

Effects of Halothane and Sevoflurane on Inhibitory Neurotransmission to Medullary Expiratory Neurons in a Decerebrate Dog Model

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Background: In canine expiratory bulbospinal neurons, 1 minimum alveolar concentration (MAC) halothane and sevoflurane reduced the glutamatergic excitatory drive at a presynaptic site and enhanced the overall γ -aminobutyric acid (GABA)-mediated inhibitory input. The authors investigated if this inhibitory enhancement was mainly caused by postsynaptic effects.

Methods: Two separate anesthetic studies were performed in two sets of decerebrate, vagotomized, paralyzed, and mechanically ventilated dogs during hypercapnic hyperoxia. The effect of 1 MAC halothane or sevoflurane on extracellularly recorded neuronal activity was measured during localized picroejection of the GABA_A receptor agonist muscimol and the GABA_A receptor antagonist bicuculline. Complete blockade of GABA_A-mediated inhibition with bicuculline was used to assess the prevailing overall inhibitory input to the neuron. The neuronal response to muscimol was used to estimate the anesthetic effect on postsynaptic GABA_A receptor function.

Results: Halothane at 1 MAC depressed the spontaneous activity of 12 expiratory neurons $22.2 \pm 14.8\%$ (mean \pm SD) and overall glutamatergic excitation $14.5 \pm 17.9\%$. Overall GABA-mediated inhibition was enhanced $14.1 \pm 17.9\%$ and postsynaptic GABA_A receptor function $74.2 \pm 69.2\%$. Sevoflurane at 1 MAC depressed the spontaneous activity of 23 neurons $20.6 \pm 19.3\%$ and overall excitation $10.6 \pm 21.7\%$. Overall inhibition was enhanced $15.4 \pm 34.0\%$ and postsynaptic GABA_A receptor function $65.0 \pm 70.9\%$. The effects of halothane and sevoflurane were not statistically different.

Conclusion: Halothane and sevoflurane at 1 MAC produced a small increase in overall inhibition of expiratory premotor neuronal activity. The increase in inhibition results from a marked enhancement of postsynaptic GABA_A receptor function that is partially offset by a reduction in presynaptic inhibitory input by the anesthetics.

VOLATILE anesthetics cause respiratory depression at clinically relevant doses. We have shown in our canine preparation that part of this depression occurs in the brainstem at the level of respiratory premotor neurons, where halothane¹ and sevoflurane (unpublished inspiratory neuronal data, A. G. Stucke, M.D., Milwaukee, WI, 2001)² reduced

the action potential discharge frequency of inspiratory and expiratory neurons to a similar extent.²

Expiratory premotor neurons in the caudal ventral respiratory group receive two major types of synaptic inputs during their active discharge phase: tonic excitatory drive inputs are primarily mediated by *N*-methyl-D-aspartate receptors.³ Tonic inhibitory inputs are solely mediated by γ -aminobutyric acid type A (GABA_A) receptors⁴ and reduce the transmission of excitatory drive to the neuron in the absence of anesthesia to approximately 50% of its original magnitude.⁵ The tonic inhibition can be completely and specifically blocked with the GABA_A antagonist bicuculline,⁶ and this will reveal the full excitatory drive these neurons receive.

Recently, we have shown that the depressive effect of halothane and sevoflurane on caudal ventral respiratory group expiratory neuron activity resulted from a decrease in overall excitatory drive by 20–30% without affecting the postsynaptic *N*-methyl-D-aspartate receptor responsiveness. Both anesthetics also enhanced the overall inhibitory input by 10–20%.^{2,5} We designed the current experiments to apportion this enhancement of overall inhibition to presynaptic and postsynaptic mechanisms and thus to come to a comprehensive understanding of the effect of these volatile anesthetics on synaptic transmission to caudal ventral respiratory group expiratory neurons.

There is evidence that halothane can reduce inhibitory input to neurons by acting at a presynaptic site. Halothane has been shown to inhibit excitation of inhibitory interneurons in mouse hippocampal slices.⁷ It also has been shown to reduce presynaptic release of glutamate from synaptosomes.^{8,9} However, this has not been demonstrated for presynaptic GABA release. On the other hand, halothane and sevoflurane have been shown to increase GABA_A receptor function and thus enhance postsynaptic inhibition.¹⁰ By measuring the effects of halothane and sevoflurane on overall inhibition and on postsynaptic GABA_A receptor function, we sought to apportion the presynaptic and postsynaptic components of the anesthetics' effect on neuronal inhibition in our *in vivo* preparation. We thus conducted two series of identical protocols with halothane and sevoflurane, respectively.

Materials and Methods

Animal Preparation and General Methodology

The research was approved by the Medical College of Wisconsin Animal Care Committee and conformed with

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standards set forth in the National Institutes of Health Guide for Care and Use of Laboratory Animals. Dogs were induced by mask with isoflurane and intubated with a cuffed endotracheal tube and from then on mechanically ventilated with oxygen. Isoflurane (1.3–1.8 minimum alveolar concentration [MAC]) was applied throughout the surgical procedures and only discontinued after completion of decerebration (1 MAC isoflurane in dogs = 1.4%¹¹). The animals were positioned in a stereotactic device (model 1530; David Kopf Instruments, Tujunga, CA) with the head ventrally flexed (30°). Bilateral neck dissections were performed. The C5 phrenic rootlet was prepared for recording, and bilateral vagotomy was performed to achieve peripheral deafferentation. Bilateral pneumothorax was performed to minimize brainstem movement and phasic inputs from chest wall mechanoreceptors. The animals were decerebrated at the midcollicular level¹² and only then paralyzed (0.1 mg/kg pancuronium followed by 0.1 mg · kg⁻¹ · h⁻¹). An occipital craniotomy was performed to expose the dorsal surface of the medulla oblongata for single neuron recording. Esophageal temperature was maintained at 38.5 ± 1°C. Mean arterial pressure was kept greater than 100 mmHg (if necessary with 0.5–5 µg · kg⁻¹ · min⁻¹ phenylephrine). Protocols were only performed during steady state conditions for blood pressure.

