



# Sodium channels as targets for volatile anesthetics

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The molecular mechanisms of modern inhaled anesthetics are still poorly understood although they are widely used in clinical settings. Considerable evidence supports effects on membrane proteins including ligand- and voltage-gated ion channels of excitable cells. Na<sup>+</sup> channels are crucial to action potential initiation and propagation, and represent potential targets for volatile anesthetic effects on central nervous system depression. Inhibition of presynaptic Na<sup>+</sup> channels leads to reduced neurotransmitter release at the synapse and could therefore contribute to the mechanisms by which volatile anesthetics produce their characteristic end points: amnesia, unconsciousness, and immobility. Early studies on crayfish and squid giant axon showed inhibition of Na<sup>+</sup> currents by volatile anesthetics at high concentrations. Subsequent studies using native neuronal preparations and heterologous expression systems with various mammalian Na<sup>+</sup> channel isoforms implicated inhibition of presynaptic Na<sup>+</sup> channels in anesthetic actions at clinical concentrations. Volatile anesthetics reduce peak Na<sup>+</sup> current ( $I_{Na}$ ) and shift the voltage of half-maximal steady-state inactivation ( $h_{\infty}$ ) toward more negative potentials, thus stabilizing the fast-inactivated state. Furthermore recovery from fast-inactivation is slowed, together with enhanced use-dependent block during pulse train protocols. These effects can depress presynaptic excitability, depolarization and Ca<sup>2+</sup> entry, and ultimately reduce transmitter release. This reduction in transmitter release is more potent for glutamatergic compared to GABAergic terminals. Involvement of Na<sup>+</sup> channel inhibition in mediating the immobility caused by volatile anesthetics has been demonstrated in animal studies, in which intrathecal infusion of the Na<sup>+</sup> channel blocker tetrodotoxin increases volatile anesthetic potency, whereas infusion of the Na<sup>+</sup> channels agonist veratridine reduces anesthetic potency. These studies indicate that inhibition of presynaptic Na<sup>+</sup> channels by volatile anesthetics is involved in mediating some of their effects.

**Keywords: sodium channels, volatile anesthetics, presynaptic, anesthetic mechanism**

## BACKGROUND

It has been over 160 years since the use of diethyl ether as a general anesthetic was publicly demonstrated, yet our mechanistic understanding of these vitally important drugs lags far behind that of most other major drug classes. Most modern inhaled anesthetics are derivatives of ether, and over the years have been developed to have improved pharmacokinetics, but they are still plagued by a lack of specificity with significant cardiovascular and respiratory side effects. It remains unclear how these drugs produce general anesthesia, a pharmacologically induced coma characterized by amnesia, unconsciousness, and immobility in response to painful stimuli (Hemmings et al., 2005b). Studies into their molecular mechanisms in the 1960s, which have their origins in the Meyer–Overton correlation of anesthetic potency with lipophilicity from 1900, led to a lipid-based theory involving a unitary mechanism of non-specific actions on the lipid bilayer (Meyer, 1899; Overton, 1901).

With technical advances in biochemistry and biophysics, specific targets were studied and identified. Pioneering studies showed that anesthetic interactions with proteins themselves, not necessarily involving lipid interactions, could explain anesthetic effects at a

biochemical level (Franks and Lieb, 1994). Animal studies showed that volatile anesthetics produce their immobilizing effects primarily by actions on the spinal cord (Antognini and Schwartz, 1993; Rampil et al., 1993), whereas unconsciousness and amnesia involve actions at supra-spinal centers (Eger et al., 2008). Membrane proteins including ion channels have been implicated as key mediators of the depressive effects of anesthetics on neuronal function. Many potential targets have been identified, and it has become clear that anesthetics act at multiple distinct targets in the central nervous system to produce the various component effects of the anesthetic state (multi-site hypothesis).

