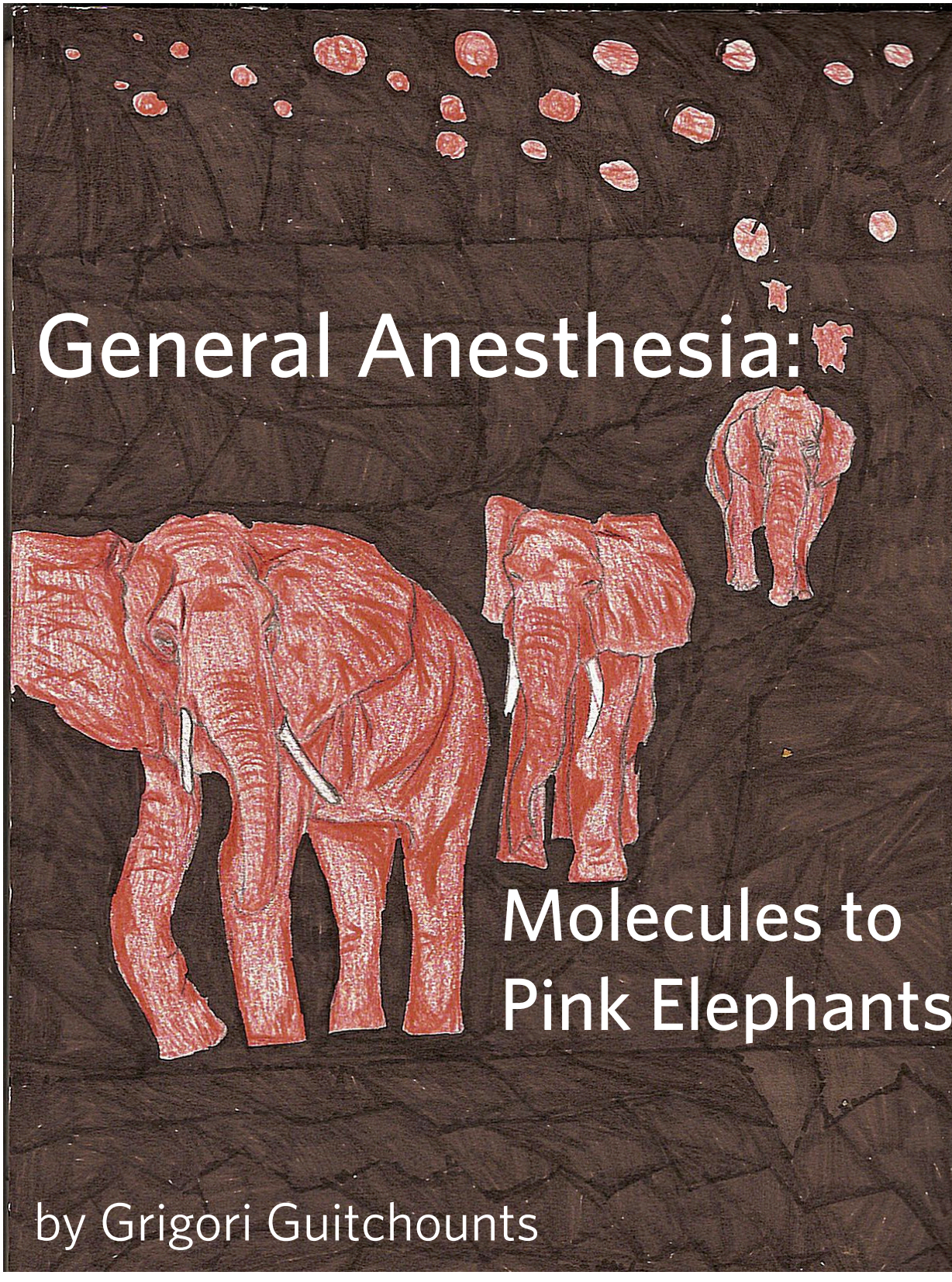
2010

General anesthesia: molecules to pink elephants


General Anesthesia:
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by Grigori Guitchounts

The Drugs and Mechanisms of General Anesthesia

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Introduction

"Gentlemen, this is no humbug," the surgeon John Warren is said to have declared to the audience at Massachusetts General Hospital after William Morton's first successful demonstration of general anesthesia on October 16th, 1846.[1] Before then, surgery was a miserable experience for both patient and surgeon. The term anesthesia – literally meaning "without sensation" – was first used by the ancient Greek surgeon Dioscorides and resurrected by Dr. Oliver Wendell Holmes after the demonstration at MGH.