Neuron Recording Technique, Data Collection, and Experimental Conditions

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 potential activity before and during pressure ejection of respective GABA-mediated (GABAergic) agonists and antagonists onto expiratory neurons of the caudal ventral respiratory group. We used the selective GABA_A receptor agonist muscimol (7.5 µM; Research Biochemicals, Natick, MA) and the GABA_A receptor antagonist bicuculline methochloride (BIC, 200 µM; Research Biochemicals), which were dissolved in an artificial cerebrospinal fluid.⁵ Single-cell expiratory neuron activity, phrenic nerve activity, picoejection marker pulses, airway carbon dioxide and volatile anesthetic concentrations, systemic blood pressure, 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 determine the respiratory phases. The tape-recorded data were digitized and analyzed offline.

The identical protocol was performed for halothane (study 1) and sevoflurane (study 2) in different sets of dogs during hyperoxic (fraction of inspired oxygen

> 0.8) and steady state hypercapnic conditions (arterial carbon dioxide tension 50–60 mmHg). A typical protocol consisted of two separate picoejection periods (part 1, muscimol; part 2, BIC) at both 0 and 1 MAC (1 MAC halothane = 0.9%; 1 MAC sevoflurane = 2.4%¹¹). In our previous studies, which included 0 MAC, 1 MAC, and 0-MAC end control, end control values consistently reached the preanesthesia control values.^{2,5} To achieve a higher yield of protocols per animal, we performed the current protocols at 0 and 1 MAC with the order randomized, which eliminated the need for end controls.

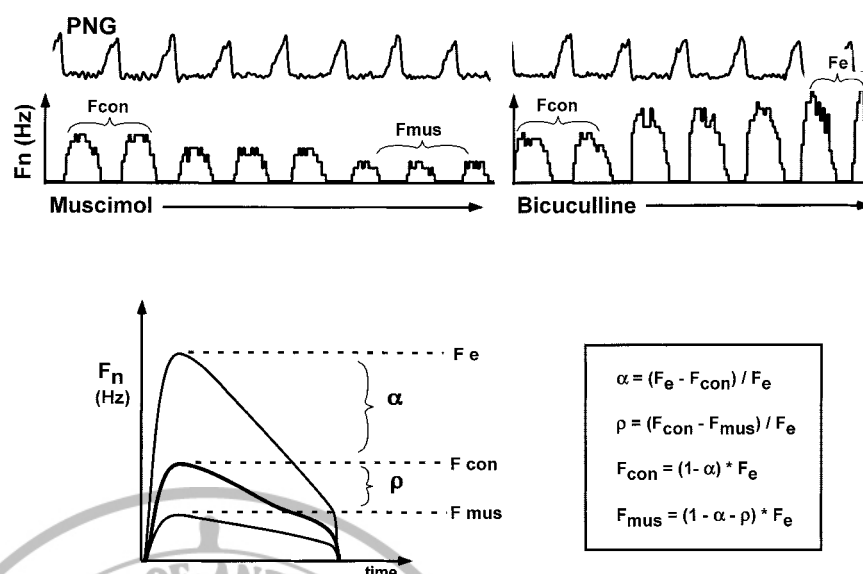
Protocol 1: Effects of Halothane and Sevoflurane on Postsynaptic GABA_Aergic Inhibition

The peak neuronal discharge frequency (F_n) was measured for 10–20 respiratory cycles during a preejection control period (F_{con}). Then the GABA_A receptor agonist muscimol was locally applied in increasing dose rates until a decrease in neuronal frequency of approximately 30–40 Hz was observed. In neurons with relatively low F_{con} , a maximal decrease that still maintained neuronal activity well above 0 Hz was chosen. Typically, picoejection durations of 8–12 min with 2–4 increasing dose rates were needed. Postejction recovery after muscimol is fast and was usually complete within 10 min.

Statistical Analysis. The effect of muscimol on expiratory neuronal frequency was quantified in the following manner. The best-fit curve was used to create dose-response curves for 0 and 1 MAC. To compare the dose-response curves at 0 and 1 MAC at an identical dose rate, we chose the lower of the two maximal muscimol dose rates of the 0 and 1 MAC picoejection runs, *i.e.*, the highest common dose rate for which data could be interpolated from both dose-response curves, and designated it D_{max} . The muscimol-induced decrease in peak F_n relative to preejection control F_{con} , *i.e.*, the net decrease (fig. 1, $F_{con} - F_{mus}$), was determined at D_{max} for 0 and 1 MAC. We then normalized the net decrease at D_{max} to the peak F_n obtained during complete block of GABA_Aergic inhibition with BIC (F_c) and designated it as the receptor response, ρ (fig. 1, bottom). During baseline conditions, the prevailing tonic GABA_Aergic input reduces F_c to F_{con} by the inhibitory factor α , where $\alpha = (F_c - F_{con})/F_c$. We previously demonstrated that GABAergic inhibition reduces neuronal discharge frequency, not by means of a simple additive-subtractive mechanism, but through a multiplicative mechanism.⁴ The relation between F_{con} and F_c is $F_{con} = (1 - \alpha) \cdot F_c$ (fig. 1). Likewise, the additional GABAergic receptor stimulation by exogenous muscimol reduces F_n in a multiplicative fashion, *i.e.*, $F_{mus} = (1 - \alpha - \rho) \cdot F_c$.

