REVIEW ARTICLE

Julien F. Biebuyck, M.B., D.Phil., Editor

Anesthesiology
78:757–776, 1993

J. B. Lippincott Company, Philadelphia

The Role of the $GABA_A$ Receptor/Chloride Channel Complex in Anesthesia

Darrell L. Tanelian, M.D., Ph.D., Peter Kosek, M.D.,† Istvan Mody, Ph.D.,‡ M. Bruce MacIver, Ph.D.§*

CONTENTS

ANESTHESIA can be defined as a behavioral state associated with loss of awareness and absence of pain. The mechanism(s) underlying anesthesia are not well elucidated and remain controversial. Two general hypotheses, unitary *versus* agent-specific action, compete as explanations for anesthetic action. It is clear, however, that the primary target for anesthetic action is the brain, and as such, numerous endogenous neuromodulatory systems (*e.g.*, ion channels, neurotransmitters and their receptors, intracellular second messenger systems) could serve as targets for anesthetic agents. Simply put, an anesthetic state can be achieved by enhancing neuronal inhibition, by decreasing neuronal excitation, or by a combination of both. In this article, we review the literature on anesthetic modulation of the brain's primary inhibitory neurotransmitter system, gamma-aminobutyric acid (GABA). Recently, there has been considerable interest in GABAergic mechanisms of anesthesia since numerous classes of anesthetic agents (volatile, barbiturate, benzodiazepine, steroid, and others) have been shown to enhance endogenous $GABA_A$-mediated inhibition in the mammalian central nervous system (CNS).

Basic Pharmacology

Approximately 50 yr ago, GABA and its synthesizing enzyme glutamic acid decarboxylase were discovered

* Associate Professor of Anesthesiology and Pain Management.

† Fellow in Anesthesia.

‡ Assistant Professor of Neurology and Neurological Sciences.

§ Assistant Professor of Neurophysiology in Anesthesia.

Received from the Department of Anesthesia, Stanford University School of Medicine, Stanford, California. Accepted for publication December 21, 1992.

Address reprint requests to Dr. Tanelian: Department of Anesthesiology and Pain Management, University of Texas, Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235-9068.

Key words: α-adrenergic agents. Anesthetic mechanisms. Barbiturates. Benzodiazepines. Ethanol. Etomidate. Propofol. Steroid anesthetics. Volatile anesthetics.

in the gray matter of the mammalian CNS (fig. 1).[1-3] Not long after its discovery, GABA was shown to be an inhibitory neurotransmitter in the mammalian CNS.[2,4] The quantification of its action was made possible by early electrophysiologic studies using the crayfish stretch receptor preparation.[1,3] Today, it is clear that GABA is the major inhibitory neurotransmitter of the mammalian brain and is responsible for most fast synaptic inhibition of neurons.[2,4-7] Bloom and Iversen in 1971[8] estimated that about a third of all synapses in the CNS are GABAergic. It is, therefore, no surprise that virtually every neuron of the mammalian brain is responsive to GABA.

Postsynaptic GABA receptors obey the principle of divergence in neurotransmitter action.[9,10] This principle states that the same neurotransmitter may have different actions depending on the nature of postsynaptic receptors. The early electrophysiologic studies of GABA action have established that GABA receptor activation causes an increase in the chloride permeability of neurons (fig. 1).[2,4,5] Activation of this receptor can be blocked by the competitive GABA antagonist bicuculline. More recent studies clarified that GABA has an additional action even in the presence of bicuculline, which consists of the opening of potassium channels.[4,5,10-12] Thus, the former bicuculline-sensitive GABA receptors have been termed $GABA_A$ receptors and the latter potassium channel-modulating receptors are called $GABA_B$ receptors.[12] It is now clear that, in the human brain, activation of $GABA_A$ receptors by synaptically released GABA is responsible for the fast inhibitory postsynaptic potential, whereas activation of $GABA_B$ receptors underlies a much slower and longer lasting inhibition.[7] The two receptors not only are functionally distinct, but they belong to separate classes of receptor families. The $GABA_A$ receptor is a ligand-gated ion channel, whereas the $GABA_B$ receptor is a G protein-coupled receptor.[10,12]

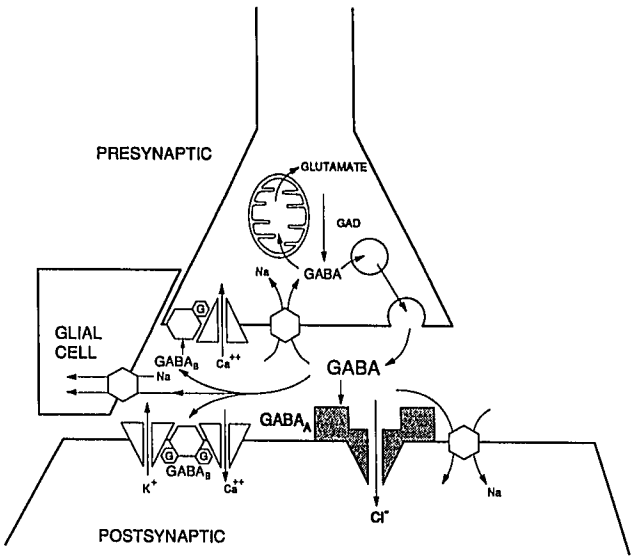

Fig. 1. Diagram of a GABAergic synapse, showing synthesis and release from a presynaptic terminal and GABA actions on a postsynaptic neuron. GABA is synthesized from glutamate *via* glutamic acid decarboxylase and stored for release. Once released, GABA can act on pre- and postsynaptic $GABA_B$ receptors, which inhibit Ca^{2+} channels or activate K^+ channels *via* G proteins. Presynaptic actions on $GABA_B$ receptors modulate the release of GABA from nerve terminals. The major postsynaptic action of GABA, especially in higher brain regions, is the activation of $GABA_A$ receptor/chloride channels. Chloride ions moving into the postsynaptic cell cause an increased membrane conductance that inhibits postsynaptic action potential discharge and depresses excitatory synaptic responses. Synaptic inhibition is terminated when GABA is removed from the synaptic cleft by sodium-coupled active transport into pre- and postsynaptic neurons and into glial cells.

The purpose of the present review is to provide a comprehensive picture of the $GABA_A$ receptor as a site for anesthetic action. Compounds that impair or enhance $GABA_A$ receptor function will effectively offset the normal balance between excitation and inhibition in the CNS. A shift of this balance in the favor of excitation results in hyperexcitability, leading to abnormal discharges of neurons such as observed in epilepsy.[13] In contrast, tilting the balance in favor of inhibition will yield a reduced state of neuronal excitability, which may be responsible for many of the effects of clinically used anesthetics and CNS depressants.

The Structure of $GABA_A$ Receptor Channels

The cloning of the nicotinic acetylcholine, glycine, and the $GABA_A$ receptors[14] has provided the basis for identification of superfamilies of receptors in the CNS. The similarities between these three functionally distinct receptors have shown that they are part of a family of ligand-gated receptor channels.[6,15-17] The channel-forming receptors in this class are composed of several glycoprotein subunits that assemble to form a functional channel with an agonist recognition site (fig. 2). Binding of agonist to the receptor will open the channel, hence the term ligand-gated to distinguish them from channels that are gated by transmembrane voltage changes.[6,15-17]

The initial cloning of the $GABA_A$ receptor channel provided evidence for two different but related subunits (alpha and beta).[14] Since that time, molecular neuro-

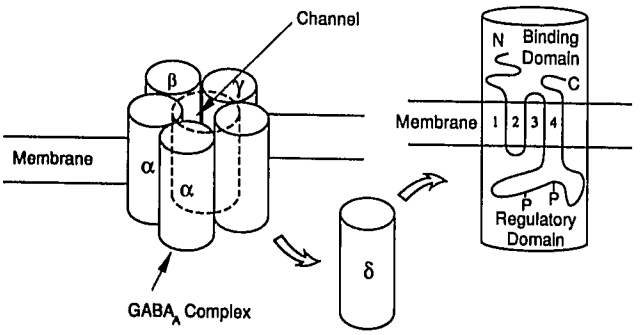

Fig. 2. Diagram of a GABA$_A$ receptor/chloride channel complex, showing subunit composition and membrane association for a representative subunit protein. Each complex consists of five subunits that come together to form the channel and receptor complex. Different combinations of subunits, in different stoichiometry, can produce receptors with different binding affinities and channel kinetics. Each subunit consists of an N-terminus region on the extracellular surface, four transmembrane-spanning regions, and an intracellular regulatory domain that can be modified by phosphorylation. These different subtypes are differentially expressed in different brain regions.

science has characterized more than 15 different GABA$_A$ receptor subunits, which are labelled α_{1-6}, β_{1-3}, γ_{1-3}, δ, ϵ, ρ. All of these subunits have four membrane-spanning domains with a relatively high degree of conservation (fig. 2).[6,14-20] Each subunit is formed by approximately 450–550 amino acid residues, and five subunits assemble to form a functional GABA$_A$ receptor channel. The conservation of positively charged residues in the transmembrane segments of each subunit, particularly in transmembrane region 2, has been suggested to serve a particular function in the channel. This region of the molecule provides the positive charges to form the selectivity filter for the negatively charged chloride ion to pass through the channel.[16]

A combination of molecular biologic and electrophysiologic techniques has allowed the expression of various combinations of GABA$_A$ receptor subunits in cells that normally do not express these receptors. Thus, it was possible to study the gating, desensitization, conductance, and rectification properties of GABA$_A$ receptor channels composed of experimentally controlled subunits in human kidney cell lines or in Xenopus oocytes.[16] It is beyond the scope of the present review to engage in detailed description of the role of each individual subunit. Several recent publications are available on this topic.[6,14-20] The triple subunit combination alpha, beta, and gamma is the simplest receptor to show high-affinity benzodiazepine binding. It also has a set of biophysical properties that resemble native GABA$_A$ receptor channels in hippocampus, neocortex, and other higher brain structures, except cerebellum. It is not clear at present how many "native" GABA$_A$ receptor complexes exist, or their brain regional distribution or sensitivity to anesthetics. All of these have important implications for the development of "targeted" GABA$_A$ receptor therapeutic agents (see Discussion).