## MECHANISMS OF GENERAL ANESTHETIC EFFECTS ON THE CENTRAL NERVOUS SYSTEM

The idea of general anesthetics acting both on excitatory and inhibitory synaptic transmission has led to many studies pointing out the complexity of anesthetic mechanisms (Rudolph and Antkowiak, 2004; Hemmings et al., 2005b; Franks, 2006). General anesthetics, including both volatile and intravenous anesthetics, enhance synaptic inhibition via postsynaptic  $\gamma$ -aminobutyric acid type A (GABA<sub>A</sub>) receptor modulation (Nicoll et al., 1975;

Zimmerman et al., 1994). More recent studies also point out the importance of extrasynaptic GABA<sub>A</sub> receptors as a target of anesthetics by potentiating tonic inhibitory currents (Orser, 2006; Rau et al., 2009) and by enhancing the release of GABA by a presynaptic increase in miniature inhibitory postsynaptic current (mIPSC) frequency (Nishikawa and MacIver, 2001). Depression of excitatory transmission by presynaptic effects is another target of anesthetic action (Perouansky et al., 1995; MacIver et al., 1996; Ouanonou et al., 1999; Wakasugi et al., 1999). Both volatile and intravenous anesthetics reduce excitatory postsynaptic potentials (EPSPs) in neurons, an effect most likely due to presynaptic mechanisms (Weakly, 1969; Richards and White, 1975; Kullmann et al., 1989; Berg-Johnsen and Langmoen, 1992). Recent evidence suggests that inhibition of glutamatergic synaptic transmission through *N*-methyl-D-aspartate (NMDA)-type glutamate receptor blockade by inhaled anesthetics might also contribute to depression of excitatory transmission (Dickinson et al., 2007; Haseneder et al., 2008).

It is now evident that ligand-gated ion channels are major targets for general anesthetics (Franks and Lieb, 1994). Both inhibition of excitatory NMDA receptors and potentiation of inhibitory GABA<sub>A</sub> and glycine receptors have come under scrutiny as important targets for both intravenous and inhaled anesthetic effects on synaptic transmission (Franks, 2006). These receptors are found throughout the central nervous system and are major transducers of excitatory and inhibitory neurotransmitter signaling.

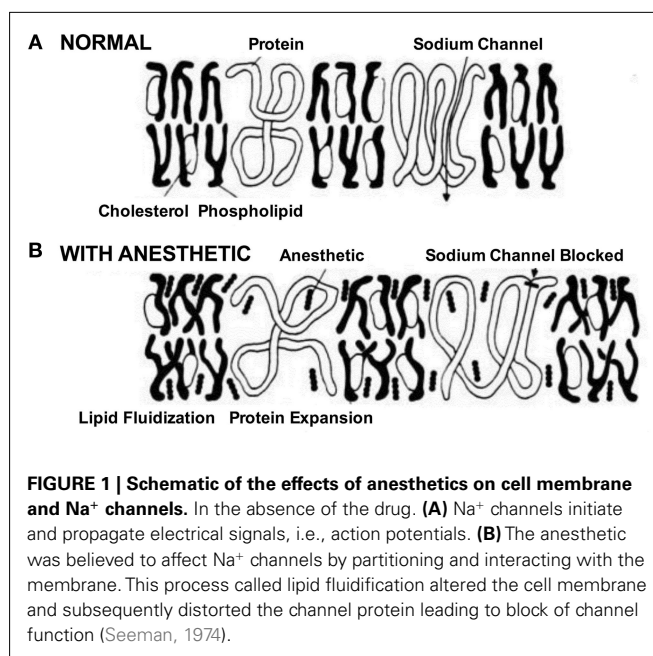
Second-messenger regulated protein phosphorylation of Na<sup>+</sup> channels has been implicated as another possible target of volatile anesthetics. Halothane increases both purified (Hemmings and Adamo, 1994) and endogenous (Hemmings and Adamo, 1996) brain protein kinase C (PKC) activity. Phosphorylation of Na<sup>+</sup> channels by PKC and PKA reduces Na<sup>+</sup> channel activity by altering channel kinetics, for example by slowing inactivation, and is therefore an important component of neuromodulation (Cantrell and Catterall, 2001). It is possible that some of the inhibitory effects of volatile anesthetics on Na<sup>+</sup> channel activity are mediated through PKC phosphorylation.

More recent studies have extended the range of likely anesthetic targets to include neuronal nicotinic acetylcholine receptors (Flood et al., 1997), two pore domain K<sub>2P</sub> channels and K<sup>+</sup> leak channels (Patel and Honore, 2001; Sirois et al., 2002), and presynaptic voltage-gated Na<sup>+</sup> channels. This review considers Na<sup>+</sup> channels as targets for the effects of volatile anesthetics (inhaled alkane and ether derivatives).

