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Different Actions of General Anesthetics on the Firing Patterns of Neocortical Neurons Mediated by the $GABA_A$ Receptor

*Bernd Antkowiak, Ph.D.**

Background: **In cultured slice preparations of rat neocortical tissue, clinically relevant concentrations of volatile anesthetics mainly decreased action potential firing of neurons by enhancing γ-aminobutyric acid ($GABA_A$) receptor–mediated synaptic inhibition. The author's aim was to determine if other anesthetic agents are similarly effective in this model system and act *via* the same molecular mechanism.**

Methods: **The actions of various general anesthetics on the firing patterns of neocortical neurons were investigated by extracellular single-unit recordings.**

Results: **Pentobarbital, propofol, ketamine, and ethanol inhibited spontaneous action potential firing in a concentration-dependent manner. The estimated median effective concentration (EC_{50}) values were close to or below the EC_{50} values for general anesthesia. Bath application of the $GABA_A$ antagonist bicuculline (100 μM) decreased the effectiveness of propofol, ethanol, halothane, isoflurane, enflurane, and diazepam by more than 90%, indicating that these agents acted predominantly *via* the $GABA_A$ receptor. The depressant effects of pentobarbital and ketamine were not significantly reduced by bicuculline treatment. Drugs acting mainly *via* the $GABA_A$ receptor altered the firing patterns of neocortical cells in different manners. Diazepam reduced the discharge rates by decreasing the number of action potentials per burst, leaving the burst rate unaffected. In contrast, muscimol, GABA, propofol, and volatile anesthetics decreased the burst rate.**

Conclusions: **Although several anesthetic agents acted nearly exclusively *via* the $GABA_A$ receptor, they changed the discharge patterns of cortical neurons in different ways. This finding is explained by GABA-mimetic or benzodiazepine-like molecular interactions. (Key words: γ-Aminobutyric acid; brain; ethanol; ketamine; neocortex; pentobarbital; propofol; rat; receptors.)**

GENERAL anesthetics reduce neuronal activity in various parts of the mammalian central nervous system. A considerable number of mechanisms was suggested to mediate this depressant effect. However, it is still a matter of debate as to which molecular targets are truly relevant in producing the anesthetic state.[1-4] In recent studies, the causal relationship between the actions of volatile anesthetics occurring on the molecular level and the corresponding effects on the firing patterns and discharge rates of central neurons were investigated. Volatile anesthetics depressed action potential firing of cells in cerebellar and neocortical brain slices mainly by increasing synaptic inhibition.[5-8] This result was supported by the observations that during voltage-clamp recordings volatile anesthetics enhanced γ-aminobutyric acid ($GABA_A$) receptor-mediated synaptic currents and that the tested agents were significantly less effective in depressing spontaneous action potential firing in preparations in which $GABA_A$-mediated synaptic transmission was pharmacologically blocked. In this work, the effects of pentobarbital, propofol, ketamine, and ethanol on the firing patterns of neurons in neocortical slice cultures were analyzed. In view of data published on the molecular mechanisms by which these drugs act, I expected pentobarbital, propofol, and ethanol but not ketamine to reduce the discharge rates at least in part by enhancing $GABA_A$ receptor function.

Material and Methods

Neocortical Slice Cultures

Neocortical slice cultures were prepared from 0- to 1-day-old Sprague-Dawley rats as previously described.[9] Animals of both sexes were deeply anesthetized with enflurane and decapitated. Cortical hemispheres were aseptically removed and stored in ice-cold Gey's balanced salt solution, consisting of (in millimoles per liter): $CaCl_2$ 1.5, KCl 5, KH_2PO_4 0.22, $MgCl_2$ 11, $MgSO_4$ 0.3, NaCl 137, $NaHCO_3$ 0.7, and glucose 33. Hemispheres were glued onto a Teflon block and sagittal slices 300-400 μm in thickness were cut. The tissue was stored in

* Research Assistant.

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Address reprint requests to Dr. Antkowiak: Max-Planck-Institut für biologische Kybernetik, Spemannstr. 38, 72076 Tübingen, Germany. Address electronic mail to: bernd.antkowiak@tuebingen.mpg.de

a refrigerator at 4°C for 30-45 min. About 20-40 slices were obtained from a single rat. Slices were then transferred onto clean glass coverslips and embedded in a plasma clot (20 μl of heparinized chicken plasma coagulated by 20 μl of a thrombine solution). The roller-tube technique was used for culturing the tissue. After 1 day in culture, antimitotics (10 μM 5-fluoro-2-deoxyuridine, 10 μM cytosine-b-d-arabino-furanoside, 10 μM uridine) were added. The suspension and the antimitotics were renewed twice a week.

Extracellular Recordings

During extracellular recordings, slices were continuously perfused with an artificial cerebrospinal fluid (ACSF) consisting of (in millimoles per liter): NaCl 120, KCl 3.3, NaH_2PO_4 1.13, $NaHCO_3$ 26, $CaCl_2$ 1.8, and glucose 11. ACSF was bubbled with 95% oxygen and 5% carbon dioxide. ACSF-filled glass electrodes with resistances of about 5 MΩ were positioned on the surface of the slices. Electrodes were advanced into the tissue until extracellular spikes exceeding 100 μV in amplitude were visible, and a single unit could be clearly discriminated. The noise amplitude was usually between 20 and 50 μV. All experiments were conducted 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, which was heated by passing an appropriate direct current through the heating wire.

EC_{50} Values for General Anesthesia and Application of Test Solutions

Aqueous concentrations of 3 μM ketamine, 0.4 μM propofol, 50 μM pentobarbital, and 425 μM ethanol were regarded to be equivalent to the estimated median effective concentration (EC_{50}) for general anesthesia. The EC_{50}s for pentobarbital, propofol, and ethanol were estimated from investigations on mice, humans, and tadpoles.[10] The EC_{50} for ketamine is based on the observation that 3 μM ketamine induced spinal analgesia in rat.[11,12] To enable easy comparison, anesthetic concentrations are also given as multiples of the EC_{50} for general anesthesia (MEC_{50}).