Anesthesia has indeed transformed medicine and while great improvements have been made in the clinical aspect of anesthesia since the 1840's, the mechanism by which anesthesia produces reversible changes in central nervous system function have remained a mystery until recently.

In the past decade, anesthesia has grown to be used in various medical settings outside the operating room. Children undergoing Magnetic Resonance Imaging (MRI), Computed Tomography (CT) scans, or endoscopy are often sedated or anesthetized to assure cooperation and the required immobility[2,3]. Likewise, electroconvulsive therapy, long considered more torture than treatment, is now being used with the aid of general anesthesia as an effective treatment for medication-resistant depression and schizophrenic affective disorders.[4]

However, despite the multitude of benefits to medical procedures that anesthesia has provided, it remains a somewhat dangerous and toxic procedure, usually involving postoperative delirium[5] and sometimes cognitive dysfunction[6]; even more frightening is the fact that one in 750 patients remain aware during general anesthesia[7]. The intravenous general anesthetics etomidate and propofol are widely appreciated for their anesthetizing properties, but are also associated with unfavorable toxicities. Patients develop "propofol infusion syndrome" after prolonged exposure to the drug; this is associated with dysrhythmias, lipemia, fatty liver, metabolic acidosis, and rhabdomyolysis[8]. Etomidate causes adrenal suppression, putting patients at high risk of mortality[9]. More common side effects include respiratory depression, hypotension, and postoperative nausea and vomiting.

It is therefore critical for safety and the progress of medicine that better drugs and methods be developed for use in general anesthesia. This review focuses on the goals of general anesthesia, the theories behind its mechanism of action, and studies of the structure and function of the GABAA receptors that mediate anesthetic action.

Goals of General Anesthesia

The basic idea of general anesthesia (GA) is to make the patient unaware of the surgical trauma and unmoving, and to allow the surgeon to perform his job with precision. More specifically, the goals of GAs are to reversibly induce immobility, amnesia, and hypnosis (unconsciousness) in the patient; a fourth and often debated goal is analgesia, which some argue is an irrelevant measure since the patient is unconscious during anesthesia.

The three goals have dissociable correlates in the brain; amnesia, consciousness, and immobility are mediated by different neural systems and therefore respond differentially to drugs. Potency of inhaled anesthetics is assessed using a scale of concentra-

tions required to suppress patient response to certain stimuli. Potency is inversely related to the amount of anesthetic required – as measured by alveolar concentration – to reach certain behavioral goals like immobility after a noxious stimulus or no response to spoken commands. The most commonly used scale is the Median Alveolar Concentration (MAC or MAC-immobility), which measures end-respiratory concentration of anesthetic for which half of the patients do not withdraw in response to a surgical incision[10]. Other common scales include MAC-awake, a concentration that prevents physical response to spoken comments, such as to squeeze the doctor's finger; and MAC-BAR, a concentration of anesthetic that blocks autonomic responses like blood pressure or heart rate changes to a surgical incision.

That the behavioral end-goals of amnesia, unconsciousness, and immobility require different concentrations of anesthetics (i.e. they have different MAC values) and that the ratios of MAC-awake to MAC-immobility vary among different volatile anesthetics10, suggest that the three behavioral end-goals are mediated by different neural mechanisms.