### PRESYNAPTIC Na<sup>+</sup> CHANNELS AS ANESTHETIC TARGETS

Na<sup>+</sup> channels play a crucial role in cell-to-cell communication, as they are involved in initiating and propagating action potentials in excitable cells throughout the nervous system (Hodgkin and Huxley, 1952). Early reports in the 1970s associated the effects of volatile anesthetics on lipid bilayer properties to alterations of certain membrane bound ion channels, in particular voltage-gated Na<sup>+</sup> channels (Figure 1).

These reports were among the first to hypothesize a specific ion channel (Na<sup>+</sup> channels) as a potential target of volatile anesthetics, though at that time no specific binding site or specific mechanism could be identified. Early studies on the effects of general



**FIGURE 1 | Schematic of the effects of anesthetics on cell membrane and Na<sup>+</sup> channels.** In the absence of the drug. **(A)** Na<sup>+</sup> channels initiate and propagate electrical signals, i.e., action potentials. **(B)** The anesthetic was believed to affect Na<sup>+</sup> channels by partitioning and interacting with the membrane. This process called lipid fluidification altered the cell membrane and subsequently distorted the channel protein leading to block of channel function (Seeman, 1974).

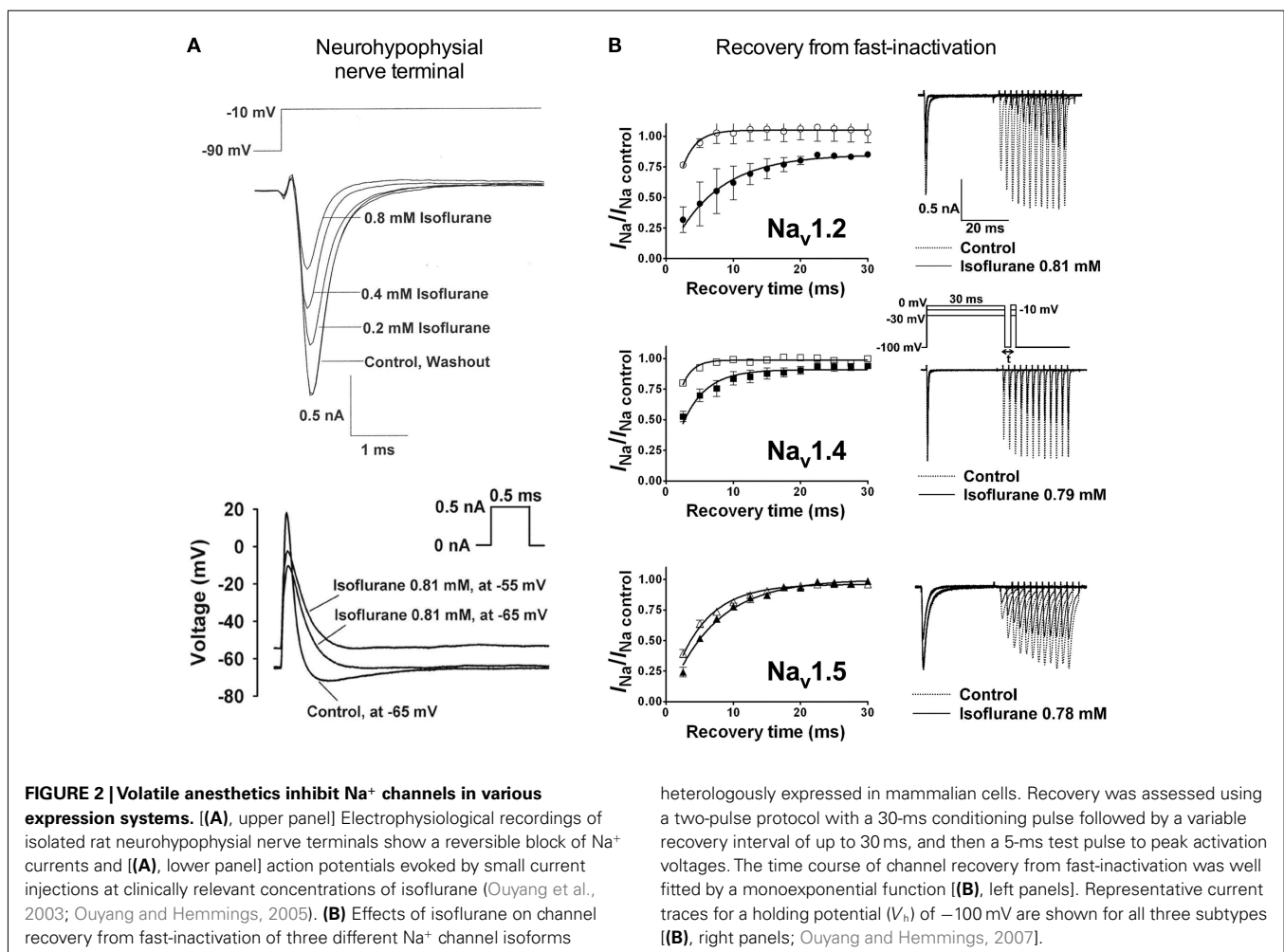
anesthetics on Na<sup>+</sup> and K<sup>+</sup> currents in the crayfish or squid giant axon showed inhibition of peak Na<sup>+</sup> (*I*<sub>Na</sub>) current and effects on channel recovery, but in these preparations inhibition occurred at relatively high concentrations (Bean et al., 1981; Haydon and Simon, 1988). Subsequent studies examined the effects of various volatile anesthetics on mammalian brain derived Na<sup>+</sup> channels heterologously expressed in mammalian cell lines (Rehberg et al., 1996). Inhibition of peak *I*<sub>Na</sub> due to stabilization of the inactivated state of Na<sup>+</sup> channels was evident as a hyperpolarizing “left-shift” in steady-state (or *h*<sub>∞</sub>) inactivation. These experiments were among the first to demonstrate inhibition of neuronal Na<sup>+</sup> channels by volatile anesthetics. The sensitivity of Na<sup>+</sup> channels to clinically relevant concentrations of volatile anesthetics was confirmed in various *in vitro* expression systems and was subsequently extended to more physiologically relevant neuronal preparations.