Anesthetics were applied *via* bath perfusion using syringe pumps (ZAK, Marktheidenfeld, Germany), which were connected *via* Teflon tubing (Lee, Frankfurt, Germany) to the experimental chamber. The flow rate was approximately 1 ml/min. When switching from ACSF to drug-containing solutions, the medium in the experimental chamber was replaced by at least 95% within 2 min. Effects on the spike patterns were stable about 5 min later. To ensure steady-state conditions, recordings during anesthetic treatment were carried out 10-15 min after commencing the change of the perfusate. The time required for recovery increased with the concentration tested. With 0.5-2 MEC_{50}, full recovery was reached after 12-15 min, and with 2-6 MEC_{50} after 30-60 min. For a single application, stable recording for about 1 h was necessary. Example recordings, showing the complete time course of the firing patterns during changing the solutions, have been provided in a number of previous publications.[5,6,8]

Data Analysis

Data were bandpass-filtered (30 Hz/3-10 kHz) and acquired on a personal computer with the Digidata 1200 AD/DA interface and pClamp 6 software (Axon Instruments, Foster City, CA). Records were simultaneously stored on a Sony data recorder (PC 204A, Racal Elektronik, Bergisch Gladbach, Germany) for further analysis. Extracellularly recorded spikes were counted on- or offline using software event detectors. Average spike rates were measured as the mean of spikes occurring during a period of 180-300 s. Interspike interval and burst analyses were performed by the pClamp program package. The time criterion distinguishing between bursts and interbursts was set at 250 ms. For statistical analysis, the paired Student *t* test and analysis of variance were used. As described in a previous publication, concentration-response curves were fitted by Hill equations.[8] The estimated EC_{50} values were derived from these fits.

Results

Effects of General Anesthetics on the Discharge Patterns

The effects of general anesthetics were investigated in cultured brain slices derived from the neocortex of postnatal rat pups as already described.[8] All recordings were carried out at 34-36°C. Action potential firing of neocortical neurons was induced by removing Mg^{2+} ions from the extracellular solution. Under these conditions, spontaneous activity was commonly observed after 10 days in culture. Recordings were carried out between 12 and 45 days *in vitro*, because the average firing rates (6.1 ± 4.3 Hz, mean ± SE; n = 281) stayed roughly constant during this period. Figure 1A presents typical firing patterns of a single neocortical cell, monitored before, during, and after bath application of pentobarbi-

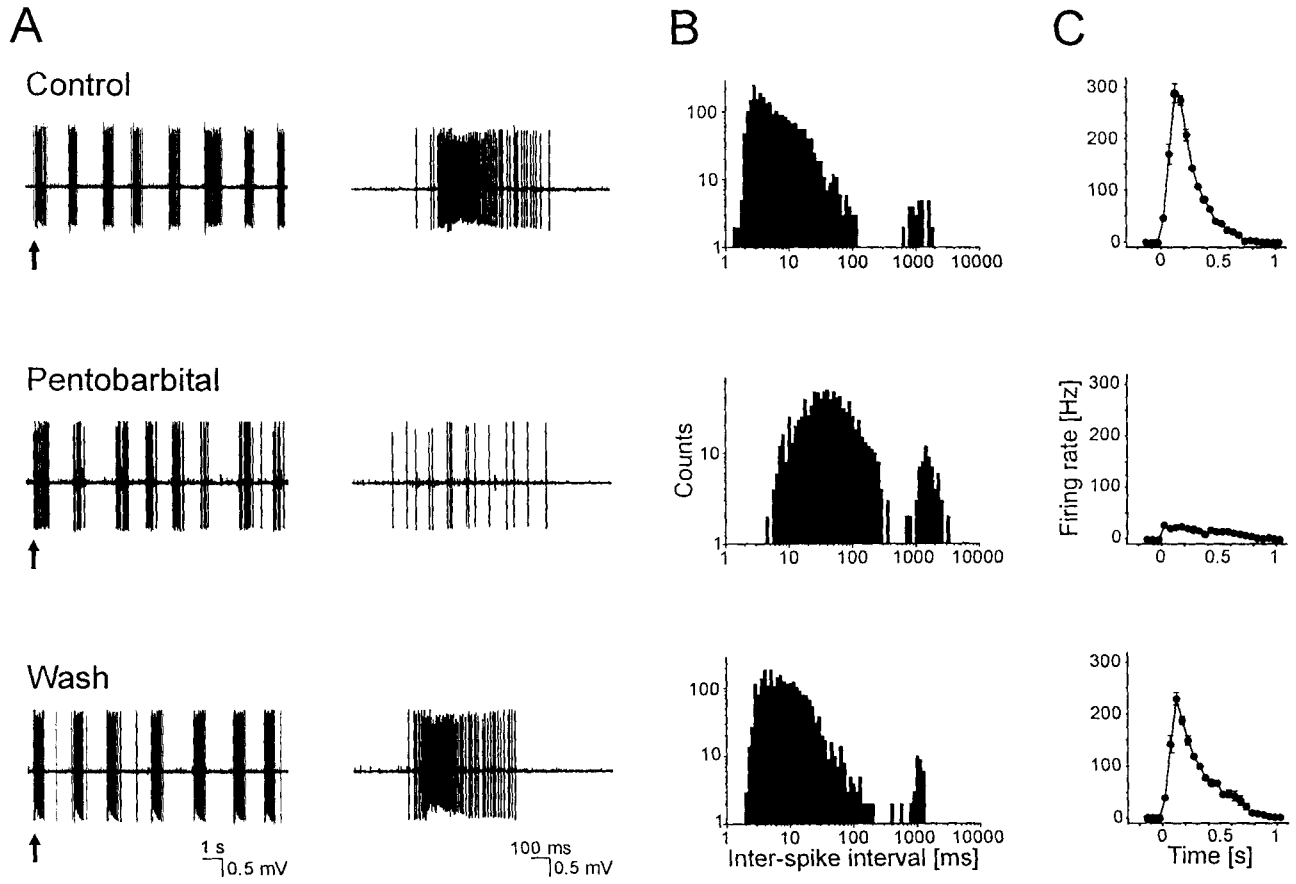

Fig. 1. Effects of pentobarbital (75 μM) on spontaneous action potential firing of a neocortical neuron. (*A*) Extracellularly monitored firing patterns before (*Control*), 12 min after the onset of pentobarbital application (*Pentobarbital*), and 25 min after removing the anesthetic (*Wash*). The traces on the left show typical records about 15 s in duration. On this time scale, it is difficult to resolve single action potentials. The bursts indicated by the arrow are shown on an extended time scale (*right*). At this resolution, the effect of the anesthetic is evident. (*B*) Interspike interval histograms derived from the firing patterns recorded before (*upper trace*), during (*middle trace*) and after (*lower trace*) pentobarbital application. The data are plotted on a log-log scale. (*C*) The time course of the intraburst discharge rates were calculated from averaged bursts in the absence and presence of pentobarbital. Results are given as mean values ± SE.

tal. Under control conditions, the cell fired action potentials that were grouped into bursts. Interspike interval histograms exhibited prominent peaks at 2–4 ms and smaller ones at 1000 ms (fig. 1B). These peaks corresponded to interspike intervals within the bursts and the interburst durations. Application of 75 μM pentobarbital (1.5 MEC_{50}) shifted interspike intervals occurring within bursts toward 20–50 ms, whereas interburst durations remained virtually unaffected. The depressant effect of pentobarbital on intraburst firing is also evident in figure 1C. Here, discharge rates within bursts were calculated by averaging about 30 bursts recorded in the absence and presence of the anesthetic. Without pentobarbital, the peak discharge rates ranged between 200 and 300 Hz but decreased to less than 50 Hz during treatment. Table 1 summarizes the results obtained from a detailed analysis of firing patterns before, during, and after pentobarbital application. Because pentobarbital did not considerably alter average burst length and burst frequency, the depression in the mean firing rate was predominantly caused by a decrease in the intraburst firing rate.

