Correlating the clinical actions and molecular mechanisms of general anesthetics

Ken Solt[a,b] and Stuart A. Forman[a,b]

Purpose of review

To summarize recent in-vitro and in-vivo research on molecular mechanisms of general anesthetics' actions.

Recent findings

Classes of general anesthetics with distinct clinical profiles appear to induce amnesia, hypnosis, and immobility via different molecular targets. Propofol, etomidate, and barbiturates produce profound amnesia and hypnosis, but weak immobility, by enhancing the activity of specific gamma-aminobutyric acid typeA receptors. In contrast, nitrous oxide, xenon, and ketamine produce analgesia, but weak hypnosis and amnesia, by inhibiting glutamate and nicotinic receptors and activating potassium 'leak' channels such as TREK-1. Volatile halogenated anesthetics show little selectivity for molecular targets. They act on all the channels mentioned above, and other targets such as glycine receptors and mediators of neurotransmitter release.

Summary

Several clinically distinct 'anesthetic states' are induced by different classes of drugs acting on neuronal circuits via different molecular targets. Understanding the mechanisms underlying the therapeutic and toxic actions of general anesthetics helps us reframe the 'art' of anesthesia into more of a 'science'. These studies also enhance efforts to develop new drugs with improved clinical utility.

Keywords

amnesia, gamma-aminobutyric acid receptor, general anesthesia, glutamate receptor, hypnosis, immobility

Curr Opin Anaesthesiol 20:300–306. © 2007 Lippincott Williams & Wilkins.

[a]Department of Anesthesia and Critical Care, Massachusetts General Hospital, Boston, USA and [b]Department of Anesthesia, Harvard Medical School, Boston, USA

Correspondence to Stuart A. Forman, MD, PhD, Department of Anesthesia and Critical Care, Massachusetts General Hospital, 55 Fruit Street, Gray 444, Boston, MA 02114, USA
Tel: +1 617 724 5156; fax: +1 617 724 8644;
e-mail: saforman@partners.org

Current Opinion in Anaesthesiology 2007, 20:300–306

Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
EEG	electroencephalogram
$GABA_A$	gamma-aminobutyric acid typeA
LORR	loss of righting reflexes
MAC	minimum alveolar concentration
NMDA	*N*-methyl-D-aspartate

0952-7907

Introduction

One hundred and sixty years after inhaled general anesthetics were widely adopted into clinical practice, the mechanisms underlying their therapeutic actions remain uncertain. Starting with Meyer and Overton's observations and continuing until a few decades ago, general anesthetics were thought to act indirectly, through perturbation of neuronal membrane lipids. This theory derived from Claude Bernard's Unitary Hypothesis, which postulated that anesthetics with widely varying chemical structures all acted through a common mechanism.

The hypothesis that all anesthetics produce the same neurobiological state is demonstrably false. General anesthesia includes three essential neurobiological effects: amnesia, hypnosis, and immobility. Respectively, these actions represent ablation of first, antegrade memory formation, second, perceptive awareness (responses to nonnoxious stimuli), and third, movement in response to painful stimuli. Each of these actions is produced at distinct anesthetic drug concentrations, demonstrating that general anesthesia is not a simple 'all-or-nothing' state change induced in the nervous system. Different anesthetic agents also display different relative potencies and efficacies for these distinct neurobiological actions.

This article reviews recent data regarding molecular and cellular mechanisms of anesthetic actions. Research on the molecular targets for general anesthetics has shifted from lipids to ion channels and receptors that rapidly alter neuronal excitability. Molecular genetics has enabled both in-vitro studies of specific putative targets, as well as in-vivo studies of transgenic animals that may lack a specific target (i.e. 'knock-outs') or contain a mutation in that target (i.e. 'knock-ins'). Recent studies reveal that different classes of general anesthetics act via different sets of target molecules, emphasizing the existence of 'multiple mechanisms and multiple sites' [1].

Clinical classification of general anesthetics

The clinical actions of general anesthetics are not all equal. Potencies for many clinically used anesthetics have been evaluated for two major therapeutic actions: immobility and hypnosis. The Minimum Alveolar Concentration for inhaled anesthetics producing immobility (MAC-immobility) is considered a standard measure of anesthetic potency, and plasma concentrations for intravenous agents (Cp50-immobility) can be evaluated in a similar manner. Analogous potency measurements for hypnosis

Table 1 General anesthetic classification based on clinical features and molecular targets

