Effects of the Volatile Anesthetic Enflurane on Spontaneous Discharge Rate and GABA_A-Mediated Inhibition of Purkinje Cells in Rat Cerebellar Slices

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Antkowiak, Bernd and Detlef Heck. Effects of the volatile anesthetic enflurane on spontaneous discharge rate and GABA_A-mediated inhibition of Purkinje cells in rat cerebellar slices. J. Neurophysiol. 77: 2525–2538, 1997. The effects of the volatile anesthetic enflurane on the spontaneous action potential firing and on γ-aminobutyric acid-A (GABA_A)-mediated synaptic inhibition of Purkinje cells were investigated in sagittal cerebellar slices. The anesthetic shifted the discharge patterns from continuous spiking toward burst firing and decreased the frequency of extracellularly recorded spontaneous action potentials in a concentration-dependent manner. Half-maximal reduction was observed at a concentration corresponding to 2 MAC (1 MAC induces general anesthesia in 50% of patients and rats). When the GABA_A antagonist bicuculline was present, 2 MAC enflurane reduced action potential firing only by 13 ± 8% (mean ± SE). In further experiments, inhibitory postsynaptic currents (IPSCs) were monitored in the whole cell patch-clamp configuration from cells voltage clamped close to −80 mV. At 1 MAC, enflurane attenuated the mean amplitude of IPSCs by 54 ± 3% while simultaneously prolonging the time courses of monoeponential current decays by 413 ± 69%. These effects were similar when presynaptic action potentials were suppressed by 1 μM tetrodotoxin. At 1–2 MAC, enflurane increased GABA_A-mediated inhibition of Purkinje cells by 97 ± 20% to 159 ± 38%. During current-clamp recordings, the anesthetic (2 MAC) hyperpolarized the membrane potential by 5.2 ± 1.1 mV in the absence, but only by 1.6 ± 1.2 mV in the presence, of bicuculline. These results suggest that enflurane-induced membrane hyperpolarizations, as well as the reduction of spike rates, were partly caused by an increase in synaptic inhibition. Induction of burst firing was related to other actions of the anesthetic, probably an accelerated activation of an inwardly directed cationic current and a depression of spike afterhyperpolarizations.

INTRODUCTION

Volatile anesthetics are commonly used in surgery to induce the state of general anesthesia. These compounds depress neuronal activity in various parts of the mammalian CNS (Fujiwara et al. 1988; Nicoll 1972; Nicoll and Madison 1982; Richards 1973; Richards and White 1975; Richards et al. 1975). The underlying cellular mechanisms are still a matter of discussion. Volatile anesthetics have been shown to hyperpolarize central neurons. This hyperpolarization is probably related to an increase in K⁺ conductance, as suggested from studies on hippocampal, neocortical, spinal, and thalamic neurons (El-Beheiry and Puil 1989; Miu and Puil 1989; Sugiyama et al. 1992; Takenoshita and Takahashi 1987). An additional mechanism that may contribute to neuronal depression concerns the effects reported on γ-aminobutyric acid-A (GABA_A)-mediated synaptic inhibition. There is growing evidence that volatile anesthetics affect GABA_A receptor channels and potentiate GABA-induced Cl⁻ currents (Hall et al. 1994a; Longoni et al. 1993; Moody et al. 1993; Nakahiro et al. 1989; Scholfield 1980; Tanelian et al. 1993; Wakamori et al. 1991). These effects occur at clinical concentrations and follow the Meyer-Overton rule (Jones et al. 1992), which correlates the potency of general anesthetics with their fat solubility (Overton 1901). On the basis of these criteria, the GABA_A receptor–ion channel complex is believed to play an important role in causing the state of general anesthesia.

The volatile anesthetic enflurane exhibits depressing but also excitatory actions (Black 1979; Collins et al. 1995; Stevens et al. 1984). Seizure-like biphasic electroencephalogram patterns during enflurane anesthesia were observed in cats (Stevens et al. 1984). Similar to epileptogenic drugs, enflurane caused bursts of population spikes in the hippocampal slice preparation (MacIver and Kending 1989). The cellular mechanisms that underlie enflurane-induced central excitation are not known. It has been proposed that a depression of GABA-mediated synaptic events may be involved (Pearce 1993). In line with this hypothesis, a recent study on cultured hippocampal neurons showed that enflurane caused dramatic reductions in the amplitudes of evoked inhibitory postsynaptic currents (IPSCs) (Jones and Harrison 1993). However, in another publication on the effects of enflurane in the hippocampus, the excitatory effects of the anesthetic were attributed to enhanced synaptic excitation rather than to a depression of GABA_A-mediated inhibition (MacIver and Kending 1989).

In the present work we tested the hypothesis that enflurane-induced alterations in the firing patterns can be largely explained by the effects on synaptic inhibition. Cerebellar Purkinje cells were chosen as a model system because they exhibit spontaneous activity even in acutely isolated brain slices (Jaeger and Bauer 1994; Llinas and Sugimori 1980a,b), thus offering the possibility of analyzing the effects of enflurane on the spike patterns and GABA_A-mediated inhibition in the same preparation.

