British Journal of Anaesthesia **103 (1):** 61–9 (2009)
doi:10.1093/bja/aep144 Advance Access publication June 9, 2009

BJA

Sodium channels and the synaptic mechanisms of inhaled anaesthetics

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General anaesthetics act in an agent-specific manner on synaptic transmission in the central nervous system by enhancing inhibitory transmission and reducing excitatory transmission. The synaptic mechanisms of general anaesthetics involve both presynaptic effects on transmitter release and postsynaptic effects on receptor function. The halogenated volatile anaesthetics inhibit neuronal voltage-gated Na^+ channels at clinical concentrations. Reductions in neurotransmitter release by volatile anaesthetics involve inhibition of presynaptic action potentials as a result of Na^+ channel blockade. Although voltage-gated ion channels have been assumed to be insensitive to general anaesthetics, it is now evident that clinical concentrations of volatile anaesthetics inhibit Na^+ channels in isolated rat nerve terminals and neurons, as well as heterologously expressed mammalian Na^+ channel α subunits. Voltage-gated Na^+ channels have emerged as promising targets for some of the effects of the inhaled anaesthetics. Knowledge of the synaptic mechanisms of general anaesthetics is essential for optimization of anaesthetic techniques for advanced surgical procedures and for the development of improved anaesthetics.

Br J Anaesth 2009; **103**: 61–9

Keywords: anaesthetics volatile; anaesthetics volatile, halogenated hydrocarbons; nerve, neurotransmitters; pharmacology, anaesthetic action; pharmacology, neurotransmission

The pharmacology and toxicology of general anaesthetics are remarkably incomplete for such a widely used and clinically important class of drugs. Despite their widespread clinical use, our understanding of the molecular and cellular mechanisms of general anaesthetic action in the CNS is insufficient to explain how any anaesthetic produces amnesia, unconsciousness, or immobilization (at increasing doses), the cardinal clinical features of general anaesthesia. Early optimism that potentiation of ligand-gated ion channel receptors for inhibitory neurotransmitters like γ-aminobutyric (GABA) acid and/or glycine might underlie the actions of all anaesthetics has given way to current concepts of multiple agent-specific mechanisms underlying the diverse features of anaesthesia.[27 81] It is now clear the general anaesthetics act at multiple anatomic sites in the nervous system to produce these distinct behavioural effects involving actions on multiple molecular targets.[64 75] For example, there is now convincing evidence that volatile anaesthetics produce immobilization by effects in the spinal cord, while amnesia and unconsiousness involve distinct supraspinal mechanisms.[1 15 66]

Ion channels have emerged as the most likely molecular targets for general anaesthetics. Neurotransmitter-gated ion channels, in particular $GABA_A$, glycine, and *N*-methyl-D-aspartate (NMDA)-type glutamate receptors, are leading candidates due to their appropriate central nervous system (CNS) distributions, essential physiological roles in inhibitory and excitatory synaptic transmission, and sensitivities of one or more of these channels to clinically relevant concentrations of all anaesthetics.[15 27 102 103] Broadly speaking, general anaesthetic targets vary between the major classes of anaesthetics. Two classes of inhaled anaesthetics can be distinguished based on their distinct pharmacological. properties: (i) the potent inhaled (volatile) anaesthetics exhibit positive modulation of $GABA_A$ receptors, and also produce significant anaesthesia-compatible effects on a number of other receptors/channels including enhancement of inhibitory glycine receptors, inhibition of excitatory NMDA-type glutamate and neuronal nicotinic acetylcholine receptors, activation of two-pore domain K_{2P} channels and leak K^+ channels,[59 79] and inhibition of presynaptic Na^+ channels (see in what follows); and (ii) the gaseous inhaled anaesthetics, which include cyclopropane, nitrous oxide, and xenon, are inactive at $GABA_A$ receptors, but block NMDA receptors and activate certain K_{2P} channels at clinical concentrations.[23] Intravenous anaesthetics like propofol and etomidate represent more potent and specific positive modulators of $GABA_A$

receptors, and the i.v. anaesthetic ketamine is a more potent and specific blocker of NMDA receptors.[103] Here I review accumulating evidence that suggests that some of the effects of volatile anaesthetics are mediated by inhibition of neuronal voltage-gated Na^+ channels, an ion channel family often overlooked as a putative target for general anaesthesia (though widely recognized as the principal target for local anaesthetics).

