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Review

General anaesthetic actions on ligand-gated ion channels

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Abstract. The molecular mechanisms of general anaesthetics have remained largely obscure since their introduction into clinical practice just over 150 years ago. This review describes the actions of general anaesthetics on mammalian neurotransmitter-gated ion channels. As a result of research during the last several decades, ligand-gated ion channels have emerged as promising molecular targets for the central nervous system effects of general anaesthetics. The last 10 years have witnessed an explosion of studies of anaesthetic modulation of recombinant ligand-gated ion channels, including recent studies which utilize chimeric and mutated receptors to identify regions of ligand-gated ion channels important for the actions of general anaesthetics. Exciting future directions include structural biology and gene-targeting approaches to further the understanding of general anaesthetic molecular mechanisms.

Key words. General anaesthesia; ligand-gated ion channels; GABA; glutamate; acetylcholine; glycine; serotonin; electrophysiology.

Introduction

Since their introduction into clinical practice just 150 years ago, general anaesthetics have become some of the most widely used and important therapeutic agents. However, despite over a century of research, the molecular mechanisms of action for general anaesthetics in the central nervous system (CNS) remain elusive. As a result of research during the last several decades, the ligand-gated ion channels have emerged as promising molecular targets to mediate the CNS effects of general anaesthetics. In this review, we aim to describe the actions of general anaesthetics on mammalian neurotransmitter-gated ion channels.

We will begin by summarizing the history of general anaesthesia and the chemical classes of general anaesthetics and then provide background on the physiology and pharmacology of ligand-gated ion channels. We will briefly look at experimental methodology and review the pharmacological criteria which can help define which proteins represent plausible molecular targets for general anaesthetics. We will then describe the actions of general anaesthetics on the ligand-gated ion channels. The last 10 years have witnessed an explosion of studies of anaesthetic modulation of ligand-gated ion channels, and we will focus in particular on recent studies which utilize recombinant chimeric and mutated receptors to identify regions of ligand-gated ion channels important for the modulatory actions of general

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anaesthetics. Lastly, we will discuss future directions in this area of research.

What is a general anaesthetic?

General anaesthetics include a startling range of structurally diverse molecules that can be roughly, and somewhat arbitrarily, divided into volatile anaesthetics, anaesthetic gases, alcohols and intravenous anaesthetics (fig. 1). A surprisingly elusive question is what defines a general anaesthetic, since anaesthesia is a behavioral state easily recognized but difficult to describe precisely. Depending on the clinical procedure, effective anaesthesia requires varying degrees of immobility, amnesia, unconsciousness/hypnosis, analgesia, muscle relaxation and depression of autonomic reflexes [1]. No general anaesthetic provides all of these effects, although immobility, unconsciousness/hypnosis and amnesia are behavioral hallmarks of most general anaesthetics [2]. Modern anaesthesia involves not only general anaesthetics but also the use of multiple supplemental agents including analgesics (e.g. opiates) and neuromuscular blockers.

Specific versus nonspecific mechanisms of anaesthetic action

The observation that a spectrum of chemically dissimilar agents produce general anaesthesia greatly influenced the thinking of early investigators seeking to explain anaesthetic mechanisms of action. A landmark series of experiments reported independently by Hans Meyer and Charles Ernest Overton around the turn of the century determined that the potencies of general anaesthetic molecules correlated well with their water/oil partition coefficients [3–5]. The so-called Meyer-Overton correlation was later extended to embrace the concept that certain molecules produce general anaesthesia by a nonspecific mechanism. The traditional view since the time of Meyer and Overton has been that general anaesthetics exert their primary effects by dissolving in cell membranes, particularly in the CNS [6–8]. The presence of general anaesthetic molecules is thought to perturb the structural and dynamic properties of the lipid bilayer (a 'nonspecific' action), so that the function of crucial but unspecified membrane proteins is affected. 'Specific' actions of anaesthetics generally refer to direct effects of general anaesthetics

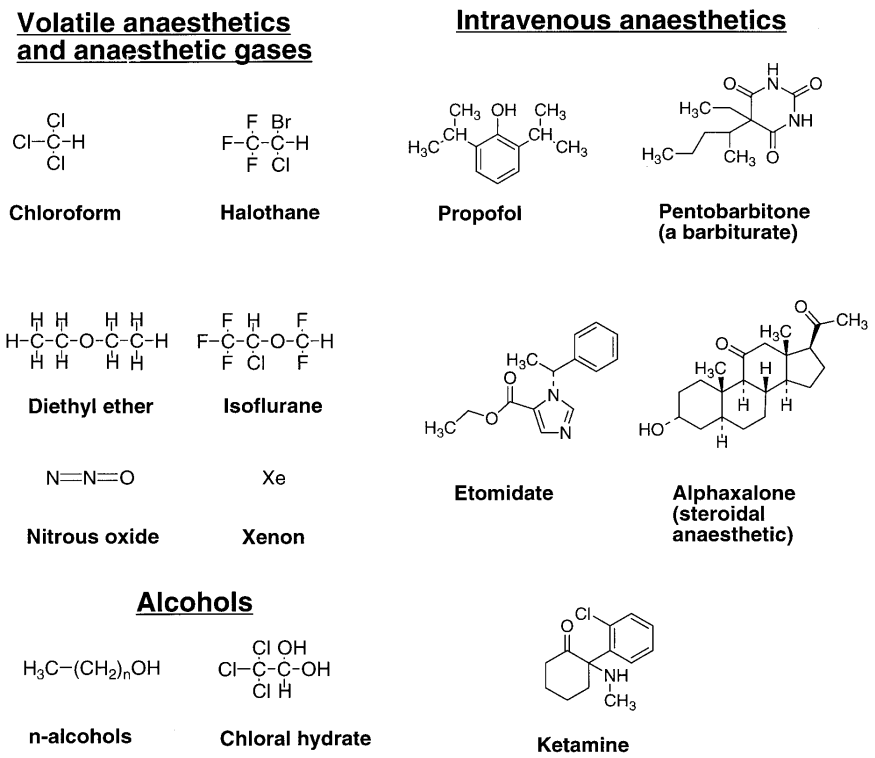

Figure 1. Chemical structures of selected general anaesthetics. Nitrous oxide is a molecule that exists in three resonating linear structures (although often misdrawn as a cyclical structure). For simplicity, we have drawn only one of the resonance forms.

on known protein molecules which result in reversible alterations in the function of the protein (e.g. increased probability of opening of an ion channel) [9].

Research within the last several decades has demonstrated numerous inconsistencies between experimental observations and nonspecific theories of general anaesthesia [9–12]. The main problems include the following [1, 13]: (i) Some chemical compounds are predicted by nonspecific theories to be anaesthetics but, in fact, do not produce anaesthesia; (ii) nonspecific theories of anaesthesia cannot account for the stereoselectivity demonstrated by some anaesthetic isomers; and (iii) anaesthetic effects on lipids (such as alterations in membrane bilayer fluidity), when measured experimentally, are often negligible at clinically relevant concentrations, and are easily reproduced by very small increases in ambient temperature. In contrast, decreases in body temperature mimic the behavioral effects of general anaesthetics [13–15]. Despite the numerous inconsistencies between the experimental evidence and nonspecific theories of anaesthesia, there have been attempts in the last decade to present modified nonspecific theories. The interested reader is best referred to some of the more recent experimental investigations and review articles in this area [16–21]. Some prescient investigators recognized a number of decades ago that anaesthetics may act instead on specific targets. For example, Sir John Eccles and colleagues studied spinal synaptic reflexes in animals under pentobarbitone anaesthesia [22, 23] and raised the possibility of anaesthetic actions at neurotransmitter receptors important in synaptic transmission.

Ligand-gated ion channels

This review summarizes recent progress in the understanding of general anaesthetic actions on receptor proteins important in synaptic transmission in the CNS. A number of excellent reviews over the last decade have summarized work on the actions of general anaesthetics on receptor proteins in the CNS [1, 13, 24–37]. We aim here to expand and update these prior reviews, with particular reference to recent studies documenting general anaesthetic actions on recombinant ligand-gated ion channels. Ligand-gated ion channels are certainly not the only possible molecular targets for general anaesthetics; other neuronal proteins such as voltage-gated ion channels and G-protein-coupled receptors may also play a role in the overall behavioral spectrum of action of general anaesthetics. However, extensive research has arrived at an almost universal consensus; voltage-gated ion channels are, in general, relatively insensitive to clinically relevant concentrations of general anaesthetics [13]. Detailed studies of general anaesthetic actions on G-protein-coupled receptors are scarce, and it can be difficult to distinguish effects on the receptor per se versus general anaesthetic perturbations of second messengers or effector molecules such as protein kinases and phospholipases.

The ligand-gated ion channels have emerged as strong candidates as molecular mediators of the CNS effects of general anaesthetics [13, 26, 27]. The ligand-gated ion channels include the gamma aminobutyric acid type A ($GABA_A$), glycine, serotonin-3 ($5\text{-}HT_3$) and nicotinic acetylcholine (ACh) receptors, along with the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-, kainate- and NMDA-sensitive subtypes of ionotropic glutamate receptors (note: gamma aminobutyric acid (GABA), glutamate, 5-HT and ACh also act on 'slow' neurotransmitter receptors, e.g. $GABA_B$, muscarinic acetylcholine and metabotropic glutamate receptors, which are coupled to second messenger systems). $GABA_A$, glycine, $5\text{-}HT_3$ and nicotinic ACh receptors form part of an evolutionarily related ligand-gated ion channel gene superfamily [38]. Ionotropic glutamate receptors were originally thought to be part of this superfamily but are now thought to belong to a distinct ion channel class (see below). All members of the ligand-gated ion channel superfamily appear to have the basic subunit topology diagrammed in figure 2, with a large N-terminal extracellular domain, four putative membrane-spanning regions (TM1–TM4), a heterogeneous intracellular loop between TM3 and TM4, and a short extracellular C-terminal domain. Residues within the extracellular N-terminal domain form the agonist binding domains [39–41], whereas amino acid residues within TM2 line the ion channel pore [42, 43] (see figs 2 and 3). Native receptors are composed of pentameric arrangements of individual receptor subunits [44, 45] (see fig. 2).

