Published in final edited form as:

Anesth Analg. 2008 November ; 107(5): 1579–1586. doi:10.1213/ane.0b013e318184b966.

Effects of Volatile Aromatic Anesthetics on Voltage-Gated Na⁺ Channels Expressed in *Xenopus* Oocytes

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Abstract

Background—Many inhaled anesthetics inhibit voltage-gated sodium channels at clinically relevant concentrations, and suppression of neurotransmitter release by these agents results, at least partly, from decreased presynaptic sodium channel activity. Volatile aromatic anesthetics can inhibit *N*-methyl-D-aspartate (NMDA) receptor function and enhance γ -amino butyric acid A (GABA_A) receptor function, but these effects depend strongly on the chemical properties of the aromatic compounds. The present study tested whether diverse aromatic anesthetics consistently inhibit sodium channel function.

Methods—We studied the effect of eight aromatic anesthetics on Na_v1.2 sodium channels with β_1 subunits, using whole-cell, two-electrode voltage-clamp techniques in *Xenopus* oocytes.

Results—All aromatic anesthetics inhibited I_{Na} (sodium currents) at a holding potential which produce half-maximal current ($V_{1/2}$) (partial depolarization); inhibition was modest with 1,3,5-trifluorobenzene ($8 \pm 2\%$), pentafluorobenzene ($13 \pm 2\%$), and hexafluorobenzene ($13 \pm 2\%$), but greater with benzene ($37 \pm 2\%$), fluorobenzene ($39 \pm 2\%$), 1,2-difluorobenzene ($48 \pm 2\%$), 1,4-difluorobenzene ($31 \pm 3\%$), and 1,2,4-trifluorobenzene ($33 \pm 1\%$). Such dichotomous effects were noted by others for NMDA and GABA_A receptors. Parallel, but much smaller inhibition, was found for I_{Na} at a holding potential which produced near maximal current (-90 mV) (V_{H-90}), and hexafluorobenzene caused small ($6 \pm 1\%$) potentiation of this current. These changes in sodium channel function were correlated with effectiveness for inhibiting NMDA receptors, with lipid solubility of the compounds, with molecular volume, and with cation- π interactions.

Conclusion—Aromatic compounds vary in their actions on the kinetics of sodium channel gating and this may underlie their variable inhibition. The range of inhibition produced by MAC concentrations of inhaled anesthetics indicates that sodium channel inhibition may underlie the action of some of these anesthetics but not others.

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Dr. Eger is a paid consultant to Baxter Healthcare Corp.

Implications Statement

Aromatic anesthetics can block NMDA receptors and enhance GABA_A receptors responses. The present report finds various effects of these compounds on sodium channels. Although volatile aromatic anesthetics are not used clinically, exploring their effects on ion channels may aid in understanding mechanisms of clinical inhaled anesthetic actions.

Introduction

Voltage-gated sodium channels have an important role in the action potential initiation and propagation in excitable cells of nerve and muscle. They are established targets of local anesthetics, but have been considered to be resistant to general anesthetics.¹ However, recent reports suggest that clinical concentrations of volatile anesthetics significantly suppress sodium channel function in neurons,^{2,3} and in recombinant mammalian sodium channels.^{4–6} Moreover, blocking of presynaptic sodium channels provides a mechanism for suppression of neurotransmitter release by volatile anesthetics.^{7–10}

N-methyl-D-aspartate (NMDA) and γ -aminobutyric acid A (GABA_A) receptors are targets for general anesthetics.^{11–14} Although not used as clinical anesthetics, volatile aromatic compounds have industrial applications, are drugs of abuse, and have anesthetic effects.¹⁵ They inhibit NMDA receptor function,¹⁶ and potentiate GABA_A receptor function,¹⁷ doing so in a reciprocal manner: the aromatic anesthetics which inhibit NMDA receptors best enhance GABA_A receptor function the least. The importance of aromatic structure to an effect on sodium channels is unknown and such knowledge might be important to an understanding of potential targets and mechanisms of volatile anesthetics.