Synaptic actions of general anaesthetics

General anaesthetics *depress* fast excitatory and *enhance* fast inhibitory synaptic transmission mediated primarily by glutamate and GABA (γ-aminobutyric acid), respectively.[27 62 81] The relative importance of anaesthetic effects on excitatory *vs* inhibitory synaptic transmission and the mechanisms involved are less clear. Prolongation of synaptic inhibition by modulation of postsynaptic $GABA_A$ receptor function at GABAergic synapses is recognized as an important component of the depressant effects of volatile anaesthetics and of several i.v. anaesthetics at clinical concentrations,[21 30 49 108] and significant progress has been made in identifying critical anaesthetic binding sites on $GABA_A$ receptors.[27] More recent studies have implicated anaesthetic actions on tonic inhibitory currents mediated by extrasynaptic $GABA_A$ receptors as well as enhanced release of GABA mediated by a presynaptic increase in miniature inhibitory postsynaptic current (mIPSC) frequency (see in what follows). Evidence also supports depression of excitatory transmission at clinical concentrations of many general anaesthetics.[40 53 61 91] The molecular mechanisms of these depressant effects on excitatory transmission are less clear, but could include depressed membrane excitability,[64] depressed presynaptic action potential conduction,[4 44 54 100] inhibition of transmitter release,[43 77] and/or blockade of postsynaptic receptors. The latter is the principal mechanism for ketamine[7] and for the gaseous anaesthetics xenon,[103] nitrous oxide,[42] and cyclopropane.[80] Blockade of postsynaptic glutamate receptors has been shown to contribute to the effects of volatile anaesthetics at some synapses.[12 25] The roles of enhanced inhibitory transmission *vs* reduced excitatory transmission to the overall depression of neuronal activity in anaesthesia likely vary between specific networks.[64 85]

Presynaptic *vs* postsynaptic anaesthetic effects

The relative contributions of presynaptic *vs* postsynaptic anaesthetic effects on synaptic transmission have been difficult to resolve.[11 19] Electrophysiological evidence supports both presynaptic (release) and postsynaptic (receptor) mechanisms for the synaptic actions of general anaesthetics. Intravenous anaesthetics and volatile anaesthetics decrease excitatory postsynaptic potentials (EPSPs) in spinal[33 93] and hippocampal neurons,[5 40 61 74] which has been attributed indirectly to a presynaptic mechanism, and decrease cortical neuron sensitivity to applied glutamate, a postsynaptic mechanism.[45 73 90 104] General anaesthetics also decrease depolarization-induced glutamate release from brain slices,[8 16 35 36] but it is difficult to localize drug effects in such intact polysynaptic neuronal circuits. Volatile anaesthetics have limited effects on cloned α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) or *N*-methyl-D-aspartic acid (NMDA) glutamate receptors but potentiate kainate receptors,[13 45] consistent with a predominantly presynaptic mechanism for inhibition of glutamatergic synapses, although recent evidence suggests that NMDA receptor blockade could contribute to inhaled anaesthetic effects.[12 25] Most i.v. anaesthetics are remarkable for their relatively potent and selective potentiation of postsynaptic GABA responses.[103] In contrast to glutamatergic synapses, augmentation of GABAergic responses by most general anaesthetics is mediated primarily by potentiation of postsynaptic and extrasynaptic $GABA_A$ receptors.[27] Anaesthetics also have presynaptic effects at GABA terminals to increase IPSC frequency and GABA release,[2 46 52 64] which combine with prolongation of action-potential-evoked IPSCs to increase inhibitory charge transfer and net inhibitory tone.[6]

