuron synapse or further upstream at the level of the rhythm generator or premotor network.

Nevertheless, our data in which ZD7288 was focally applied at 100 μM to the XII nucleus (approximately equivalent to 10 μM bath application) are consistent with data from late neonatal mice (P6–P11), indicating that bath application of 1–10 μM ZD7288 is without effect on rhythm or motor output (41). These data, however, are inconsistent with data from slightly older mice (P7–P22) in which bath application of 100 μM ZD7288 increased frequency and potentiated burst amplitude (64). Strain or developmental differences may account for some of the discrepancy between these two studies. However,
it is also possible that the alteration of respiratory activity in the latter study reflects the higher concentration of ZD7288 and actions via mechanisms other than inhibition of \( I_h \). For example, it is now apparent that ZD7288 also inhibits voltage-gated Na\(^+\) channels, reduces AMPA and N-methyl-D-aspartate glutamate receptor currents (9), reduces T-type calcium currents in expression systems (17), and shortens the interspike interval in neurons (38), possibly by modulating calcium-dependent K\(^+\) channels.

Regardless of the reasons underlying the differences between these earlier studies in mice, our data demonstrate at the level of the XII nucleus that modulation of \( I_h \) does not contribute to inspiratory burst pattern formation in the neonatal rat. XII burst amplitude was unaffected by concentrations of ZD7288 that almost completely blocked \( I_h \). Effects on inspiratory output were only observed when the concentration of ZD7288 was elevated to the point that it inhibited action potential amplitude and repetitive firing.

Whether a role for \( I_h \) in controlling inspiratory activity of XII motoneurons develops postnatally is not known. Certainly, the density of the \( I_h \) current increases developmentally (5). Developmental changes in the voltage dependence of activation would impact how \( I_h \) influences XII inspiratory activity, but this property does not change postnatally (5). Our measurements for the voltage dependence of \( I_h \) activation in neonatal rats appear somewhat more hyperpolarized than previously reported for juveniles. Some of this may reflect an artifact of whole cell recording. However, the majority of it likely reflects that our voltage protocol stemmed from \(-60\) to \(-75\) to \(-90\) mV. In many motoneurons, a slowly developing inward current was apparent with the step to \(-75\) mV. Thus \( I_h \) began to activate between \(-60\) and \(-75\) mV, consistent with the value of approximately \(-65\) mV reported previously (5).

Independent of changes in \( I_h \) itself, developmental changes in membrane, synaptic, and network properties, particularly the maturation of inhibitory systems and the hyperpolarization of the Cl\(^-\) reversal potential (60), mean it is possible that respiratory-related oscillations in membrane potential will fall into more hyperpolarized ranges and \( I_h \) will play a more conspicuous role in controlling inspiratory motor output. Alternatively, \( I_h \) may contribute to nonrespiratory behaviors of the XII motoneurons, such as coughing or swallowing (53), in which stronger inhibitory inputs may lower membrane potential into the relevant window.

\( \alpha_2 \)-Receptor-mediated modulation of XII motoneuron excitability? The objective of this study was to determine whether the previously demonstrated \( \alpha_2 \)-receptor-mediated inhibition of XII inspiratory output (58) was due to presynaptic inhibition of glutamate release or modulation of \( I_h \) (50). Because we used high concentrations of clonidine and clonidine affected \( I_h \), we were confident that adequate drug was delivered to the target area and therefore that neither of these two proposed mechanisms contribute to the noradrenergic modulation of XII excitability. Dialysis of intracellular components critical in signal transduction is always a concern with whole cell recording that can account for false negative data. However, dialysis was not a factor in our conclusions regarding the potential involvement of these two mechanisms. First, a presynaptic \( \alpha_2 \)-receptor-mediated inhibitory mechanism would not have been altered by dialysis of the postsynaptic cell. Second, in the context of \( I_h \), clonidine inhibited \( I_h \) under whole cell conditions, indicating that the necessary signaling cascades were still, at least partially, intact. The possibility that a clonidine-mediated potentiation of tonic or phasic inspiratory GABAergic inhibitory inputs to XII motoneurons (8, 56) contributed to the inhibition was also eliminated by the observation that the clonidine inhibition was similar in (+)bicuculline and control. However, this leaves the question as to the mechanism underlying the clonidine-mediated inhibition.

In neurons of the dorsal motor nucleus of the vagus (19), substantia gelatinosa (44), and sympathetic preganglionic neurons (26), clonidine, via \( \alpha_2 \)-receptors, induces a hyperpolarization that is accompanied by decreased R\(_N\) and has a reversal potential near equilibrium potential for K\(^+\) (\( E_{K^+} \)) that shifts with changing extracellular K\(^+\) as predicted by the Nernst equation for a K\(^+\) conductance. Although such a mechanism might contribute in XII motoneurons, we found no evidence for a clonidine-induced outward current that reduces input resistance and reverses near \( E_{K^+} \).