Nine distinct pore-forming α subunits of sodium channels have been identified.^{18,19} Each α subunit has a different pattern of development and localization and demonstrates subtle differences in electrophysiological characteristics.²⁰ We examined the effect of several structurally distinct volatile aromatics on Na_v1.2, a sodium channel expressed primarily in the central nervous system, including the spinal cord, a major site of inhaled anesthetic action.²¹ We used the oocyte expression system to explore the mechanism of effect by volatile anesthetics on Na_v1.2.

Methods

The Animal Care and Use Committees of the University of Texas approved this study.

Adult female *Xenopus laevis* frogs were obtained from Xenopus Express, Inc. (Plant City, FL). All aromatic anesthetics were purchased from Sigma-Aldrich (St. Louis, MO). We thank the following researchers for providing the subunit clones: cDNA for rat Na_v1.2 α subunit (Dr. W. A. Catterall, University of Washington, Seattle, WA), cDNA for human β_1 subunit (Dr. A.L. George, Vanderbilt University).

Linearized cDNA templates with *Cl*aI (Na_v1.2 α subunit), *E*coRI (β_1 subunit) were transcribed using T7 (Na_v1.2 α subunit), T6 (β_1 subunit) RNAPolymerase of mMESSAGE mMACHINE kit (Ambion, Ausin, TX). Preparation of *Xenopus laevis* oocytes and microinjection of the cRNA were performed as described previously.⁵ Stage IV–VI oocytes were manually isolated from an ovary. Oocytes were treated with collagenase (0.5 mg/ml) for 10 min and Na_v1.2 α subunit cRNA was co-injected with the β_1 subunit cRNA at a ratio of 1:10 (total volume was 20 ~ 40 ng/50 nl). Injected oocytes were incubated in modified Barth's solution (88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO₃, 10 mM HEPES, 0.82 mM MgSO₄, 0.33 mM Ca(NO₃)₂, 0.91 mM CaCl₂, adjusted to pH 7.5) supplemented with 10,000 units of penicillin, 50 mg of gentamicin, 90 mg of theophylline, and 220 mg of sodium pyruvate per liter (incubation medium) at 19 °C.

Two to six days after injection, electrophysiological recordings were performed at room temperature (23 °C) using the whole-cell, two-electrode, voltage-clamp technique. Oocytes were placed in a 100 μ l recording chamber and impaled with recording electrodes prepared with borosilicate glass using a puller (P-97; Sutter Instruments Company, Novato, CA) and filled with 3M KCl / 0.5% low melting point agarose and with resistances between 0.3 and 0.5

MΩ. The oocytes were perfused at 2 ml/min with Frog Ringer solution containing NaCl 115 mM, KCl 2.5 mM, HEPES 10 mM, CaCl₂ 1.8 mM, pH 7.2 using a peristaltic pump (Cole-Palmer Instrument Co., Chicago, IL). The whole-cell voltage clamp was achieved through these two electrodes using a Warner Instruments model OC-725C (Hamden, CT). Currents were recorded and analyzed using pCLAMP software (Axon Instruments, Foster City, CA). The sodium current amplitudes were typically 2 – 15 μA. Transients and leak currents were subtracted using the P/N procedure.

The voltage dependence of activation was determined by 50-ms depolarizing pulses from a holding potential of –90 mV (V_{H-90}) or from a holding potential causing half-maximal current ($V_{1/2}$) (approximately from –50 mV to –60 mV) to 60 mV in 10-mV increments. $V_{1/2}$ value was determined individually for each oocyte in each experiment. Normalized activation data were fitted to a Boltzmann equation: $G/G_{\max} = 1/(1 + \exp(V_{1/2} - V)/k)$, where G is the voltage-dependent sodium conductance, $G_{\max}$ is the maximal sodium conductance, $G/G_{\max}$ is the normalized fractional conductance, $V_{1/2}$ is the potential at which activation is half maximal, k is the slope factor. G values for each oocyte were calculated using: $G = I/(V_t - V_r)$, where I is the peak sodium current, V_t is the test potential, and V_r is the reversal potential. V_r for each oocyte was estimated by extrapolating the linear ascending segment of the current voltage relationship (I – V) curve to the voltage axis. To measure steady-state inactivation, currents were elicited by a 50-ms test pulse to –20 mV after 200-ms prepulses ranging from –140 mV to 0 mV in 10-mV increments from a holding potential of –90 mV. Steady-state inactivation curves were fitted to a Boltzmann equation: $I/I_{\max} = 1/(1 + \exp(V_{1/2} - V)/k)$, where $I_{\max}$ is the maximal sodium current, $I/I_{\max}$ is the normalized current, $V_{1/2}$ is the voltage of half-maximal inactivation, and k is the slope factor.