While the study of the relationship between subunit compositions and GABA$_A$ receptor function has yielded valuable information about the possible function of each individual receptor subunit, the picture in the intact brain is more complex. Different neuronal populations possess different types of subunits, and the expression of messenger RNAs for the various subunits is dramatically altered during different stages of development.[18,19] There are also species differences in the distribution of the various GABA$_A$ receptor subunits in various brain regions. This, variability should be noted in comparing and contrasting results of studies we review here, which were obtained from different preparations and several species.

Identification of GABAergic Agonists and Antagonists

Table 1 identifies the various GABAergic agonists and antagonists referred to in this article.

An issue that cannot be neglected when reviewing the action of drugs on GABA$_A$ receptors is that the functional consequences of GABA$_A$ receptor activation upon a given cell will depend directly on the chloride reversal potential. Several studies recently reviewed by Cherubini *et al.* have indicated that, during early embryonic development and during the first postnatal week in the rat, activation of GABA$_A$ receptors produces a depolarization due to the large concentration of chloride in neurons.[21] If the GABA-induced depolarization does not reach threshold for activation of voltage-dependent conductances, then activation of GABA$_A$ receptors may be considered inhibitory. A subthreshold depolarization still would produce a shunting of excitatory synaptic currents by the increase in chloride conductance across the nerve cell membrane. However, as is the case during early development, the depolarization following GABA$_A$ receptor activation is large enough to activate voltage-dependent calcium channels that allow calcium entry into the neurons. Therefore, caution should be taken in interpreting some of the findings presented in our review with regard to the site of GABA action, the age of the preparation at issue, and

Table 1. GABA-ergic Agonists and Antagonists

- $GABA_A$ receptor agonists
  - GABA (γ-aminobutyric acid)
  - Muscimol (5-aminomethyl-3-hydroxy-izoxalole)
  - Isoguvacine (1,2,5,6-tetrahydroisonicotinic acid)
  - Isonipecotic acid (hexahydroisonicotinic acid)
  - THIP hydrochloride (4,5,6,7-tetrahydroisoxazolo[5,4,-c]pyridin-3-ol HCl)
  - THPO hydrate (4,5,6,7-tetrahydroisoxazolo[5,4,-c]pyridin-3-ol hydrate)
- Competitive $GABA_A$ receptor antagonists
  - (−) Bicuculline methiodide, methyl chloride, methyl bromide
  - SR-95531 (2-(3'-carboxy-2'-propyl)-3-amino-6*p*-(4-methoxyphenyl)pyridazinium bromide)
- Noncompetitive $GABA_A$ receptor antagonists–Cl^- channel blockers
  - Picrotoxin (isolated from the seed of *Anamirta occulus*)
  - (?) TBPS (*t*-butyl-bicyclo-phosphorothionate)
  - (?) Penicillin
  - (?) Pentilenetetrazole, metrazole
- Benzodiazepine site
  - Agonists (augment the response of GABA)
    - CGS 9896
    - Chlordiazepoxide
    - Clonazepam
    - Flunitrazepam
    - Flurazepam
    - Diazepam
    - Midazolam
    - Zolpidem
  - Inverse agonists (decrease in response to GABA)
    - Ro 19-4603
    - DMCM (methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate)
    - β-CCE (ethyl β-carboline-3-carboxylate)
    - β-CCM (methyl β-carboline-3-carboxylate)
    - Ro 15-4513
    - FG 7142 (N-methyl-β-carboline-3-carboxamide)
  - Antagonists (no effect on their own, but block effect of both agonists and antagonists)
    - β-CCP (propyl β-carboline-3-carboxylate)
    - Flumazenil (Ro 15-1788)
    - Ro 14-7437
- Barbiturate site
  - Agonists
    - Pentobarbital (5-ethyl-5-(1-methylbutyl)barbituric acid)
    - Phenobarbital (5-ethyl-5-phenobarbituric acid)
    - (−)MPPB ((−)1-methyl-5-phenyl-5-propylbarbituric acid)
  - Antagonists
    - (+)MPPB ((+)1-methyl-5-phenyl-5-propylbarbituric acid)
    - (?) TBPS (*t*-butyl-bicyclo-phosphorothionate)

? = the precise site and/or action is not known for the agent.

the possible subunit composition of the $GABA_A$ receptors underlying the various drug effects.

The modulation of $GABA_A$ receptor function by drugs, such as anesthetics, may occur directly or through several different second messenger systems[22–27] that affect the opening of $GABA_A$ receptor channels. In addition, a number of neurotransmitter systems have been shown to selectively innervate GABAergic interneurons,[28,29] and increased GABAergic function may result from the selective increases in interneuronal excitability.

GABA and Anesthesia

The involvement of GABA in anesthesia has been studied by administering GABA or GABA analogs to animals and humans and observing alteration of sleep time, sedation, and anesthesia. GABA is not effective when given systemically because it cannot cross the blood-brain barrier. Therefore, the GABA analog THIP has been used to study the CNS effects of GABA. In humans, the primary side effects of THIP are sedation and dizziness; although anesthesia has not been described.[30–33] In rats and mice, THIP produced analgesia, sedation, and loss of righting reflex.[34] Recovery was complete and took about 1.5 h. Local perfusion of the thalamus *in vivo* with GABA using an indwelling microdialysis probe produces a significant increase in sleep and induces long-lasting inhibition of somatosensory event-related potentials in cats.[35] Further studies in humans are required to determine the extent to which activation of GABAergic systems can contribute to analgesia, sedation, hypnosis, or anesthesia; this will probably await the development of selective, potent, and lipophilic $GABA_A$ receptor agonists. It will be evident from this review that anesthetics can influence GABAergic inhibition by different mechanisms. Some agents directly activate the $GABA_A$ receptor, others enhance GABA binding to the receptor, some can enhance coupling between receptor activation, and yet others directly influence chloride channel opening. Many anesthetic agents appear to act by more than one of the above mechanisms, and it is not yet known which actions are most important for sedation, hypnosis, and anesthesia.

Table 2 provides a description of the most common techniques used to study anesthetic actions at the $GABA_A$ receptor/chloride channel complex. Experimental parameters and specific measures are provided, together with a cursory discussion of the advantages and disadvantages of each technique. It is clear that no single technique or measure can be relied upon in isolation; rather, the results derived from multidisciplinary approaches are necessary to understand anesthetic effects at the $GABA_A$ receptor/chloride channel complex (and any other proposed site). Discrepancies exist be-

Table 2. Measurement of Anesthetic Effects on the $GABA_A$–Cl^- Channel Complex

Experimental Method	Parameter Evaluated	Measurement	Advantage	Disadvantage
Behavioral and genetic studies	Loss of righting reflex Analgesia Sleep time	Inability to regain upright position within specified time following drug administration Paw or tail withdrawal latency, hemodynamic or muscular response to noxious stimuli Duration of sleep or sleep pattern EEG following drug administration	Closest representation of human behavioral response to anesthetic drugs	Impossible to validate drug effects upon memory Does not allow identification of predominant site of anesthetic action (*e.g.*, brain region, receptor, ion channel)
Receptor binding assay	Receptor affinity (K_D) Receptor number (B_{max})	Displacement/modulation of known agonist or antagonist binding	Provides a measure of drug interaction at effector site and classification of effector site	Does not evaluate functional significance of the receptor–ligand interaction No behavioral correlate
Chloride ion flux	$^{36}Cl^-$ flux through cell membranes	Uptake or efflux of $^{36}Cl^-$	Quantitative measure of chloride ionophore activity	Low signal-to-noise ratio Many studies use high drug concentrations (beyond clinically effective range) to show effect
Cellular and synaptic electrophysiology	Inhibitory postsynaptic current (IPSC) Inhibitory postsynaptic potential (IPSP) Response to exogenous application of agonist	Amplitude Decay time constant Frequency Conductance Receptor desensitization	Functional measure of synaptic inhibition Allows regional differentiation between brain regions	Incomplete neuronal circuitry and modulatory mechanisms No behavioral correlate
Single-channel electrophysiology	Current flow through single-ion channels	Probability of channel opening Mean open time Burst time Single-channel conductance	Detailed measure of kinetics and mechanism of drug action at identified single channels	Does not measure synaptic contribution to drug action No behavioral correlate

tween results obtained using different experimental approaches, and these should be viewed in light of the advantages and disadvantages described for each technique.