In figure 2, example recordings for the actions of propofol, ethanol, and ketamine are shown. The effects of propofol on the discharge patterns (fig. 2A) were clearly distinguishable from those of pentobarbital, because the former anesthetic depressed the mean discharge rate exclusively by reducing the burst frequency, but intraburst firing rates remained unaffected (fig. 2D). In the case of ethanol, the depression of the burst rate and the reduced number of action potentials per burst

Table 1. Effects of Pentobarbital (75 μM) on the Firing Patterns

	Mean Spike Rate (Hz)	Intraburst Firing Rate (Hz)	Number of Spikes per Burst	Burst Length (ms)	Burst Rate (Hz)
Control	18.97 ± 0.61	57.38 ± 1.70	38.35 ± 0.85	685 ± 32	0.506 ± 0.020
Pentobarbital	3.34 ± 0.33	9.06 ± 0.21	6.73 ± 0.41	750 ± 30	0.457 ± 0.023
Wash	16.82 ± 1.93	45.17 ± 1.04	34.68 ± 1.19	775 ± 30	0.545 ± 0.018
% Depression	82.39	84.21	82.45	−9.46	9.68

Values are mean values ± SE. The time criterion to choose between burst and interburst periods was set at 250 ms. Pentobarbital treatment slightly decreased the burst rate while simultaneously increasing the burst length by about the same amount. Thus, the overall time spent within bursts remained constant and the depression of the mean spike rate was exclusively caused by the effect on the intra burst firing rate.

contributed equally to the decrease in the mean discharge rate (fig. 2B and 2D). Similarly, ketamine reduced both the burst frequency and, to a much smaller extent, the average number of action potentials per burst (fig. 2C and 2D).

Effects of General Anesthetics on the Average Discharge Rates

The concentration-dependent effects of ketamine, propofol, pentobarbital, and ethanol on the average discharge rates of neocortical neurons are summarized in figure 3. The depressant effects of the tested agents were obtained by comparing the firing rate of the same cell before and during the treatment. All concentration-response relationships were fitted by Hill equations. The estimated EC_{50} values and Hill coefficients derived from the fitted curves are summarized in table 2. The EC_{50} values determined for pentobarbital and propofol were close to the EC_{50} for general anesthesia. Ketamine was about five times more potent than expected from this criterion. Similarly, ethanol was about 10 times more potent than *in vivo*.

Effects of General Anesthetics in the Presence of the $GABA_A$ Antagonist Bicuculline

In a previous work, the effects of various drugs affecting either glutamatergic or GABA-ergic synaptic transmission were investigated in the presence and absence of the specific $GABA_A$ antagonist bicuculline.[8] A decreased efficacy in bicuculline-treated slices was shown to be indicative for an agent acting on the $GABA_A$ receptor-ion channel complex. Similar results were obtained in studies on the effects of general anesthetics in hippocampal and cerebellar brain slices.[5,13] However, in cultured neocortical brain slices 20 μM bicuculline reduced the efficacy of volatile anesthetics and diazepam only by about 70%. Thus it remained to be elucidated whether this incomplete reduction of the anesthetic effect by bicuculline was caused by an incomplete blockade of $GABA_A$ receptors or by further molecular actions of these agents. I investigated the effects of various general anesthetics and diazepam at two different concentrations of bicuculline (20 and 100 μM). In figure 4A the hypothesis is tested that the depressant effects of anesthetic agents on action potential firing of neocortical cells do not differ in the presence and absence of bicuculline. This hypothesis was rejected in the case of diazepam, ethanol, propofol, halothane, isoflurane, and enflurane, but not in the case of pentobarbital and ketamine (*t* test and analysis of variance, $P < 0.05$). Bicuculline decreased the depressant actions of pentobarbital by about 30%, but this effect was not statistically significant. In figure 4B and 4C the hypothesis is tested that in bicuculline-treated slices the mean discharge rates did not differ, regardless of whether anesthetic agents or a drug-free solution was applied. At 20 μM bicuculline, this hypothesis was rejected in the cases of diazepam, isoflurane, pentobarbital, and ketamine (*t* test and analysis of variance, $P < 0.05$). Increasing the bicuculline concentration to 100 μM markedly decreased the residual effects of diazepam, ethanol, propofol, and volatile anesthetics but not of pentobarbital and ketamine. These results clearly indicate that 20 μM bicuculline was not sufficient to block the $GABA_A$ receptor-mediated conductance completely. The data further show that, except for pentobarbital and ketamine, the tested agents acted predominantly *via* the $GABA_A$ receptor.

Ketamine-induced depression of action potential firing remained unchanged in the presence of 20 μM bicuculline and was decreased by only 5% if slices were treated with 100 μM bicuculline. Thus, ketamine acts nearly exclusively on targets distinct from the $GABA_A$ receptor.

