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Is a New Paradigm Needed to Explain How Inhaled Anesthetics Produce Immobility?

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Abstract

A paradox arises from present information concerning the mechanism(s) by which inhaled anesthetics produce immobility in the face of noxious stimulation. Several findings, such as additivity, suggest a common site at which inhaled anesthetics act to produce immobility. However, two decades of focused investigation have not identified a ligand- or voltage-gated channel that alone is sufficient to mediate immobility. Indeed, most putative targets provide minimal or no mediation. For example, opioid, 5-HT3, gamma-aminobutyric acid type A and glutamate receptors, and potassium and calcium channels appear to be irrelevant or play only minor roles. Furthermore, no combination of actions on ligand- or voltage-gated channels seems sufficient. A few plausible targets (e.g., sodium channels) merit further study, but there remains the possibility that immobilization results from a nonspecific mechanism.

Introduction

Immobility of the surgical patient is a cardinal feature of general anesthesia. All inhaled anesthetics (including all clinically useful anesthetics) share the capacity to produce immobility in the face of noxious stimulation. Immobility forms part of the standard unit of anesthetic potency, MAC, the minimum alveolar concentration of inhaled anesthetic that abolishes movement in response to noxious stimulation in 50% of subjects.[1–3] The mechanism of inhaled anesthetic action provides one of the oldest problems in pharmacology, and the most challenging. The present essay reviews the evidence for possible mechanisms by which inhaled anesthetics produce immobility.

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A few observations might serve the reader before embarking on that review. As will be emphasized below, the spinal cord is the primary site affected by inhaled anesthetics to produce immobility.[4,5] However, immobility does not result from a lack of processed input into the cord, nor is it necessarily consequent to the ability of the cord to respond to impulses from the brain that would provoke movement. Although the amnesia produced by inhaled anesthetics precludes extracting from surgical patients whether they felt pain during surgery, a surrogate measure, autonomic responses to noxious stimulation, indicates that immobility is not a consequence of a limitation on sensory input. At 1.0 MAC, incision produces an increase in arterial blood pressure and heart rate,[6] and it increases ventilation, even at twice MAC.[7] MAC-BAR for sevoflurane exceeds MAC by a factor of 2.2.[8] Sensory-evoked potentials in humans can be recorded at concentrations well in excess of MAC.[9] Transmission of impulses through the dorsal horn of rats continues during halothane, isoflurane and propofol anesthesia,[10,11] and propofol anesthesia does not prevent spinal cord c-fos expression in mice subjected to an intraplantar injection of formalin.[12] Similarly, immobility is not a consequence of paralysis, nor does it necessarily result from the inability of motor nerves in the anterior horn to respond to impulses from the brain. At least some studies find that motor-evoked potentials continue at 1.0 MAC.[13]

If inhaled anesthetics at MAC allow both transmission of sensory input and cerebral control over motor movement, how do they suppress movement in response to noxious stimulation? How do they accomplish that at the level of the spinal cord? The answer is not known for certain, but one possibility is that they depress central pattern generators in the cord, local command-posts that coordinate movement.[14] Consistent with this notion, studies with nitrous oxide suggest that its action lies in ventral portions of the cord.[15]

The essay first considers evidence suggesting that inhaled anesthetics act at a common site to produce immobility. The evidence includes the absence of synergy in inhaled anesthetic interactions, similarities in steric and electrostatic properties of inhaled anesthetics, the correlation of MAC with affinity to the membrane bilayer interface or its surrogate, and the evolutionary conservation of the site at which anesthetics act.

The essay continues with an examination of the potential contributions of specific ligand-gated channels, concluding that one or two such channels (e.g., glycine) might play a role, but that present evidence suggests that no one channel can explain more than a portion of anesthetic-induced immobility. Voltage-gated potassium channels seem unable to explain the production of immobility, but the voltage-gated sodium channels remain a plausible candidate. How inhaled anesthetics act to block this and other sites remains a mystery, but some new concepts are proposed.

Evidence for a Common Site of Inhaled Anesthetic Action

Additivity

Observations made below indicate that inhaled anesthetic actions on potassium channels or a single ligand-gated channel can explain only a minor part of the capacity of inhaled anesthetics to produce immobility. Even summing the effects of inhaled anesthetics on several channels appears to be insufficient to explain immobility. Could synergistic inhaled anesthetic effects on ligand- and voltage-gated channels magnify their actions sufficiently to produce immobility?

We have found[16] that inhaled anesthetic pairs that act on different channels (i.e., at MAC, anesthetic A acts potently on receptor X but weakly on receptor Y, whereas anesthetic B acts weakly on X but potently on Y) combine in an additive, never synergistic, manner to produce immobility (Fig. 1). As the recent review by Hendrickx et al. shows,[17] this is unusual. Most

drugs that act on separate channels (i.e., as anesthetics A and B) show synergy when combined. A recent theoretical analysis of additivity and synergy demonstrated that there are only two mechanisms by which additivity can be observed: two drugs competing at the same site of action, or two drugs acting at different sites of action at concentrations causing very low levels of receptor occupancy.[18] The implication is that, if there is more than one biological target for inhaled anesthetic action, then binding of an inhaled anesthetic to those sites of action must be very weak. This effectively excludes high affinity targets as potential sites of anesthetic action.

Since clinical concentrations are typically in the range of 0.3 mM (especially see Table 1 of the following cited article),[19] it follows that if anesthetics act at more than one site of action, then Kd must exceed 0.3 mM, possibly many-fold. This is weak binding compared to IV anesthetics, which are typically effective at μM or nM concentrations,[20] and have Kd values also in the nM range.[21,22] Such observations effectively exclude the relatively high affinity targets of IV anesthetic action as targets of inhaled anesthetic action.

Although additivity argues for a single site of action, it is not definitive. Although synergy more commonly results from concurrent actions of drugs on different sites, such pairs can produce additivity.[17] A report concerning the anesthetic actions of propofol plus sevoflurane provides a example.[23] Thus, additivity constitutes but part of a broader argument for a single site.

Common Steric and Electrostatic Properties Define Anesthetic Action

Bertaccini et al. found common chemical motifs within various anesthetic binding sites.[24] Similarly, Sewell and Sear asked if volatile halogenated anesthetics have electrostatic (charge) and steric (shape and size) properties that define their potencies as anesthetics, specifically their capacities to produce immobility.[25] They applied comparative molecular field analysis (CoMFA) to 69 structurally diverse halogenated anesthetics, randomly divided into a training-set (N=52) used to derive their model and a test-set (N=17) used to independently assess the model's predictive power. The method maximized similarity in molecular shape and electrostatic potential. The predicted and observed activities of the training set had a correlation coefficient squared of 94% (i.e., the model explained 94% of the variance in the observed activities) and 70%–84% of the test-set. Similar correlations were found for non-halogenated volatile anesthetics,[26] with considerable overlap, particularly for certain steric characteristics. The demonstration that CoMFA can predict anesthetic potency from anesthetic structures suggests that potency and binding affinity (which depends on anesthetic structure) are tightly linked as it is unlikely that one could develop a single 3-D pharmacophoric map for MAC with high predictive ability if there was little relationship between anesthetic binding affinity and potency. The CoMFA results might also be taken to imply a single rather than multiple sites at which inhaled anesthetics might act. However, Sewell and Sear caution that "It should be noted that a common molecular basis for immobilizing activity does not necessarily imply a common site of action."

Implications of Stereoselectivity

The (+) isomer of isoflurane is 53% more potent than the (−) isomer in rats, "consistent with a receptor-mediated anesthetic mechanism by volatile anesthetics. "[27] Small stereoselective effects are found with 2-butanol and 2-pentanol (but not 2-hexanol or 2-heptanol.)[28] Although stereoselective effects can be found *in vitro* (in frog oocytes) for the actions of these alcohols on TRESK potassium channels, gamma-aminobutyric acid type A ($GABA_A$) receptors, and/or N-methyl-D-aspartate (NMDA) receptors, the effect does not correlate with the *in vivo* differences in MAC.[29] Stereospecificity is an important determinant of potency for anesthetics such as ketamine.[30] It implies a binding site, and that inhaled anesthetics conform to a specific pharmacophore. If stereoselectivity is important to inhaled anesthetics, does that indicate a

specific (read single) site of action? How would xenon, a nicely rounded simple atom, dock in a protein in the same highly specific manner that more complex ligands would dock? Does xenon bind, and cause profound effects, at the same place isoflurane binds? Perhaps so,[31] and yet a single portion of a protein acting stereoselectively would seem an unlikely site for a common place at which xenon and isoflurane would act; perhaps a lipid bilayer (which has chiral centers) would form a more attractive site.