	Group 1	Group 2	Group 3
General anesthetics	Etomidate, propofol, pentobarbital	Nitrous oxide, ketamine, xenon, cyclopropane	Halogenated ethers (e.g. isoflurane, sevoflurane, desflurane) and alkanes (e.g. halothane, chloroform)
Clinical features	Strong hypnotics Strong amnestics Weak immobilizers Slow cortical EEG	Weak hypnotics Weak immobilizers Potent analgesics No EEG slowing	Strong hypnotics Strong amnestics Strong immobilizers Slow cortical EEG
Ratio of MAC-immob. to MAC-awake	4 (propofol)	1.5 (N_2O)-2 (Xe)	2 (halothane)-3 (halogenated ethers)
Molecular targets	$GABA_A$ receptors (β_3 and β_2 subunits)	NMDA receptors AMPA receptors Neuronal nAChRs 2-pore K^+ channels	$GABA_A$ receptors Glycine receptors Glutamate receptors (NMDA and AMPA) Neuronal nAChRs 2-pore K^+ channels

EEG, electroencephalogram; NMDA, *N*-methyl-D-aspartate; $GABA_A$, gamma-aminobutyric acid subtypeA; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid.

(MAC-awake or Cp50-awake) have been documented for many anesthetics [2]. To date, potencies for amnesia are not as thoroughly studied. Other anesthetic actions, such as production of analgesia, protection from ischemia, cardiovascular effects, and postoperative nausea and vomiting (PONV) also vary widely among anesthetic agents.

Clinical observations, as well as comparison of potencies for different essential actions, indicate that general anesthetics can be broadly classified into three groups (Table 1) [3•]. Etomidate, propofol, and barbiturates (group 1) are all intravenous drugs that are efficacious sedative/hypnotic agents, but are relatively weak immobilizers. For propofol, hypnosis is achieved at plasma concentrations around 3 μg/ml, whereas immobility during skin incision requires four-fold higher concentrations [4]. All of these drugs cause slowing of the cortical EEG, and EEG-based anesthetic depth monitors can be used to monitor their effects [5]. In stark comparison to propofol is the gaseous anesthetic nitrous oxide (N_2O), which shares its clinical action profile with ketamine, xenon (Xe), and cyclopropane (Group 2). These drugs are weak hypnotics and immobilizers, but strong analgesics (an action that is not demonstrable in other general anesthetics). Group 2 anesthetics are also associated with cardiovascular stability and a high frequency of reported dreamlike experiences. N_2O produces hypnosis at about 0.7 atm, and immobility is estimated to occur at 1.05 atm [2]. Thus, the ratio of MAC-immobility to MAC-awake for N_2O is only 1.5. Moreover, group 2 drugs either increase or do not alter cortical EEG frequencies, so anesthetic depth monitoring is not sensitive to the hypnotic effects of these agents [6,7]. Volatile halogenated anesthetics (group 3: halothane, enflurane, isoflurane, sevoflurane, desflurane, etc.) are notable for their efficacy at inducing amnesia, hypnosis and immobility in a predictable manner. The ratio of MAC-immobility to MAC-awake for group 3 drugs is between that for propofol and N_2O, ranging from 2 to 3 [2]. Group 3 drugs are also known to produce amnesia at concentrations (partial pressures) lower than those that produce hypnosis. Volatile anesthetics slow the cortical EEG and anesthetic depth monitoring works well with these agents.

Etomidate, propofol, and barbiturates (group 1)

Molecular studies have now demonstrated that hypnosis and immobility produced by etomidate, propofol, and barbiturates are mediated by specific gamma-aminobutyric acid typeA ($GABA_A$) receptors. $GABA_A$ receptors are the major inhibitory neurotransmitter-gated ion channels in the human brain. Each receptor consists of five homologous subunits arranged symmetrically around a gated chloride channel. Each subunit shares a topology with all members of the cys-loop ligand-gated ion channel superfamily: a large extracellular N-terminal domain, four transmembrane domains (TM1-4), and a large intracellular loop between TM3 and TM4. Genetic techniques have identified 18 different $GABA_A$ receptor subunits: α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, π, and ρ_{1-3}. The most common $GABA_A$ receptor subunit composition and stoichiometry is $2\alpha{:}2\beta{:}1\gamma$. When $GABA_A$ receptors are activated in the presence of group 1 anesthetics and submaximal concentrations of GABA, channel activation is enhanced. GABA-mediated inhibitory postsynaptic currents are also prolonged in the presence of these anesthetics. Thus, group 1 anesthetics decrease neuronal excitability by enhancing the activity of inhibitory $GABA_A$ receptors.

A number of correlative studies support the hypothesis that $GABA_A$ receptors mediate anesthesia in the presence of group 1 drugs. Etomidate has a chiral carbon and R(+)-etomidate is 10-fold more potent than S(−)-etomidate at inducing loss of righting reflexes (LORR, a surrogate test for hypnosis) in tadpoles and mice, and exactly the same

degree of stereoselectivity is observed in molecular studies of $GABA_A$ receptor modulation by etomidate [8]. Picrotoxin is a convulsant that acts by selectively blocking $GABA_A$ receptors, and when it is administered intrathecally, Cp50-immobility for propofol in mice increases four-fold [9].