Bicuculline treatment did not reduce the potency of pentobarbital by more than about 30%, indicating that this drug acts, at least at the tested concentration (37 μM), mainly *via* a molecular mechanism not involving

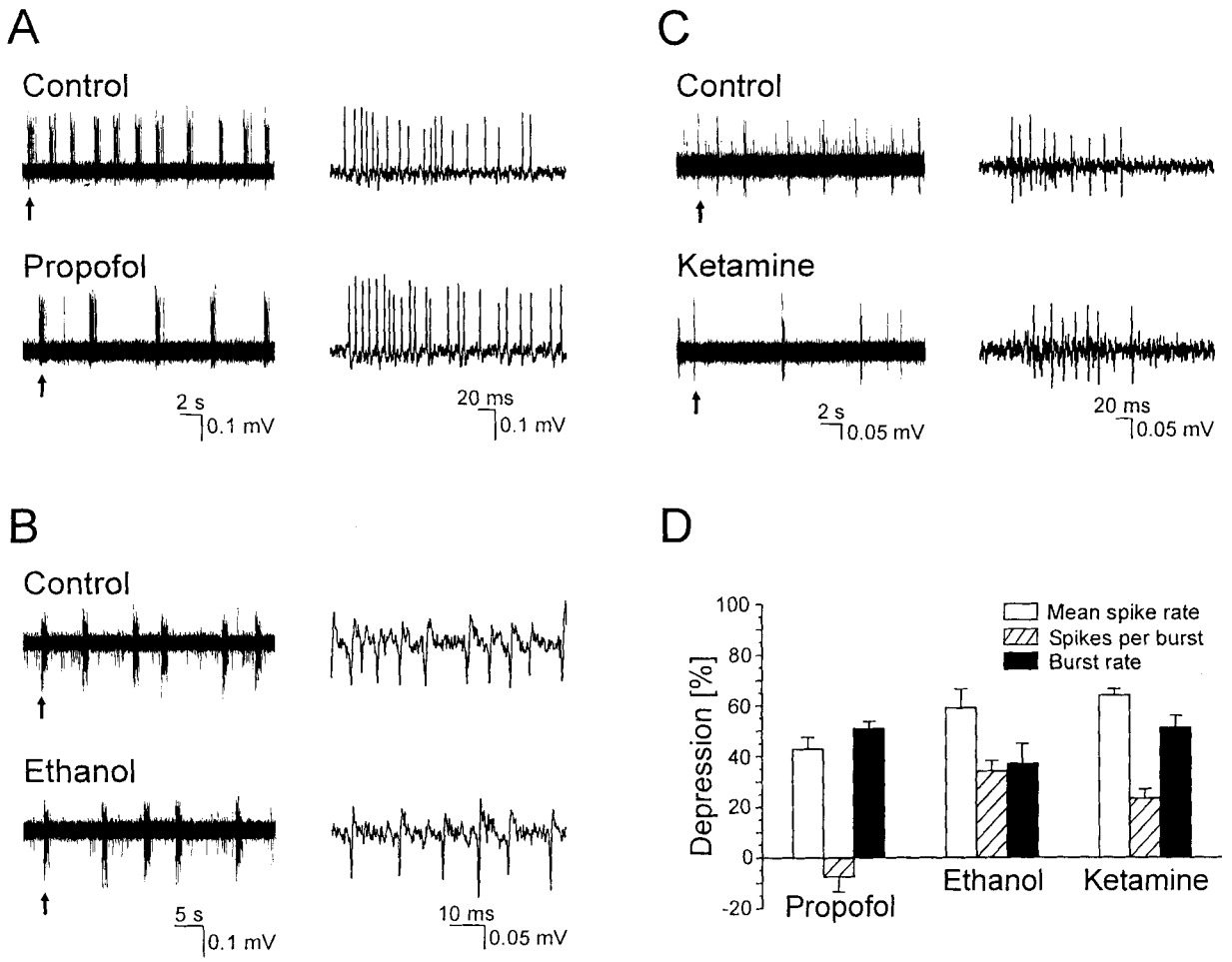

Fig. 2. (*A–C*) Example records for the effects of propofol (1 μM), ethanol (30 mM), and ketamine (0.8 μM) on the discharge patterns of neocortical neurons. In (*B*) and (*C*), two single units can be clearly discriminated by the different amplitudes. Only the larger units were used for the spike pattern analysis in (*D*). The bursts indicated by the arrows are shown on an extended time scale (*right*). (*D*) Comparison of the effects of the tested agents on the mean discharge rates, the burst rate, and the average number of action potentials per burst for the spike patterns shown in (*A–C*). Results are given as mean values ± SE. They were obtained by averaging nine successive time segments every 20 s.

the $GABA_A$ receptor. However, from the data in figure 4A it cannot be excluded that actions of pentobarbital at the $GABA_A$ receptor also contributed to the depression of action potential firing. This possibility was supported by the finding that bicuculline (20 μM) decreased the efficacy of pentobarbital by 44 ± 9% (mean ± SE, n = 8) when the anesthetic was applied at a higher concentration (75 μM instead of 37 μM pentobarbital in fig. 4A).

Effects of Anesthetic Agents on the Firing Patterns

On comparing the effects of ethanol and propofol, it is remarkable that, although both agents largely mediate their depressant effect *via* the $GABA_A$ receptor (fig. 4), their actions on the discharge patterns were clearly different (fig. 2). How are we to explain this finding? Do they affect this receptor differently? This possibility was tested by investigating the effects of GABA, muscimol, and diazepam on the discharge patterns of neocortical cells. Bath application of GABA was used to simulate the molecular action of a GABA-mimetic drug inducing a persistent bicuculline-sensitive Cl^- current. Benzodiazepines are known to operate in a qualitatively different manner at the same receptor. For example, diazepam alters the shape of synaptic events but does not induce tonic inhibition.[14] The recordings in figure 5A indeed show that bath application of GABA and diazepam altered the firing patterns in different ways. The effects of diazepam closely resembled those of pentobarbital, be-

