Anesthesiology 2004; 101:1167–76

Propofol and Sevoflurane Depress Spinal Neurons In Vitro via *Different Molecular Targets*

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Background: **The capacity of general anesthetics to produce immobility is primarily spinally mediated. Recently, compelling evidence has been provided that the spinal actions of propofol involve γ-aminobutyric acid type A ($GABA_A$) receptors, whereas the contribution of glycine receptors remains uncertain. The relevant molecular targets of the commonly used volatile anesthetic sevoflurane in the spinal cord are largely unknown, but indirect evidence suggests a mechanism of action distinct from propofol.**

Methods: **The effects of sevoflurane and propofol on spontaneous action potential firing were investigated by extracellular voltage recordings from ventral horn interneurons in cultured spinal cord tissue slices obtained from embryonic rats (embryonic days 14–15).**

Results: **Propofol and sevoflurane reduced spontaneous action potential firing of neurons. Concentrations causing half-maximal effects (0.11 μM propofol, 0.11 mM sevoflurane) were lower than the median effective concentration immobility (1–1.5 μM propofol, 0.35 mM sevoflurane). At higher concentrations, complete inhibition of action potential activity was observed with sevoflurane but not with propofol. Effects of sevoflurane were mediated predominantly by glycine receptors (45%) and $GABA_A$ receptors (38%), whereas propofol acted almost exclusively *via* $GABA_A$ receptors (96%).**

Conclusions: **The authors' results suggest that glycine and $GABA_A$ receptors are the most important molecular targets mediating depressant effects of sevoflurane in the spinal cord. They provide evidence that sevoflurane causes immobility by a mechanism distinct from the actions of the intravenous anesthetic propofol. The finding that propofol acts exclusively *via* $GABA_A$ receptors can explain its limited capacity to depress spinal neurons in the authors' study.**

IMMOBILITY is an important aspect of anesthesia. It is now well accepted that ablation of spontaneous or stimulus-induced movements by general anesthetics is primarily spinally mediated.[1–3] In knock-in mice carrying a subtle mutation in the β_3 subunit of the γ-aminobutyric acid type A ($GABA_A$) receptor, which renders receptors containing this subunit largely insensitive to propofol and etomidate, both anesthetics fail to suppress withdrawal reflexes.[4] This result indicates that propofol and etomidate cause immobility predominantly by modulating $GABA_A$ receptors.[3] The latter conclusion is supported by the observation that the noncompetitive $GABA_A$ receptor antagonist picrotoxin increases the ED_{50} immobility for propofol in rats by 200–400%.[5] However, several studies on glycine receptors have shown potentiating effects of propofol.[6,7] Therefore, it is uncertain to what extent glycine receptors contribute to the effects of propofol on spinal neurons. For volatile anesthetics, $GABA_A$ receptors seem to be a target of minor importance in the spinal cord because picrotoxin only produces a small increase in minimum alveolar concentration (MAC) immobility.[5] Recent studies on evoked neuronal responses in spinal cord slices have shed some light on the molecular mechanisms by which enflurane depresses spinal excitability.[8–10] In contrast to propofol and etomidate, enflurane decreases excitability of spinal neurons by acting *via* multiple molecular targets, including glycine, $GABA_A$, and glutamate receptors.

In the current investigation, we studied the effects of propofol and sevoflurane on neurons in tissue cultures derived from the spinal cord of rats. Instead of analyzing drug actions on evoked neuronal responses as done by Cheng and Kendig[8,10] to evaluate the effects of enflurane, we induced spontaneous network activity by lowering extracellular Mg^{2+} ions. Previous studies on neocortical slices have shown that this kind of network activity is sensitive to clinically relevant anesthetic concentrations.[11,12] Inspired by the literature cited above, two questions are addressed: (1) Do sevoflurane and propofol reduce excitability by similar mechanisms? (2) Are the depressant effects of propofol restricted to $GABA_A$ receptors and thus different from the effects of sevoflurane?

Materials and Methods

Spinal Slice Cultures

All procedures were approved by the animal care committee (Eberhard-Karls-University, Tuebingen, Germany) and were in accordance with the German law on animal experimentation. Preparation of spinal cord slices was performed according to the method described by Braschler *et al.*[13] Pregnant Sprague-Dawley rats (day 14–15) were anesthetized, and the uterus was aseptically removed into a sterile Petri dish. Embryos were freed from the uterus and the amniotic sac and stored in ice-cold Gey's balanced salt solution consisting of 1.5 mM $CaCl_2$, 5 mM KCl, 0.22 mM KH_2PO_4, 11 mM $MgCl_2$, 0.3 mM $MgSO_4$, 137 mM NaCl, 0.7 mM $NaHCO_3$, and 33 mM glucose. In a following step, the embryos were decapitated, and the spinal column was freed from the inner organs and the limbs. The spinal column was then em-

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Received from the Section of Experimental Anesthesiology, Department of Anesthesiology and Intensive Care, Eberhard-Karls-University, Tuebingen, Germany. Submitted for publication January 21, 2004. Accepted for publication July 24, 2004. Supported by fortune fund (1079-0-0), Eberhard-Karls-University, Tuebingen, Germany.

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bedded into an agar block that was glued onto a Teflon (Lee, Frankfurt, Germany) block and was cut transversely into 300-μm slices using a vibratome. Afterward, slices including the spinal cord dorsal root ganglions were placed on a coverslip and embedded in a plasma clot consisting of 20 μl heparin-treated chicken plasma and coagulated by 20 μl of a thrombin solution. The coverslips were inserted into plastic tubes containing 0.75 ml nutrient fluid including 10 nM nerve growth factor (Sigma, Taufkirchen, Germany) and incubated at 5% carbon dioxide at 36.0°C for 1–2 h. The roller tube technique described by Gahwiler[14] was used to culture the tissue. After 1 day in culture, antimitotics (10 μM 5-fluoro-2-deoxyuridine, 10 μM cytosine-b-d-arabinofuranoside, 10 μM uridine) were added to reduce proliferation of glial cells. The nutrient fluid and the antimitotics were renewed twice a week. Slices were used after 12 days *in vitro* for extracellular recordings.

