Isoflurane Attenuates Resonant Responses of Auditory Thalamic Neurons

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Tennigkeit, Frank, Craig R. Ries, Dietrich W. F. Schwarz, and Ernest Puil. Isoflurane attenuates resonant responses of auditory thalamic neurons. J. Neurophysiol. 78: 591–596, 1997. In thalamo-cortical neurons, sensory signals are transformed differently during various states of consciousness. We investigated the effects of a general anesthetic, isoflurane, on the frequency responses of neurons in the ventral medial geniculate body, the primary nucleus of the auditory thalamus. Using slice preparations, whole cell current-clamp recording techniques, and frequency-domain analyses with oscillatory inputs, we observed a resonance in the hyperpolarized voltage range, implying a frequency preference near 1 Hz in the subthreshold frequency responses of medial geniculate neurons. As in other thalamocortical neurons, an interaction of a T-type Ca2+ current with passive membrane properties generates the resonant responses. The frequency preference shapes the input-output signal transformation, coupling oscillatory inputs at preferred frequencies to firing. Thus resonance may contribute to the rhythmic synchronization of the output to the cortex. In a concentration range of 0.5–3%, isoflurane application reversibly decreased the resonant responses of medial geniculate neurons. Throughout the subthreshold voltage range, it reduced impedance at frequencies <10 Hz. At depolarized potentials near −60 mV, isoflurane reduced the low-pass filter selectivity of the neuron membrane. At rest near −70 mV or at hyperpolarized potentials, isoflurane had a greater effect on resonance (centered at ~1 Hz), reducing the peak impedance more than the magnitudes at other frequencies. At concentrations of ≥2%, isoflurane completely blocked the resonance peak, thereby imposing low-pass characteristics of poor quality throughout the subthreshold voltage range. Application of isoflurane reversibly increased membrane conductance and the current threshold for firing evoked by depolarizing pulses from potentials between −60 and −90 mV. The neurons discharged in a tonic pattern on depolarization from about −60 mV and in a phasic (burst) mode from potentials negative to about −70 mV. An increase in current amplitude compensated the suppression of tonic firing much more readily than that of the burst firing on a low-threshold Ca2+ spike. Although a reduction in T-type Ca2+ channel activation may occur during isoflurane application, the depression of resonance is consistent with an interaction of a greatly increased leak conductance with the low-threshold Ca2+ current and the membrane capacitance. In the intact animal, this would tend to disrupt synchronized neural oscillations and the transfer of auditory information.

INTRODUCTION

General anesthetics induce an unconscious state that anesthesiologists view as an insensitivity to stimuli from the external environment. Anesthetics decrease neuronal firing and transmission in many regions (Krnjević and Puil 1997), disrupting the rhythmic activities of the brain that characterize conscious states (Moruzzi and Magoun 1949). The cortico-thalamocortical system has a crucial involvement in the responses to auditory, tactile, and visual stimuli as well as the rhythms during awareness and sleep (Barth and MacDonald 1996; Steriade et al. 1990, 1996). In humans, for example, anesthetics markedly attenuate the middle-latency response of auditory evoked potentials (Galambos et al. 1981; Madler et al. 1991) and the auditory steady-state responses to rapidly presented stimuli (40 per s) (Plourde 1993, 1996; Plourde and Villemure 1996). These potentials arise mainly from neuron circuits of the primary auditory cortex and medial geniculate body of the thalamus.

The effects of anesthetics on neurons of the thalamus have attracted recent interest (Angel and LeBeau 1992; Puil et al. 1996; Ries and Puil 1993; Sugiyama et al. 1992; see also Steriade et al. 1996) because anesthetics may disorganize the temporary relationship between the sensory responses and coherent oscillations or reduce the background synchronous activity implicated in awareness and cognition (Plourde 1993; Plourde and Villemure 1996). Normally, a transfer of sensory information to the cortex results from synaptic stimuli interacting with intrinsic membrane properties of thalamocortical neurons. In thalamic slices, this integrative activity produces a frequency selectivity, identified with alternating current inputs as resonance (Hutcheon et al. 1994; Puil et al. 1994b; Ströhmann et al. 1994, 1995) or viewed overtly as voltage oscillations in a subthreshold range (McCormick and Pape 1990). This frequency selectivity, which is likely subject to modulation during behavioral and anesthetic states (cf. Angel 1993; McCormick 1992; Neuman et al. 1996), acts as a filter in the conversion of synaptic inputs to output firing. We have investigated the effects of a volatile anesthetic, isoflurane (IFL), on the frequency preferences of neurons in the ventral partition of the medial geniculate body (MGBv). Understanding anesthetic effects on resonance is important in the physiology of the auditory system, where neurons sharply modify their pattern of synaptically evoked discharge depending on the type of anesthesia (Zurita et al. 1994). The effects of IFL, which we describe here, differ markedly from the effects of an anesthetic barbiturate, pentobarbital (Puil et al. 1996).