Investigations of the molecular targets responsible for these synaptic effects of general anaesthetics have focused on voltage-gated and ligand-gated ion channels, many of which have been cloned and are accessible to direct electrophysiological analysis.[19 27] $GABA_A$ receptors in particular are potentiated by most general anaesthetics, which accounts for their postsynaptic actions on inhibitory synaptic transmission. Potentiation of $GABA_A$ receptors is clearly an important mechanism for i.v. anaesthetics such as propofol, the immobilizing effects of which can be antagonized by intrathecal injection of the $GABA_A$ receptor antagonist bicuculline[6] or by a β3 receptor N265M knock-in mutation *in vivo*.[31] In contrast, potentiation of $GABA_A$ receptors is insufficient to explain the actions of volatile anaesthetics, since bicuculline does not antagonize immobilization by isoflurane.[37 107] Thus volatile anaesthetic actions are less specific than those of most i.v. anaesthetics, and other sites of action must be involved. In addition to possible effects on glutamate receptors, convincing evidence exists for volatile anaesthetic effects on voltage-gated Na^+ channels,[54 58 71 100] two-pore-domain background K^+ (K_{2P}) channels,[18 22 60] nicotinic cholinergic receptors,[17 89] voltage-gated Ca^{2+} channels,[32 50 84] and presynaptic SNARE proteins.[47] An important goal is to identify the relevant molecular mechanisms for the presynaptic effects of volatile anaesthetics from among the multiple potential targets.

Anaesthetic effects on neurotransmitter release

The basic mechanisms underlying release are conserved among different neurotransmitters.[86] However, transmitter- and nerve-terminal-specific specializations exist, such as

transmitter-specific involvement of individual Ca^{2+} channel types in release and the modulation of release by presynaptic receptors.[39 41 72 98] Transmitter release is tightly coupled to the concentration of presynaptic cytoplasmic Ca^{2+}. The relationship between extracellular Ca^{2+} concentration and neurotransmitter release is exponential. Thus, Ca^{2+} entering through presynaptic voltage-gated Ca^{2+} channels acts in a highly cooperative manner to evoke exocytosis, with a degree of cooperativity (Hill slope) of ~3–4 depending on the synapse.[14 99] Factors that influence the modulation of neurotransmitter release include the amount of Ca^{2+} entering the presynaptic terminal, the efficiency with which Ca^{2+} controls exocytosis (Ca^{2+}-secretion coupling), the cooperativity of Ca^{2+} action, and the maximal amount of release reflected in the number of docked and primed vesicles (the readily releasable pool).

Depolarization and Ca^{2+} entry determine the amount of transmitter released, which is regulated by presynaptic ion channels (e.g. Na^+, Ca^{2+}, and K^+ channels), modulatory presynaptic receptors, and cell signalling mechanisms (e.g. second messengers and protein phosphorylation). Potential presynaptic targets for general anaesthetic action include: nerve terminal excitability/depolarization, Ca^{2+} influx (by direct effects on Ca^{2+} channels, indirectly through Ca^{2+} modulatory pathways, or via other ion channels), synaptic vesicle availability and mobilization (by effects on the cytoskeleton and/or synaptic vesicle-associated proteins), coupling between Ca^{2+} and exocytosis, fusion/exocytosis mediated by SNARE proteins, and vesicle endocytosis/recycling. The involvement of these and possibly other targets in the presynaptic actions of general anaesthetics on neurotransmitter release is not clearly established.

The presynaptic effects of anaesthetics on transmitter release can be measured directly in isolated nerve terminals (synaptosomes) without interference from intrinsic neuronal networks or somatic effects that limit interpretation of results obtained using brain slices or cultured neurons.[48] Volatile anaesthetics inhibit pharmacologically evoked release of endogenous glutamate from cortical nerve terminals, indicating a direct presynaptic site of action.[38 43 77] These results are supported by recent studies using advanced techniques to measure presynaptic anaesthetic effects. A high-resolution optical technique for imaging exocytosis showed that isoflurane inhibits action potential-evoked synaptic vesicle exocytosis in cultured rat hippocampal neurons.[28] Electrophysiological techniques have also been used to show that isoflurane inhibits glutamatergic transmission in the unusually large rat calyx of Held synapse.[63 100] Inhibition of transmitter release by volatile anaesthetics is conserved in the phylogenetically distant organisms *Caenorhabditis elegans*[88] and *Drosophila*.[51 76] Volatile anaesthetics also inhibit evoked GABA release from rat cortical nerve terminals, but with lower potency compared with glutamate release (see in what follows).[95 97] The mechanism for this selectivity/differential sensitivity is intriguing but currently unknown. In addition, volatile anaesthetics enhance basal GABA release while inhibiting basal glutamate release, consistent with reports of increased IPSC and decreased excitatory postsynaptic current (EPSC) frequency in hippocampal slices.[52 64]

