Mechanisms of General Anesthesia

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In this paper I will briefly review the current understanding of how general anesthetic drugs cause the behavioral state of anesthesia. Research into the subject is at present very vigorous; and has benefited greatly from the recent explosion of novel experimental neuroscience methods—such as neuroimaging, drug action, and genetic methods. Proper understanding of mechanisms of general anesthesia involves correctly linking the observed effects of anesthetic drugs on target molecules and neuronal dynamics, with the behavioral change in state from "esthesia" to "anesthesia." Therefore any coherent explanation must include plausible descriptions of phenomena at each scale of measurement. It has also become clear that the state of drug-induced unresponsiveness to the environment is not a single state; but includes a spectrum of different neurobehavioral components namely: 1) immobility, 2) amnesia, and 3) sedation/hypnosis—the "anesthesia syndrome." The separation of these sub-components of anesthesia is slightly artificial. For example, it is hard to form a declarative memory without being awake at the time it is formed. But as described below, it is possible to use specific drugs or genetic alterations to induce a partial anesthetic syndrome—e.g., an experimental animal may exhibit sedation, but not be immobile. These observations have appreciable clinical consequences; both in terms of avoiding "unbalanced" anesthesia, and the potential for reducing clinical adverse effects from the anesthesia. Although not mentioned in the recent reviews, the idea of the anesthesia syndrome should be extended to include some mention of arousal blockade. After all, this is what distinguishes general anesthesia from natural sleep, in which there exists a state of: hypnosis, amnesia, and (some) immobility—but there is potential for a quite minor stimulus to cause behavioral arousal. If the autonomic effects of surgical stimuli were not obtunded by general anesthesia, many patients would die from an intraoperative sympathetic storm. In this brief review, I will describe how different classes of general anesthetic drugs act at a molecular level, how these effects are translated into neuronal dysfunction, and subsequently how some of these actions may cause the different sub-components of the anesthesia syndrome. For more detailed discussion, and references on this large topic, I would direct the reader to several recent excellent reviews (1–12).

WHICH OF THE PUTATIVE MOLECULAR EFFECTS ARE IMPORTANT IN CAUSING THE "ANESTHESIA SYNDROME" IN THE INTACT ANIMAL?

At the risk of oversimplification, it is opportune to be quite skeptical and rigorous in evaluating the various competing theories of anesthesia. In the spirit of Karl Popper, any claim must submit to falsification. It is reasonable to propose that any claim that: "something causes anesthesia" should satisfy the following five criteria:

1. The dose–response of the putative molecular target should be similar to the clinical dose–response in the patient. It should be noted that this is not a trivial problem; because determining the true free anesthetic drug concentration at the effect site is confused by many other nonneural binding sites.
2. The effect should be logically sufficient. For example, if we make the statement: "gap junction blockade causes unresponsiveness . . . ," we must immediately ask ourselves the question: "Do nonanesthetic gap junction blockers exist?" The answer is yes—quinine is a nonanesthetic gap junction blocker. We must therefore conclude that gap junction blockade is not completely *sufficient* for unresponsiveness. (We may also have other subsidiary questions regarding dosage, penetration across the blood brain barrier, etc).
3. The effect should be logically necessary. Using the example of gap junction blockade, we must ask the question: "Do we have examples of anesthetic drugs that don't block gap junctions?" If we can find an example of such a drug then gap junction blockade is not *necessary* for general anesthesia to be achieved.
4. There should be consistent "scale-coherence." For example, the molecular dose–response (e.g., impairment of drug-protein binding) should agree with the changes in neuronal dynamics (e.g., prolongation of synaptic potentials), and this should also agree with behavioral observations (e.g., MAC). Furthermore, if the dose–responses are modified by use of different drug isomers, or by the use of receptor mutations with different sensitivity; this should agree with changes in behavioral endpoints. As an extreme

example R(+) etomidate potentiates $GABA_A$ receptor channels 5–10 times more potently that the S(−) enantiomer. A similar potency ratio is found for "clinical" immobility (albeit in tadpoles).
5. There should be agreement on anatomical/regional localization. The anatomical sites at which the putative receptors are found should agree with other methods of investigation (e.g., fMRI changes with anesthesia).