Extracellular Recordings

Spinal cord slices were continuously perfused with an artificial cerebrospinal fluid (ACSF) consisting of 120 mM NaCl, 3.3 mM KCl, 1.13 mM NaH_2PO_4, 26 mM $NaHCO_3$, 1.8 mM $CaCl_2$, and 11 mM glucose. The ACSF was bubbled with 95% oxygen and 5% carbon dioxide. Glass electrodes with a resistance of approximately 2–5 MΩ were filled with ACSF and positioned on the surface of the slices. Electrodes were advanced into the tissue until extracellular spikes exceeding 100 μV in amplitude were visible and single- or multiple-unit activity could be clearly identified. The noise amplitude was approximately 50 μV. All experiments were performed at 34°–36°C. The recording chamber consisted of a metal frame with a glass bottom. A heating wire was glued onto the metal frame to achieve appropriate temperature during the experiments.

Preparation and Application of Test Solutions

Test solutions including sevoflurane were prepared as described previously for other volatile anesthetics[12] by dissolving the liquid form of sevoflurane in the ACSF, which was equilibrated with 95% oxygen and 5% carbon dioxide. A closed, air-free system was used to prevent evaporation. After the test solutions were stirred for at least 120 min, they were transferred into gas-tight glass syringes avoiding air exposure. Anesthetic concentrations are given as multiples of MAC. These MAC values refer to the plasma or blood concentrations of volatile anesthetics in mammals at 37°C. We used the median effective concentration (EC_{50}) values for general anesthesia proposed by Franks and Lieb.[15] Thus, we assume that 1 MAC corresponds to an aqueous concentration of 0.35 mM sevoflurane. Propofol was obtained from Tocris (Cologne, Germany). Propofol was dissolved in dimethyl sulfoxide (Sigma) to produce a 1 M stock solution. This stock solution was diluted in ACSF to achieve the appropriate concentrations. When dimethyl sulfoxide was tested at the same concentration as that present in the highest propofol concentration (10 μl/l), spontaneous action potential firing remained unchanged (depression of 4.7 ± 12.2%, not significant, Student *t* test, n = 6).

Anesthetics were applied *via* bath perfusion using gas-tight syringe pumps (ZAK, Marktheidenfeld, Germany), which were connected to the experimental chamber *via* Teflon tubing (Lee). The flow rate was approximately 1 ml/min. Switching from ACSF to drug-containing solution replaced the medium in the experimental chamber by at least 95% within 2 min. Effects on spike patterns were stable approximately 5 min later. To ensure steady state conditions, recordings during anesthetic treatment were performed 10–15 min after commencing the change of the perfusate. The time required for recovery depended on the drug tested. With 0.15–1.5 MAC sevoflurane, full recovery occurred after 12–15 min and with 0.075–1.5 μM propofol after 30–60 min. For a single application, stable recording for approximately 1 h was necessary.

Data Analysis

Data were low-pass filtered between 3 and 10 kHz as acquired on a personal computer using the Digidata 1200 AD/DA interface (Axon Instruments, Union City, CA). Records were in addition stored on a Sony data recorder PC 204A (Racal Elektronik, Bergisch Gladbach, Germany). Further analysis was performed using self-written software in OriginPro version 7 (OriginLab Corporation, Northampton, MA) and MATLAB version 6 (The MathWorks Inc., Natick, MA).

After close inspection of the data, a threshold was set manually to avoid artifacts produced by baseline noise (fig. 1A). The mean firing rate was obtained from single- or multiple-unit recordings and defined as the number of action potentials breaking the threshold divided by the recording time of 180 s. As shown in figure 1A action potentials appeared in bursts, separated by silent periods. The burst rate was calculated from the number of bursts occurring during the recording period (180 s). For quantifying the peak firing rate, burst durations were subclassified into 50-ms bins. The peak firing rate represents the highest firing frequency within a single burst. For statistical analysis, the Student *t* test was used. Unless otherwise stated, results are given as mean ± SEM. Concentration–response curves were fitted by Hill equations as previously described.[12] Estimated EC_{50} values were derived from these fits.

Results

Action Potential Firing in Cultured Spinal Cord Slices

Spinal cord slices were taken from embryonic rats and cultured. After 6–8 days *in vitro*, spontaneous neuronal

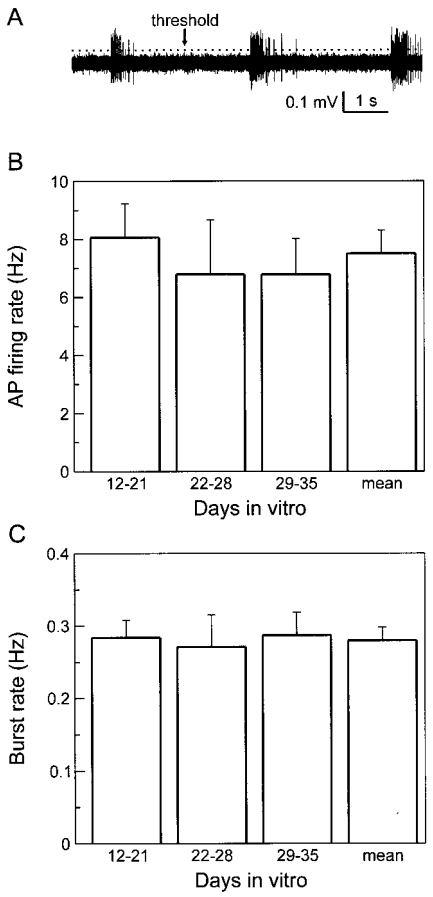

Fig. 1. Characteristics of spontaneous neuronal activity in spinal cord tissue slices in the absence of drugs. (*A*) Extracellular recording from the ventral horn area. Action potentials appear in groups (bursts). The threshold used for detecting single action potentials is indicated by the *dotted line*. (*B*) Action potential (AP) firing rate remained constant between 12 and 35 days *in vitro*. Within this time window, tissue cultures were used. The mean action potential firing rate was 7.51 ± 0.80 Hz (n = 58). (*C*) The mean burst rate was calculated to be 0.28 ± 0.02 Hz (n = 58) and showed no significant differences between 12 and 35 days *in vitro*.

