

Effects of Halothane on Synaptic Neurotransmission to Medullary Expiratory Neurons in the Ventral Respiratory Group of Dogs

Eckehard A. E. Stuth, M.D.,* Mirko Krolo, M.D.,† Mislav Tonkovic-Capin, M.D.,* Francis A. Hopp, M.S.,‡ John P. Kampine, M.D., Ph.D.,§ Edward J. Zuperku, Ph.D.||

Background: The activity of canine expiratory neurons is primarily dependent on *N*-methyl-D-aspartic acid (NMDA)-receptor mediated excitatory chemodrive inputs and a powerful inhibitory gain modulatory mechanism mediated via γ -aminobutyric acid_A (GABA_A) receptors. We examined whether the depressant effect of halothane on expiratory neuronal activity is primarily caused by a reduction in glutamatergic excitation or a potentiation of the inhibitory mechanism.

Methods: Experiments were performed in halothane-anesthetized, vagotomized, paralyzed, and mechanically ventilated dogs during hypercapnic hyperoxia. The effect of a halothane dose increase from one minimum alveolar concentration (MAC) to 2 MAC on extracellularly recorded expiratory neuronal activity was studied before and during complete GABA_A receptor blockade by localized picroejection of bicuculline close to the neuron. Complete blockade of the inhibitory mechanism allowed differentiation between the effects of halothane on overall NMDA-mediated excitation and on GABA_A-mediated inhibition.

Results: The spontaneous activity of 12 expiratory neurons was significantly depressed (18.1%) by the 1-MAC halothane dose increase. Overall glutamatergic excitation was depressed

38.3 ± 12.3% (mean ± SD) by the 1-MAC halothane increase. The prevailing GABA_Aergic attenuation of neuronal output decreased significantly from 49.5 ± 10 to 32.0 ± 10.4%. Thus overall inhibition was reduced by halothane by 33.5 ± 17.2%.

Conclusions: These results suggest that the depressive effect of a 1-MAC halothane dose increase on expiratory neuronal activity in our *in vivo* preparation with an intact neural network was mainly caused by a reduction of synaptic excitatory mechanisms and not an enhancement of synaptic inhibitory mechanisms. (Key words: Brainstem; GABA; glutamate; ionotropic neurotransmitters; NMDA; respiratory centers; volatile anesthetics.)

CLINICALLY relevant concentrations of volatile anesthetics alter neuronal excitability primarily by affecting ligand-gated¹⁻³ and voltage-gated⁴⁻⁶ ion channels. Based on *in vitro* techniques in reduced preparations, it has been hypothesized that anesthetic depression of many central nervous system functions is caused by enhancement of γ -aminobutyric acid (GABA)⁷- and glycine receptor-mediated neuronal inhibition or reduction in glutamate receptor-mediated excitation.³ However, the relative importance of these effects has not been adequately tested in the central nervous system *in vivo*.

In reduced *in vitro* systems, interpretation of results is often limited by the use of neuronal tissue with mixed or poorly defined functions and the presence of highly artificial experimental conditions. This makes extrapolation of the findings to the whole mammalian central nervous system difficult. Examination of the effects of anesthetics on synaptic transmission in a spontaneously active neuronal network, in which neurotransmitters are released at levels that are present under physiologic conditions during normal function, would provide insight on the relative importance of the various underlying mechanisms in that particular network.

The brainstem circuitry for the generation and control of breathing⁸⁻¹⁰ provides a system that is spontaneously active, both during wakefulness and anesthesia. Within this system inspiratory and expiratory bulbospinal premotor neurons provide the major sources of input to the

* Assistant Professor of Anesthesiology.

† Research Fellow in Anesthesiology.

‡ Biomedical Engineer.

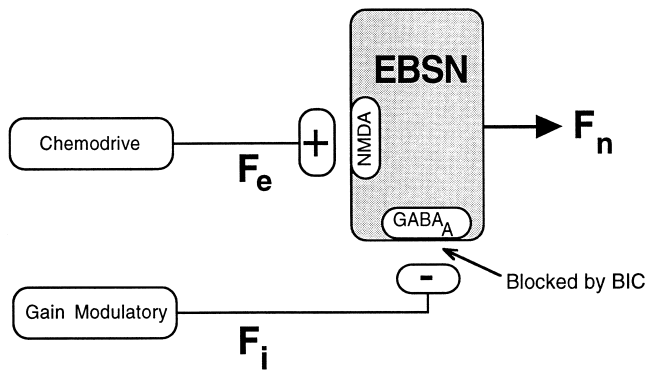
§ Professor and Chairman of Anesthesiology.

||Research Professor of Anesthesiology.

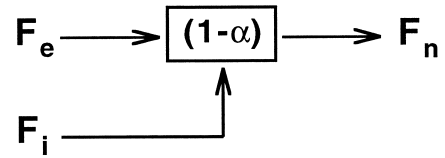
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Address reprint requests to Dr. Zuperku: Research Service/151, Zablocki VA Medical Center, Milwaukee, Wisconsin 53295. Address electronic mail to: ezuperku@mcw.edu

Synaptic Inputs to Expiratory Bulbospinal Neurons (EBSNs)



Gain Modulation Model



$$F_n = (1-\alpha)F_e$$

$$\alpha \propto F_i$$

During complete GABA_A block:
 $\alpha = 0$ and $F_n = (1-0)F_e = F_e$

Fig. 1. Synaptic scheme that was used to analyze the effects of halothane on overall synaptic neurotransmission in expiratory VRG neurons (EBSNs) during their active discharge phase in the vagotomized dog. (*Left*) The spontaneous expiratory neuron activity, F_n , is determined by the strength of excitatory (F_e) and inhibitory (F_i) inputs. (*Right*) The excitatory glutamatergic input F_e is modulated by the inhibitory GABA_A receptor input F_i in a multiplicative fashion that results in an attenuation of the neuron activity by the factor $(1 - \alpha)$, where α is an inhibitory constant that characterizes the strength of the inhibitory input. During complete block of the inhibitory input with bicuculline, α becomes zero. Thus, during complete bicuculline block, F_n reflects the neuronal activity during full transmission of the excitatory input F_e , (i.e., $F_n = F_e$). Comparing the neuron activities before and during complete bicuculline block at two different levels of anesthesia allows the calculation of the effects of the anesthetic dose increase on overall excitatory and inhibitory neurotransmission (see also table 1).