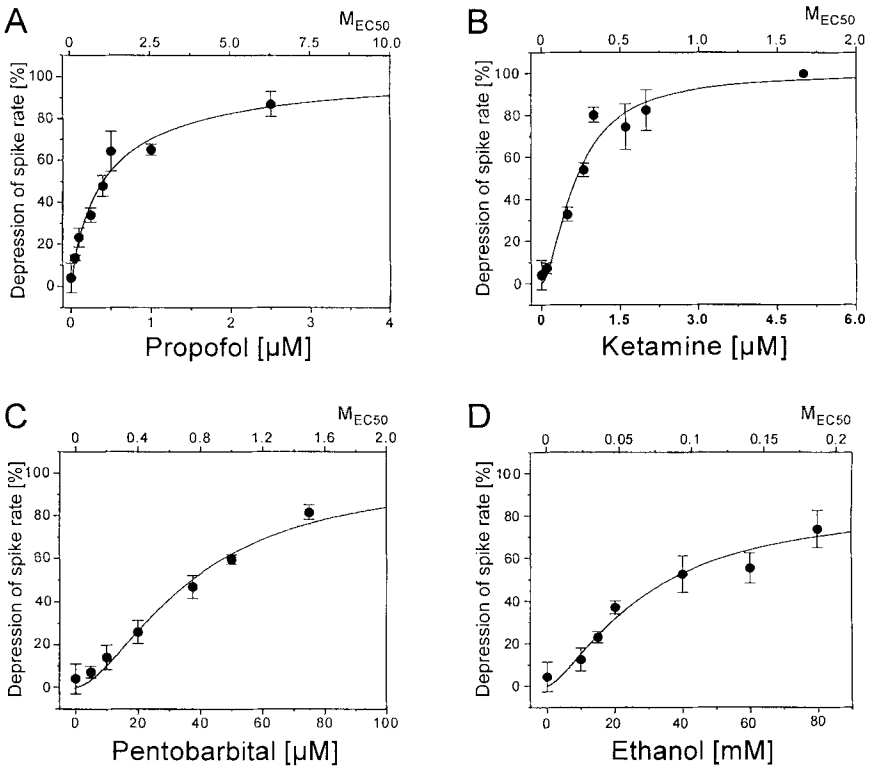

Fig. 3. Concentration–response relationships of anesthetic-induced depression of mean spike rates for (*A*) propofol, (*B*) ketamine, (*C*) pentobarbital, and (*D*) ethanol. The data points represent mean values ± SE. Concentrations are also given as multiples of the *in vivo* median effective concentrations (MEC_{50}) at the top of each graph. The effects of the tested anesthetics were calculated by comparing action potential discharge rates before and during the treatment. For each tested concentration, the mean value was calculated from 5–12 cells. The curves were fitted with Hill equations to the data points. The median effective concentrations and the Hill coefficients derived from the fits are summarized in table 2.

cause the depression of the discharge rate was exclusively caused by a decrease in the number of action potentials per burst, whereas the burst rate remained nearly unaffected. In contrast, GABA and muscimol largely decreased the burst frequency. The effects of these and other agents on the discharge rate, the average burst frequency, and the number of action potentials per burst are presented in figure 5B. This analysis was restricted to recordings in which anesthetic treatment reduced the average firing rates between 40 and 60%. The specific $GABA_A$ receptor agonist muscimol inhibited spontaneous action potential firing exclusively by decreasing the burst frequency. The differential effects of GABA and muscimol can be explained by their molecular targets. Whereas GABA activates both $GABA_A$ and $GABA_B$ receptors, muscimol acts only on $GABA_A$ receptors. This explanation was supported by the observation that the $GABA_B$ receptor agonist saclophen (50 μM) decreased the number of action potentials per burst but left the burst frequency nearly unaffected (n = 5). Thus it can be concluded that the decrease in burst frequency observed during GABA treatment mainly results from $GABA_A$ receptor activation, whereas the decrease in the number of action potentials per burst is related to effects at $GABA_B$ receptors. Unexpectedly, the effects of propofol and volatile anesthetics, which depressed the average discharge rates mainly by enhancing $GABA_A$ receptor-mediated synaptic inhibition, closely resembled the ac-

Table 2. Comparison on *In Vivo* and *In Vitro* Potencies as Estimated from the Concentration–Response Curves in Figure 3

	Ketamine	Propofol	Pentobarbital	Ethanol
EC_{50}, *in vivo*	3 μM	0.4 μM	50 μM	425 mM
EC_{50}, fitted	0.67 ± 0.1 μM	0.44 ± 0.24 μM	37.07 ± 2.2 μM	29.25 ± 1.75 mM
MEC_{50}, fitted	0.22 ± 0.03	1.10 ± 0.06	0.74 ± 0.04	0.069 ± 0.004
Hill coefficient	1.65 ± 0.45	0.88 ± 0.25	1.67 ± 0.19	1.44 ± 0.66
EC_{50}, linear interpolation	0.67 μM	0.41 μM	37.58 μM	36.21 mM

Half-maximal depression of the average discharge rates (EC_{50} ± SE, fitted) and Hill coefficients were estimated from the concentration–response curves in figure 3. The fitted EC_{50} values are also given as multiples of the *in vivo* EC_{50}. Note that fitting and linear interpolation yield very similar results.

EC_{50} = median effective concentration; MEC_{50} = multiples of the EC_{50} *in vivo*.