activity was detectable. We performed extracellular recordings from spinal interneurons visually identified in the ventral horn area. A typical recording is shown in figure 1A. Single action potentials can be identified as vertical deflections. Action potentials were grouped in bursts separated by silent periods lasting approximately 2–3 s. Two parameters of spontaneous neuronal activity, the mean firing rate and the burst rate, were quantified to characterize firing patterns over a period of 12–35 days *in vitro*. Under drug-free conditions, the mean burst rate was 0.28 ± 0.02 Hz (n = 58), which is in accord with previously published data.[16,17] The average firing rate was 7.51 ± 0.80 Hz (n = 58). Between 12 and 35 days *in vitro*, the mean firing rates and the mean burst rates remained constant (figs. 1B and C). Cultures of the corresponding age were used to analyze the effects of sevoflurane and propofol.

Effects of Sevoflurane and Propofol on the Discharge Patterns

Representative examples of the effects of sevoflurane and propofol on the discharge patterns of spinal neurons are given in figures 2 and 3. Original recordings are shown on the left, with corresponding binned spike data on the right. Both anesthetics decreased spontaneous action potential firing in a concentration-dependent manner. Figure 2 displays the effects of sevoflurane on the discharge patterns. At 0.75 MAC, sevoflurane decreased the mean discharge rate by reducing the number of spikes per bin. At 1.5 MAC, not only the number of spikes per bin but also the burst rate was diminished. Figure 3 presents original recordings in the absence and presence of 0.25 and 0.5 μM propofol. At both concentrations, the decrease of the mean discharge rate resulted from a concentration-dependent reduction of the number of spikes per bin. Figure 4 shows the effects of sevoflurane and propofol on the peak firing rate and the burst rate. Both anesthetics decreased the mean firing rate predominantly by reducing the peak firing rates (figs. 4A and C). At low concentrations, sevoflurane and propofol accelerated the burst rate, whereas at concentrations above 1 MAC, sevoflurane depressed the burst rate (figs. 4B and D). At concentrations above 0.1 μM, propofol left the burst rate unchanged.

Effects of Sevoflurane and Propofol on the Average Discharge Rates

The concentration-dependent effects of sevoflurane and propofol on the average discharge rates of spinal neurons are summarized in figure 5. Concentration–response relations were fitted by Hill equations. The estimated EC_{50} values and Hill coefficients are given in table 1. With both anesthetics, half-maximal depression was observed at concentrations below the EC_{50} for general anesthesia. Surprisingly, full depression of spontaneous activity was only achieved with sevoflurane. With propofol, the concentration–response curve showed an upper limit close to 60% (fig. 5B).

Effects of the $GABA_A$ Receptor Antagonist Bicuculline and the Glycine Receptor Antagonist Strychnine

Figure 6 summarizes the effects of the $GABA_A$ receptor antagonist bicuculline (100 μM), the glycine receptor antagonist strychnine (1 μM), and a combination of both antagonists on ongoing neuronal activity. The effectiveness of 1 μM concentration of strychnine in saturating glycine receptors has previously been shown in the rat spinal cord in accord with the high receptor affinity of this antagonist.[18] This was confirmed by a study on organotypic spinal cord slices, where, in the presence of bicuculline, the concentration of strychnine was increased from 1 to 10 μM, and no additional change in bursting pattern was observed, thus suggesting that 1 μM strychnine had already saturated

