

Isoflurane inhibits calcium currents in neocortical neurons

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Abstract

We used voltage-clamp techniques to assess the effects of isoflurane anaesthesia in slices of sensorimotor cortex of guinea pigs and neonatal rats. Isoflurane (0.5–4%) depressed inward Ca^{2+} -currents evoked by depolarizing commands from -50 mV. With additional blockade of K^{+} -currents by internal Cs^{+} , an early component of the sustained inward currents was a high voltage-activated current whereas the delayed component was an unclamped Ca^{2+} -current; this was consistent with a simple compartmental model. Isoflurane decreased the magnitude of the high voltage-activated current.

Key words: Isoflurane; Anaesthesia; Cerebral cortex; Arousal; High threshold calcium currents

In bringing about the unconscious state, anaesthetics depress neuronal excitability, as evident from the reduced spontaneous and synaptically-evoked firing [9]. Increasingly, there is evidence that the clinically effective inhalation anaesthetics, halothane and isoflurane, depress low and high voltage-activated (LVA and HVA) Ca^{2+} -currents in hippocampal [8], thalamic [14], dorsal root ganglion neurons [18] and pituitary GH_3 cells [5]. These currents are fundamental to neurotransmitter release and postsynaptic excitability. While neocortical neurons possess multiple subtypes of Ca^{2+} -currents [1,15], there are no descriptions in the literature of anaesthetic interactions with them. In this report, we examined the effects of isoflurane application on neocortical neurons in slices. Although the complex cytoarchitecture of cortical neurons results in unclamped Ca^{2+} -currents (see results), these currents are likely present *in vivo* and may represent critical targets for anaesthetic actions. We found that therapeutic concentrations of isoflurane markedly depressed HVA Ca^{2+} -currents, providing a

plausible molecular mechanism for synaptic depression in neocortex during anaesthesia [2].

We used whole cell patch-clamp techniques in slices of cerebral cortex from rats (7–14 days old). In some cases, we also used sharp microelectrodes to voltage-clamp cortical neurons of adult guinea pigs. There are previous descriptions of these techniques, including the coronal slices ($\approx 400 \mu\text{m}$ thick) of sensorimotor cortex [2,7,8]. The artificial cerebrospinal fluid (aCSF) contained (in mM): NaCl, 124; KCl, 4; KH_2PO_4 , 1.25; CaCl_2 , 1; MgCl_2 , 2; NaHCO_3 , 26; dextrose, 10; tetraethylammonium, 10; 4-aminopyridine, 5; Cs^{+} , 3; and 300–500 nM tetrodotoxin (TTX). The aCSF, aerated with 95% O_2 and 5% CO_2 had a flow rate of 2.5 ml/min into a submersion type of chamber (volume = 1.6 ml).

Recordings were made (Axoclamp 2A amplifier) at 22–25°C using electrodes with resistances in either the 4–6 $\text{M}\Omega$ (patch) or 40–80 $\text{M}\Omega$ (sharp) range. The sharp electrodes contained either 3 M KCl or 3 M CsCl (to block K^{+} currents). The patch pipettes contained (in mM): NaCl, 15; Cs or K-gluconate or -methylsulfonate, 140; HEPES, 10; CaCl_2 , 1; Mg-ATP, 2; GTP, 0.3; EGTA, 11. The final $[\text{Cs}^{+}]$ or $[\text{K}^{+}]$ was 140 mM. The pipette solution had a pH of 7.2 and a $[\text{Ca}^{2+}] = 10$ nM.

Computer simulations of clamped and unclamped

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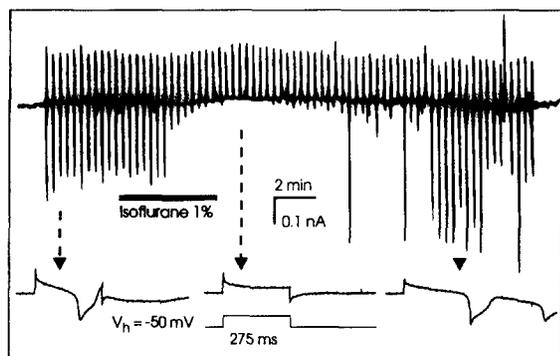


Fig. 1. Isoflurane at low concentrations blocks unclamped Ca^{2+} -spikes. The upper record is a chart recording of currents evoked by test pulses to -27 mV from a holding potential of -50 mV in the presence of K^+ - and Na^+ -current blockers. Lower records are individual traces (arrowheads) on an expanded scale. Note multiple firing on recovery.

