General Anesthetics and Molecular Mechanisms of Unconsciousness

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Unconsciousness is the Sine Qua Non of General Anesthesia

The term "anesthesia" was originally used by the ancient Greek surgeon Dioscorides and resurrected by Dr Oliver Wendell Holmes to describe the insensible state produced by inhalation of ether.[1,2] The goals of general anesthesia include amnesia, unconsciousness (also termed hypnosis), and immobilization. By definition, general anesthetics reversibly produce all 3 of these therapeutic effects.[3,4] General anesthetic drugs include inhaled gases and intravenous agents. Other classes of drugs may be used by anesthetists to achieve specific clinical goals during surgery.[5] For example, anesthetists often use drugs that selectively inhibit neuromuscular transmission to reduce patient movement and facilitate surgery. Benzodiazepines may be used to provide anxiolysis and anterograde amnesia, and opioids provide analgesia (an action that is produced by only a few general anesthetics). However, among the many drugs used by anesthetists, general anesthetics are uniquely used to produce unconsciousness.

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In contrast to early hypotheses that it represents a single global state change in the central nervous system (CNS), we now view general anesthesia as a combination of specific behavioral effects.[2] Indeed, the ability of general anesthetics to produce distinct therapeutic end points is not equal. Explicit memory is the most sensitive target of inhaled general anesthetics; consciousness (assessed by appropriate response to spoken commands) is preserved in the absence of memory during exposure to low doses. Unconsciousness is produced by lower concentrations of most general anesthetics than those that ablate movement in response to noxious stimuli.[6] Moreover, different drugs display distinct relative potencies in their ability to produce different components of general anesthesia. Thus, the ratio of the drug concentration producing immobility during incision to that producing unconsciousness is 1.5 for nitrous oxide (N_2O), about 3 for most of the inhaled halogenated ethers, and over 4 for the intravenous anesthetic propofol.

Distinguishing among the clinical goals of general anesthesia is not simply of academic interest. An immobile patient is desired by surgeons to improve exposure and precision, whereas patients primarily wish for oblivion and amnesia during surgery, creating the potential for divergent goals. Cases of unintended and undesired consciousness occur in about 1/750 general anesthetics, due largely to the use of selective neuromuscular blockade together with inadequate general anesthetic doses.[7] Thus, there is interest in identifying and monitoring neurophysiologic correlates of consciousness during general anesthesia, to titrate dosing precisely to patient needs.

Recent molecular and transgenic animal studies have identified a number of targets where general anesthetics act to produce their effects, revealing that different types of general anesthetics act through distinct mechanisms, and perhaps even different neural circuits. This review focuses on the general anesthetic drugs and their molecular targets, specifically those linked to consciousness [Research linking general anesthetic targets to behavior has been carried out in laboratory animals, such as transgenic mice. Using animals requires measuring correlates of human behavior. For example, the most commonly used surrogate for unconsciousness in animals is loss of righting reflexes (LORR), a test of response to a non-noxious postural change. Whether these experimental observations in mice are relevant to the nuances of human consciousness is not known].

■ Distinct Clinical Groups of General Anesthetics

We and others have classified the general anesthetics into 3 groups based on their relative potencies for different clinical end points and their impact on electroencephalogram (EEG). This clinical classification

of general anesthetics has been shown to correlate remarkably well with studies identifying molecular targets of these drugs, particularly those targets associated with loss of consciousness (or its correlate in animals).[8,9] Group 1 consists of etomidate, propofol, and barbiturates, intravenous drugs that are much more potent at producing unconsciousness than immobilization. For propofol, which has been studied more than other group 1 agents, hypnosis is achieved at plasma concentrations around 3 μg/mL, whereas immobility during skin incision requires 4-fold higher concentrations.[10] These drugs shift cortical EEG toward lower frequencies, enabling reproducible correlation with anesthetic depth.[11] Group 2 includes the gaseous anesthetics N_2O, xenon (Xe), and cyclopropane, along with ketamine, an intravenous agent. Clinically, these drugs produce significant analgesia, whereas their potency as hypnotics and immobilizers are relatively weak. In fact, no analgesia is demonstrable using groups 1 and 3 general anesthetics at concentrations below those producing unconsciousness. Cardiovascular stability and a high frequency of reported dreamlike experiences are also features associated with group 2.[12] The ratio of doses (alveolar concentrations) producing immobility versus unconsciousness for N_2O is only 1.5, whereas that for Xe is 2.[13] N_2O and ketamine may increase cortical EEG frequencies, and EEG-based anesthetic depth monitoring is not reliable for detecting the effects of N_2O and ketamine.[14,15] There is conflicting data on whether EEG-based monitoring is useful for Xe.[16,17] Group 3 consists of the volatile halogenated anesthetics: halothane, enflurane, isoflurane, sevoflurane, and desflurane. These drugs induce amnesia, hypnosis, and immobility in a predictable manner.[4,18] The ratio of doses producing immobility to those producing unconsciousness for group 3 drugs is between that for propofol and N_2O, ranging from 2 to 3.[6] Group 3 drugs produce amnesia at doses (partial pressures) lower than those that produce unconsciousness. Volatile anesthetics reduce the spectral edge frequency of cortical EEG and anesthetic depth monitors produce reliable correlations with the level of consciousness.