Anaesthetic effects on transmitter release from isolated nerve terminals

Isolated CNS nerve terminals (synaptosomes) provide an *in vitro* model to study the pharmacology of neurotransmitter release that has allowed a detailed pharmacological analysis of presynaptic anaesthetic pharmacology. Synaptosomes contain all the cellular machinery necessary for the generation and maintenance of ion gradients, and for synthesis, storage, uptake, and release of transmitters.[48] They are depleted of glial and neuronal cell body elements, and are therefore devoid of cellular and network interactions, and can be prepared from various CNS regions that differ in their transmitter content and modulatory mechanisms. Release from synaptosomes can be evoked chemically by several methods, each of which involves activation of distinct components of the endogenous release mechanisms (Fig. 1). Thus, synaptosomes provide an unexcelled system for analyzing the presynaptic mechanisms of anaesthetic effects on synaptic transmission in isolation of indirect effects present in intact neural networks. Since transmitter release is coupled to changes in the activity of various ion channels, presynaptic receptors, and second messenger pathways, many of which have been implicated as anaesthetic targets, this system provides a functional assay for analyzing the mechanisms of anaesthetic effects at a number of potential target sites.

The presynaptic actions of volatile anaesthetics likely vary between transmitters due to differences in presynaptic physiology and release mechanisms, and between volatile anaesthetics due to their distinct pharmacological profiles. Recent studies on glutamate and GABA release from cortical nerve terminals provide a paradigm for studying the mechanisms of these effects and those of anaesthetics on other transmitters and in other CNS regions. Previous studies with rat cortical nerve terminals demonstrated that volatile anaesthetics (at clinically relevant concentrations) and propofol (at supraclinical concentrations) inhibit Ca^{2+}-dependent glutamate release evoked by secretogogues that require Na^+ channel activation (veratridine or 4-aminopyridine) with greater potency than Na^+ channel-independent release (evoked by depolarization with elevated extracellular KCl which bypasses Na^+ channels) (Fig. 2).[38 68 77 95] Taken together with independent neurochemical[67 69] and electrophysiological[58 70 71] evidence of anaesthetic blockade of neuronal Na^+ channels, these findings suggest that volatile anaesthetics inhibit glutamate release by blocking presynaptic Na^+ channels,