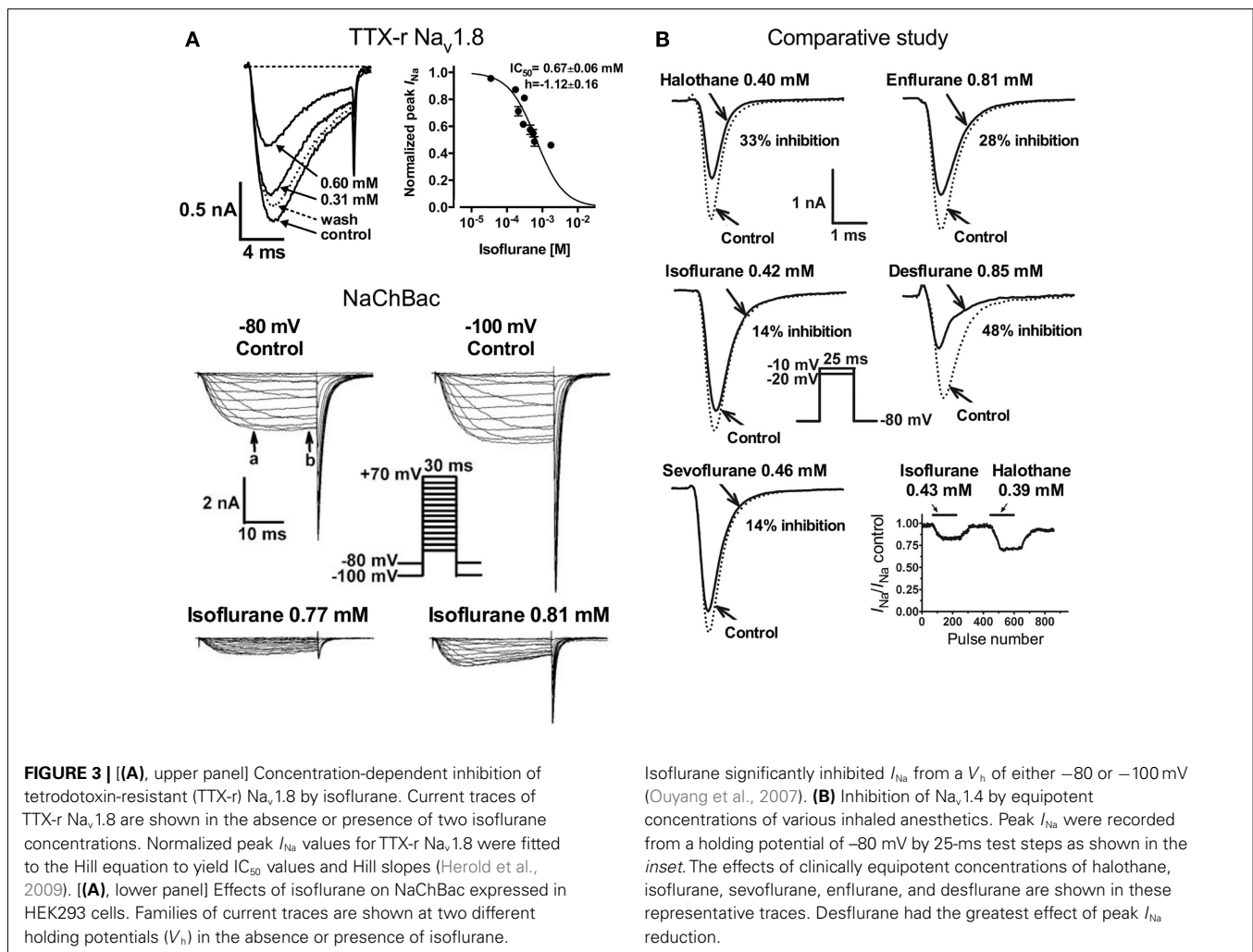
Electrophysiological recordings performed in isolated rat neurohypophysial nerve terminals, an experimentally accessible nerve terminal preparation, showed that clinically relevant concentrations of isoflurane inhibited peak *I*<sub>Na</sub> in nerve terminals in a concentration- and voltage-dependent manner (Ouyang et al., 2003; Figure 2A, upper panel). Similar to heterologous expression systems, a left-shift in the voltage-dependence of steady-state inactivation demonstrated stabilization of the fast-inactivated state. These results support the hypothesis that volatile anesthetics depress excitatory synaptic transmission by inhibiting presynaptic voltage-gated Na<sup>+</sup> channels. In addition, in the rat neurohypophysial nerve terminal preparation, isoflurane inhibited action potential amplitude and increased action potential half-width (Ouyang and Hemmings, 2005; Figure 2A, lower panel). The underlying current mediating the fast and rising depolarizing phase of the action potential is carried by tetrodotoxin (TTX)-sensitive Na<sup>+</sup> channels, which were inhibited by isoflurane using a voltage-stimulus based on an averaged action potential. The effects of non-immobilizers (structurally similar compounds without