Etomidate, Propofol, and Barbiturates (Group 1)

LORR and immobility produced by etomidate, propofol, and barbiturates are mediated by a subset of γ-aminobutyric acid type A ($GABA_A$) receptors. The $GABA_A$ receptors are neurotransmitter-gated chloride channels that are members of the ligand-gated ion channel superfamily that also contains nicotinic acetylcholine receptors, glycine receptors, and serotonin type 3 receptors. $GABA_A$ receptors are located both postsynaptically and extrasynaptically on neurons and when activated, they reduce neuronal excitation. Genetic techniques have identified 18 different $GABA_A$ receptor subunits: 6α's, 3β's, 3γ's, δ, ε, π,

and 3ρ's.[19] Typical postsynaptic $GABA_A$ receptors contain α, β, and γ subunits, whereas extrasynaptic channels often contain δ. Group 1 anesthetics enhance GABA-mediated channel activation and prolong postsynaptic inhibitory currents, suppressing neuronal excitability.

Evidence supporting a major role for $GABA_A$ receptors in unconsciousness caused by group 1 drugs is plentiful. Etomidate has a chiral carbon and R(+)-etomidate is 20-fold more potent at inducing LORR than S(−)-etomidate.[20] Stereospecificity of the same degree is observed in molecular studies of $GABA_A$ receptor modulation by etomidate. Critical studies linking specific types of $GABA_A$ receptors to anesthetic-induced unconsciousness evolved from identification of a role for the β subunit in sensitivity to etomidate and propofol. $GABA_A$ receptors containing β2 and β3 subunits are sensitive to etomidate and propofol, but not those containing β1 subunits.[21] Researchers identified amino acids on β2 and β3 that, when mutated to their homologs on β1, reduced receptor modulation by etomidate and propofol.[22] Mutations were introduced into the β2 and β3 genes of mice (knock-in transgenic animals). To induce LORR in mice containing the β3(N265M) mutation requires at least 4-fold higher propofol or etomidate doses than those that produce LORR in wild-type litter-mates.[23,24] The β3(N265M) transgenic mice are also resistant to LORR produced by pentobarbital.[25] Intriguingly, mice containing the β2(N265S) mutation show normal etomidate and propofol sensitivity for LORR, but they are less sedated than wild-type mice at low drug doses.[26] Thus, group 1 anesthetics induce LORR, the most commonly used correlate of unconsciousness in animals, through $GABA_A$ receptors containing β3 subunits, whereas sedation seems linked to $GABA_A$ receptors containing β2 subunits.

Other studies have suggested that propofol-induced unconsciousness may be caused by indirect activation of cannabinoid receptors.[27] Propofol was found to inhibit fatty acid amide hydrolase, an enzyme that degrades the endogenous cannabinoid receptor agonist, anandamide. This mechanism may also contribute to the unusual antiemetic properties of propofol.

N_2O, Xe, Cyclopropane, and Ketamine (Group 2)

At clinical concentrations, the group 2 drugs N_2O, Xe, cyclopropane, and ketamine have little or no effect on $GABA_A$ receptors. Instead, these anesthetics potently inhibit *N*-methyl-D-aspartate (NMDA) receptors,[28–31] which are excitatory cation channels activated by the amino acid glutamate. Glutamate receptors are the major excitatory neurotransmitter-gated ion channels in mammalian brain, and are tetramers formed from a group of 7 homologous subunits: NR1, NR2 (A to D), and NR3 (A and B). In the presence of N_2O, Xe, cyclopropane, or

ketamine, NMDA receptor-mediated excitatory postsynaptic currents are markedly inhibited, and presumably reduced excitatory signals in critical neuronal circuits causes unconsciousness.