The aromatic anesthetics were examined at the concentrations that are equivalent to 1MAC or 2MAC in rats.^{16,22} (MAC is the minimum anesthetic concentration needed to produce immobility in response to noxious stimulation in 50% of rats; it is equivalent to the EC₅₀ for producing surgical immobility). All aromatic anesthetics were directly dissolved in the Frog Ringer, and were infused for 3 min to produce equilibrium. The loss of concentration from vial to bath was approximately 60–80% for all aromatic anesthetics, as determined by gas chromatography. Bath/vial concentrations of the four compounds that were tested were 1.1/2.5 mM (benzene), 0.40/1.4 mM (1,3,5-trifluorobenzene), 0.65/2.1 mM (pentafluorobenzene), and 0.12/0.58 mM (hexafluorobenzene).

Data Analysis

All values are presented as the mean ± SEM. The n values refer to the number of oocytes studied. Each experiment was performed with oocytes from at least two frogs. Statistical analyses were performed using a one-way analysis of variance (ANOVA) for multiple comparisons and a t test using GraphPad Prism software (GraphPad Software Inc., San Diego, CA).

Results

Effects of aromatic anesthetics on the peak Na⁺ inward currents elicited from two different holding potentials

All eight aromatic anesthetics suppressed I_{Na} at the $V_{1/2}$ holding potential, and the magnitudes of these effects varied widely (Fig. 1B,D, Fig. 2). At $V_{1/2}$, these anesthetics reduced I_{Na} by 37 ± 2% (benzene), 39 ± 2% (fluorobenzene), 48 ± 2% (1,2-difluorobenzene), 31 ± 3% (1,4-difluorobenzene), 33 ± 1% (1,2,4-trifluorobenzene), 8 ± 2% (1,3,5-trifluorobenzene), 13 ± 2% (pentafluorobenzene), and 13 ± 2% (hexafluorobenzene) (Fig. 2). Effects on I_{Na} at V_{H-90} were smaller than at $V_{1/2}$ holding potential, and the magnitudes of the effects varied widely, with

inhibitions of $8 \pm 1\%$ (benzene), $10 \pm 1\%$ (fluorobenzene), $15 \pm 2\%$ (1,2-difluorobenzene), $6 \pm 0.5\%$ (1,4-difluorobenzene), and $3 \pm 0.7\%$ (1,2,4-trifluorobenzene) (Fig. 1A, Fig. 2). 1,3,5-trifluorobenzene did not affect these currents. In contrast, pentafluorobenzene and hexafluorobenzene potentiated I_{Na} at V_{H-90} by $2 \pm 1\%$, $6 \pm 1\%$, respectively (Fig. 1C, Fig. 2). Thus, there are two effects of aromatic anesthetics on sodium channels: All produced inhibitory effects at $V_{1/2}$ holding potential, albeit by widely varying amounts. At V_{H-90} , benzene, fluorobenzene, 1,2-difluorobenzene, 1,4-difluorobenzene and 1,2,4-trifluorobenzene produced inhibition but pentafluorobenzene and hexafluorobenzene caused slight potentiation.