A two-way repeated-measures analysis of variance with main factors of anesthesia level (0 or 1 MAC) and neurotransmitter status (preejection control *vs.* muscimol response) was used to examine the anesthetic effect on the muscimol-induced receptor response.

Fig. 1. (Top) Effect of γ -aminobutyric acid type A (GABA_A)-mediated stimulation and blockade on expiratory neuronal firing pattern. The upper two traces show short record segments of the neuronal response to application of increasing dose rates of muscimol (left) and bicuculline (right), together with the simultaneously recorded phrenic neurogram (PNG). Changes in neuronal discharge frequency (F_n) produced by GABA_A receptor agonism or antagonism follow a multiplicative mechanism. (Bottom, left) Illustration of how the physiologically prevailing GABA-mediated inhibition (α) and the effect of muscimol on the GABA_A receptor (ρ) were calculated for each expiratory neuron. During control conditions, the overall excitatory drive to the neuron (F_e) is attenuated by the inhibitory factor α to F_{con} (bold). Application of the GABA_A agonist muscimol reduces F_{con} to F_{mus} . The neuronal response to receptor stimulation (ρ) is expressed as the net decrease from F_{con} , normalized to overall excitation, i.e., $\rho = (F_{con} - F_{mus})/F_e$. The comparison of ρ at an identical dose rate for 0 and 1 MAC is used to assess the effect of the anesthetic on the postsynaptic GABA_A receptor.



Protocol 2: Effects of Halothane and Sevoflurane on Overall Synaptic Neurotransmission

After complete recovery from muscimol, the GABA_A antagonist BIC was picroejected until complete block of GABA_Aergic inhibition occurred, i.e., when an increase in picroejection dose rate did not evoke any further increase in neuronal discharge frequency. Typically, picroejection durations of 5–10 min with several increasing dose rates were required. After the BIC run, complete postinjection recovery was awaited, which required 30–45 min. Then the randomized state of anesthesia (i.e., 0 or 1 MAC) was switched, and after a minimum equilibration time of 15 min, both steps of the protocol were repeated in the same fashion. It was ascertained that the peak BIC dose rate at the second state of anesthesia reached that at the first state of anesthesia, where state of anesthesia refers to either 1 MAC anesthesia or absence of anesthesia (0 MAC).

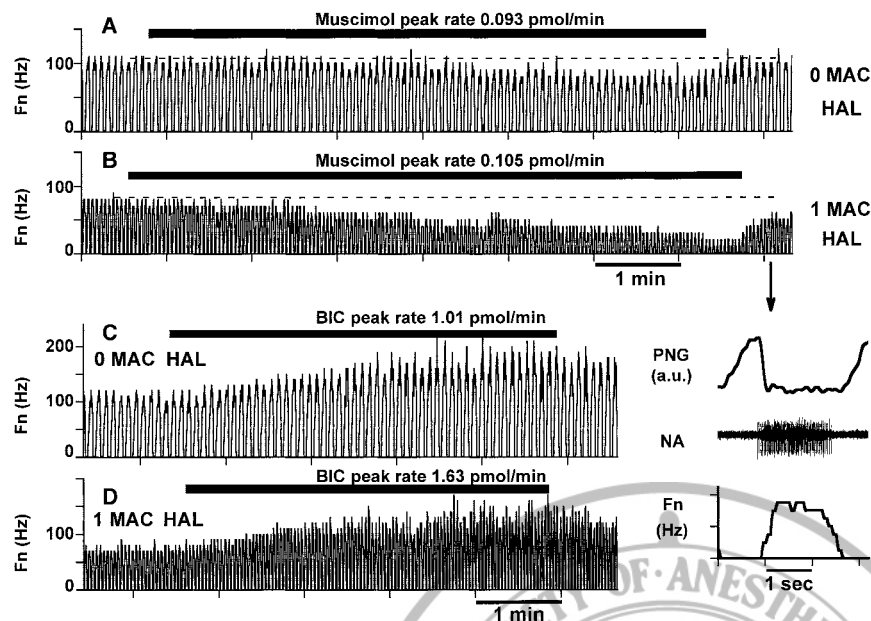
Statistical Analysis. During complete GABA_Aergic block, F_e is a measure of the uninhibited overall excitatory drive to the neuron, while F_{con} represents this drive reduced by the prevailing GABA_Aergic inhibition. To calculate the change in overall excitatory drive, the data were normalized to F_e at the 0-MAC level, which was assigned a value of 100%. A two-way repeated-measures analysis of variance was used with main factors of anesthesia level (0 or 1 MAC) and neurotransmitter status (preejection control *vs.* maximal BIC block).

The values for F_e and F_{con} were obtained for the 0-MAC level (F_{con0} , F_{e0}) and the 1-MAC level (F_{con1} , F_{e1}) from the experimental runs. They were then used in the calculation of the anesthetic induced effects on overall excitation ($\Delta F_e = [F_{e1} - F_{e0}] / F_{e0}$) and overall inhibition ($\Delta \alpha = [\alpha_1 - \alpha_0] / \alpha_0$).