Ca^{2+} -currents were performed using the program PHASEPLANE and the Hodgkin-Huxley parameters for HVA Ca^{2+} -currents were taken from Brown et al. [1].

The results were obtained from observations on a total of 75 layer II/III neurons in slices of frontoparietal cortex. With patch electrodes and application of K^+ - and Na^+ -channel blockers for 0.5–2 h, the resting potentials averaged -50 ± 9 mV and the input resistances, 425 ± 107 M Ω ($n = 45$). With sharp electrodes, the resting potentials averaged -69 ± 7 mV (S.D.; $n = 29$) and the input resistances calculated from the voltage responses to hyperpolarizing current pulse injections averaged 62 ± 11 M Ω . The neurons produced fast rising, overshooting action potentials of 60 to 90 mV amplitude when tested with current pulse injections. In the presence of TTX and external K^+ -blockers, the slow Ca^{2+} -spikes viewed under current-clamp exhibited properties similar to those reported previously [3].

Under these pharmacological conditions, depolarizing voltage commands (275–400 ms duration) of 10–40 mV from holding potentials near -50 mV produced partially clamped, slow inward currents that had a spike-like component (Fig. 1). We studied the effects of Ca^{2+} -channel blockade in several neurons. Application of La^{3+} (10 or 20 μM ; $n = 2$) or replacement of Ca^{2+} by Mg^{2+} in the aCSF ($n = 3$) completely blocked these currents. In addition, they were reduced by Ni^{2+} (1 mM; $n = 3$) or nimodipine (500 nM; $n = 2$). In the absence of the blockade, the inward currents exhibited a fade, typical of HVA Ca^{2+} -currents recorded with patch electrodes [5]. For the above reasons, we consider, likely, a primary involvement of Ca^{2+} in the currents.

Isoflurane was administered by perfusion using aCSF equilibrated with the anaesthetic vapour at specified concentrations [2]. Isoflurane markedly depressed the spike-like Ca^{2+} -currents within 3 min of commencing the application. Low concentrations (0.5 or 1%) of isoflurane

depressed the peak amplitudes by 20% or more after 3–6 min. The extent of blockade was variable and in some sensitive neurons, for example, 1% isoflurane completely blocked the action currents (Fig. 1). Concentrations greater than 2% greatly reduced or annihilated the inward currents after 3–4 min and revealed a net outward current. The effects of isoflurane were slowly reversible, usually requiring >4 min for partial recovery. In several neurons, recovery was additionally characterized by a period of heightened excitability that lasted for 3–5 min, before stabilizing to a constant amplitude (Fig. 1).

When internal Cs^+ was used to additionally block outward currents, we observed sustained inward currents, consisting of 2 components that declined very slowly (Fig. 2A). The early component of the inward current activated in a smoothly graded manner with depolarization whereas the late component had a much steeper voltage dependence (Fig. 2A). This is consistent with an early current under voltage control and a later, unclamped component flowing through channels that are electrotonically distant from the electrode, and therefore under only partial voltage control. This situation can be simulated by a simple compartmental model with theoretical Ca^{2+} -conductances in the soma and one dendritic compartment (Fig. 2B).

Isoflurane (0.5–4%) rapidly and reversibly reduced both components of the inward current. Substantial blockade of the early component occurred with 1–2% isoflurane (Fig. 2A). Since the early component represented a clamped HVA current, we quantified the effects of 5 isoflurane concentrations on the graded currents at a point 20 ms after the onset of the voltage command. The results of these experiments are shown in Figure 2C. Application of 2–4% isoflurane significantly inhibited the inward currents relative to the spontaneous rundown in a control group of neurons at all time points tested.