$GABA_A$ and glycine receptors

$GABA_A$ and glycine receptors are chloride-selective ion channels. These are generally considered to be inhibitory neurotransmitter receptors, since in most cells, opening of chloride channels results in membrane hyperpolarization and/or stabilization of the membrane potential away from the threshold for firing action potentials [46]. GABA and glycine are the primary fast inhibitory neurotransmitters in the CNS, with glycine abundant in the spinal cord and brainstem [40, 47] and GABA predominant in higher brain regions [46]. It has been estimated that one-third of all synapses in the CNS are GABA-ergic [48].

Subunit heterogeneity creates extensive diversity among the inhibitory ligand-gated ion channels. Multiple subunits have been cloned for $GABA_A$ (α_{1-6}, β_{1-4}, γ_{1-4}, δ, ε and π) [49–56] and glycine (α_{1-4}, β) [40, 47, 57, 58] receptors. $GABA_A$ receptors in vivo predominantly consist of α, β and γ subunits with a proposed stoichiometry of 2α: 2β: 1γ [59, 60] (see fig. 2). The exis-

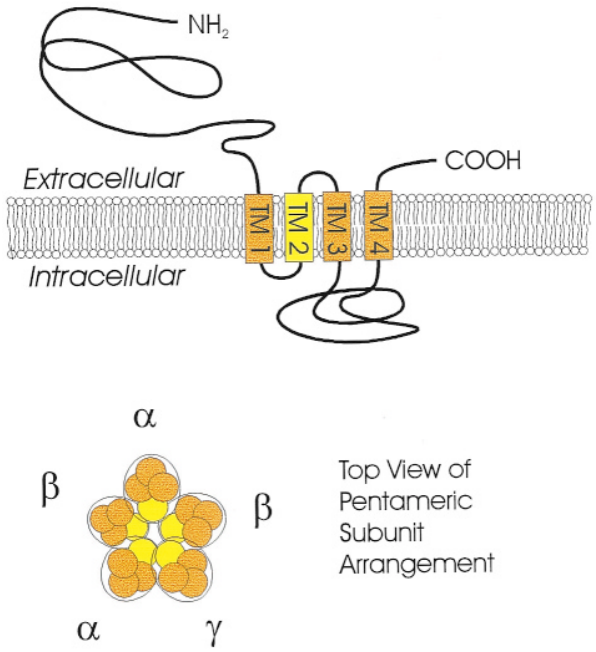

Figure 2. Illustration of the general subunit topology and pentameric structure of receptors from the ligand-gated ion channel superfamily (nicotinic ACh, $GABA_A$, $GABA_C$ ρ, glycine and $5\text{-}HT_3$ receptors). Basic features of ligand-gated ion channel subunit topology (top panel) include an extensive extracellular N-terminal domain which binds the endogenous agonist, four putative transmembrane domains, a diverse intracellular linker between TM3 and TM4, and a short extracellular C-terminal domain. Other features not illustrated include critical disulfide linkages for some receptor subunits in the N-terminal extracellular domain and consensus phosphorylation sites for some subunits in the TM3–TM4 intracellular linker. The bottom panel depicts a schematic top view of a $GABA_A$ $\alpha\beta\gamma$ receptor complex. For all ligand-gated ion channels, TM2 from each subunit (yellow) is thought to line the central ion channel pore.

tence of six α-subunit isoforms enables considerable anatomical and functional diversity of $GABA_A$ receptors [61–63]. In particular, the α-subunit isoform may influence agonist potency [64, 65], agonist efficacy [66], regulation by benzodiazepines [67] and channel kinetics [68, 69]. The most common neuronal subunit combination is $\alpha_1\beta_2\gamma_2$ [52, 56]. $GABA_A$ receptors are blocked competitively by bicuculline and noncompetitively by picrotoxinin and Zn^{2+} [39] (see fig. 3).

Strychnine-sensitive glycine receptors in vivo consist of both α homomers and $\alpha\beta$ heteromers, with a switch from homomeric to heteromeric receptors occurring during development [40, 57, 58]. The best-described role for glycine receptors is in Renshaw cell inhibition of motor neurones in the spinal cord; however, glycine receptors are also widely expressed in the brainstem and throughout higher regions of the neuraxis [57, 58].

$GABA_C$ receptors are formed from ρ subunits (ρ_{1-3}) [70–72]. $GABA_C$ receptors show greatest expression in the retina but are also found in other areas of the brain [73]. The designation of '$GABA_C$' for ρ subunits, while potentially confusing [56], follows from their extensive pharmacological differences from $GABA_A$ receptors, including insensitivity to the classical $GABA_A$ competitive antagonist bicuculline [70–72].

Nicotinic acetylcholine receptors

Nicotinic ACh receptors may be divided into two main groups: the 'muscle' subtype expressed in skeletal muscle [74, 75] and in the electroplaque of *Torpedo* [76], and 'neuronal' nicotinic ACh receptors found throughout the CNS and at autonomic ganglia [41, 77–79]. These receptors contain a nonselective cation channel. Multiple subunit isoforms have been described for muscle (α_1, β, γ, δ, ε) and neuronal (α_{2-9}, β_{2-4}) nicotinic ACh receptors [41, 75, 79, 80]. The muscle nicotinic ACh receptors mediate synaptic excitation at the neuromuscular junction [74, 75]. The physiological roles of neuronal nicotinic ACh receptors are currently an area of intense inquiry. These receptors seem likely to participate in nicotine addiction, and may perhaps be involved in neurological and psychiatric disorders, in addition to their more traditional role in the function of the sympathetic and parasympathetic nervous systems [41, 77–79]. Neuronal nicotinic ACh receptors occur at both pre- and postsynaptic loci in the CNS. Presynaptic nicotinic ACh receptors modulate the release of neurotransmitters such as GABA, 5-HT, dopamine, norepinephrine, glutamate and ACh [81]. The existence of postsynaptic neuronal nicotinic ACh receptors at sympathetic ganglia has been known for many decades [79]; more recent research has also demonstrated functional postsynaptic neuronal nicotinic ACh receptors in the CNS [82].

$5\text{-}HT_3$ receptors

$5\text{-}HT_3$ receptors are ligand-gated cation channels that are expressed in a number of central and peripheral nervous system areas, including the hippocampus, brainstem, dorsal root ganglia (cell bodies of sensory neurones) and vagal axons [83]. $5\text{-}HT_3$ receptors are expressed efficiently as homomers in heterologous expression systems such as *Xenopus* oocytes, but there is evidence that $5\text{-}HT_3$ receptors in vivo may be heteromeric, indicating that additional subunits or splice variants have yet to be characterized [84]. The most unambiguous physiological role for $5\text{-}HT_3$ receptors in humans is in the medullary circuitry subserving the vomiting reflex, which is consistent with robust expression of the $5\text{-}HT_3$ receptor in the nucleus tractus solitarius and area postrema [83, 85]. $5\text{-}HT_3$ receptor antagonists such as ondansetron are used clinically to prevent nausea and vomiting [86]. Activation of $5\text{-}HT_3$ receptors also modulates extracellular dopamine levels in

the nucleus accumbens, and this may be involved in the rewarding properties of certain drugs of abuse [87]. 5-HT_3 receptors may also play a role in nociception [85]; for example, some nociceptive primary afferents express 5-HT_3 receptors, and activation of these receptors facilitates the response of some dorsal horn neurones to noxious stimuli [88].

Ionotropic glutamate receptors

The ionotropic glutamate receptors consist of the AMPA-, NMDA- and kainate-sensitive subtypes. Glutamate receptors were originally assumed to have a membrane topology and pentameric structure similar to that proposed for the original ligand-gated ion channel superfamily. More recent work suggests instead a subunit topology that includes a reentrant loop forming the ion channel pore [89–92] analogous to the reentrant pore loop associated with the S5–S6 'signature sequence' of the voltage-gated potassium channels [93]. Tetrameric structures have been proposed for AMPA [94] and NMDA receptor [95] complexes. Once again, the proposed tetrameric structure is much more similar to the voltage-gated potassium channels [96] than to the members of the ligand-gated ion channel gene superfamily discussed above.