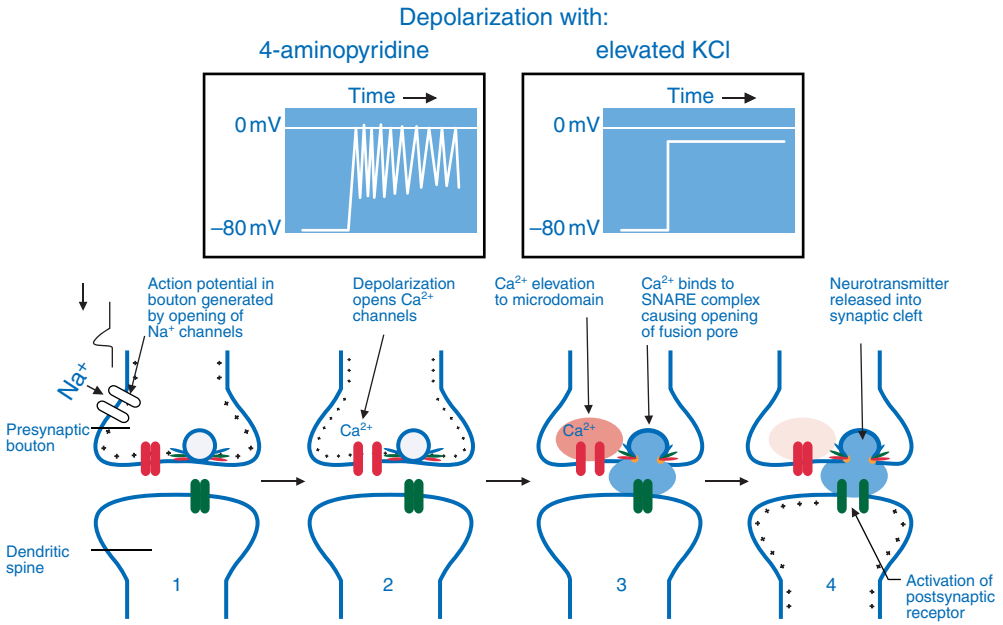

Fig 1 Steps in the process of synaptic transmission. The action potential invades the presynaptic bouton (1) leading to depolarization mediated by voltage-gated Na^+ channels. This can be mimicked in isolated nerve terminals by the chemical secretogogue 4-aminopyridine. The depolarization activates voltage-gated Ca^{2+} channels closely coupled to docked and releasable synaptic vesicles. (2) Increased extracellular K^+ can depolarize the membrane potential independent of Na^+ channel involvement to an extent sufficient to activate Ca^{2+} channels and transmitter release. The local elevations in intracellular Ca^{2+} concentration bind to the SNARE vesicle fusion complex, leading to exocytosis of transmitter. (3) The transmitter enters the synaptic cleft where it diffuses to the postsynaptic cell, activates synaptic and extrasynaptic receptors, and thereby modifies the excitability of the postsynaptic cell. (4) Anaesthetics can potentially disrupt this process at multiple points. Considerable evidence implicates inhibition of presynaptic voltage-gated Na^+ channels as a probable site of inhaled anaesthetic action, particularly at excitatory glutamatergic synapses.

thereby inhibiting nerve terminal depolarization. Additional evidence for Na^+ channel involvement is provided by observations that the model anaesthetic 1-chloro-1,2,2-trifluoro-cyclobutane (F3) inhibits Na^+ channel-dependent glutamate release and blocks neuronal Na^+ channels, while the structurally similar non-immobilizer 1,2-dichlorohexafluorocyclobutane (F6) is ineffective at predicted anaesthetic concentrations.[70] This indicates a greater anaesthetic sensitivity of presynaptic Na^+ channels than of the Ca^{2+} channels coupled to glutamate release, and is consistent with the observation that the predominant Ca^{2+} channel coupled to neurotransmitter release at hippocampal glutamatergic synapses (P/Q-type) is relatively insensitive to isoflurane.[24] However, these findings do not preclude involvement of additional presynaptic targets in depression of transmitter release by volatile anaesthetics and do not explain the greater sensitivity of glutamate *vs* GABA release or the differential effects on basal release.[95–97] Other presynaptic mechanisms that have been proposed include enhanced glutamate uptake[35] or actions on the vesicle fusion process.[88] However, enhanced glutamate uptake is insufficient to explain inhibition of glutamate release,[94] and isoflurane effects on exocytosis in hippocampal neurons occur primarily upstream of vesicle fusion.[20 28 77] A contribution of anaesthetic effects on the vesicle fusion process is possible,[47] but has yet to be directly demonstrated in a vertebrate synapse.

The complexity of the presynaptic terminal is evident in the modulation of nerve terminal excitability by numerous ion channels and presynaptic receptors, and the multiple molecular interactions involved in vesicle mobilization, docking, fusion, and recycling.[86] The small size of most nerve terminals in the CNS (<1 μm diameter) precludes direct electrophysiological analysis of presynaptic events. Isolated neurohypophysial nerve terminals[92] have served as a useful model for studying the electrophysiological effects of general anaesthetics on nerve terminal ion currents by whole-terminal patch-clamp recording.[54 58] Isoflurane inhibits nerve terminal Na^+ currents and action potential amplitude through Na^+ channel blockade in isolated rat neurohypophysial terminals. A similar approach used on the rat calyx of Held giant synapse also showed that isoflurane inhibits glutamatergic transmission presynaptically by a mechanism involving a reduction in Na^+ channel-mediated action potential amplitude rather than Ca^{2+} channel blockade. Isoflurane significantly depressed action potential-evoked synaptic vesicle exocytosis and EPSC amplitude with only a small reduction in presynaptic action potential amplitude and no direct effect on Ca^{2+} current in the rat calyx of Held synapse.[100] Simulated reductions in action potential amplitude reproduced this highly nonlinear relationship between peak Na^+ current inhibition and exocytosis. These findings identify presynaptic Na^+ channels as important anaesthetic targets