Modulation of the $GABA_A$ Receptor Complex by Anesthetics

Barbiturates

Barbiturates form a large and diverse class of drugs, and an extensive literature concerning their effects on the $GABA_A$ receptor complex is available. In addition to their effects on $GABA_A$ receptor activity, barbiturates produce many other effects, including inhibition of glutamate and adenosine receptor activity, as well as release of norepinephrine, serotonin, and acetylcholine.[36,37] Although clinically useful barbiturates are sedative-hypnotics or anticonvulsants, another subgroup of barbiturates are proconvulsant. These diverse clinical effects have been correlated with chemical structures and specific neuropharmacologic properties.[38] This comparative pharmacology has helped identify the neurophysiologic and binding characteristics necessary to predict and design clinically useful barbiturates with minimal side effects.

***In Vivo* and Genetic Studies.** Pharmacologic studies of barbiturates can be divided into effects on sedation-hypnosis and on convulsions. Pentobarbital-induced loss of righting reflex is prolonged by the $GABA_A$ agonists muscimol and THIP.[30,34] This suggests that hypnosis is at least partly mediated by activation of the $GABA_A$ receptor complex. This theory is strengthened by the finding that pentobarbital-induced loss of righting reflex is decreased by the $GABA_A$ antagonist bicuculline,[39] demonstrating that blocking $GABA_A$ receptors is antihypnotic. More evidence implicating the $GABA_A$ system in sedation-hypnosis comes from genetic studies. Rats specifically bred for short or long duration of

ethanol-induced sleeping time showed corresponding differences in barbiturate- and benzodiazepine-induced sleeping time,[40] indicating that a common site may underlie barbiturate-, benzodiazepine-, and ethanol-induced sedative-hypnotic actions.

The anticonvulsant barbiturate phenobarbital effectively blocks seizures induced by the chloride channel blocker picrotoxin.[39] This effect is not modified by benzodiazepine antagonists[41] or inverse agonists,[42] indicating that the anticonvulsant effect of barbiturates does not depend on the benzodiazepine receptor. An interesting corollary is that the $GABA_A$ blocker picrotoxin has a protective effect against a lethal dose of pentobarbital in animals.[43] However, since picrotoxin is a potent convulsant, this pharmacologic reversal is of no clinical utility. At present, results derived from *in vivo* studies support the possibility that barbiturates produce their sedative-hypnotic effects *via* actions at the $GABA_A$ receptor complex, but definitive data in humans is lacking and will be difficult to acquire given the proconvulsant effects of $GABA_A$ receptor blockers.

Binding Studies. Barbiturates modulate the binding of other ligands to the $GABA_A$ receptor. The interactions between the binding of GABA, $GABA_A$ blocking convulsants, barbiturates, and benzodiazepines with respect to their distinct binding sites on the $GABA_A$ complex are not easily explained with a simple model. These ligands bind to any of four known domains and modulate binding at other sites presumably *via* allosteric interactions.[44] Binding studies have proved useful in understanding the behavioral effects of barbiturates, since the potencies for a series of barbiturates as anesthetics correlate well with their ability to modulate GABA and benzodiazepine binding.[45,46]

Barbiturates markedly potentiate the affinity of GABA binding to the $GABA_A$ receptor in a manner dependent on the presence of chloride ions.[41,47–49] Scatchard analysis of binding data indicates that barbiturates appear to increase the number of available GABA receptors[49] and slow the dissociation of GABA from receptors.[50] The increased number of sites available for GABA binding may be explained by a shift of very low-affinity binding sites (which are difficult to measure) to a higher affinity conformation.[51] This very low-affinity GABA binding site also binds the GABA antagonist bicuculline.[52,53] Barbiturates selectively enhance only agonist binding by decreasing the affinity of bicuculline binding to this very low-affinity GABA binding site.[53]

The binding to $GABA_A$ receptors of convulsant $GABA_A$ blockers (*e.g.*, picrotoxin, TBPS) is inhibited by barbiturates. Barbiturates markedly accelerate dissociation of the caged convulsant TBPS from its recognition site.[54,55] The relative potencies of barbiturates to inhibit this binding correlate well with their ability to enhance GABAergic synaptic transmission.[46]

Barbiturates increase the binding of benzodiazepines to the $GABA_A$ receptor by increasing their affinity in a chloride-dependent manner.[45] This capacity to increase GABA and benzodiazepine binding correlates well with the anesthetic potencies of the barbiturates studied.[56] Picrotoxin, a chloride channel antagonist, and the convulsant barbiturate (±)-5-(1,3,-dimethylbutyl)-5-barbituric acid ((±)-DMBB) block this potentiation of benzodiazepine binding indicating that they also allosterically modulate receptor conformation.[41,45,54,57] Barbiturates decrease the affinity of benzodiazepine inverse agonists (discussed in more detail later) for the benzodiazepine receptor,[53] thereby selectively enhancing only agonist binding *via* allosteric interaction.

Chloride Flux Studies. The development of biochemical assays for GABA-mediated $^{36}Cl^-$ flux in various neuronal preparations[58–60] has provided the basis for analytical studies of $GABA_A$ receptor function. In the absence of GABA, pentobarbital increases $^{36}Cl^-$ uptake into synaptoneurosomes.[61] In addition, pentobarbital markedly potentiates $^{36}Cl^-$ uptake induced by GABA and the GABA agonist muscimol.[62,63] The anesthetic pentobarbital is approximately ten times as potent as the anticonvulsant phenobarbital in this effect,[61] suggesting that chloride flux is related to the sedative rather than the anticonvulsant properties of barbiturates.

The convulsant barbiturate +MPPB (S-(+)-1-methyl-5-phenyl-5-propyl barbiturate) inhibits GABA-stimulated chloride flux as do the $GABA_A$ receptor antagonists bicuculline and TBPS.[61] The benzodiazepine inverse agonist Ro 15-4513 had no effect on pentobarbital-enhanced GABA-stimulated $^{36}Cl^-$ flux.[63]

This is in accordance with the binding study data (see above), which suggests that barbiturates and benzodiazepines act at two distinct binding sites on the receptor complex.

Electrophysiologic Studies. In 1961, Eccles *et al.* demonstrated that anesthetic concentrations of pentobarbital prolong inhibitory postsynaptic potentials in the spinal cord.‖ This effect is blocked by the $GABA_A$ antagonist picrotoxin and occurs in various regions of

‖ Eccles JC: The mechanism of synaptic transmission. Ergebnisse Physiology 51:299–430, 1961.

the brain. Single cell electrophysiologic studies have provided considerable insight into the mechanisms underlying barbiturate modulation of $GABA_A$ receptor function. The $GABA_A$ channel opens in bursts consisting of multiple single openings in response to GABA. The net chloride flux is a composite of the single channel current, the frequency of bursts, the number of openings per burst and the duration of each opening. Pentobarbital and secobarbital applied in the presence of GABA increase both the mean channel open time of the $GABA_A$ complex, and the number of openings per burst.[64-66] At similar concentrations, the anticonvulsant barbiturate phenobarbital has no effect on the duration of the open state but increases the number of single channel openings.[64]

Voltage clamp studies on cultured rat hippocampal neurons demonstrate that the convulsant barbiturates 5-ethyl-5-(3-methylbut-2-enyl) barbituric acid (3M2B) and (±)-DMBB also potentiated GABA-mediated chloride currents and prolonged the duration of GABAergic inhibitory postsynaptic currents in a manner indistinguishable from the sedative-hypnotic barbiturates,[67] suggesting that the proconvulsant properties of these barbiturates may not be mediated by the $GABA_A$ receptor. Care should be taken in the interpretation of these data, since they derive from only a single study. Furthermore, results from binding studies and chloride flux measurements have provided consistent evidence that convulsant and anesthetic barbiturates can produce the expected opposite effects (see above).

Benzodiazepines

Three pharmacologic classes of benzodiazepine ligands have been identified: agonists, inverse-agonists, and antagonists. Benzodiazepine agonists are potent sedative-hypnotics, anticonvulsants, and anxiolytics. In 1980, the first endogenous compound with high affinity for the benzodiazepine receptor ethyl 3-carboethoxy-β-carboline (β-CCB, a β-carboline) was extracted from human urine.[68] Although this compound may have been formed during the extraction and identification procedure, another β-carboline, 3-carbobutoxy-β-carboline (β-CCE), with high affinity for the benzodiazepine receptor subsequently was identified in pig brain.[69] These compounds produce behavioral effects opposite those of benzodiazepine agonists (discussed in more detail below) and are the prototypes of benzodiazepine receptor "inverse-agonists." The benzodiazepine receptor antagonist flumazenil (Ro 15-1788) was first described in 1981 at Hoffmann La Roche, in Basel, Switzerland. This compound has a high affinity for the benzodiazepine receptor, blocks the effects of both benzodiazepine agonists and inverse-agonists, and has minimal intrinsic activity. Agonists increase the chloride conductance, inverse-agonists decrease it, and antagonists have no effect but block the actions of agonists and antagonists at the $GABA_A$ receptor.