NMDA receptors (NR1, NR2A-2D, NR3A) [97–100] are ligand-gated cation channels with several unusual

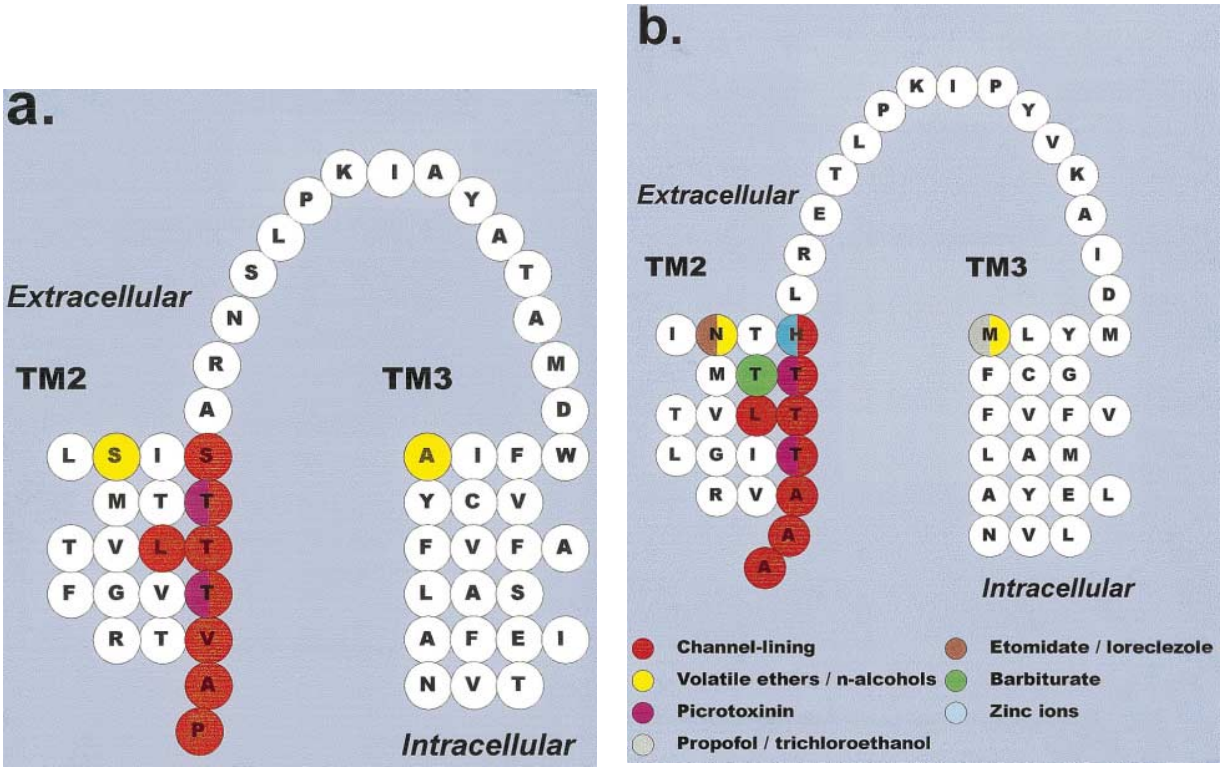

Figure 3. Location of amino acid residues within TM2 and TM3 of (*a*) human $GABA_A$ α_1 [280] and (*b*) human $GABA_A$ β_2 [281] receptor subunits that are critical for general anaesthetic modulation or block by the noncompetitive antagonists picrotoxinin and Zn^{2+}, in addition to amino acid residues which are thought to line the ion channel pore. $GABA_A$ α_1 and β_2 subunit isoforms are chosen since they represent the most common neuronal α and β subunit isoforms [52, 56]. The residue positions are from published studies: channel-lining residues [42, 282], volatile ethers (enflurane [219] and isoflurane [206, 219]), n-alcohols [219], picrotoxinin [283], propofol [206], trichloroethanol [227], etomidate [228, 229], loreclezole [284], barbiturate (pentobarbitone) [261] and zinc ions [285]. Note that some of the residue positions highlighted were actually first described in α- or β-subunit isoforms different from α_1 or β_2. To date, detailed three-dimensional structural information about TM2, the TM2–TM3 linker, and TM3 is lacking, so that the ordering of residues is hypothetical only. For example, the spatial interrelationship between TM2 and TM3 in a functional $GABA_A$ receptor complex is currently unknown.

features including, most notably, high Ca^{2+} permeability [101] and strong voltage-dependent block by Mg^{2+} ions [102–104]. NMDA receptors have attracted a great deal of attention due to their involvement in the induction of long-term potentiation in the CA1 subfield of the hippocampus and other areas of the cerebral cortex [105–107]. NMDA receptors are proposed to be involved in learning and memory [108, 109]. NMDA receptors are also implicated in certain forms of neurotoxicity and in the etiology of several neurodegenerative disorders [110–113]. The pharmacology of the NMDA receptors has been extensively characterized, and a number of substances modify NMDA receptor function, including the coagonist glycine [114, 115], polyamines [116, 117], Zn^{2+} [118, 119], protons [120, 121], fatty acids [122, 123] and oxidizing/reducing agents [124].

AMPA receptors, represented in neurones by combinations of the gene products GluR1–4, appear to serve as the major fast excitatory neurotransmitter receptors at most synapses in the CNS [113, 125]. Synaptic AMPA receptors respond to glutamate quickly and transiently, due to diffusion and rapid removal of glutamate from the synaptic cleft, in addition to fast receptor desensitization [126]. AMPA receptors are therefore ideally suited for their role in excitatory transmission on a millisecond timescale. The physiological roles of kainate glutamate receptors (GluR5–7, KA1, KA2) are less clear at present, even though kainate receptors are widely distributed throughout the brain and spinal cord. Recent work provides strong evidence for the synaptic activation of kainate receptors at both pre- and postsynaptic sites [127, 128]. The study of AMPA and kainate receptors in the CNS was hampered for a long time by a lack of selective antagonists. Recent development of selective AMPA receptor antagonists has remedied this problem to some extent [128, 129]. An experimental obstacle to study of some of the kainate and AMPA receptors is desensitization on the millisecond to submillisecond timescale [130–132]. This complicates the interpretation of many studies, for example those employing heterologous expression of kainate and AMPA receptors in *Xenopus* oocytes, in which agonist may be applied for seconds to minutes.

Pharmacological criteria that a candidate receptor must meet to be considered as a reasonable general anaesthetic target

Before discussing the actions of specific agents on ligand-gated ion channels, it is worthwhile to define specific criteria that an anaesthetic target (receptor protein or otherwise) must fulfill in order to qualify as a candidate in mediating the behavioral actions of the general anaesthetics [1, 13].

1) The general anaesthetic must alter the function of the receptor at clinically relevant concentrations.
2) The receptor must be expressed in the appropriate anatomical locations to mediate the specific behavioral effects of the anaesthetic.
3) If an anaesthetic molecule shows stereoselective effects in vivo, these should be mirrored by the in vitro actions at the receptor.
4) The hydrophobicity of a compound within a homologous series of anaesthetics should correlate with the in vivo anaesthetic potency and that at the target receptor.

The general anaesthetic must alter the function of the receptor at clinically relevant concentrations. What is the 'clinically relevant concentration' for a general anaesthetic? For an inhaled anaesthetic such as isoflurane, 1 minimum alveolar concentration (MAC) conventionally refers to the concentration of inhaled anaesthetic that produces immobility in 50% of animals studied [133, 134]. Immobility, a lack of purposeful response to a noxious stimulus, represents an easily determined endpoint across a large variety of different animal species. The use of immobility as an experimental endpoint is helpful in that, for most general anaesthetics, anaesthetic concentrations two- to fourfold above the EC_{50} (concentration of a compound which produces 50% of the maximal effect) for producing immobility are invariably lethal [13]. The anaesthetic concentrations that produce significant inhibition of cognitive functions and cortical activity, assessed using EEG-derived indicators, are lower than those required for producing immobility [135–137]. Thus, anaesthetic concentrations severalfold greater than those that produce immobility define the upper boundary of the concentration range that is clinically relevant. For a target to have any relevance for anaesthesia, it must at least be sensitive to sublethal but immobilizing concentrations of anaesthetics. This issue of relevant concentrations alone poses a severe challenge to the plausibility of 'lipid' theories of anaesthetic action, since 'nonspecific' effects of general anaesthetics (e.g., disruption of lipid bilayer fluidity) appear to be negligible at clinically relevant concentrations [13–15].

While the issue of relevant concentrations is obviously of paramount importance to molecular studies of general anaesthetics, the physicochemical and pharmacokinetic properties of the various anaesthetic drugs pose some obstacles to the determination of relevant concentrations. We will therefore outline the basic issues involved in the determination of accurate clinically relevant anaesthetic concentrations. This will provide a background to our later discussion of those ligand-gated ion channels that are modulated by clinically relevant concentrations of general anaesthetics.

Volatile anaesthetic potency is usually quantified in terms of MAC [133, 134]. MAC values (often expressed in the operating room in terms of percentage of anaesthetic gas

by volume) can be converted to 'aqueous' MAC equiva lent concentrations' by use of the approgated ion channels that are modulated by clinically relevant concentrations of general anaesthetics.

Volatile anaesthetic potency is usually quantified in terms of MAC [133, 134]. MAC values (often expressed in the operating room in terms of percentage of anaesthetic gas by volume) can be converted to 'aqueous' MAC equivalent concentrations' by use of the appropriate water/gas (or blood/gas) partition coefficients [28, 138]. This provides an estimate for the concentration of anaesthetic in the blood that is in equilibrium with the inspired partial pressure of anaesthetic in the gas phase. Franks and Lieb [28, 138] have thoroughly discussed the conversion of MAC values to aqueous equivalents, including the nettlesome issue of experimental temperature [138, 139]. Aqueous MAC equivalents are often used as guides for in vitro experiments which involve the study of volatile anaesthetics in aqueous solution [13, 28, 138].

The issue of clinically relevant concentrations for the intravenous anaesthetics and the alcohols in mammals is considerably more complicated because of pharmacokinetic aspects of these drugs and the difficulty of ascertaining steady-state drug concentrations in the brain [13]. In some cases (e.g. for propofol and the barbiturates), detailed pharmacokinetic studies have addressed these issues, and reasonable free anaesthetic concentrations in brain can be estimated [13]. In other cases (e.g. ketamine and the steroid anaesthetic alphaxalone), only total anaesthetic concentrations in blood are known, thus invariably underestimating anaesthetic potency in the brain of this class of anaesthetics, often by as much as one to two orders of magnitude [140, 141].

Table 1 lists concentrations of general anaesthetics that represent the EC_{50} value for producing immobility in a variety of animal species. There is a growing database of studies that determine anaesthetic concentrations needed to produce other anaesthetic endpoints involving higher cortical functions [136, 137, 142]. However, such data are not yet available for all anaesthetics. In some cases (see table 1 and accompanying legend), no mammalian data are available, or the mammalian data are likely to be incorrect, due to significant pharmacokinetic issues. In these cases, we have reported the values for tadpoles, in which pharmacokinetic impediments are considerably attenuated [13, 143].