The main effect of isoflurane observed in this study was a decrease in the magnitude of depolarization-induced inward currents mediated by Ca^{2+} . We recently demonstrated that isoflurane prevented Ca^{2+} -dependent spike bursting or the voltage-gated transitions to repetitive spike firing in thalamocortical neurons [14]. An increased leak current accounted for the reduced firing capability and partly accounted for the observed blockade of the T-type Ca^{2+} -current that generates the slow bursts [14]. In the neocortical neurons here, changes in leak conductance due to isoflurane were assessed from responses to small hyperpolarizing commands from -50 mV; these were not statistically significant, except for an increase at 4% isoflurane ($P = 0.089$; one-tailed test). The effects of isoflurane at 1%, corresponding to <1 MAC (minimum alveolar concentration) [2], on Ca^{2+} -currents were not so clear-cut whereas the effects of higher concentrations were unambiguous (Fig. 2C).

Many of our recordings, obtained with blockade of rectifying currents, are consistent with at least two clus-

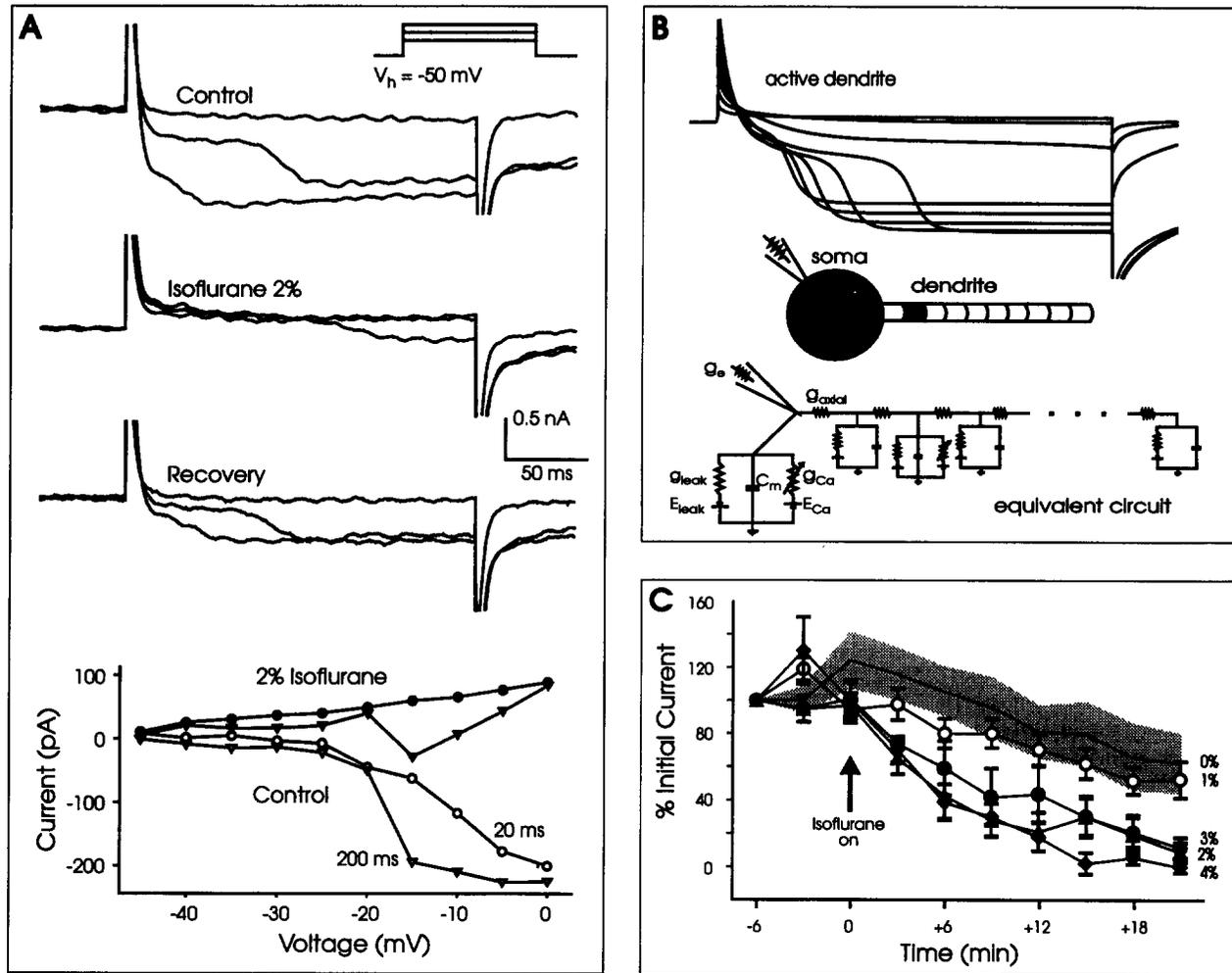


Fig. 2. Isoflurane reduces inward currents evoked in the presence of 140 mM internal Cs^+ . A: upper records show 2 sustained components of the inward current evoked by voltage steps to -40 , -35 , and -30 mV, unmasked by internal Cs^+ and their attenuation by isoflurane. Graph at bottom shows current/voltage relations for the same neuron. The early component (measured 20 ms after the onset of the test pulse; circles), and not the late component (measured at 200 ms; triangles), is graded. For the graph, a linear leak current, measured in control conditions, was subtracted from all data. Isoflurane (solid symbols) decreased the early inward current and produced an outward current. B: a compartmental model shows that the 2 components of the inward current can be interpreted as contributions from spatially separated regions containing similar non-inactivating high-threshold conductances. The