An Updated Meyer-Overton Relationship

More than one-hundred years ago, Meyer[32] and Overton[33] demonstrated a correlation between anesthetic affinity for lipid and anesthetic potency. This correlation guided studies of anesthetic mechanisms for 80 years, focusing work on the lipid bilayer. Several investigators, notably Franks and Lieb,[34] shifted that focus to proteins. Part of the shift resulted from the failure of a bilayer focus to produce a verifiable theory. Part resulted from evidence against the correlation. For example, non-immobilizers are inhaled compounds that do not produce immobility despite possessing a lipophilicity that would indicate anesthetic capability.[35] However, if another factor, polarity, is added to the calculus of factors determining potency, a modified Meyer-Overton relationship remains defensible.[36,37] That is, the anesthetic interaction with proteins implies amphipathicity.[24] The correlation can be much improved by selecting a solvent that has an element of polarity. Abraham et al. suggested methanol.[38] The correlation also can be much improved by selecting a lipid-like phase that more closely resembles the membrane bilayer [e.g., one that includes phospholipoproteins].[39,40] If such a modified Meyer-Overton relationship is correct, it implies similar sites of action for multiple receptor/channels. It would be remarkable if specific pockets in various channel proteins (both inhibitory and excitatory) share relatively common characteristics.

Go a step further and demand a correlation with a slope of 1.0. This results in MAC times some index of solubility (e.g., solubility in methanol or some other solvent) equals a constant. A key point is not that anesthetic potency has something to do with methanol, but that exactly the same number of anesthetic molecules at the site of action are required to produce MAC and that site of action resembles methanol. How could that be across 5–7 orders of MAC values, unless some fundamental, highly conserved, process was at work?

Evolution and Conservation of the Anesthetic Site of Action

MAC or its equivalent varies little (perhaps two- or three-fold) among different vertebrate classes, again suggesting conservation of the site at which anesthetics act. Such a site has no apparent survival benefit since anesthetics do not form part of the natural environment. Thus, the constancy implies that the susceptibility to anesthetics develops from a parallel process, some essential aspect of ion channel function that fortuitously leads to this sensitivity and confers a survival benefit. It is difficult to see how this might result from an invariant protein structure of inhibitory and excitatory receptors. Is it reasonable to believe that there is a single such structure in all plausible channels? These issues are discussed in a recent symposium in this issue of *Anesthesia & Analgesia*.[41–46]

In that symposium, Sonner hypothesized that one-celled organisms selected a beneficial trait that also fortuitously produced the capacity of inhaled anesthetics to increase in currents through inhibitory channels, and decrease currents through excitatory channels.[41] The beneficial trait arose as a response to compounds present in the environment that influenced the conformational equilibrium of ion channels and otherwise would have promoted the entry of positive charges that might damage the cell. That is, the trait increased the fitness (survival) of the organism by limiting the effect of compounds present in the environment that might otherwise reduce electrochemical potentials across the cell membrane through their effects on channel function. Consistent with this view, exposure to inhaled anesthetics changes the

membrane composition of one-celled organisms.[47–50] The finding that surfactants modulate anesthetic-sensitive channels in a manner similar to inhaled anesthetics[51] is consistent with the notion that the response to anesthetics arose as an adaptation to environmental conditions which influenced channel function by perturbing bilayer properties.

This evolutionary narrative correctly predicted that certain nonvolatile compounds have anesthetic-like modulatory effects on ion channels and in animals. These may include endogenous compounds increased in disease [e.g., ammonia[52] and ketoacids.][53] Such compounds modulate ion channel function in a manner similar to inhaled anesthetics.[51] Cantor proposed that the slow adsorption and desorption of high (higher than those arising at synapses?) concentrations of neurotransmitter onto and off the membrane may produce a parallel, membrane-mediated effect manifested as receptor desensitization.[54] This process may provide a selective pressure for receptors to respond to membrane-mediated effects of inhaled anesthetics in multicellular organisms. The anesthetic-like modulatory effect of non-native neurotransmitters on receptors (e.g., acetylcholine on an NMDA receptor) supports this hypothesis.[55]

Tests of Relevance

Inhaled anesthetics affect many ligand- and voltage-gated ion channels in ways that plausibly explain immobility (e.g., enhancement of inhibitory channels; blockade of excitatory channels). How can we test whether plausible translates to relevant? Exclusion or inclusion of a target as a likely mediator of anesthesia may require the concurrence of results from several tests of relevance.

Location of effect

An explanation for MAC must consider the importance of the spinal cord in this process. Two seminal studies, differing in experimental design, demonstrated that the spinal cord, not the brain, is the primary site at which inhaled anesthetics produce immobility.[4,56,57] One study finds that direct application of sevoflurane to the spinal cord can produce reversible immobility in response to noxious stimulation of the hindlimbs,[58] and a second study finds that epidural administration of an emulsion of 8% isoflurane can produce a reversible epidural anesthetic in rabbits but does not appear to affect the level of consciousness.[59] A limitation of these investigations is that one cannot tell if the cord anesthetic concentrations far exceed those needed during conventional anesthesia.

Non-Immobilizers

Some inhaled compounds do not produce immobility in the face of noxious stimulation, nor do they decrease the requirement for anesthesia by conventional inhaled anesthetics.[35] These "non-immobilizers" do not produce or contribute to anesthesia despite having a lipophilicity that predicts that they should.[32,33] Non-immobilizers should not influence a relevant site of anesthesia.

Correlation of Physiological Changes on the Channel and on MAC

Physiological variables that affect a channel should produce an effect that is consistent with the effect of those variables on MAC. For example, an increase in temperature can open an inhibitory potassium channel.[60] An increase in inhibitory current should decrease MAC but, in fact, MAC increases with temperature,[61] diminishing the likelihood that this channel is a relevant mediator.

Agonists and Antagonists as Pharmacological Probes

Drugs that block or enhance putative targets of inhaled anesthetic action can be used to test the relevance of those targets. An advantage of this approach over some genetic approaches is that the effects leave less time for compensation. However, the interpretation of pharmacological interactions can be complex.

Suppose administration of a given receptor antagonist does not affect MAC. Blockade of that receptor by the inhaled anesthetic cannot be the sole cause of anesthesia or the antagonist would have produced anesthesia. The initial interpretation of an absence of blocker effect might be that the receptor is not relevant. However, what if MAC concentrations potently block the receptor in question, but concurrent blockade of a second receptor is required for immobility? Blockade of both receptors would be needed to produce anesthesia. Thus, administration of a blocker of one might not decrease the need for the inhaled anesthetic to block the second, leaving the concentration required for anesthesia unchanged.

To find that the receptor was irrelevant might require demonstration that blockade had no effect on an anesthetic whose action was known to be caused solely by alteration of a different receptor. Inhaled anesthetics block specific neuronal acetylcholine receptors at concentrations much less than MAC.[62] Neuronal nicotinic acetylcholine receptors are probably irrelevant to immobilization because administration of mecamylamine (an antagonist of neuronal nicotinic acetylcholine receptors) does not affect MAC for several inhaled anesthetics, and because mecamylamine does not influence the anesthetic requirement for etomidate (which produces immobility solely by enhancing the effect of GABA on $GABA_A$ receptors).[63]

Parallel arguments may apply to inhibitory receptors. Consider GABA, the major inhibitory transmitter in the nervous system, as an example. Suppose blockade of $GABA_A$ receptors (e.g., with picrotoxin) increases the MAC of test anesthetics. One might argue that MAC increased because anesthetic-induced enhancement of the $GABA_A$ receptor response to GABA had been abolished. However, relevance demands that the increases in MAC consequent to blockade be proportional to the separately measured capacity of the anesthetic to enhance the receptor response *in vitro*. In other words, suppose that one MAC of test anesthetic X minimally enhances $GABA_A$ receptors *in vitro*, but one MAC of anesthetic Y has a major effect. This would mean that the anesthetic effects of X do not result from enhancement of $GABA_A$ receptors, but the anesthetic effects of Y might, in part, be explained by their action on $GABA_A$ receptors. If that were true, then blockade of $GABA_A$ receptors might minimally increase the MAC of X, but substantially increase the MAC of Y. That is what is found for blockade of glycine receptors with strychnine but not for blockade of $GABA_A$ receptors with picrotoxin (see below). Thus $GABA_A$ receptors are not relevant as mediators of immobility, but glycine receptors could be.