Definitive experiments linking $GABA_A$ receptors to anesthesia grew out of molecular studies identifying mutations that selectively altered receptor sensitivity to anesthetics. Varying the subunits in $GABA_A$ receptors expressed in cells led to observations that receptors containing β_2 and β_3 subunits were sensitive to etomidate and propofol, while those containing β_1 subunits were insensitive. Using molecular genetics, chimeric subunits containing parts of β_1 and $\beta_{2/3}$ were created, and researchers identified several amino acids that, when mutated, determined receptor sensitivity to etomidate and propofol [10]. Mutations that changed the asparagine (N) at position 265 in the β_2 or β_3 peptide sequences to methionine (M) or serine (S) were introduced into the genome of mice (i.e. 'knock-in' transgenic animals). Mice containing the β_3(N265M) mutation are insensitive (at least a four-fold reduction in sensitivity) to both propofol and etomidate anesthesia, specifically limb withdrawal to pinch (a surrogate test for immobility) and LORR [11,12]. Mice containing the β_2(N265S) mutation show normal sensitivity to anesthetics for hypnosis and immobility, but at low concentrations of anesthetics they are less sedated than wild-type mice [13]. These results indicate that hypnosis and immobility are mediated by $GABA_A$ receptors containing β_3 subunits, while sedation is linked to receptors containing β_2 subunits.

Transgenic mice lacking $GABA_A$ receptor α_5 subunits exhibit resistance to the amnestic (but not hypnotic) effects of etomidate [14•], providing further evidence that specific anesthetic endpoints are produced at distinct molecular sites. An anesthetic photolabel analog of etomidate has been synthesized and was recently found to covalently modify both α and β subunits of purified bovine $GABA_A$ receptors [15••]. If mutations that selectively reduce etomidate sensitivity can be identified in the photolabeled region of α subunits, we may learn more about how different anesthetic binding sites on $GABA_A$ receptors mediate the specific neurobiological effects of anesthetics.

The transgenic β_3(N265M) mice have revealed that some of the undesirable effects of propofol and etomidate anesthesia are also linked to the β_3 $GABA_A$ receptor subunits. Respiratory depression is one of these. Recently, immobility and hypnosis induced by pentobarbital were shown to be dramatically reduced in β_3(N265M) mice but, surprisingly, respiratory depression was not [16•].

Other important clinical effects of propofol and etomidate are clearly not linked to $GABA_A$ receptors. Etomidate inhibits adrenal cortisol synthesis, an effect that has been linked to inhibition of 11β-hydroxylase and 17α-hydroxylase enzymes. One of the clinical advantages provided by etomidate is cardiovascular stability. This unique feature may be due to etomidate activation of α_{2B} adrenergic receptors [17]. In wild-type mice, etomidate raises blood pressure, whereas in mice lacking α_{2B} adrenoreceptors, no increase in blood pressure occurs. Propofol is often selected for patients at risk for PONV, because it apparently has antiemetic activity. Recent studies suggest that this unique feature of propofol anesthesia may be caused by indirect activation of cannabinoid receptors [18]. Propofol was found to inhibit fatty acid amide hydrolase, an enzyme that degrades the endogenous cannabinoid receptor agonist, anandamide.

Nitrous oxide, xenon, cyclopropane and ketamine (group 2)

Unlike group 1 drugs, N_2O, Xe, cyclopropane and ketamine have minimal effects on $GABA_A$ receptors at clinically relevant concentrations. All of these anesthetics are potent inhibitors of *N*-methyl-D-aspartate (NMDA) receptors, however [19–22]. NMDA receptors belong to the family of excitatory ionotropic glutamate receptors that also include α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptors. Glutamate receptors constitute the major excitatory neurotransmitter-gated ion channels in the human brain. Seven NMDA receptor subunits have been identified: NR1, NR2 (A through D), and NR3 (A and B). Unlike the pentameric $GABA_A$ receptor, the NMDA receptor is composed of only four subunits, and within each subunit the second 'transmembrane' region (TM2) is actually a re-entrant loop that does not traverse the cell membrane. Thus NMDA receptor subunits possess only three true transmembrane regions (TM1, TM3 and TM4), and the C-terminal domain resides intracellularly. In the presence of N_2O, Xe, cyclopropane or ketamine, NMDA receptor-mediated excitatory postsynaptic currents are markedly inhibited.

Correlative studies suggest that ketamine anesthesia is mediated (at least in part) by NMDA receptor inhibition. Like etomidate, ketamine possesses a chiral carbon, and in voltage-clamped rat hippocampal neurons S-ketamine was 1.9 times more potent than R-ketamine at inhibiting NMDA receptors, mirroring the relative stereoselectivity of the anesthetic's actions *in vivo* [21].