***In Vivo* Studies.** β-CCB has a high affinity for the benzodiazepine receptor, but this compound is ineffective as an anticonvulsant or anxiolytic and has proconvulsant activity. Many other compounds of this β-carboline class have been synthesized and have similar behavioral pharmacology.[70-73] The anxiogenic and proconvulsant effects of benzodiazepine inverse agonists β-CCB and β-CCE are blocked by the benzodiazepine antagonist flumazenil. The behavioral activity of these inverse agonists is due to an antagonism of GABA responses.[74-76]

Binding Studies. As discussed in the barbiturate section, mutual allosteric interactions are observed between the binding of GABA, benzodiazepines, barbiturates, and convulsants to the $GABA_A$ receptor.[77-83] Benzodiazepine agonists enhance the binding of GABA to the very low-affinity GABA binding sites on the $GABA_A$ receptor complex.[84] The binding of benzodiazepines to the $GABA_A$ receptor complex is enhanced by GABA,[85,86] barbiturates,[57] and chloride ions. Picrotoxin inhibits basal, GABA-stimulated, and pentobarbital-stimulated [^{3}H] flunitrazepam binding in a manner dependent on the presence of chloride ions in homogenized rat forebrain but has no effect on binding in the cerebellum.[87] GABA agonists have no effect on the binding of the benzodiazepine antagonist flumazenil,[88,89] and inhibit the binding of the inverse agonist β-CCM.[90] As discussed in the barbiturate section, the potency of barbiturates as anesthetics but not as anticonvulsants correlates well with their ability to enhance benzodiazepine binding to the $GABA_A$ receptor.[44,91] Barbiturates also inhibit the binding of β-CCM to rat brain membranes.[53] Similar to the effect of barbiturates, benzodiazepine agonists decrease the binding of the caged convulsant TBPS,[92] whereas inverse agonists increase this binding.[93]

Chloride Flux Studies. Benzodiazepines alone have no effect on $^{36}Cl^-$ flux into synaptoneurosomes in the absence of an exogenously applied $GABA_A$ agonists.[94] In the presence of GABA or muscimol, benzodiazepine agonists increase $^{36}Cl^-$ uptake in a concentration-dependent manner.[63] Benzodiazepine agonists shift the concentration-response curve for muscimol-stimulated

$^{36}Cl^-$ uptake to the left without decreasing V_{max}.[63] This increase in $^{36}Cl^-$ uptake is blocked completely by the benzodiazepine antagonist flumazenil.[63] The benzodiazepine inverse agonist β-CCE inhibited muscimol-stimulated $^{36}Cl^-$ flux in rat brain synaptoneurosomes in a competitive fashion, shifting the concentration-response curve to the right.[63] In contrast, in spinal cord neurons, β-CCE and DMCM act as noncompetitive inhibitors of GABA.[95] In hippocampal slices,[96] DMCM does not alter muscimol-stimulated $^{36}Cl^-$ flux. The reasons for these discrepancies may lie in regional differences in the subunit composition of the $GABA_A$ receptor complex, but taken together, the results on $^{36}Cl^-$ flux suggest that the major action of benzodiazepines is to enhance agonist binding to the receptor, with little or no direct effect on chloride channel gating.[44]

Electrophysiologic Studies. Benzodiazepine agonists potentiate $GABA_A$-induced chloride channel opening in a manner consistent with increasing the association of GABA with its receptor. Single channel recordings in mouse spinal cord neurons have demonstrated that benzodiazepines enhance the probability of GABA channel openings in long-duration bursts.[66] Benzodiazepine agonists also enhance GABAergic synaptic transmission and the response to exogenously applied GABA.[97–99] These results are consistent with data obtained from binding and chloride flux studies, but differ from the actions produced by barbiturates in that direct effects on the channel (*i.e.*, bypassing the receptor) were not observed with benzodiazepines.

Steroid Anesthetics

Binding and Chloride Flux Studies. Metabolites of the steroid hormones progesterone (3α-hydroxy-5α-dihydroprogesterone) and deoxycorticosterone (3α,5α-tetrahydrocorticosterone) have been shown to act as barbiturate-like ligands at the $GABA_A$ receptor-chloride ion channel complex.[100–102] At nanomolar to low micromolar concentrations, these steroid derivatives can stimulate [^{3}H]flunitrazepam and [^{3}H]muscimol binding and displace the convulsant [^{35}S]TBPS from its binding site in an allosteric manner.[100] Structure-activity relationship data demonstrated that the essential features of the active structures are a 5a- or 5b-reduced pregnane skeleton with a hydroxyl at C3 in the a-position and a ketone group at C20.[100]

Further *in vitro* studies in the rat brain revealed the most potent steroid, 5α-pregnan-3α-ol-20-one, modulates [^{35}S]TBPS binding in a regionally dependent manner.[103] The steroids that were most active at the $GABA_A$ receptor-chloride channel were not active at the intracellular progestin receptor, demonstrating functional differentiation of these steroid compounds.[103]

Most recently, steroid anesthetics (*e.g.*, alphaxalone) and other naturally occurring analogs have been shown to modulate the $GABA_A$ receptor complex at a site distinct from the barbiturates.[104–106] These studies also suggest the existence of a specific binding site for the steroids in the $GABA_A$ receptor complex.

Electrophysiologic Studies. In the rat cuneate nucleus slice, alphaxalone potentiated depolarizing responses to perfused GABA and muscimol but not to glycine.[107] Further work in cultured rat spinal cord neurons substantiated these findings by showing alphaxalone, at submicromolar concentrations, to increase the amplitude and duration of chloride-dependent responses to GABA.[108] The nonanesthetic 3B-hydroxy-alphaxalone analog was without effect.[108] Higher alphaxalone concentrations, similar to anesthetic concentrations, increased membrane conductance in the absence of exogenous GABA, suggesting alphaxalone increases chloride conductance.[108] These steroid effects resemble the "direct" channel-enhancing actions produced by the barbiturates.

Whole-cell patch clamp studies of cultured rat hippocampal neurons also have demonstrated alphaxalone to potentiate chloride conductance responses elicited by GABA as well as to prolong evoked GABA-mediated postsynaptic currents.[101] The decay time constant of these inhibitory postsynaptic currents was prolonged by five to eight times, with no increase in peak amplitude. In rat dorsal root ganglia, alphaxalone (10–60 μM) was shown to activate a chloride conductance, and this effect was blocked by picrotoxin.[109] In another preparation, bovine chromaffin cells, alphaxalone, and other pregnane steroids directly activated the $GABA_A$ receptor at a site distinct from the barbiturate and benzodiazepine allosteric sites.[110] In this study, intracellularly applied alphaxalone had no effect on the $GABA_A$ receptor, suggesting that the steroid binding site is better accessed extracellularly. Given the high lipid solubility of steroids and the difficulties of single cell "pharmacokinetics," it remains to be determined where the steroid binding site resides on the $GABA_A$ receptor.

Ethanol

The sedative, hypnotic, and anesthetic effects of ethanol, one of the oldest and most widely consumed drugs, are comparable to those of the barbiturates and benzodiazepines. The cellular and molecular mechanisms of ethanol's actions are not fully understood, and

numerous sites of action have been proposed and recently reviewed by Dietrich *et al.*[111] These sites include voltage-dependent sodium and potassium channels, chloride channels, calcium channels, intracellular calcium homeostasis, the acetylcholine system, the NMDA glutamate system, biogenic amines, adenosine, and protein phosphorylation. Interest in the effects of ethanol on the $GABA_A$ system have grown steadily, as shown in a number of recent investigations. The results of these studies are presented in the following sections.

Behavioral and Genetic Studies. Behavioral studies, examining anesthesia, ataxia, and punished responding, have shown that GABAmimetic drugs can increase the actions of ethanol, whereas GABA antagonists reduce them.[112] Behavioral studies also have addressed the pharmacodynamic interactions between ethanol, benzodiazepines, and barbiturates. For example, the benzodiazepine inverse agonist Ro 15-4513, which is a good antagonist of benzodiazepine actions, also reduces many of the actions of ethanol.[113–115] Genetic studies with long sleep/short sleep (LS/SS) mice and rats (AT/ANT), selected for ethanol sensitivity, display differences in benzodiazepine sensitivity as well; LS mice sleep longer in response to a given dose of benzodiazepine compared with SS mice.[116–118] Mice selected for differences in diazepam-induced ataxia, the diazepam sensitive/diazepam insensitive (DS/DR) mouse lines, display differences in their response to ethanol. DS mice exhibited ataxia at lower brain concentrations of ethanol (1.1 mg/g) compared with DR mice (1.4 mg/g), and ethanol had a longer duration of action in DS than DR mice.[119] These studies implicate the $GABA_A$ system in the actions of ethanol but require cellular and molecular level support (discussed in the following section) to substantiate the indirect conclusions. It also would be helpful if similar concentration ranges and common endpoints were adopted in behavioral studies, to allow direct comparisons between experiments and with intoxicating and/or anesthetic concentrations for humans.

Chloride Flux Studies. The effect of ethanol was studied on chloride flux through $GABA_A$-activated chloride channels by measuring uptake of $^{36}Cl^-$ into cultured spinal cord neurons or isolated brain membrane vesicles (synaptosomes). Ethanol increases $GABA_A$-mediated $^{36}Cl^-$ flux in cultured spinal cord cells at concentrations of 5–50 mM, and this effect can be blocked by picrotoxin and bicuculline.[40,60,114,116,120–122] Based upon the behavioral genetic studies described above, one would suspect that chloride flux would differ in response to ethanol in accordance with genetically determined behavioral effects. Studies with LS/SS and DS/DR mice demonstrated that $GABA_A$-activated chloride flux was augmented by both ethanol and benzodiazepines in LS and DS but not SS and DR mice.[40,123,124] In addition, comparable effects on chloride flux were demonstrated in the high and low acute sensitivity rats selected for differences in ethanol-induced loss of righting reflex and the long- and short-sleeping heterogeneous stock mice selected for differences in ethanol sleep time.[124]

Another area of investigation centers around the ability of benzodiazepine inverse agonists (Ro 15-4513 and FG 7142) to reduce the behavioral effects of ethanol. In these studies, Ro 15-4513 and FG 7142 antagonized ethanol's effect on chloride flux.[120,121,125] It should be noted that the benzodiazepine antagonists can reverse the effects of ethanol only partially, suggesting that ethanol can act on other (nonbenzodiazepine) sites. This is true also at the behavioral level (see above), but the consistency between data obtained from both experimental approaches strengthens the argument for ethanol's ability to enhance GABAergic synaptic inhibition.