One might also suppose blockade of an excitatory receptor decreases MAC. In itself, that proves nothing since many factors that do not mediate anesthesia (e.g., administration of opioids) can modulate anesthesia. As with blockade of inhibitory receptors, relevance demands that the effect on MAC be inversely proportional to the capacity of the test anesthetics to block the excitatory receptor *in vitro*. For example, if the anesthetic completely blocks the receptor at one MAC, then administration of a blocker cannot increase blockade and cannot affect MAC. In contrast, administration of a blocker can decrease MAC of an anesthetic that minimally affects the receptor because the blockade can add to the decrease in excitatory neurotransmission. By this test, we can exclude NMDA receptors as relevant mediators of the effects of conventional inhaled anesthetics (see below).

Genetic Tests

Global knockouts—If global knockout (genetic inactivation) of a receptor markedly changes MAC, we might suspect that the receptor mediates anesthesia. While global knockout studies have advanced our understanding of anesthetic mechanisms,[64] two problems can confound results obtained using this approach. First, gene inactivation can alter expression of other genes and thereby compensate for the loss of the targeted gene. Second, global knockout affects all neurons and thus can influence MAC by an effect on cerebral rather than spinal cord neurons, an effect we believe is irrelevant since the brain, at most, provides only a minor contribution to the immobility produced by inhaled anesthetics.

Knockins—An alternative approach relies on the production of receptor knockins, wherein an "engineered" receptor that responds normally to its ligand (e.g., GABA) but is not influenced by anesthetics replaces the natural ("wild-type) receptor. If a receptor knockin animal no longer responds to anesthetics, that receptor likely mediates anesthesia. A major advantage of this strategy is that it should not provoke compensatory effects because it permits a normal response to the natural ligand. The knockin mouse has powerfully contributed to our appreciation of the $GABA_A$ receptor to the action of anesthetics such as etomidate.[64] Of note, this knockin does not materially alter isoflurane MAC,[65] but the knockin approach can have problems. The replacement can have a different distribution from the wild-type receptor and this may provoke compensation, or the engineered receptor might not have completely normal function. Finally, both the knockin and knockout approaches usually alter only one subtype of a receptor (although multiple mutations are possible). A given family (e.g., $GABA_A$ receptors) can include tens or hundreds of receptors and perhaps the critical subtype was not chosen!

Variations on a Theme—Some genetic approaches minimize the potential for compensation. For example, the receptor can be targeted for expression of a receptor deletion (induction of deletion) at a specific time in life or in a specific region of the body/brain.

Cautions

Absence of movement in response to noxious stimuli underlies MAC, which has obvious clinical importance. Other end-points, such as loss of righting reflex, do not substitute for immobility. Loss of righting reflex results from cerebral as well as spinal cord actions. There is no necessary correlation between the concentrations that suppress immobility versus righting.[66,67] Similar restrictions limit the usefulness of animals that have the attraction of allowing considerable genetic manipulation (e.g., fruit flies and worms).

Relevance of Plausible Specific Targets (Alphabetical Order) to MAC

A present consensus holds that multiple ligand-gated and/or voltage-gated ion channels and possibly other targets mediate the immobility produced by inhaled anesthetics. These include receptors for GABA, glycine, acetylcholine (neuronal nicotinic), various glutamate (e.g., NMDA and AMPA-kainate), opioid, adrenergic, and serotonin receptors, nitric oxide synthase (NOS) (and nitric oxide), sodium and potassium channels, and gap junctions. The present essay will not assemble the considerable evidence for the plausibility of these targets as mediators of immobility; we[68] and others[20,69–71] have done that previously, with suggestions of specific receptor locations needed to produce anesthesia.[72,73] Instead we will discuss evidence that suggests that no single target can explain immobility produced by inhaled anesthetics.

Acetylcholine Receptors

In rats, blockade of nicotinic (mecamylamine) or muscarinic (atropine or scopolamine) receptors does not modify anesthetic potency, either *in vivo*[74,75] or *in vitro*.[76] Co-administration of large doses of mecamylamine and atropine does not affect the cerebral

concentration of etomidate required to produce immobility.[63] Intrathecal administration of atropine does not alter MAC of isoflurane.[75] Similarly, in mice, administration of nicotine [at concentrations that produce behavioral effects][77] does not alter isoflurane MAC.[74] 1,1,2-trichlorocyclobutane (an anesthetic) and 1,2-dichlorohexafluorocyclobutane (a non-immobilizer) both inhibit neuronal nicotinic receptors.[78–80] These observations suggest that acetylcholine receptors do not mediate inhaled anesthetic-induced immobility.

Acid-Sensitive Ion Channels (ASICs)

ASICs are proton-gated members of the family of degenerin channels[81] and are expressed in the spinal cord,[82] particularly in the dorsal horn.[83] They are important to the modulation of nociception.[84] Acidosis opens such channels, increasing excitatory currents but also increasing the rate of inactivation.[83] The latter may decrease repetitive firing. If these channels mediate the capacity of inhaled anesthetics to produce immobility, one might expect that the acidosis produced by inhalation of carbon dioxide might increase MAC by opening such channels. However, a rectilinear decrease in MAC is found.[85]

Adenosine Receptors

Adenosine receptors have been proposed as mediators of the capacity of inhaled anesthetics to produce immobility. Administration of adenosine decreases MAC of halothane in dogs,[86] and intrathecal administration of the adenosine agonist R-phenylisopropyl-adenosine (R-PIA) decreases halothane MAC in rats.[87] However, aminophylline, an adenosine receptor blocker, does not increase halothane MAC in dogs,[88] rats,[88] or humans.[89] Similarly, administration of the A_1 adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) does not alter halothane MAC in rats, but DPCPX administration does prevent the decrease in MAC that otherwise is produced by the adenosine receptor agonist R-PIA.[87] Thus, adenosine receptors do not appear to mediate the immobility produced by inhaled anesthetics.

Adenosine Triphosphate Receptors

Administration of 100 $\mu g*kg^{-1}*min^{-1}$ adenosine triphosphate to patients does not alter MACawake or MAC of sevoflurane in humans.[90]

α-2 Adrenoreceptors

Results from studies of α-2 adrenoreceptors expressed in Xenopus oocytes suggest that halothane does not act via such receptors.[91] N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (a catecholamine depleter) does not change halothane MAC in rats,[92] and the α-2 adrenoreceptor blocker tolazoline does not affect halothane MAC in dogs.[93] The blocking drugs yohimbine and atipamezole at 0.8 mg/kg–1.0 mg/kg increase the MAC of isoflurane in rats by approximately 10%, but larger doses decrease MAC, particularly as lethal doses are reached.[94] The 10% increase in MAC probably results from suppression of the effect of normal tonic stimulation of α-2 adrenoreceptors. Since blockade and depletion of α-2 adrenoreceptors minimally affects MAC, these receptors are unlikely mediators of the capacity of inhaled anesthetics to produce immobility.

Calcium Channels

Although some studies support a role for Ca^{++} channels,[95] several do not. Enflurane, halothane, and isoflurane concentrations exceeding MAC by an order of magnitude minimally affect Ca^{++} channels *in vitro*.[96,97] Halothane and sevoflurane exert opposite effects on Ca^{++} release from sarcoplasmic reticulum,[98] but are nearly exactly additive in their capacities to produce immobility.[16] Knockout of the α_{IG} T-type calcium channel does not alter MAC in mice.[99] Ca^{++} channels seem unlikely mediators of immobility.