Transgenic mice lacking the NMDA receptor ε_1 subunit (homologous to the human NR2A subunit) show resistance to the LORR produced by ketamine [23]. The same mice also demonstrate resistance to immobility and LORR produced by N_2O [24]. Nagele *et al.* [25] showed that in

the nematode *Caenorhabditis elegans*, a null mutation of the gene nmr-1 (encoding an NMDA-type receptor) renders the organism resistant to the behavioral effects of N_2O. The altered behavior in the presence of N_2O was noted to be distinct from that produced by halogenated anesthetics. Furthermore, a mutation that confers resistance to halogenated anesthetics did not confer resistance to N_2O, proving that N_2O and halogenated anesthetics have distinct mechanisms of action in *C. elegans*. The behavioral effects of Xe in wild-type *C. elegans* were found to be similar to those produced by N_2O, but were only attenuated when a different, non-NMDA glutamate receptor (glr-1, most similar to the mammalian AMPA receptor) was deleted [26], suggesting that non-NMDA glutamate receptors may also play a role in mediating anesthetic effects. Electrophysiological experiments using heterologously expressed receptors demonstrate that Xe and N_2O inhibit both human NMDA and AMPA receptors [27,28].

A recent study by Colloc'h *et al.* [29•] demonstrated that N_2O and Xe bind to the same hydrophobic sites in the model proteins urate oxidase and annexin V, causing expansion of the binding cavity and thus altering protein conformation. These results suggest the possibility of a common binding site and molecular mechanism of action for N_2O and Xe at their target receptor(s).

Although NMDA receptor blockade may play a role in mediating the immobility produced by N_2O, Xe, cyclopropane and ketamine, it is insufficient by itself to achieve immobility. The selective NMDA receptor antagonist MK-801 reduces MAC for anesthetics, yet does not produce immobility when administered by itself [30]. The failure of selective NMDA receptor antagonists to produce immobility proves that other molecular targets must be involved in this action of group 2 general anesthetics.

Group 2 anesthetics have also been shown to inhibit $\alpha_4\beta_2$ neuronal nicotinic acetylcholine receptors [22,27,31]. Stereospecific actions of ketamine that correlate with in-vivo potency have also been demonstrated in nicotinic acetylcholine receptors [32]. Although these receptors do not contribute significantly to immobility [33], they may be important mediators of anesthetic-induced amnesia [34]. N_2O, Xe and cyclopropane also activate TREK-1, a member of the two-pore (2P) domain potassium channel family [35]. TREK-1 is a 'background leak' K^+ channel that is highly expressed in the brain and spinal cord, and regulates the resting membrane potential of neurons. In addition to gaseous and volatile anesthetics, TREK-1 channels are also activated by heat, membrane stretch, intracellular acidosis and local anesthetics. Opening of TREK-1 channels increases potassium conductance, clamping membrane voltages near their resting values and decreasing excitability of neurons. A TREK-1 knockout mouse has been reported, but it is unknown whether the loss of this potential target reduces sensitivity to group 2 anesthetics.

Halogenated volatile anesthetics (group 3)

The halogenated volatile anesthetics used in modern clinical practice (namely isoflurane, sevoflurane and desflurane) all share common clinical characteristics, including reliable amnesia, hypnosis and immobility. They all have similar MAC-immobility/MAC-awake ratios near 3 [2]. Although differences exist among these anesthetics in terms of their uptake and distribution kinetics, it is reasonable to hypothesize that these chemically similar agents (all halogenated ethers) share sites and mechanisms of actions. Halothane (a halogenated alkane) is similar to halogenated ethers in many respects but it possesses a significantly lower MAC-immobility/MAC-awake ratio of 2 (similar to Xe) [2], suggesting that there may be differences in the molecular mechanisms by which halogenated alkanes and ethers achieve hypnosis and immobility. Although all anesthetics presumably produce amnesia and hypnosis in the brain, studies have demonstrated that volatile agents produce immobility primarily via actions in the spinal cord [36]. A recent study by Antognini *et al.* [37•], however, showed that MAC-immobility for o-difluorobenzene, a volatile anesthetic that potently inhibits NMDA receptors, does not change when the anesthetic is delivered selectively to the brain. This result suggests that the selectivity of anesthetics for specific molecular targets also determines their anatomic sites of action.

Halogenated ethers and alkanes constitute a group of anesthetics that are notable for their lack of selectivity for potential anesthetic target molecules. Volatile anesthetics modulate $GABA_A$ receptors, although with less selectivity than group 1 anesthetics, and they also affect the targets associated with group 2 anesthetics.

Halogenated volatile anesthetics have been shown to enhance the function of many types of inhibitory $GABA_A$ and glycine receptors. Using receptor subunit chimeras that combined portions of the anesthetic-sensitive glycine receptor α_1 subunit and the anesthetic-insensitive $GABA_A$ receptor ρ_1 subunit, Mihic *et al.* [38] determined that amino acid residues in TM2 and TM3 are critical for anesthetic sensitivity. Subsequently, specific amino acids in both α and β subunits of $GABA_A$ receptors were shown to affect anesthetic sensitivity, supporting the idea that volatile anesthetics bind to receptor sites to produce their effects.