Immobility

A common assumption has been that all anesthetic effects are mediated by the central nervous system (CNS) above the brainstem. However some studies have implicated the spinal cord as key to producing immobility. Experiments in goats, whose unique circulatory system makes it possible to isolate the forebrain's circulation from that of the brainstem and spinal cord, have shown that immobility is in fact produced primarily by the spinal cord.[11] Selective administration of isoflurane to the brain raises the concentration required to suppress movements in response to noxious stimuli threefold[12]. In contrast, administration of isoflurane to only the body (brainstem and below) induces immobility at the concentrations required for whole animals. So while the brain is able to induce immobility, it is not required, nor is it the primary actor.

Hypnosis

Hypnosis is defined as the impairment of perception and awareness, or loss of consciousness – a critical component of general anesthesia[10]. Loss of consciousness in anesthesia is remarkably similar to stages of sleep. Functional magnetic resonance imaging (fMRI) studies show that the brain regions affected by sleep or anesthesia overlap significantly[13]. Studies of the brain during sleep may therefore inform what we know of GA-induced unconsciousness.

The thalamus acts as a major relay center in the brain, passing sensory information from the outside world up to cortex[20]. In the awake brain, thalamocortical neurons receive depolarizing input from arousal nuclei in the ventrolateral preoptic area, VLPO, which allows them to faithfully pass along information to cortical neurons in a single-spike tonic firing fashion[21]. In contrast, during sleep the ascending arousal input to thalamocortical cells is inhibited. Thalamocortical cells lacking this depolarization enter an oscillatory firing mode, generated by I_h and I_{Ca}[20], which subsequently prevents the transmission of signals to cortex.

The most obvious similarities between sleep and loss of consciousness under anesthesia aside, the two states share nearly identical EEG signatures (i.e. spindles and delta waves), suggesting that the thalamus is involved in sleep as well as anesthetic-induced loss of consciousness[13]. However, general anesthetics do have widespread molecular targets, such as GABAA receptors, which are found in a large number of inhibitory cortical interneurons. It remains unclear whether their effects in the thalamus (specifically on the reticular system) are necessary and/or sufficient for loss of consciousness.

Amnesia

The hippocampus and surrounding medial temporal lobe (MTL) structures are responsible for the formation and consolidation of episodic memories[22]. The hippocampus is therefore a likely target of general anesthetics. The formation of memory depends on the integrity of Long Term Potentiation (LTP), which requires functioning NMDA receptors. It follows that inhibition of NMDA receptors by general anesthetics, such as cyclopropane and ketamine, would impair memory. Some studies also implicate the amygdala, which processes affective information and is strongly connected to the hippocampus[23].

THEORIES OF ACTION

Lipid vs. Protein

At the beginning of the 20th century, Hans Meyer and Charles Overton observed a correlation between the potency of anesthetic agents and their solubility

in olive oil. The Meyer-Overton rule predicts that the more hydrophobic the anesthetic, the higher its potency. From the Meyer-Overton rule, it followed that all anesthetics must act through a common mechanism, probably in the cell membrane, which is a hydrophobic lipid bilayer, and that anesthetics somehow disrupt that bilayer. And while most people agreed that the final result of the perturbation of the cell membrane was a conformational change in the structure of membrane proteins, none of the lipid theories explained how this could happen[13].

It was not until the latter half of the 20th century that research emphasis turned to the interactions between anesthetics and proteins themselves. In an important set of experiments, Franks and Lieb[14] showed that the lipid-free enzyme luciferase could be inhibited by anesthetics following the Meyer-Overton rule: the higher the hydrophobicity of the anesthetic, the more potently it inhibited luciferase. While anesthetics target diverse molecules, such as PKC[10], transmembrane ion channels have received the most attention. Research since then has focused mostly on the hypothesis that anesthetics enhance the action of inhibitory ion channels (GABA and glycine receptors) and inhibit excitatory ion channels (Serotonin type 3; nicotinic acetylcholine; and glutamate receptors). For example, electrophysiological studies have shown that the GABAA receptor is a major target of intravenous general anesthetics, such as propofol and etomidate[15].