Catecholamine Receptors

Dopamine receptors do not materially mediate the immobility produced by inhaled anesthetics in rats.[100] However, depletion of catecholamine neurotransmitters (including norepinephrine, and epinephrine) with reserpine or other drugs decreases MAC for halothane and/or cyclopropane in dogs[101,102] and rats.[103,104] The decrease varies, one study finding a 20% decrease,[102,103] a second a maximum of 30%,[101] and others of 40%,[102–104] but one study found no change in cyclopropane MAC in rats given a single dose of 10 mg/kg reserpine,[105] a dose that decreased whole brain norepinephrine to 3% of control values. Such results suggest that neurotransmission via catecholamine receptors might explain a minor portion of the immobility produced by inhaled anesthetics, but the decrease could be an epiphenomenon related to the nearly complete depletion of an excitatory neurotransmitter(s).

$GABA_A$ Receptors

Propofol, alfaxalone and etomidate produce immobility by enhancing $GABA_A$ receptor function (i.e., by increasing the effect of a given concentration of GABA).[106] A point mutation that minimizes the effect of propofol or etomidate on the $GABA_A$ receptor also minimizes the capacity of these compounds to produce anesthesia.[64] Since inhaled anesthetics also enhance the action of GABA on $GABA_A$ receptors,[107] a parallel effect would seem plausible. However, present evidence argues against the importance of this receptor to the immobility produced by inhaled anesthetics.

Not all drugs that augment the *in vivo* action of GABA produce immobility. Gabaculine, a GABA-transaminase inhibitor, produces a dose-related loss of righting reflex in mice but does not alter the MAC of halothane, enflurane, isoflurane, or sevoflurane.[66] The absence of a correlation of loss of righting reflex and MAC is consistent with the finding that the concentration of an inhaled anesthetic required to impair righting does not bear a constant relationship with the MAC for that anesthetic.[67] Others have noted a differential effect of sevoflurane on $GABA_A$ receptors in the cortex versus the spinal cord, minimally affecting the latter.[108]

In rats, the noncompetitive $GABA_A$ receptor antagonist picrotoxin differs in its effects on the immobilization produced by propofol versus inhaled anesthetics. The direct antagonism of $GABA_A$ receptors by picrotoxin progressively increases the immobilizing ED_{50} of propofol up to at least 400%, showing no ceiling for this effect.[109] In contrast, picrotoxin (or gabazine, a competitive antagonist at $GABA_A$ receptors) administration equally increases isoflurane MAC and the immobilizing ED_{50} of the nonGABAergic anesthetic ketamine to a ceiling of approximately 60% (reflecting an indirect antagonism of the effect of naturally occurring tonic GABA release).[109]

Cyclopropane and xenon minimally enhance the response of $GABA_A$ receptors to GABA *in vitro*, whereas isoflurane causes substantial enhancement.[20,110,111] If $GABA_A$ receptors mediate the capacity of inhaled anesthetics to suppress movement in response to noxious stimuli, then blockade of $GABA_A$ receptors should increase the MAC of isoflurane more than the MAC of xenon or cyclopropane. However, intrathecal administration of picrotoxin increases MAC equally for all three anesthetics (Fig. 2),[68,112] indicating that $GABA_A$ receptors do not mediate immobility produced by inhaled anesthetics.

Evidence from genetically engineered mice adds to the notion that enhancement of the effect of GABA does not underlie MAC. Global knockout of the α-1 $GABA_A$ receptor does not change isoflurane MAC.[113] Knockout of the β3 subunit of the $GABA_A$ receptor increases enflurane MAC by 26% but only increases halothane MAC by 9%,[114] and even these small increases could be attributable to compensation for lack of the subunit. Such minor increases

agree with those found by Jurd et al. for β3 N265M knockin mice unresponsive to the anesthetic effects of propofol but "normally" sensitive to GABA:[64] enflurane MAC was 15% greater than in wild-type mice, and halothane MAC was 21% greater. More importantly, this mutation equally increased the MAC of isoflurane and cyclopropane despite the enormous differences in enhancement of $GABA_A$ receptors by these anesthetics.[65]

Similarly, MAC for fluorinated alkanols[115] does not correlate with their capacity to enhance the response of $GABA_A$ receptors.[116] Also, enflurane and halothane enhance GABA-mediated chloride conductance in rat hippocampal neurons,[117] but a given MAC-multiple of enflurane has twice the effect of halothane (i.e., the result is not quantitatively consistent across anesthetics.)

Thus results from studies of blocking drugs and of knockin and knockout animals do not support a role for $GABA_A$ receptors as mediators of the immobility produced by inhaled anesthetics.

Gap Junctions

Gap junctions are protein channels that form electrical synapses by directly connecting the cytosol of neighboring cells. Although these may be plausible targets of drugs, such as thiopental and propofol, 10 MAC, but not 2 MAC, halothane can block gap junction coupling in hippocampal slices.[118] Others similarly report a low sensitivity of gap junctions to the effects of inhaled anesthetics.[119]

Glycine Receptors

Many IV anesthetics enhance glycine receptor function.[107,120,121] Their spinal localization and their enhancement by volatile anesthetics suggest a role as a mediator of MAC. Evidence in rats supports this notion. Intravenous and intrathecal administration of strychnine, a glycine receptor antagonist, increases MAC.[122,123] In contrast to the similar effect of intrathecal picrotoxin on the MAC of cyclopropane, xenon and isoflurane (see above), the increase in the MAC of cyclopropane, isoflurane, and halothane produced by intrathecal strychnine administration correlates with the enhancing effect of these anesthetics on glycine receptors *in vitro* (Fig. 2).[68,123]

Results from studies of mice with genetic alterations provide conflicting evidence for the importance of glycine receptors to MAC. Spastic mice have decreased glycine receptor expression[124] and show a 30% increase in enflurane MAC but no increase in halothane MAC.[125] A missense mutation in the glycine receptor α1 subunit decreases the sensitivity of spasmodic mice to glycine,[126] but these mice show no difference in enflurane or halothane MAC from control mice.[125]

Overall, current data indicate that glycine receptors might mediate part of the immobility produced by some inhaled anesthetics (e.g., halothane and isoflurane) but not by other anesthetics (e.g., cyclopropane).

Glutamate Receptors

AMPA Receptors—Blockade of AMPA receptors can decrease MAC by approximately 60%,[127] a maximum decrease similar to that found with NMDA receptor blockade.[128,129] AMPA receptor blockade can augment the capacity of blockade of NMDA receptors to decrease MAC.[130] Enflurane inhibits the postsynaptic action of glutamate on AMPA receptors in mouse spinal cord,[131] and halothane similarly affects the hippocampus, but at higher MAC values.[132] Also, clinically-relevant concentrations of volatile anesthetics partially inhibit native and recombinant AMPA receptors activated by exogenous agonists.[131,133–136]

However, results from studies of genetically modified mice do not support the notion that AMPA receptors mediate the immobility produced by inhaled anesthetics. GluR1-GluR4 subunit combinations form AMPA receptors. Mice lacking the GluR2 subunit have MAC values for halothane, isoflurane and sevoflurane similar to wild type littermates, despite altered concentrations for loss of righting and antinociception.[135] Clinical concentrations of isoflurane and halothane minimally inhibit AMPA receptors *in vitro* (both GluR2-containing and deficient).[135] Motor neurons in the ventral spinal cord (i.e., that could mediate MAC) lack GluR2 subunits.[137–139] Thus, knockout of the GluR2-containing receptors should not change MAC but might change righting reflex and antinociception.