The availability of paired data on overall inhibition (α) and on the postsynaptic receptor function (ρ) for the same neuron allowed us to estimate the anesthetic effect on presynaptic inhibitory input. We assume that the transmission of overall inhibition is proportional to the product of its presynaptic (pre) and postsynaptic (post) components, i.e., $\alpha \propto \text{pre} \cdot \text{post}$. Our model does not allow us to assess the absolute magnitude of either the presynaptic or postsynaptic inhibitory component. However, it is possible to calculate the effect of the anesthetic on these components, since we assume that the ratio of the receptor response to identical dose rates of muscimol at 0 and 1 MAC (ρ_1/ρ_0) is representative of the ratio of the postsynaptic inhibitory component ($\text{post}_1/\text{post}_0$). The change in receptor function by the anesthetic can thus be assessed without knowledge of the absolute magnitude of postsynaptic receptor function. The change in presynaptic inhibition caused by 1 MAC of the anesthetic (Δpre) can then be calculated using the ratio of overall inhibition (α_1/α_0) and of the muscimol-induced postsynaptic inhibition (ρ_1/ρ_0), where the subscripts indicate the anesthetic levels of 0 and 1 MAC. The estimated presynaptic effect has the form $\Delta \text{pre} = [(\alpha_1/\alpha_0)/(\rho_1/\rho_0) - 1]$ (see Appendix for details). All results are given as mean \pm SD, and $P < 0.05$ was used to indicate significant differences unless stated otherwise.

Results

Experiments were performed on 14 animals in study 1 (halothane) and 24 animals in study 2 (sevoflurane), yielding 12 and 23 complete neuron protocols, respectively.



Effects of Halothane and Sevoflurane on Postsynaptic GABA_Aergic Inhibition

Figure 2 shows a representative example of an E neuronal response to increasing picoejection dose rates of the GABA_A receptor agonist muscimol at 0 and 1 MAC halothane (traces A and B) and the GABA_A receptor antagonist BIC at 0 and 1 MAC halothane (traces C and D). The maximally ejected dose rates for muscimol were 0.093 pmol/min without anesthesia (0 MAC) and 0.105 pmol/min at 1 MAC halothane. Thus, the maximal dose rate that could be interpolated from both dose-response curves, D_{max} , was 0.093 pmol/min. At 0 MAC the net decrease at D_{max} was 23 Hz, and the net decrease at 1 MAC was 53 Hz (fig. 3). Normalization to F_c (203 Hz at 0 MAC and 176 Hz at 1 MAC) yielded a receptor

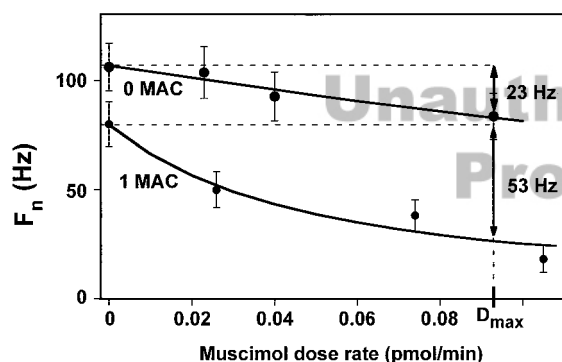


Fig. 3. Method used to analyze the effect of halothane on postsynaptic inhibition caused by picoejection of the γ -aminobutyric acid type A (GABA_A) receptor agonist muscimol onto the neuron in figure 2. The best-fit curve was used to create dose-response curves for muscimol at 0 and 1 minimum alveolar concentration (MAC) halothane. The net decrease from neuronal control frequency (\downarrow) was determined for both states of anesthesia at the highest muscimol dose rate achieved at both anesthetic states (D_{max}). The net decreases were then normalized to overall excitation as described in the text.

Fig. 2. Dose-response of an expiratory neuron to the γ -aminobutyric acid type A (GABA_A) agonist muscimol at 0 and 1 MAC halothane (A and B) and neuronal activity at saturation with the GABA_A antagonist bicuculline at 0 and 1 MAC (C and D). The picoejection response curves are shown as the rate-meter recordings of the neuronal discharge frequency F_n (in hertz). The duration of the picoejection is marked. Only the maximally ejected dose rates are indicated. Note the more pronounced response to muscimol (dotted line = F_{con}) at similar maximal dose rates at 1 MAC compared with 0 MAC. (Insert) The bottom trace shows a time-expanded view of (B). The simultaneously recorded phrenic neurogram (PNG, in arbitrary units [a.u.], top) identifies the neuron as expiratory. The neuronal raw activity directly appears on the oscilloscope as a train of action potential spikes (NA, middle), which is also displayed as the neuronal rate-meter recording in spikes per second or hertz (bottom).

response ρ of 11% at 0 MAC and 30% at 1 MAC, respectively. Halothane at 1 MAC thus enhanced the receptor response to muscimol by 173%.

The pooled normalized net decreases in peak F_n , induced by muscimol, are summarized in figure 4. At D_{max} , the average receptor response (ρ) values were $17 \pm 7\%$ (0 MAC) and $28 \pm 10\%$ (1 MAC) for halothane and $24 \pm 11\%$ (0 MAC) and $35 \pm 12\%$ (1 MAC) for sevoflurane. On average, 1 MAC halothane enhanced the GABA_A receptor response at D_{max} by $74.2 \pm 69.2\%$ ($\Delta\rho$; fig. 5). Sevoflurane at 1 MAC enhanced GABA_A receptor response by $65.0 \pm 70.9\%$. Interpolation of ρ at the relative dose rates $0.25 D_{max}$, $0.5 D_{max}$, $0.75 D_{max}$, and D_{max} from the individual dose-response curves showed that the receptor response to muscimol was linear in the applied dose range (fig. 4). The effect of both anesthetics was not significantly different (unpaired t test).