The most problematical of these criteria is that of logical necessity. The failure of proving logical necessity is one of the strongest arguments against a unitary theory of anesthesia. If there is a single final common pathway, then blockade of this pathway should be the only way to induce general anesthesia. This holy grail has not been found to date; and thus we assume that there are many paths to achieve anesthesia.

POSSIBLE ANESTHETIC DRUG TARGETS

At a molecular level anesthetic drugs have been shown to have effects on a wide range of putative targets. These include:

1. Ligand-gated ion channels, (slow/indirect/G-protein activated (metabotropic) and/or fast/directly activated (ionotropic) receptors).
2. Other ion channels (K^+, Na^+, Ca^{2+}: leak and voltage-gated channels, ATP-activated channels).
3. Other intracellular functions (mitochondrial function, quantum coherence in microtubules).

At a neuronal level the sites of action could be: axon and/or synapse, excitatory and/or inhibitory synapses, presynaptic and/or postsynaptic, or intracellular. At the macroscopic level, anesthetics could act in specific regions in the central nervous system such as the levels of the spinal cord, brainstem, thalamus, or cerebral cortex. It now seems to be clear that different sub-components of the anesthesia syndrome are mediated in different anatomical regions.

If general anesthetic drugs are classified according to their receptor actions, they fall broadly into three groups.

1. The commonly used IV induction drugs (barbiturates, propofol, etomidate, benzodiazepines, and neurosteroids), are relatively potent and clean, and have been shown to mainly act on the various forms of the γ-aminobutyric acid-A ($GABA_A$) receptor. Because of the intense interest in this area of research, and the fact that some relatively unequivocal results have been obtained, the bulk of this review will deal with these effects.
2. The volatile anesthetic agents (ethers, substituted hydrocarbons) are less potent and have multiferous low-affinity binding effects. As a result their actions are much less well understood than the IV drugs. A variety of molecular targets (including the $GABA_A$, glycine, and *N*-methyl-D-aspartate (NMDA) receptors, and potassium channels) play a significant role in the anesthetic effects of the volatile drugs.
3. Xenon and nitrous oxide and ketamine form a third group, which is differentiated by the fact that they have minimal effects on the $GABA_A$ receptor, but marked blockade of NMDA actions.

DOES THE PREVAILING $GABA_A$R THEORY OF ANESTHESIA PASS THE FIVE TEST CRITERIA?

It has been shown that $GABA_A$ receptors are sensitive to most general anesthetic drugs—which act primarily to open the chloride channels and prolong the inhibitory postsynaptic potentials (for synaptic receptors), or hyperpolarize the neuronal membrane (for nonsynaptic receptors). Thus, $GABA_A$ receptor opening seems to be *almost necessary* for general anesthesia (the exceptions being xenon and nitrous oxide and ketamine as described above). The theory is also *probably sufficient*—volatile convulsant "anesthetic-like" drugs do not open the chloride channel, and may even block it (13). However, there do exist some GABAergic drugs that are not anesthetic—e.g., muscimol, and some antiseizure drugs like vigabactrin. Whether these anomalies can be explained by the effective level of GABA augmentation, or action on different sub-types of GABA receptor, remains to be seen.