Electrophysiologic Studies. Ethanol is able to facilitate electrophysiologic responses associated with GABAergic neurotransmission. In patch clamp studies using rat dorsal root ganglion neurons, ethanol at concentrations of 30–300 mM augmented the peak current induced by GABA without causing a change in the steady-state current.[126] The use of longer chain alcohols (n-butanol-C4, n-hexanol-C6, and n-octanol-C8) for which hypnotic effects increase with chain length[127] augmented the peak current induced by GABA in a concentration-dependent manner.[128] The potency, on a logarithmic scale, was linearly related to carbon atom number and membrane/buffer partition coefficients of these alcohols. In cultured mouse hippocampal neurons, ethanol (1–80 mM) also potentiated GABA-activated chloride currents in a concentration-dependent manner.[129]

The effects of ethanol occur over the concentration range (10–300 mM) that corresponds to intoxicating and anesthetic blood levels in humans; thus, the electrophysiologic data, together with results from binding and chloride flux studies, further strengthen the involvement of GABAergic inhibition as a site of action for ethanol.

Volatile Anesthetics

Behavioral Studies. There is evidence for interactions between GABAergic drugs and volatile anesthetic

action in both animals and humans. In mice, Ro 15-4513 (a high-affinity ligand of the benzodiazepine receptor with partial inverse agonist qualities) produced a dose-dependent reduction in the sleep time of mice exposed to the inhalation anesthetic methoxyflurane.[130] Sleep time measures, though, do not address anesthesia, and studies investigating modification of volatile agent MAC using the benzodiazepine antagonist flumazenil have produced differing results. In rats anesthetized with halothane, flumazenil (0.1 and 1.0 mg/kg) was found to have no effect on the MAC of halothane.[131] However, in dogs, flumazenil (0.15–0.45 mg/kg) significantly decreased halothane MAC.[132] In another study, Geller *et al.* pretreated mice intraperitoneally with flumazenil (1–10 mg/kg) and found no effect on the MAC of halothane, but demonstrated that, on emergence from anesthesia, the flumazenil-treated mice recovered spontaneous motor activity more quickly.[133] The current behavioral studies do not provide clear evidence for an interaction between the site of volatile anesthetic action and the benzodiazepine receptor.

Chloride Flux Studies Inhalation anesthetics such as ether, enflurane, halothane, and methoxyflurane have been shown to increase $^{36}Cl^-$ uptake into rat cerebral cortical synaptosomes in a concentration-dependent, picrotoxin-sensitive fashion.[134] The same study also demonstrated volatile anesthetic inhibition of TBPS binding to cortical membranes. In this study, the concentration of volatile anesthetics required to produce major effects on chloride uptake and TBPS binding were approximately 5–15 times higher than their clinical 1 MAC potency. In mice selectively bred for sensitivity to diazepam (DS), halothane enhanced GABA-gated chloride flux to a much greater extent than in the diazepam resistant (DR) strain.[135] Detailed studies of this nature will be required to sort out the extent to which volatile agents (and other anesthetics) act at GABA/benzodiazepine/barbiturate receptors. Taken together, these studies indicate that the volatile anesthetics interact with the $GABA_A$ receptor, but the interaction may be more complex than for other $GABA_A$ modulators. For example, Longoni and Olsen have demonstrated that enhancement by halothane of muscimol-stimulated $^{36}Cl^-$ efflux in rat cortical slices requires extracellular calcium.[136] A similar calcium dependency for volatile anesthetics has been noted in electrophysiologic studies (see below).

Studies on GABA Metabolism Another method of increasing GABAergic neurotransmission is to increase the amount of GABA functionally available in the synaptic cleft. Halothane at 3 vol% increases the GABA content in the transmitter pool of rat cerebral cortical slices by more than 100%.[137] Using radioactive GABA, it was shown that 3% halothane did not affect the high-affinity uptake or the release of GABA but did inhibit the catabolism of GABA.[138] Further work by Cheng and Brunner studied GABA disposal in synaptosomes by measuring the conversion of [1-^{14}C]GABA to $^{14}CO_2$ and concluded that chloroform, halothane, enflurane, and ether inhibit this process.[139] Although high concentrations were studied, volatile agents appear to increase GABA levels in the brain by blocking break down, and this could lead to enhanced inhibition by elevating GABA concentrations at the inhibitory synapse.

Electrophysiologic Studies Halothane has been shown to potentiate GABA-mediated inhibition in the CNS.[140–142] In hippocampal slices, maintained at room temperature, halothane (1–5 vol%; 0.2–1.0 mM) was shown to prolong the decay time constant of spontaneous inhibitory postsynaptic currents in a reversible fashion.[143] At 3% halothane, inhibitory postsynaptic current decay time constants were prolonged twofold, whereas rise time and amplitude were not affected.[143]

Using whole-cell patch-clamp recording in hippocampal slices, halothane (1.2 vol%) prolonged the decay time constant of $GABA_A$-mediated spontaneous inhibitory postsynaptic currents by 275%.[144,145] These results have been confirmed by independent laboratories.[146,147] Intracellular administration of the calcium chelator BAPTA or the calcium-release inhibitor dantrolene to the hippocampal neurons significantly reduced halothane's effect.[144,145] In extracellular recordings, the halothane-induced depression of hippocampal population spike amplitude was blocked by the $GABA_A$ antagonist bicuculline. Taken together, these findings suggest that a major depressant effect of halothane involves the enhancement of $GABA_A$-mediated inhibition through release of intracellular calcium. Similar prolongation of the $GABA_A$-mediated inhibitory postsynaptic current decay time constant has been shown for isoflurane and enflurane.[144,148,149] However, the requirement of intracellular calcium for the volatile anesthetic potentiation of GABA-activated chloride current may not be applicable to all forms of $GABA_A$ receptors. The action of halothane on $GABA_A$ receptors expressed in Xenopus oocytes does not appear to require an elevation of intracellular calcium.#

In cultured or acutely dissociated rat dorsal root gan-

Lin H, Harris RA: General anesthetics potentiate GABA actions on $GABA_A$ receptors expressed by xenopus oocytes: Lack of involvement of intracellular calcium. Soc Neurosci Abstr 17:798, 1991.

glion cells, the volatile anesthetics halothane, enflurane, and isoflurane, at 2 MAC concentrations, enhanced the $GABA_A$-activated current.[150,151] Single channel analysis attributed this effect to a 30-pS GABA-activated channel whose open probability and mean open time were increased by volatile anesthetics.[150]

Propofol (2,6-Diisopropylphenol)

Binding and Chloride Flux Studies. Propofol was found to inhibit [^{35}S]TBPS binding to membrane preparations from rat cerebral cortex in a concentration-dependent fashion.[152,153] Propofol, in the presence of either alphaxalone or pentobarbital, produced an additive inhibition of [^{35}S]TBPS binding that was greater than that produced by either agent alone, suggesting separate sites of action for these drugs. Propofol also enhanced [^{3}H]GABA binding in the rat cerebral cortex and in cortical membrane preparations.[152] In addition, propofol potentiates muscimol-induced stimulation of Cl uptake in membrane vesicle preparations.[152] These findings suggest that propofol may exert its effects by enhancing the function of the $GABA_A$ receptor-activated chloride channel.

Electrophysiologic Studies. Recent electrophysiologic studies have provided the strongest evidence that clinically relevant concentrations of propofol (10–50 μM) can increase GABAergic inhibition. In rat olfactory cortex, propofol (50 μM) potentiates GABA-mediated pre- and postsynaptic inhibition with no effect on monosynaptically evoked excitatory transmission.[154] In bovine chromaffin cells, known to have neuronal-like $GABA_A$ receptors, propofol (1.7 μM) increased the size of GABA-activated currents by 500%.[155,156] In the same studies, propofol-activated single Cl^- channels recorded in cell-attached configuration, increased the amplitude of whole-cell GABA-mediated chloride currents, and these effects were blocked by bicuculline (1 μM). Recordings from the CA1 region of the hippocampus and isolated rat spinal cord have shown propofol to depress the hippocampal population spike amplitude and spinal cord monosynaptic reflex response in a dose-dependent fashion.[157] When GABA-mediated inhibition was blocked by picrotoxin, the propofol-induced depression of these responses was abolished, suggesting that propofol attenuates synaptic transmission by enhancing GABA-mediated inhibition.

Etomidate

Binding and Chloride Flux Studies. In rat brain synaptosomal membranes, etomidate enhanced [^{3}H]GABA binding by increasing the number of high-affinity binding sites rather than by altering receptor affinity.[158,159] Etomidate also has been shown to enhance [^{3}H]-diazepam binding to rat cerebral cortical membranes.[159,160] This effect on [^{3}H]diazepam binding was shown to be brain region-specific, occurring in the rat forebrain but not in the cerebellum.[160] There are specific populations of $GABA_A$ receptor subunit complexes, demonstrating regional brain distributions selective for forebrain *versus* cerebellum. Etomidate may act selectively on such a subpopulation of $GABA_A$ receptors.