Although the above data suggest that the GluR2 subunit does not affect MAC, this cannot exclude the possibility that other subunits of AMPA receptors contribute to MAC. This concern appears to be addressed by the failure of the intrathecal injection of a blocker of AMPA receptors to change the MAC of isoflurane.[140]

Kainate Receptors—GluR5-7, KA1 and KA2 subunits combine to form the kainate subtype of ionotropic glutamate receptors.[141] Although inhaled anesthetics enhance currents mediated by kainate receptors containing GluR6 *in vitro*,[142] GluR6 knockout mice have normal desflurane, halothane and isoflurane MAC values.[143] GluR6 editing mutant mice also do not demonstrate consistent changes in MAC values for these anesthetics.[143] However, the findings for GluR6 mutations do not conclusively eliminate kainate receptors as mediators of immobility because kainate receptors can be assembled from other subunits, even in the absence of the GluR6 subunit. Finally, the non-immobilizer F6 blocks mGluR5 (a metabotropic receptor),[78,79] a finding inconsistent with a role for this class of receptors as mediators of immobility. Intrathecal injection of a blocker of metabotropic glutamate receptors does not change the MAC of isoflurane.[140]

NMDA Receptors—Blockade of NMDA receptors, can markedly decrease MAC.[129,144,145] Ketamine, which largely produces anesthesia by inhibiting NMDA receptor function [but also acts on acetylcholine receptors],[146] can abolish movement in response to noxious stimulation. However, blockade of NMDA receptors alone does not appear to cause immobility.[129] Several lines of evidence suggest that NMDA receptors do not mediate the immobility produced by inhaled anesthetics. For example, knockout of the ε1 subunit in mice does not change the concentration of isoflurane or sevoflurane[147] or of ethanol[148] producing loss of righting. Approximately 10% of Caucasians are missing the NMDA receptor subunit NR3B,[149] but determinations of MAC do not reveal outliers and, clinically, 10% of Caucasian patients do not appear to have major decreases in inhaled anesthetic requirement.

Other indirect evidence further supports the notion that NMDA receptors do not mediate immobility. Like ketamine[150] and nitrous oxide,[151] xenon potently inhibits NMDA receptors *in vitro*,[152] but 1 MAC xenon decreases cerebral metabolism in humans[153], whereas ketamine[154] and nitrous oxide[155] increase metabolism. On the basis of such discrepancies, the authors of a human study of xenon suggested that "…inhibition of the glutamatergic system is likely to be of minor significance for the anesthetic action of xenon *in vivo*."[153]

A shortening of intervals between repeated stimuli increases the collective effect of stimulation (temporal summation) and increases the likelihood of a motor response to stimulation.[128] Studies with isoflurane indicate that approximately 40% of the generation of movement evoked by noxious stimulation (MAC) depends on interstimulus interval, suggesting the persistence of temporal summation and transmission via NMDA pathways.[156] That is, if temporal summation persists, then administration of the NMDA blocker MK-801 should and does abolish summation.[156] However, another interpretation is possible: perhaps isoflurane causes suppression of NMDA receptor transmission, and blockade with MK-801 simply substitutes

for the blockade produced by isoflurane. Consistent with the interpretation that isoflurane does not block temporal summation, electrophysiologic studies of neuronal wind-up show that temporal summation can occur during anesthesia.[157]

Finally, administration of drugs that block NMDA receptors can decrease MAC for conventional inhaled anesthetics by more than 60%. This decrease does not correlate with the extent of functional blockade that these anesthetics produce at MAC, although it does correlate with blockade that fluorinated aromatic anesthetics produce at MAC.[158,159] This failure of correlation plus the evidence from temporal summation and knockout mice leads to the conclusion that NMDA receptors do not mediate the immobility produced by conventional inhaled anesthetics.

NOS and Nitric Oxide

Knockout of the type I NOS isoform significantly increases isoflurane MAC.[160] The neuronal NOS inhibitors L-NAME[161–163] or 7-NI[160,162,164,165] can decrease MAC by 35%–95%, but not all investigators find an effect of L-NAME on MAC.[166,167] Peculiarly, 7-NI decreases MAC to the same extent in wild-type and NOS knockout mice,[160] and arginine reverses the effect of the 7-NI by the same amount in wild-type and NOS knockout mice.[160] Thus, diverse interactions may govern the anesthetic effects of changes in transmission mediated by NOS and nitric oxide, but evidence for a role as a mediator of the immobility produced by inhaled anesthetics is limited.

Opioid Receptors

Analgesia is thought to accompany anesthesia by inhaled anesthetics. Thus, inhaled anesthetics might enhance the release of endogenous opioids, and/or enhance sensitivity of opioid receptors and thereby contribute to the anesthetic state. However, inhaled anesthetics do not appear to increase endogenous opioid concentrations in cerebrospinal fluid,[168] and they do not prevent autonomic or ventilatory responses to surgical stimulation at concentrations that suppress movement.[7] Small doses of opioids markedly decrease inhaled anesthetic concentrations that prevent movement.[169] That is, opioids supply something (analgesia) that inhaled anesthetics do not produce or minimally produce. Finally, several reports suggest that administration of naloxone, a μ opioid receptor antagonist, does not affect the MAC of various inhaled anesthetics.[104,170–177] A single report suggested that mice lacking the μ opioid receptor gene ($\mu OR^{-/-}$) had a sevoflurane MAC 20% greater than wild-type mice bearing the normal ($\mu OR^{+/+}$) gene (3.3±0.5% vs. 2.7±0.2% sevoflurane), and that 0.1 mg/kg naloxone did not increase MAC in the knockout mice but increased MAC 18% in wild-type mice.[178] However, in a replication of this study, 0.1 and 1.0 mg/kg naloxone did not change sevoflurane MAC in the same strain of wild-type mice.[179] This replication added a control group of mice, and MAC was determined in a blinded manner. We conclude that opioid receptors can modulate, but do not mediate, the immobility produced by inhaled anesthetics.

Potassium Channels

Knockout of the TREK-1 potassium channel increases the MAC of chloroform, desflurane, halothane and sevoflurane.[180] However, the increase is not consistent, varying from 7% (desflurane) to 48% (halothane). This effect on MAC does not parallel the effect of these and other anesthetics on the capacity of those anesthetics to increase opening (current through) the channels *in vitro* (Fig. 3),[180,181] a disparity that is inconsistent with a causal connection. Finally, an increase in temperature opens TREK-1 channels,[60] and the consequent increase in inhibitory current should decrease MAC. However, an increase in body temperature increases MAC in diverse mammals,[61,182–184] further diminishing the likelihood that this channel is a relevant mediator.

Knockout of the TASK-3 channel significantly increases halothane MAC (by 18%) but not isoflurane MAC (a nonsignificant 9% increase is found). [185] Increases in pH increase the opening of TASK potassium channels,[186] but decreases in $PaCO_2$ and thus increases in pH do not decrease MAC.[85] Reducing pH blocks TASK channels,[186] and thus should increase MAC, but decreasing pH by increasing $PaCO_2$ decreases MAC rectilinearly.[187]

MAC does not increase in mice lacking KNCK5 or Kir3.2 potassium channels.[188] Intracerebroventricular administration of cromakalim or pinacidil [ATP-sensitive potassium channel (K_{ATP}) blocking drugs] does not affect isoflurane MAC,[189] but this does not indicate what action would result from application to the spinal cord. Systemic administration of the K^+ channel blocker 4-aminopyridine does not decrease halothane MAC in rats.[87] Administration of the TASK-1 or TASK-3 potassium channel blocker doxapram to mice does not increase MAC.[190]

Intravenous and intrathecal infusions of riluzole (a nonspecific activator of KCNK potassium channels) decreases isoflurane MAC in rats,[191] but a given dose produces the same change regardless of the route of administration. This result indicates that activation of potassium channels might affect anesthetic requirement, but does so primarily by an effect on higher centers rather than the spinal cord. The non-specificity of riluzole further complicates the issue. Riluzole even more potently blocks non-inactivating sodium channels,[192] channels important to repetitive firing (i.e., sustained activity)[193] and thus to MAC.

If potassium channels are important, an increase in extracellular potassium ion concentration might increase MAC by decreasing polarization. In dogs, an increase in serum K^+ from 3.8 ±0.2 mEq/L to 7.4±0.5 mEq/L did not change halothane MAC (1.09±0.04% and 1.09±0.04%).[194] Although the concurrent increase in cerebrospinal fluid K^+ was statistically significant, it was too small (2.5±0.1 mEq/L to 2.7±0.1 mEq/L) to test the importance of K^+. Changes in intrathecal K^+ in rats induced by infusing artificial cerebrospinal fluid with altered KCl concentrations ranging from zero to 24 times normal did not increase MAC.[195] The highest concentration infused caused post-infusion impairment of hindlimb function.

Overall, these findings are not consistent with a role for potassium channels as mediators of the capacity of inhaled anesthetics to produce immobility.