METHODS

The preparation and maintenance of thalamic slices for recording were similar to those described previously (Tennigkeit et al. 1996).
Sprague-Dawley rats (16–21 days old) were decapitated during deep halothane anesthesia. The brain was rapidly removed from the cranium and submerged in cold (4°C) artificial cerebrospinal fluid (ACSF). The ACSF consisted of (in mM) 124 NaCl, 26 NaHCO3, 10 glucose, 4 KCl, 2 CaCl2, 1 MgCl2, and 1.25 KH2PO4, pH maintained at 7.3 by continuous saturation with 95% O2-5% CO2. Using a Vibroslicer (Campden Instruments, London, UK), we cut 300- to 400-μm-thick coronal slices of the medial geniculate body. Before recording, we incubated the slices at 22–25°C for ≥3 h. Whole cell patch-clamp electrodes were pulled (Narishige, Model PP83) from borosilicate glass (WP Instruments, Sarasota, FL). They contained an electrolyte solution (pH 7.3) consisting of (in mM) 130 potassium gluconate, 15 KCl, 10 Na-[N-2-hydroxyethylpiperazine-N(2-ethanesulfonate)], 1 Mg-ATP, 10 ethylene glycol-bis-(β-aminoethylether) -N,N,N',N'-tetraacetic acid, and 1 CaCl2 (10 nM Ca2+, calculated with the use of Max Chelator). Current-clamp recordings were made with an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) in slices at 22–25°C. Data acquisition, storage, and analysis were controlled with the use of pClamp 5 software (Axon Instruments) with an IBM-compatible computer. Recordings with access resistance compatible computer. Recordings with access resistance

**RESULTS**

The results described here were obtained from measurements on a total of 14 medial geniculate neurons. We applied only one concentration of IFL to each neuron, all in different slices, except in the case of four neurons (cf. Fig. 2).

We assessed the effects of IFL on the frequency responses in 11 neurons at membrane voltages that reflect wakefulness and sleep (Steriade et al. 1990). First, to simulate the tonic firing mode, we used DC to depolarize the neurons to about −60 mV (cf. Fig. 3A), and then injected subthreshold as well as threshold ZAP stimuli. The smaller stimulus displaced the membrane potential by 8–10 mV, whereas the larger stimulus evoked firing at low frequencies (Fig. 1A). The top frequency response curves in Fig. 1A, right, corresponding to the subthreshold and threshold ZAP responses, show the low-frequency preference typical for depolarized MGBv neurons under control conditions. Application of IFL (1%) reduced the overall ZAP voltage responses to subthreshold and threshold currents, abolished the action potentials, and reduced the impedance magnitude <10 Hz (Fig. 1A). Although firing returned when the ZAP current magnitude was 20 Hz. Increase in ZAP current amplitude produced firing in resonant frequency range (10 Hz, cf. frequency response curves at right) and suppressed firing, which required increased ZAP current amplitude. Note that impedance magnitude was not affected by changed ZAP current amplitude and contamination of voltage response by action potential firing. B: at hyperpolarized membrane potential (−70 mV), ZAP current (0.1–20 Hz) evoked resonance in voltage responses at ~1 Hz. Increase in ZAP current amplitude produced firing in resonant frequency range (−70 Hz, cf. frequency response curves at right) and suppressed firing, which required increased ZAP current amplitude. Note that impedance magnitude was not affected by changed ZAP current amplitude and contamination of voltage response by action potential firing. C: sine-wave current inputs (same amplitudes as ZAP current inputs in B) at −70 mV elicited action potential firing at resonant frequency, not above or below. Note different time scales and truncated action potentials. On application of IFL (1%), larger-amplitude input (same as large ZAP current input in B) also elicited frequency-selective firing. Scale bars: (A and B) voltage, 40 mV; current, 200 pA; (C) voltage, 20 mV; current, 200 pA.

**IFL blockade of resonant responses and frequency-selective firing**

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increased, the impedance profile remained reduced. The impedance did not change at frequencies >10 Hz, implying that IFL did not greatly affect membrane capacitance (cf. Puil and Gimbarzevsky 1987).