current responses for 8 test potentials between -30 mV and -10 mV from a holding potential of -40 mV show qualitative agreement with traces in (A). C: effects of isoflurane on early (20 ms) components of leak-subtracted inward currents evoked by $+20$ mV pulses from holding potentials near -50 mV. 2–4% isoflurane reliably blocked inward currents compared to spontaneous rundown (vertical bars and grey area indicate ± 1 standard error; $n = 5$). The effect of 1% isoflurane was not statistically significant.

ters of Ca^{2+} -current densities, as simulated by a model with active Ca^{2+} -conductances in somatic and dendritic compartments (Fig. 2). Recent investigations in neocortical neurons have shown that Ca^{2+} -electrogenesis occurs in the dendrites and a region electrotonically closer to the recording electrode [13]. When visualized with monoclonal antibodies, L-type Ca^{2+} -channels predominate in the soma and proximal portion of the dendrites [19] and N-type Ca^{2+} -channels have a patch-like distribution in the dendrites [20]. Our studies do not identify the distinct types of Ca^{2+} -current affected by isoflurane. Why Ca^{2+} -currents should be susceptible is not clear although there is some evidence that phosphorylation sites of Ca^{2+} -

channel proteins [4] or second messenger activity [16] are vulnerable to anaesthetic actions.

Synaptic transmission in the neocortex, as elsewhere in the CNS [10], may involve the activation of HVA Ca^{2+} -currents. During anaesthesia, the reductions in excitatory postsynaptic potentials (EPSPs) and spontaneous firing of action potentials [9] can result from potentiated GABA-activated Cl^- [6] or induced extrasynaptic, hyperpolarizing K^+ -currents [14,17]. In neocortex, these mechanisms do not operate at all clinical concentrations of isoflurane [2]. A depression of HVA Ca^{2+} -currents would attenuate presynaptic release [5,9] and postsynaptic responsiveness to excitatory transmitter [11,12], pro-

viding an alternative mechanism for the reduced EPSPs [2,17].