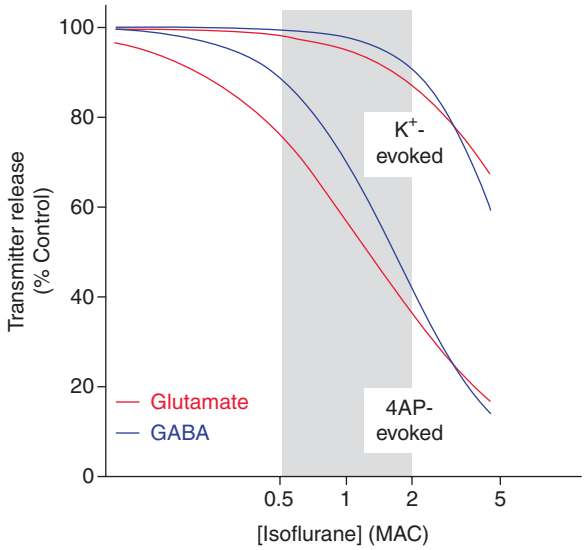

Fig 2 Selective inhibition of Na^+ channel-dependent glutamate release by isoflurane. Nerve terminals isolated from rat cerebral cortex were pre-labelled with radio-labelled glutamate and GABA, and release was measured following stimulation with either 4-aminopyridine (4AP) or elevated extracellular K^+ as described in Figure 1.[95] Na^+ channel-dependent release of both glutamate (IC_{50}=0.44 mM) and GABA (IC_{50}=0.58 mM) evoked by 4AP was more sensitive to inhibition by isoflurane than release of glutamate (IC_{50}=2.6 mM) or GABA (IC_{50}=1.9 mM) evoked by elevated K^+, consistent with a greater sensitivity of presynaptic Na^+ channels than Ca^{2+} channels or other downstream processes. Moreover, 4AP-evoked release of the excitatory transmitter glutamate was inhibited at significantly lower concentrations than release of the inhibitory transmitter GABA within the clinical concentration range (0.5–2 MAC, or minimum alveolar concentration, which corresponds to the mean effective dose) indicated by gray shading. IC_{50}, concentration for 50% inhibition. Unpublished data from H.C.H. and R.I. Westphalen.

for volatile anaesthetics. These approaches yielded the first direct recordings of anaesthetic actions on presynaptic ion channels, and helped bridge the gap between neurochemical studies of anaesthetic effects on transmitter release, studies of the molecular actions of anaesthetics on isolated targets, and the effects of anaesthetics on synaptic physiology.

Na^+ channel pharmacology

Voltage-gated Na^+ channels are responsible for the rapid depolarization phase of the action potential in electrically excitable cells such as nerve, muscle and heart.[9] Voltage-gated channels respond to changes in the plasma membrane potential by opening to allow the passive flux of ions down their electrochemical gradient into or out of the cell. Their modular architecture allows interactions between multiple regions of the channel to orchestrate gating, rapid channel opening and closure. A dynamic model of receptor gating has been developed to explain the pharmacological response of this channel and its ion-selective conductance. A variety of drugs and toxins, including local anaesthetics, class I anti-arrhythmic drugs, and class I anti-epileptic drugs, exhibit voltage-dependent and frequency-dependent block of Na^+ channels as described by the modulated receptor hypothesis.[65] According to this model, these properties are conferred by different drug affinities for the various functional states of the Na^+ channel: resting, open, and inactivated. *Voltage-dependent inhibition* is explained by drug binding to the inactivated state of the channel. This impedes the voltage-dependent transition of the channel from its inactivated state back to its resting state, which effectively reduces the number of resting channels available for activation in response to depolarization. *Frequency-dependent inhibition* is explained by selective drug binding to the open state of the channel. Na^+ channels stimulated with increased frequency are statistically more likely to be in the open state. This allows increased drug binding, but does not prevent subsequent channel inactivation. Local anaesthetics, which show frequency-dependent inhibition, enter from the intracellular side and bind to the inner pore of the Na^+ channel with high affinity.