The receptor must be expressed in the appropriate anatomical locations to mediate the specific behavioral effects of the anaesthetic. This is a more difficult issue to discuss, since there is considerable debate about precisely which synaptic circuits are responsible for the various behavioral functions perturbed by general anaesthetics. The immobility produced by general anaesthetics, perhaps not surprisingly, appears to involve depression of spinal reflex pathways, since it is independent of drug actions in the brain [144–146]. Receptors such as $GABA_A$ and AMPA receptors are promising general anaesthetic targets due to

Table 1. Clinically relevant concentrations of general anaesthetics.

a. Volatile anaesthetics and anaesthetic gases	Anaesthetic concentration
Chloroform	0.86 mM [286–288]
Diethyl ether	10.5 mM [286–288]
Enflurane	0.49 mM [287–289]
Halothane	0.20 mM [133, 288, 289]
Isoflurane	0.26 mM [133, 288, 289]
Methoxyflurane	0.27 mM [286–288]
Nitrous oxide	27.4 mM [290]
Sevoflurane	0.26 mM [291–293]
Xenon	3.7 mM [242]
b. Intravenous agents	**Anaesthetic concentration**
Alphaxalone (steroid anaesthetic)	<5.8 μM [140] (humans) 3.4 μM [294] (tadpoles)
Etomidate	<8.7 μM [275] (human) 3.4 μM [15] (tadpoles)
Propofol	0.4 μM [13] (humans)
Ketamine	9.3 μM [277] (humans) 59 μM [295] (tadpoles)
Methohexitone (barbiturate)	11–38 μM [276] (humans)
Pentobarbitone (barbiturate)	50 μM [13] (mice)
Thiopentone (barbiturate)	25 μM [13] (humans)
c. Alcohols	**Anaesthetic concentration**
C1 (methanol)	441 mM [143, 278]
C2 (ethanol)	138 mM [143, 278, 296]
C3 (propanol)	64 mM [143, 296]
C4 (n-butanol)	10 mM [143, 278, 296]
C5 (n-pentanol)	2.9 mM [143]
C6 (n-hexanol)	690 μM [143, 278, 296]
C7 (n-heptanol)	230 μM [143]
C8 (n-octanol)	48 μM [143, 278, 296]
C9 (n-nonanol)	37 μM [143]
C10 (n-decanol)	13 μM [143, 296]
C11 (n-undecanol)	8.1 μM [143]
C12 (n-dodecanol)	5.1 μM [143, 296]
C13 (n-tridecanol)	inactive [143, 297]
Trichloroethanol (metabolite of chloral hydrate)	0.2–1 mM [298–300] (mammals)

For the volatile ethers and halothane, anaesthetic concentrations are averages of multiple MAC determinations in mammals [13, 28, 138]. Solubility coefficients and methods for converting MAC values (in partial pressures) to aqueous MAC equivalents at 20 °C are described by Franks and Lieb [28, 138]. Mammalian anaesthetic values for alphaxalone [140], etomidate [275] and methohexitone [276] reflect total anaesthetic concentrations in blood with the extent of plasma protein binding uncertain. It is also unclear whether steady-state levels were reached in these studies. The stated concentrations thus likely substantially underestimate anaesthetic potency, possibly by as much as two orders of magnitude. The human anaesthetic value for ketamine [277] indicates stable plasma concentrations in patients maintained on ketamine anaesthesia supplemented with nitrous oxide. The anaesthetic concentration for ketamine alone may be double this value, since ketamine was combined with nitrous oxide. Anaesthetic concentrations for n-alcohols are averages of multiple studies. All values for n-alcohols are from tadpoles except for one study in rats [278].

their ubiquitous distribution and essential physiological roles as the major fast transmitters of the CNS. However, given the uncertainty concerning the exact anatomy of the synapses that are disrupted to produce the constellation of behavioral effects seen during general anaesthesia, receptors with more limited distribution (e.g. 5-HT_3 receptors) may certainly play major roles as molecular mediators of the general anaesthetic state.

If an anaesthetic molecule shows stereoselective effects in vivo, these should be mirrored by the in vitro actions at the receptor. Stereoselectivity represents an important test for the relevance of a putative anaesthetic target [13, 147]. A number of general anaesthetic molecules possess a chiral carbon atom, and some pairs of stereoisomers exert different anaesthetic potencies in vivo. Stereoselectivity for producing immobility has been documented for the isomers of etomidate [15, 148] (see fig. 4), the barbiturates [149], isoflurane [150, 151] (although see [152]), ketamine [153, 154] and steroid anaesthetics [155]. The potency differences are greatest for stereoisomers of etomidate and ketamine (greater than 10-fold), with smaller potency differences (sometimes only 2-fold or less) seen for other anaesthetic isomers. The formulation of these anaesthetics is usually based on the racemic mixture due to the difficulty of separating enantiomers in large quantities (an exception is etomidate, which is prepared by a chiral synthesis [148]). Production of pure enantiomers perhaps would improve the clinical profile for some general anaesthetics [156], although cost considerations probably preclude such an outcome.

General anaesthetic stereoselectivity poses the most severe challenge yet to the 'traditional' lipid theories of anaesthetic action. The optical isomers of isoflurane [157] and etomidate [15], despite significant differences in their in vivo potency (see fig. 4), behave identically with respect to their ability to disorder lipid bilayers. In contrast, stereoselectivity supports the plausibility of the $GABA_A$ receptor as a target in mediating the actions of etomidate [15], barbiturates [158, 159], isoflurane [160, 161], and the steroid anaesthetics [155, 162], since in vivo potency and activity at the $GABA_A$ receptor display identical trends. The in vivo stereoselectivity of ketamine stereoisomers is paralleled by the inhibitory action of the isomers at the NMDA receptor [163]. Interestingly, two enantiomers of pentobarbitone display opposing stereoselectivity for inhibition of the muscle nicotinic ACh receptor relative to their in vivo potency [164]. The structure-activity relationships for barbiturate inhibition of the muscle nicotinic ACh receptor also correlate poorly with in vivo potency [165] which effectively eliminates the muscle-type nicotinic ACh receptor as a plausible target for barbiturate action. This is perhaps not surprising, since barbiturates (and, indeed, most other general anaesthetics) do not inhibit neuromuscular transmission to any substantial degree, suggesting little or no functional block of the muscle nicotinic ACh receptor at anaesthetic concentrations [2, 13]. The probable lack of relevance of muscle nicotinic ACh receptors for the actions of general anaesthetics certainly does not rule out the possibility that general anaesthetic actions on neuronal nicotinic ACh receptors may play a major role in the behavioral actions of general anaesthetics. The muscle and neuronal nicotinic ACh receptors, despite sharing a common agonist, have quite distinct structural and functional properties. Despite the rewards of studying general anaesthetic stereoisomers, exemplified by the etomidate work outlined above [15] (see fig. 4), the stereoselectivity approach has been underutilized, mainly due to the limited supply and expense of purified stereoisomers [156]. Furthermore, only limited anaesthetic endpoints (mainly immobility) have been assessed for the anaesthetic stereoisomers. It would be quite interesting to know whether the additional neurobiological actions of anaesthetics (e.g. amnesia, analgesia) display similar patterns of stereoselectivity.

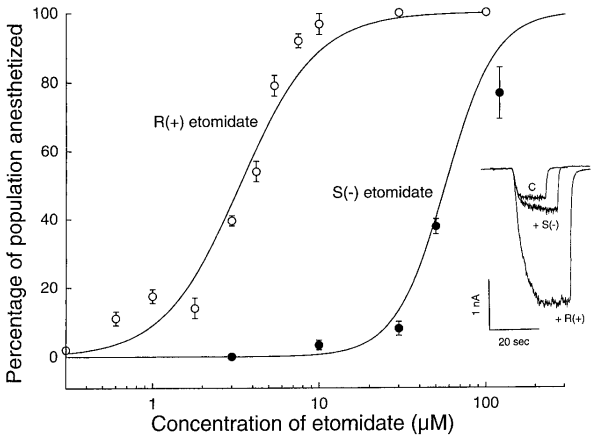

Figure 4. The selectivity of etomidate optical isomers for producing general anaesthesia in tadpoles mirrors the selectivity for potentiation of $GABA_A$ receptor function. The main graph illustrates the concentration-response curves for immobility produced by etomidate stereoisomers in *Rana temporaria* tadpoles. Note that the in vivo potency of R(+) etomidate is approximately one order of magnitude greater than that of S(−) etomidate (also see [148]). The inset depicts electrophysiological traces from GABA responses at bovine $GABA_A$ $\alpha_1\beta_1\gamma_{2L}$ receptors stably transfected in mouse L-cell fibroblast cells. Coapplication of R(+) etomidate produces a vastly greater enhancement of the control submaximal GABA response (C) than coapplication of S(−) etomidate. Figure from: Tomlin S. L., Jenkins A., Lieb W. R. and Franks N. P. (1998) Stereoselective effects of etomidate optical isomers on gamma-aminobutyric acid type A receptors and animals. Anesthesiology **88:** 708–717. Reproduced in adapted form with permission of the authors and Lippincott-Raven Publishers, 227 East Washington Square, Philadelphia, PA 19106-3708, USA.

The hydrophobicity of a compound within a homologous series of anaesthetics should correlate with the in vivo anaesthetic potency and that at the target receptor. The so-called Meyer-Overton hypothesis, which led to the adoption of the traditional dogma concerning lipid mechanisms of anaesthesia, arose from the fundamental observation that the in vivo potency of general anaesthetics rises in parallel with increasing hydrophobicity of the anaesthetic molecules. This trend is most noticeable with the homologous series of n-alcohols (see table 1c) but also holds true for diverse anaesthetic molecules with oil/water partition coefficients varying over numerous orders of magnitude [3–5]. General anaesthetic actions at a plausible receptor target should, therefore, exhibit similar trends.