Effects of aromatic anesthetics on activation of sodium currents

Because of the different effects of aromatic anesthetics noted above, we examined actions of these two types compounds on gating of sodium channels. We selected benzene and 1,2-difluorobenzene as compounds with potent inhibitory effects at $V_{1/2}$ holding potential plus smaller inhibitory effects at V_{H-90} , and hexafluorobenzene as the compound with a much smaller inhibitory effect at $V_{1/2}$ holding potential and slight potentiating effect at V_{H-90} . The voltage dependence of activation was determined by 50-ms depolarizing pulses from a holding potential of -90 mV to 60 mV in 10 -mV increments or from a holding potential of $V_{1/2}$ to 60 mV in 10 -mV increments. The activation curves were derived from the $I-V$ curve (see Methods). Benzene and 1,2-difluorobenzene (2 MAC) reduced the peak I_{Na} at V_{H-90} and $V_{1/2}$ (Fig. 3A, B). However, hexafluorobenzene (2 MAC) potentiated the peak I_{Na} at V_{H-90} , especially in the depolarizing region where channel opening begins (around $-40 \sim -30$ mV) and suppressed the sodium currents at $V_{1/2}$ more weakly than benzene or 1,2-difluorobenzene (Fig. 3C). Benzene and 1,2-difluorobenzene did not affect voltage dependence of activation at V_{H-90} or $V_{1/2}$ (Fig. 4A, B, Table 1), but hexafluorobenzene shifted the midpoint of steady-state activation, $V_{1/2}$ in hyperpolarizing direction by 4.9 and 2.2 mV at V_{H-90} and $V_{1/2}$ (Fig. 4C, Table 1).

Effects of aromatic anesthetics on inactivation of sodium currents

The effects of benzene, 1,2-difluorobenzene and hexafluorobenzene on steady-state inactivation were also investigated. Benzene and 1,2-difluorobenzene shifted $V_{1/2}$ in the hyperpolarizing direction significantly by 7.4 and 8.9 mV, respectively and also reduced sodium currents in the hyperpolarizing potential range (Fig. 5, Table 1). Hexafluorobenzene also significantly shifted $V_{1/2}$ in the hyperpolarizing direction by 4.6 mV although the shift was smaller than that by benzene and 1,2-difluorobenzene, whereas hexafluorobenzene potentiated sodium current in the hyperpolarizing potential range slightly (Fig. 5, Table 1). The effects of benzene, 1,2-difluorobenzene and hexafluorobenzene in the hyperpolarizing range were consistent with the effects of these compounds on I_{Na} at V_{H-90} , and the effects on these compounds on activation curve at V_{H-90} .

Discussion

We found that structurally distinct aromatic anesthetics differently affected I_{Na} at V_{H-90} and $V_{1/2}$ holding potentials. At $V_{1/2}$ holding potential, 1 MAC benzene, fluorobenzene, 1,2-difluorobenzene, 1,4-difluorobenzene, 1,2,4-trifluorobenzene suppressed I_{Na} by $30\text{--}50\%$. These inhibitory effects parallel those of other inhaled anesthetics. For example, clinically relevant concentrations ($0.25\text{--}0.4$ mM) of isoflurane suppress I_{Na} by $30\text{--}40\%$ in several preparations: Chinese hamster ovary cells expressing $Na_v1.2$,⁴ isolated rat neurohypophysial nerve terminals,³ and an oocyte system expressing $Na_v1.2$, $Na_v1.4$, or $Na_v1.6$.⁵

Solt et al. separated these aromatic anesthetics into two groups based on the inhibition of NMDA receptors produced by 1 MAC.¹⁶ Less fluorinated compounds with the limited fluorination – benzene, fluorobenzene and 1,2-difluorobenzene – inhibited strongly (Group 1),

and compounds with greater fluorination – 1,4-difluorobenzene, 1,2,4-trifluorobenzene, 1,3,5-trifluorobenzene, pentafluorobenzene, hexafluorobenzene – inhibited weakly (Group 2). Solt et al. also studied the effect of these compounds on GABA_A receptors,¹⁷ and found that aromatic anesthetics which inhibit NMDA receptor currents most (Group 1) potentiate GABA_A receptor currents least, and anesthetics that inhibit NMDA receptor currents least (Group 2) potentiate GABA_A receptor currents most. These findings suggest that distinct molecular properties determine the action of anesthetics on NMDA and GABA_A receptors.