motoneurons of the respiratory musculature, such as the diaphragm, intercostal,⁸ and abdominal¹¹ muscles. The physiologic and pharmacologic responses of these respiratory premotor neurons in dogs are well characterized.¹²⁻¹⁸ The discharge activity of these neurons is highly dependent on excitatory synaptic inputs,^{15,16} and the control of the bursting discharge pattern is mediated by inhibitory synaptic inputs.^{17,18} The silent phase of inspiratory and expiratory premotor neurons is produced primarily by GABA_A receptor-mediated, phasic inhibition.¹⁹

The relatively large size, restricted location, and functional characterization of canine expiratory premotor neurons allow for detailed studies of their reflex inputs and responses *in vivo*.¹²⁻¹⁴ In addition, the types of neurotransmitters that are involved in these responses have been identified by combining extracellular recordings of single neurons *in vivo* with localized pressure picroejection of neurotransmitter agonists and antagonists. These studies showed that only certain receptor types are endogenously activated in these neurons. For example, the *in vivo* activity of canine expiratory premotor neurons is primarily dependent on NMDA receptor-mediated excitatory inputs from both central and peripheral chemosensors.¹⁵ Antagonists of the non-

NMDA ionotropic glutamate receptors were without effect. In addition, the spontaneous and reflex activities of these neurons were not affected by glycinergic, serotonergic, adrenergic, cholinergic, or GABA_B receptors antagonists^{17,18}, suggesting that these receptor subtypes were not endogenously activated in these neurons. Thus, expiratory premotor neurons appear to receive excitatory drive inputs exclusively through the NMDA subtype of glutamate receptor.

Most importantly, both the spontaneous and reflexively altered activities of these canine expiratory premotor neurons are powerfully modulated to a similar degree by an inhibitory input. This inhibitory input appears to be mediated by GABA_A receptors that are blocked by the competitive GABA_A antagonist bicuculline, but not by the Cl⁻ channel blocker picrotoxin.¹⁹ The type of this inhibition is gain modulation rather than hyperpolarization.¹⁷ Gain modulation is a multiplicative process whereby the output discharge frequency of a neuron, F_n , is the product of its underlying neuronal discharge frequency, F_e , and a modulation coefficient, $(1 - \alpha)$. The mathematical model for gain modulation is summarized in figure 1, right, in which the inhibitory constant, α , is proportional to the net inhibitory input (F_i). The model states that the output discharge frequency, F_n , is propor-

tional to the uninhibited (unattenuated) discharge frequency, F_e . F_e can only be assessed if α is reduced to zero *via* synaptic blockade (*i.e.*, $F_e = F_n$ if $\alpha = 0$). F_e is an index of the total excitatory drive to the neuron.

Thus, in the vagotomized dog during the expiratory phase, the activity of these expiratory neurons is mainly dependent on two inputs, F_e and F_i (fig. 1, left). This type of simplified model allows for the detailed study of the effects of anesthetics on synaptic transmission *in vivo*.

The objective of the present study was to test the hypothesis that halothane-induced depression of these brainstem neurons is produced mainly by the depression of glutamatergic excitatory neurotransmission rather than enhancement of GABA_Aergic inhibitory neurotransmission. We determined the amount of anesthetic-induced alterations in overall glutamatergic synaptic excitation and GABA_Aergic inhibition to these neurons in a halothane-anesthetized canine model. We found that an increase in halothane concentration from one minimum alveolar concentration (MAC) to 2 MAC decreased both the excitatory and inhibitory inputs to a similar extent, and thus the net neuronal activity was less depressed than either of the synaptic inputs. Furthermore, these data do not support the notion that anesthetic-induced depression is mediated *via* enhancement of GABA_Aergic neurotransmission in this type of preparation.

Materials and Methods

Animal Preparation and General Methodology

This research was approved by the Medical College of Wisconsin Animal Care Committee and conformed with standards set forth in the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Fifteen adult mongrel dogs (weight 8–16 kg) were studied under halothane as the sole anesthetic agent. Anesthesia in dogs was induced by mask with halothane and maintained with the same agent throughout the surgical preparation and the experimental protocols. No intravenous anesthetic agents were used in any of the studies. The trachea was intubated with a cuffed endotracheal tube without the help of muscle relaxants. During surgery the animals were artificially ventilated without the use of muscle relaxants with O₂-air-halothane mixtures under normocapnic conditions. Airway CO₂ and volatile agent concentrations were continuously recorded with an infrared analyzer (POET II, Criticare Systems, Waukesha, WI). The femoral artery was cannu-

lated for continuous blood pressure monitoring (Gould-Statham P23 ID transducer) and periodic arterial blood gas sampling. A triple-lumen catheter was placed in the femoral vein for drug administration and for continuous infusion of maintenance fluids (lactated Ringer's solution at 4 ml · kg⁻¹ · h⁻¹). Additional sodium bicarbonate to correct metabolic acidosis was given as determined by blood gas analysis but was rarely required. In addition, 3 ml of the maintenance solution was infused to replace each milliliter of estimated blood loss. Phenylephrine (0.5–5.0 μg · kg⁻¹ · min⁻¹) was infused if necessary to keep mean arterial pressure above 75 mmHg.^{20,21} Phenylephrine was only required for anesthetic doses of 2.0 MAC in a few animals. The animals were monitored for signs of inadequate anesthesia, including movement, salivation, lacrimation, or increases in blood pressure and heart rate. The anesthetic depth was increased immediately if such signs were present. In general, anesthetic depths of 1.3–1.7 MAC halothane were found adequate for surgery. Esophageal temperature was measured and maintained within a target range of 37.5–38.5°C with a servocontrolled heating pad.