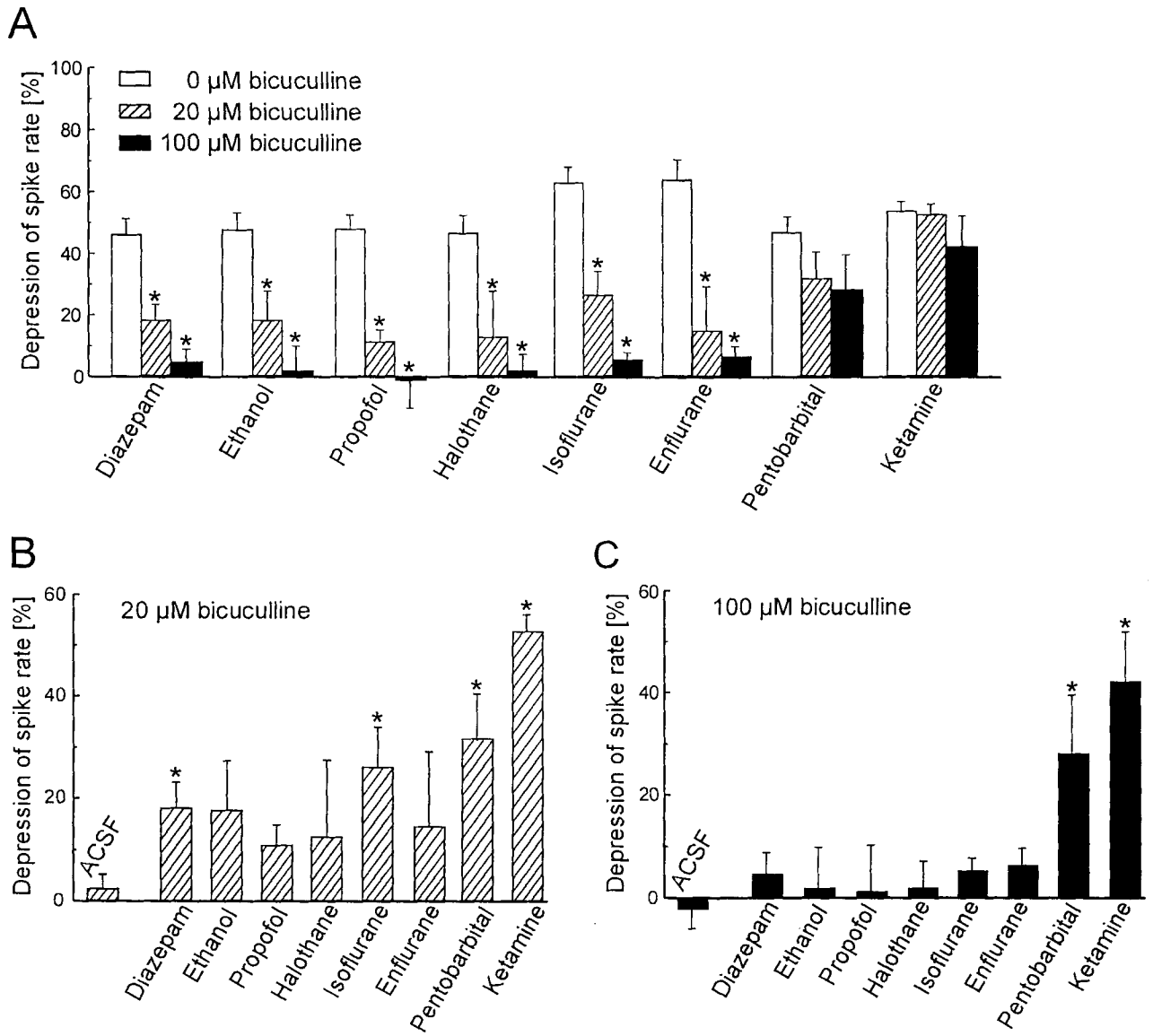

Fig. 4. (*A*) Effects of diazepam (10 μM), ethanol (20 mM), propofol (0.4 μM), halothane (125 μM), isoflurane (160 μM), enflurane (310 μM), pentobarbital (37 μM), and ketamine (0.8 μM) on the discharge rates in the presence of 0, 20, and 100 μM bicuculline. The number of cells tested (without bicuculline/with 20 μM bicuculline/with 100 μM bicuculline) were: diazepam, 10/8/8; ethanol, 7/7/8; propofol, 8/6/7; halothane, 9/6/6; isoflurane, 10/8/7; enflurane, 9/4/7; pentobarbital, 7/8/8; ketamine, 6/9/5. Statistically significant effects are indicated by asterisks (by *t* test and analysis of variance, $P < 0.05$). (*B,C*) Comparison of the effects of various anesthetics with a drug-free test solution (artificial cerebrospinal fluid) in the presence of 20 μM and 100 μM bicuculline. When slices were treated with 100 μM bicuculline, only pentobarbital and ketamine caused a significant decrease in action potential firing (*t* test and analysis of variance, $P < 0.05$). The number of tested cells as in (*A*). All results are given as mean values ± SE.

tions of muscimol, indicating a GABA-mimetic mechanism of action at the $GABA_A$ receptor.

Correlation between In Vitro *and* In Vivo *Potencies*

In order to compare *in vitro* and *in vivo* potencies, EC_{50} values obtained from the fitted concentrations response curves in figure 3 were plotted against *in vivo* EC_{50} values for general anesthesia. The latter were taken from published literature values for mammals[10,12] and, in the case of ethanol, for tadpoles.[15] Previous results concerning the effects of the volatile anesthetics halothane, isoflurane, and enflurane were

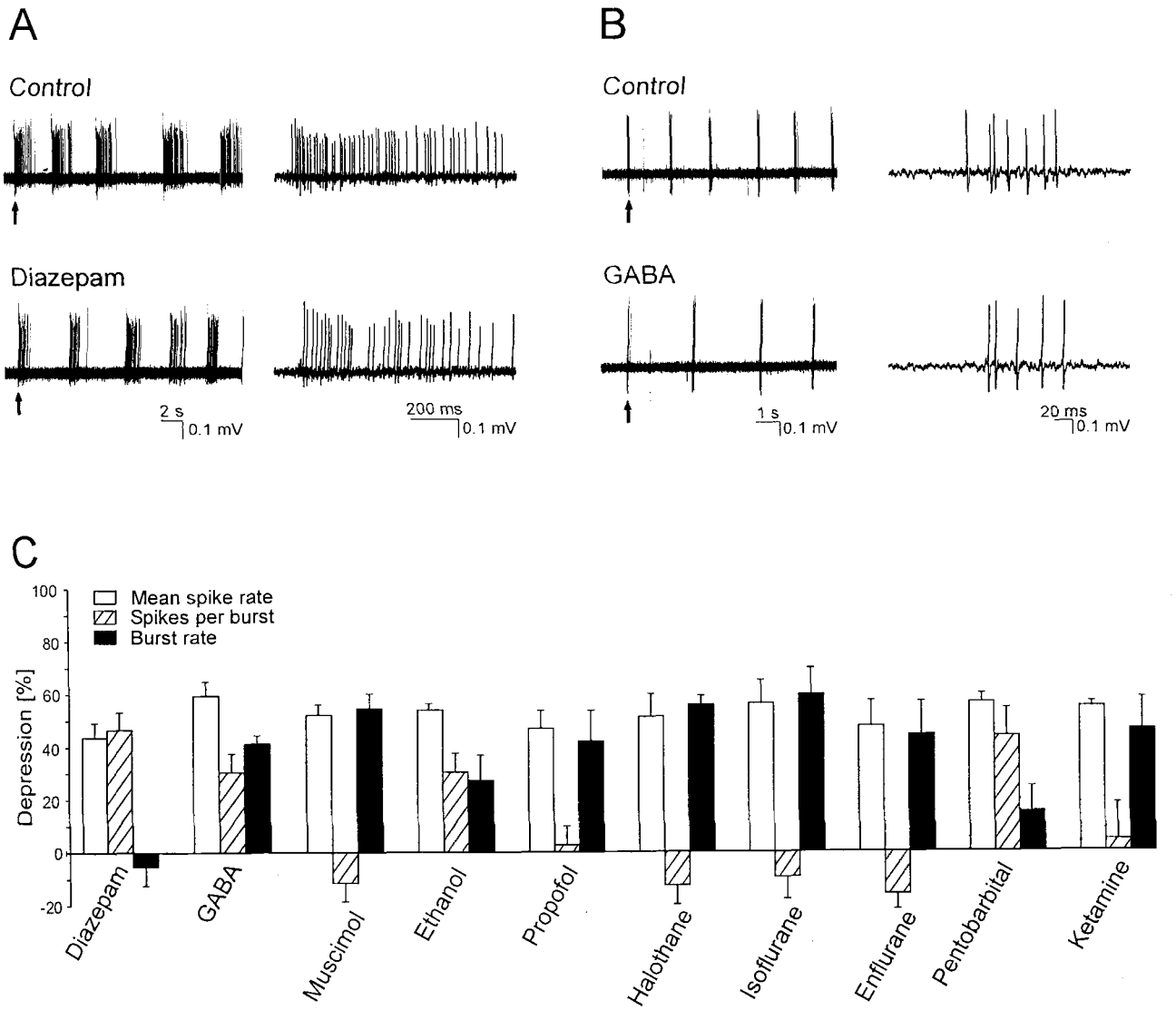

Fig. 5. (*A* and *B*) Representative recordings showing the effects of diazepam (10 μM) and GABA (50 μM) on the discharge patterns of neocortical neurons. Single bursts indicated by arrows are shown on an expanded time scale on the right. (*C*) Effects of various agents on the discharge rate, the burst rate, and the number of action potentials per burst (mean values ± SE). The concentrations and the number of tested cells were: diazepam, 10 μM, n = 6; GABA, 50 μM, n = 7; muscimol, 0.5 μM, n = 6; ethanol, 20 mM, n = 7; propofol, 0.4 μM, n = 5; halothane, 125 μM, n = 6; isoflurane, 160 μM, n = 6; enflurane, 310 μM, n = 5; pentobarbital, 37 μM, n = 6; ketamine, 0.8 μM, n = 7.