Effects of Halothane and Sevoflurane on Overall Synaptic Neurotransmission

Further analysis of the sample neuron (fig. 2, traces C and D) showed that at 0 MAC, BIC increased peak F_n from 106 to 203 Hz, yielding an inhibitory factor $\alpha_0 = 0.48$. At 1 MAC halothane, BIC increased peak F_n from 80 to 176 Hz, yielding an α_1 value of 0.55. Halothane at 1 MAC thus enhanced overall GABAergic inhibition in this neuron by 15%. The overall excitatory drive, F_c , measured at complete block of GABAergic input, decreased from 203 to 176 Hz or by 13% with 1 MAC halothane.

The pooled data for the effect of BIC is shown in figure 5. Halothane at 1 MAC increased overall inhibition by $14.1 \pm 17.9\%$ ($\Delta\alpha$). At the same time, overall excitation was depressed by $14.5 \pm 9.0\%$ (ΔF_c). In addition, F_{con} was significantly reduced by $22.2 \pm 14.8\%$ (ΔF_{con}).

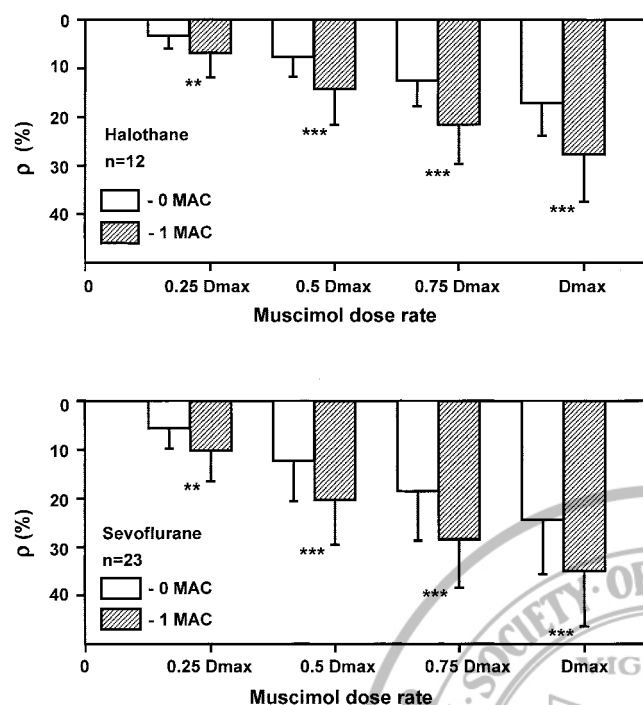


Fig. 4. Pooled summary data for protocol part 1, postsynaptic receptor response to muscimol for halothane (top) and sevoflurane (bottom). The graphs present the inhibitory receptor response (ρ in %). ρ was calculated at D_{max} and its 0.25, 0.5, and 0.75 fractions. At all dose rates, ρ was significantly greater at 1 minimum alveolar concentration (MAC) (filled bar) than at 0 MAC (open bar) for both anesthetics (** $P < 0.01$, *** $P < 0.001$). The receptor responses were linear in the studied dose range. The applied muscimol dose rate and the actual net decreases in F_n were different for each individual neuron. Therefore, the relative change in ρ ($\Delta\rho$) was used to compare the effects of halothane and sevoflurane on postsynaptic γ -aminobutyric acid type A receptor function (see $\Delta\rho$, fig. 5).

Sevoflurane at 1 MAC increased overall inhibition by $15.4 \pm 34.0\%$. Overall excitation was reduced by $10.6 \pm 21.7\%$, and F_{con} was reduced by $20.6 \pm 19.3\%$. All measured parameters were not significantly different between the two anesthetics (unpaired t test).

Estimation of the change in presynaptic inhibition (Δpre) yielded a decrease in presynaptic inhibition by $22.2 \pm 43.5\%$ at 1 MAC halothane and a decrease in presynaptic inhibition by $9.5 \pm 73.3\%$ at 1 MAC sevoflurane. Because these estimated values did not appear to follow a normal distribution, we used the nonparametric Wilcoxon test to test for significance. For both anesthetics, Δpre did not reach significance (halothane: $P = 0.06$; sevoflurane: $P = 0.07$).

Discussion

The current studies show that 1 MAC halothane and sevoflurane reduced the activity of expiratory premotor neurons in the brainstem of decerebrate dogs by depression of glutamatergic excitation and by enhancement of overall inhibition. The enhancement of overall inhibition

was the product of a pronounced increase in the postsynaptic GABA_A receptor function together with a decrease in presynaptic inhibitory input. There were no significant differences in effect between the two anesthetics. Figure 6 provides a synopsis of our current knowledge on the effects of halothane and sevoflurane on synaptic neurotransmission in our expiratory neuronal model.

