Respiratory Rhythm Generation and Synaptic Inhibition of Expiratory Neurons in Pre-Bötzinger Complex: Differential Roles of Glycinergic and GABAergic Neural Transmission

XUESI M. SHAO AND JACK L. FELDMAN

Departments of Neurobiology and Physiological Science, Systems Neurobiology Laboratory, University of California, Los Angeles, California 90095-1527

Shao, Xuesi M. and Jack L. Feldman. Respiratory rhythm generation and synaptic inhibition of expiratory neurons in pre-Bötzinger complex: differential roles of glycinergic and GABAergic neural transmission. J. Neurophysiol. 77: 1853–1860, 1997. A key distinction between neural pacemaker and conventional network models for the generation of breathing rhythm in mammals is whether phasic reciprocal inhibitory interactions between inspiratory and expiratory neurons are required. In medullary slices from neonatal rats generating respiratory-related rhythm, we measured the phasic inhibitory inputs to expiratory neurons with the use of whole cell patch clamp in the hypothesized rhythm generation site, the pre-Bötzinger complex (pre-BötC). Expiratory neurons, which generate tonic impulse activity during the expiratory period, exhibited inhibitory postsynaptic potentials (IPSPs) synchronized to the periodic inspiratory bursts of the hypoglossal nerve root (XIIIm). Bath application of the glycine receptor antagonist strychnine (STR; 5–10 μM) reversibly blocked these inspiratory-phase IPSPs, whereas the γ-aminobutyric acid-A (GABA A) receptor antagonist bicuculline (BIC; 10–100 μM) had no effect on these IPSPs. Replacing the control in vitro bathing solution with a Cl−-free solution also abolished these IPSPs. Respiratory-related rhythmic activity was not abolished when inspiratory-phase IPSPs were blocked. The frequency and strength of XIIIm rhythmic activity increased and seizure-like activity was produced when either STR, BIC, or Cl−-free solution was applied. Inspiratory-phase IPSPs were stable after establishment of whole cell patch conditions (patch pipettes contained 7 mM Cl−). Under voltage clamp, the reversal potential of inspiratory-phase inhibitory postsynaptic currents (IPSCs) was −75 mV. The current-voltage (I–V) curve for IPSCs shifted to the right when extracellular Cl−concentration was reduced by 50% (70 mM) and the reversal potential was reduced to −60 mV, close to the new Cl−Nernst potential. In tetrodotoxin (0.5 μM) under voltage clamp (holding potential = −45 mV), local application of glycine (1 mM) over pre-BötC induced an outward current and an increase in membrane conductance in expiratory neurons. The effect was blocked by bath application of STR (0.8–1 μM). Local application of the GABA A receptor agonist 4,5,6,7-tetrahydroisoaxazolo[5,4-c]pyridin-3-ol (THIP, 1 mM) induced an outward current and an increase in membrane conductance that was blocked by BIC (10–100 mM). Under voltage clamp (holding potential = −45 mV), we analyzed spontaneous IPSCs during expiration in expiratory neurons. Bath application of BIC (10 μM) reduced the IPSC frequency (from 2.2 to 0.3 per s), whereas the inspiratory-phase IPSCs did not change. Bath application of STR (8–10 μM) abolished both IPSCs. These results indicate that 1) reciprocal inhibition of expiratory neurons is glycinergic and mediated by a glycine-activated Cl−channel that is not required for respiratory-related rhythm generation in neonatal rat medullary slices; 2) endogenous GABA and glycine modulate the excitability of respiratory neurons and affect respiratory pattern in the slice preparation; 3) both glycine and GABA A receptors are found on pre-BötC expiratory neurons, and these receptors are sensitive to STR and BIC, respectively; 4) glycine and GABA A inhibitory mechanisms play different functional roles in expiratory neurons: both glycine and GABA A receptors modulate neuronal excitability, whereas glycinergic transmission alone is responsible for reciprocal inhibition; and 5) intracellular Cl−concentration in these neonatal expiratory neurons is similar to that in adults.