Pharmacologic and genetic studies, however, suggest that $GABA_A$ receptors play a limited role in mediating volatile anesthetic effects. Intrathecal infusion of the $GABA_A$ receptor inhibitor picrotoxin increases the MAC for isoflurane in rats by about 40% [39]. Intrathecal picrotoxin also increases MAC by about 40% for Xe and cyclopropane,

neither of which appreciably enhance $GABA_A$ receptors *in vitro* [40]. Thus, the MAC increase for volatiles observed with picrotoxin is probably due to an indirect effect.

Transgenic mice lacking the $GABA_A$ receptor β_3 subunit require higher concentrations of halothane and enflurane to achieve immobility compared with wild-type mice, although the increases in MAC are small (9% and 26%, respectively) [41]. No change in anesthetic requirement was observed for LORR in β_3 subunit knockout mice, suggesting that volatile anesthetic-induced immobility and hypnosis are produced at different sites. Transgenic mice bearing the point mutation N265M in the β_3 subunit are largely insensitive to the effects of propofol and etomidate, but MAC for isoflurane, enflurane and halothane in these mice is only increased by about 20% [12]. Surprisingly, these β_3(N265M) mice also exhibit a 13% increase in MAC for cyclopropane.

A point mutation in the $GABA_A$ receptor α_1 subunit (serine to histidine at position 270) eliminates receptor enhancement by isoflurane and desflurane (but not halothane) *in vitro*. Knock-in mice with the α_1S270H mutation and another L277A mutation (that normalizes GABA sensitivity) show modestly increased requirements for isoflurane and enflurane (but not halothane) to induce LORR [42••]. MAC-immobility did not differ from wild-type mice, however, suggesting that α_1 subunit-containing $GABA_A$ receptors mediate isoflurane and enflurane hypnosis, but not immobility.

Pharmacologic studies suggest that glycine receptors play a role in mediating volatile anesthetic-induced immobility. Intrathecal administration of strychnine (a glycine receptor inhibitor) increases MAC for inhaled anesthetics, correlating with the relative magnitudes of glycine receptor enhancement *in vitro* (halothane > isoflurane>cyclopropane) [43]. Even in combination, however, intrathecal administration of strychnine and picrotoxin reduce MAC for isoflurane only by about 40% [39], suggesting that targets other than $GABA_A$ and glycine receptors contribute to isoflurane immobility.

Many of the two-pore domain K^+ channels, including TREK-1, TREK-2, TASK-1, TASK-2, TASK-3 and TRESK, are activated by clinical concentrations of volatile anesthetics [35,44,45]. TREK-1 knockout mice show modestly increased volatile anesthetic MAC-immobilities (up to a 48% increase for halothane) [46], suggesting that TREK-1 contributes to this action. Other volatile anesthetic-sensitive K^+ channels such as TASK-2 do not appear to contribute to immobility [47], although they may play roles in mediating other anesthetic effects.

Like N_2O, Xe and cyclopropane, the volatile anesthetics also inhibit excitatory glutamate receptors in the CNS, but at concentrations equivalent to 1 MAC, the halogenated anesthetics inhibit NMDA receptors less than the gaseous anesthetics [48•]. Additional evidence linking these receptors to volatile anesthetic actions is lacking. NMDA receptor ε_1 subunit knockout mice that exhibit resistance to ketamine and N_2O show no resistance to sevoflurane [24]. Similarly, kainate receptor GluR6 subunit knockout mice exhibit no change in MAC for isoflurane, desflurane or halothane [49].

A variety of other ion channels are sensitive to volatile anesthetics, including neuronal nicotinic acetylcholine receptors [50], serotonin type 3 receptors [51], Na^+ channels [52], mitochondrial ATP-sensitive K^+ channels [53], and cyclic nucleotide-gated HCN channels [54]. These receptors have been proposed to play important roles in hypnosis, amnesia, immobility, and protection from ischemia, as well as undesirable side effects of volatile anesthetics.

Taken together, current data suggest that the volatile group 3 anesthetics act nonselectively at a number of molecular targets to produce each of the essential clinical effects (amnesia, hypnosis, and immobility). Optimistic investigators hypothesize that a modest number of important targets can be identified, which may be demonstrated in molecular genetic experiments.