Electrophysiologic Studies. Several studies have shown that etomidate can produce depression of neuronal excitability, consistent with an enhancing action on GABAergic inhibition. In the *in vivo* rat preparation (+)etomidate depressed the firing rate of caudal medulla neurons, an effect antagonized by bicuculline, suggesting a GABAmimetic action for etomidate.[161] In the guinea pig hippocampus, (+)etomidate produced a dose-related, stereospecific, reversible increase in paired-pulse inhibition by increasing the effectiveness of GABA in a chloride-dependent fashion.[162] Studies on the recurrent GABAergic inhibitory pathway in the CA1 region of rat hippocampal slices have found that (+)etomidate (10 μM) markedly increased the duration of the bicuculline-sensitive IPSP and frequently increased its amplitude as well.[163] The rat brain hypnotic dose of etomidate and the clinical serum concentration of etomidate in humans (8.2 μM)¶ is in the same range as used in these studies. In a recent patch clamp study of primary cultures of dissociated rat hippocampal neurons, etomidate (8.2 μM) potentiated $GABA_A$-gated currents by 185%.[164] The use of clinically defined concentrations lends greater support to these studies, and the almost fourfold increase in GABAergic inhibition produced by etomidate suggests that this may be one of the most important actions of this anesthetic.

α-Adrenergic Agents

Behavioral Studies. Since 1914, adrenergic agents consistently have been shown to produce analgesia and anesthesia in animals. The first demonstration of this phenomenon was that sleep could be produced in dogs by injection of adrenalin into the brain.[165] In the 1940s, adrenergically induced anesthesia and analgesia were demonstrated in several species, including humans.[166–168] Subsequently, it has been shown that α_2-

¶ Heykants J: The distribution, metabolism and excretion of etomidate in the rat, Biological Research Reports. Janssen Pharmaceutica, 1974.

adrenergic receptors mediate the sedative/anesthetic effects of these drugs.[169–171]

GABA Release Studies. Noradrenaline and the α_2-adrenoreceptor agonist clonidine enhance, in a concentration-dependent manner, the release of endogenous GABA from rat hippocampal synaptosomes.[172,173] This effect is blocked by the α_2-adrenoreceptor antagonist yohimbine but not by the α_1-antagonist prazosin. From these studies, it has been concluded that GABAergic nerve terminals in rat hippocampus possess α_2-adrenoreceptors whose activation causes enhancement of GABA release.

Electrophysiologic Studies. In the rat hippocampal slice preparation, exogenously applied norepinephrine and α-adrenergic agonists slowed or stopped spontaneous interictal discharges, suggesting potential enhancement of inhibition.[174] Other electrophysiologic studies in rat hippocampus have demonstrated that norepinephrine can inhibit complex spike (pyramidal) neurons through an α_1 receptor while exciting interneurons by activation of an α_2 receptor.[175,176] Recently, it has been shown that adrenergic agonists excite interneurons and thereby increase the frequency of spontaneous $GABA_A$ receptor-mediated inhibitory postsynaptic potentials (IPSPs),[177] suggesting that α-adrenergic agents increase GABAergic inhibition by presynaptically increasing endogenous GABA release.

Discussion

$GABA_A$ Receptor Involvement in Anesthesia

There is now compelling evidence implicating anesthetic actions at the $GABA_A$ receptor/chloride channel complex in the neuronal mechanism of anesthesia. Anesthetics, at clinically relevant concentrations, enhance GABAergic inhibition by at least two primary actions: 1) an increase in agonist affinity for the $GABA_A$ receptor and 2) a prolongation or augmentation of the Cl^- conductance that is gated by this receptor. Either of these actions result in CNS depression, because excitatory synaptic potentials would be shunted and neuronal excitability would decrease, as the membrane potential of neurons would not reach the threshold for action potential discharge. It is noteworthy that anesthetics comprising several chemically distinct classes of agents can enhance GABAergic inhibition without an apparent structural requirement for the molecule involved. A lack of structural requirement has long been recognized among anesthetics and has been attributed to a lack of a specific anesthetic receptor. This view can be modified now in light of anesthetic interaction with the $GABA_A$ receptor/chloride channel complex, where the fact that multiple receptor subtypes exist may explain the apparent lack of structural requirement for anesthetic molecules.

The importance of anesthetic actions at the $GABA_A$ complex is evident from several interdependent findings. First, $GABA_A$ antagonists have been shown to reverse some of the depressant actions of anesthetics on synaptic and neuronal responses and also can reverse anesthesia, as measured by behavioral observations in animals and humans. Second, anesthesia can be enhanced by $GABA_A$ receptor agonists that act selectively on $GABA_A$ receptors. Third, and most compelling, evidence from molecular, cellular, and intact animal levels all support an important action for anesthetics on the $GABA_A$ receptor complex. The extent to which actions on the $GABA_A$ complex contribute to the overall phenomenon of clinical anesthesia remains to be determined.

This problem has several compounding components. Increased $GABA_A$ receptor activity necessarily will produce an apparent depression of excitatory synaptic activity, but some anesthetics also are known to produce direct depressant effects on excitatory synaptic responses[178,179] and on postsynaptic ionic conductances contributing to the resting membrane potential.[180,181] In other words, anesthetic effects on excitatory synaptic currents can add to direct effects on the $GABA_A$ receptor complex. For example, the volatile anesthetic enflurane has been shown to depress excitatory synaptic inputs to cortical pyramidal neurons and to increase $GABA_A$ postsynaptic inhibitory synaptic potentials in these same neurons.[182] Yet, the inhibitory postsynaptic potentials also depend on excitatory synaptic drive onto inhibitory interneurons. Thus, the increased inhibitory potentials recorded in the presence of enflurane must occur despite a reduced excitatory drive to inhibitory interneurons, and therefore, underestimate the increased $GABA_A$-mediated inhibition. Similarly, the enflurane-induced depression of excitatory synaptic responses can be attributed to shunting of dendritic excitation by an increased GABA-gated chloride conductance (but see below).

This problem of interdependent actions produced by anesthetics is even more pronounced in behavioral and whole animal studies, where simultaneous effects on many synaptic pathways and transmitter systems can produce additive, synergistic, or antagonistic actions

on the relatively few parameters that can be measured in any given study. For this reason, the use of *in vitro* preparations will be required to establish the relative importance of specific actions on $GABA_A$ inhibition *versus* actions on excitatory synaptic responses. Several studies have addressed this by using simple invertebrate neuromuscular preparations[183-185] or by selectively eliminating the $GABA_A$ system using specific antagonists in the hippocampal slice preparation.[144,145] The latter approach indicated that effects on the $GABA_A$ receptor complex can account for a major component (approximately 70%) of the overall depressant effects of halothane on neuronal excitability, since only 25–30% of the depressant effect of halothane remained when $GABA_A$ inhibition was blocked totally by bicuculline. Similar studies will provide a basis for determining the fractional contribution of enhanced $GABA_A$ inhibition to the depressant effects for each class of agents. At present, it is likely that actions at the $GABA_A$ complex are the dominant effect for barbiturates, benzodiazepines, steroids, and some other agents (*e.g.*, halothane, propofol), but also may be important for opiates and dissociative anesthetics (*e.g.*, ketamine). Both opiates[186] and ketamine[143] have been shown to block GABAergic inhibition; however, GABAergic effects of these agents have not been researched well to date.

Sensitivity of the $GABA_A$ Receptor Complex to Anesthetics

As pointed out above, the $GABA_A$ receptor complex is not the only target for general anesthetics but appears to be an important site of action for several classes of agents that enhance its activity *via* agent-specific mechanisms (fig. 3). This reflects the paramount physiologic role that GABAergic inhibition plays in controlling CNS excitability; the $GABA_A$ receptor is an important target for anesthetics because it is subject to a number of convergent modulatory influences that can be altered by anesthetics. GABAergic inhibition is critical to the normal function of all brain regions studied to date. Localized disruption of GABAergic inhibition results in a loss of center-surround organization, pattern formation, and rhythmicity that underlie many aspects of neuronal information processing. A more global loss of GABAergic tone results in a complete disruption of normal CNS function, hyperexcitability, and seizure activity.

The $GABA_A$ receptor complex is modulated by several second messenger systems that converge at this site to enhance inhibition. The complex is subject to phosphorylation *via* several protein kinase systems and also appears to be regulated by intracellular Ca^{++} levels. GABAergic inhibition also is modulated by several neurotransmitter systems, including: acetylcholine, catecholamines, indolamines, excitatory amino acids, steroids, opiates, and other peptides. Receptors for each of these transmitter systems have been localized on inhibitory GABAergic interneurons in various brain regions and have clearly been shown to modulate GABAergic inhibition.[187-189] Anesthetic actions on any of these other transmitter systems will be reflected in changes in GABAergic tone. In addition, the complex structure of the $GABA_A$ receptor/chloride channel, with its numerous membrane-spanning regions (figs. 2 and 3), provides optimal lipid soluble sites for perturbation by anesthetics. This structural complexity may impart a higher degree of lability, relative to other membrane-associated proteins that do not appear to be sensitive to low, clinically effective concentrations of anesthetics