INTRODUCTION

The pre-Bötzinger complex (pre-BötC) in the rostroventrolateral medulla is hypothesized to be the site for respiratory rhythm generation (Feldman and Smith 1995; Smith et al. 1991, 1995). There is considerable data consistent with this hypothesis (Connelly et al. 1992; Funk et al. 1993; Johnson et al. 1994; Ramirez et al. 1996; Schwarzacher et al. 1995; Smith et al. 1991). How this rhythm is generated is a matter of intense investigation. The recent focus has been on distinguishing between two classes of models: hybrid-pacemaker versus conventional network models (Cohen 1970; Duffin et al. 1995; Feldman et al. 1990; Feldman and Smith 1995; Johnson et al. 1994; Richter et al. 1986; Smith et al. 1991). In en bloc in vitro brainstem–spinal cord preparations of neonatal rat, respiratory-related rhythm persists after synaptic inhibition is disrupted by antagonists of inhibitory neural transmitters γ-aminobutyric acid (GABA) and glycine or ion substitution affecting Cl−ion currents (Feldman and Smith 1989; Onimaru et al. 1990). These results are inconsistent with present network models, which require mutual synaptic inhibition between inspiratory and expiratory neurons to produce oscillations (Duffin 1991; Feldman and Smith 1995). In an in situ arterially perfused adult rat, reduction of synaptic inhibition by reducing Cl−concentration of artificial blood (Hayashi and Lipski 1992) alters and eventually abolishes respiratory rhythm. This result is consistent with either model (q.v. Feldman and Smith 1995). In none of these experiments were the effects of inhibitory blockade measured at the cellular/synaptic level, making interpretation (especially of the latter result) difficult (Feldman and Smith 1995). Similar results have been reported in in vivo mice, where strychnine (STR) abolishes respiratory rhythm in adults but is ineffective in neonates (Paton and Richter 1995). Two questions follow. 1) What neurotransmitter(s) and what ionic mechanism(s) are responsible for reciprocal inhibition? 2) Do antagonists of...
GABA or glycine receptors or ionic substitution really block reciprocal inhibition in neonates or in adults? We reported (Feldman and Shao 1995) that reciprocal inhibition of expiratory neurons is glycnergic and not required for rhythm generation in neonatal rat. In older mice, glycnergic mechanisms modulate but are not essential for respiratory rhythm generation (Ramirez et al. 1996). This paper extends the study of inhibitory mechanisms affecting expiratory neurons and their role in rhythm generation.

A medullary slice including pre-BötC generates respiratory-related rhythmic activity (Smith et al. 1991). This preparation allows physiological classification of neurons as respiratory related, and, compared with in vivo models, is more accessible for pharmacological manipulation. The pre-BötC contains numerous expiratory and inspiratory neurons including pacemaker neurons (Connelly et al. 1992; Johnson et al. 1994). In this paper we patch clamped expiratory neurons in pre-BötC and studied endogenously generated inhibitory postsynaptic potentials (IPSPs). We identified the ionic mechanisms of these IPSPs, the inhibitory neurotransmitter receptors on expiratory neurons, and the obligatory role of synaptic inhibition plays in respiratory rhythm generation.

METHODS

Slice preparation

Experiments were performed on a medullary slice preparation that retains functional respiratory networks and generates respiratory rhythm (Smith et al. 1991). Briefly, Sprague-Dawley neonatal rats (0–3 days old) were anesthetized with ether and decerebrated and the neuraxis was isolated. The cerebellum was removed and the brain stem was pinned down with the ventral surface facing upward and mounted in the specimen vise of a Vibratome (Technical Products International, VT 1000) oriented vertically (rostral end upward). The brain stem was sectioned serially in the transverse plane until the landmarks (e.g., nucleus ambiguous, inferior olive) at the rostral boundary of pre-BötC were visible. One transverse slice (600–750 μm thick) containing the pre-BötC was cut. The slice was transferred to a recording chamber (volume 8±10 ml) and pinned down on a Sylgard elastomer. The dissection and slicing were performed in a standard bath solution containing (in mM): 128 NaCl, 3.0 KCl, 1.5 CaCl2, 1.0 MgSO4, 23.5 NaHCO3, 0.5 NaH2PO4, and 30 glucose plus 1.0 ascorbic acid bubbled with respiratory-related pre-BoÈtC neurons are categorized as expiratory. Methods of superfusate flowed from dorsal to ventral to minimize the spread of KOH. In some experiments, 120 mM potassium gluconate was replaced by 120 mM cesium gluconate. Intracellular signals were amplified with a patch-clamp amplifier (AXOPATCH 1D, Axon Instruments). Electrodes were mounted on a microdrive and positioned over the pre-BötC under a surgical microscope. Positive pressure (150–180 mmHg) was applied to the electrodes as they were advanced 100 μm below the surface of the slice. The positive pressure was then released and the electrode was advanced in 1-μm steps until a rapid increase in electrode resistance was observed. A slight negative pressure was applied to form a gigahm seal (>1 GΩ). Cells were then ruptured with the use of short duration negative pressure pulses. The whole cell capacitance was compensated and the series resistance was compensated 80–95%. Cells with large, unstable access resistances (>40 MΩ), indicating inadequate membrane rupture, were not used. A −10-mV junction potential potential between bath solution and pipette filling solution was determined experimentally; reported values of potential are corrected values. To avoid variation in junction potential between the bath solution and an AgCl grounding probe, a 3 M KCl-agar bridge was used in experiments in which Cl− concentration was changed.