Conclusion

Data from both in-vitro molecular studies and transgenic animals demonstrate different degrees of target selectivity for different groups of anesthetics. Group 1 drugs (etomidate, propofol, and barbiturates) act primarily through specific $GABA_A$ receptors associated with different subunit types. Group 2 drugs (N_2O, Xe, ketamine, and cyclopropane) appear to act via a small number of targets, including glutamate receptors and two-pore potassium channels. Group 3 volatile anesthetics are the least selective group, affecting a wide range of plausible molecular targets. Some important correlations between molecular targets and clinical features of anesthetics are also emerging. Strong hypnosis and amnesia appear to be linked to enhancement of $GABA_A$ receptors for both group 1 and 3 anesthetics. Group 1 and 3 drugs also produce graded slowing of cortical EEG frequencies, providing useful methods for assessing anesthetic depth. Analgesia, although not an essential component of anesthesia, is a desirable effect of group 2 anesthetics that correlates strongly with NMDA receptor inhibition. Links between molecular targets and other therapeutic and toxic effects of various anesthetics have also been revealed by molecular studies. In addition, pharmacological structure–function studies are revealing features that influence which anesthetics act at important targets. The powerful combination of modern molecular and pharmacological approaches will undoubtedly continue to identify the targets responsible

for anesthetic actions, and the development of more specific agents with improved side-effect profiles will likely follow.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 389–390).

1 Grasshoff C, Rudolph U, Antkowiak B. Molecular and systemic mechanisms of general anaesthesia: the 'multisite and multiple mechanisms' concept. Curr Opin Anaesthesiol 2005; 18:386–391.

2 Eger EI 2nd. Age, minimum alveolar anesthetic concentration, and minimum alveolar anesthetic concentration-awake. Anesth Analg 2001; 93:947–953.

3 • Grasshoff C, Drexler B, Rudolph U, Antkowiak B. Anaesthetic drugs: linking molecular actions to clinical effects. Curr Pharm Des 2006; 12:3665–3679.

This review proposes a similar grouping of anesthetics as that used here, and provides a thorough summary of data up to 2006.

4 Smith C, McEwan AI, Jhaveri R, *et al.* The interaction of fentanyl on the Cp50 of propofol for loss of consciousness and skin incision. Anesthesiology 1994; 81:820–828.

5 Kuizenga K, Wierda JM, Kalkman CJ. Biphasic EEG changes in relation to loss of consciousness during induction with thiopental, propofol, etomidate, midazolam or sevoflurane. Br J Anaesth 2001; 86:354–360.

6 Anderson RE, Jakobsson JG. Entropy of EEG during anaesthetic induction: a comparative study with propofol or nitrous oxide as sole agent. Br J Anaesth 2004; 92:167–170.

7 Goto T, Nakata Y, Saito H, *et al.* Bispectral analysis of the electroencephalogram does not predict responsiveness to verbal command in patients emerging from xenon anaesthesia. Br J Anaesth 2000; 85:359–363.

8 Belelli D, Muntoni AL, Merrywest SD, *et al.* The in vitro and in vivo enantioselectivity of etomidate implicates the $GABA_A$ receptor in general anaesthesia. Neuropharmacology 2003; 45:57–71.

9 Sonner JM, Zhang Y, Stabernack C, *et al.* GABA(A) receptor blockade antagonizes the immobilizing action of propofol but not ketamine or isoflurane in a dose-related manner. Anesth Analg 2003; 96:706–712.

10 Belelli D, Lambert JJ, Peters JA, *et al.* The interaction of the general anesthetic etomidate with the gamma-aminobutyric acid type A receptor is influenced by a single amino acid. Proc Natl Acad Sci U S A 1997; 94:11031–11036.

11 Jurd R, Arras M, Lambert S, *et al.* General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA(A) receptor beta3 subunit. FASEB J 2003; 17:250–252.

12 Liao M, Sonner JM, Jurd R, *et al.* Beta3-containing gamma-aminobutyric acidA receptors are not major targets for the amnesic and immobilizing actions of isoflurane. Anesth Analg 2005; 101:412–418.

13 Reynolds DS, Rosahl TW, Cirone J, *et al.* Sedation and anesthesia mediated by distinct GABA(A) receptor isoforms. J Neurosci 2003; 23:8608–8617.

14 • Cheng VY, Martin LJ, Elliott EM, *et al.* Alpha5GABAA receptors mediate the amnestic but not sedative-hypnotic effects of the general anesthetic etomidate. J Neurosci 2006; 26:3713–3720.

Mice lacking the alpha5 subunit of the $GABA_A$ receptor were found to be resistant to the amnestic effects of etomidate, but not the sedative/hypnotic effects, demonstrating that distinct $GABA_A$ receptor subtypes mediate specific drug actions.

15 •• Li GD, Chiara DC, Sawyer GW, *et al.* Identification of a GABAA receptor anesthetic binding site at subunit interfaces by photolabeling with an etomidate analog. J Neurosci 2006; 26:11599–11605.

This is the first study to report direct photolabeling of $GABA_A$ receptors with a photoactivatable anesthetic. Azi-etomidate binds to both alpha and beta subunits, suggesting an intersubunit binding pocket. A role for alpha subunits in etomidate binding was previously unknown.

16 • Zeller A, Arras M, Jurd R, Rudolph U. Identification of a molecular target mediating the general anesthetic actions of pentobarbital. Mol Pharmacol 2007; 71:852–859.