Enhancement of GABA_A Receptor Function

The GABA_A receptor has long been regarded as one of the main effector sites of anesthetic action in the brain.¹³ A binding site for small volatile anesthetic molecules, *i.e.*, isoflurane, halothane, and chloroform, has been described on the transmembrane segments of the α subunit.¹⁴ Whole cell patch clamp studies in rat CA1 pyramidal neurons showed that the increase in receptor function was caused by an increase in decay time of the inhibitory postsynaptic current (IPSC) that more than offset a slight decrease in IPSC amplitude.¹⁵ Our data agree with *in vitro* whole cell patch clamp studies by Nishikawa and MacIver¹⁶ and Jenkins *et al.*¹⁰, where the enhancement of GABA_A receptor function was similar for halothane and sevoflurane.

Depression of Presynaptic Inhibition

In our *in vivo* preparation, presynaptic inhibitory drive appears to be inhibited by 1 MAC halothane and

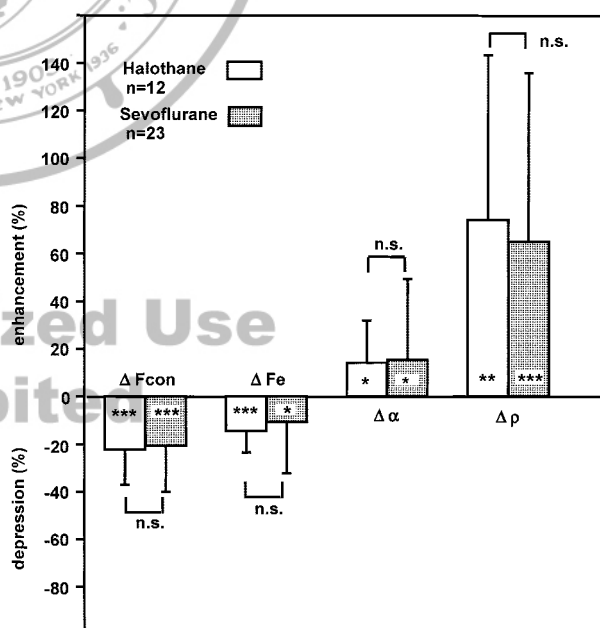


Fig. 5. Pooled summary data. Mean changes \pm SD for neuronal control frequency (ΔF_{con}), overall excitation (ΔFe), overall inhibition ($\Delta \alpha$), and inhibitory receptor response ($\Delta \rho$) by 1 minimum alveolar concentration (MAC) halothane and sevoflurane, respectively (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, relative to no change). The anesthetic-induced changes were not different between 1 MAC halothane and 1 MAC sevoflurane (nonsignificant).

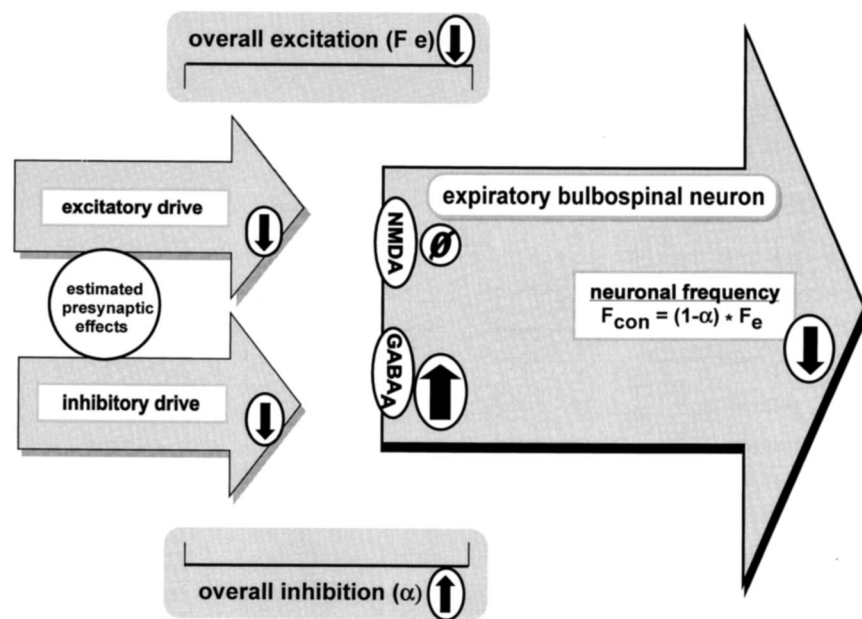


Fig. 6. Summary scheme of the effect of 1 minimum alveolar concentration (MAC) halothane and sevoflurane on synaptic transmission to expiratory premotor neurons in the caudal ventral respiratory group. The overall glutamatergic excitatory drive is reduced (\downarrow) without change in *N*-methyl-D-aspartate (NMDA) receptor activity (\emptyset). Overall inhibition is increased (\uparrow) as a result of a strong increase (\uparrow) in γ -aminobutyric acid type A (GABA_A) receptor function, whereas presynaptic inhibitory drive appears reduced. The combined effects lead to a decrease in control discharge frequency (F_{con}) of the premotor neuron.