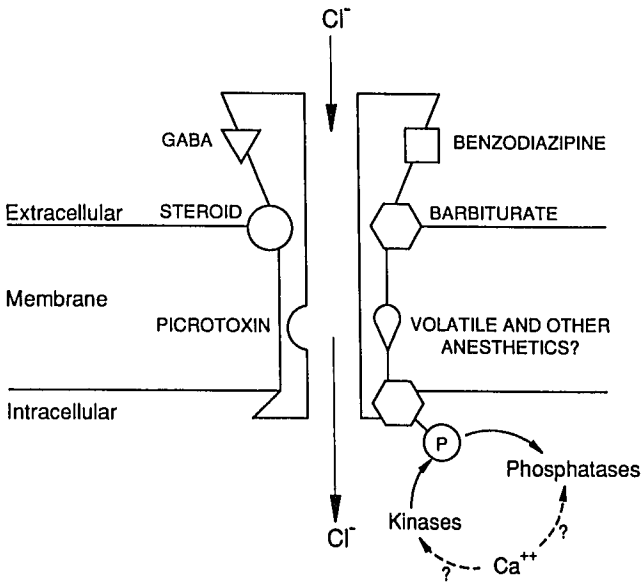

Fig. 3. Diagram of $GABA_A$ receptor/chloride channel complex, showing proposed sites of action for anesthetics and other agents (see table 1). GABA acting on an extracellular receptor site opens the chloride selective channel. Agents acting on several distinct sites (benzodiazepine, steroid, and barbiturate) can enhance GABA binding and/or chloride conductance. Agents acting at the picrotoxin site block channel conductance directly. It is unclear at this time whether there are specific binding sites for the volatile anesthetics, propofol, ETOH, and etomidate. GABA channel kinetics also can be modulated by phosphorylation/dephosphorylation of intracellular regulatory sites, some of which may depend on intracellular calcium levels. Volatile anesthetics may increase intracellular calcium to enhance activity at the GABA channel.

(*e.g.*, Na^+ channels, A-type potassium channels, most enzymes studied to date). As further research unlocks the remaining mysteries of anesthetic actions at the $GABA_A$ receptor complex, an explanation of why this particular protein is so sensitive to anesthetics will emerge.

Anesthetic Agents Enhance GABAergic Inhibition by Different Mechanisms

GABAergic inhibition can be increased by several different mechanisms in the CNS. Potential mechanisms include: enhanced GABA release presynaptically, decreased GABA reuptake, decreased GABA metabolism, enhanced GABA receptor binding, and modification of the $GABA_A$ chloride channel. It appears that the different anesthetic agents enhance GABAergic inhibition by using several of these mechanisms. The benzodiazepines enhance GABAergic inhibition by increasing binding of endogenously released GABA; barbiturates, etomidate, propofol, and volatile agents modify the $GABA_A$ receptor-chloride channel such that it remains open longer after binding of GABA; and the α-adrenergic agents enhance presynaptic GABA release. In addition, volatile agents have been shown to inhibit GABA disposal. Prolonged opening of the $GABA_A$ receptor-chloride channel can be achieved by different mechanisms as well. For example, this process appears to require an elevation of intracellular calcium for volatile anesthetics but not for the barbiturates. All of the possible GABAergic enhancing mechanisms have not been investigated for each anesthetic agent, and future research is needed to determine the predominant mechanism for each class of anesthetic.

Can Different Anesthetic Behavioral Effects Be Explained by a Common Neurotransmitter Mechanism?

How can different behavioral effects of different anesthetic agents be explained by a common GABAergic mechanism? One could postulate that the different behavioral effects seen at low anesthetic concentrations of different agents are due to multiple selective effects upon different neurotransmitter or neuromodulatory systems. However, these differences also could arise from heterogeneity of GABA receptors and their differential expression in the brain.[14-20,190] It is likely that anesthetic agents have different affinities for the different $GABA_A$ receptor subtypes, and at low concentrations, these different affinities could result in agent-specific behavioral effects. When the concentrations of anesthetic agents are sufficient, receptor specificity is functionally lost, and all of the agents appear to produce a similar behavioral state—anesthesia.

$GABA_A$ Inhibition and Theories of Anesthesia

It has been recognized for a number of years that "unitary" theories of anesthetic action cannot account for the diverse, agent-specific effects observed at a molecular, cellular, and whole animal level.[179,191,192] Typically, unitary theories propose a common molecular mechanism of action for all anesthetics[193-195] and most often emphasize the common physiochemical property of lipid solubility as a primary determinant for anesthetic activity.[196,197] While lipid solubility is correlated with anesthetic potency, it rarely is noted that this correlation is highly exponential. Small changes in lipid solubility are associated with large differences in potency for agents that are not very lipid soluble (*e.g.*, ethanol, ether), but large differences in solubility have little effect on potency for agents that are most lipid soluble (*e.g.*, alphaxalone, decanol, chlorpromazine). A much better, and linear, correlation ($r = 0.89$) exists for anesthetic depression of neuronal and synaptic responses *versus* clinical potency.[179,180,192] It is not yet known whether anesthetic potencies for effects on the $GABA_A$ receptor complex obey the lipid solubility rule; however, observations of anesthetic effects on the $GABA_A$ receptor complex have clearly demonstrated that different classes of anesthetics act *via* distinct molecular mechanisms.

The diverse anesthetic actions seen at the $GABA_A$ receptor complex are consistent with a multisite agent-specific theory of anesthesia that predicts that chemically different classes of anesthetics will act at distinct sites and *via* unique mechanisms to produce CNS depression. Support for a multisite agent-specific theory comes from the observation that stereoisomers of pentobarbital and isoflurane differ markedly in potency,[198-200] indicating that the site of action for these anesthetics is structurally selective to a degree that cannot be accounted for by interactions with membrane lipids.[200]

The multisite agent-specific theory of anesthesia also predicts that a common action (*e.g.*, enhanced GABAergic inhibition) can be produced *via* completely different mechanisms. Such is the case for the presynaptic actions of α-adrenergic anesthetics (increased GABA release) compared with the postsynaptic actions of barbiturates and benzodiazepines. Both actions result in an increased GABAergic tone and, hence, CNS

depression, but involve completely different sites and mechanisms of action. Even agents that act postsynaptically do so *via* different mechanisms that come about through drug-receptor interactions at distinct sites on the same protein complex. The major advantage provided by a multisite agent-specific theory is the prediction that new anesthetics can be developed that exhibit structure-activity relationships based on selective interactions with discrete receptor-effector systems. This holds promise for the development of safer, more specific therapeutic agents.

We conclude that actions on the $GABA_A$ receptor complex can account for the dominant CNS depressant effects of several chemically distinct classes of anesthetics. Future research will elucidate fundamental GABAergic mechanisms of action for anesthetics, which will, in turn, lead to the rational design of better agents. Recent studies already have provided a more detailed analysis of anesthetic mechanisms of action and promoted a more enlightened view for a theory of anesthesia.

References

1. Florey E: Nervous Inhibition. New York, Pergamon, 1960

2. Roberts E, Chase TN, Tower DB: GABA in Nervous System Function. New York, Raven, 1976

3. Roberts E, Baxter CF, Van Harrefeld A, Wiersma CAG, Adey WR: Inhibition in the Nervous System and Gamma-aminobutyric Acid. New York, Plenum, 1960

4. Bowery NG: Actions and Interactions of GABA and benzodiazepines. New York, Raven, 1983

5. Bormann J: Electrophysiology of $GABA_A$ and $GABA_B$ receptor subtypes. Trends Neurosci 11:112–116, 1988

6. Levitan ES, Schofield PR, Burt DR, Rhee LM, Wisden W, Kohler M, Fujita N, Rodriguez HF, Stephenson A, Darlinson MG, Barnard EA, Seeburg PH: Structural and functional basis for $GABA_A$ receptor heterogeneity. Nature 335:76–79, 1988

7. McCormick DA: GABA as an inhibitory neurotransmitter in human cerebral cortex. J Neurophysiol 62:1018–1027, 1989

8. Bloom FE, Iversen LL: Localizing [^{3}H]GABA in nerve terminals of cerebral cortex by electron microscopic autoradiography. Nature 229:628–630, 1971

9. McCormick DA, Williamson A: Convergence and divergence of neurotransmitter action in human cerebral cortex. Proc Natl Acad Sci U S A 86:8098–8102, 1989

10. Nicoll RA: The coupling of neurotransmitter receptors to ion channels in the brain. Science 241:545–551, 1988

11. Bowery NG, Hill DR, Hudson AL, Doble A, Middlemiss DN, Shaw J, Turnbull M: (−)-Baclofen decreases neurotransmitter release in mammalian CNS by an action at a novel GABA receptor. Nature 283:92–94, 1980

12. Bowery N: $GABA_B$ receptors and their significance in mammalian pharmacology. Trends Pharmacol Sci 10:401–407, 1989

13. Roberts E: Failure of GABAergic inhibition: A key to local and global seizures, Basic Mechanisms of Epilepsies: Molecular and Cellular Approaches, Advances in Neurology. Edited by Delgado-Escueta AV, Ward AA, Woodbury DM, Porter RJ. New York, Raven, 1986, pp 319–341

14. Schofield PR, Darlison MG, Fujita N, Rodriguez H, Burt DR, Stephenson FA, Rhee IM, Ramachandran J, Glencorse TA, Reale V, Seeburg PH, Barnard EA: Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family. Nature 328:221–227, 1987

15. Olsen RW, Tobin AJ: Molecular biology of $GABA_A$ receptors. FASEB J 4:1469–1480, 1990

16. Seeburg PH, Wisden W, Verdoorn TA, Pritchett DB, Werner P, Herb A, Luddens H, Sprengel R, Sakmann B: The $GABA_A$ receptor family: Molecular and functional diversity. Cold Spring Harb Symp Quant Biol 55:29–40, 1991