We found that benzene, fluorobenzene, and 1,2-difluorobenzene (Group 1 compounds), strongly inhibited sodium currents at $V_{1/2}$, whereas 1,3,5-trifluorobenzene, pentafluorobenzene, and hexafluorobenzene (Group 2 compounds), inhibited weakly. But unlike the NMDA results, we found that 1,4-difluorobenzene and 1,2,4-trifluorobenzene (Group 2 compounds) inhibited sodium currents (Fig. 6A). Our previous study of isoflurane⁵ found similar sodium channel inhibition as for 1,4-difluorobenzene and 1,2,4-trifluorobenzene, and these three compounds are effective on both sodium channels and GABA_A receptors (Fig. 6B).

Raines et al. showed that cation- π interactions predict the actions of aromatic anesthetics on NMDA receptors.²³ In order to explore the physical properties important for actions of these compounds on sodium channels, we correlated drug effects with several parameters (Fig. 6C,D, and E). Sodium channel inhibition was correlated with cation- π interactions, but this relationship was weaker than seen for cation- π interactions and NMDA receptor inhibition by Raines et al.²³ Inhibition of sodium channels was strongly correlated with oil/gas partition coefficients. These results suggest that aromatic anesthetics interact with different channels by distinct mechanisms. We recently reported that *n*-alcohols inhibit sodium channel function and their potency increase with carbon number up to a cut-off at nonanol,²⁴ suggesting that hydrophobic and steric interactions determine the potencies of *n*-alcohols. Hydrophobic and steric interactions might also be important for sodium channel inhibition by aromatic anesthetics, with a smaller contribution from cation- π interactions. Some fluorinated compounds are either not as potent anesthetics as would be predicted from their lipid solubility or do not produce anesthesia (nonimmobilizers).²⁵ The nonimmobilizing fluorinated cyclobutanes do not inhibit sodium channels,²⁶ indicating that factors other than lipid solubility are also important for channel inhibition by some compounds.

Analysis of gating parameters revealed differences in the actions of benzene, 1,2-fluorobenzene and hexafluorobenzene. Other volatile anesthetics generally enhance inactivation with no effect on activation.^{5,6} We also found this to be true for benzene and 1,2-difluorobenzene. However, hexafluorobenzene enhanced peak I_{Na} at V_{H-90} , shifted activation in the hyperpolarizing direction and increased sodium currents in the hyperpolarizing range of inactivation. These changes indicate that hexafluorobenzene shifts channel gating equilibrium toward the open channel state. No other anesthetics have been reported to have an activating effect on sodium channels. This action of hexafluorobenzene on channel gating might offset the effects on the inactivated state and explain the small inhibition at $V_{1/2}$ holding potential relative to the other compounds. Pentafluorobenzene, another Group 2 compound, may shift GABA_A receptor gating toward the open channel state because pentafluorobenzene decreases the GABA EC_{50} and activates GABA_A receptors directly.¹⁷ This observation suggests some commonalities in the action of aromatic compounds on sodium channels and GABA_A receptors.

In conclusion, we find that volatile aromatic anesthetics inhibit Na_v1.2 sodium channel function but the magnitude of the inhibition varies widely among the compounds. Differences in the actions of these compounds on sodium channel gating are likely responsible for the range of inhibitory effects. Although numerous inhaled anesthetics and *n*-alcohols inhibit sodium

channel function, some inhibit weakly and others strongly (Group 1 versus Group 2) at concentrations corresponding to MAC. We applied aromatic compounds in the present study because they reciprocally affect GABA_A and NMDA receptors; we tested whether the sodium channel might provide more consistent responses. However, neither the aromatics nor clinical agents (e.g., halothane, isoflurane) provide consistent inhibition of sodium channels. This indicates that sodium channel inhibition may be important for some anesthetics but not others as a mediator of the capacity of inhaled anesthetics to produce immobility in the face of noxious stimulation.