Drug application

For experiments with local application of GABA, or glycine agonists, double-barreled pressure ejection pipettes (tip diameter 6–9 μm per barrel, pressure 8–12 psi) were placed over the rostral surface of pre-BötC. The slices were oriented so that the direction of superfusate flowed from dorsal to ventral to minimize the spread of the drug to the XII motor nucleus and neuron populations dorsal to pre-BötC. Because recorded neurons were 100–200 μm below the slice surface and the bath solution was perfused continuously, the drug concentration at the cell was estimated to be 10% of that in the pipette (Liu et al. 1990). The composition of 0.0 mM Cl− bath solution was as follows (in mM): 128 sodium isethionate, 9.0 potassium isethionate, 0.5 NaH2PO4, 3.0 (1/2 calcium propionate), 1.0 MgSO4, 23.5 NaHCO3, and 30 glucose. The GABA A agonist 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) hydrochloride was from RBI and all other drugs [BIC, STR, glycine, and tetrodotoxin (TTX)] were from SIGMA.

RESULTS

A medullary slice from neonatal rat containing pre-BötC generates behaviorally relevant respiratory-related rhythmic motor outflow on XIln (Smith et al. 1991). In these slices, respiratory-related pre-BötC neurons are categorized as expiratory or inspiratory by their discharge pattern and membrane potential relative to rhythmic inspiratory bursts of XIln. We recorded 40 expiratory neurons in this study. These neurons generated tonic impulse activity during the period between, and were hyperpolarized by IPSPs during inspiratory bursts of XIln. The impulse activity and membrane potential were relatively constant during the expiratory period. The spiking frequency increased if we injected depolarizing current, whereas it decreased if we injected hyperpolarizing current into a neuron. To identify the neurotransmitter producing the inspiratory-phase, i.e., reciprocal, IPSPs, we bath applied 1) STR (5–10 μM), which reversibly blocked these IPSPs (n = 13, Fig. 1B); 2) BIC (10–100 μM), which had no effect on IPSPs (n = 9, Fig. 1A); for eight of these nine expiratory neurons whose IPSPs were unaffected by BIC, we then applied STR (5–10 μM), which completely blocked the IPSPs; and 3) a 0.0 mM Cl− solution (isethionate substituted...
FIG. 1. Effects of bicuculline (BIC), strychnine (STR), or Cl⁻-free solution on inspiratory-phase inhibitory postsynaptic potentials (IPSPs) of a current clamped pre-Bötzinger complex (pre-BötC) expiratory neuron and on the respiratory-related rhythmic motor outflow of the hypoglossal nerve root (XIIrn). Each record for the effect was taken 7–10 min after drug application. Action potentials are truncated. A: slice anatomy, electrophysiological recording setup, and effects of bath application of 10 μM BIC. XII, hypoglossal nucleus; NA, nucleus ambiguus. B: effects of bath application of 10 μM STR. Vm, membrane potential. C: effects of Cl⁻-free bath solution.
for Cl\(^{-}\)), which also blocked reciprocal IPSPs (n = 5, Fig. 1C). Both BIC and STR induced seizurelike activity superimposed on uninterrupted XIIIn respiratory-related rhythm, which increased in frequency and amplitude, i.e., integral of the inspiratory burst on XIIIn (Fig. 2). Increase in frequency for STR was not statistically significant (P = 0.07, n = 6, paired t-test for the effects of antagonists compared with its control for each neuron). Long-lasting intense seizurelike activity was induced by 0.0 mM Cl\(^{-}\) solution; nevertheless, regular respiratory-related rhythmic activity was still present. Reciprocal IPSPs were not blocked when cesium gluconate was substituted for potassium gluconate in the patch electrode. The amplitudes of inspiratory-phase inhibitory postsynaptic currents (IPSCs) increased when the cell membrane was voltage clamped to levels positive to end-expiratory membrane potential and decreased at more negative levels, reversing at around −75 mV (Fig. 3A). When we reduced extracellular Cl\(^{-}\) concentration by 50% (isethionate replaced Cl\(^{-}\) to 70 mM Cl\(^{-}\)), the IPSC I-V curve shifted to the right and the reversal potential was reduced to −60 mV (Fig. 3B), close to the new Cl\(^{-}\) Nernst potential. Thus the inhibitory input to expiratory neurons during inspiration is glycineric and not GABAergic, mediated by a glycine-activated Cl\(^{-}\) current. Because rhythmic outflow of XIIIn was not abolished when IPSPs were totally blocked, inspiratory-phase postsynaptic inhibition on expiratory neurons was not required for respiratory rhythm generation. The results also suggest that endogenous GABA and glycine modulate the excitability of respiratory neurons and affect respiratory pattern in neonatal medullary slice. Blockade of either GABA\(_{\text{A}}\) or glycine receptors or Cl\(^{-}\)-dependent processes induced seizurelike activity.