The Meyer-Overton correlation was traditionally interpreted to suggest nonspecific mechanisms of action for general anaesthetics in membrane lipids; however, an alternative explanation is that anaesthetics bind to hydrophobic domains of receptor proteins [9, 13]. For example, amino acid residues of hydrophobic character within the transmembrane domains of ligand-gated ion channels would be likely candidates to interact with general anaesthetics. As will be discussed below, a number of amino acid residues have been identified within ligand-gated ion channels that are critical for the modulatory actions of some general anaesthetics. Many of these amino acid residues are proposed to lie either within a transmembrane domain or else at the membrane interface.

A major problem for traditional theories arose with the discovery of hydrophobic compounds that disobey the Meyer-Overton 'rule' [166]. These nonanaesthetics or nonimmobilizers would be expected to partition extensively into the lipid bilayer yet produce no general anaesthetic action. The nonimmobilizers provide additional clues as to which receptor targets might underlie the behavioral actions of general anaesthetics (see below).

Experimental approaches to studying general anaesthetic actions at ligand-gated ion channels

General anaesthetic actions at ligand-gated ion channels have been studied using a variety of methodologies, including protein chemistry, radioligand binding, ion flux studies and electrophysiology [13, 24, 27]. We will focus mainly on electrophysiological studies since these, in general, provide superior time resolution and also offer the possibility of analyzing isolated cells or even single ion channels. The general anaesthetics have properties which limit the utility of other experimental techniques. For example, specific binding of radiolabeled general anaesthetics to ligand-gated ion channels has proven exceedingly difficult to demonstrate due to low affinity and high nonspecific binding to neuronal membranes [13, 24, 27], although allosteric effects of general anaesthetics have been monitored using radioligand binding of drugs to other sites on the ligand-gated ion channels (e.g. [167, 168]). In addition, limited progress has been made in developing anaesthetic congeners useful for photoaffinity labeling or other covalent modification of receptors (although see [169]). These limitations contrast starkly with the studies of other classes of agents at ligand-gated ion channels. For instance, the high-affinity benzodiazepine binding site on the $GABA_A$ receptor has been mapped out in some detail due to the ability to perform both specific radioligand binding and photoaffinity labeling [170, 171], which powerfully complements the extensive body of literature on electrophysiological actions of benzodiazepines at $GABA_A$ receptors [170].

Another exciting tool in the quest to establish the in vivo significance of a putative anaesthetic target is the use of targeted gene manipulations in mice [172]. A variety of manipulations are possible, including introducing a gene not normally present (transgenic mice), removing an endogenous gene ('knockout mice'), or replacing an endogenous gene with an altered copy ('knock-in mice') [172]. Gene targeting in mice has already been very valuable for elucidating the mechanism of action for some drugs. Knockout of the $GABA_A$ γ_2 receptor subunit gene resulted in mice which were effectively insensitive to the sedative/hypnotic actions of benzodiazepines such as diazepam [173]. The γ_2 subunit gene knockout, in conjunction with the dependence of benzodiazepine modulation of the $GABA_A$ receptor on the presence of a γ subunit [174], effectively demonstrates the $GABA_A$ receptor as a major target mediating the sedative/hypnotic actions of benzodiazepines. Another gene-targeting experiment in mice involved the replacement of the α_{2a}-adrenoreceptor with a dysfunctional receptor mutant. These knock-in mice failed to show analgesic and sedative responses to α_{2a}-adrenoreceptor agonists such as dexmedetomidine and clonidine [175].

Knockout mice lacking subunit genes for $GABA_A$ (α_6, β_3, γ_2, γ_{2L}) [173, 176–179], neuronal nicotinic ACh (α_7, β_2) [180, 181], AMPA (GluR2) [182], NMDA (NR1, NR2A, NR2C, NR3A) [183–186] and kainate receptors (GluR6) [187] have already been created, and the study of such mice has enhanced understanding of the physiological roles of the particular receptor subunit. For example, mice homozygous for a deletion of the $GABA_A$ receptor β_3 subunit gene exhibit cleft palate, absence seizures, hyperexcitability [177, 188] and some resistance to the immobilizing actions of intravenous and volatile anaesthetics [189].

Actions of general anaesthetics at ligand-gated ion channels

General anaesthetics act as positive or negative allosteric modulators of agonist actions at ligand-gated

ion channels. Among the ligand-gated ion channels, there is no known case in which the anaesthetic competes for the same binding site as the endogenous neurotransmitter. The most extensively examined ligand-gated ion channel target for general anaesthetics has been the $GABA_A$ receptor [13, 24, 27]. Virtually every general anaesthetic tested enhances the function of the $GABA_A$ receptor at clinically relevant concentrations [13, 27, 190] (except for ketamine [191], xenon [192] and possibly nitrous oxide [193–195]). General anaesthetic enhancement of $GABA_A$ receptor function is evident in single-cell electrophysiological experiments as potentiation of a submaximal GABA response (see fig. 5) or, at the synaptic level, as prolongation of inhibitory postsynaptic potentials [196, 197] or currents (see fig. 6) [160, 198–200]. Potentiation of submaximal GABA-induced currents remains the most popular assay for electrophysiological experiments since it is easily reproducible and can be used to study native $GABA_A$ receptors in dissociated neurones or recombinant receptors expressed in mammalian cell lines or *Xenopus* oocytes [13, 24, 27].

Some anaesthetics, particularly the intravenous agents, open the $GABA_A$ receptor chloride channel in the absence of agonist [201–214]. This 'direct activation' by general anaesthetics involves a binding site completely distinct from that for classical $GABA_A$ receptor agonists such as GABA and muscimol [215]. Although direct activation usually occurs at supraclinical concentrations, direct activation effects do sometimes occur at lower concentrations for some anaesthetics (e.g. propofol), suggesting possible clinical relevance. Direct activation by anaesthetics has been observed in other ligand-gated ion channels (e.g. for the anaesthetic isoflurane at the strychinine-sensitive glycine receptor [216]) but is most pronounced at the $GABA_A$ receptor.

The cloning of multiple subunit isoforms for the ligand-gated ion channels in the last decade has precipitated an explosion of studies of general anaesthetic actions on recombinant receptors. Table 2 summarizes the electrophysiological effects of general anaesthetics on a range of ligand-gated ion channels studied in neurones or in various expression systems.

A difficult issue to address is how much alteration of receptor function by a general anaesthetic is necessary to produce certain behavioral actions. For example, even though the EC_{50} or IC_{50} (concentration of antagonist that reduces the response to a sub-maximal concentration of agonist by 50%) for alteration of the function of a particular receptor by an anaesthetic may be well outside the clinically relevant range (the upper limit of this range is defined by the anaesthetic concentration that produces immobility in 100% of subjects), the anaesthetic may nevertheless produce slight alteration of receptor function within the clinically relevant concentration range [13]. Thus, in table 2 we have distinguished between complete and relative lack of sensitivity of a particular

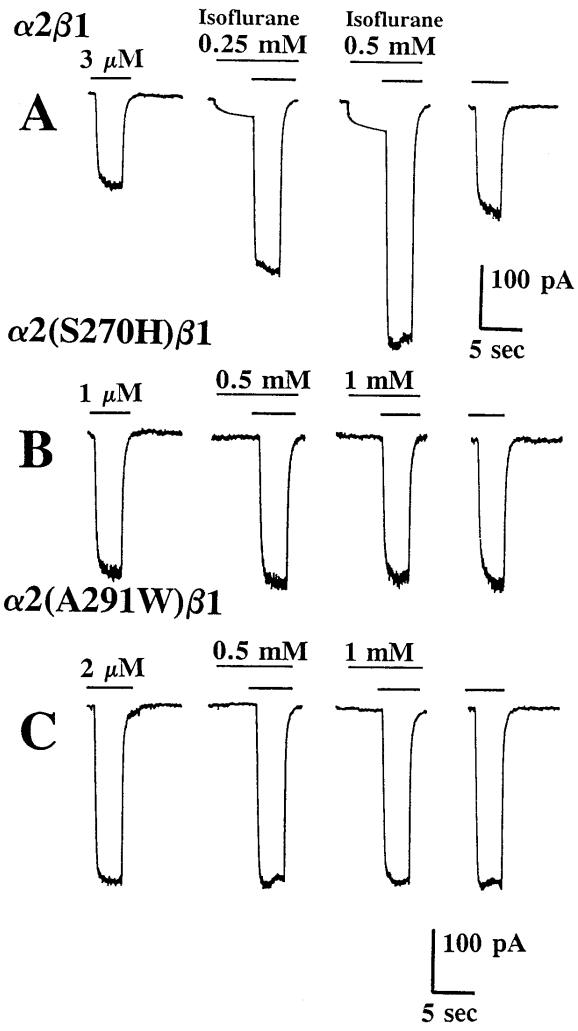

Figure 5. Specific mutations in TM2 or TM3 of the human $GABA_A$ α_2 subunit abolish positive allosteric modulation by the volatile anaesthetic isoflurane at $GABA_A$ $\alpha_2\beta_1$ receptors. (*A*) Submaximal GABA currents in wild-type $GABA_A$ $\alpha_2\beta_1$ receptors are strongly enhanced (i.e. potentiated) by coapplication of clinically relevant concentrations of isoflurane. (*B*, *C*) In contrast, submaximal GABA currents in α_2(S270H)β_1 or α_2(A291W)β_1 mutant receptors are not enhanced by coapplication of isoflurane concentrations up to 1 mM. Thus, these mutant receptors are insensitive to GABA potentiation by isoflurane even at supraanaesthetic concentrations. Individual whole-cell voltage-clamp recordings from human embryonic kidney 293 cells transfected with cDNAs encoding the indicated subunit combination. Figure from: Krasowski M. D., Koltchine V. V., Rick C. E., Ye Q., Finn S. E. and Harrison N. L. (1998) Propofol and other intravenous anesthetics have sites of action on the γ-aminobutyric acid type A receptor distinct from that for isoflurane. Mol. Pharmacol. **53:** 530–538. Reproduced with permission of the authors and the American Society for Pharmacology and Experimental Therapeutics, 9650 Rockville Pike, Bethesda, MD 20814-3995, USA.

receptor to clinically relevant anaesthetic concentrations. In order to qualify for inclusion in table 2, a study had to (i) assess the effects of several different anaesthetic concentrations in order to derive an estimate for the EC_{50} or IC_{50} (concentration of antagonist that reduces the response to a sub-maximal concentration of agonist by 50%) for modulation and (ii) offer a reasonable certainty of examining a 'pure' receptor population. The latter concern is especially acute with the AMPA and kainate subtypes of glutamate receptors, for which there has been until recently a relative dearth of selective agonists and antagonists. Kainate itself activates both AMPA and kainate receptors, and this may confound electrophysiological studies which utilize kainate application to neurones.