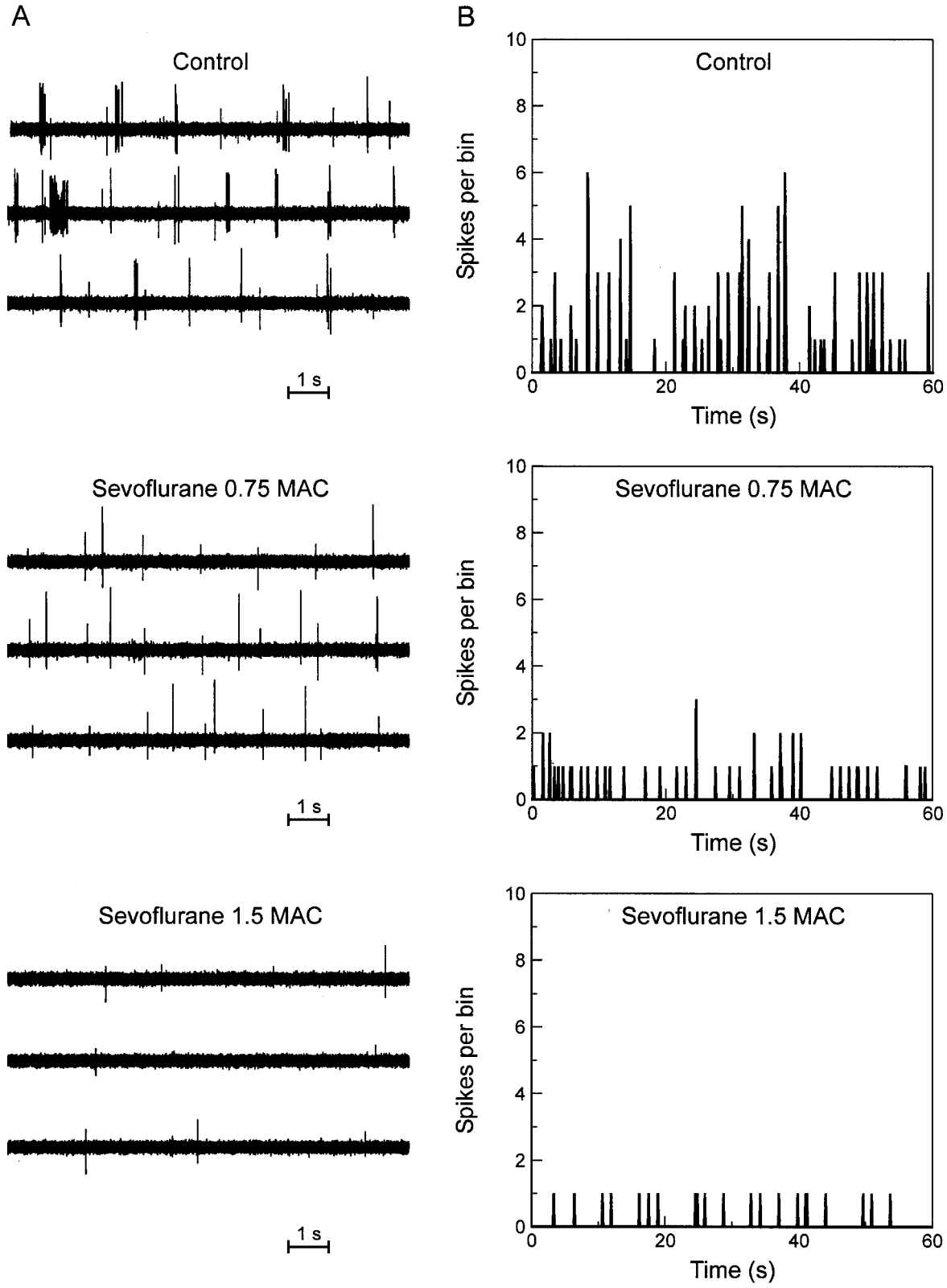

Fig. 2. Concentration-dependent actions of sevoflurane on the discharge patterns of spinal neurons. Sevoflurane was applied at a concentration of 0.26 mM (0.75 minimum alveolar concentration [MAC]) and 0.52 mM (1.5 MAC). (*A*) Original recordings in the absence and presence of the anesthetic. (*B*) Corresponding binned data derived from the recording in *A*. Spikes were binned at 50-ms intervals. The average firing rates were 1.96 Hz (control), 0.71 Hz (0.26 mM), and 0.32 Hz (0.52 mM). Burst rates were calculated to be 0.44 Hz (control), 0.43 Hz (0.26 mM), and 0.32 Hz (0.52 mM). A depression of the burst rate could be observed at a concentration above 1 MAC.