To verify that the effect of STR on IPSPs was mediated by glycine receptors on expiratory neurons, and also to verify the presence of GABA receptors, we locally applied agonists to preparations bathed in a solution containing 0.5 μM TTX. Under voltage clamp at −45 mV, local application of glycine (1 mM) induced an outward current (18.9 ± 8.5 pA, mean ± SD, n = 5) that was associated with an increase in membrane conductance and antagonized by bath application of STR (0.8–1.0 μM, Fig. 4A). Local application of the GABA\(_{\text{A}}\) receptor agonist THIP (1 mM) induced an outward current (27.3 ± 12.6 pA, n = 9) that was associated with an increase in membrane conductance and antagonized by BIC (10–100 μM, bath application, Fig. 4B). Chemical synaptic transmission can be blocked by high concentrations of Mg\(^{2+}\) (Henderson 1993). When we raised the Mg\(^{2+}\) concentration to 12 mM by adding MgCl\(_{2}\) to the bath, rhythmic activity from XIIIn gradually terminated within 3 min. When expiratory neurons were synaptically isolated with this solution, we obtained the same results as with TTX (n = 2, Fig. 4A).

In addition to reciprocal IPSCs synchronized to the inspiratory discharge of XIIIn, there are spontaneous background IPSCs, readily visible during expiration in expiratory neurons. We used patch pipettes filled with cesium gluconate solution to examine spontaneous IPSCs. BIC (bath; 10 μM) reduced the frequency of these IPSCs (from 2.2 to 0.3 per s, n = 2, Fig. 5B), whereas the inspiratory-phase IPSCs did not change. This suggests that BIC blocks the spontaneous IPSCs mediated by GABA\(_{\text{A}}\) receptors while the spontaneous and inspiratory-phase IPSCs mediated by glycine receptors remain. When we applied STR to the bath (8–10 μM), all IPSPs disappeared (n = 4, Fig. 5A). We presume that this effect results from the action of STR on glycine receptors as well as its cross action on GABA\(_{\text{A}}\) receptors (Shirasaki et al. 1991; Takahashi et al. 1994). Thus the actions of GABAergic and glycnergic synapses on individual pre-BöC expiratory neurons are functionally distinct, each contributing to a tonic inhibitory input that modulates neuronal excitability, whereas glycineric transmission alone is responsible for reciprocal inhibitory input.

**DISCUSSION**

We studied inhibitory synaptic mechanisms of expiratory neurons in pre-BöC of medullary slices. We found that the reciprocal, i.e., inspiratory-phase, inhibition of expiratory neurons is glycineric, mediated by postsynaptic glycine-gated Cl\(^{-}\) channels, and is not necessary for respiratory-related rhythm generation. This result is consistent with the hybrid-pacemaker model for respiratory rhythm generation (Feldman et al. 1990; Smith et al. 1995). We also found both GABA\(_{\text{A}}\) and glycine receptors present in expiratory neurons; each plays a different functional role in affecting expiratory neuron firing behavior. GABA\(_{\text{A}}\) and glycine receptors mediate tonic inhibitory modulation, whereas glycine receptors alone are responsible for reciprocal inhibition. Blockade of either class of inhibitory receptors generates seizurelike activity and increases the frequency and strength of the rhythmic activity from XIIIn.