anesthetic properties) in rat dorsal root ganglion neurons showed that compound F3, an anesthetic fluorinated cyclobutane, inhibited  $\text{Na}^+$  channels similar to the effects of conventional volatile anesthetics, but the non-anesthetic (non-immobilizer) fluorinated cyclobutane F6 had only minimal effects (Ratnakumari et al., 2000; **Figure 4A**). These findings support the role of  $\text{Na}^+$  channels as molecular targets for volatile anesthetic action.

Studies investigating subtype-specific effects of volatile anesthetics revealed small, but potentially significant, differences in isoflurane potency with  $\text{IC}_{50}$  values ranging from 0.45 to 0.7 mM (at  $V_h$   $-70$  mV) on  $\text{Na}_v1.2$ ,  $\text{Na}_v1.4$ ,  $\text{Na}_v1.5$  expressed in Chinese hamster ovary cells (Ouyang and Hemmings, 2007). Despite the small potency differences, there were differences between isoforms in recovery from fast-inactivation tested by a double-pulse protocol. The effect of isoflurane on channel recovery was greatest in  $\text{Na}_v1.2$ , a major brain isoform (**Figure 2B**). Another study in which subtypes  $\text{Na}_v1.2$ ,  $\text{Na}_v1.4$ ,  $\text{Na}_v1.6$ , and TTX-resistant  $\text{Na}_v1.8$  were expressed (with and without  $\beta 1$  subunit co-expression) in *Xenopus* oocytes also revealed that  $\text{Na}_v1.2$ ,  $\text{Na}_v1.4$ ,  $\text{Na}_v1.6$  were sensitive to isoflurane, whereas the TTX-resistant subtype  $\text{Na}_v1.8$ , which is highly expressed in dorsal root ganglion nociceptive neurons, was insensitive (Shiraishi and Harris, 2004). Nerve terminals