Acknowledgements

We thank Dr. Michael Laster for determining the concentrations of anesthetics used in this study.

Supported by National Institutes of Health Grants GM47818 and AA06399 (to R.A.H.).

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Figure 1.

Effects of benzene and hexafluorobenzene on sodium currents at different holding potentials in oocytes expressing $Na_v1.2$ with β_1 . (A) The effect of benzene at -90 mV (V_{H-90}) holding potential. Left panel; traces of sodium currents evoked by a 50-ms depolarizing pulse to -20 mV from a holding potential of V_{H-90} in the absence and presence of benzene. Right panel; time course figure of benzene effect at V_{H-90} holding potential on sodium currents. Currents were elicited by a 50-ms depolarizing pulses to -20 mV applied every 10 s from -90 mV. Normalized current is shown. Filled circles represent control and washout, open circles represent currents during 3 min benzene treatment. (B) The effect of benzene at a holding potential which induced half maximal current ($V_{1/2}$). Left panel; traces of sodium currents evoked by a 50-ms depolarizing pulse to -20 mV from $V_{1/2}$ holding potentials in the absence and presence of benzene. Right panel; time course of benzene action at $V_{1/2}$ holding potential. Currents were elicited by a 50-ms depolarizing pulses to -20 mV applied every 10 s from $V_{1/2}$ holding potential. (C) Effect of hexafluorobenzene at V_{H-90} holding potential. (D) Effect of hexafluorobenzene at $V_{1/2}$ holding potential. Concentrations of anesthetics were equivalent to 1 MAC.

Figure 2.

Effects of aromatic anesthetics on sodium currents at different holding potentials. Percent inhibitions were calculated from time course figure. Open columns represent the effect at V_{H-90} holding potential. Closed columns indicate the effect at $V_{1/2}$ holding potential. Concentrations of anesthetics were equivalent to 1 MAC. $V_{1/2}$ value was -53.5 ± 0.4 mV. Data were obtained from 6 oocytes and are mean $\pm$ S.E.M. ($n = 6$). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (one-way ANOVA).

Figure 3.

Effects of benzene (A), 1,2-difluorobenzene (B), and hexafluorobenzene (C) on I–V relations of sodium currents at V_{H-90} and $V_{1/2}$ holding potentials in oocytes expressing $Na_v1.2$ with β_1 subunits. Left panel; representative I_{Na} traces in absence and presence of aromatic anesthetics at a V_{H-90} holding potential. Currents were elicited by 50-ms depolarizing steps between -80 and 60 mV in 10 -mV increments from holding potentials of -90 mV. Center panel; the effects of aromatic anesthetics on I–V curves elicited from a V_{H-90} holding potential (closed circles, control; open circles, aromatic anesthetics; cross, washout). The peak currents were normalized to the maximal currents which were observed at -20 or -30 mV. Right panel; the effects of aromatic anesthetics on I–V curves elicited from a $V_{1/2}$ holding potential. Currents were elicited by 50-ms depolarizing steps between -60 and 60 mV in 10 -mV increments from a $V_{1/2}$ holding potential. Maximal currents were observed at -20 mV. Concentrations of anesthetics were equivalent to 2 MAC. $V_{1/2}$ value was -53.3 ± 0.4 mV. Data were obtained from 5 oocytes and shown as mean $\pm$ S.E.M. ($n = 5$).

Figure 4.

Effects of benzene (A), 1,2-difluorobenzene (B), and hexafluorobenzene (C) on channel activation in oocytes expressing Na_v1.2 with β_1 from a V_{H-90} holding potential (upper) or $V_{1/2}$ holding potential (down). Closed circles represent control, open circles indicate the effect of aromatic anesthetics, and cross indicate washout. Data shown as mean $\pm$ S.E.M. ($n = 5$). Activation curves were fitted to a Boltzmann equation and $V_{1/2}$ are shown in Table 1. Concentrations of anesthetics were equivalent to 2 MAC.