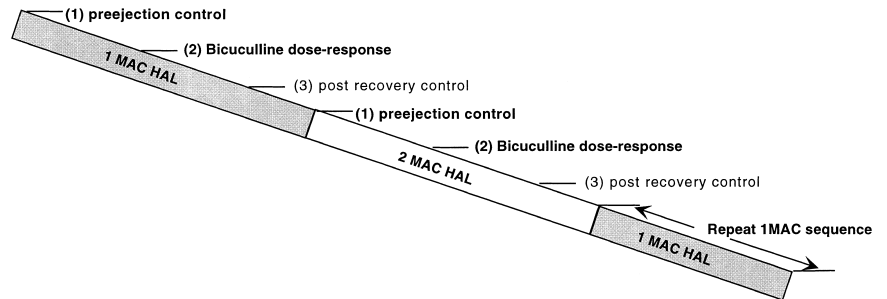
The animals were positioned in a stereotaxic device (model 1530; David Kopf Instruments, Tujunga, CA) with the head ventrally flexed (30 degrees). Bilateral dorsolateral neck dissections were performed. The vagus and phrenic nerves (right C5 central rootlet) were isolated and prepared for recording (phrenic) and sectioning. Some peripheral inputs, including those from the pulmonary stretch receptors and aortic arch chemoreceptors, were then removed by bilateral cervical vagotomies. Bilateral pneumothorax was performed to minimize brainstem movement and phasic inputs from chest wall mechanoreceptors. An occipital craniotomy was performed and the dura mater was cut along the midline and retracted to expose the medulla oblongata. After completion of the occipital craniotomy the animals were maintained at 1 MAC halothane level as baseline anesthesia. Pancuronium (bolus of 0.1 mg/kg, followed by a continuous infusion of 0.1 mg · kg⁻¹ · h⁻¹) was then given to avoid motion artifacts during respiratory neuronal recordings.

Neurotransmitter Pressure Picoejection and Neuron Recording Technique

Expiratory neurons of the caudal ventral respiratory group were located within the dorsal medulla 2–5 mm caudal to the obex, 2.5–4.5 mm lateral to the midline, at a depth of 2–4.5 mm as described previously.^{21,22} Over 90% of these neurons have been demonstrated to be

Protocol Sequence for Halothane-Induced Effects on Overall Synaptic Transmission in Expiratory Neurons

Fig. 2. Experimental protocol that was used to examine the effects of halothane (HAL) on overall excitatory and inhibitory synaptic mechanisms in expiratory VRG neurons. The neuronal activity of single expiratory neurons was extracellularly recorded under the conditions that are indicated in the flow diagram. MAC = minimum alveolar concentration.



bulbospinal premotor neurons.¹² Multibarrel compound glass electrodes consisting of a recording barrel containing a 7 μm carbon filament and three drug barrels were used to simultaneously record extracellular neuronal action potentials before and during pressure picroejection of the GABA_A neurotransmitter antagonist bicuculline methyl chloride (50–200 μM ; Research Biochemicals, Natick, MA), at a constant, steady-state dose rate on the neuron of interest.^{15,17} The neurotransmitter antagonist was prepared in an artificial cerebrospinal fluid consisting of 124 mM NaCl, 2 mM KCl, 2 mM MgCl₂, 1.3 mM KH₂PO₄, 0.9 mM CaCl₂, 26 mM NaHCO₃, and 11 mM glucose in which the pH was adjusted to 7.2–7.4 by aeration with 5% CO₂ and 95% O₂. The drug-free artificial cerebrospinal fluid vehicle served as a control. Compressed nitrogen gas was used for pressure picroejection of the neurotransmitter antagonist and artificial cerebrospinal fluid. Ejection was accomplished with the “pico-spritzer” technique that allowed controlled drug volume application by regulation of ejection pressure, duration, and frequency of the ejection pressure pulses. Drug dose delivery rates were determined *in vivo* by reading the change per unit time of the meniscus level in the drug barrel of the capillary glass electrode with a 50 \times monocular microscope equipped with a calibrated reticule (resolution 2 nl). To minimize any transient effects of the pharmacokinetics or pharmacodynamics of the neurotransmitter antagonist on neuronal response patterns, steady-state dose (constant ejection rate) picroejection techniques were used.

Data Recording and Collection

Single-cell expiratory neuronal activity, phrenic nerve activity, picroejection marker pulses, airway CO₂ and volatile anesthetic concentrations, systemic blood pres-

sure, and airway pressure were recorded on a digital tape system (model 3000A; A. R. Vetter Co., Rebersburg, PA). These variables or their time averages were also continuously displayed on a polygraph (model 7; Grass Instruments, Quincy, MA) during the experimental runs. Timing pulses at the beginning and end of neural inspiration were derived from the phrenic neurogram and were used to aid in data analysis. The tape-recorded data were digitized and analyzed off-line.

Experimental Protocols

All experiments were performed under hyperoxic (inspired oxygen fraction > 0.8) and steady-state hypercapnic conditions (target arterial carbon dioxide tension: 50–60 mmHg) in vagotomized, paralyzed, and mechanically ventilated preparations. This level of hypercapnia was chosen to ensure adequate phrenic activity even at the higher level (2 MAC) of anesthesia. Target arterial carbon dioxide tension was held constant for all experimental runs within an animal but was allowed to differ within the set target range between animals to prevent apnea in each animal at the 2 MAC level. The experimental protocols were designed to determine the amount of overall anesthetic-induced alterations of glutamatergic excitation and GABA_Aergic inhibition of expiratory neurons.

Procedure

The experimental protocol is outlined in figure 2 and was as follows: A minimum of 1 h was allowed for preparation stabilization before data collection. Once a stable signal from a single expiratory neuron was obtained, the neuronal activity was continuously recorded throughout the entire protocol. The peak neuronal dis-

charge frequency, F_n , was measured for 10–20 respiratory cycles during the preejection control period (F_{con}). The GABA_A antagonist, bicuculline, was then used to completely block the endogenous GABA_Aergic inhibition to the recorded neuron. The dose rates for bicuculline were increased in a stepwise manner until an additional increase in dose rate did not lead to a further increase in peak F_n compared with the prior dose rate. Typically picroejection durations of 5–10 min with increasing dose rates were required to achieve complete GABA_Aergic block, as evidenced by saturation of the neuronal response. Then complete postejection recovery was awaited. Control conditions were typically reestablished 20–30 min postejection of bicuculline. Thereafter the anesthetic depth was increased from 1.0 MAC halothane to 2.0 MAC and the same procedures were repeated at the new anesthetic depth once steady-state anesthesia (30 min equilibration) had been reached. The complete protocol, including anesthetic state 1 (1-MAC level), the anesthetic washin and washout between anesthetic states, anesthetic state 2 (2-MAC level), and return to anesthetic state 1 took about 4 h to complete.