also included.[8] As can be seen in figure 6A, a highly significant correlation was obtained ($r = 0.99$, $P < 0.0001$).

Correlation between the Depressant Effects on Action Potential Firing and Potentiation of $GABA_A$-Mediated Synaptic Inhibition

In the present and a previous study, evidence was provided that halothane, isoflurane, enflurane, ethanol, and propofol all reduced action potential firing of neocortical cells by enhancing $GABA_A$ receptor-mediated inhibition. If this conclusion is correct, the effectiveness of the previously mentioned agents in depressing the discharge rates in neocortical slice cultures should then also correlate with their effectiveness in enhancing $GABA_A$ receptor-mediated conductances. Anesthetic potencies at the $GABA_A$ receptor given in the literature were calculated from those anesthetic concentrations

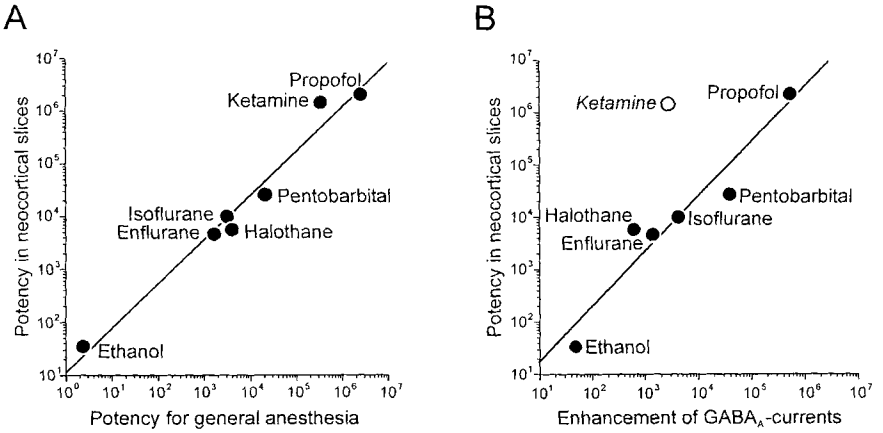

Fig. 6. Comparison of the potencies of various general anesthetics in neocortical slice cultures with *in vivo* potencies, as derived from the depression of motor responses to painful stimuli (*A*) and with the potencies at the $GABA_A$ receptor (*B*). Potencies were defined as reciprocals of aqueous ED_{50} concentrations (in liters per mole). The slope of the regression lines and correlation coefficients were 0.84 and 0.99 in (*A*) and 1.04 and 0.96 in (*B*).

that caused an approximately twofold increase in the Cl^- current in response to bath-applied GABA.[16,17] With the exception of ketamine, figure 6B shows a good correlation between the potentiating actions at the $GABA_A$ receptor and the depression of the mean discharge rates of neocortical neurons ($r = 0.96$, $P < 0.0028$).

Discussion

Molecular Mechanisms of Anesthetic Action

In the present work, evidence has been provided that propofol and ethanol inhibit spontaneous action potential firing of neocortical neurons mainly by increasing $GABA_A$-mediated synaptic inhibition. This conclusion is in accordance with the observation that, at similar concentrations, these agents enhance $GABA_A$ receptor-mediated conductances.

The effects of propofol on the $GABA_A$ receptor–chloride ion channel complex have been analyzed in dissociated hippocampal pyramidal neurons[18,19] and hippocampal brain slices.[14] The authors reported that, even in the absence of GABA, propofol induced a tonic $GABA_A$ receptor-mediated chloride current. Furthermore, the anesthetic enhanced the response to bath-applied GABA. Significant effects already occurred between 0.1 and 1.0 μM, which matches the EC_{50} determined in the present work quite well (0.44 μM).

In the case of ethanol, also, the concentrations found to decrease action potential firing in neocortical slices compare well with those found to enhance $GABA_A$ receptor-mediated currents. In oocytes expressing whole brain mRNA, 20–30 mM ethanol increased the current response evoked by bath application of GABA by twofold.[17] This value is close to the EC_{50} reported here (29 mM). However, besides the actions on $GABA_A$ receptors, the depressant effects of ethanol have also been attributed to a blockade of *N*-methyl-D-aspartate (NMDA) receptors.[20,21] Single-channel recordings revealed that ethanol concentrations exceeding 80 mM are necessary to block NMDA receptor-mediated currents by about half.[22] From the latter results, it can be concluded that the ethanol-induced decrease in spontaneous firing of neocortical cells is predominantly caused by an increase in synaptic inhibition and not by a blockade of NMDA receptors. This suggestion is indeed in accordance with the observation that the depressant effects of ethanol were significantly lowered by bicuculline treatment (fig. 4A).

The hypothesis that reported actions of volatile anesthetics, ethanol, and propofol at the $GABA_A$ receptor are causally related to a decrease in neuronal activity is not universally accepted.[4] In most of the studies mentioned previously, the actions of general anesthetics at the $GABA_A$ receptor–ion channel complex have been investigated by comparing the current responses to bath-applied GABA in the presence and absence of anesthetic agents. It has been argued that, from this type of experiment, the effects at intact synapses are difficult to predict, because the applied GABA concentrations may not match those in the synaptic cleft.[23] A further complication arises from the fact that the amount of GABA-mediated synaptic inhibition in brain slices is influenced by a number of uncontrolled factors. For example, neocortical pyramidal cells directly excite GABA-ergic interneurons. A decrease in the overall network activity should also decrease excitation of GABA-ergic interneurons, thereby depressing synaptic inhibition.[4] Finally, it has

been suggested that general anesthetics might directly inhibit GABA-ergic interneurons, thus canceling the potentiating effects at the $GABA_A$ receptor.[24] The present work involved these various complications, which inevitably arise when anesthetic actions are investigated at the neuronal-network level. However, the data shown in figure 4 provide clear evidence that in the case of volatile anesthetics, propofol, and ethanol, the depression of the mean discharge rate is largely related to enhanced $GABA_A$ receptor function.