of nociceptive sensory neurons are the (main) origin of neuro-pathic and inflammatory pain signals (Dib-Hajj et al., 2010), but the pro- or anti-nociceptive effects of volatile anesthetics are not clearly defined. It is evident that these nociceptive neurons carry a distinct selection of  $\text{Na}^+$  channel subtypes related to pain signaling (e.g.,  $\text{Na}_v1.7$ ,  $\text{Na}_v1.8$ ,  $\text{Na}_v1.9$ ; see review Dib-Hajj et al., 2010). Subsequently,  $\text{Na}_v1.8$  expressed in mammalian neuronal cells revealed concentration- and voltage-dependent inhibition of  $\text{Na}_v1.8$  by clinically relevant concentrations of isoflurane similar to other subtypes (Herold et al., 2009; **Figure 3A**, upper panel). This demonstrates the importance of choosing a suitable expression system for pharmacological studies of ion channels. In this case the neuronal cell line ND7/23, a hybrid cell line between rat dorsal root ganglion neurons and mouse neuroblastoma cells, may have provided auxiliary  $\beta$ -subunits or other neuron-specific signaling pathways that are important for inhibition by anesthetics. A comparative study showing the effects of several different volatile anesthetics on heterologously expressed  $\text{Na}^+$  channels in mammalian cells revealed that desflurane, a highly fluorinated inhaled anesthetic, had the strongest effect on peak  $I_{\text{Na}}$  inhibition, but all agents in this class were effective at clinically relevant concentrations (Ouyang et al., 2009; **Figure 3B**). In contrast, the





intravenous anesthetic propofol inhibits  $\text{Na}^+$  channels only at supratherapeutic concentrations (Rehberg and Duch, 1999).

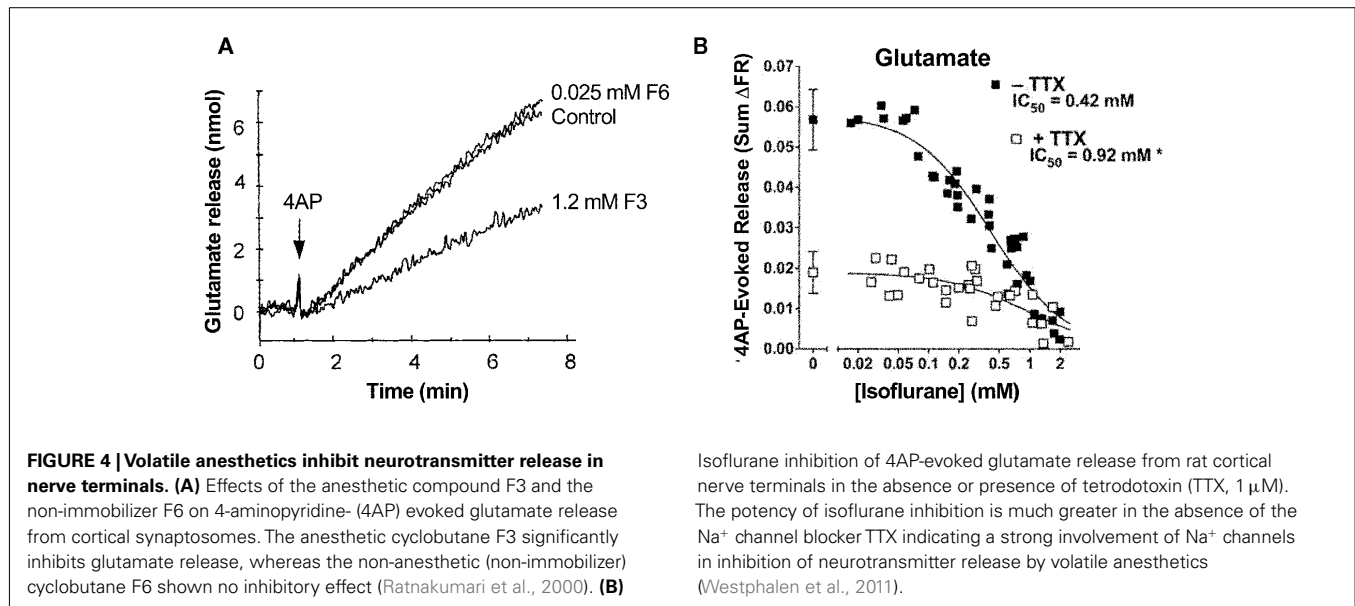
The prototypical halogenated ether isoflurane also inhibits the prokaryotic voltage-gated  $\text{Na}^+$  channel of *Bacillus halodurans* (NaChBac; Ouyang et al., 2007; Figure 3A, lower panel). This was the first prokaryotic channel shown to be inhibited by an anesthetic, and demonstrates impressive evolutionary conservation of the mechanism responsible for this pharmacological effect. As with mammalian channels, inhibition of peak  $I_{\text{Na}}$  was concentration- and voltage-dependent, and was associated with a positive shift in the voltage-dependence of activation and a negative shift in the voltage-dependence of steady-state fast-inactivation. Furthermore use-dependent block occurred due to slowed recovery from inactivation. Despite the evolutionary difference between prokaryotic and eukaryotic voltage-gated  $\text{Na}^+$  channels, the mechanisms by which volatile anesthetics act on the channel seem remarkably similar.