Methods of Data Analysis

The effect of halothane on peak discharge frequency, F_n , prior to and during GABA_Aergic blockade with bicuculline was quantified in the following manner: The averaged peak F_n values for each condition were normalized to the peak activity of the neuron during maximal bicuculline block (F_e) at the lowest anesthetic dose (1 MAC). This represented the maximal discharge frequency obtained for each respective neuron and was assigned a value of 100%. A two-way, repeated measures analysis of variance, with main factors of anesthetic state (*i.e.*, 1 or 2 MAC halothane) and neurotransmitter status (preejection control *vs.* maximal bicuculline block), was used to test for significant differences.

To analyze the effects of halothane on the overall excitatory and inhibitory synaptic transmission we used the neuronal model of figure 1. The index of overall inhibition, α , is obtained from the model definition (fig. 1, right) as follows: $\alpha = [F_e - F_{con}]/F_e$, where F_{con} is the peak F_n of the neuron during the prevailing basal control condition of GABA_Aergic attenuation. The values for F_e and F_{con} were obtained for both anesthetic levels and were then used in the calculation of anesthetic-induced effects on the overall excitatory (ΔF_e) and the overall inhibitory neurotransmission ($\Delta\alpha$) as summarized by equations 1 and 2

Table 1. Procedure Used To Quantify the Effects of Halothane on the Excitatory and Inhibitory Components of Neurotransmission

Anesthetic Level	Measurement of Synaptic Inputs	Variable	Picroejection Procedure
1 MAC	Excitatory	F_{e1}	Inhibitory antagonist GABA _A block (bicuculline)
	Inhibitory	α_1	
2 MAC	Excitatory	F_{e2}	Inhibitory antagonist GABA _A block (bicuculline)
	Inhibitory	α_2	
Calculations for anesthetic induced changes Δ	Overall excitatory component	$\Delta F_e = (F_{e2} - F_{e1})/F_{e1}$	Eq 1
	Overall inhibitory component	$\Delta\alpha = (\alpha_2 - \alpha_1)/\alpha_1$ where $\alpha = [F_e - F_{con}]/F_e$	Eq 2

MAC = minimum alveolar concentration; GABA_A = γ -aminobutyric acid_A; F_e = peak discharge frequency of the neuron during complete GABA_A receptor block with BIC; F_{con} = control peak discharge frequency during the prevailing basal conditions of GABA_Aergic attenuation. The level of prevailing GABA_Aergic inhibition is described by the inhibitory constant α .

of table 1. All data values are given as mean \pm SD, and $P < 0.05$ was used to indicate significant differences unless stated otherwise.

Results

Effects of Halothane on Synaptic Neurotransmission

Figure 3 illustrates the response of an expiratory neuron to picroejection of the GABA_A antagonist bicuculline at 1 and 2 MAC halothane. Comparison of the time-expanded views of this data for the preejection control period (upper left) and during maximal bicuculline block (upper right) show that antagonism of GABA_A receptors produces an increase in the expiratory-phase activity. Local application of bicuculline typically results in a discharge pattern that is an amplified replica of the underlying control pattern, which is a manifestation of gain modulation. The bicuculline dose-dependent responses (bottom records) show a steeper increase and a greater maximum increase in peak neuronal discharge frequency, F_n , at the maximal level of GABA_Aergic block for 1 MAC compared with 2 MAC. Quantification of these data is shown in figure 4, in which at 1 MAC halothane, the maximum bicuculline dose increased the peak discharge frequency, F_n , from 150 ± 9.9 Hz (F_{con1})