Current work is focused on the structure-function relationships of the target ligand-gated ion channels (i.e. how the molecular structure of a receptor mediates its ion-conducting function) in order to elucidate the molecular mechanism by which such channels open and close; and the sites at which anesthetic agents bind and affect those channels. While the former has been of interest to the entire neurobiology community since the 1950's, when Hodgkin and Huxley proposed a model of the action potential that depended on a mystical conduction of ions across the cell membrane[16], the latter promises the more practical improvement of clinical outcomes for patients undergoing general anesthesia. With the elucidation of the pharmacologic interactions between proteins and anesthetics, researcher-physicians may soon have at their disposal better drugs that produce minimal side effects and toxicity.

As many as one in 750 patients remain aware during general anesthesia

THREE GROUPS OF ANESTHETICS

General anesthetics have been classified into three groups based on their relative potencies for behavioral endpoints and effects on the EEG.

Group 1: Etomidate, propofol, and barbiturates

Etomidate, propofol, and barbiturates produce amnesia and hypnosis at doses far lower than those for immobility (reviewed in ref 17). These drugs act via GABAA receptors, the most abundant inhibitory ligand-gated ion channels in the brain. Convincing evidence for their action via GABAA receptors comes from mutation and transgenic animal studies.

Etomidate's action via GABAA receptors has been confirmed in studies utilizing its chirality[18]. R(+)-etomidate is 20 times more potent at inducing Loss of Righting Reflex (LORR) in animals, a surrogate test of consciousness, than S(-)-etomidate. Likewise, the R(+) enantiomer modulates GABAARs 20 times more potently than does the S(-) enantiomer.

Moreover, mutations on the GABAAR subunits affect GABAAR electrophysiology and in vivo responses to anesthetics similarly. For example, mice with a methionine residue in place of asparagine at the 265 position on the β3 subunit exhibited a four-fold reduction in sensitivity to propofol and etomidate anesthesia, specifically immobility. The same point mutation reduces anesthetic modulation of the receptors in electrophysiology studies. The same mutation on the β2 subunit did not affect immobility or hypnosis, but reduced sedation in mice[17]. Furthermore, mice lacking α5 GABAAR subunits were insensitive to emotidate's amnesic effects[19]. And while the β3(N265M) mutation reduces hypnosis and immobility response, it preserves the undesirable effect of respiratory depression, showing that GABAARs are not etomidate's only target in the body.

Group 2: Nitrous oxide, cyclopropane, and ketamine

Drugs in Group 2 act mostly via glutamatergic NMDA receptors, as opposed to GABA receptors. Glutamate is the most abundant excitatory neurotransmitter in the brain, so it's no surprise that inhibition

of glutamate receptors would produce anesthetic effects, which among the Group 2 drugs are mostly analgesia, weak hypnosis and immobility[17].

As with etomidate, ketamine chirality has been used to correlate its anesthetic effects with NMDA receptor modulation. S-ketamine was 1.9 times more potent in inhibiting NMDA receptors in hippocampal slice preparations than was R-ketamine; the same effect was observed in vivo[17].

Horace Wells noted nitrous oxide's (NO2, "laughing gas") analgesic properties while attending a demonstration in 1845; Wells' own demonstration of nitrous oxide's anesthetic properties at MGH was deemed a failure. Transgenic animal studies have shown that NO2 also acts via NMDA receptors. Transgenic mice lacking the ε1 subunit (homolog of the human NR2A) of the NMDA receptor do not exhibit immobility or loss of righting reflex under NO2 administration[24].

However, using an NMDA antagonist MK-801, Stabernack et al. showed that inhibition of NMDA receptors is not sufficient to produce anesthesia. Administration of MK-801 alone reduced MAC but did not produce immobility[25].

Group 3: Halogenated volatile anesthetics

The halogenated volatile anesthetics, usually isoflurane, sevoflurane, desflurane, and halothane, are the least selective of the general anesthetics. These anesthetics affect the GABAARs, two-pore K+ channels, NMDA and 5HT3 receptors, Na+ channels, and other targets[26].