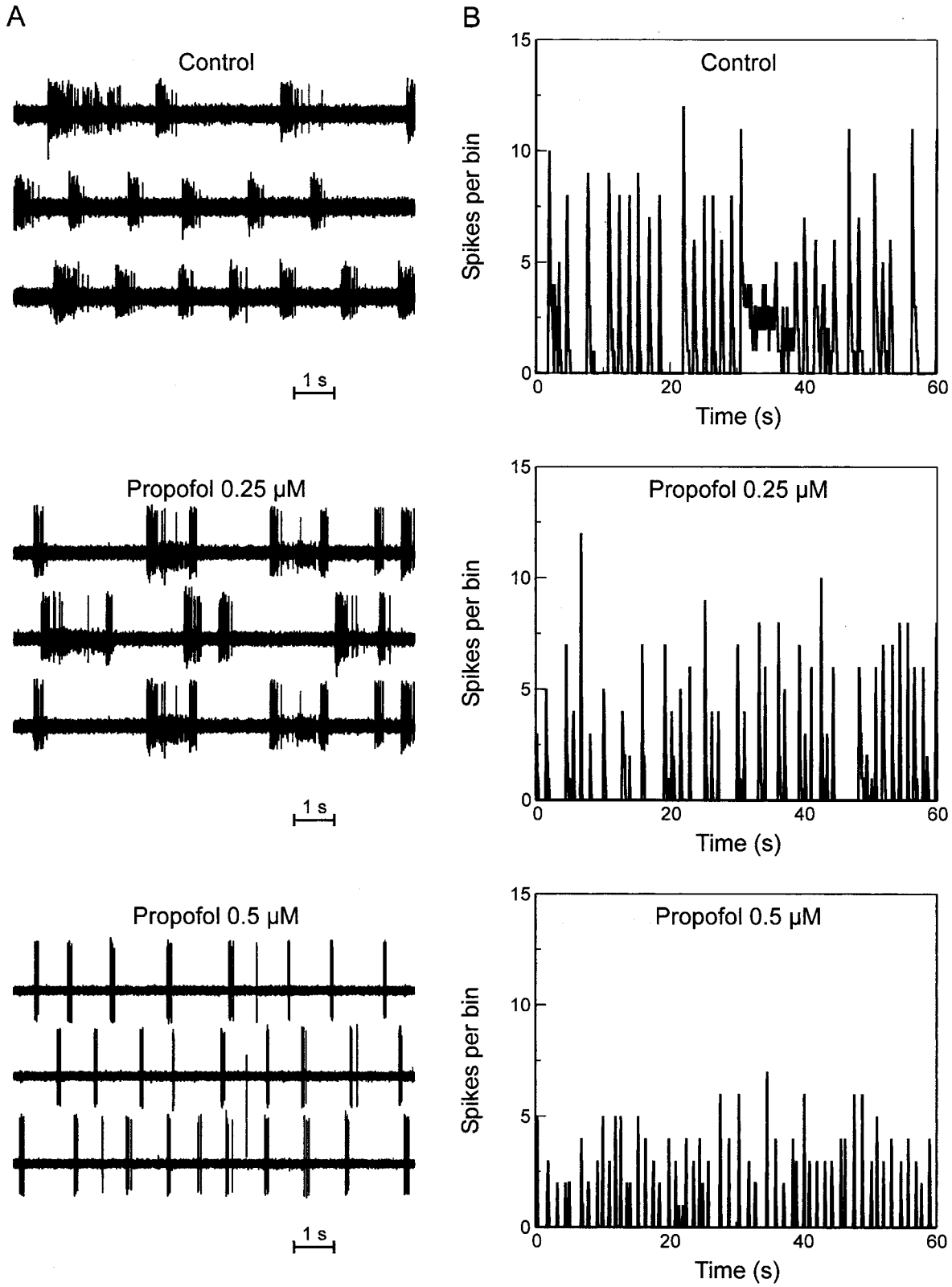

Fig. 3. Effects of propofol on spontaneous action potential firing of spinal neurons. Propofol was applied at concentrations of 0.25 and 0.5 μM. (*A*) Original recordings in the absence and presence of the anesthetic. (*B*) Corresponding binned data derived from the recording displayed in *A*. Spikes were binned at 50-ms intervals. Mean spike rates were 13.13 Hz (control), 8.14 Hz (0.25 μM), and 4.39 Hz (0.5 μM). Burst rates were 0.24 Hz (control), 0.33 Hz (0.25 μM), and 0.46 Hz (0.5 μM).

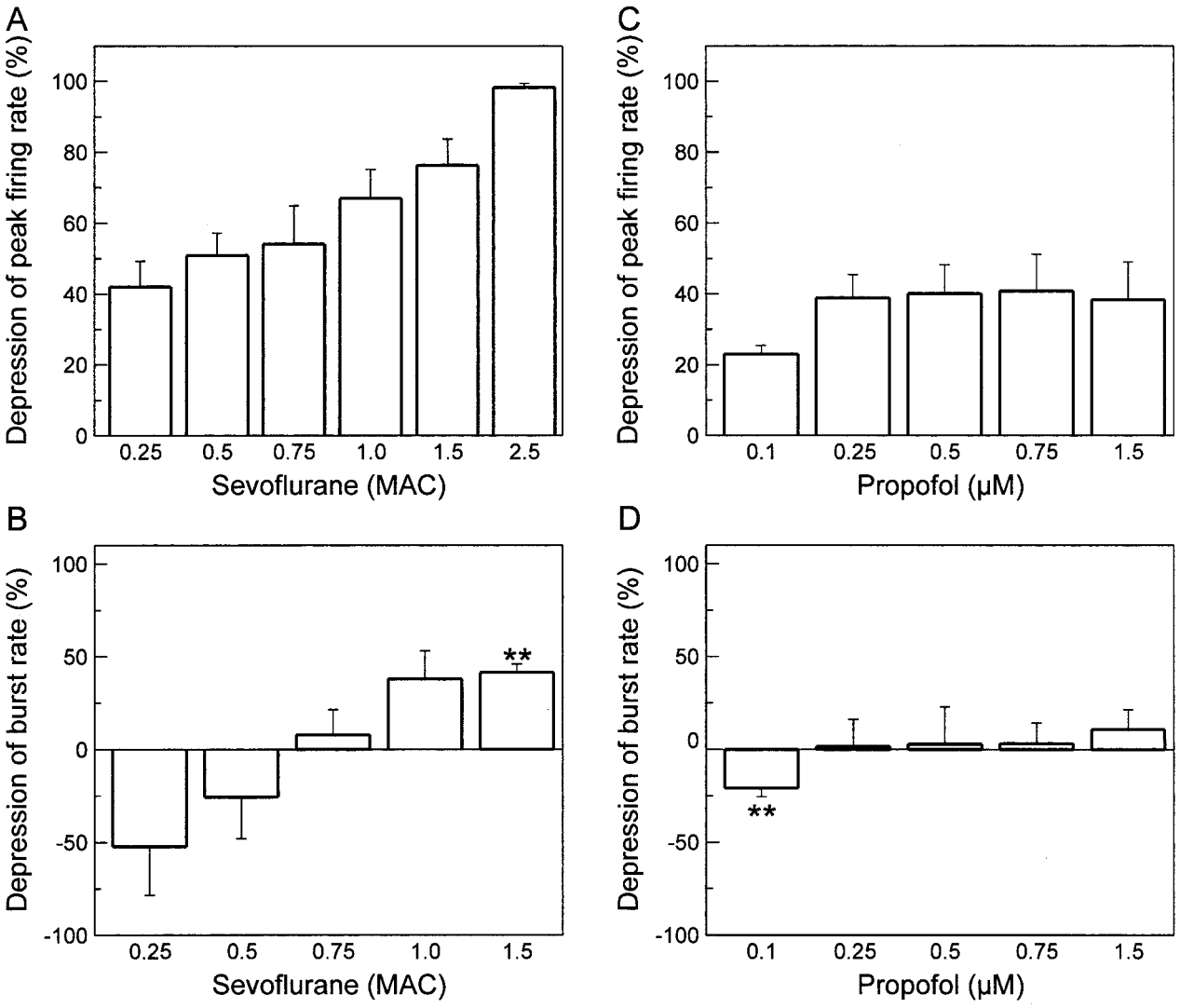

Fig. 4. Changes in firing patterns caused by sevoflurane and propofol. (*A*) Sevoflurane induced a concentration-dependent depression of the peak firing rate (n = 6–9). (*B*) The burst rate was accelerated at low concentrations of sevoflurane but significantly depressed at 1.5 minimum alveolar concentration (MAC) (*t* test, ** $P < 0.01$, n = 6–10). (*C*) Propofol depressed the peak firing rate in a concentration–dependent manner, reaching a maximum at 0.25 μM (n = 6–10). (*D*) Propofol induced a significant increase in the burst rate at 0.1 μM (*t* test, ** $P < 0.01$). At higher concentrations, there was no significant change in the burst rate (n = 6–10).

glycine receptors.[19] Because a bicuculline concentration of 20 μM was not sufficient to block $GABA_A$ receptors in cultured neocortical slices completely, we used a bicuculline concentration of 100 μM, which was shown to exert a complete blockade of $GABA_A$ receptor–mediated conductance in organotypic neocortical slices.[11]