We then tested the ZAP responses of neurons in the phasic (burst) firing mode near rest or hyperpolarized beyond about −70 mV with DC injection (Figs. 1B and 2, B and C). A ZAP analysis with a subthreshold ZAP current revealed a broad resonant hump that peaked at ∼1 Hz (Fig. 1B). At large ZAP current amplitudes, LTSs and action potentials contributed to the voltage responses when the input swept through the resonant frequencies (centered near 1 Hz). This frequency-selective firing was confirmed with the use of sine-wave current inputs, shown in Fig. 1C. Application of IFL (1%) to neurons in this hyperpolarized state reduced the low-frequency bulge in the voltage response and blocked firing (Fig. 1B). Despite this marked reduction in impedance, some frequency preference remained in the same frequency range while the neuron was in this hyperpolarized state. The suppression of firing was surmountable by increasing the amplitude of the ZAP current, which reestablished frequency-selective firing independent of the input waveform (Fig. 1, B and C).

**Effects of IFL on the ZAP**

Application of IFL (0.5–3%) reversibly decreased the amplitude of frequency responses at membrane voltages between about −100 mV and action potential threshold (about −50 mV, n = 11). Normally, MGBv neurons at depolarized potentials exhibited low-pass filter characteristics. In a concentration-dependent manner, IFL reduced the amplitude of the frequency responses (<10 Hz) of neurons in this membrane potential range. During application of 2% IFL, the maximum in the frequency response of a DC-depolarized neuron was reduced to ∼50% of control (Fig. 2A, n = 8). The effects of 2% IFL on the frequency responses were relatively greater in neurons at potentials near rest where they displayed a resonance; in the example of Fig. 2B, the maximal amplitude in the frequency response was reduced to ∼40% of control. The amplitude of the resonance peak near ∼1 Hz was increased at DC-hyperpolarized potentials in neurons under control conditions (Fig. 2C). At concentrations of 0.5–3%, IFL produced a marked depression of this resonant response. Application of IFL at 2% to a neuron in this hyperpolarized state reduced the amplitude of the maximal frequency response to ∼30% of control and eliminated the voltage-dependent resonant responses (n = 8). Administration of IFL at 3% produced the same effect (n = 2). As a result, higher IFL concentrations abolished the differences in the frequency responses observed at different membrane potentials, imposing low-pass characteristics of low quality at all membrane voltages.

**Effects of IFL on subthreshold responses to current pulses and firing**

The frequency response curves near zero frequency indicated that IFL increased input conductance. Because the observed changes in frequency preference depend on an altered interaction of the resting conductance and T-type Ca²⁺ current activation, we examined the effects of IFL on the voltage responses to depolarizing and hyperpolarizing current pulses in 14 neurons.

**SUBTHRESHOLD RESPONSES.** We observed that application of IFL hyperpolarized MGBv neurons and increased input conductance, measured with small hyperpolarizing current pulses that displaced the membrane potential from rest (about −68 mV) by <10 mV. These changes were concentration dependent. At 0.5%, IFL (n = 3) elicited a hyperpolarization of 1–2 mV and increased conductance by 8.8, 9.5, and 43.5%. At 1%, IFL hyperpolarized three neurons by 3–4 mV, and, despite DC compensation, increased conductance by 22.5, 38.6, and 61.8% (cf. Fig. 1). Application of 2% IFL increased conductance by 76.6 ± 13.5% (mean ± SE, n = 6, Fig. 3, A and B). A hyperpolarization of 4–6 mV, requiring DC compensation, accompanied this conductance increase.

**TONIC FIRING MODE.** IFL reversibly reduced the subthreshold voltage responses and tonic firing evoked by square pulse current injections into neurons in the DC-depolarized state. As with the increased ZAP input, current pulses of larger amplitude elicited tonic firing during IFL application (Fig. 3A).

**BURST FIRING MODE.** When the neurons were at rest, IFL application (2%) suppressed the LTS burst firing at the offset of hyperpolarizing pulses (Fig. 3B). Larger-amplitude pulses that hyperpolarized the neurons to about −110 mV...
Our major finding is that IFL reversibly attenuated membrane impedance, in particular a low-frequency resonance in MGBv neurons. At the lowest concentration (0.5%), which corresponds to ~1% at 37°C (see METHODS), IFL reduced the resonant hump and increased steady-state input conductance. At higher concentrations, IFL eliminated the resonance, creating a flat band-pass function between 0.1 and 10 Hz, and markedly increased input conductance. In MGBv neurons of the intact animal, the effects on resonance and conductance would alter the transformation, or prevent relay, of signals to the primary auditory cortex. For example, a depression of transmission through the ventrolateral and lateral geniculate thalamus occurs during administration of halothane or pentobarbital in cats (Marshall and Murray 1980).