STRUCTURE AND FUNCTION OF CYS-LOOP LIGAND-GATED ION CHANNELS

Studies show that the GABAA receptor is a major target of halogenated volatile anesthetics and intravenous anesthetics such as etomidate and propofol. The GABAAR is a member of a large family of ligand-gated ion channels known as the cys-loop family for the cysteine loop that all family members share; other members include the 5HT3, glycine, and nACh receptors. Most of what is known about the structure of the GABAAR comes from studies of the nicotinic acetylcholine receptor.

Nicotinic Acetylcholine Receptor

Serious investigation into the structure and function of the nAChR began with the studies of the Torpedo electric ray nAChR[27,28,29,30]. Electron microscopy revealed that the receptor is composed of five subunits organized around a central pore. Molecular cloning showed that each subunit consists of four α-helical transmembrane domains (TM1-4). TM2 lines the pore, TM1 and 3 are next to it, and TM4 faces the membrane. Importantly, TM2 is able to interact with hydrophilic molecules since it faces the water-permeable pore, while the other transmembrane domains interact with the hydrophobic cell membrane[31].

Advances in genomics have produced large libraries of eukaryotic and prokaryotic genes that are available for analysis. Interestingly, these libraries have brought to light a large number of cys loop / pentameric LGIC genes conserved in prokaryotes[32]. One such advance was the discovery of the Acetylcholine Binding Protein (AChBP) found in the snail Lymnaea stagnalis, where it mediates neurotransmission. Even though AChBP is water-soluble and lacks the transmembrane domain of mammalian nAChRs, it nonetheless forms a homopentamer with distinct acetylcholine and nicotine binding sites[33]. As expected from prior experiments, ligand binding occurs at the interface between subunits, with different affinities for acetylcholine, nicotine, and the agonist carbamylcholine. While ligand binding induces local conformational changes, studies of signal transduction in a system that lacks the transmembrane domain that is integral for the study of channel gating have to be interpreted with caution.

In contrast with AChBP, which is a good model for the extracellular domain of the nAChR, the Erwinia chrysanthemi (ELIC) and Gloebacter violaceus (GLIC) channels reveal the minimal structural requirements for a functional LGIC in the Cys-loop family.

Even though ELIC and GLIC share only 16% and 20%, respectively, of the sequence with the nAChR, their structures are remarkably similar[34,35]. GLIC is activated by protons, while ELIC's activating ligand has not been discovered yet. The X-ray crystallography structures of ELIC and GLIC are valuable because they are thought to show the closed and open conformations of the protein, respectively. Hilf and Dutzler suggest that the difference in the two conformations can explain the mechanism of gating (channel opening). Specifically, it is the difference in orientation of TM2 and TM3 α helices and the extracellular β sheet between the GLIC and ELIC structures that Hilf and Dutzler base their hypothesis on.

Similar to the pores of other cation channels, the

GLIC pore-lining TM2 domain is composed of hydrophobic bulky residues; the intracellular half is filled with polar residues such as serine and threonine, making this part of the pore hydrophilic. Cation selectivity arises most likely from a conserved ring of glutamate residues on the intracellular end of the pore; this "intermediate ring of charges" is also found in the Torpedo and muscle nAChR[36].

$GABA_A$ Receptors

The GAB_AA receptor is the major inhibitory ligand-gated ion channel in CNS neurons, found in all layers of the cerebral cortex, the hippocampus and the rest of the limbic system, the cerebellum, thalamus, and brainstem[37]. The receptor is composed of five subunits arranged pseudosymmetrically around a central ion-conducting pore. Nineteen subunit varieties have been cloned to date, which theoretically allows for a tremendous amount of diversity of expressed receptors[38]. However, not all possible combinations have been found assembled, and indeed the majority of the neuronal receptors are composed of α, β, and γ subunits[37]. Nevertheless, the abundance of subunit varieties, as well as subunit-dependent localization of the receptors in the brain make GABAARs a promising target for drug discovery[39].