There is a large body of experimental work that demonstrates a consistent (and almost complete) trail of dose–response effects, seen at all scales of measurement. We can describe how and where the drugs interact with the protein moiety, how this causes IPSP prolongation, which in turn causes reduction in spike-rate, interneuronal communication, EEG changes, and clinical unresponsiveness. The molecular and behavioral dose–responses are consistent for different isomers of the drugs, and various anesthetic-resistant mutations in mice (although this relationship is much less clear in invertebrates). The anatomic distribution of the various receptor subtypes is also consistent with their putative actions—e.g., receptors that mediate immobility are found in the spinal cord, and receptors that mediate hypnosis in the cerebral cortex and thalamus. There are a large number of different combinations of $GABA_A$ receptor subunits (6α, 3β, 3γ, 1δ, 1ϵ, 1θ, and 3ρ), with the majority of $GABA_A$ receptors composed of α, β, and γ subunits. Because different combinations of subunits make up receptors that are found in anatomically different brain regions, it means that drugs that specifically bind to one subunit, will have different clinical effects when compared with drugs that bind to another subunit. For example, zolpidem causes sedation (acting via $\alpha 1$ $GABA_A$ receptors) but does not cause much amnesia because it

does not bind to α5 $GABA_A$ receptors in the hippocampus. In contrast benzodiazepines, which bind strongly to α5 $GABA_A$ receptors, are more amnesic.

We will now examine in more detail the specific sub-components of anesthesia.

IMMOBILITY

Over the last 15 years, a series of experiments were done in which different concentrations of anesthetic drugs were delivered to the head when compared with the rest of the experimental animal. They have consistently shown that spinal concentrations of the drugs contribute strongly to immobility (and EEG arousal) in response to a nociceptive stimulus in the experimental animal (14,15). It must be noted that there were significant differences between drugs. The spinal immobilizing effect of thiopentone was less than half the spinal effect of halothane. However, given a high enough concentration to the brain, eventually immobility could be obtained. There is a complex interplay between the spinal cord, the brainstem arousal mechanisms, and the cortex. Because we accustomed to thinking about the anatomy in terms of a "telegraph system" of sensory pathways, the role of cortico-fugal, and ponto-fugal pathways in modifying efferent and afferent blockade and spinal reflex excitability is underestimated (16).

How do the molecular-scale investigations tie-in with these anatomical-level observations. The most impressive (and almost unique) work has been done using etomidate. Jurd and others engineered a knock-in point-mutant mouse in which asparagine at position 265 of the β3 subunit of the $GABA_A$ receptor, was changed to methionine (β3N265M) (17). The mouse was normal in all respects except that etomidate and propofol became ineffective (even at huge doses) in preventing the hind-limb withdrawal reflex to a noxious stimulus (a relative pure behavioral measure of immobility). The mice could be rendered sedated (although less than the wild-type mice), but were not immobile. The study also showed that the inhibitory currents in the β3N265M $GABA_A$ receptor were not potentiated by etomidate, and there was less depression of neuronal cortical action potentials. It is presumed that the partial sedation was mediated by the actions of etomidate on the, still active, β2 $GABA_A$ receptors. Because these investigators have apparently shown a *complete* failure to produce immobility with etomidate in these mice; this would suggest that the etomidate-induced immobility is *entirely* dependent on the interaction between the drug and the β3 $GABA_A$ receptor subtype, i.e., a functioning β3 $GABA_A$ receptor subtype is *completely necessary and sufficient* for etomidate (or propofol) to produce immobility. It does not require additional actions at other targets. It has also been shown that the β3 $GABA_A$ receptor subtype is the predominant $GABA_A$ receptor subtype expressed in dorsal root ganglia, and the superficial dorsal horn of the spinal cord—and thus fulfils the criterion for anatomic consistency. It is clear that the explanation of immobility caused by volatile anesthetics is more complex; with measurable contributions to immobility by actions on glycine receptors, voltage-dependent potassium channels (TREK-1), and glutamate receptors (see Sonner et al. for detailed review) (12).