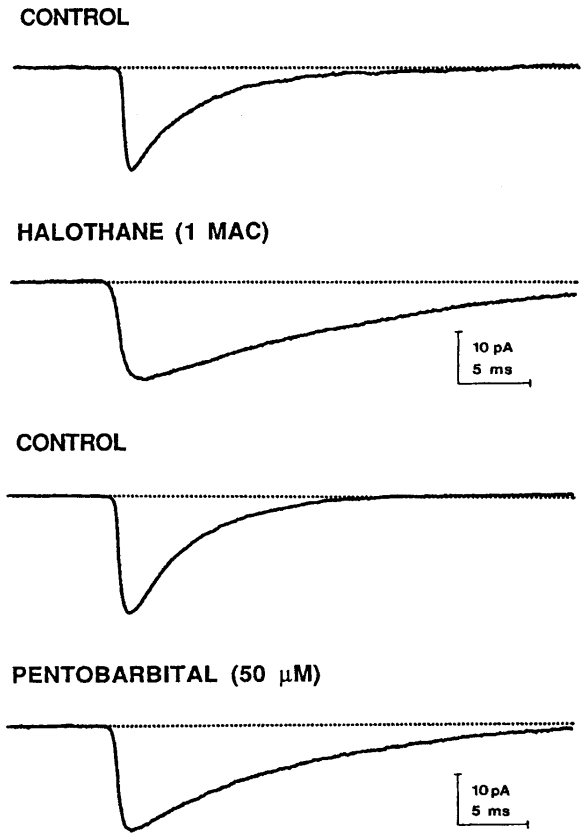

Figure 6. Both the volatile anaesthetic halothane and the intravenous anaesthetic pentobarbitone prolong inhibitory postsynaptic currents (IPSCs) mediated by $GABA_A$ receptors. Data were obtained from whole-cell patch-clamp recordings of rat hippocampal neurones from brain slices. Average records from whole-cell voltage-clamp recordings in hippocampal neurones of 100 individual spontaneous IPSCs for each trace show the prolongation of the decay phase of the IPSC produced by halothane and pentobarbitone. Data from halothane and pentobarbitone are from different neurones and preparations. Figure from: MacIver M. B., Tanelian D. T. and Mody I. (1991) Two mechanisms for anesthetic-induced enhancement of $GABA_A$-mediated neuronal inhibition. Ann. N. Y. Acad. Sci. **625:** 91–96. Reproduced with permission of the authors and the *Annals of the New York Academy of Sciences*, 655 Madison Avenue, New York, NY 10021, USA.

The advent of cloning and recombinant expression techniques has greatly accelerated and facilitated attempts to classify ligand-gated ion channel sensitivity to general anaesthetics. Molecular biology techniques may now be used to determine which regions of ligand-gated ion channels are critical for anaesthetic modulation. Sensitivity to general anaesthetics varies considerably, sometimes even among closely related receptors (table 2), and this forms the basis for the use of 'chimeric' receptors to isolate regions of a receptor essential for anaesthetic modulation. Chimeric receptors are created by joining together, at the complementary DNA (cDNA) level, complementary fragments of receptor subunits, in which the parental subunits exhibit markedly different anaesthetic pharmacologies. The analysis of chimeric receptors can be used to delimit a region of a receptor essential for general anaesthetic modulation, after which site-directed mutagenesis can be used to identify key residues. Chimeric receptors constructed to date include panels of $GABA_A$/glycine [217], $GABA_A$/$GABA_C$ ρ [218], glycine/$GABA_C$ ρ [219, 220], neuronal nicotinic ACh/5-HT_3 [221, 222] and AMPA GluR3/kainate GluR6 [223] receptors. The most extensive sets of chimeras created and functionally expressed for analysis of anaesthetic modulation are glycine/$GABA_C$ ρ [219, 220] and GluR3/GluR6 [223] chimeras.

Several problems may arise in the study of such chimeric receptors, including (i) lack of functional expression (greatly reduced or absent responses to agonist), (ii) chimeric receptor function that differs radically from the constituent parent receptors, and/or (iii) ambiguous pharmacological data. The first problem has substantially limited the utility of $GABA_A$/$GABA_C$ [224] and neuronal nicotinic ACh/5-HT_3 chimeras [225]; for instance, chimeras formed between the nicotinic ACh α_7 subunit and the 5-HT_3 receptor show functional expression only when the nicotinic α_7 receptor subunit provides the N-terminal half but not vice versa [225]. Lack of functional chimeric receptor responses could potentially be due to protein folding or assembly problems, impaired ion permeation leading to very low single-channel conductance and/or a minuscule probability of opening following agonist binding (i.e. a defect in ion channel gating). Folding and assembly problems probably predominate and seem especially likely to occur when blending heteromeric with homomeric receptors (e.g. $GABA_A$ with $GABA_C$ receptors). Despite these potential pitfalls, the use of chimeric receptors has already helped to define putative sites of general anaesthetic action on some of the ligand-gated ion channels (see below).

Table 2. Modulatory effects of general anaesthetics on ligand-gated ion channels.

a. Volatile anaesthetics and anaesthetic gases	$GABA_A$	Glycine	GABA ρ_1	Muscle nAChR	Neuronal nAChR	$5\text{-}HT_3$	AMPA (GluR1–4)	Kainate (GluR5–7; KA1, KA2)	NMDA
Chloroform	+++ [190, 218]	+++ [301]	0 [218]	unknown	−−− [279]	unknown	−−− [302]	unknown	0 [302]
Diethyl ether	+++ [303]	+++ [301]	−/0*	−/0 [304]	−−− [279]	+++ [305]	−−− [302]	unknown	−−− [302]
Enflurane	+++ [190, 219, 232, 303, 306–309]	+++ [216, 219, 233, 307]	−/0 [219, 234]	−−− [239, 304, 310]	−−− [279]	+++ [311]	−−− [237, 307, 312]	+++ [31, 223, 237, 312]	−/0 [31, 307, 312]
Halothane	+++ [139, 190, 303, 307–309, 313]	+++ [216, 233, 301, 307, 314]	−/0 [234]	−/0 [304, 310, 315]	−−− [222, 279, 315, 316]	+++ [222, 235, 311]	−−− [30, 237, 302, 307, 317]	+++ [223, 237]	−/0 [30, 31, 302, 307, 317]
Isoflurane	+++ [139, 161, 190, 206, 303, 308, 309, 313, 318, 319]	+++ [216, 318]	−/0 [234, 318]	−/0 [304, 310, 315, 320]	−−− [222, 236, 279, 315, 316, 321]	+++ [222, 235, 311]	−−− [302]	+++ [223]	−/0 [31, 302]
Methoxyflurane	+++ [139, 313]	+++ [216]	0*	−−− [304, 310]	−−− [279, 316, 322]	+++ [311]	unknown	unknown	unknown
Nitrous oxide	+++ [193] 0 [194, 195]	+/0 [301]	unknown	−−− [310]	unknown	unknown	−/0 [194]	unknown	−−− [194, 195]
Sevoflurane	+++ [139, 313, 323, 324]	+++ [216]	0*	−/0 [315, 320]	−−− [315]	unknown	unknown	unknown	unknown
Xenon	0 [192]	unknown	unknown	unknown	unknown	unknown	0 [192]	unknown	−−− [192]

Table 2. (Continued)

b. Intravenous agents	$GABA_A$	Glycine	GABA ρ_1	Muscle nAChR	Neuronal nAChR	$5\text{-}HT_3$	AMPA (GluR1–4)	Kainate (GluR5-7; KA1, KA2)	NMDA
Barbiturates (e.g. methohexitone, pentobarbitone or thiopentone)	+++ [64, 190, 213, 217, 218, 243, 261, 325–328]	+/0 [217, 233, 243, 301]	0 [218, 234, 329]	−/0 [330, 331]	−−− [322]	−/0 [311, 332]	−−− [30, 237, 257, 302, 317]	−−− [30, 223, 237, 257, 317]	0 [30, 302, 317]
Etomidate	+++ [15, 204–206, 213, 228, 229, 243, 244, 333, 334]	+/0 [233, 243, 301]	0*	−/0 [335]	−/0 [322]	0 [336]	unknown	unknown	unknown
Ketamine	+/0 [191, 303]	0 [233]	unknown	−/0 [335, 337]	−−− [264]	+/0 [338]	0 [264, 302]	0 [264]	−−− [163, 263–265, 302]
Propofol	+++ [190, 206, 207, 210, 211, 213, 243, 245, 246, 339–341]	+++ [207, 233, 243, 301, 314]	0 [234]	−/0 [304, 315, 335]	−/0 [315, 321]	0 [235]	−/0 [342]	0 [342, 343]	−/0 [342, 343]
Steroidal anaesthetics (alphaxalone)	+++ [33, 190, 205, 212, 213, 243, 249–251, 325, 326, 344]	0 [233, 243, 250, 344, 345]	0 [234, 346]	−/0 [335]	−/0 [251]	−/0†	0 [29]	0 [29]	0 [29]