Serotonin (5HT) Receptors

5-HT2A Receptors—Although inhaled anesthetic concentrations of approximately 1 MAC block the *in vitro* effect of 5-HT on 5HT2A receptors[78] and the 5HT2A receptor blocker ketanserin[196–204] can decrease nociception by supraspinal and spinal[200,203,205] effects, other evidence suggests that 5HT2A receptors, and serotonin receptors in general, play a minimal role in producing immobility.

Systemic administration of ketanserin does not change[206] or may decrease[207,208] MAC by up to 60%, larger doses proving lethal,[208] but the same study finds that intrathecal administration of ketanserin decreases MAC only 20%–25%. If 5HT2 receptors mediate MAC, then intrathecal injection should produce the greater effect. The 20%–25% decrease in MAC could result from absorption and an effect on higher centers (i.e., this would indicate that spinal 5HT2 receptors are not important mediators of the immobility produced by inhaled anesthetics).

Halothane and the nonimmobilizer 1,2-dichlorohexafluorocyclobutane equally affect the 5HT2 receptor *in vitro*, at 1 MAC or concentrations predicted to equal 1 MAC (1,2-dichlorohexafluorocyclobutane).[78] Finally, administration of parachlorophenylalanine, which

depletes serotonin, does not decrease halothane MAC in dogs.[209] Such a finding, alone, suggests the lack of relevance of 5HT3 receptors as mediators of MAC.

5-HT2C Receptors—Both 1,1,2-trifluorocyclobutane (an anesthetic) and 1,2-dichlorohexafluorocyclobutane (a non-immobilizer) inhibit 5-HT2C receptors,[78,79] and thus these receptors are unlikely mediators of immobility.

5-HT3 Receptors—Blockade of the 5-HT3 receptor by systemic[210] or intrathecal (Pamela Flood, personal communication) administration of ondansetron does not affect the MAC of isoflurane.

Summary Regarding Ligand- and Voltage-Gated Channels as Mediators

No ligand- or voltage-gated channel or other targets discussed above appears capable of explaining more than a minor part of the immobility produced by inhaled anesthetics. One response to this observation is that it is the combined actions of numerous small effects on diverse targets that explains immobility. Perhaps 20 such targets, each contributing 5% to the production of anesthesia, add to produce a 100% effect, i.e., produce MAC. What does this hypothesis imply? Given that the spinal cord mediates the immobility produced by inhaled anesthetics,[4] each of the 20 targets would have to be present in appreciable numbers in the spinal cord or, if in small numbers, such a target would have to contribute mightily. Anesthetics would have to affect each target at relevant (anesthetizing) concentrations in a way that plausibly explained anesthesia. Note the enormous variation in the differences in functional potencies, acetycholine receptors nearly completely blocked at 0.1–0.2 MAC[62] and NMDA receptors affected, the majority blocked, by some anesthetics only at concentrations 2–3 times MAC.[159] Targets affected at sub-MAC concentrations would contribute less than those requiring supra-MAC concentrations so that at MAC each would contribute just 5% to the MAC. That is, fortuitously, each would, indeed, make a 5% contribution without any one target providing a large, obvious, contribution (i.e., one that would exceed the 5% average contribution, and therefore be measurable). That is, the 20-target theory would have to argue that no one target would stand out, even for one anesthetic. The 20 targets would have to add to each other's effect and not be synergistic (lest we see wide differences in potency/MAC among anesthetics that did not reflect the Meyer-Overton correlation). Thus, we see the 20-target theory as possible, but unlikely.

Another response is that we have not found the channel that mediates a major part of the immobility produced by inhaled anesthetics. The sodium channel might fit that argument (see below), but we are at a loss to deal with the researcher who says there are tens or thousands of X (e.g., potassium) channels that have not been explored. We can only answer that we cannot disprove the existence of dragons.[211]

Sodium Channels as the Mediators of Immobility

Why Sodium Channels?

The sodium channel presents an attractive target in that it potentially affects all ligand-gated ion channels because depolarization of the nerve terminal (a process governed by the sodium channel) underlies neurotransmitter release at those terminals. The diversity of sodium channels adds to their attractiveness, and the difficulty of ascribing anesthesia to an action on a particular channel. Fast inactivating sodium channels mediate the fast depolarization-repolarization that underlies action potentials, while other slowly inactivating sodium channels contribute to repetitive firing (i.e., sustained activity)[193] and perhaps temporal summation, and thus to MAC. Still other sodium channels on dendrites influence the response to release of synaptic ligands.[212] Thus, an effect on sodium channels allows for a multiplicity of

anesthetic actions that depends on the distribution of effects on various sodium channels. If immobility results from an action on sodium channels and, thereby, on multiple ligand-gated channels simultaneously, that might be consistent with the steep dose (anesthetic concentration)-response (movement) relationship seen for MAC,[213] although a more likely explanation for the steepness of the dose-response relationship that underlies MAC is low inter-subject variability in sensitivity.

Evidence Supporting Sodium Channels as Mediators of Immobility

Some findings indirectly support a role for sodium channels. An increase in central nervous system extracellular sodium rectilinearly increases MAC and conversely a decrease decreases MAC.[194] Systemic administration of the sodium channel blocker lidocaine progressively decreases MAC for several conventional anesthetics in rats.[214] Lidocaine shares one other property with some inhaled anesthetics [e.g., enflurane[215] and sevoflurane],[216] the capacity to produce convulsions.[217] Could this result from differential effects on the various sodium channels (e.g., decrease inhibitory output relative to excitatory output)? Intrathecal administration of veratridine, a compound that sustains the open state of sodium channels, increases isoflurane MAC in rats by a maximum of 21%.[218]

Sodium channels have been given little attention because sodium channel-dependent axonal conduction continues at all levels of anesthesia, and many levels of synaptic transmission remain intact. However, this ignores the greater vulnerability of the bare nerve terminal in contrast to the considerable reserve in conduction in myelinated nerves. In further support of the importance of sodium channels, inhaled anesthetics inhibit multiple isoforms of Nav α-subunits (rat $Na_v1.2$,[219] human $Na_v1.5$,[220] rat $Na_v1.2$, rat 1.6, and human $Na_v1.4$),[221] with small differences in anesthetic potencies and mechanism. Isoflurane at clinically relevant concentrations inhibits rat neuronal ($Na_v1.2$), skeletal muscle ($Na_v1.4$), and cardiac muscle ($Na_v1.5$) voltage-gated Na^+ channel α subunits heterologously expressed in Chinese hamster ovary cells with subtle isoform-dependent differences.[222] Although an earlier study found that the human $Na_v1.8$ isoform heterologously expressed in *Xenopus* oocytes was insensitive,[221] recent evidence indicates that isoflurane inhibits $Na_v1.8$ expressed in a neuroblastoma cell line (HCH, unpublished observations).

Potency differs among anesthetics with inhibition of rat $Na_v1.2$ expressed in *Xenopus* oocytes by 2 MAC cyclopropane being two-thirds that of 2 MAC isoflurane and by 2 MAC halothane being one-third.[221] Rehberg et al. found that isoflurane and halothane were equally potent but that enflurane was much less potent.[219] The non-immobilizer 1,2-dichlorohexafluorobutane has no effect.[221]

Sodium channel inhibition, and a consequently decreased nerve action potential, decreases release of neurotransmitters such as glutamate,[223] with 1 MAC isoflurane having a greater effect than 1 MAC halothane, but at 2 MAC, the difference disappears. Both isoflurane and propofol produce a dose-related inhibition of sodium channels in neurohypophysial nerve terminals, approximately a 30% inhibition at 1 MAC or its equivalent,[224] and progressively greater inhibition at larger concentrations. The non-immobilizer 1,2-dichlorohexafluorobutane does not affect sodium-channel-dependent glutamate release.[225]

A few factors suggest caution regarding the relevance of sodium channels. As noted above, the effect of inhaled anesthetics on most sodium channels is limited at MAC. *In vitro* studies suggest that the effect at MAC differs among conventional inhaled anesthetics and that propofol acts like isoflurane in decreasing current.[224] If propofol acts solely by enhancing $GABA_A$ receptors what does this imply? A partial answer might be that propofol is a much weaker blocker than isoflurane. A similar concern applies to barbiturates which, like propofol, act by enhancing the response of $GABA_A$ receptors. Barbiturates can block sodium channels by a

pH-dependent mechanism acting on the inside of the cell.[226] Finally, as noted above, IV and intrathecal infusions of riluzole equally decrease isoflurane MAC in rats.[191] Riluzole potently blocks both fast inactivating and non-inactivating sodium channels,[192] the latter channels being important to repetitive firing (i.e., sustained activity).[193] This result indicates that blockade of such sodium channels might affect anesthetic requirement by acting on higher centers rather than the spinal cord, the site of inhaled anesthetic action. This says nothing concerning sodium channels responsible for rapid nerve conduction. Finally, none of this indicates how anesthetics might block the sodium channel. Do they do it by an action directly on the channel or indirectly through an action on the membrane bilayer?