Upon activation by GABA, the receptor pore conducts Cl- and bicarbonate ions, hyperpolarizing the neuron and thereby reducing the action potential firing rate. GABAARs are thought to be the major target of several general anesthetics, including etomidate, propofol, alcohols[40,41], halogenated volatile anesthetics[42], and barbiturates[43]. The understanding of how these and other drugs affect the GABA receptor is essential for improvements in anesthetic drugs and treatments of CNS diseases.

Structure

Using a forced subunit assembly method, Sigel and colleagues determined that receptors composed of α1β2γ2 subunits (the major isoform) are arranged γ2β2α1β2α1 counterclockwise when viewed from the synaptic cleft[44]. Each of the five subunits making up a GABAAR contains a large N-terminal domain, four transmembrane domains (TM1-4) and an intracellular domain between TM3 and TM4[45]. The receptor conducts ions in an all-or-none fashion, transitioning among the open, closed, and desensitized states46.

Ligand binding is described in terms of an allosteric model in which ligands bind and affect the protein at sites distinct (and sometimes far) from the GABA site. These allosteric sites may be found in the extracellular and transmembrane domains of the GABAAR.

Extracellular Domain

GABAARs have two binding sites for GABA, each in the extracellular domain at the interface between the α and β subunits47; GABA binding at these sites opens the Cl- pore. Baumann et al used point mutations on linked subunits to differentiate the contributions of each site to channel gating (using free subunits does not indicate which of the two α/β interfaces has the desired mutation), showing that the α/β site where α is adjacent to γ is more sensitive to GABA; and conversely that the α/β site where β is adjacent to γ is more sensitive to the competitive antagonists bicuculline and muscimol[48].

Classic benzodiazepines enhance inward currents produced by GABA binding[37]. Benzodiazepines bind with high affinity at the interface between α and γ subunits, sites homologous to GABA's at the two αβ interfaces. Rusch and Forman showed that benzodiazepines enhance GABA currents by enhancing GABA gating as opposed to binding. Using the Monod-Wyman-Changeux allosteric model, they were able to conclude that benzodiazepines are high-affinity but low-efficacy co-agonists, in stark contrast to general anesthetics such as etomidate, which are low-affinity high-efficacy agonists[43]. The differences between benzodiazepine and etomidate agonism are further highlighted by single-channel studies. These studies show that etomidate lengthens the mean single-channel open time, whereas benzodiazepines do not; although single channels did open more frequently in the presence of benzodiazepines or etomidate[49,50].

Transmembrane Domain

A diverse set of organic compounds has been shown to act by binding to sites in the GABAAR transmembrane domain. These include alcohols[40], neurosteroids[51], barbiturates[52], volatile anesthetics[42], etomidate, and propofol[53,54].

Alcohols

Alcohols enhance GABA-induced currents in αβγ GABAARs[40], and reduce currents in receptors with

ρ1 subunits[41]. Mihic et al (1997) constructed chimeric receptors containing sequences from the GABA ρ subunit and the glycine receptor α1 subunit (a close homolog of αβγ GABAAR). They were able to identify a region of 45 amino acids between TM2 and TM3 that are necessary and sufficient for enhancement of currents by ethanol and enflurane. Specifically, the α1S270 and β1S265 in the TM2, as well as α2A291, β1M286, and β3N265 in the TM3, were critical to ethanol's enhancement of GABA currents.

Barbiturates

Barbiturates are CNS depressants, and have been used through the 20th century to treat conditions such as insomnia, anxiety and epilepsy; and in the OR as inducers of anesthesia (their history is reviewed in ref 55). Like etomidate, pentobarbital enhances GABA currents at low concentrations and activates GABAARs directly at high concentrations[52]; at even higher concentrations (millimolar) pentobarbital blocks GABA currents.