Table 2. (Continued).

c. Alcohols	$GABA_A$	Glycine	GABA ρ_1	Muscle nAChR	Neuronal nAChR	$5\text{-}HT_3$	AMPA (GluR1–4)	Kainate (GluR5–7; KA1, KA2)	NMDA
Short-chain (methanol to propanol)	+++ [219, 271, 347–353]	+++ [219, 220, 226, 233, 314, 349, 354, 355]	−−− [220, 234]	−/0 [304, 356]	−−− [221, 266, 279] +++/−−− [357]	+++ [221, 235, 305, 311, 358, 359]	−−− [30, 271, 317]	−−− [30, 223, 271, 317, 360, 361]	−−− [30, 271, 317, 360, 362–365]
Long-chain (hexanol and higher)	+++ [271, 351, 352, 366]	+++ [220, 233]	−−− [220, 234]	−/0 [304, 356, 367]	−−− [279]	+++/−−− [311]	−−− [271]	−−− [271, 360]	−−− [271, 360, 368, 369]
'Alcohol cut-off'‡	cutoff at C10–C12 [271, 366]	cutoff at C10–C12 [220, 233]	cutoff at C7 [220, 234]	cutoff at C11–C12 [304, 367]	cutoff at C12 [279]	potentiation cutoff at C6; inhibition cut off at C12–C13 [311]	cutoff at C7–C8 [271]	cutoff at C7–C8 [271]	cutoff at C7–C8 [368]
Chloral hydrate/ trichloroethanol	+++ [190, 211, 227, 243, 370–372]	+++ [227, 243]	−−− [227]	+++§	unknown	+++ [305, 373–375]	−/0 [30, 317]	−/0 [30, 317, 376]	−−− [30, 317, 376]

+++and −−− indicate potentiation or inhibition of agonist actions at the receptor by the anaesthetic with an EC_{50} or IC_{50} no greater than threefold higher than the ED_{50} for producing immobility (see table 1). +/0 and −/0 indicate little potentiation or inhibition, except at concentrations greater than threefold the EC_{50} for immobility. 0 signifies no effect at any concentration tested. For a few receptors, either potentiation or inhibition has been documented at clinically relevant anaesthetic concentrations (designated as +++/−−−), depending on which receptor subunit combination is present (e.g. short-chain alcohol actions on neuronal nicotinic ACh receptors [267]). All receptors are mammalian except for one study of snail neuronal nicotinic ACh receptors [279]. This study was included since there are no published data of the effects of some general anaesthetics on mammalian neuronal nicotinic ACh receptors.

* M. D. Krasowski and N. L. Harrison, unpublished data.

† J. J. Lambert and J. A. Peters, unpublished data.

‡ The 'cutoff' for modulation of the ligand-gated ion channels by the n-alcoholds (see text for details).

§ A. Ravindran, A. Ghazafari and F. F. Weight, unpublished data.

Actions of general anaesthetics at ligand-gated ion channels

Volatile anaesthetics and anaesthetic gases

Volatile anaesthetics (e.g. halogenated ethers such as isoflurane and alkanes such as halothane) alter the function of many ligand-gated ion channels at reasonable concentrations. In general, submaximal agonist responses at $GABA_A$, glycine, 5-HT_3 and GluR6 receptors are enhanced by volatile anaesthetics, whereas agonist responses at neuronal nicotinic ACh and GluR3 receptors are inhibited (table 2a). The low potency and physicochemical properties of the volatile anaesthetics pose some technical challenges for in vitro experiments [13, 27, 28, 138]. Nevertheless, recent years have witnessed a steady increase in the quality and quantity of careful studies of volatile anaesthetic actions on ligand-gated ion channels.

Considerable progress has been made in identifying amino acid residues within $GABA_A$, glycine and kainate receptors that are critical for volatile anaesthetic potentiation of agonist-induced currents. The use of a panel of glycine α_1/$GABA_C$ ρ_1 chimeric receptors allowed the implication of a 45-amino acid region encompassing TM2 and TM3 of the glycine α_1 receptor as both necessary and sufficient for potentiation of agonist-induced currents by the volatile ether enflurane [219]. Extensive site-directed mutagenesis of glycine α_1 and $GABA_A$ α_2 and β_1 subunits determined that specific amino acid positions within TM2 and TM3 are also critical for agonist potentiation by isoflurane [206, 219] (see figs 3 and 5), n-alcohols (including ethanol) [219, 220, 226] and trichloroethanol [227] (see fig. 3). Agonist potentiation by propofol [206] and etomidate [228, 229] is also influenced by some or all of these amino acid positions (see fig. 3). In contrast to the situation at $GABA_A$ and glycine receptors, in TM4 of GluR6 kainate receptors, residue G819 is critical for volatile anaesthetic (e.g. isoflurane, enflurane, halothane) enhancement but not ethanol or pentobarbitone inhibition of submaximal kainate responses [223].

An obvious extension of the work described above with $GABA_A$ and glycine receptors is to determine whether homologous residues in the evolutionarily related neuronal nicotinic ACh and 5-HT_3 receptors also play crucial roles in volatile anaesthetic actions. Preliminary evidence suggests that such is indeed the case at the 5-HT_3 receptor. Some mutations in TM2 of the 5-HT_3 receptor abolish the agonist-potentiating actions of volatile ethers such as enflurane and isoflurane (S. J. Mihic, personal communication).

Most halogenated alkanes and ethers containing six or fewer carbons have anaesthetic properties, but some notable exceptions to this rule exist. The work of Eger, Koblin and colleagues has demonstrated that certain highly lipid-soluble halogenated cyclobutanes and alkanes are unable to produce immobility at concentrations predicted by the Meyer-Overton correlation to be in the anaesthetic range [166]. These compounds, originally called nonanaesthetics, are now more properly referred to as nonimmobilizers, since although they do not produce immobility [166] or analgesia [230], they may interfere with learning and memory [231]. The nonimmobilizers, which are often heavily halogenated compounds (e.g. 1,2-dichlorohexafluorocyclobutane), elicit convulsions at higher concentrations [166]. The nonimmobilizers have no modulatory actions at $GABA_A$ [232], glycine [233], $GABA_C$ ρ [234], 5-HT_3 [235], neuronal nicotinic ACh [236], AMPA or kainate receptors [237]. The nonimmobilizers have, however, been shown to alter the function of muscarinic ACh [238], muscle nicotinic ACh [239] and metabotropic glutamate receptors [240] at concentrations of the nonimmobilizers predicted to be anaesthetic. These results would seem to exclude the muscle nicotinic ACh muscarinic ACh and metabotropic glutamate receptors as viable molecular targets for producing immobility. These receptors may certainly play a role in other actions important in general anaesthesia such as amnesia, since nonimmobilizers and general anaesthetics share some behavioral actions in common.

The anaesthetic gases nitrous oxide and xenon have a pattern of action on the ligand-gated ion channels different from the volatile ethers and alkanes (see table 2a). This is perhaps not surprising since the clinical effects of xenon and nitrous oxide vary from that of the ethers and alkanes; for instance, unlike the ethers and alkanes, nitrous oxide is a potent analgesic with only weak immobilizing activity [2]. Nitrous oxide inhibits agonist responses at NMDA receptors [194, 195] but has only weak potentiating actions at $GABA_A$ receptors [193–195]. Very recently, xenon has been demonstrated to inhibit NMDA receptors at clinically relevant concentrations but does not modulate the function of $GABA_A$ or AMPA receptors [192]. The anaesthetic properties of xenon and krypton have long presented a challenge for molecular theories of anaesthesia, since these noble gases are among the simplest of molecules that produce anaesthesia [241]. Intriguingly, argon, xenon and krypton all possess anaesthetic properties, whereas the smaller noble gases helium and neon do not produce anaesthesia even at hyperbaric concentrations [242]. The NMDA receptor inhibition produced by xenon and nitrous oxide, with a lack of potent actions on $GABA_A$ receptors, resembles the actions of the 'dissociative anaesthetic' ketamine at ligand-gated ion channels (see below).

Intravenous agents

Etomidate and propofol both appear to be relatively selective for the $GABA_A$ receptor (table 2b). The $GABA_A$ receptor fulfills all the criteria as a plausible target underlying the anaesthetic actions of these compounds. Propofol and etomidate do not modulate other ligand-gated ion channels at clinically relevant concentrations with the exception of propofol actions at the strychnine-sensitive glycine receptor [207, 233, 243]. Amino acid residues within the β subunit of the $GABA_A$ receptor have been identified that are essential for potentiation of $GABA_A$ receptor function by etomidate [228, 229, 244] and propofol [206] (see fig. 3), consistent with previous studies suggesting that the β subunit of the $GABA_A$ receptor was likely to contain binding sites for these compounds [211, 245, 246].

Many steroid anaesthetics such as alphaxalone are relatively selective for the $GABA_A$ receptor, although certain steroids have potent actions on other ligand-gated ion channels (see table 2b). For the steroid anaesthetics, structure-activity studies comparing in vivo and in vitro potencies support a role for $GABA_A$ receptors in the actions of these compounds [32, 33, 247–249]. For example, the nonanaesthetic isomer structural betaxalone does not modulate the $GABA_A$ receptor [250, 251]. There have been extensive (although as yet not completely fruitful) attempts to synthesize steroid anaesthetics with improved therapeutic properties over the prototype alphaxalone, and many of these analogs have been tested at the $GABA_A$ receptor [252–254]. Critical residues for modulation by alphaxalone or other steroid anaesthetics have not yet been identified within any ligand-gated ion channel, although studies of $GABA_A$/glycine chimeric receptors suggest a major contribution of the N-terminal extracellular domain of the $GABA_A$ receptor to GABA potentiation by alphaxalone [255].