What Might a Nonspecific Mechanism Be?

Do inhaled anesthetics act by affecting surface properties of the membrane bilayer? Polyhydroxyalkanes are compounds confined to the membrane surface by virtue of their multiple hydroxyl groups, yet they have anesthetic effects in tadpoles.[227] Their size also would preclude entrance of the whole molecule into the pockets hypothesized as binding sites for inhaled anesthetics. Still, other large alcohols can cause anesthesia.[228,229] The molecular excess that does not fit into the pocket might simply extend from the pocket.

Cantor has suggested that anesthetics might act at the bilayer/membrane interface to alter the pressure profile within the membrane and thereby alter the function of proteins that reside there.[230] Roth et al.[231] propose a different theory, one that has elements of Meyer and Overton's thesis. They suggest that water vapor naturally forms bubbles in small tubes (i.e., ion channels) and that organisms make use of such formation in the regulation of conduction through channels. Anesthetics influence the formation of the bubbles, thereby altering conduction and producing anesthesia. One appeal of this theory is that it would affect many channels, an action known for inhaled anesthetics.

Conclusions

The preceding discussion suggests that despite many plausible candidates, at present, no single target, including ligand- and voltage-gated channels, can explain the immobility produced by inhaled anesthetics, nor can any combination of effects currently explain immobility. (We consider "plausible candidates" to be channels conducting excitatory impulses that inhaled anesthetics block or channels conducting inhibitory impulses that anesthetics enhance.) As an aside, we note that none of the discussion necessarily applies to the capacity of inhaled anesthetics to suppress learning and memory. Future studies will continue to test the relevance of particular ligand- and voltage-gated channels to immobility and other anesthetic end-points. Future studies might need to test radically different theories, perhaps resurrecting Meyer and Overton’s ideas in a drastically different form. Even if anesthetics act on lipid bilayers, they will exert their action through changes induced in protein and ion current conduction. No matter where they act, we still must explain how conformational changes in lipid or protein structure leads to anesthesia.

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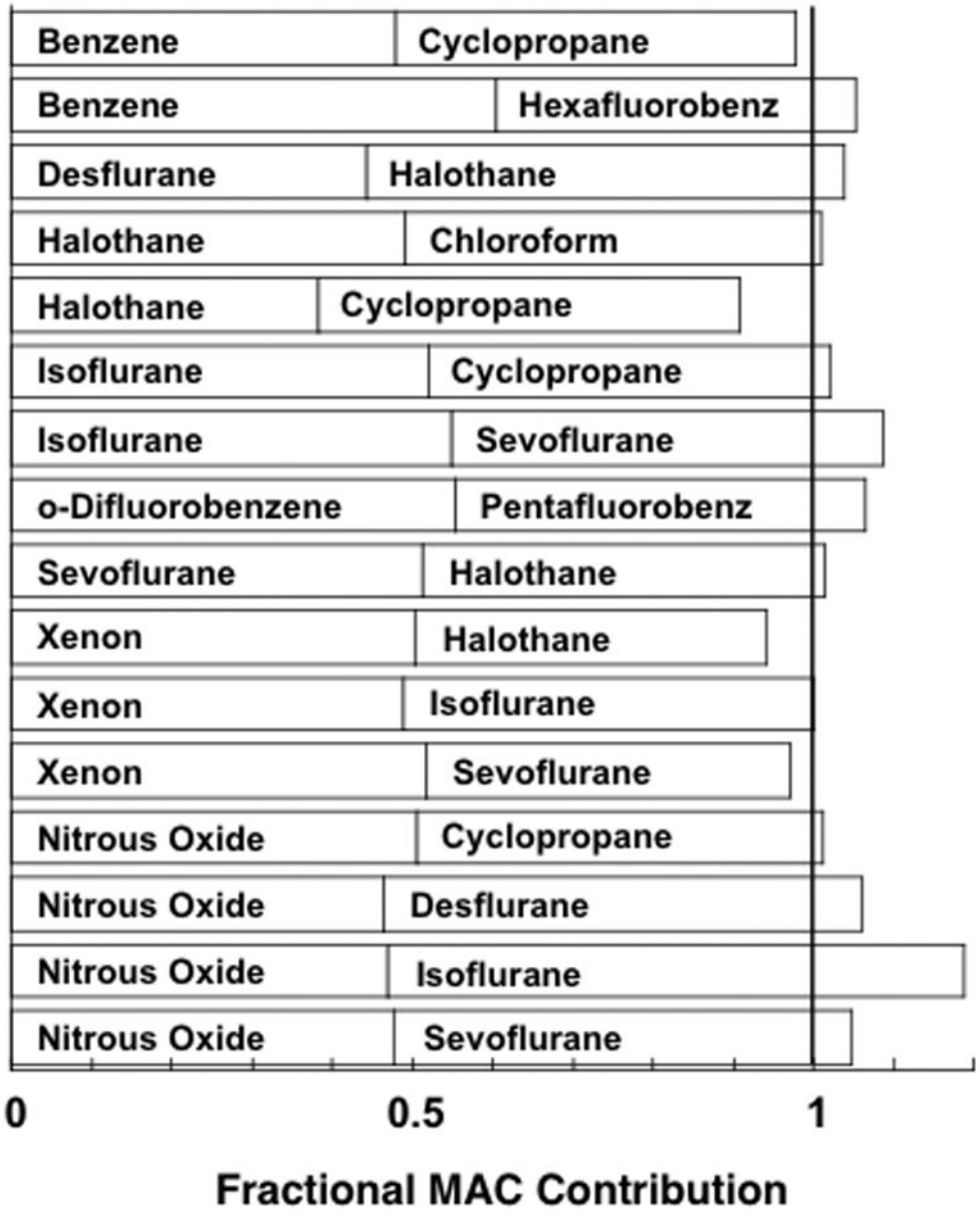

Figure 1.

This graph indicates the fraction of MAC for each anesthetic of a pair that, in combination, produces immobility in response to noxious stimulation in rats. Each anesthetic pair was usually chosen because the two anesthetics differed in their capacities to inhibit or enhance the response of a specific channel (e.g., cyclopropane minimally enhances the response of gamma-aminobutyric acid $(GABA)_A$ receptors to GABA, whereas halothane markedly enhances the response). The sum of the fractional contributions never was less than 0.9, an a priori value assigned as the boundary between additivity and synergism (i.e., no pair acted synergistically). The data are taken from Eger et al.[16]