METHODS

Slice preparation

Slices were prepared according to procedures similar to those described by Edwards et al. (1989). In brief, 13- to 16-day-old
FIG. 1. Extracellularly recorded firing patterns of Purkinje cells observed before (control), during 1.3 MAC (1 MAC induces general anesthesia in 50% of patients and rats), and after (wash) enflurane application. Recordings during and after enflurane treatment were carried out 12 min after switching between the solutions. At that time, discharge patterns arrived at the new steady state. A: enflurane reduced the action potential discharge rate by introducing periods of quiescence. Mean spike rates were 11.3 Hz before, 6.7 Hz during, and 9.8 Hz after enflurane treatment. B: in this cell, enflurane evoked bursts of spikes with decreasing amplitudes. The small unit exhibited a discharge pattern very similar to the large one (not shown). Note the different time scales. C: spike rates and interspike intervals before, during, and after enflurane treatment. Top row: number of spikes recorded within 5-s time intervals. Bottom row: distributions of interspike intervals peaking at 65, 46, and 57 ms before, during, and after enflurane treatment. Inter spike intervals lasting >150 ms were ignored. Enflurane reduced spontaneous firing of Purkinje cells by introducing long periods into the discharge patterns when the cell stayed silent, but not by increasing the interspike intervals observed within bursts of spikes. Mean firing rates were 12.4 Hz before, 6.3 Hz during, and 11.2 Hz after enflurane treatment.

Sprague-Dawley rats of either sex were deeply anesthetized with enflurane, isoflurane, or halothane and decapitated, and the brains were quickly removed. Systematic effects on the quality of the slices or on the characteristic discharge patterns of Purkinje neurons were not observed with regard to the anesthetic chosen. Brains were stored for 5–10 min in ice-cold artificial cerebrospinal fluid (ACSF) consisting of (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 1 MgCl2, 26 NaHCO3, 2 CaCl2, and 25 glucose. The cerebellum was then glued onto a Teflon block and 250- to 300-μm-thick sagittal slices were prepared with a vibratome (Campden, Loughborough, UK). Slices were stored in a bath of ACSF at 21–23°C bubbled with 95% O2-5% CO2. They were then transferred to the recording chamber 1–6 h later and continuously perfused with ACSF at a flow rate of ~1 ml/min.

Control of experimental temperature
Experiments were carried out at either 21–23 or 34–36°C. The recording chamber consisted of a metal frame with a glass bottom.
A heating wire was glued onto the metal frame. In cases in which experiments were carried out at 34–36°C, the frame was heated to 36°C by passing an appropriate direct current through the heating wire. Furthermore, the bathing solutions were heated to 36°C before entering the recording chamber.

**Extracellular recordings**

Cerebellar slices were viewed under a low-power dissection microscope. ACSF-filled glass electrodes with resistances of ~5 MΩ were positioned on the surface of the Purkinje cell layer. Electrodes were advanced into the slices until extracellular spikes >300 μV in amplitude were visible and a single unit could be clearly discriminated. The noise amplitude was between 20 and 100 μV.

**Whole cell voltage-clamp and current-clamp recordings**

Experiments were carried out with a setup similar to that described by Stuart et al. (1993). Patch pipettes were pulled from thin-walled borosilicate capillaries (1.5 mm OD) and coated with Sylgard (Dow Corning). After fire polishing, resistances were 1.5–3.5 MΩ. For voltage-clamp recordings, pipettes were filled with an intracellular solution containing (in mM) 145 CsCl, 1 MgCl₂, 5 ethylene glycol-bis-(β-aminoethyl ether) N,N,N',N'-tetraacetic acid, 2 ATP, and 10 N-2-hydroxyethyl-piperazine-N'-2-ethanesulphonic acid (HEPES), pH 7.3. Input resistance of Purkinje cells was 337 ± 12 (SD) MΩ (n = 41). The access resistance ranged within 5–12 MΩ and was compensated by ~80% with the series resistance compensation of the LM/EPC (List, Germany), as described in Llano et al. (1991b). Purkinje cells (n = 12), with the soma close to the surface of the slice, were stained with the fluorescent dye Lucifer yellow (Sigma, Deisenhofen, Germany). They showed intact dendritic arborizations within the molecular layer. Cells lying deeper (10–30 μm) in the slice were cleaned before establishment of the gigaseal, as described by Edwards et al. (1989). Current-clamp recordings were carried out with the use of an Axoclamp-2A amplifier. Patch pipettes were filled with 140 potassium gluconate, 5 HEPES, 3 NaCl, 1 MgCl₂, 0.5 CaCl₂. In some experiments, 0.3 mM guanosine 5’-triphosphate was added. Under these conditions, input resistances of Purkinje cells were 187 ± 35 (SD) MΩ (n = 33).

**Stimulating presynaptic fibers**

Glass electrodes with tip diameters between 2 and 10 μm were used for stimulating presynaptic fibers. In these recordings, 15 μM 6-cyano-nitroquinoloxaline-2,3-dione (Sigma) and 20 μM d-t.-2-amino-5-phosphonononic acid (Sigma) were added to the bath solution. In some experiments, bipolar stimulus electrodes pulled from double-barreled theta glass capillaries (Hilgenberg, Malsfeld, Germany) were used (Vincent et al. 1992). Currents of 15–35 μA and 0.1 ms in duration were sufficient to elicit synaptic currents with peak amplitudes of ~0.2–2 nA. The stimulation frequency was 0.5 Hz.