For pentobarbital a depression of glutamatergic synaptic transmission and an enhancement of GABAergic synaptic inhibition have been reported to occur at clinically relevant concentrations.[3] When recording from the same neuron, Sawada and Yamamoto[25] showed that the depression of glutamatergic synaptic transmission occurs at slightly lower concentrations than necessary to potentiate responses to bath-applied GABA. This finding can explain that at a concentration (37 μM) decreasing action potential firing of neocortical cells by half,[7,8] the depressant effect of pentobarbital is only slightly antagonized by bicuculline. At 100 μM pentobarbital, $GABA_A$ receptor-mediated currents are enhanced by twofold to fourfold.[17,26,27]

In contrast to propofol, ethanol, and volatile anesthetics, the decrease in action potential firing induced by ketamine could not be attributed to an increased $GABA_A$ receptor-mediated conductance, because this anesthetic was equally efficient, regardless of whether synaptic inhibition was present or blocked (fig. 4). Potentiating effects of ketamine at the $GABA_A$ receptor have been reported in the literature, but these effects occur at concentrations 1,000-fold higher than those necessary to depress action potential firing in neocortical slices.[26] The EC_{50} for ketamine determined in this study (0.67 μM) roughly matched the concentrations reported *in vivo* (3 μM)[12] and those necessary to block NMDA receptors (1–10 μM).[28,29] The high efficacy of ketamine in organotypic slice cultures of neocortex may be attributed to the fact that experiments were conducted at 0 Mg^{2+}, a condition that enhances NMDA receptor function in this preparation considerably.[8]

Correlation between In Vivo *and* In Vitro *Potencies*

In the present and a previous work,[8] an excellent correlation between *in vitro* and *in vivo* potencies was observed in the cases of pentobarbital, propofol, and volatile anesthetics. This finding was surprising, because the *in vivo* potencies in figure 6 had been determined from the blockade of movements evoked by painful stimuli. This response is mediated by spinal neurons and is independent of forebrain structure.[30] In contrast to pentobarbital and propofol, the potency of ethanol was about 10-fold higher than expected from the *in vivo* EC_{50} derived from experiments with tadpoles. What is the reason for this discrepancy? Investigations on the effects of enflurane and isoflurane revealed that, at anesthetic concentrations close to the EC_{50} for general anesthesia, $GABA_A$ receptor function is enhanced by about two to four-fold. Other studies have shown that, at 30 mM, ethanol enhanced $GABA_A$ receptor currents by about the same amount. The latter results indicate that general anesthesia should occur at ethanol concentrations close to 30 mM and not at 425 mM. Thus, the effect of ethanol at the $GABA_A$ receptor is possibly balanced by an unknown excitatory action. Alternatively, there may be substantial differences between the potencies of ethanol in tadpoles and mammals.

Except for ketamine, figure 6B shows a good correlation between the potentiating actions of the tested agents at the $GABA_A$ receptor and their efficacy in neocortical brain slices. It is interesting that pentobarbital fits well into this correlation, because concentrations causing half maximal depression of action potential firing in neocortical slices (37 $\pm$ 2 μM, mean $\pm$ SE) and enhancing $GABA_A$ receptor function reported in other studies (50–100 μM) lie close together. However, pentobarbital decreases action potential firing in neocortical slices mainly by a mechanism not involving the $GABA_A$ receptor, although the latter mechanism may have contributed in some extent to the depressant effect of the anesthetic. Because general anesthetics frequently affect a number of molecular targets, the correlation shown in figure 6B and a similar one published by Zimmerman *et al.*[16] should be interpreted with caution. As demonstrated by the effects of pentobarbital, such correlations do not prove a causal relation between anesthetic actions at the $GABA_A$ receptor and the state of general anesthesia.

Diverse Anesthetic Actions on the Firing Patterns of Central Neurons

In a recent study we investigated the effects of the volatile anesthetics halothane, isoflurane, and enflurane on the spontaneous discharge patterns of cerebellar Purkinje cells.[5,6] The tested agents, when applied at equivalent minimum alveolar concentrations, altered the firing patterns differently. Similar observations were made in studies on hippocampal[31] and

spinal neurons.[32] From these findings we concluded that, besides the actions at the $GABA_A$ receptor, further side effects are involved. There is indeed some experimental evidence for such a suggestion.[6] However, from the data presented here, a further possibility emerges. Anesthetic agents may alter the kinetic properties of $GABA_A$ receptor-mediated currents in a specific manner. In an exceptional case, some of these may induce a persistent inhibitory current, whereas others exclusively alter the shape of inhibitory postsynaptic events. Such different actions at the $GABA_A$ receptor were mimicked by applying either GABA or diazepam experimentally. The evoked effects were clearly distinct (fig. 5).

Evidence for the proposal that either tonic activation or benzodiazepine-like actions at $GABA_A$ receptors result in very different changes in the activity patterns of central neurons has also been provided by *in vivo* studies on the sleep electroencephalogram. Midazolam, which belongs to the class of benzodiazepines, but not the specific $GABA_A$ agonist muscimol increased the time spent in non-rapid eye movement sleep.[33] Furthermore, both agents affected the electroencephalogram in this sleep stage in a different fashion. A further example of specific actions of benzodiazepines and $GABA_A$ receptor agonists on central nervous system functions concerns the processing of painful stimuli. It has been reported that benzodiazepines induce hyperalgesia and antagonize the effects of opioids,[34-36] whereas muscimol and 4,5,6-tetrahydroisoxazolo[5,4-C]pyridin-3-ol cause analgesia.[37,38]

Taken together, these results strongly support the idea that tonic activation of $GABA_A$ receptors and prolongation of inhibitory postsynaptic currents can influence the firing patterns of central neurons in distinct manners. In view of these findings, the actions of general anesthetics appear amazingly complex. The data of MacIver *et al.*[39,40] showed that a single anesthetic agent can cause qualitatively different effects at the $GABA_A$ receptor and consequently on the firing patterns of neocortical neurons when applied at increasing concentrations. On the other hand, there is evidence that different anesthetic compounds alter the kinetic properties of inhibitory postsynaptic currents in a different manner even when applied at corresponding MEC_{50} values.[5,7,14,41-43] Given that the $GABA_A$ receptor-ion channel complex is deeply involved in the anesthetic state, there is no uniform action on the molecular level by which general anesthesia can be explained.

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