Aromatic compounds such as fluorobenzene, hexafluorobenzene, and 1,2-difluorobenzene have been shown to inhibit  $\text{Na}_v1.2a$  expressed in *Xenopus* oocytes. Inhibition of peak  $I_{\text{Na}}$  as well as a shift in the  $V_{1/2}$  of fast-inactivation occurs in an agent-dependent manner (Horishita et al., 2008). The exact mechanism

of the differential effects of these structurally different compounds has yet to be elucidated. Differences also exist in the potency of volatile anesthetic inhibition of specific  $\text{Na}^+$  channel subtypes (Ouyang et al., 2009), but again the mechanisms for these differences have to be studied in more detail. Such differences might underlie region-specific presynaptic effects of volatile anesthetics on neurotransmitter release (Westphalen et al., 2010, 2011).

### **$\text{Na}^+$ CHANNEL INHIBITION LEADS TO INHIBITION OF NEUROTRANSMITTER RELEASE BY ANESTHETICS**

A physiological consequence of presynaptic  $\text{Na}^+$  channel inhibition is depression of presynaptic action potential generation and conduction. Considerable evidence indicates that volatile anesthetics inhibit neurotransmitter release, and that this is due in part to inhibition of presynaptic  $\text{Na}^+$  channels. Volatile anesthetics preferentially inhibit 4-aminopyridine (4AP)-evoked release of glutamate compared to GABA from isolated rat cortical nerve terminals (Westphalen and Hemmings, 2006). Action potential-evoked depolarization and release can be pharmacologically mimicked by 4AP, a  $\text{K}^+$  channel blocker, while  $\text{Na}^+$  channel independent release can be elicited by depolarization with elevated extracellular  $\text{K}^+$  (Tibbs et al., 1989). Using this approach, 4AP-evoked



release is significantly more sensitive to inhibition by volatile anesthetics as compared to KCl-evoked release, supporting a role for blockade of presynaptic Na<sup>+</sup> channels in the inhibitory effects of the anesthetics (Schlame and Hemmings, 1995; Westphalen and Hemmings, 2003). Interestingly, inhibition of glutamate release occurs with about 50% greater potency than inhibition of GABA release, consistent with pharmacologically relevant transmitter-specific specializations in neurotransmitter release regulation, perhaps involving differential coupling to Na<sup>+</sup> channels (Westphalen et al., 2010; **Figure 4B**). There is also evidence that volatile anesthetics inhibit neurotransmitter release in a brain region-specific manner (Westphalen et al., 2011), which suggests diversity in presynaptic Na<sup>+</sup> channel subtype expression and/or coupling to release (Westphalen et al., 2010).