**Preparation and application of volatile anesthetics**

 Defined concentrations of volatile anesthetics dissolved in ACSF were prepared according to the method described by Tas et al. (1989). In brief, 500 ml ACSF were bubbled at a flow rate of 200 1/h with a vapor containing the volatile anesthetic. The desired vapor concentration was delivered by calibrated vaporizers (Draeger, Lubeck, Germany). Temperature was 22–23°C. After ~30 min, an equilibrium between the gas and liquid phase was reached (Tas et al. 1989). About 60 min later, samples were taken with the use of gas-tight syringes. Continuous bubbling ensured that enflurane did not evaporate during this procedure. Samples were later applied during electrophysiological recordings.

Because the solubility of enflurane is twice as high at 21 ± 2°C as at 34–36°C, ACSF was bubbled with 1 vol% enflurane to obtain a concentration in the ACSF corresponding to 1 MAC (1 MAC induces general anesthesia in 50% of patients and rats). Vapor pressure concentrations delivered by the calibrated vaporizers were regarded as correct, because the MAC value determined by the suppression of the righting reflex with eight rats was close to 2.0 vol%. This is in accordance with the value given in the literature (Franks and Lieb 1993). Test solutions of 1 MAC enflurane were additionally prepared by dissolving enflurane in the ACSF to a final concentration of 0.63 mM (Franks and Lieb 1994), according to the method described by Wakamori et al. (1991).

Anesthetics were applied via bath perfusion with the use of syringe pumps (ZAK, Marktheidenfeld, Germany), which were connected via Teflon tubing to the experimental chamber. The flow rate was 1 ml/min. Slices were positioned close to the outlet of the Teflon tube in the recording chamber to minimize the loss of enflurane. In some solutions, we additionally blew a vapor containing 1 vol% enflurane across the surface of the ACSF while applying a test solution of 1 MAC enflurane and recording of IPSCs from Purkinje cells. Switching the stream of vapor on and off had no effect on the time constant of IPSCs. From this finding we conclude that the loss of enflurane before reaching the slice was negligible.

Control recordings before enflurane application were taken ~15 min after the whole cell configuration was established. After that time, the reversal potential for IPSCs was close to 0 mV, indicating complete perfusion of the cell with the pipette solution. When switching from ACSF to drug-containing solutions, the medium in the experimental chamber was replaced within 2 min by ~95%. Effects on the spike patterns and IPSCs were stable ~5 min later. This delay may be attributed to the diffusion of the test solution into the tissue. Recordings in the presence of enflurane were taken 8–15 min after switching between the solutions. The time required to observe recovery increased with the concentration tested. With 0.5–2 MAC, full recovery was reached after 12–15 min, and with 4.0 MAC it was reached after 30–60 min. Stable recording of ≥1 h was necessary to test a single enflurane concentration.

**Data analysis**

Data were low-pass filtered between 2 and 5 kHz, acquired on a 486 PC with the digidata 1200 AD/DA interface and pClamp

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**TABLE 1. Concentration-dependent effects of enflurane on the interburst and burst durations of action potential firing of Purkinje cells**

<table>
<thead>
<tr>
<th>MAC</th>
<th>0</th>
<th>0.1–0.4</th>
<th>0.5–0.9</th>
<th>1.0–1.9</th>
<th>2.0–3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>22</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Interburst duration, s</td>
<td>1.1 ± 0.8</td>
<td>2.7 ± 1.3</td>
<td>5.3 ± 3.0</td>
<td>11.1 ± 9.5</td>
<td>21.2 ± 17.2</td>
</tr>
<tr>
<td>Burst duration, s</td>
<td>55.7 ± 70.5</td>
<td>12.4 ± 23.5</td>
<td>7.2 ± 8.6</td>
<td>14.9 ± 13.7</td>
<td>7.5 ± 3.9</td>
</tr>
</tbody>
</table>

Interburst and burst duration values are means ± SE. One MAC induces general anesthesia in 50% of patients and rats. The time criterion distinguishing between burst and interbursts was 1 s.
6.1 software (Axon Instruments). Sampling rates ranged between 5 and 15 kHz. Alternatively, records were stored on a Sony data recorder for later analysis. Extra- and intracellulrly recorded spikes and synaptic events were counted on- or off-line with the use of software event detectors. Amplitudes of synaptic events were determined from cursor measurements. IPSC current decays were fitted with monoexponential functions. Fitting routines were provided by the pClamp program package. Concentration-response relationships were fitted with Hill equations with the use of the simplex algorithm. Spike rates were measured as the mean of spikes occurring in a period of 180–300 s. For statistical analysis the paired Student’s *t*-test was used. Where not otherwise stated, results are given as means ± SE.

RESULTS

Effects of enflurane on the discharge rates of Purkinje cells

Spike patterns of Purkinje cells were monitored extracellularly in sagittal cerebellar brain slices with electrodes positioned within the Purkinje cell layer. The effects of enflurane were tested with 127 cells at 22°C and 57 cells at 35°C. Before and after enflurane treatment, Purkinje cells continuously fired action potentials interrupted only by brief periods of silence, typically ~2 s in duration. When enflurane concentrations corresponding to 1–2 MAC were applied, spike patterns such those as shown in Fig. 1A emerged. The anesthetic caused the cells to fire periodic bursts of action potentials, separated by gaps ~5–10 s in duration. In the majority of tested cells, the mean spike rates transiently increased before burst firing was established (data not shown). The concentration-dependent actions of enflurane on the interburst and burst durations are summarized in Table 1. The anesthetic prolonged the interburst intervals and shortened burst durations. In Fig. 1B, application of 1.3 MAC enflurane introduced a spike pattern similar to that in Fig. 1A and simultaneously caused the neuron to fire short trains of five to seven action potentials with decreasing amplitudes. Such patterns were observed in 5 of 14 cells tested with 1.3 MAC enflurane and in 6 of 12 cells with 2.0 MAC. In the remaining cells we obtained activity patterns like those in Fig. 1A. In Fig. 1C, the effect of enflurane on the discharge rate and on the interspike intervals is given. The data are derived from the large unit of Fig. 1B. They demonstrate that the anesthetic established a highly regular pattern of activity. During the active periods the spike rates were higher than observed under control conditions. This is indicated by the peak in
the interspike intervals, which shifted from ~65 ms to 42 ms in the presence of enfluran.