HALOTHANE AND RESPIRATORY NEUROTRANSMISSION

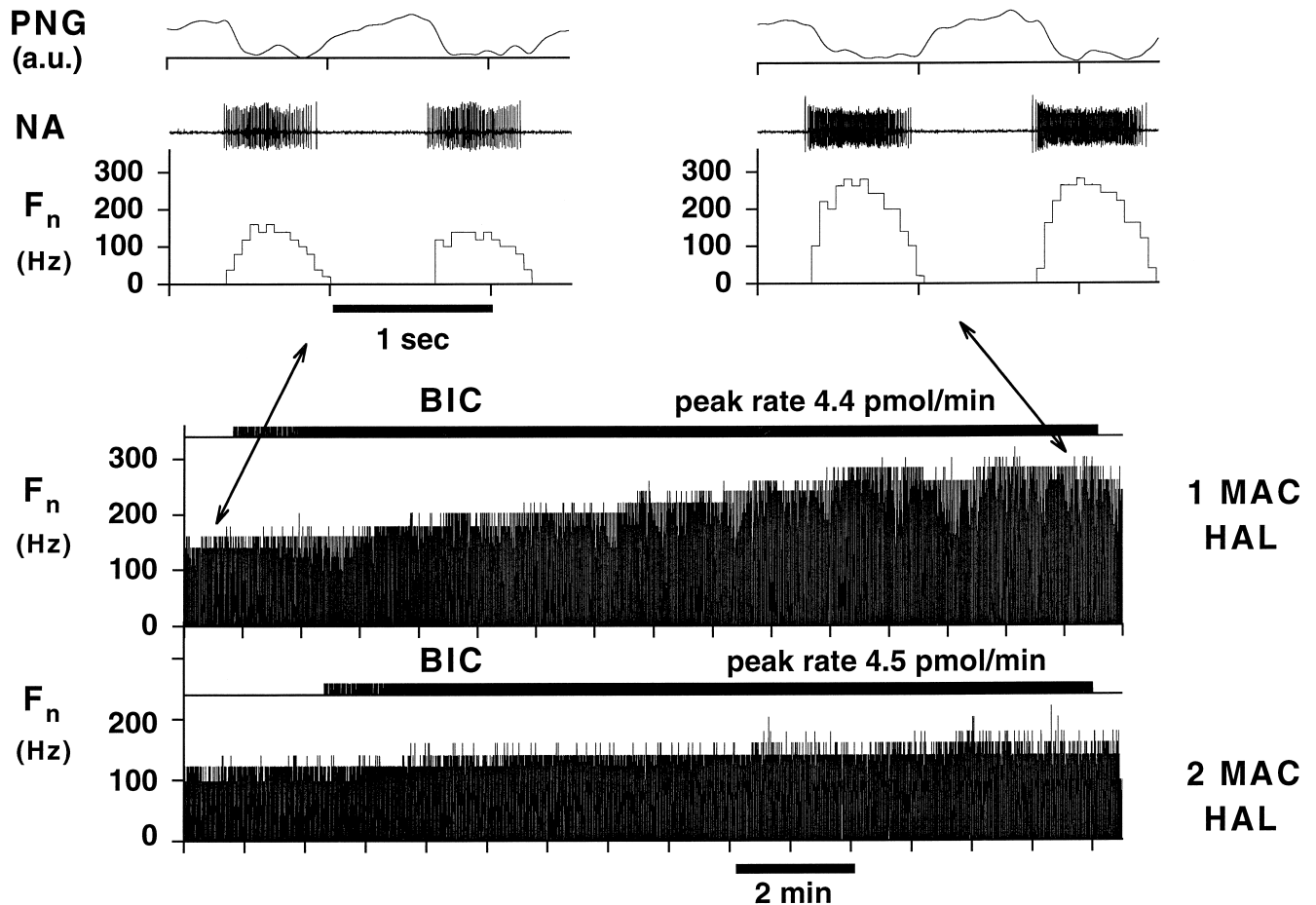


Fig. 3. Response of an expiratory neuron to picoinjection of the GABA_A receptor antagonist bicuculline (BIC) at 1 and 2 minimum alveolar concentration (MAC) halothane (HAL) doses. The full picoinjection response curves to BIC are shown in the bottom traces as rate-meter recordings of the expiratory neuronal discharge frequency F_n , which is displayed in hertz. Each BIC picoinjection run lasts more than 10 min and consists of the compression of several hundred neural breaths. To better illustrate the effects of BIC during single neural respiratory cycles, the top records show time-expanded views of the phrenic neurogram (a.u. = arbitrary units), two action potential spike trains (NA = neuronal activity) from a single expiratory neuron, and the corresponding rate-meter recordings of the neuronal spike discharge frequency (F_n in hertz) both during the 1-MAC HAL preejection control period (left traces; peak $F_n \approx 150$ Hz) and during saturation of the neuronal response at the maximum BIC picoinjection rate (right traces; peak $F_n \approx 280$ Hz). Note that the expiratory neuron discharges throughout the expiratory phase with a mainly decrementing pattern. A relatively high control discharge frequency (150 Hz) is fairly typical for these premotor neurons during hyperoxic hypercapnia at 1 MAC HAL. Although the BIC dose-response curve shows a relatively steep increase in discharge frequency at 1 MAC (from approximately 150 to 280 Hz), the curve at 2 MAC is relatively flat (from about 120 to 154 Hz). Note that the peak picoinjection dose rates for BIC are similar for both HAL doses. Saturation of the BIC response was assumed if an additional increase in dose rate did not cause a further increase in expiratory neuronal discharge frequency.

to 280 ± 12.6 Hz (F_{c1}), yielding an average α_1 value of 0.46 (i.e., $\alpha_1 = (280 - 150)/280$; see also table 1). This means that the tonic inhibition (gain modulation) attenuated the neuronal output by 46%. The corresponding peak F_n values for 2 MAC were 119 ± 7.6 Hz (F_{con2}) and 154 ± 6.4 Hz (F_{c2}), yielding an average α_2 value of 0.23. Thus, for this neuron, the 1-MAC increase in halothane dose reduced the tonic inhibition by 50% (i.e., $\Delta\alpha = (0.23 - 0.46)/0.46 = -0.50$; see table 1). At the same

time the excitatory activity, which was measured in the absence of tonic inhibition during maximal GABA_Aergic block with bicuculline, was reduced by 45% (i.e., $\Delta F_c = (154 - 280)/280 = -0.45$; see table 1). Because both excitatory and inhibitory synaptic drives were reduced by the anesthetic dose increase, overall neuronal peak, F_n , was only reduced from 150 to 119 Hz, or by 21%. This is markedly less than the 45% reduction in excitatory component of synaptic drive.

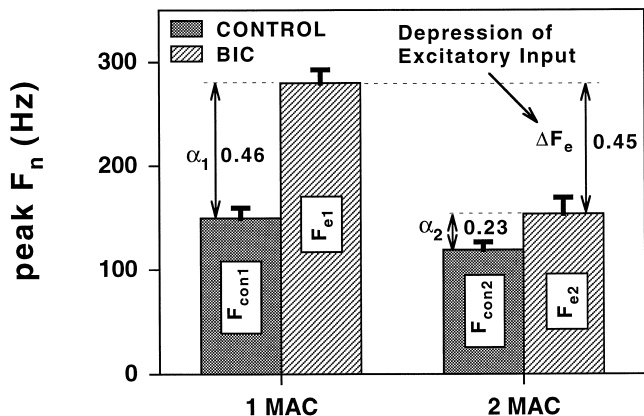


Fig. 4. Method used to quantify the effects of halothane on overall synaptic neurotransmission in the expiratory neuron protocols. Comparing the neuron activities before (F_{con}) and during complete GABA_A receptor block with bicuculline (F_e) at two different levels of anesthesia (one and two minimum alveolar concentration [MAC] halothane) allows the calculation of the effects of the anesthetic dose increase on overall excitatory and inhibitory neurotransmission (see equations in table 1). This analysis is based on the data from the neuron shown in fig. 3. For this neuron mean values \pm SD were obtained for each condition by averaging the peak activity of the neuron over 10 consecutive neural respiratory cycles (see text for details). Coefficients of variation are (left to right): 6.7, 4.5, 6.4, and 9.9%.