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- [1] Brown, A.M., Schwindt, P.C. and Crill, W.E., Voltage dependence and activation kinetics of pharmacologically defined components of the high-threshold calcium current in rat neocortical neurons, *J. Neurophysiol.*, 70 (1993) 1530–1543.
- [2] El-Beheiry, H. and Puil, E., Anaesthetic depression of excitatory synaptic transmission in neocortex, *Exp. Brain Res.*, 77 (1989) 87–93.
- [3] Franz, P., Galvan, M. and Constanti, A., Calcium-dependent action potentials and associated inward currents in guinea-pig neocortical neurons in-vitro, *Brain Res.*, 366 (1986) 262–271.
- [4] Hell, J.W., Yokoyama, C.T., Wong, S.T., Warner, C., Snutch, T.P. and Catterall, W.A., Differential phosphorylation of two size forms of the neuronal class C L-type calcium channel $\alpha 1$ subunit, *J. Biol. Chem.*, 268 (1993) 19451–19457.
- [5] Herrington, J., Stern, R.C., Evers, A.S. and Lingle C.J., Halothane inhibits two components of calcium current in clonal (GH3) pituitary cells, *J. Neurosci.*, 11 (1991) 2226–2240.
- [6] Jones, M.W., Brooks, P.E. and Harrison, N.E., Enhancement of γ -aminobutyric acid-activated Cl^- currents in cultured rat hippocampal neurones by three volatile anaesthetics, *J. Physiol.*, 449 (1992) 279–293.
- [7] Kamondi, A., Williams, J.A., Hutcheon, B. and Reiner, P.B., Membrane potential of mesopontine cholinergic neurons studied with the whole-cell patch-clamp technique: implications for behavioral state control, *J. Neurophysiol.*, 68 (1992) 1359–1372.
- [8] Krnjevic, K. and Puil, E., Halothane suppresses slow inward currents in hippocampal slices, *Can. J. Physiol. Pharmacol.*, 66 (1988) 1570–1575.
- [9] Krnjevic, K. and Puil, E., Cellular mechanisms of general anesthesia. In E.E. Bittar and N. Bittar (Eds.), *Principles of Medical Biology*, JAI Press, Greenwich, CT, 1994, in press.
- [10] Miller, R.J., Multiple calcium channels and neuronal function, *Science*, 235 (1987) 46–52.
- [11] Puil, E. and El-Beheiry, H., Anaesthetic suppression of transmitter actions in neocortex, *Br. J. Pharmacol.*, 101 (1990) 61–66.
- [12] Puil, E., El-Beheiry, H. and Baimbridge, K.G., Anesthetic effects on glutamate-stimulated increase in intraneuronal calcium, *J. Pharmacol. Exp. Ther.*, 255 (1990) 955–961.
- [13] Reuveni, I., Friedman, A., Amitai, Y. and Gutnick, M.J., Stepwise repolarization from Ca^{2+} plateaus in neocortical pyramidal cells: Evidence for nonhomogeneous distribution of HVA Ca^{2+} channels in dendrites, *J. Neurosci.*, 13 (1993) 4609–4621.
- [14] Ries, C.R. and Puil, E., Isoflurane prevents transitions to tonic and burst firing modes in thalamic neurons, *Neurosci. Lett.*, 159 (1993) 91–94.
- [15] Sayer, R.J., Schwindt, P.C. and Crill, W.E., High- and low-threshold calcium currents in neurons acutely isolated from rat sensorimotor cortex, *Neurosci. Lett.*, 120 (1990) 175–178.
- [16] Slater, S.J., Cox, K.J.A., Lombardi, J.V., Ho, C., Kelley, M.B., Rubin, E. and Stubbs, C.C., Inhibition of protein kinase C by alcohols and anaesthetics, *Nature*, 364 (1993) 82–84.
- [17] Sugiyama, K., Muteki, T. and Shimoji, K., Halothane-induced hyperpolarization and depression of postsynaptic potentials of guinea pig thalamic neurons in vitro, *Brain Res.*, 576 (1992) 97–103.
- [18] Takenoshita, M. and Steinbach, J.H., Halothane blocks low-voltage-activated calcium current in rat sensory neurons, *J. Neurosci.*, 11 (1991) 1404–1412.
- [19] Westenbroek, R.E., Ahljianian, M.K. and Catterall, W.A., Clustering of L-type Ca^{2+} channels at the base of major dendrites in hippocampal pyramidal neurons, *Nature*, 347 (1990) 281–284.
- [20] Westenbroek, R.E., Hell, J.W., Warner, C., Dubel, S.J., Snutch, T.P. and Catterall, W.A. Biochemical properties and subcellular distribution of an N-type calcium channel $\alpha 1$ subunit, *Neuron*, 9 (1992) 1099–1115.