Development of oscillatory spike activity and the depression of the action potential discharge rates were not temperature dependent. In Fig. 2A, averaged spike rates monitored at various enflurane concentrations are shown. The spontaneous spike rate measured before enflurane treatment was $10.7 \pm 4.0$ Hz ($n = 127$) at 22°C and $26.7 \pm 3.7$ Hz ($n = 57$) at 35°C. The depressant effect of enflurane was quantified by comparing the discharge rates of the same cell before and during enflurane treatment. The results are given in Fig. 2B. At either temperature, half-maximal depression was observed close to 2 MAC. Spontaneous activity of Purkinje cells was significantly reduced at $\geq 0.4$ MAC enflurane ($P < 0.01$).

Assuming that the effects on the discharge patterns of Purkinje cells are exclusively caused by a possible action on GABA$_\alpha$ channels, the anesthetic should not be effective in bicuculline-treated slices, because the GABA$_\alpha$ antagonist depressed inhibitory synaptic currents regardless of whether volatile anesthetics were present or not (see Fig. 8). To test this hypothesis, spontaneous activity of Purkinje cells was monitored and slices were then treated as follows. First, enflurane was applied during recording from cells that exhibited a stable discharge pattern. About 10 min later, bicuculline was added to a final concentration corresponding to 25 $\mu$M. Finally, enflurane and bicuculline were removed. Data from a typical recording are presented in Fig. 3. In the presence of enflurane, the same regular spike patterns emerged as already shown in Fig. 1C. When bicuculline was added, oscillatory activity remained. Within the active periods the spike rate increased. In this experiment the peak in the interspike interval was between 50 and 60 ms under control conditions and between 20 and 30 ms after application of bicuculline (data not shown). In all cells ($n = 11$) tested this way, bicuculline did not reverse oscillatory firing patterns of Purkinje cells caused by enflurane treatment.

To determine whether bicuculline altered the effects of enflurane on the mean spike rates summarized in Fig. 2, a different experimental protocol was used as illustrated in Fig. 3. In these recordings, the GABA$_\alpha$ antagonist was applied before enflurane was added. In $\sim 20\%$ of the tested neurons, bicuculline completely abolished action potential activity. These neurons were excluded from further analysis. In the remaining cells, spike rates increased from $9.4 \pm 0.9$ Hz to $11.9 \pm 1.0$ Hz ($n = 14$). On average, 2 MAC enflurane reduced the discharge rate of Purkinje cells by only $13 \pm 8\%$ in the presence of bicuculline.

![Figure 4](image_url)

**FIG. 4.** Effects of enflurane on the amplitudes of spontaneous inhibitory postsynaptic currents (IPSCs). A: effects of 1.3 and 2.0 MAC enflurane on synaptic events recorded from a voltage-clamped cell held at $-77$ mV. The cell was filled with 145 mM CsCl. An increase in GABA$_\alpha$-mediated conductance corresponds to inward currents (downward deflections). B: depression of IPSC amplitudes by enflurane is plotted against the mean IPSC amplitudes recorded before enflurane treatment. The data points are derived from cells tested with 1 MAC (■) and 2 MAC (▲). They were fitted by regression lines. No significant correlation was observed ($F$ test $P < 0.05$). C: concentration-response relationship for the depression of IPSC amplitudes. For each concentration, the number of tested cells was between 5 and 15. The solid line was fitted to the data by a Hill equation. Half-maximal inhibition was estimated to be close to 0.9 MAC enflurane.
FIG. 5. Effects of enflurane on the reversal potential of IPSCs. A: current traces recorded from the same cell at different membrane potentials before and during enflurane treatment. Membrane potentials are indicated at left. B: current-voltage relationship of mean IPSC amplitudes observed in the absence and presence of enflurane. Top graph: corresponding SDs. The data are derived from the same cell as in A. IPSC amplitudes were fitted by regression lines. The estimated reversal potential for a Cl–selective conductance is 2.5 mV.

Effects of enflurane on spontaneous and evoked IPSCs

IPSC AMPLITUDES. Spontaneous GABA<sub>A</sub>-mediated IPSCs were monitored from voltage-clamped cells held at membrane potentials between −72 and −80 mV. To improve the voltage-clamp conditions, to amplify GABA<sub>A</sub>-mediated synaptic events and block GABA<sub>B</sub> currents, the neurons were filled with 145 mM CsCl. Under these conditions, spontaneous synaptic events with amplitudes between a few picampers and several nanoampers were observed. These events were identified as spontaneous GABA<sub>A</sub>-mediated IPSCs, because they were abolished by bicuculline (25 μM, n = 7).