Pooled Data

The pooled data from eight neurons with end controls are summarized in figure 5 (top). At the 1-MAC halothane level complete GABA_Aergic block with bicuculline approximately doubled the control neuron activity both during the initial and the end control (1-MAC return) runs. The data suggest that these neurons were attenuated by endogenous GABA_Aergic inhibition by $48 \pm 12\%$ (i.e., $\alpha = 0.48 \pm 0.12$) at the 1-MAC dose. At the 2-MAC halothane dose the control neuron frequency was only moderately decreased compared with the 1-MAC level (from $51.7 \pm 11.6\%$ to $43.5 \pm 9.2\%$). Complete GABA_Aergic block at the 2-MAC level increased control neuron activity from $43.5 \pm 9.2\%$ to $64.8 \pm 11.7\%$. This amounts to an α of 0.32 ± 0.13 , which suggests that these neurons were attenuated by endogenous GABA_Aergic inhibition less at the 2-MAC dose ($\alpha = 0.32 \pm 0.13$) than at the 1-MAC dose ($\alpha = 0.48 \pm 0.12$). Thus, the anesthetic-induced change in overall inhibitory GABA_Aergic transmission, $\Delta\alpha$, to these neurons was a reduction of $33.5 \pm 17.2\%$ (fig. 5, bottom). At the same time the overall excitatory neurotransmission, ΔF_e , was reduced by $35.1 \pm 11.8\%$ (fig. 5, bottom). For 12 neurons the average reductions of total excitatory and inhibitory neurotransmission by the 1-MAC dose increase were $38.3 \pm 12.3\%$ and $34.5 \pm 16.1\%$, respectively. Four

additional neurons are included in these latter data but not shown in the graphs of figure 5 because end controls could not be obtained because of loss of the neuronal signal. Because of electrode dislodgment, data at both 1 MAC and 2 MAC could only be obtained for 12

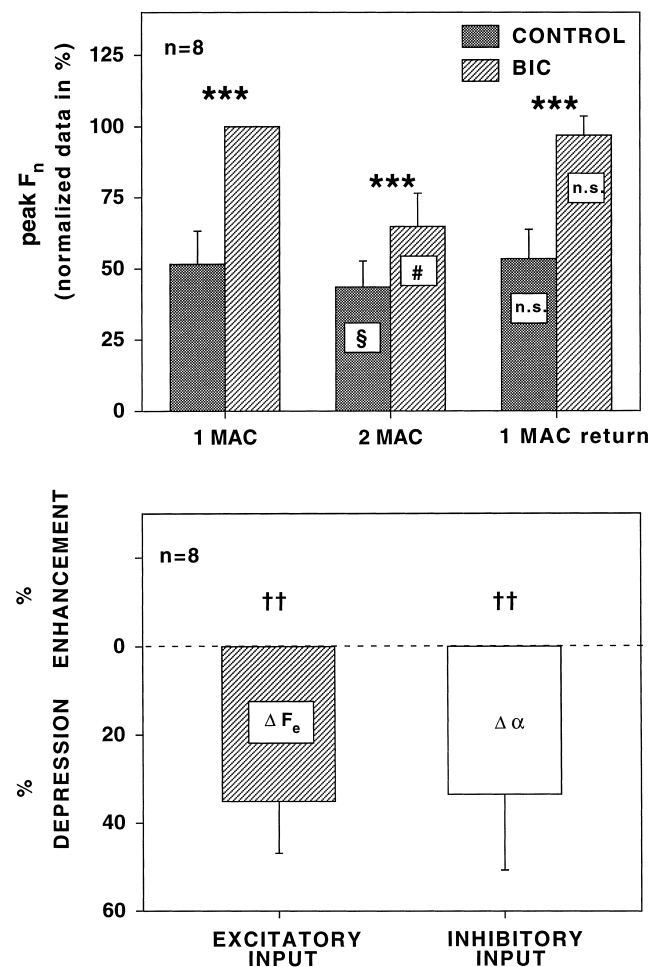


Fig. 5. Pooled summary data. (Top) Mean values \pm SD of peak neuronal discharge frequency (peak F_n) for the preejection control periods and during maximal GABA_Aergic block with bicuculline for one minimum alveolar concentration (MAC), 2 MAC, and 1 MAC end-control doses for eight neurons. The 1-MAC halothane dose increase caused a significant reduction of peak F_n during maximal GABA_Aergic block with bicuculline (BIC) ($\#P < 0.05$) as well as a reduction in the maximum response to BIC at the 2-MAC dose. This combination of effects resulted in only a small reduction in the control peak F_n at 2 MAC versus 1 MAC ($\$P < 0.05$). The 1 MAC end-control values are not significantly different from the initial 1 MAC values, indicating stability of the preparation and good reproducibility of the responses. The responses to BIC are highly significant at all halothane doses ($***P < 0.001$). (Bottom) Mean depression \pm SD of excitatory neurotransmission (ΔF_e) and inhibitory neurotransmission ($\Delta\alpha$) by the 1-MAC anesthetic dose increase in eight neurons ($\dagger\dagger P < 0.01$ relative to no change).