All experimental conditions accelerated the mean firing rate and led to a moderate depression of the burst rate. Disinhibition of spontaneous network activity by blocking $GABA_A$ receptors with 100 μM bicuculline significantly increased the firing rate by approximately 89% (*t* test, $P < 0.001$, n = 25), whereas the burst rate was depressed by 25% (*t* test, $P < 0.05$, n = 25). Disinhibition of spontaneous network activity by blocking glycine receptors with 1 μM strychnine increased the firing rate by approximately 108% (*t* test, $P < 0.01$, n = 15) and reduced the burst rate by 30% (*t* test, $P < 0.001$, n = 15). When blocking $GABA_A$ and glycine receptors simultaneously by bicuculline (100 μM) and strychnine (1 μM), the mean firing rate was increased by approximately 132% (*t* test, $P < 0.001$, n = 12), whereas the burst rate was depressed by 32% (*t* test, $P < 0.01$, n = 9).

Effects of Sevoflurane and Propofol in the Presence of Antagonists of $GABA_A$ or Glycine Receptors

Equieffective concentrations of sevoflurane and propofol were used for the drug interaction experiments. The effects of 0.5 MAC sevoflurane (corresponding to 0.175 mM) or 0.5 μM propofol on discharge rates were analyzed in the presence or absence of the specific receptor antagonist bicuculline, to test the involvement of $GABA_A$ receptors, or strychnine for glycine receptors. For example, a decreased efficacy of an anesthetic to reduce the mean firing rate in the presence of a $GABA_A$

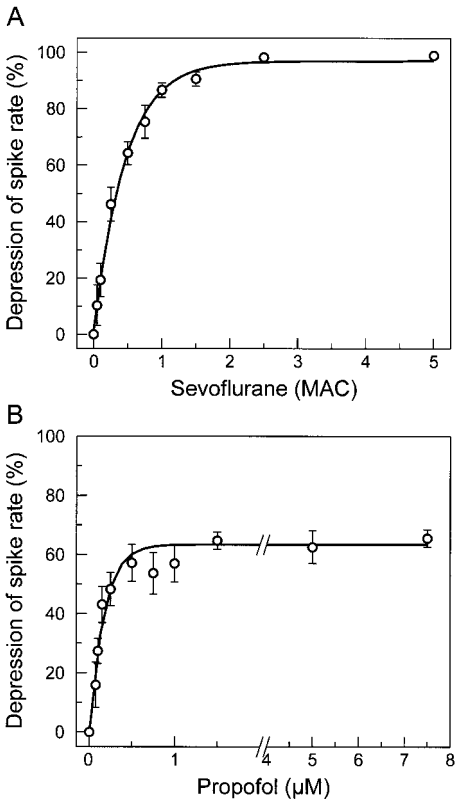

Fig. 5. Concentration–response relations of anesthetic-induced depression of mean firing rates for sevoflurane (*A*) and propofol (*B*). For each concentration, the mean value and SE were obtained from 6–12 cells. The effects of sevoflurane and propofol were calculated by comparing the spike rates before and during treatment. The curves were fitted with Hill equations. Table 1 shows the median effective concentration (EC_{50}) values and Hill coefficients. MAC = minimum alveolar concentration.

receptor antagonist is indicative for the anesthetic to act *via* the $GABA_A$ receptor–ion channel complex.[3,5,11]

Figure 7 presents the effects of sevoflurane and propofol in the absence or presence of bicuculline (100 μM), strychnine (1 μM), or a combination of both antagonists. We tested the hypothesis that the depressant effects of 0.5 MAC sevoflurane (corresponding to 0.175 mM) or 0.5 μM propofol on discharge rates did not differ in the presence or absence of bicuculline. This hypothesis had to be rejected for both anesthetics, although the extent of depression differed. Bicuculline decreased sevoflurane-induced depression of ongoing activity by approximately 38% (*t* test, $P < 0.01$, n = 13; fig. 7A), whereas suppression of action potential firing by propofol was completely abolished (*t* test, $P < 0.001$, n = 9; fig. 7B).

Table 1. Half-maximal Depression of Average Spike Rates and Hill Coefficients, as Calculated from the Concentration–Response Fits in Figure 5

	Sevoflurane, mM	Propofol, μM
EC_{50}	0.11 ± 0.00 (0.32 ± 0.01 MAC)	0.11 ± 0.01
Hill coefficient	1.23 ± 0.06	1.86 ± 0.38
Goodness of fit (R^2)	0.997	0.958

EC_{50} = median effective concentration; MAC = minimum alveolar concentration.

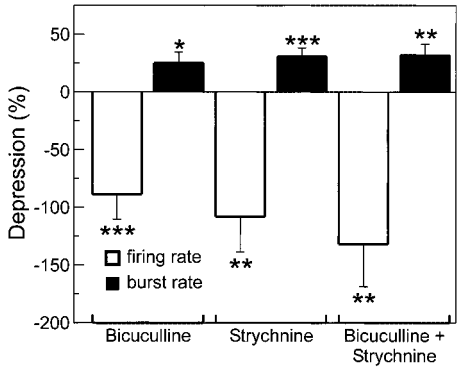

Fig. 6. Effects of bicuculline, strychnine, or both agents on spontaneous action potential firing of spinal neurons. Bicuculline (100 μM) or strychnine (1 μM) or a combination of both antagonists accelerated the mean firing rate (action potential firing rate), whereas they depressed the burst rate (*t* test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