The effects of enflurane on the amplitudes of spontaneous IPSCs are shown in Fig. 4A. In this particular experiment, two different concentrations were applied to the same cell. The anesthetic decreased the amplitude of IPSCs in a concentration-dependent manner. Setting the amplitude for event detection at 20 pA, the mean amplitudes were 248 ± 232 pA before enflurane, 167 ± 111 pA in the presence of 1.3 MAC, 46 ± 42 pA in the presence of 2.0 MAC, and 205 ± 148 pA following washout.

Mean IPSC amplitudes varied considerably between cells. We calculated an average of 203 ± 245 (SD) pA (n = 41). Similar results were reported by Farrant and Cull-Candy (1991), Llano et al. (1991a), and Puia et al. (1994). Despite this variability, depression of IPSC amplitudes by enflurane did not depend on the mean IPSC amplitudes that were recorded before enflurane treatment. In Fig. 4B, the effects of enflurane were quantified by dividing the mean IPSC amplitude recorded in the presence of the anesthetic by the amplitude observed before enflurane treatment. These values were plotted against the IPSC amplitudes measured before enflurane application. With concentrations corresponding to 1 and 2 MAC, no statistically significant correlation was observed (P < 0.05, F test).

In Fig. 4C, the concentration-response relationship is given. The solid curve was fitted with a Hill equation to the data points. Half-maximal reduction was estimated at 0.9 MAC. Depression of the mean IPSC amplitude was significant at 1.0 MAC (P < 0.01).

The reduction of IPSC amplitudes shown in Fig. 4 can be explained by at least two different mechanisms. Either the membrane conductance during IPSCs is reduced or, alternatively, a shift in the reversal potential toward more negative values is involved. When Purkinje cells were voltage clamped at values between +80 and −80 mV, anesthetic concentrations corresponding to 1 and 2 MAC enflurane depressed IPSC amplitudes at all tested voltages, whereas the reversal potential of IPSCs remained unaffected. In Fig. 5B, the mean IPSC amplitudes monitored at different membrane potentials were fitted by a straight line. The conductances estimated from the slope of the regression line were reduced to 53% of the control value with 1 MAC enflurane and to 23% with 2 MAC. The results from five cells tested in this way are summarized in Table 2. They indicate that depression of IPSC amplitudes is caused by a change in conductance and not by a shift in the reversal potential.

DECAY OF IPSCs. IPSC decays recorded from Purkinje cells can be well fitted with monoeXponential functions (Puia et al. 1994; Vincent et al. 1992). A time constant of 10.4 ± 0.5 ms (n = 79) was calculated from recordings carried out before enflurane treatment at 21–23°C. This is close to the value given elsewhere (Puia et al. 1994; Vincent et al. 1992).

In hippocampal pyramidal cells, the volatile anesthetic halothane prolonged the current decays of evoked and spontane-
TABLE 2. Effects of enflurane on the amplitude of GABA<sub>A</sub>-mediated conductances as obtained from current-voltage relationships

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1 MAC</th>
<th>2 MAC</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>Conductance, nS</td>
<td>2.32 ± 0.25</td>
<td>1.14 ± 0.1</td>
<td>0.59 ± 0.07</td>
<td>5</td>
</tr>
<tr>
<td>Reversal potential, mV</td>
<td>6.4 ± 2.6</td>
<td>8.9 ± 2.0</td>
<td>3.8 ± 1.4</td>
<td>5</td>
</tr>
</tbody>
</table>

Values, except n values, are means ± SE. Conductances were calculated from the slopes of regression lines, as shown in Fig. 5. The same fits were used to estimate the reversal potentials of inhibitory postsynaptic currents (IPSCs). Reversal potentials obtained in the presence of enflurane did not differ significantly from the control values (P < 0.02). GABA<sub>A</sub>, γ-aminobutyric acid-A; for explanation of MAC, see Table 1.

In the experiment shown in Fig. 6A, IPSCs were simultaneously evoked by electrical stimulation of afferent fibers (bottom traces). For stimulation, a second patch electrode was placed on the surface of the slice at the border between the Purkinje cell layer and granule cell layer. A current of 18 μA, 0.1 ms in duration, was used to evoke postsynaptic IPSCs. Average time constants of 9.7 ± 2.7 (SD) ms (n = 27) and 26.1 ± 5.3 ms (n = 26), respectively, were fitted to evoked IPSCs recorded before and during enflurane treatment (0.5 MAC).

In Fig. 6B, the time constants of current decays were averaged once a minute before, during, and after enflurane treatment to estimate the time required to obtain stable effects. At 1 MAC, enflurane increased the time constant from 9 to 47 ms. Steady state was reached 6–7 min after starting enflurane perfusion. Complete recovery was observed ~11 min after removal of the anesthetic.

In Fig. 6C, the concentration-response curve for the time constants of current decays estimated from spontaneous IPSCs is shown. At concentrations corresponding to 0.5 MAC, current decays were significantly prolonged (P < 0.01). With 2 and 4 MAC, time constants increased ~10-fold. From the fit in Fig. 6C, half-maximal potentiation was observed at 1.7 MAC.