Both pharmacologic and molecular evidence supports a role for NMDA receptors in mediating unconsciousness produced by group 2 drugs. Ketamine possesses a chiral carbon, and S-ketamine has been shown to be 4 times more potent than R-ketamine in human volunteers. In hippocampal neurons, S-ketamine also inhibits NMDA receptors more potently than R-ketamine,[30] reflecting the relative stereoselectivity of in vivo actions. Transgenic mice lacking the NMDA receptor ε1 subunit (homologous to the human NR2A subunit) show resistance to LORR produced by ketamine[32,33] and by N_2O.[34] In the nematode *Caenorhabditis elegans*, a null mutation of the NMDA-type receptor gene nmr-1 produces resistance to the effects of N_2O,[35] which are distinct from those produced by halogenated anesthetics. Furthermore, a mutation that confers resistance to halogenated anesthetics did not produce resistance to N_2O (and vice versa). These behavioral observations parallel those in humans and mice, whereas the genetic experiments demonstrate that N_2O and halogenated anesthetics have distinct molecular targets in *C. elegans*. The behavioral effects of Xe in wild-type *C. elegans* are similar to those produced by N_2O, and are attenuated by knock-out of a non-NMDA glutamate receptor, glr-1.[36] This gene encodes a homolog of the human alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor. Xe and N_2O inhibit both human NMDA and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors.[37]

Group 2 anesthetics also inhibit $\alpha_4\beta_2$ neuronal nicotinic acetylcholine receptors,[31,38] which modulate synaptic release of neurotransmitters. Ketamine stereospecificity has also been demonstrated in these receptors.[39] Neuronal nicotinic receptors have not been linked to LORR, but they may be important mediators of anesthetic-induced amnesia.[40] Group 2 general anesthetics also affect members of the 2-pore (2P) domain potassium channel family.[41] TREK-1 is an anesthetic-sensitive "background leak" K^+ channel that regulates the resting membrane potential of neurons. TREK-1 channels are also activated by volatile anesthetics[42] and TREK-1 knockout mice have been created.[43] The knock-out of TREK-1 reduces sensitivity to volatile anesthetic-induced LORR (see below), but testing against group 2 drugs has not been reported.

Halogenated Volatile Anesthetics (Group 3)

Group 3 general anesthetics, comprising the halogenated ethers and alkanes, lack significant selectivity for general anesthetic target molecules.[18] Group 3 drugs positively modulate $GABA_A$ receptors and closely

related inhibitory glycine receptors, while also activating 2P potassium channels and inhibiting a variety of excitatory cation channels. There is also evidence that group 3 drugs act at presynaptic targets.

Volatile general anesthetics enhance the function of inhibitory $GABA_A$ and glycine receptors. Studies of $GABA_A$ receptors have identified amino acid residues located in the transmembrane domains of both α and β subunits that determine anesthetic sensitivity. Pharmacologic and genetic studies, however, suggest that volatile anesthetics produce unconsciousness via different $GABA_A$ receptor subunits than those targeted by the group 1 drugs. Transgenic mice that lack the $GABA_A$ receptor β3 subunit show no change in their volatile anesthetic requirement for LORR.[44] Transgenic mice bearing the point mutation β3(N265M) also show little change in volatile anesthetic concentrations associated with LORR,[23] whereas this β3 mutation significantly reduces in vitro receptor modulation by group 3 drugs. Surprisingly, β3(N265M) mice also show a small reduction in sensitivity for cyclopropane, which has minimal effects on $GABA_A$ receptors in vitro. Another well-studied mutation on $GABA_A$ receptors is α_1 serine to histidine at position 270, which eliminates in vitro receptor enhancement by isoflurane and desflurane, but not halothane.[45] Knock-in mice with the α_1(S270H) mutation require moderately increased concentrations of isoflurane and enflurane to induce LORR.[46] These transgenic animals showed no change in sensitivity to halothane, and volatile anesthetic concentrations producing immobility in α_1(S270H) mice matched those in wild-type mice. Thus, α_1 subunit-containing $GABA_A$ receptors contribute to group 3 LORR, but not immobility.

Many 2P domain K^+ channels, including TREK-1, TREK-2, TASK-1, TASK-2, TASK-3, and TRESK are activated by group 3 general anesthetics.[42,47] TREK-1 and TASK-3 knockout mice required increased amounts of volatile anesthetic to produce both LORR and immobility.[43,48] However, other volatile anesthetic-sensitive K^+ channels such as TASK-2 seem to play little or no role in anesthesia,[49] though they may produce some side effects.

Like N_2O, Xe, and cyclopropane, the volatile anesthetics also inhibit excitatory glutamate receptors in the CNS.[50] However, NMDA receptor ε_1 subunit knockout mice that exhibit resistance to ketamine and N_2O show no resistance to sevoflurane.[34] Similarly, kainate receptor GluR6 subunit knockout mice exhibit minimal changes in volatile anesthetic sensitivity for immobility or fear conditioning.[51] The latter requires both consciousness and learning. Other glutamate receptor subunits may mediate actions of volatile anesthetics.