Mice with the point mutation $GABA_A$ beta3(N265M) are resistant to the immobilizing and hypnotic actions of pentobarbital, but not the respiratory depressant effects. This contrasts with similar studies on etomidate and propofol, where respiratory depression was linked to the beta3 subunit. This suggests that the respiratory effects of pentobarbital are mediated by a distinct pathway.

17 Paris A, Philipp M, Tonner PH, *et al.* Activation of alpha 2B-adrenoceptors mediates the cardiovascular effects of etomidate. Anesthesiology 2003; 99:889–895.

18 Patel S, Wohlfeil ER, Rademacher DJ, *et al.* The general anesthetic propofol increases brain N-arachidonylethanolamine (anandamide) content and inhibits fatty acid amide hydrolase. Br J Pharmacol 2003; 139:1005–1013.

19 Jevtovic-Todorovic V, Todorovic SM, Mennerick S, *et al.* Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin. Nat Med 1998; 4:460–463.

20 Franks NP, Dickinson R, de Sousa SL, *et al.* How does xenon produce anaesthesia? Nature 1998; 396:324.

21 Zeilhofer HU, Swandulla D, Geisslinger G, Brune K. Differential effects of ketamine enantiomers on NMDA receptor currents in cultured neurons. Eur J Pharmacol 1992; 213:155–158.

22 Raines DE, Claycomb RJ, Scheller M, Forman SA. Nonhalogenated alkane anesthetics fail to potentiate agonist actions on two ligand-gated ion channels. Anesthesiology 2001; 95:470–477.

23 Sato Y, Kobayashi E, Hakamata Y, *et al.* Chronopharmacological studies of ketamine in normal and NMDA epsilon1 receptor knockout mice. Br J Anaesth 2004; 92:859–864.

24 Sato Y, Kobayashi E, Murayama T, *et al.* Effect of *N*-methyl-D-aspartate receptor epsilon1 subunit gene disruption of the action of general anesthetic drugs in mice. Anesthesiology 2005; 102:557–561.

25 Nagele P, Metz LB, Crowder CM. Nitrous oxide (N(2)O) requires the *N*-methyl-D-aspartate receptor for its action in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 2004; 101:8791–8796.

26 Nagele P, Metz LB, Crowder CM. Xenon acts by inhibition of non-*N*-methyl-D-aspartate receptor-mediated glutamatergic neurotransmission in Caenorhabditis elegans. Anesthesiology 2005; 103:508–513.

27 Yamakura T, Harris RA. Effects of gaseous anesthetics nitrous oxide and xenon on ligand-gated ion channels. Comparison with isoflurane and ethanol. Anesthesiology 2000; 93:1095–1101.

28 Plested AJ, Wildman SS, Lieb WR, Franks NP. Determinants of the sensitivity of AMPA receptors to xenon. Anesthesiology 2004; 100:347–358.

29 • Colloc'h N, Sopkova-de Oliveira Santos J, Retailleau P, *et al.* Protein crystallography under xenon and nitrous oxide pressure: comparison with in vivo pharmacology studies and implications for the mechanism of inhaled anesthetic action. Biophys J 2007; 92:217–224.

A recent study investigating the binding interactions of two small anesthetics, N_2O and Xe, with two model proteins. The authors found that both anesthetics bind to the same sites in both proteins, and that anesthetic-induced volume expansion correlates with anesthetic potency.

30 Stabernack C, Sonner JM, Laster M, *et al.* Spinal *N*-methyl-D-aspartate receptors may contribute to the immobilizing action of isoflurane. Anesth Analg 2003; 96:102–107.

31 Coates KM, Flood P. Ketamine and its preservative, benzethonium chloride, both inhibit human recombinant alpha7 and alpha4beta2 neuronal nicotinic acetylcholine receptors in Xenopus oocytes. Br J Pharmacol 2001; 134:871–879.

32 Friederich P, Dybek A, Urban BW. Stereospecific interaction of ketamine with nicotinic acetylcholine receptors in human sympathetic ganglion-like SH-SY5Y cells. Anesthesiology 2000; 93:818–824.

33 Eger EI 2nd, Zhang Y, Laster M, *et al.* Acetylcholine receptors do not mediate the immobilization produced by inhaled anesthetics. Anesth Analg 2002; 94:1500–1504.

34 Raines DE, Claycomb RJ, Forman SA. Nonhalogenated anesthetic alkanes and perhalogenated nonimmobilizing alkanes inhibit alpha(4)beta(2) neuronal nicotinic acetylcholine receptors. Anesth Analg 2002; 95:573–577.

35 Franks NP, Honore E. The TREK K2P channels and their role in general anaesthesia and neuroprotection. Trends Pharmacol Sci 2004; 25:601–608.