The Na^+ channel family consists of nine homologous pore-forming α subunits with distinct cellular and subcellular distributions (Table 1).[9] The principle pore forming component of Na^+ channels in mammalian brain is the 260 kilodalton glycoprotein α-subunit. It is a transmembrane protein with large intracellular N- and C-termini. The subunit contains four internally homologous repeated domains (I–IV) with over 50% sequence identity. Each domain consists of six segments (S1–S6) that form transmembrane α-helices. Four additional integral membrane glycoprotein subunits have been identified. The β1 and β3 (36 kDa) subunits interact non-covalently with the α-subunit, while the β2 and β4 subunits (33 kDa) are attached via a disulfide bond. There are at least nine isoforms of the α-subunit which vary in species and tissue expression. The α-subunit is sufficient to carry out the basic function of the channel. Coexpression of β-subunits accelerates inactivation and shifts voltage dependence toward more negative membrane potentials.

Several potent toxins have been used to classify, purify, and define functional domains of Na^+ channels.[10] The puffer fish poison tetrodotoxin (TTX) and the dinoflagellate toxin saxitoxin bind to an extracellular site (site 1) of the α-subunit. These toxins block Na^+ permeability with high potency (K_I=1–10 nM) to TTX-sensitive Na^+ channels, and have enabled the identification of outer pore structures and the selectivity filter. Tissue selectivity is evident in the 200-fold lower affinity of TTX for TTX-insenstive Na^+ channels (Table 1). Lipid soluble steroids such as the frog-skin toxin batrachotoxin and the plant alkaloids aconitine and veratridine bind to site 2. These toxins have a high affinity for the open state of the channel and lead to channel activation by slowing inactivation.

Table 1 Voltage-gated Na^+ channel family members. DRG, dorsal root ganglion; CNS, central nervous system; PNS, peripheral nervous system

Channel α-subunits	Tissue expression	Modulators
$Na_V1.1$	CNS, PNS	Antagonists: Tetrodotoxin, Saxitoxin, μ-Conotoxin,
$Na_V1.2$	CNS	Sea-anemone toxin, Local anaesthetics
$Na_V1.3$	CNS	Activators: Veratridine, Batrachotoxin, α/β-Scorpion
$Na_V1.4$	Skeletal muscle	toxins
$Na_V1.5$	Heart, skeletal muscle	
$Na_V1.6$	CNS, PNS	
$Na_V1.7$	PNS, Schwann cells	
$Na_V1.8$	PNS (DRG)	
$Na_V1.9$	PNS	
Na_x	Heart, uterus, skeletal muscle, astrocytes, DRG	

Direct evidence for Na^+ channel block by volatile anaesthetics

Voltage-gated Na^+ channels are critical to axonal conduction, synaptic integration, and neuronal excitability. Axonal action potentials were initially reported to be relatively resistant to clinical concentrations of volatile anaesthetics,[34] which was consistent with the relative insensitivity of Na^+ currents in squid[26] and crayfish[3] giant axons to volatile anaesthetics. This established the widespread notion 'that clinical concentrations of general anaesthetics almost certainly do not act by blocking Na^+ channels' or any other voltage-gated ion channel.[19] However, axonal conduction in small (0.1–0.2 μm) unmyelinated hippocampal axons is significantly depressed by inhaled anaesthetics,[4 44] and other small diameter structures such as nerve terminals might also be sensitive. Patch clamp recordings of accessible nerve terminals have shown that isoflurane inhibits action potential amplitude,[54] and that reductions in nerve terminal action potential amplitude have significant effects on transmitter release and hence on postsynaptic responses.[100]