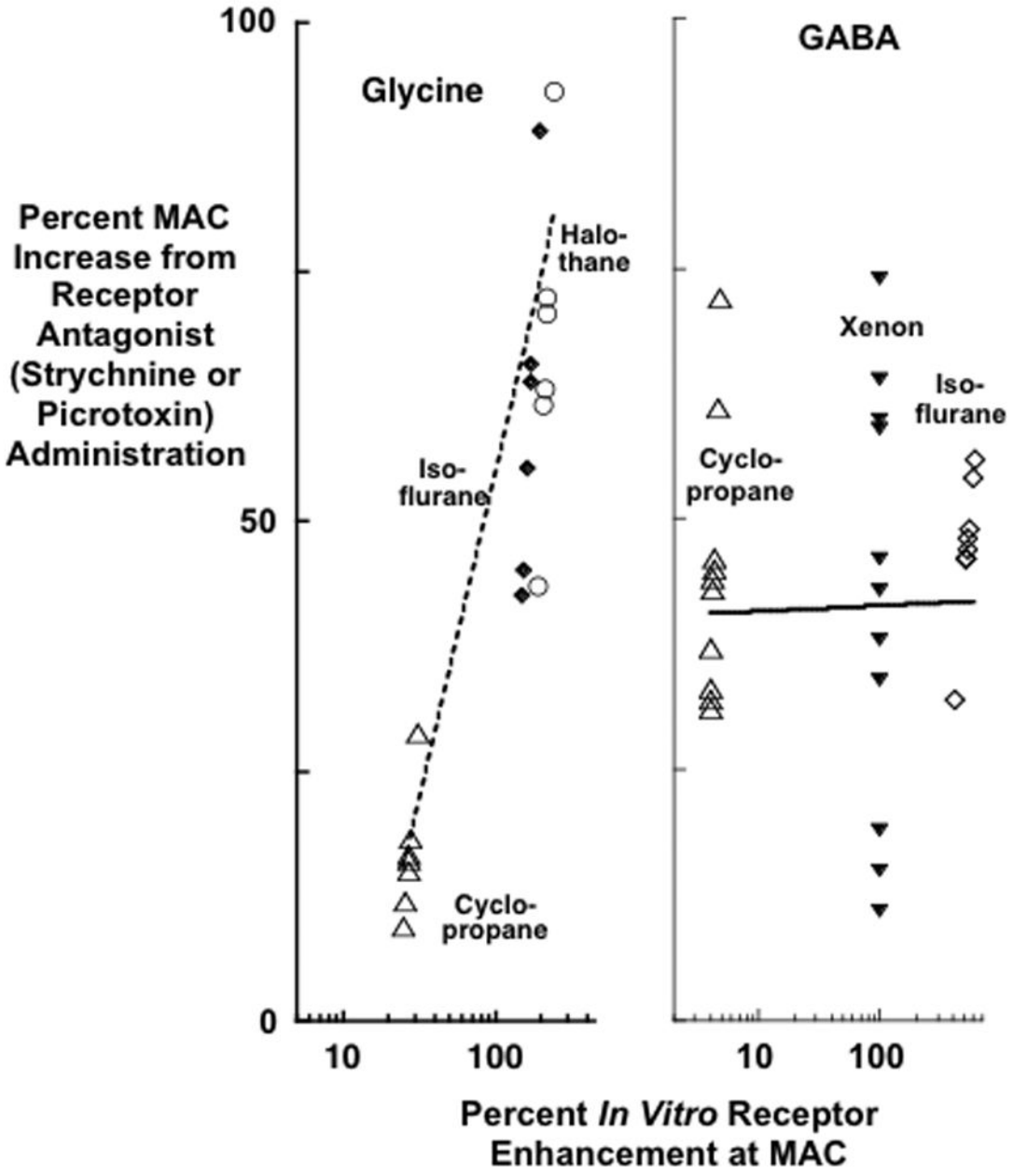

Figure 2.

In rats, MAC for various anesthetics was determined in the presence of intrathecal infusions of strychnine [a glycine receptor blocker;[123] left panel] or picrotoxin [a gamma-aminobutyric acid $(GABA)_A$ receptor blocker;[112] right panel]. For infusions that produced maximal increases in MAC, the increases correlated with the capacity of particular anesthetics to enhance the response of glycine receptors *in vitro* but did not correlate with the capacity of particular anesthetics to enhance the response of $GABA_A$ receptors *in vitro* (the *in vitro* capacity is indicated for each abcissa). Such results are consistent with a role for glycine receptors as the mediators of the anesthetic effect of some (e.g., halothane – see open circles) but not other (e.g., cyclopropane – see open triangles) anesthetics. Data for isoflurane are shown

as closed diamonds, and data for isoflurane are shown as open circles. Such results also are not consistent with a role for $GABA_A$receptors as mediators of immobility.

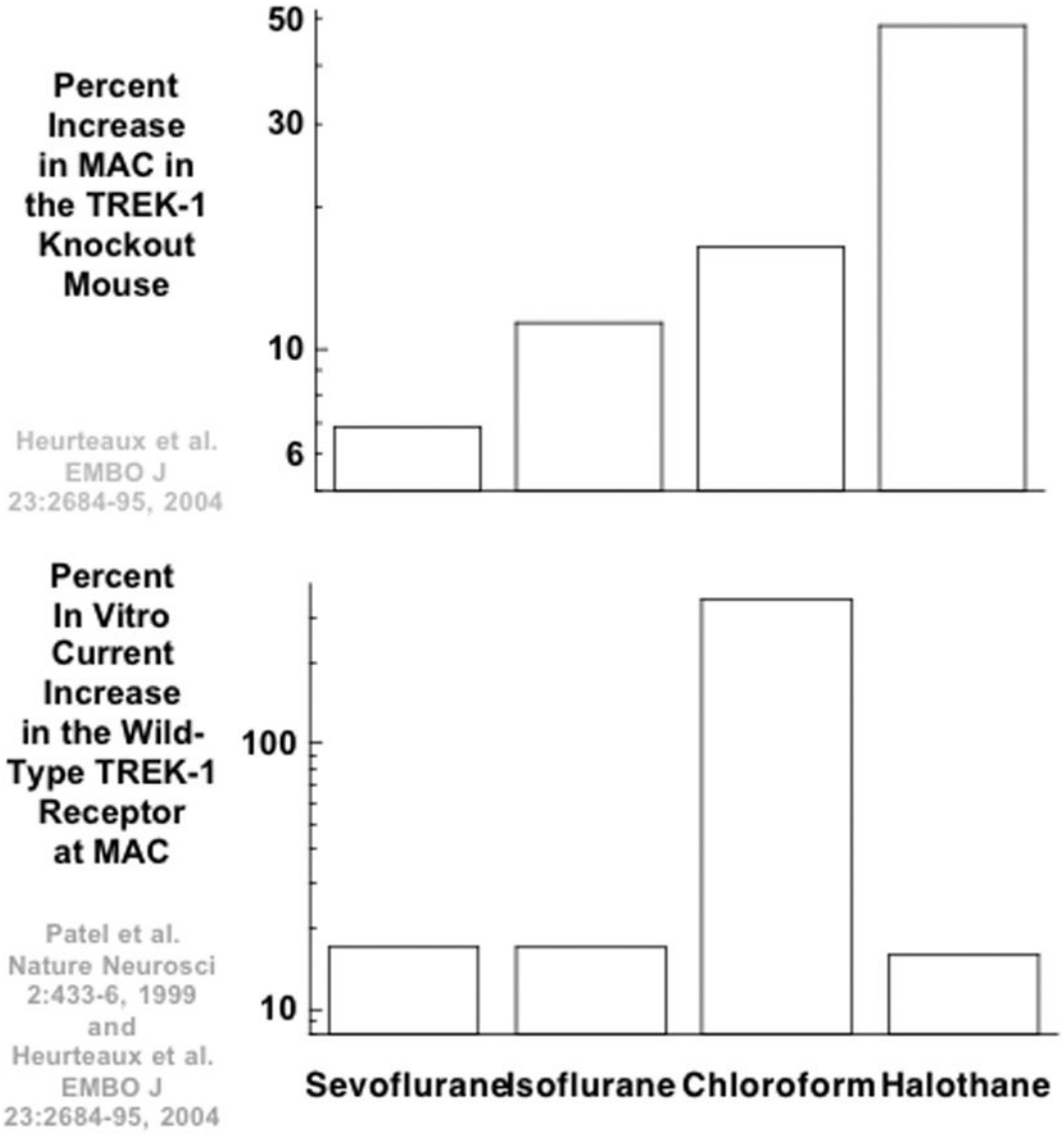

Figure 3.

TREK-1 knockout mice have greater MAC values than their wild-type littermates. The upper graphs indicate the percentage increase in MAC that attends knockout.[180] The increases vary by nearly an order of magnitude (e.g., 7% for desflurane, 15% for sevoflurane, and 47% for halothane), but do not correlate with the capacity of these anesthetics to enhance the response of the TREK-1 channel *in vitro* (bottom graph).[180,181] The failure of the correlation calls into question the relevance of TREK-1 channels as mediators of the immobility produced by inhaled anesthetics.