Further experiments have examined the effects of volatile anesthetics on synaptic vesicle exocytosis, detected using fluorescence imaging, in cultured rat hippocampal neurons. This preparation allows electrical stimulation of release, and showed concentration-dependent and reversible inhibition of action potential-evoked exocytosis by isoflurane. Involvement of presynaptic Na<sup>+</sup> channels is supported by the observation that exocytosis, evoked by depolarization with elevated extracellular K<sup>+</sup> (which is insensitive to TTX), was relatively insensitive to isoflurane (Hemmings et al., 2005a). Isoflurane has also shown to inhibit excitatory postsynaptic currents (EPSCs) in the rat calyx of Held due to inhibition of neurotransmitter release caused by a reduction of presynaptic action potential amplitude (Wu et al., 2004). These effects of volatile anesthetics on synaptic transmission result primarily from inhibition of action potential-evoked synaptic vesicle exocytosis, most likely as a result of Na<sup>+</sup> channel blockade upstream of Ca<sup>2+</sup> entry and exocytosis.

*In vivo* studies on rodents have implicated spinal Na<sup>+</sup> channels in immobilization, a major component of general anesthesia. Intravenous infusion of lidocaine, a classical local anesthetic, or intrathecal administration of riluzole, another potent Na<sup>+</sup> channel inhibitor, significantly increases the potency of volatile anesthetics

Isoflurane inhibition of 4AP-evoked glutamate release from rat cortical nerve terminals in the absence or presence of tetrodotoxin (TTX, 1 μM). The potency of isoflurane inhibition is much greater in the absence of the Na<sup>+</sup> channel blocker TTX indicating a strong involvement of Na<sup>+</sup> channels in inhibition of neurotransmitter release by volatile anesthetics (Westphalen et al., 2011).

as immobilizers (Xing et al., 2003; Zhang et al., 2007). The role of Na<sup>+</sup> channels in volatile anesthetic-mediated immobility is further supported by the observation that intrathecal infusion of the Na<sup>+</sup> channel activator veratridine, a plant neurotoxin that binds to site 2 and stabilizes the open state (Ulbricht, 1998), reduces the potency of isoflurane (Zhang et al., 2008), while intrathecal infusion of TTX increases the potency of isoflurane, and reverses the effect of veratridine (Zhang et al., 2010). Taken together, these results indicate that inhibition of spinal voltage-gated Na<sup>+</sup> channels by inhaled anesthetics is likely an important mechanism in anesthetic immobility.

### NON-ANESTHETIC EFFECTS OF VOLATILE ANESTHETICS

A major side effect of volatile anesthetics is cardiovascular depression. Multiple ion channel types expressed in cardiomyocytes contribute to action potential conduction and myocardial contractility. Inhibition of L-type Ca<sup>2+</sup> currents or voltage-gated transient and sustained outward K<sup>+</sup> currents by volatile anesthetics can lead to reduced contractility and delayed repolarization with mismatch of action potential duration (Huneke et al., 2004). In cardiac Na<sup>+</sup> channels (Na<sub>v</sub>1.5), volatile anesthetics at clinically relevant concentrations inhibit peak I<sub>Na</sub> and affect steady-state fast- as well as slow-inactivation (Stadnicka et al., 1999; Ouyang and Hemmings, 2007). This can, in combination with other cardiodepressant drugs, slow conduction and lead to tachyarrhythmias. Na<sup>+</sup> channels have also been implicated as potential targets for neuroprotection by volatile anesthetics (Hemmings, 2004). The possible role of voltage-gated Na<sup>+</sup> channels and other beneficial and detrimental side effects of volatile anesthetics in brain and other organs cannot be excluded.

### CONCLUSION

Both electrophysiological and functional studies indicate that presynaptic voltage-gated Na<sup>+</sup> channels are inhibited by clinically used concentrations of volatile anesthetics. This leads to reductions in evoked neurotransmitter release that is both brain

region and neurotransmitter selective. The selective inhibition of glutamate release underlies a reduction in excitatory synaptic transmission with resultant nervous system depression. Detailed information regarding the presynaptic localization, function, and regulation of specific Na<sup>+</sup> channel subtypes is currently lacking. Further studies are necessary to identify the roles of specific presynaptic Na<sup>+</sup> channel subtypes in mediating neurotransmitter

release and its inhibition by volatile anesthetics and other Na<sup>+</sup> channel inhibitors.

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