Evidence that mammalian voltage-gated Na^+ channels are sensitive to clinically relevant concentrations of general anaesthetics has come from careful analysis of anaesthetic effects on heterologously expressed channels. Analysis of volatile anaesthetic effects on heterologously expressed channels indicates that mammalian voltage-gated Na^+ channels are sensitive to clinically relevant concentrations of general anaesthetics. One neuronal isoform ($Na_v1.2$) is inhibited by multiple potent inhaled anaesthetics through a voltage-independent block of peak current and a hyperpolarizing shift in the voltage dependence of steady-state inactivation.[71] Isoflurane and other inhaled anaesthetics inhibit multiple mammalian Na^+ channel isoforms[55] including $Na_v1.2$,[71] $Na_v1.4$ and $Na_v1.6$,[56 78] $Na_v1.5$,[82] and $Na_v1.8$. Initial reports suggested that the peripheral tetrodotoxin-resistant isoform $Na_v1.8$ expressed in amphibian oocytes was resistant to inhaled anaesthetics,[78] but more focused studies in neuronal cells indicates that $Na_v1.8$ is inhibited by isoflurane at concentrations similar to those that inhibit other Na_v isoforms.[29]

Potent inhaled anaesthetics also inhibit native Na^+ channels in isolated nerve terminals[58 69] and dorsal root ganglion neurons, while the non-immobilizer F6 is ineffective.[70] In contrast, xenon has no detectable effect on Na^+, Ca^{2+}, or K^+ channels in isolated cardiomyocytes.[83] Recent studies indicate that xenon can in fact block neuronal Na^+ channels at clinically relevant concentrations (H.C.H. and K.F. Herold unpublished data). Two principal mechanisms contribute to Na^+ channel inhibition by isoflurane: voltage-independent block of peak currents and enhanced inactivation due to a hyperpolarizing shift in the voltage dependence of steady-state fast inactivation, with significant differences between isoform in the contributions of each mechanism to overall inhibition.[55 71] Volatile anaesthetics, but not non-immobilizers, also inhibit native neuronal and nerve terminal Na^+ channels, lending support to the notion that depression of synaptic neurotransmitter release occurs by Na^+ channel blockade.[55 70] The recent demonstration that NaChBac, a prokaryotic homologue of voltage-gated Na^+ channels, is also inhibited by volatile anaesthetics opens the way for structure-function studies of these channels.[57] Anaesthetic interactions with NaChBac might ultimately allow co-crystallization with anaesthetic for three-dimensional structure determinations by X-ray crystallography, as achieved for voltage-gated K^+ channels, to determine the site(s) of interaction of anaesthetics with a voltage-gated ion channel. It is also intriguing that the binding sites for anaesthetics on ion channels exist in prokaryotic homologues, indicating a remarkable evolutionary conservation.

Testing the relevance of Na^+ channel block as an anaesthetic target

Voltage-gated Na^+ channels have received short shrift as anaesthetic targets, largely because early studies failed to demonstrate significant effects on action-potential conduction in myelinated axons. However smaller diameter unmyelinated fibres and bare nerve terminals are more sensitive to Na^+ channel block and do not possess the considerable reserve in conduction seen in myelinated nerves.

Numerous studies summarized earlier demonstrate that inhaled anaesthetics partially impair Na^+ channel function at MAC (minimum alveolar concentration). Moreover, a variety of evidence supports a role for Na^+ channels in general anaesthesia *in vivo*. An increase in cerebrospinal fluid Na^+ concentration increases MAC (equivalent to ED_{50}), while a reduction decreases MAC, in rats.[87] Intravenous administration of the Na^+ channel blocker lidocaine reduces MAC for several volatile anaesthetics in rats,[105] and i.v. or intrathecal infusions of riluzole, a potent inhibitor of Na^+ channels and glutamate release, decrease isoflurane MAC in rats.[101] Finally, intrathecal but not intraventricular administration of veratridine, a toxin that maintains Na^+ channels in their open state, increases the MAC for isoflurane in rats.[106] Collectively, these results point to anaesthetic inhibition of Na^+ channels as a plausible mechanism for the mediation of immobility produced by inhaled anaesthetics.

Acknowledgement

The invaluable contribution of R.I. Westphalen to the preparation of Figure 2 is acknowledged.

Funding

Supported by a grant from the U.S. National Institutes of Health (GM 58055).

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