Etomidate and Propofol

At clinically relevant concentrations, the potent intravenous anesthetic etomidate enhances activation of GABAARs by GABA (lowers EC50) . This interaction directly activates GABAARs at higher concentrations, in the absence of GABA[56]. These two types of activation suggest that GABAARs have two types of etomidate sites – high-affinity sites that enhance GABA sensitivity and low-affinity sites that directly activate the receptor. These observations suggest an orthosteric model where etomidate binds to the same site as GABA to directly activate the receptor (and an allosteric site to enhance GABA activation). However, using electrophysiological methods and the Monod-Wyman-Changeux allosteric model, Rusch et al. (2004) showed that a single class of sites can explain both enhancement and direct activation, concluding that etomidate's affinity for the receptor changes depending on the receptor's state (open or closed); the open receptor has a high affinity for etomidate, whereas the closed receptor has a low affinity).[56]

Evidence that etomidate acts at the interface of the α and β subunits comes from experiments using a photoactivatable etomidate analog, [3H]azi-etomidate, which labels the amino acids αM236 (TM1) and βM286 (TM3).[57]

Mutations of these residues to tryptophan (which is bulky and hydrophobic like etomidate), α1M236W and β2M286W, mimic etomidate presence. Stewart et al used these mutations to show that one class of sites is sufficient to explain etomidate's effects.[58] The β2M286W mutant was insensitive to etomidate direct activation and had zero GABA enhancement by etomidate, consistent with one type of modulatory site. In contrast, the α1M236W mutant showed reduced GABA potentiation by etomidate compared to wild-type but increased direct activation. These opposite effects seem to suggest two classes of etomidate sites, but are nevertheless consistent with the interpretation of one class of sites: etomidate's efficacy is reduced in the α1M236W mutant, but direct activation appears to be enhanced because the mutant has high spontaneous activity, which lowers the energy barrier required for channel opening.

Propofol has been thought to act near the TM2 domain at α1Ser270/β2Asn265 and TM3 domain at α1A291/β2M286. Cysteine protection experiments showed that propofol protects all four of these residues, but only β2M286 in a dose-dependent manner, implicating this residue as the propofol binding site.[59] However, Siegwart et al showed that the mutants β3M286W as well as β3N265M significantly reduce propofol enhancement of GABA currents and direct activation by propofol.[53] Propofol's varying effects on receptors composed of different forms of the β subunit may be useful in drug discovery or modification.

New or Improved Drugs

Going forward, two major branches of research must be pursued to ensure success in the development of new anesthetic drugs. These are the study of the structure and function of LGICs, as well as their subunit compositions and localization in the human brain; and drug discovery, including high-throughput assays of novel agents and chemical modification of existing drugs such as etomidate.

Efforts to improve etomidate include methoxy carbonyl- (MOC-)etomidate, which is designed to be metabolized quicker than etomidate[60] and carboetomidate, which is a significantly weaker inhibitor of adrenal cortisol synthesis[61]. The goal of these chemical modifications is to remove etomidate's toxic properties while preserving the anesthetizing ones.

Another approach is drug discovery. Recently, Lea et al (2010) reported a high-throughput screening of compounds that interact with apoferritin, a soluble protein that mimics the GABAAR's interaction with an-

esthetics.[62] Of 1280 compounds, about 1% were hits. Obviously further electrophysiological, metabolic, and clinical characterizations are necessary before any of those compounds can be used as general anesthetics.

Conclusion

While surgeons and patients have enjoyed the availability of general anesthesia for over 150 years, scientists have not made progress in elucidating its mechanism of action until quite recently. That paradigm shift came in the 1980s, when Franks and Lieb showed that anesthetics act via proteins, not the lipid membrane; research since then has focused primarily on ligand-gated ion channels that mediate fast synaptic transmission in the CNS.

Numerous techniques are available for the study of the structure and function of LGICs, including electron microscopy, cysteine substitution, point mutagenesis, and x-ray crystallography. Our current conception of the 3D structure of the GABAAR is largely based on x-rays of the nicotinic acetylcholine receptor and its homologs. While the overall structure appears to be conserved among the Cys-loop receptors, the fine details (including single amino acids) are different. Until we have a definite idea of GABAAR's 3D structure, including the orientation of the transmembrane domains where general anesthetics act, our interpretations of experimental data will remain murky. Nevertheless, the past decade's progress in research on new or improved anesthetics is bound to continue in the next.

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