36 Antognini JF, Schwartz K. Exaggerated anesthetic requirements in the preferentially anesthetized brain. Anesthesiology 1993; 79:1244–1249.

37 • Antognini JF, Raines DE, Solt K, *et al.* Hexafluorobenzene acts in the spinal cord, whereas o-difluorobenzene acts in both brain and spinal cord, to produce immobility. Anesth Analg 2007; 104:822–828.

MAC for o-difluorobenzene, a strong NMDA receptor inhibitor like Group 2 drugs, increases by only 37% when it is selectively delivered to the brain. In contrast, MAC for hexafluorobenzene, a weak NMDA receptor inhibitor and strong $GABA_A$ receptor modulator like Group 3 drugs, increases 2.5-fold. Thus, the anatomic sites of anesthetic-induced immobility appear to depend on selectivity for different molecular targets.

38 Mihic SJ, Ye Q, Wick MJ, *et al.* Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. Nature 1997; 389:385–389.

39 Zhang Y, Wu S, Eger EI 2nd, Sonner JM. Neither GABA(A) nor strychnine-sensitive glycine receptors are the sole mediators of MAC for isoflurane. Anesth Analg 2001; 92:123–127.

40 Zhang Y, Sonner JM, Eger EI 2nd, *et al.* Gamma-aminobutyric acidA receptors do not mediate the immobility produced by isoflurane. Anesth Analg 2004; 99:85–90.

41 Quinlan JJ, Homanics GE, Firestone LL. Anesthesia sensitivity in mice that lack the beta3 subunit of the gamma-aminobutyric acid type A receptor. Anesthesiology 1998; 88:775–780.

42 •• Sonner JM, Werner DF, Elsen FP, *et al.* Effect of isoflurane and other potent inhaled anesthetics on minimum alveolar concentration, learning, and the righting reflex in mice engineered to express alpha1 gamma-aminobutyric acid type A receptors unresponsive to isoflurane. Anesthesiology 2007; 106:107–113.

The authors engineered mice with a double mutation in the $GABA_A$ receptor alpha1 subunit that confers insensitivity to isoflurane in vitro, and found that the mice were selectively resistant to the hypnotic effects, but not amnestic or immobilizing actions of isoflurane. This demonstrates that $GABA_A$ receptors containing alpha1 subunits mediate the hypnotic actions of isoflurane.

43 Zhang Y, Laster MJ, Hara K, *et al.* Glycine receptors mediate part of the immobility produced by inhaled anesthetics. Anesth Analg 2003; 96:97–101.

44 Gray AT, Zhao BB, Kindler CH, *et al.* Volatile anesthetics activate the human tandem pore domain baseline K+ channel KCNK5. Anesthesiology 2000; 92:1722–1730.

45 Liu C, Au JD, Zou HL, *et al.* Potent activation of the human tandem pore domain K channel TRESK with clinical concentrations of volatile anesthetics. Anesth Analg 2004; 99:1715–1722.

46 Heurteaux C, Guy N, Laigle C, *et al.* TREK-1, a K+ channel involved in neuroprotection and general anesthesia. EMBO J 2004; 23:2684–2695.

47 Gerstin KM, Gong DH, Abdallah M, *et al.* Mutation of KCNK5 or Kir3.2 potassium channels in mice does not change minimum alveolar anesthetic concentration. Anesth Analg 2003; 96:1345–1349.

48 • Solt K, Eger EI 2nd, Raines DE. Differential modulation of human *N*-methyl-D-aspartate receptors by structurally diverse general anesthetics. Anesth Analg 2006; 102:1407–1411.

This study probes the structural and biophysical properties of anesthetics with different efficacies for NMDA receptor inhibition.

49 Sonner JM, Vissel B, Royle G, *et al.* The effect of three inhaled anesthetics in mice harboring mutations in the GluR6 (kainate) receptor gene. Anesth Analg 2005; 101:143–148.

50 Flood P, Role LW. Neuronal nicotinic acetylcholine receptor modulation by general anesthetics. Toxicol Lett 1998; 100–101:149–153.

51 Stevens RJ, Rusch D, Davies PA, Raines DE. Molecular properties important for inhaled anesthetic action on human 5-HT3A receptors. Anesth Analg 2005; 100:1696–1703.

52 Roch A, Shlyonsky V, Goolaerts A, *et al.* Halothane directly modifies Na+ and K+ channel activities in cultured human alveolar epithelial cells. Mol Pharmacol 2006; 69:1755–1762.

53 Nakae Y, Kohro S, Hogan QH, Bosnjak ZJ. Intracellular mechanism of mitochondrial adenosine triphosphate-sensitive potassium channel activation with isoflurane. Anesth Analg 2003; 97:1025–1032.

54 Chen X, Sirois JE, Lei Q, *et al.* HCN subunit-specific and cAMP-modulated effects of anesthetics on neuronal pacemaker currents. J Neurosci 2005; 25:5803–5814.