Unlike propofol, etomidate and the steroid anaesthetics, the barbiturates are much less selective. In addition to their actions at $GABA_A$ receptors, barbiturates also potently inhibit AMPA, kainate and neuronal nACh receptors (table 2b). The inhibition of AMPA receptors by barbiturates is voltage- and use-dependent [256, 257]. Studies of recombinant AMPA receptors have revealed that the potency of pentobarbitone block is critically dependent on a glutamine/arginine site in the pore-forming loop of GluR2 subunits [258]. The presence of a glutamine or arginine at this site is determined by specific RNA editing of the GluR2 RNA and strongly influences the ion selectivity and permeation properties of receptors containing the GluR2 subunit [259, 260]. The observation that pentobarbitone block depends on the glutamine/arginine site, together with the voltage- and use-dependence of the block, indicates penetration of barbiturates deep into the ion-conducting pore of AMPA receptors. Optical isomers of pentobarbitone display the same order of potency for modulatory actions at the $GABA_A$ receptor as for their in vivo anaesthetic actions [13, 159]. A residue within TM2 of the β_1 subunit of the $GABA_A$ receptor has been suggested to be necessary for GABA potentiation by pentobarbitone [261] (see fig. 3). Agonist potentiation by barbiturates is not altered by mutations in $GABA_A$ receptors that abolish potentiation by volatile anaesthetics, n-alcohols, propofol or trichloroethanol [206, 219, 227]. Similarly, a mutation within TM4 of the kainate GluR6 receptor that ablates volatile anaesthetic enhancement of submaximal kainate responses does not alter inhibition by barbiturates [223].

Compared with other intravenous anaesthetic agents discussed above, the 'dissociative anaesthetic' ketamine has a very different in vivo and in vitro profile of action (table 2b). Ketamine and related arylcycloalkylamines such as phencyclidine produce an atypical state of 'dissociative' anaesthesia, characterized by sedation, immobility, amnesia, marked analgesia and a feeling of dissociation from the environment, without true unconsciousness [262]. These compounds can also produce intense hallucinations that limit their clinical use, especially in adults [2]. In contrast with most other general anaesthetics, ketamine does not potentiate $GABA_A$ receptor function at clinically relevant concentrations [191]. Ketamine appears instead to produce anaesthesia by inhibition of NMDA receptors [163, 250, 263–265], although ketamine is also a potent inhibitor of neuronal nicotinic ACh receptors so contributions from these receptors cannot be ruled out [264]. NMDA receptors satisfy all of the pharmacological criteria expected of molecular targets for ketamine and phencyclidine, including stereoselectivity [163]. A site of ketamine action on the NMDA receptor has not yet been elucidated, although single-channel studies have explored the mechanism of ketamine inhibition at NMDA receptors in detail [263].

Alcohols

The alcohols display very little selectivity among the ligand-gated ion channels considered in this review are modulated by anaesthetic concentrations of most alcohols (table 2c). This obfuscates attempts to dissect the molecular underpinnings underlying the diverse behavioral actions of the alcohols. Neuronal nicotinic ACh receptors appear to be exquisitely sensitive to alcohols, in some cases showing modulation by ethanol concentrations as low as 1–10 mM [266, 267]. Mammalian blood alcohol concentrations in this range produce only mild intoxication [268]. As described above, residues within TM2 and TM3 of $GABA_A$ and glycine receptors are critically important for the agonist-potentiating actions of the n-alcohols, trichloroethanol and the volatile ether anaesthetics [206, 219, 220, 226, 227] (see figs 3 and 5).

The demonstration of a 'cutoff' phenomenon for the in vivo actions of the straight-chain alcohols has presented challenges for many molecular theories of anaesthesia. Potencies of the primary alcohols in producing immobility increase with increasing number of carbon atoms (n), but only up to a certain size (the cutoff), after which alcohols with longer carbon chains decline in potency or remain equipotent with the $(n-1)$-alcohols [143, 269, 270]. We have followed previous suggestions [13, 220] in defining cutoff as the point at which the potency of the n-alcohol no longer increases with increasing carbon chain length. As with stereoselectivity, the alcohol cutoff poses severe problems for nonspecific theories of anaesthetic action, since there appears to be no cutoff for the disordering actions of n-alcohols on lipid bilayers [14]. In general, the immobilizing actions of n-alcohols show a cutoff around dodecanol (C12) [143, 269, 270], although the limited aqueous solubility of long-chain alcohols can complicate matters [271]. The alcohol cutoff for the ligand-gated ion channels varies between receptors (see table 2c), and this could be useful in implicating or eliminating specific receptors in the various biological effects of the alcohols.

Alcohol cutoff has recently been applied to the study of glycine and $GABA_C$ ρ_1 receptors harboring mutations in TM2 and TM3. It was first noted that mutation of a smaller to a larger amino acid residue in TM2 of the glycine α_1 subunit (serine-267 to glutamine) reduced the alcohol cutoff for the glycine receptor from dodecanol (C12) to propanol (C3) [220]. In contrast, a double mutation of larger to smaller residues in TM2 and TM3 of the $GABA_C$ ρ_1 receptor extended the alcohol cutoff from heptanol (C7) to beyond dodecanol (C12) [220]. These data provide strong evidence that mutation of selected residues within TM2 and TM3 of glycine and $GABA_C$ receptors may actually alter the dimensions of a binding pocket for n-alcohols.

Discussion and future directions

Substantial progress has been made in the last decade in defining the actions of general anaesthetic agents on ligand-gated ion channels, particularly in the areas of molecular biology, pharmacology and electrophysiology. The coming years will surely witness more major advances, perhaps most notably from the application of structural biology and gene-targeting approaches. The use of site-directed mutagenesis and chimeric receptors has proven very helpful in identifying regions of ligand-gated ion channels that play critical roles in modulation by general anaesthetics. However, more definite evidence of the existence of general anaesthetic binding pockets probably awaits the resolution of three-dimensional structures for the ligand-gated ion channels. Structural biology approaches have already been applied to the study of general anaesthetic interactions with model soluble proteins [20], including the recent report of the 2.2-Å resolution three-dimensional structure of firefly luciferase complexed with the general anaesthetic bromoform [272].

In common with other many integral membrane proteins, ligand-gated ion channels have proved recalcitrant to structural biology approaches. However, the crystallization and determination of a high-resolution structure for a bacterial potassium channel [273] surely foreshadows the eventual determination of the three-dimensional structure of the ligand-gated ion channels. A more immediate possibility is the determination of the structure of limited domains of ligand-gated ion channels; indeed, researchers have very recently succeeded in resolving the structure of the extracellular domain of an ionotropic glutamate receptor complexed with kainate [274]. Even in the absence of detailed structures, molecular modeling may be of use in making preliminary predictions that can be tested experimentally.

Targeted gene manipulations in mice will also provide hypothesis-driven tests of the in vivo roles of certain ligand-gated ion channels in mediating the diverse behavioral actions of general anaesthetics. As described above, researchers over the last 5 years have created 'global knockout mice' for various subunits of the ligand-gated ion channels. Given the abundance of ligand-gated ion channel knockout mice (and the commercial availability of some of these knockouts), it would be a logical step to test anaesthetic sensitivity in some or all of these mice. However, while knockout mice may provide initial clues as to the nature of anaesthetic targets, such mice can be very difficult to analyze for anaesthetic sensitivity if they exhibit grossly abnormal motor behavior, lethality or aberrations in neural development. These problems with knockout mice may be circumvented by 'conditional' gene knockouts, in which the gene of interest is disrupted only in limited brain regions and/or specified developmental time periods [172].

Another elegant example of gene targeting is a 'knock-in' mouse. One possibility is the introduction of a mutated receptor subunit that is insensitive to anaesthetic modulation in place of the normal endogenous receptor subunit (e.g. see [175]). This type of approach has recently been applied to the benzodiazepines. These studies utilized knock-in mice expressing a mutant $GABA_A$ receptor α_1 subunit that confers insensitivity to benzodiazepine modulation, in place of the benzodiazepine-sensitive wild-type α_1 subunit. These preliminary studies have not only demonstrated the importance of the $GABA_A$ α_1 subunit isoform for the behavioral actions of benzodiazepines but also have suggested that distinct $GABA_A$ receptor α subunit isoforms mediate different actions of the benzodiazepines, with the α_1 subunit isoform necessary for sedative and anticonvulsant effects and other α-subunit isoforms critical for myorelaxant and anxiolytic actions (U. Rudolph, F. Crestani, H. Möhler, personal communication).

Knock-in mouse experiments potentially provide an elegant bridge between in vitro experiments and whole animal behavior. Ideally, the mutated receptor subunit would differ from the normal subunit only in terms of general anaesthetic modulation (i.e. agonist binding, channel gating, voltage dependence, kinetics etc. of the receptor would be relatively normal). Recently described mutations within TM2 and TM3 of $GABA_A$ (see fig. 3) and glycine receptors, which confer insensitivity to volatile ether anaesthetics [206, 219], n-alkanols [219, 220, 226], propofol [206], trichloroethanol [227], pentobarbitone [261] and etomidate [228, 229] essentially fit this qualification, as do point mutations within GluR6 kainate receptors that abolish volatile anaesthetic potentiation [223]. A complication to gene-targeting experiments is the presence of multiple subunit isoforms for the ligand-gated ion channels. For example, there are at least 17 gene products for $GABA_A$ receptor subunits; if multiple $GABA_A$ subunit isoforms play a role in general anaesthesia, then targeting of multiple genes may be required to obtain an unambiguous change in anaesthetic sensitivity. General anaesthetics produce a range of behavioral effects in animals and humans. It appears overly simplistic to ascribe all of these to a single receptor. Current and future research should eventually define the specific receptors that underlie each of the diverse behavioral actions of every class of general anaesthetics. The upcoming decade will undoubtedly be an exciting time for research into the molecular mechanisms of general anaesthetics.

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