Unlike some expiratory neurons recorded from caudal medulla of adult cat in vivo (Anders et al. 1991; Ballantyne and Richter 1986), the expiratory neurons located in the rostral medulla in this study showed no augmenting or decrementing
patterns during expiration. This difference may be due to the developmental changes in network properties, different conditions in in vivo and in vitro preparations, and/or species differences. The early expiratory decrementing wave of expiratory neuron membrane potential in cat in vivo depends on the level of anesthesia and stimulation (Ballantyne and Rich-

**FIG. 3.** A: endogenous inspiratory-phase inhibitory postsynaptic currents (IPSCs) of an expiratory neuron voltage clamped at different potentials. Bars under each trace: concurrent inspiratory bursts in XIIh. B: current-voltage curves of IPSCs with standard and reduced-Cl$^-$ (70 mM) bathing solution. Cl$^-$ was substituted by equimolar isethionate. Each data point is the mean of 3 peak IPSCs at the same voltage. Solid lines: regression lines.

**FIG. 4.** Whole cell currents induced by local application of the $\gamma$-aminobutyric acid-A (GABA$_A$) receptor agonist 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) and glycine under tetrodotoxin (TTX) or high concentration of Mg$^{2+}$ in bath solution. Cells were voltage clamped at $-45$ mV. Bars under each agonist name: time of agonist application. THIP and glycine concentrations were 1 mM in the double-barreled ejection pipette (See METHODS for the estimated agonist concentration around the cell). A: effect of bath application of 100 $\mu$M BIC on outward currents with 12 mM Mg$^{2+}$ in the bath solution to block synaptic transmission (Henderson 1993). B: effects of bath application of 0.8 $\mu$M STR and 0.8 $\mu$M STR plus 10 $\mu$M BIC in presence of 0.5 $\mu$M TTX (cesium gluconate solution in patch electrode).
FIG. 5. Effects of bath-applied STR (A) and BIC (B) on reciprocal IPSCs and spontaneous IPSCs during expiration. The patch electrode was filled with cesium gluconate solution. Cells were voltage clamped at −45 mV. Each record was triggered by XIIIn inspiratory activity. Six records were superimposed for each trace. Bar under each trace: inspiratory period. Upward spikes after inspiratory period: spontaneous IPSCs. Downward spikes: excitatory postsynaptic currents. Notice that the excitatory postsynaptic currents do not change in frequency and amplitude in either A or B.

Our results shown in Fig. 3 are consistent with the results of Mitchell and Herbert (1974) and Bianchi et al. (1988) on expiratory neurons in rostral ventral medulla of adult cat in vivo showing that inspiratory-related IPSPs are Cl− dependent. In dorsal medulla of adult cat, glycine and GABA mediate tonic postsynaptic inhibition of respiratory neurons (Haji et al. 1992). Our results on pre-BötC expiratory neurons are consistent with these findings.

In previous studies researchers have attempted to identify whether inhibitory synaptic interactions between inspiratory and expiratory neurons are required for respiratory rhythm generation (in mammals) by the application of GABA and glycine receptor antagonists or ionic substitutions (Feldman and Smith 1989; Hayashi and Lipski 1992; Onimaru et al. 1990; Paton and Richter 1995; Ramirez et al. 1996; Schmid et al. 1991). An STR-sensitive mechanism affecting respiratory rhythm generation undergoes developmental changes during early life in rodents (Paton and Richter 1995; Paton et al. 1994; Ramirez et al. 1996), with the rhythm relatively insensitive to blockade of glycine receptors in neonates. However, whether this is due to lack of glycine receptors on neonatal expiratory neurons, insensitivity of glycine receptors to STR, high intracellular Cl− concentration, or a rhythm generation process that does not require a glycine-mediated mechanism in neonates is unknown. High Cl− concentrations in spinal cord and brain stem neurons in perinates may underlie depolarizing responses to glycine or GABA (Kandler and Friauf 1995; Reichling et al. 1994; Wang et al. 1994; Wu et al. 1992). While measuring the inspiratory-phase IPSPs from neonatal raphe neurons in pre-BötC, we established that 1) there are STR-sensitive glycine receptors on expiratory neurons of neonatal rats that underlie reciprocal inhibitory neurotransmission; 2) rhythmic respiratory-related motor outflow continues unabated when this reciprocal inhibition is blocked; and 3) intraneuronal Cl− concentration was similar to adult levels. Our patch pipettes contained 7.0 mM Cl−. Intracellular Cl− concentration in adult neurons is estimated to be 5−15 mM (Alvarez-Leefmans 1990; Thompson et al. 1988). If the intracellular Cl− concentration was much higher than 7 mM, we should have observed, but did not, a gradual change from excitatory postsynaptic potentials to IPSPs or increases in amplitude of phasic IPSPs due to diffusion of the patch pipette solution into the cell following establishment of whole cell patch condition.