As mentioned previously, within motoneurons there is considerable evidence for \( \alpha_2 \)-receptor-mediated inhibition of excitability (3), including inhibition of XII inspiratory activity in neonatal mice (58). However, a similar \( \alpha_2 \)-mediated inhibition of inspiratory output in rat is difficult to reconcile with several observations. First, inhibition is only produced when high concentrations of clonidine are used. Moreover, UK14304 has no effect even when applied to the same regions where clonidine produces an inhibition. The attenuation of the clonidine inhibition by rauwolscine supports an \( \alpha_2 \) mechanism because it, unlike idazoxan, is selective for \( \alpha_2 \) and not \( I_1 \) receptors (16). However, the rauwolscine data are compromised by the fact that rauwolscine itself inhibits XII output, which means that the actions of clonidine in control and antagonist are not directly comparable. The rauwolscine-mediated inhibition of XII output is also surprising for two reasons. First, most sources of endogenous noradrenergic inputs to XII motoneurons are not included in the rhythmic slice (1). Thus an effect of the antagonist alone is unexpected. Second, clonidine inhibits XII inspiratory output. If this effect is due to activation of \( \alpha_2 \)-receptors, inhibition of an endogenous \( \alpha_2 \)-receptor-mediated effect should manifest as an increase in burst amplitude. Thus either there is an endogenous \( \alpha_2 \)-receptor-mediated potentiation of inspiratory activity that accounts for the rauwolscine-mediated inhibition or there is an unidentified action of these antagonists on inspiratory burst amplitude that is not mediated through \( \alpha_2 \)-receptors. The former possibility is unlikely because \( \alpha_2 \)-agonists do not increase amplitude. Nonspecific actions are supported by the fact that the three \( \alpha_2 \)-antagonists used here, yohimbine, rauwolscine, and idazoxan, inhibited XII inspiratory output to different degrees. The inhibition may reflect that all three compounds can act as agonists at 5HT\(_{1A}\) receptors (11, 35, 40). In turn the greater efficacy of yohimbine in inhibiting XII inspiratory output may reflect that it, unlike rauwolscine and idazoxan, can also act as an agonist at 5HT\(_{1B}\) receptors (40), which act presynaptically to inhibit glutamatergic inputs to XII motoneurons (59) and glutamatergic inspiratory inputs to phrenic motoneurons (33).

Additional evidence suggesting that \( \alpha_2 \)-receptors are not involved in the inhibition is that at high concentration, clonidine has non-\( \alpha_2 \)-receptor-mediated effects. As described previously in rabbit (13, 22), we demonstrated in every XII
motoneuron examined that clonidine inhibited either action potential amplitude or repetitive firing. This inhibition, combined with the fact that the time course of the effect on repetitive firing was similar to the inhibitory actions of clonidine on XII inspiratory burst amplitude, suggests that a major mechanism underlying the inhibitory actions of clonidine on XII burst amplitude in neonatal rat is via nonspecific inhibition of Na⁺ channels.

Imidazoline receptor involvement must be considered because clonidine can activate I₁ receptors (16). Indeed, imidazoline receptors, as defined by [³H]rilmenidine binding, are present in the XII nucleus (31). Pharmacological data are most consistent with the activation of I₁ receptors by clonidine. First, the effects of clonidine were attenuated by the I₁ antagonist efaroxan. Second, the I₁ agonist, rilmenidine, inhibited XII inspiratory output. Note, however, that both rilmenidine and efaroxan can also act on α₂-receptors (16, 25). Inhibition of clonidine effects by rauwolscine, an α₂-antagonist that does not affect I₁ receptors (16), also questions I₁ receptor involvement, but the inhibition may have resulted from the nonspecific actions of rauwolscine. Thus, while pharmacological data more strongly support clonidine activation of I₁ receptors, data must be interpreted cautiously. It is also possible that inhibition of voltage-gated calcium currents by α₂-receptor activation, as seen in mitral cells of Xenopus and in mammalian sympathetic and raphe neurons (10, 32, 57), contributes to the inhibition of XII burst amplitude.

Thus multiple mechanisms are likely to contribute to the clonidine-mediated inhibition of XII inspiratory burst amplitude. A contribution from α₂- and I₁ receptor cascades and inhibition of voltage-gated calcium channels is possible. However, a major factor contributing to the inspiratory inhibition appears to be the disruption of repetitive firing.

In summary, these data reinforce the view that the dominant effect of NE within the XII nucleus is mediated through an α₁-receptor mechanism that increases motoneuron excitability and potentiates inspiratory output. Both mechanisms through which α₂-receptors were hypothesized to act within the XII nucleus were shown not to influence inspiratory activity. First, there is no α₂-receptor mechanism acting presynaptically to inhibit glutamate release from the inspiratory premotor-to-XII-motoneuron synapse. Second, inhibition of I₁ does not impact on the inspiratory behavior of XII motoneurons. What then is the role of α₂-receptors in the XII nucleus (54, 63)? First, the role of I₁ and its modulation may change developmentally owing to increases in Ih current density (5), as well as the maturation of membrane and network properties. α₂-Receptors may also control nonrespiratory activity of XII motoneurons or the activity of other neuromodulatory systems. Certainly, a role for α₂-receptor mechanisms in the presynaptic regulation of NE release is well established (69). It is also possible, as suggested by the observation that repeated hypercapnia leads to an α₁-receptor-dependent long-term depression of inspiratory activity in both XII and phrenic nerves (3), that an α₂-receptor-mediated effect exists but is only expressed under specific conditions or patterns of input.

ACKNOWLEDGMENTS

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