Problems and possible errors that are associated with pre-
TABLE 3. Comparison of the effects of differently prepared test solutions on the time constant of IPSCs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Test</th>
<th>Test/Control</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>1 vol%</td>
<td>10.7 ± 1.4</td>
<td>54.1 ± 3.3</td>
<td>5.12 ± 0.78</td>
<td>12</td>
</tr>
<tr>
<td>2 vol%</td>
<td>10.6 ± 0.86</td>
<td>173.2 ± 26.6</td>
<td>16.04 ± 2.31</td>
<td>15</td>
</tr>
<tr>
<td>0.63 mM</td>
<td>10.0 ± 0.61</td>
<td>51.0 ± 3.01</td>
<td>5.20 ± 0.42</td>
<td>9</td>
</tr>
<tr>
<td>1.26 mM</td>
<td>10.2 ± 0.81</td>
<td>159.1 ± 5.3</td>
<td>15.81 ± 0.79</td>
<td>9</td>
</tr>
</tbody>
</table>

Values, except n values, are means ± SE. Test solutions corresponding to 1 and 2 vol% enflurane were prepared either by bubbling the artificial cerebrospinal fluid (ACSF) with 1 and 2 vol% enflurane at 21–23°C or by dissolving the anesthetic to final concentrations of 0.63 and 1.26 mM, respectively. Mean values at corresponding MACs did not differ significantly (P < 0.01). For abbreviations, see Tables 1 and 2.

paring test solutions of volatile anesthetics and with estimating MAC values have been analyzed and discussed by Franks and Lieb (1993). To exclude such errors, we prepared the test solutions either by bubbling the ACSF with a vapor containing the anesthetic or by diluting the anesthetic to the desired concentration. The effects of the differently prepared solutions on the IPSC time constant are compared in Table 3. The data suggest that both methods yielded very similar anesthetic concentrations in the ACSF.

IPSC FREQUENCY. Figure 6D summarizes the actions of enflurane on the frequency of synaptic events. Between 0.5 and 2 MAC, enflurane reduced the frequency of IPSCs in a concentration-dependent manner. With 4 MAC, IPSCs were nearly abolished. Because the IPSC amplitudes were largely depressed with this high concentration, it seems possible that synaptic events could not be resolved from the baseline current.

ESTIMATION OF THE OVERALL EFFECT OF ENFLURANE ON GABA A-MEDIATED INHIBITION. Presynaptic GABA release causes the opening of postsynaptic GABA A channels, subsequently allowing Cl− ions to flow across the postsynaptic membrane. The GABA A-mediated inhibitory input received by a Purkinje cell thus depends on the number of ions transferred in the time course of synaptic events and on the frequency of these events. In Fig. 7A, idealized synaptic events observed under control conditions at 22°C and with different enflurane concentrations are shown. Idealized IPSCs were constructed from the amplitudes and time constants in Figs. 4C and 6C. Enflurane depressed the amplitudes of IPSCs and simultaneously prolonged current decays. Because of the monoexponential current decays, the transferred charge per IPSC can be approximated by multiplying the IPSC amplitudes and the corresponding time constants of current decays. The results are given in Fig. 7B. They show that, despite the amplitude depressing effect, the increase in the transferred charge per averaged IPSC is well approximated by a straight line.

We estimated the mean inhibitory current received by Purkinje cells at different enflurane concentrations by multiplying the charge transfer per average synaptic event and the frequency of these events. The data show that, because of the concentration-dependent decrease of synaptic events, the increase in GABA A-mediated inhibition is less steep compared with the transferred charge per IPSC. Between 0.5 and 2 MAC, GABA A-mediated inhibition of Purkinje cells has doubled and remains more or less constant. Without enflurane, synaptically mediated inhibition of Purkinje cells corresponded to a conductance of 0.63 ± 0.17 nS.

EFFECTS OF BICUCULLINE ON BASELINE CURRENTS. In a study on cultured hippocampal neurons, it was suggested that volatile anesthetics also gate GABA A channels in the absence of GABA (Yang et al. 1992). We examined our data with regard to the question whether a persistent GABA A-mediated current was induced during enflurane treatment. The results of Fig. 8 were obtained before and during enflurane application. In both cases bicuculline was added to the bathing solution. To resolve the effects on the baseline current, a cell with a low synaptic input was chosen. Enflurane application did not cause an inward current, as is to be expected when persistent Cl− current is induced. Application of bicuculline abolished synaptic events, but did not affect the baseline current. Similar results were obtained with all cells investigated (n = 7). From these findings, it must be concluded that in our preparation enflurane did not gate GABA A channels in the absence of GABA.

EFFECTS OF TEMPERATURE. In further experiments, the effects of enflurane were compared at different temperatures. The results are summarized in Fig. 9. Raising the temperature from 22 to 35°C shortened the IPSC time constant and increased the frequency of synaptic events. The mean IPSC amplitude was hardly affected. The effects of enflurane on the time constant of current decays and on the mean IPSC amplitudes were similar at 22 and 35°C.

EFFECTS OF ENFLURANE IN THE PRESENCE OF TETRODOTOXIN. Llano et al. (1991a) demonstrated that the mean amplitude of IPSCs recorded from voltage-clamped Purkinje cells is
strongly reduced if presynaptic action potentials are abolished by tetrodotoxin (TTX). This finding raises the question of whether the amplitude-depressing effect of enflurane summarized in Fig. 4 results from a depression of presynaptic action potentials. TTX treatment alone reduced the mean amplitude of IPSCs to 66.1 ± 4.4 pA (n = 12) and decreased the frequency of synaptic events to 4.1 ± 0.3 Hz. Figure 10 shows that the effects of 1 and 2 MAC enflurane on the IPSC amplitude were similar, regardless of whether TTX was present or not. Enflurane did not cause a decrease in the frequency of synaptic events in TTX-treated slices.