sevoflurane, although these values did not reach statistical significance because of the variability of the data. Although substantial evidence indicates that anesthetics inhibit synaptic glutamatergic excitation by decreasing presynaptic glutamate release,^{8,17} this mechanism has not been shown for GABA. On the contrary, intravenous anesthetics were shown to increase the release of ³H-GABA from rat cortical synaptosomes.¹⁸ In a rat hippocampal slice preparation, Nishikawa and MacIver¹⁹ demonstrated that 1 MAC halothane increased the frequency of miniature IPSCs in voltage clamped inhibitory interneurons and CA1 pyramidal neurons by 100%. This indicated a halothane-evoked, action potential-independent, presynaptic release of GABA to these neurons. An anesthetic-induced presynaptic increase in spontaneous GABA release is not necessarily inconsistent with our conclusion that the total presynaptic inhibitory input may be reduced by the anesthetics in our preparation. For example, Nishikawa and MacIver¹⁹ showed that, in inhibitory interneurons, 1 MAC halothane reduced the stimulus-evoked excitatory postsynaptic potential amplitude to 50% and increased the inhibitory postsynaptic potential amplitude to 220%. This is likely to cause a proportional depression of action potentials in the interneuron and thus a pronounced reduction of action potential-dependent GABA release to postsynaptic pyramidal neurons. In the preparation of Nishikawa and MacIver,¹⁹ miniature IPSCs in pyramidal neurons had an amplitude of 100–200 pA, whereas IPSCs evoked by stimulation of the Schaffer collaterals had an amplitude of 600 pA. It is conceivable that in a spontaneously active *in vivo* network with tonic GABAergic inhibition, as in our preparation, a small increase in spontaneous presynaptic GABA release by 1 MAC halothane will be

counterbalanced by decreased activity of the presynaptic inhibitory neurons.

In our decerebrate *in vivo* preparation, expiratory premotor neurons receive a tonic inhibitory input⁴ that reduced the overall glutamatergic drive by 40–50% in the absence of anesthesia (α for both studies 0.43 ± 0.10). We assume that the anesthetic-induced decrease in presynaptic inhibitory drive that our data suggest is caused by inhibition of antecedent inhibitory GABAergic neurons that is of a greater magnitude than any possible anesthetic-induced, non-action potential-dependent increase in GABA release by these neurons.

The above line of reasoning supports the hypothesis⁵ that, at 1 MAC, halothane and sevoflurane enhance overall inhibition because of the pronounced anesthetic-induced increase in postsynaptic GABA_A receptor function (fig. 6, bottom). This enhanced postsynaptic inhibition more than offsets any decrease in presynaptic inhibitory input. However, at higher anesthetic doses, overall inhibition may be depressed. For example, overall inhibition was depressed 34% when halothane was increased from 1 to 2 MAC in an otherwise comparable, but neuraxis-intact canine preparation.²⁰ This hypothesis is also supported by findings in rat hippocampal slices, where the increase in GABA_A receptor function as well as the anesthetic-induced GABA release were maximal between 1 and 2 MAC for enflurane and isoflurane.¹⁵ In contrast, the depression of stimulus-evoked excitatory postsynaptic potentials in interneurons in rat hippocampal slices continually increased with the increasing halothane dose.⁷

Halothane and Sevoflurane Affect Overall Inhibition and Excitation

In cultured rat neocortical slice preparations, GABA_A antagonism with bicuculline could reverse 90% of the

depressant effect of halothane, suggesting that the anesthetic effect was predominantly mediated by GABA_A receptors.²¹ On the other hand, Eilers *et al.*⁹ showed that isoflurane and enflurane reduced depolarization-evoked glutamate release in rat cortical brain slices, presenting another possible mechanism of volatile anesthetic-mediated depression. The investigators could demonstrate that structurally similar halogenated alkanes without clinical anesthetic properties did not affect the glutamate release, indicating that this release mechanism might specifically mediate anesthetic action. Nishikawa and MacIver¹⁹ found that, in the interneurons and pyramidal neurons of a hippocampal slice preparation, 1 MAC halothane led to both an enhancement of GABAergic inhibition and a depression of the glutamatergic excitatory postsynaptic potential. The combined effects resulted in a 2.5-fold decreased probability of evoking a postsynaptic action potential.¹⁹ The results of our *in vivo* preparation agree with the observations of Nishikawa and MacIver,¹⁹ *i.e.*, at 1 MAC halothane and sevoflurane, neuronal activity was depressed by both a presynaptic⁵ depression of glutamatergic excitation and an enhancement of overall GABAergic inhibition.

The In Vivo Model Puts Single Receptor Effects into Clinical Perspective

Voltage clamp studies in single rat pyramidal cells show direct anesthetic effects on GABA_A receptors and a strong enhancement of the receptor response to GABA.²² In our *in vivo* preparation, we also observed a strong (65–75%) enhancement of GABA_A receptor function by 1 MAC halothane and sevoflurane. However, the corresponding increase in overall synaptic inhibition was much smaller. This shows that observations on single receptors in isolated cells do not necessarily have clinical significance because they cannot predict how anesthetics affect neurons in an intact neuronal network during physiologic conditions. Our setup allows the assessment of these receptor effects in single neurons in an *in vivo* network model that is closer to the clinical situation. In addition, quantitative and qualitative differences in anesthetic mechanisms between volatile anesthetics that have been shown in the patch clamp preparation²³ were not reflected in the magnitude of enhancement of receptor function by 1 MAC halothane and sevoflurane in our *in vivo* network.

Interestingly, at 1 MAC halothane and sevoflurane, the relative depression of excitation and the enhancement of inhibition were of a similar magnitude, *i.e.*, approximately 15%. Consequently, for the development of new anesthetic substances, *e.g.*, with less respiratory side effects, it may not be sufficient to focus only on one transmitter system.

In the anesthetic-free state, overall excitatory drive to expiratory premotor neurons was tonically inhibited by 43% ($\alpha = 0.43 \pm 0.1$). In neurons that are subject to a

larger baseline tonic inhibition, a 15% enhancement of inhibition by an anesthetic would translate into a more pronounced effect on the depression of neuronal activity. It remains to be determined if differences exist between the anesthetic effect on inspiratory and expiratory neurons.