Lack of Effect of aCSF Vehicle

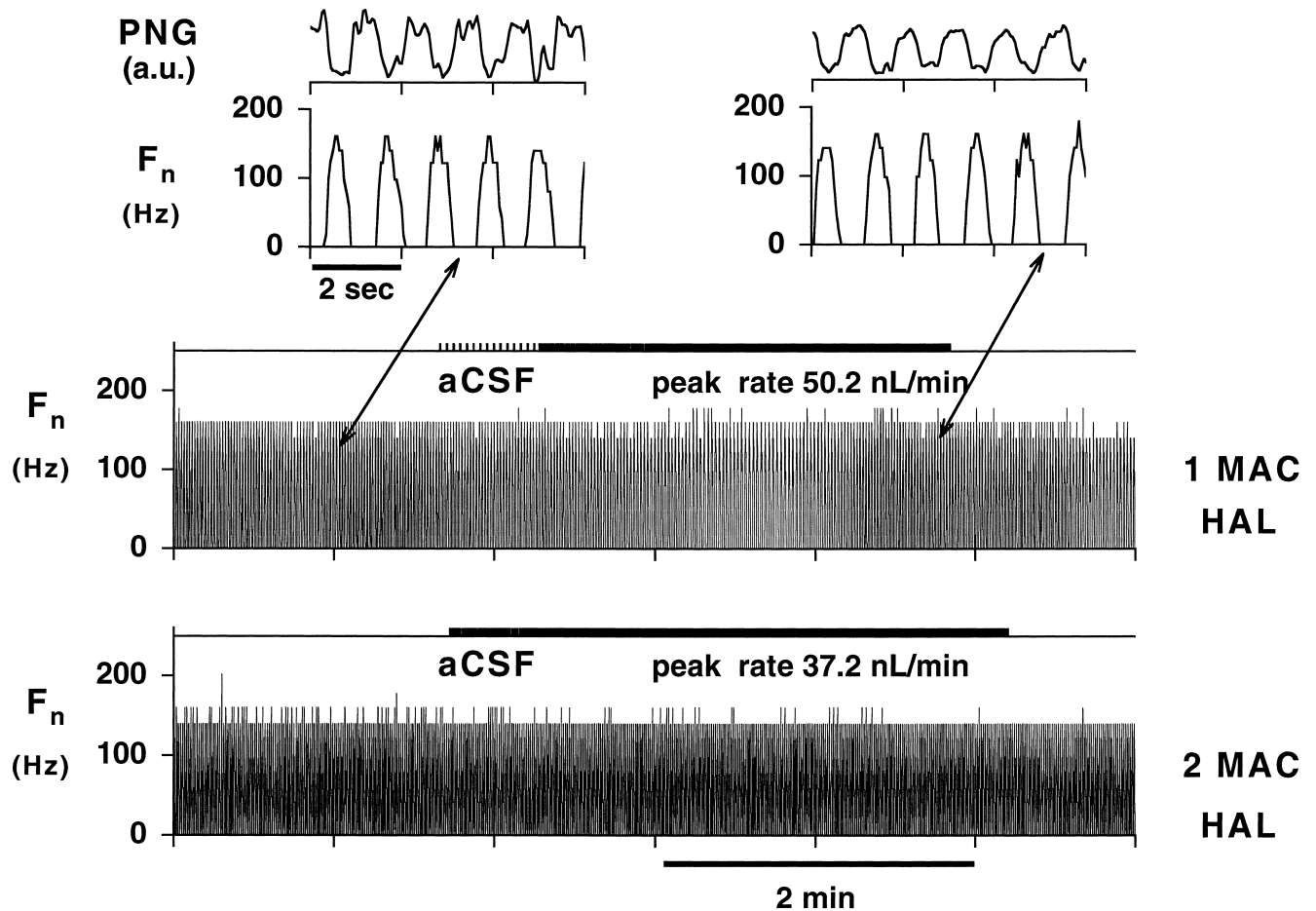


Fig. 6. Lack of response of an expiratory neuron to picoejection of the artificial cerebrospinal fluid (aCSF) vehicle at the one minimum alveolar concentration (MAC) and the 2-MAC halothane doses. The aCSF dose-response curves (bottom) show no change in peak F_n during picoejection rates of aCSF that are similar to the peak volume rates during maximal GABA_Aergic block. (Top) Time-expanded views of the phrenic neurogram (PNG) and the F_n rate-meter output at the 1-MAC level for the preejection control period (left) and during maximal aCSF application (right).

neurons in 15 dogs. Thus, these results suggest that the simultaneous reduction in inhibition by the 1-MAC halothane dose increase was able to preserve neuronal activity at levels much higher than would be expected after the anesthetic dose increase. There was no net enhancement of inhibition.

Effect of the Artificial Cerebrospinal Fluid Vehicle

The vehicle in which the neurotransmitter antagonist bicuculline was dissolved did not show any effects on neuronal activity if picoejected at similar peak volume

rates as bicuculline (e.g., fig. 6). This demonstrates that not only the ejection rate but also the constituents of the artificial cerebrospinal fluid vehicle itself did not alter F_n and bias our results. Such runs are important controls and were routinely performed for each experimental preparation.

Discussion

These studies confirm our hypothesis that halothane's depressive effect on expiratory neurons *in vivo* is caused

by a depression of excitatory neurotransmission. Because the excitation of these expiratory neurons is mainly dependent on NMDA receptor activation,¹⁵ halothane appears to depress glutamatergic neurotransmission mechanisms. No enhancement of GABA_Aergic inhibitory mechanisms could be demonstrated with increasing depth of anesthesia from 1 to 2 MAC. These conclusions are based on our previously stated model assumptions that during the expiratory phase the main contributors to the discharge pattern are F_e and F_i (see fig. 1), and that the effects of F_i were completely antagonized by bicuculline (also see below). These results suggest that at deep levels of halothane (2 MAC) both excitatory and inhibitory neurotransmissions are depressed to a similar extent. This contrasts with *in vitro* studies of strictly "postsynaptic" preparations that show that halothane enhances GABA_Aergic inhibitory currents²³⁻²⁶ but has little or no effect on glutamatergic receptor function at clinically relevant doses.^{3,23} Taken together, these findings suggest that halothane may alter presynaptic mechanisms that result in decreased neurotransmitter release of both glutamate and GABA *in vivo*. The combined effects of halothane on presynaptic and postsynaptic neuronal mechanisms determine the overall depressant effect of halothane on respiratory neuron activity. Although the present studies provide new information about the effects of halothane on overall excitatory and inhibitory synaptic control of respiratory neurons, clearly additional studies are necessary to determine the relative effects on presynaptic *versus* postsynaptic mechanisms in this model.

Methodological Limitations

Although *in vivo* intracellular recordings have the advantage of detecting excitatory and inhibitory postsynaptic potentials, even during quiescent periods, such recordings are difficult to maintain under the best of conditions. Although extracellular recordings of spike discharge patterns reflect the excitability of a neuron only above its firing threshold,²⁷ such recordings are much easier to obtain and hold for long (3-6-h) periods. It is possible, therefore, to study the effects of many different, systematically controlled interventions such as drug picrojections, changes in anesthetic depth, or alterations of afferent inputs. Usually such manipulations produce transient changes in blood pressure, which make intracellular recordings of these events very difficult. Although the picrojection technique can result in very localized agent application, it is recognized that presynaptic neurons may also be affected, which is also true for microiontophoresis or *in vitro* bath application.