It should also be noted that there are potent endogenous systems that induce immobility; for example the profound skeletal muscular paralysis (with diaphragmatic sparing) is a feature of REM sleep. The neural pathways that induce these states, are not yet fully understood, but clearly involve an interaction between GABAergic and cholinergic systems in the upper pons that send efferent fibers to potently depress spinal motor neurons. Devor made barbiturate microinjections in the pons, lateral to the peri-aqueductal gray matter and medial to the pedunculopontine nucleus (the so-called mesopontine tegmental anesthesia locus), and induced immobility (18). They claimed that they had induced a state similar to general anesthesia. However, in similar experiments, Voss found that the rats became atonic and unresponsive (with thiopentone), but the EEG did not show any signs of cortical slowing or hypnosis. It is more likely that they were observing a sort of drug-induced catalepsy (19). Subsequent work has demonstrated neuroanatomical connections from the mesopontine tegmental anesthesia locus to brainstem areas that are known to produce REM-induced atonia (20).

The issue of immobility (without the use of specific muscle relaxants) is of some clinical significance. It has been reported that perhaps a quarter of intraoperative awareness incidents occur in patients who have not received specific muscle relaxant drugs (21). These are situations of immobility with failed hypnosis and amnesia. It is hard to explain (or even believe) these incidents unless we are familiar with anesthetic drug actions. There are at least three possible explanations.

1. It may be that the patients had powerful analgesia (they could not be bothered to move), but had an inadequate dose of hypnotic drug.
2. The patients have a genetic variation in which they have variants of the GABA subunits that are resistant to sedation/hypnosis, but have β3GABA (or other unknown variants of glycine, or perhaps TREK-1 receptors) that are very sensitive to general anesthetic actions, i.e., there is a genetic imbalance between the hypnotic and immobilizing components of the general anesthesia syndrome.
3. The third possibility (and most likely) is that the patients suffer from a drug-induced "sleep-paralysis." In this condition subjects achieve conscious awareness waking from REM sleep, but they still have the "pseudo-REM" induced skeletal muscle paralysis, i.e., the parenteral anesthetic agents have somehow emulated the pontine microinjection experiments.

AMNESIA

Mechanisms of amnesia are of great importance to the practicing clinician. Failure of intraoperative amnesia results in cases of recall, and prolonged postoperative amnesia contributes to postoperative delirium and cognitive impairment. There is a massive literature describing how neural activity alters synaptic weights, and hence memory formation. The framework for laying down memories includes formation of localized synaptic modifications (working memory) and incorporation of these constructs into distributed permanent synaptic modifications (long-term declarative memory). The specific processes involved are still poorly understood, but are believed to involve at least the following:

1. Working memory may be thought of as spontaneous prolonged synaptic activity (outlasting the stimulus-evoked response) occurring in a subset of neurons.
2. Some of the working memory will become consolidated via a series of (parallel and redundant) calcium-dependent cellular processes, including activation of NMDA receptors and protein kinase-C pathways. These processes activate calcium-calmodulin protein kinase II; and hence modify synaptic connectivity by increasing the trafficking and insertion of AMPA receptors in synapses and new synapse formation.
3. The reinforcement and consolidation of very long-term memory then involves a complex interplay between different brain regions, principally between the frontal cortex and the hippocampus/limbic system, and changes in neuronal gene expression.

It is clear that, even at low doses, most anesthetic agents act to disrupt the sequence of events underlying stable memory formation, thus preventing conscious recall of intraoperative events. Both propofol and isoflurane have been shown to eliminate the phenomenon of long-term potentiation. Patients who are woken intraoperatively for various surgical purposes hardly ever have recollection of this experience. This indicates that the consolidation of short-term memory to long-term memory is more sensitive to anesthetic disruption than short-term memory (that is required for conscious awareness) *per se*. It would seem that anesthetic drugs may act indirectly and/or directly on GABAergic and cholinergic systems to impair calcium influx and activation of calcium-calmodulin protein kinase II. However this area requires more research.