Methodological Considerations

Possible Indirect Effects of the Picoejection. Extracellular recording with microelectrodes allows stable *in vivo* recording of single neuronal activity over long periods of time, even during changes of the state of anesthesia when changes in blood pressure may cause movements of the tissue that make prolonged intracellular recordings *in vivo* practically impossible. One of the concerns of the picoejection method is that the ejected drug may diffuse into the tissues beyond the neuron of interest and possibly affect adjacent neurons, which may alter the activity of the recorded neuron in an unpredictable way. However, simulations suggest that the ejected concentrations that are applied in close proximity to the recorded neuron decrease steeply with increasing distance from the ejection site.²⁴ We consider this an advantage of localized pressure ejection compared with *in vitro* bath application, where all neurons of the preparation are exposed to the same concentration of the drug, and therefore the activity of the whole network may be altered. Microiontophoresis can also be applied more locally, but dose-response curves comparable to the picoejection method are difficult to obtain because it is not possible to directly measure the amount of ejected drug. The picoejection of bicuculline causes a relatively large increase in neuronal firing frequency, but the highly consistent drug response suggests that the drug effect is mainly postsynaptic. Significant additional effects on surrounding interneurons would be expected to cause inconsistent or poorly reproducible responses in the neuron of interest. We did not observe such behavior. The neuronal responses to picoejected drugs were always consistent with the expected postsynaptic effects of the picoejected drugs. In this context it is important to note that both the concentrations of the drugs as well as the total volumes of our picoejections are considerably smaller than those that are microejected into brain regions to cause reflex changes that can be observed as whole system responses.

Possible Desensitization of the GABA_A Receptor. Application of higher GABA concentrations, *e.g.*, greater than 50 μ M bath concentrations for single hippocampal neurons, has been reported to cause a desensitization of the GABA_A receptor.²² We did not observe any diminished efficacy with picoejection of the GABA_A agonist muscimol. Continuous muscimol picoejection over 8–12 min reproducibly achieved a steady state decrease in neuronal frequency, which suggests that desensitization of the respiratory neuron to this agonist in our *in*

vivo model does not play an important role. During control conditions, these neurons receive tonic inhibitory drive that can be antagonized with GABA_A antagonists.⁴ Thus, the neuronal GABA_A receptors are continuously exposed to endogenous GABA. Because the observed dose-response to muscimol (7.5 μM) was linear, we assume that this additional GABAergic stimulus was in a concentration range, where physiologically relevant desensitization did not occur. We speculate that this agonist concentration range may be comparable to low GABA concentrations of approximately 10 μM or less where the Cl⁻-current dose-response is linear.²² In addition, the neuronal response to muscimol was similar to the response of the GABA uptake inhibitor nipecotic acid.⁶ We thus conclude that our experiments stimulated the GABA_A receptor in a range that was close to physiologic conditions.

In summary, 1 MAC halothane and sevoflurane produced a small increase in overall inhibition of expiratory premotor neuronal activity. This increase of overall inhibition by the anesthetics can be explained by the observed marked enhancement of GABA_A receptor function that appears to be partially offset by a reduction in presynaptic inhibitory inputs.

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Appendix: Estimation of the Presynaptic Inhibitory Component

We assumed that the magnitude of overall inhibition (α) is proportional to the product of the cascaded presynaptic (pre) and postsynaptic (post) components of inhibitory neurotransmission:

$$\text{at 0 MAC: } \text{pre}_0 * \text{post}_0 \propto \alpha_0 \quad (1)$$

$$\text{at 1 MAC: } \text{pre}_1 * \text{post}_1 \propto \alpha_1 \quad (2)$$

The ratio of overall inhibition for the two anesthetic levels (α_1/α_0) is a measure of the anesthetic effect on this mechanism. Thus:

$$\alpha_1/\alpha_0 = (\text{pre}_1 * \text{post}_1)/(\text{pre}_0 * \text{post}_0), \text{ or} \quad (3a)$$

$$\alpha_1/\alpha_0 = (\text{pre}_1/\text{pre}_0) * (\text{post}_1/\text{post}_0) \quad (3b)$$

We further assumed that the anesthetic effect on the muscimol response at D_{max} (ρ) is an index of the anesthetic effect on the postsynaptic receptor function, *i.e.*

$$\text{post}_1/\text{post}_0 = \rho_1/\rho_0 \quad (4)$$

Thus, equation 3b becomes:

$$\alpha_1/\alpha_0 = (\text{pre}_1/\text{pre}_0) * (\rho_1/\rho_0), \quad (5)$$

and rearranging yields :

$$\text{pre}_1/\text{pre}_0 = (\alpha_1/\alpha_0)/(\rho_1/\rho_0), \quad (6)$$

Anesthesia-induced changes (Δ) were expressed as the ratio of the difference between 1-MAC and 0-MAC value, relative to the 0-MAC value. This means for the presynaptic inhibitory component Δpre :

$$\Delta\text{pre} = (\text{pre}_1 - \text{pre}_0)/\text{pre}_0 = [(\text{pre}_1/\text{pre}_0)-1] \quad (7)$$

Substituting equation 6 into equation 7 yields :

$$\Delta\text{pre} = [(\alpha_1/\alpha_0)/(\rho_1/\rho_0)-1], \quad (8)$$

which estimates the anesthetic effect on the presynaptic component based on the effects on overall inhibition and on the postsynaptic component of neurotransmission.