A detailed discussion of these limitations can be found elsewhere.^{19,28}

Our assumption that bicuculline produced nearly a complete block of the gain modulating input, F_i (fig. 1), has an effect on the accuracy of the amount of halothane-induced depression of both inputs. We feel relatively confident that the block was nearly complete because the bicuculline dose rate was increased until no further increase in F_n was observed compared to the prior dose rate. At the 2-MAC level the maximal dose rate was at least as large or larger than the maximal dose rate used at 1 MAC. It is possible that changes in halothane concentration may alter the affinity of bicuculline at the GABA_A receptor. However, it is known that halothane binds to a site (or sites) on a GABA_A receptor subunit that is distinct from the GABA recognition site.^{29,30} Thus, direct competition between halothane and bicuculline or GABA is not expected to occur. It has been suggested that the enhancement of GABA at the GABA_A receptor by volatile anesthetics is caused by an increased affinity of GABA at its recognition site.³¹ It may be possible that the competitive antagonist, bicuculline, may be similarly affected.

Effects of Volatile Anesthetics on Other Respiratory Neurons

Only a few studies describe the effects of volatile anesthetics on brainstem respiratory neurons.^{21,22,32-37} In most cases, well-defined, steady-state conditions, in terms of volatile agent concentration, were rarely established. Doi's group^{36,37} compared the dose-dependent depressant effects of three volatile anesthetics on inspiratory brainstem neurons in the cat, but the exact function of these neurons was not ascertained. Tabatabai *et al.*³³ found that halothane decreased the discharge frequency of the inspiratory neurons in decerebrate cats throughout the anesthetic exposure and the anesthetic effects were readily reversible. Takeda *et al.*^{34,35} used short, 90-s exposures of 2% halothane in decerebrate cats while performing both intra- and extracellular recordings of propriobulbar respiratory neurons. This transient halothane exposure caused only a 25% depression in peak phrenic nerve activity, which is only a third of the magnitude of depression produced during steady-state conditions. Halothane decreased synaptic noise and membrane potential fluctuations in all of the respiratory neurons that were tested. This included both expiratory and inspiratory propriobulbar neurons, but their exact functions were not determined. Furthermore, the membrane responses were not uniform. A third of neurons were depolarized by the anesthetics even though neuronal

discharge frequency decreased. About 15% of neurons were hyperpolarized and exhibited a uniform decrease in neuronal discharge frequency. However, in the majority of neurons, no change in membrane potential was observed. Grelot and Bianchi³² found that the combination of background anesthesia with a volatile anesthetic exerts profound depressant effects on distinct subgroups of brainstem respiratory neurons, where many of the neurons stopped firing already at halothane concentrations below 1 MAC.

In studies that employed anesthesia with a volatile agent as the sole anesthetic under steady-state conditions, Stuth *et al.* delineated the dose-dependent depressant effects of halothane²¹ and isoflurane²² on the activity of canine respiratory premotor neurons at different levels of central chemodrive. These studies showed that the phrenic nerve discharge was depressed to a greater extent than that of the premotor neurons. A 1-MAC increase (1 MAC to 2 MAC) reduced peak phrenic activity by about 75%; peak neuronal activity was reduced to a much lesser extent.²¹

Halothane and Synaptic Transmission in Other Central Nervous System Neurons

Ion-channel studies, performed with patch-clamp techniques in neuronal membrane patches or in isolated neurons *in vitro*, have conclusively shown that halothane prolongs GABA_A receptor-mediated chloride currents.³⁸ This can enhance inhibition of neurons that receive GABA_Aergic inputs.

In addition, studies in isolated cerebral synaptosomes, a subcellular fraction containing pinched-off nerve terminals that retain the ability to take up, store, and release various neurotransmitters, have shown that halothane and also other volatile anesthetics inhibit endogenous glutamate release.³⁹ This points to presynaptic inhibition of excitatory neurotransmitter release as a potential mechanism of anesthetic action. Halothane appeared to affect glutamate release at a step proximal to calcium influx that controls transmitter release,³⁹ but this issue remains controversial.⁴⁰ The overall effects of halothane are more variable in preparations in which both presynaptic and postsynaptic neural elements remain intact. For example, halothane depressed rather than enhanced GABA_Aergic inhibitory synaptic potentials in spinal motoneurons⁴¹ and in CA1 pyramidal cells,⁴² possibly by presynaptically inhibiting evoked GABA release. Recently, Perouansky *et al.*⁴³ have shown in a hippocampal slice preparation that halothane blocked glutamate receptor-mediated activation of GABA_Aergic inhibitory interneurons. Both NMDA and non-NMDA subtypes of ionotropic glutamate receptors were nearly equally af-

ected. In a similar preparation, this group previously showed that halothane had similar effects on both subtypes of glutamate receptor-mediated excitatory postsynaptic currents in CA1 pyramidal cells.⁴⁴ They concluded that halothane indiscriminately reduces glutamatergic excitation, both of inhibitory interneurons and of principal excitatory neurons in the hippocampus. The studies also showed that only glutamatergic excitatory postsynaptic currents that were evoked by a presynaptic stimulus were depressed by halothane. Halothane did not reduce exogenously applied glutamatergic agonist-induced currents and did not alter the rise or decay times of non-NMDA or NMDA excitatory postsynaptic currents. This strongly suggests that the observed effects of halothane on excitatory neurotransmission occur at a presynaptic site. Findings suggestive of a presynaptic action of halothane were also observed in spinal motoneurons of decerebrate cats, in which halothane depressed excitatory postsynaptic potentials without changing their decay rate, indicative of a decrease in neurotransmitter release.⁴⁵

In summary, picroejection of the GABA_A receptor antagonist bicuculline in conjunction with extracellular recordings from brainstem ventral respiratory group expiratory neurons, *in vivo*, demonstrates that a 1-MAC increase in halothane concentration depresses both excitatory glutamatergic and inhibitory GABA_Aergic synaptic transmission. The data do not support the notion that the halothane-induced depression of respiratory neuronal activity is mediated *via* enhancement of GABA_Aergic neurotransmission in this preparation.

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