It is possible that tonic GABAergic currents are important in anesthetic-induced amnesia. α5 subunit-containing $GABA_A$ receptors are present predominantly in the hippocampus. This anatomical localization suggests an important role in memory consolidation. They have been shown to be very sensitive to benzodiazepines, propofol, and isoflurane. For example, approximately 0.1 MAC concentration of isoflurane causes 50% increase in tonic $GABA_A$ current (22,23). Cheng et al. investigated the effects of etomidate on α5 null mutant knockout mice (24). In the wild type mice they showed that etomidate (at low doses of 0.1 μM) had no effect on synaptic currents (mIPSP prolongation), but increased the tonic current by 156%, impaired long-term potentiation, and impaired behavioral measures of memory performance. The α5 null mutant mice showed similar sedation dose–responses to the wild type mice, but had very little etomidate-induced amnesia, long-term potentiation, and tonic current augmentation. Whether some patients who suffer intraoperative awareness episodes have a polymorphism that is functionally equivalent to the α5 null mutant mice is at present unknown.

SEDATION AND HYPNOSIS

The component of the anesthesia syndrome that is most subtle and complex is that of sedation (defined as slower and less complex responses), or hypnosis (defined as lack of response to verbal command). Even the anatomical loci of consciousness are a subject of debate and disagreement. It is thought that conscious awareness requires the simultaneous binding of information from spatially separate areas of the brain. However, neuroactive drugs injected into localized areas of the brainstem and midbrain seem to have effects on conscious state that are disproportionate to the dose given (18,25). Traditionally, activation of the reticular formation and thalamus was considered to be a prerequisite for the cortex to achieve the conscious state based on the theory that thalamo-cortico-thalamic feedback loops are required to synchronize the different brain systems (26). However it is difficult to impair consciousness with even extensive thalamic lesions (27), and some anesthetic drugs (etomidate) have no effect on the thalamus (28)—thus thalamic disruption is probably not necessary for hypnosis. Similarly, destruction of the posterior hypothalamus and mesencephalic reticular formation initially induces hypersomnia; but after 1–2 wk the animals develop alternative pathways for arousal and resume normal activities (29). Although it is clear that anesthetics do act partly to target endogenous subcortical sleep systems, a good case can be made for a primarily cortical locus of action for hypnosis (30). As with amnesia and immobility, our understanding of the volatile anesthetic molecular targets of sedation are much less clear than for the IV anesthetics, which seem to specifically involve β2 and β3 sub-types of the $GABA_A$ receptors. The β2N265S point mutation prevents the binding of etomidate to $GABA_A$ receptors. Reynolds et al. found that wild-type mice ($GABA_A$ receptors with an etomidate-sensitive β2 subunit) are associated with much more prolonged sedation period, when compared with the

same dose of etomidate given to the β2N265S mutant mice. It is presumed that the vastly shortened period of etomidate sedation in the mutant mice is mediated through β3 receptors, which have less affinity for etomidate than would the wild-type β2 receptors.

Why do we not have a pharmacological antagonist of propofol or etomidate hypnosis? There are a number of antagonists at the $GABA_A$ receptor, such as bicuculline and picrotoxin. However, there are conflicting reports as to the efficacy of bicuculline/picrotoxin antagonism of anesthetic effects (31,32). What are the reasons for the lack of consistent efficacy? At a molecular level of explanation, it must be remembered that propofol acts to decrease protein mobility (or change the energy states of the protein) at a site distant from the ion channel. Bicuculline does not compete directly for the binding site, but instead acts as a sort of physiological antagonist. The other explanation is that propofol and etomidate have other unknown mechanisms of action.

CONCLUSION

1. General anesthesia is a behavioral syndrome (a collection of related responses: amnesia, hypnosis, immobility, and arousal blockade).
2. To date, no theory of anesthesia completely satisfies five simple criteria of causation, but the GABAergic theory comes closest (at least for IV anesthetic drugs).
3. Although immobility is mainly spinally-mediated, the role of descending pontine control of atonia is unresolved. It may be the cause of some non-muscle relaxant intraoperative awareness cases.
4. General anesthetic drugs act mainly to prevent stabilization of long term memory—possibly via the α5 $GABA_A$ receptors in the hippocampus.
5. Sedation is predominantly a cortical action of general anesthetic drugs, although endogenous hypnotic systems are strongly activated by the anesthetic drugs, and contribute to the impairment of consciousness.

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