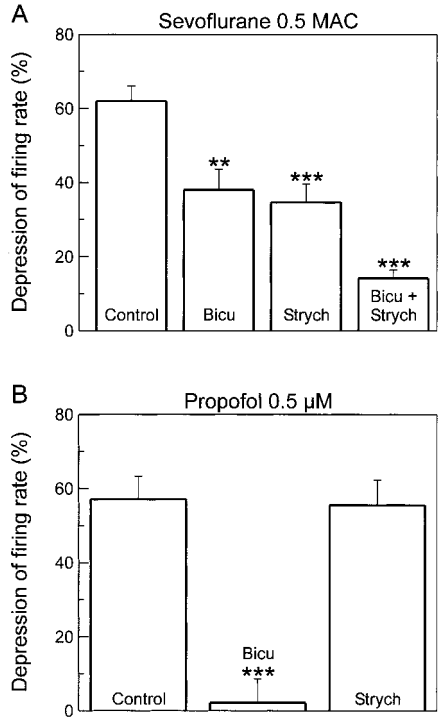

Fig. 7. Effect of sevoflurane and propofol on spontaneous action potential firing of spinal neurons in the presence of bicuculline (Bicu), strychnine (Strych), or bicuculline and strychnine (Bicu + Strych). (*A*) Depression of the mean firing rate by 0.175 mM sevoflurane (0.5 minimum alveolar concentration [MAC]) was reduced almost to the same amount by either 100 μM bicuculline (38%) or 1 μM strychnine (45%). A combination of both antagonists reduced the mean firing rate even further (two-sided *t* test, ** $P < 0.01$, *** $P < 0.001$, n = 9–13). (*B*) Depression of the mean firing rate by 0.5 μM propofol was completely prevented in the presence of 100 μM bicuculline (*t* test, *** $P < 0.001$, n = 9). The presence of strychnine did not affect the action of propofol (*t* test, n = 7).

From this result, we conclude that enhanced $GABA_A$-mediated synaptic inhibition contributed to the decrease in neuronal activity induced by 0.5 MAC sevoflurane and was the predominate mechanism mediating the depressive effects of 0.5 μM propofol.

In analogy to the experiments with bicuculline described above, a decreased efficacy in strychnine-treated slices is indicative for sevoflurane or propofol to act on the glycine receptor–ion channel complex.[10,20] We tested the hypothesis that depression of average firing rates by 0.5 MAC sevoflurane or 0.5 μM propofol did not differ in the presence or absence of strychnine. This hypothesis was rejected for sevoflurane because strychnine reduced the effects of sevoflurane on the mean firing rate by 45% (*t* test, $P < 0.001$, n = 11; fig. 7A). In contrast to sevoflurane, the hypothesis could not be rejected for propofol (fig. 7B) because strychnine did not affect the efficacy of propofol in reducing neuronal activity.

The combination of bicuculline and strychnine reduced the depression of action potential firing of 0.5 MAC sevoflurane by 78% (*t* test, $P < 0.001$, n = 9), indicating that enhancement of γ-aminobutyric acid–mediated and glycinergic synaptic transmission was responsible for a large part but not for all depressive actions of the anesthetic. Figure 8 provides an estimation of how $GABA_A$, glycine, and further, not yet identified receptors contribute to the overall effects of sevoflurane and propofol on ongoing activity. Relative fractions were calculated from the data shown in figure 7.

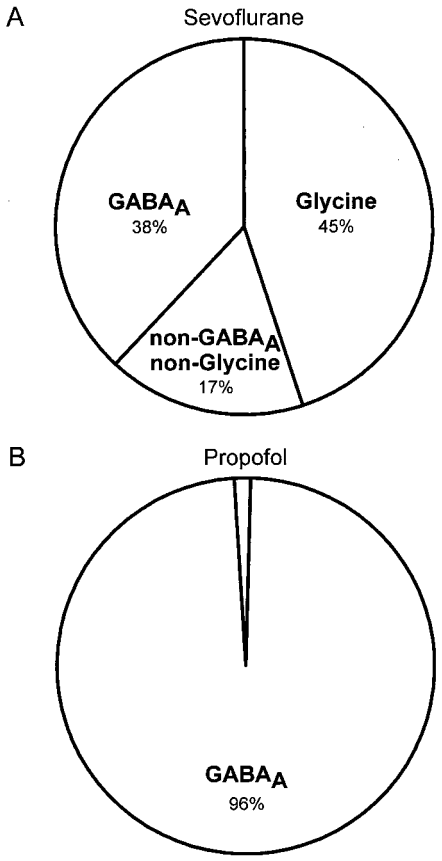

Fig. 8. Estimated contributions of molecular targets to the effects of sevoflurane (*A*) and propofol (*B*) on the mean firing rate. Concentrations of 0.5 minimum alveolar concentration sevoflurane (corresponding to 0.175 mM) and 0.5 μM propofol were used because, at these concentrations, both anesthetics depressed spontaneous network activity by approximately 60% and were therefore equieffective. The effects of sevoflurane and propofol on spontaneous action potential firing were measured in the absence or presence of bicuculline (100 μM), strychnine (1 μM), or a combination of both antagonists. Effects of the anesthetics in the absence of bicuculline and strychnine were taken as 100%. Bicuculline decreased sevoflurane-induced depression of ongoing activity by approximately 38%; suppression of action potential firing by propofol was almost completely abolished (γ-aminobutyric acid type A [$GABA_A$]). Strychnine reduced the effects of sevoflurane on the mean firing rate by 45% and did not affect the efficacy of propofol in reducing neuronal activity (glycine).

Discussion

Spontaneous Activity in Organotypic Spinal Cord Slices

In the current investigation, we studied the effects of sevoflurane and propofol on ongoing action potential activity in spinal slice cultures. A wide variety of movements are initiated by rhythmic pattern-generating circuits, including stereotyped movements such as breathing, or locomotor rhythms such as running.[21] Cultures of spinal cord slices have been established to investigate isolated pattern-generating networks *in vitro*.[13,22] It was recently shown that the sources of bursting activity are situated ventrally on both sides of the central fissure.[23] Furthermore, it has been suggested that the characteristics of the rhythmic activity are comparable to those seen in intact spinal cord during fictive locomotion.[24] Cultures are prepared at embryonic days 14–15 and grown *in vitro* for up to 5 weeks. The experiments were performed at an *in vitro* age (2–5 weeks) corresponding to an *in vivo* postnatal age of 1–4 weeks.[25] We assume that the anesthetic-induced changes in ongoing activity reflect corresponding changes in the excitability of spinal neurons *in vitro*.