**EFFECTS OF ENFLURANE DURING CURRENT-CLAMP RECORDINGS.** When treated with enflurane, Purkinje cells began to fire bursts of action potentials (Fig. 1). Studies on thalamic neurons demonstrated that a transition in the discharge patterns from singular spiking toward burst firing occurred as the membrane potential was hyperpolarized (for a review, see Steriade et al. 1993). A transient Ca$$^{2+}$$ current and a hyperpolarization-activated cationic current ($I_h$) were identified as the most important components in causing these changes. Because the question arose of whether the effects of enflurane on Purkinje cells involved a similar mechanism, current-clamp experiments were carried out with the use of patch pipettes filled with potassium gluconate. Five to ten minutes after the whole cell configuration was obtained, 21 of 33 neurons fired action potentials spontaneously. Membrane resting potentials ranged between −55 and −69 mV. When injecting hyperpolarizing currents through the recording electrode, the discharge rates decreased, but, unlike in thalamic neurons, burst firing was not induced (Fig. 11A). Exposure to the anesthetic (2 MAC) transiently increased the mean spike rate and caused burst firing (Fig. 11A2). During current-clamp recordings, 2 MAC enflurane reduced firing rates by 61 ± 14%, which is close to the value calculated from extracellular recordings.

The effects of depolarizing currents, injected before and during enflurane treatment, are illustrated in Fig. 11B. Under control conditions without enflurane, current injections shortened interspike intervals and increased the spike rate. Enflurane application evoked burst firing and, as the cell was depolarized, burst durations lengthened and interburst intervals shortened (Fig. 11, B2 and B5). The discharge rate within the bursts was little affected by the amount of injected current (Fig. 11B4).

A representative example for the effects of the anesthetic on action potentials, the membrane resting potential and the input resistance is presented in Fig. 12. At 2 MAC, enflurane reduced the spike amplitude by 5 mV (7% of spike amplitude) and depressed spike afterhyperpolarizations by 3 mV (4%). The threshold for spike generation, as well as the width of action potentials, remained unaffected. On average, enflurane treatment hyperpolarized Purkinje cells by 5.2 ± 1.1 mV (n = 13), but only by 1.6 ± 1.2 mV (n = 7), when GABA$_A$-mediated synaptic transmission was blocked. Bicuculline treatment alone (25 μM) increased the input

**FIG. 8.** Effects of 25 μM bicuculline on the baseline current in the absence and presence of 1 MAC enflurane. All traces were recorded from the same neuron. The Purkinje cell was held at −72 mV. A: before enflurane treatment, application of bicuculline (bicu) abolished synaptic events while leaving the baseline current unaltered. B: in the presence of 1.0 MAC enflurane, inhibitory postsynaptic potentials were abolished when bicuculline was added. Again, the baseline remained unchanged.

**FIG. 9.** Effects of enflurane on spontaneous IPSCs at different temperatures. Current decays were recorded at 22°C (A) and at 35°C (B). For each bar, the number of cells tested ranged between 9 and 15. C: mean IPSC amplitudes as observed at 22 and 35°C.
resistance of Purkinje cells by \( \sim 10\% \) and depolarized the membrane potential between \( 0 \) and \( 3 \) mV. Enflurane-induced membrane hyperpolarizations reversed at membrane potentials between \( -77 \) and \( -86 \) mV. This was close to the Nernst potential for Cl\(^-\) ions \( (-80 \) mV).

During enflurane application, the input resistance, determined from the peaks of voltage deflections in response to hyperpolarizing current pulses, transiently increased, reaching a maximum \( \sim 3-4 \) min after the onset of enflurane perfusion, but then approached a steady state close to that recorded before enflurane administration (Fig. 12C). Under steady-state conditions, input resistances did not differ significantly, regardless of whether the anesthetic was present or not.

Similar to observations made in a previous study (Chang et al. 1993), hyperpolarizing currents activated \( I_h \), thus causing the sag in the voltage traces of Figs. 12A and 13A. Activation of \( I_h \) was considerably accelerated by enflurane (Fig. 13B).

The voltage records shown in Fig. 13A were obtained when regenerative action potential activity and synaptic inhibition were blocked by TTX and bicuculline, respectively. The corresponding current-voltage relationships recorded before, during, and after enflurane application show that, under these conditions, the anesthetic slightly increased the input resistance on average of \( 8 \pm 3\% \) \( (n = 4) \).

**DISCUSSION**

In the present study we investigated the relation between the effects of enflurane on GABA\(_A\)-mediated synaptic inhibition and on the discharge patterns of Purkinje cells. The result that bicuculline increased spontaneous firing without causing synaptic excitation (Fig. 8) indicated that GABA\(_A\)-mediated inhibition in fact participated in controlling the discharge rates. There are two lines of evidence supporting the hypothesis that enflurane diminished spontaneous action potential activity by increasing synaptic inhibition. First, enflurane was more efficient in depressing spike rates in preparations with GABA\(_A\)-mediated inhibition intact than in disinhhibited slices. Second, 1–2 MAC enflurane increased synaptically mediated inhibition by a factor of 2 and simultaneously hyperpolarized the membrane potential by \( \sim 5.2 \) mV in the absence, but only by 1.6 mV in the presence, of bicuculline. Assuming an input resistance of 150 M\( \Omega \), a change in the membrane potential of 5 mV is caused by a current of \( \sim 30 \) pA. The results summarized in Fig. 11B3 demonstrate that such currents were indeed sufficient to alter the spontaneous activity of Purkinje cells considerably.