A wide range of other ion channels are sensitive to group 3 general anesthetics, including neuronal nicotinic acetylcholine receptors,[52] serotonin type 3 receptors,[53] Na^+ channels,[54] mitochondrial ATP-sensitive

K^+ channels,[55] and cyclic nucleotide-gated hyperpolarization-activated cyclic nucleotide-gated channels that mediate neuronal pacemaker currents.[56] Some of these receptors may play important roles in mediating unconsciousness, but testing in genetically altered animals has not yet occurred.

Dexmedetomidine

Dexmedetomidine is an unusual intravenous drug that produces some behavioral effects that are characteristic of general anesthetics and illustrative of how distinct behavioral effects of sedative/hypnotic drugs may be mediated by quite specific neural circuits. The molecular targets of dexmedetomidine and the neural networks involved in its sedative action have been characterized in detail. The molecular targets for dexmedetomidine are central α2-adrenergic receptors (α2-AdRs).[57] Activation of spinal α2-AdRs is linked to dexmedetomidine's antinociceptive activity, whereas α2-AdRs in sleep circuits of the brainstem, specifically the locus ceruleus (LC), mediate sedation that mimics NREM sleep. Selective ablation of the LC or injection of α2-AdR inhibitor into the LC prevents dexmedetomidine-induced sedation.[58]

In addition, the sedative actions of group 1 general anesthetics are apparently mediated by $GABA_A$ receptors in another sleep circuit nucleus of the brainstem, the tubo-mammilary nucleus (TMN).[59] A critical link between the LC and the TMN is the ventro-lateral preoptic nucleus, which is disinhibited when dexmedetomidine activates α2-AdRs in the TMN. The ventro-lateral preoptic nucleus in turn inhibits the TMN via GABA release, causing effects similar to those caused by selective injection of group 1 anesthetics or GABA agonists in this nucleus. Despite this link, dexmedetomidine is another drug that does not cause reliable slowing of cortical EEG frequencies.[60] Indeed, it does not reliably produce unconsciousness, and is therefore proving useful for "awake craniotomy" cases that require patients to communicate with surgeons during cortical mapping.[61]

■ Anesthetic Targets and Models of Drug-induced Unconsciousness

Consciousness is a complex mechanism for filtering and processing both new primary information and memory into high-fidelity subjective constructs (qualia). In the context of this functional model of the conscious mind, impaired consciousness represents reduction or elimination of subjective construct fidelity, which can be caused by (1) impaired primary sensation; (2) impaired filtering of information, or (3) impaired processing. There is little support for the idea that general anesthetics impair primary sensation (detection and reception). Group 2

drugs such as ketamine and N_2O seem to act by impairing signal filtration, perhaps altering the amounts or intensities of sensory inputs, or by allowing leakage of unconscious signals into conscious processing. This lack of filtering "jams" or overwhelms the mind with signals, leading to a "dissociative" state, frequently associated with reports of vivid dreamlike experiences. In parallel, cortical activity is maintained or increased by N_2O and ketamine, suggesting enhanced, perhaps overloaded neural activity. Interestingly, ketamine and N_2O have minimal effects on important "unconscious" processes such as breathing and blood-pressure regulation, suggesting that their actions are somewhat selective for filtering functions, which probably occur in subcortical circuits. Thus, information filtering seems to be particularly sensitive to drugs that inhibit excitatory glutamate and nicotinic acetylcholine receptors in the CNS.

In contrast, groups 1 and 3 drugs that act by enhancing $GABA_A$ receptor activity seem to impair both unconscious and conscious processing, including associative cortex and synchronous integration among multiple cortical areas. Cortical EEG activity shifts toward lower frequencies in the presence of these drugs, and large doses can lead to the total loss of coordinated neural activity (isoelectric EEG). Unconscious processes are suppressed by these general anesthetics less than the conscious mind, perhaps because they are less dependent on coordinated integration among multiple cortical areas.

■ Conclusions

General anesthetics are the sole class of drugs used by physicians for inducing unconsciousness. General anesthetics have traditionally been considered to be nonspecific drugs with widespread effects on the CNS. As a result, it has long been thought that these drugs can teach us little about the nature of consciousness or the mechanisms through which it can be inhibited. However, molecular pharmacology and transgenic animal studies are revealing that some general anesthetics are in fact quite selective for important CNS targets and structures that are critical for modulating the processes associated with consciousness. As we discover more about the plethora of molecules involved in neuronal activity and the anatomy of the circuitry in which they function, studies of general anesthetic mechanisms will likely reveal more important insights into how consciousness is modulated. At the same time, this information will be critical for designing improved anesthetic agents with more selectivity against consciousness.

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