Depressant Effects of Sevoflurane and Propofol Occur at Clinically Relevant Concentrations

The concentration of sevoflurane, reducing mean firing rates of spinal neurons by half (EC_{50}), was 0.11 mM (corresponding to 0.32 MAC). This value compares well to the concentration causing half-maximal depression in experimental settings using evoked responses, such as the dorsal root-ventral root potential (EC_{50}, 0.07 mM) or the monosynaptic reflex (EC_{50}, 0.22 mM) in the rat hemisected spinal cord preparation,[26] and are in the same order of magnitude compared with the EC_{50} values for agonist activated Cl^- currents at human $GABA_A$ $\alpha_2\beta_1$ (0.45 ± 0.1 mM) or glycine α_1 receptors (0.36 ± 0.1 mM) expressed in human embryonic kidney 293 cells.[27]

For propofol, the calculated EC_{50} value for agonist-evoked currents mediated at recombinant $GABA_A$ $\alpha_1\beta_1\gamma_{2L}$ receptors expressed in *Xenopus laevis* oocytes was reported to be 2.3 ± 0.2 μM.[6] The plasma concentration of propofol causing surgical immobility in 50% of the patients has been estimated to be between 1 and 1.5 μM.[28–30] In the same study, a plasma concentration of 0.3 μM propofol produced unresponsiveness to verbal commands.[28] Our experiments revealed an EC_{50} value of 0.11 μM for the suppression of ongoing activity in cultivated spinal cord slices. At first glance, this concentration seems surprisingly low. However, in a recent study in humans, subhypnotic concentrations of propofol depressed spinal neurons.[31] This finding provides indirect evidence that propofol decreases excitability of spinal neurons at concentrations smaller than 0.3 μM, which is consistent with our results.

Limited Capacity of Propofol in Reducing Spinal Excitability

The concentration–response relations of anesthetic-induced depression of mean firing rates showed that sevoflurane exerted a complete suppression of the mean discharge rate, whereas effects of propofol sealed to an upper limit of approximately 60%, even at a concentration as high as 7.5 μM. The limited capacity of propofol to depress spinal neurons in the current study is consistent with the findings of Jewett *et al.*,[32] who investigated the effects of propofol on the slow ventral root response in neonatal rat spinal cords. At propofol concentrations above 10 μM, they observed a suppression of approximately 80%. At smaller, clinically relevant concentrations (up to 2 μM), propofol did not exceed a 60% depression. Taken together, these data provide compelling evidence that the intravenous anesthetic propofol, in contrast to the volatile anesthetic sevoflurane, exhibits limited capacity in depressing action potential firing of spinal neurons.

Sevoflurane and Propofol Act via *Different Molecular Targets*

How can we explain that sevoflurane but not propofol caused a complete depression of spontaneous action potential firing? In view of the recent literature,[2,3,7] we hypothesize that sevoflurane acts *via* several molecular targets in the spinal cord, whereas propofol exclusively enhances γ-aminobutyric acid–mediated synaptic transmission. To test this hypothesis, we compared the effects of both anesthetics on spontaneous action potential firing of spinal neurons *in vitro* in the absence and presence of the competitive $GABA_A$ receptor antagonist bicuculline. The effectiveness in depressing the mean firing rates decreased significantly for sevoflurane and was completely abolished for propofol. These results indicate that propofol and sevoflurane develop their depressant action on the network level *via* the $GABA_A$ channel by enhancing GABA-induced Cl^- currents. We estimated that only 38% of the depressant actions of sevoflurane can be attributed to $GABA_A$ receptors. In our study, propofol acted almost exclusively through $GABA_A$ receptors, although several studies reported previously potentiating effects of propofol on glycine receptors as well.[6,7,33] Propofol concentrations shown to be effective in these studies were up to 100- to 1,000-fold higher compared with our experiments. At clinically relevant concentrations, the limitation of propofol to act *via* a single molecular target can therefore explain the limited depressive effect of the anesthetic on the mean firing rate of spinal neurons.

The volatile anesthetic sevoflurane was not only shown to depress neuronal activity in the presence of bicuculline, but also in the presence of strychnine and in the presence of a combination of bicuculline and strychnine. These results clearly indicate that sevoflurane acts on a spinal network level not exclusively *via* $GABA_A$ receptors but also *via* glycine receptors and receptors that are different from both. Sevoflurane was previously reported to be effective at glycine receptors, either in dissociated hippocampal neurons[34] or at recombinant human glycine α_1 receptors expressed in human embryonic kidney 293 cells.[27] The concentrations of sevoflurane used in these studies produced a significant decrease of neuronal activity in our study. The extent of depressive action on glycine receptors mediated by sevoflurane was approximately 45% and not significantly different from the $GABA_A$-mediated part (38%). It has been shown previously that other volatile anesthetics, such as enflurane, isoflurane, and halothane, affect glycine receptors on spinal neurons.[10] For enflurane, the estimated actions on spinal neurotransmission have been determined on a molecular basis in mice by Wong *et al.*[35] In that study, which was performed in whole spinal cords, enflurane depressed spinal neurotransmission *via* $GABA_A$ receptors (30%) and glycine receptors (20%). Although these results are qualitatively similar to our findings, they differ somewhat from those computed in figure 8. This can be explained by the different *in vitro* systems or the different parameters of measurement, as well as by the type of volatile anesthetic used. The yet unidentified molecular targets in our study probably include glutamate receptors.[26]

The current study provides evidence that sevoflurane and propofol depress spinal information processing by different mechanisms. Our finding that propofol acts exclusively *via* $GABA_A$ receptors can explain its limited capacity to depress spinal neurons in the current study. It also may provide an explanation for why propofol does not match all criteria defining a general anesthetic agent.[36,37]

The authors thank Claudia Holt (Technical Assistant, Eberhard-Karls-University, Tuebingen, Germany) for excellent technical assistance.

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