Bath perfusion of Cl−-free solution induced intense seizures like activity in XIIIn for up to 1−2 min, but respiratory-related rhythm persisted. These long-lasting seizures, which were more intense than those resulting from bath-applied STR or BIC (or both), likely resulted from multiple effects of Cl− reduction affecting such processes as intracellular pH regulation; cell volume regulation; Na+, K+, and Cl− cotransport (Alvarez-Leefmans 1990); and G protein modulation (Higashijima et al. 1987; Nakajima et al. 1992). The results indicating that perfusion with low-Cl− solution in an in situ brain stem−spinal cord preparation abolished respiratory activity (Hayashi and Lipski 1992) may also be due to these effects of low extracellular Cl− concentration in addition to any effects resulting from perturbation of inhibitory Cl− currents.

The expiratory neurons we recorded from may not themselves be part of the respiratory rhythm generator. If this were true, could a network rhythm-generating mechanism requiring postsynaptic inhibition remain unaffected when the phasic inhibition of these expiratory neurons was blocked? We consider this unlikely, because 1) the probability that some pre-BötC expiratory neurons are part of the rhythm generator but not one of them was in our sample should be
small; and 2) even if the respiratory neurons we recorded were not part of the rhythm generator, their pharmacological response to applied drugs should be representative of other pre-Bötzinger complex (pre-BötC) respiratory neurons with similar receptors. Thus one would have to assume that inhibitory mechanisms responsible for rhythm generation require neither glycine nor GABA receptors and are mediated by an ion other than Cl−. Yet, respiratory-related rhythm also persists when GABAergic and/or K+-dependent neurotransmission is significantly reduced in en bloc brain stem—spinal cord preparations (Feldman and Smith 1989).

Glycine functions as a major inhibitory neurotransmitter in spinal cord (Young and Macdonald 1983) and brain stem auditory structures (Caspar 1990; Kandler and Friauf 1995). For other parts of the brain, the role of glycine is not unequivocally established. Glycine receptors are present throughout the brain (Araki et al. 1988; Grenningloh et al. 1990; Malosio et al. 1991; van den Pol and Gorcs 1988). Exogenous glycine can elicit Cl− currents in dissociated neurons from various brain regions, including hypothalamus (Akaie and Kameda 1989), hippocampus and medulla (Krishtal et al. 1988), and cerebral cortex (Siebler et al. 1993), as well as from slices of cerebellum (Dieudonne 1995; Kameda et al. 1995) and hippocampus (Ito and Cherubini 1991). Whether the glycine receptors on these neurons are synaptic or extrasynaptic and whether they are physiologically active (Kameda et al. 1995) was not determined. Synchronous IPSPs in Golgi cells in the rat cerebellum can be blocked by STR (Dieudonne 1995), but the physiological function of these IPSPs is unclear. Our results indicate that the reciprocal inhibition of respiratory neurons in pre-BötC is glycnergic, providing clear evidence for an endogenous glycnergic synaptic transmission with known physiological function in mammalian brain.

We thank colleagues in the Systems Neurobiology Laboratory for helpful discussion and comments on the manuscript. This work was supported by National Heart, Lung, and Blood Institute Grant HL-40959. Address for reprint requests: X. M. Shao, Dept. of Physiological Science, Henderson, 21000: 1537 ± 162, 1992.

Received 26 August 1996; accepted in final form 24 December 1996.

REFERENCES