In thalamic neurons, periodic burst discharges were evoked by hyperpolarizing current injections (Steriade et al. 1993). Bursts of action potentials were generated by the interplay of \( I_h \) and a transient Ca\(^{2+}\) current. \( I_h \) acted as a pacemaker current and depolarized the membrane potential until Ca\(^{2+}\) spikes occurred. As Ca\(^{2+}\) spikes occurred, the intracellular Ca\(^{2+}\) concentration rose. Consequently, Ca\(^{2+}\)-dependent K\(^+\) conductances were activated, leading to membrane hyperpolarization. As a result, inactivation of Ca\(^{2+}\) channels was removed, \( I_h \) was activated, and the next cycle commenced. Burst discharges disappeared as the membrane potential was depolarized because, in this case, Ca\(^{2+}\) channels remained inactivated.

The following observations make it improbable that the same mechanism underlies enflurane-induced burst firing of Purkinje cells. First, bursts of action potentials were neither induced when hyperpolarizing currents were injected in the absence of enflurane nor abolished when depolarizing currents were applied in the presence of the anesthetic. Second, enflurane-induced membrane hyperpolarizations were largely blocked in the presence of bicuculline, whereas burst discharges were not.

**FIG. 10.** A: effects of 1 and 2 MAC enflurane on the frequency (top trace), the time constant (middle trace), and the mean amplitude (bottom trace) of IPSCs in the presence of 1 \( \mu \)M tetrodotoxin (TTX). The data were taken from the same cell and are given as mean values \( \pm \) SD. The frequency was calculated from the number of synaptic events counted within a period of 100 s. B–D: effects of enflurane on the frequency (B), the time constant (C), and the mean amplitude of synaptic events (D) in the absence and presence of TTX.
Effects of enflurane on Purkinje cells

**FIG. 11.** Effects of hyperpolarizing and depolarizing current injections on the spike patterns of Purkinje cells. The injected current is indicated at the top of the records. **A1:** in a spontaneously active cell, hyperpolarizing currents reduced spike rates without inducing burst firing. **A2:** enflurane (2 MAC) was applied, the spike rate transiently increased (enflurane 3 min) before the cell started bursting (enflurane 9 min). Spontaneous firing rates were 6.0 Hz before, 3.7 Hz during, and 6.7 Hz after treatment. **B1:** in a different cell with a bottom spontaneous activity (1.4 Hz), depolarizing currents increased the discharge rate in the absence (**B1**) and presence (**B2**) of the anesthetic. Enflurane reduced action potential firing by ~50% (**B3**). Without enflurane, membrane depolarization shifted the peak values of interspike interval histograms toward bottom values (**B4**). When enflurane was present (2 MAC), interspike intervals were hardly affected by the currents applied. **B5:** in the presence of enflurane, burst and interburst durations depended on the injected currents.

In Purkinje cells, the mechanisms underlying burst firing remain to be elucidated. It has been demonstrated that, when burst firing was induced by TTX treatment, blockage of \( I_h \) altered burst and interburst durations (Chang et al. 1993). Because the anesthetic accelerated activation of this current, it seems possible that \( I_h \) was indeed involved in causing burst firing in the present study. We speculate that the effects observed on \( I_h \) facilitated a shift of the membrane potential toward the plateau potentials seen during bursts (Fig. 11). Thus, similarly as in thalamic neurons, \( I_h \) may have acted as the pacemaker current. However, completely different mechanisms, e.g., those involving autonomous oscillations in the intracellular \( \text{Ca}^{2+} \) concentration and periodic activation of \( \text{Ca}^{2+} \)-dependent \( K^+ \) channels, as reported in several preparations, cannot be ruled out at present (for reviews see Meyer and Stryer 1991; Tsien 1990).

Besides the mechanisms discussed above, the depression of spike afterhyperpolarizations shown in Figs. 11 and 12A could be also involved in altering the discharge patterns. This was already suggested in studies on the effects of volatile anesthetics on neocortical and hippocampal pyramidal cells (El-Beheiry and Puil 1989; Fujiwara et al. 1988). Depression of spike afterhyperpolarizations may contribute to neuronal excitation frequently observed during enflurane anesthesia. An excitatory action of enflurane was further indicated by the persistent increase in the input resistance observed in bicuculline-treated slices. Without bicuculline, the input resistance did not differ before and during enflurane
administration. This indicates that the effect, which was unmasked in disinhibited preparations, was counterbalanced by an increase in synaptic inhibition.

The overall effects of enflurane on the discharge patterns of Purkinje cells appear to be rather complex. A typical example is given in Fig. 11B. During injection of a current of $\sim 60$ pA, the maxima of interspike intervals determined under control conditions and during enflurane-induced burst-
spike patterns. Our results demonstrate that increased GABA<sub>A</sub>-mediated inhibition reduced spontaneous spike rates of Purkinje cells, whereas burst discharges were probably caused by a parallel action of the anesthetic on $I_h$ and spike afterhyperpolarizations.

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REFERENCES