The flattened frequency responses of MGBv neurons likely relate to the effects of IFL on the membrane properties that normally generate resonance at potentials near and below the resting level. The resonant hump in thalamic neurons of mammals, but not birds (Ströhmann et al. 1994), is due to the low-threshold, T-type Ca\(^{2+}\) current with the membrane leak current and capacitance (mediodorsal neurons, Hutcheon et al. 1994; Puil et al. 1994b; lateral geniculate nucleus neurons, Jahnsen and Karnup 1994; MGBv neurons, Tennigkeit et al. 1994). An increase in input capacitance, possibly due to an increased membrane fluidity, occurs in peripheral sensory neurons during IFL anesthesia (Puil and Gimbarzevsky 1987). We did not observe any evidence that IFL application may have increased input capacitance.

A depression of the T current may contribute to the observed changes in resonance. IFL and other volatile anesthetics, or barbiturates, depress low- and high-threshold Ca\(^{2+}\) currents in various neurons (ffrench-Mullen et al. 1993; Gross and MacDonald 1988; Krnjević and Puil 1988; Puil et al. 1994a; Study 1994; Takenoshita and Steinbach 1991). We found that IFL had the greatest effect at hyperpolarized potentials at which resonance was largest, i.e., at potentials at which the slow inactivation parameter dominates the low-frequency peak in thalamocortical neurons (Hutcheon et al. 1994). Pentobarbital and the induction agent propofol enhance the apparent steady-state inactivation and accelerate the rate of inactivation, decreasing Ca\(^{2+}\) currents (ffrench-Mullen et al. 1993; Gross and MacDonald 1988; Gundersen et al. 1988; Olcese et al. 1994). IFL may have similar actions in suppressing the T current (Study 1994; Takenoshita and Steinbach 1991), but this may not be the main explanation for our observations on resonance, particularly at the higher IFL concentrations.

IFL application greatly increased membrane conductance, producing a shunt that likely reduced resonant responses. This occurred when IFL shunted firing of action potentials elicited by current pulses, as observed in ventrobasal thalamic neurons (Ries and Puil 1993). In both types of neurons, the effects of IFL on the firing of action potentials also were surmountable by greatly increasing the amplitudes of the input current. In MGBv neurons, larger-amplitude ZAP currents produced the same frequency preference compared with controls. An increase in leak current accompanied the depression of the T current by IFL in ventrobasal thalamic neurons (Ries and Puil 1993), also shown for the depression of Ca\(^{2+}\) currents in other neurons (neocortical, Puil et al. 1994a; hippocampal, Study 1994). This raises some uncertainty about the amount of reduction in the T current due to an IFL-induced decrease in Ca\(^{2+}\) channel activity, as opposed to the increase in leak conductance. Under the present conditions, the concomitant effects of IFL on resonance, action potential firing, and LTSs imply that an increased conductance, probably for K\(^+\) (cf. halothane, Nicoll and Madison 1982; Sugiyama et al. 1992; IFL, Berg-Johnsen and Langmoen 1990; Ries and Puil 1993), produced a large part of the reduction in resonance.

The decreased tendency for the membrane potential to oscillate and the flat band-pass filter function in MGBv neurons under IFL anesthesia have consequences for the centripetal transfer of auditory information. Normally, the sub-
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threshold frequency preference would couple synaptic inputs with preferred dynamic profiles and/or repetition rates to firing, filtering the output to the cortex. The in vitro slice conditions used here exclude depolarizing cortical and brain stem inputs and allow neuronal expression of a slow delta-like (1–4 Hz) rhythm (cf. McCormick and Pape 1990). Under these conditions, resonance at 1–2 Hz tunes thalamocortical neurons to activity at such frequencies from other structures and to bursts within the same frequency range. IFL anesthesia would disrupt such synchronizing mechanisms in MGBv neurons, similar to the disruption of oscillations in other thalamocortical neurons on administration of a barbiturate in vivo (cf. Fig. 11 in Curró Dossi et al. 1992). This would corrupt or prevent the transfer of auditory information. In addition, the increased conductance, hyperpolarization, and decreased LTS bursts observed in thalamic neurons (cf. Results and Pui et al. 1993) may contribute to the electroencephalographic burst suppression patterns during deep IFL anesthesia (Ogawa et al. 1992; Steriade et al. 1994).

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