Figure 5.

Effects of benzene (A), 1,2-difluorobenzene (B), and hexafluorobenzene (C) on inactivation in oocytes expressing $Na_v1.2$ with β_1 . Left panel; representative I_{Na} traces in absence and presence of aromatic anesthetics. Currents were elicited by a 50-ms test pulse to -20 mV after 200-ms prepulses ranging from -140 to 0 mV in 10 -mV increments from a holding potential of V_{H-90} . Right panel; the effects of aromatic anesthetics on inactivation curves (closed circles, control; open circles, aromatic anesthetics; cross, washout). Data shown as mean $\pm$ S.E.M. ($n = 7$). Inactivation curves were fitted to a Boltzmann equation and the $V_{1/2}$ values are shown in Table 1. Concentrations of anesthetics were equivalent to 2 MAC.

Figure 6.

(A) Relationship between inhibitory potencies on sodium channels at V_{H-90} holding potential (open circle) or $V_{1/2}$ holding potential (closed circle) and that on NMDA receptors of aromatic anesthetics at 1MAC. (B) Relationship between inhibitory potencies on sodium channels at V_{H-90} holding potential (open circle) or $V_{1/2}$ holding potential (closed circle) and potencies of enhancement of aromatic anesthetics on GABA_A receptors. NMDA receptor inhibition are from Solt et al.¹⁶, and GABA_A receptor potentiation are from Kelly et al.¹⁷ Data shown as mean $\pm$ S.E.M. ($n = 6$). Correlation between inhibitory potencies on sodium channels of aromatic anesthetics at V_{H-90} holding potential (open circle) or $V_{1/2}$ holding potential (closed circle) and their abilities to oil/gas partition coefficient (C), molecular volumes (D), or cation- π interactions (E). The lines were derived from linear regression analysis. Oil/gas partition coefficients of aromatic anesthetics are from Fang et al.,²² molecular volume and cation- π binding energy of aromatic anesthetics are from Raines et al.,²³ In C, $r^2 = 0.88$ and $p = 0.0005$ ($V_{1/2}$), $r^2 = 0.87$ and $p = 0.0006$ (V_{H-90}); in D, $r^2 = 0.55$ and $p = 0.0353$ ($V_{1/2}$), $r^2 = 0.68$ and $p = 0.0113$ (V_{H-90}); in E, $r^2 = 0.52$ and $p = 0.0445$ ($V_{1/2}$), $r^2 = 0.67$ and $p = 0.0135$ (V_{H-90}).

Table 1
Effects of Benzene, 1,2-fluorobenzene, and Hexafluorobenzene on Activation and Inactivation in Oocytes Expressing Na_v1.2 with β₁

Activation	Holding V _{H-90}			Holding V _{1/2}		
	Control	Anesthetics	Shift	Control	Anesthetics	Shift
V _{1/2} (mV)						
Benzene	-34.3 ± 0.5	-34.6 ± 0.4	-0.3	-27.3 ± 0.5	-26.9 ± 0.4	+0.4
1,2-difluorobenzene	-33.5 ± 0.3	-33.9 ± 0.6	-0.4	-27.0 ± 0.3	-27.3 ± 0.6	-0.3
Hexafluorobenzene	-34.4 ± 0.4	-39.3 ± 0.9 ^{***}	-4.9	-27.4 ± 0.5	-29.6 ± 0.2 ^{***}	-2.2
Inactivation						
Control			Shift			
V _{1/2} (mV)						
Benzene	-55.1 ± 0.4	-62.5 ± 0.5 ^{***}	-7.4			
1,2-trifluorobenzene	-55.8 ± 0.3	-64.7 ± 0.7 ^{***}	-8.9			
Hexafluorobenzene	-56.6 ± 0.4	-61.2 ± 0.4 ^{***}	-4.6			

** , $p < 0.01$;

*** $p < 0.001$, compared with control by one-way analysis of variance (mean ± S.E.M.; n = 5–7).