17. Stephenson FA: Understanding the $GABA_A$ receptor: A chemically gated ion channel. Biochem J 249:21–32, 1988

18. Gambrana C, Beattie CE, Rodriguez ZR, Siegel RE: Region-specific expression of messenger RNAs encoding $GABA_A$ receptor subunits in the rat brain. Neuroscience 45:423–432, 1991

19. MacLennan AJ, Brecha N, Khrestchatisky M, Sternini C, Tillakaratne NJK, Chiang M-Y, Anderson K, Lai M, Tobin AJ: Independent cellular and oncogenic expression of mRNAs encoding three α polypeptides of the rat $GABA_A$ receptor. Neuroscience 43:369–380, 1991

20. Shivers BD, Killisch I, Sprengel R, Sontheimer H, Kohler M, Schofield PR, Seeburg PH: Two novel $GABA_A$ receptor subunits exist in distinct neuronal subpopulations. Neuron 3:327–337, 1989

21. Cherubini E, Gaiarsa JL, Ben-Ari Y: GABA: An excitatory transmitter in early postnatal life. Trends Neurosci 14:515–519, 1991

22. Chen QX, Stelzer A, Kay AR, Wong RKS: $GABA_A$ receptor function is regulated by phosphorylation in acutely dissociated guinea-pig hippocampal neurones. J Physiol (Lond) 420:207–221, 1990

23. Gyenes M, Farrant M, Farb DH: "Run-down" of $GABA_A$ receptor function during whole-cell recording: A possible role for phosphorylation. Mol Pharmacol 34:719–723, 1988

24. Inoue M, Oomura Y, Yakushiji T, Akaike N: Intracellular calcium ions decrease the affinity of the GABA receptor. Nature 324: 156–158, 1986

25. Sigel E, Baur R: Activation of protein kinase C differentially modulates neuronal Na^+, Ca^{2+} and γ-aminobutyrate type A channels. Proc Natl Acad Sci U S A 85:6192–6196, 1988

26. Sigel E, Baur R: Allosteric modulation by benzodiazepine receptor ligands of the $GABA_A$ receptor channel expressed in *Xenopus* oocytes. J Neurosci 8:289–295, 1988

27. Stelzer A, Kay AR, Wong RKS: $GABA_A$ receptor function in hippocampal cells is maintained by phosphorylation factors. Science 241:339–341, 1988

28. Freund TF, Antal M: GABA-containing neurons in the septum control of the hippocampus via local inhibitory interneurons. Nature 336:170–173, 1988

29. Freund TF, Gulyas AI, Acsady L, Gorcs T, Toth K: Serotonergic control of the hippocampus via local inhibitory interneurons. Proc Natl Acad Sci U S A 87:8501–8505, 1989

30. Mondrup K, Pedersen E: The acute effect of the GABA-agonist, THIP, on proprioceptive and flexor reflexes in spastic patients. Acta Neurol Scand 67:48–54, 1983

31. Petersen HR, Jensen I, Dam M: THIP: A single-blind controlled trial in patients with epilepsy. Acta Neurol Scand 67:114–117, 1983

32. Kjaer M, Nielsen H: The analgesic effects of the GABA-agonist

THIP in patients with chronic pain of malignant origin: A phase 1–2 study. Br J Clin Pharmacol 16:477–485, 1983

33. Hoehn-Saric R: Effects of THIP on chronic anxiety. Psychopharmacology (Berlin) 80:338–341, 1983

34. Cheng S-C, Brunner EA: Inducing anesthesia with a GABA analog, THIP. ANESTHESIOLOGY 63:147–151, 1985

35. Juhasz G, Emri Z, Kekesi K, Pungor K: Local perfusion of the thalamus with GABA increases sleep and induces long-lasting inhibition of somatosensory event-related potentials in cats. Neurosci Lett 103:229–233, 1989

36. De Boer T, Stoof JC, van Duijn H: The effects of convulsant and anticonvulsant drugs on the release of radiolabeled GABA, glutamate, noradrenaline, serotonin and acetylcholine from rat cortical slices. Brain Res 253:153–160, 1982

37. Macdonald RL, Barker JL: Enhancement of GABA mediated postsynaptic inhibition in cultured mammalian spinal cord neurons: A common mode of anticonvulsant action. Brain Res 167:323–336, 1979

38. Saunders PA, Ho IK: Barbiturates and the $GABA_A$ receptor complex. Prog Drug Res 34:261–286, 1990

39. Sivam SP, Nabeshima T, Ho IK: Acute and chronic effects of pentobarbital in relation to postsynaptic GABA receptors: A study with muscimol. J Neurosci Res 7:37–47, 1982

40. Allan AM, Gallaher EJ, Gionet SE, Harris RA: Genetic selection for benzodiazepine ataxia produces functional changes in the γ-aminobutyric acid receptor chloride channel complex. Brain Res 452: 118–128, 1988

41. Ashton D: Diazepam, pentobarbital and d-etomidate produced increases in bicuculline seizure threshold: Selective antagonism by Ro 15-1788, picrotoxin and (±)-DMBB. Eur J Pharmacol 94:319–325, 1983

42. Nutt DJ, Lister RG: The effect of the imidazepine Ro 15-4513 on the anticonvulsant effects of diazepam, sodium pentobarbital and ethanol. Brain Res 413:193–196, 1987

43. Mendelson WB, Martin JV, Wagner R, Rosebarry C, Skolnick P, Weissman BA, Squires R: Are the toxicities of pentobarbital and ethanol mediated by the GABA-benzodiazepine receptor-chloride ionophore complex? Eur J Pharmacol 108:63–70, 1985

44. Olsen RW, Sapp DM, Bureau MH, Turner DM, Kokka N: Allosteric actions of central nervous system depressants including anesthetics on subtypes of the inhibitory γ-aminobutyric $acid_A$ receptor-chloride channel complex, Molecular and Cellular Mechanisms of Alcohol and Anesthetics. Edited by Rubin E, Miller KW, Roth SH. New York, Annals of the New York Academy of Sciences, 1991, pp 145–154

45. Leeb-Lundberg F, Snowman A, Olsen RW: Barbiturate receptor sites are coupled to benzodiazepine receptors. Proc Natl Acad Sci U S A 77:7468–7472, 1980

46. Ticku MK, Olsen RW: Interaction of barbiturates with dihydropicrotoxin binding sites related to the GABA receptor-ionophore system. Life Sci 22:1643–1652, 1978

47. Johnston G, Willow M: Barbiturates and GABA receptors. Adv Biochem Psychopharmacol 26:191–198, 1981

48. Ticku M: Interaction of depressant, convulsant, and anticonvulsant barbiturates with the [^{3}H]diazepam binding site of the benzodiazepine-GABA-receptor-ionophore complex. Biochem Pharmacol 30:1573–1579, 1981

49. Asano T, Ogasawara N: Chloride-dependent stimulation of GABA and benzodiazepine receptor binding by pentobarbital. Brain Res 225:212–216, 1981

50. Willow M, Johnston GAR: Pentobarbitone slows the dissociation of GABA from rat brain synaptosomal binding sites. Neurosci Lett 23:71–74, 1981

51. Olsen RW, Snowman AM: Chloride-dependent enhancement by barbiturates of γ-aminobutyric acid receptor binding. J Neurosci 2:1812–1823, 1982

52. Olsen R, Snowman AM: [^{3}H]Bicuculline methochloride binding to low affinity γ-aminobutyric acid receptor sites. J Neurochem 41: 1653–1663, 1983

53. Wong EHF, Snowman AM, Leeb-Lundberg LMF, Olsen RW: Barbiturates allosterically inhibit GABA antagonist and benzodiazepine inverse agonist binding. Eur J Pharmacol 102:205–212, 1984

54. Maksay G, Ticku MK: Dissociation of [^{35}S]*t*-butylbicyclophosphorothionate binding differentiates convulsants and depressant drugs that modulate GABAergic transmission. J Neurochem 44:480–486, 1985

55. Trifiletti RR, Snowman AM, Snyder SH: Barbiturate recognition site on the GABA/benzodiazepine receptor complex is distinct from the picrotoxin/TBPS recognition site. Eur J Pharmacol 106:441–447, 1984

56. Asano T, Ogasawara N: Stimulation of GABA receptor binding by barbiturates. Eur J Pharmacol 77:355–357, 1982

57. Leeb-Lundberg F, Olsen RW: Interactions of barbiturates of various pharmacological categories with benzodiazepine receptors. Mol Pharmacol 21:320–328, 1982

58. Harris RA, Allan A: Functional coupling of the γ-aminobutyric acid receptors to chloride channels in rat membranes. Science 228: 1108–1110, 1985

59. Thampy KG, Barnes EMJ: γ-Aminobutyric acid-gated chloride channels in cultured cerebral neurons. J Biol Chem 259:1753–1757, 1984

60. Ticku MK, Lowrimore P, Lehoullier P: Ethanol enhances GABA-induced ^{36}Cl-influx in primary spinal cord cultured neurons. Brain Res Bull 17:123–126, 1986

61. Allan AM, Harris RA: Anesthetic and convulsant barbiturates alter γ-aminobutyric acid-stimulated chloride flux across brain membranes. J Pharmacol Exp Ther 238:763–768, 1986

62. Akaike N, Hattori K, Inomata N, Oomura Y: γ-Aminobutyric-acid and pentobarbitone-gated chloride currents in internally perfused frog sensory neurons. J Physiol (Lond) 360:367–386, 1985

63. Morrow LA, Suzdak PD, Paul SM: Benzodiazepine, barbiturate, ethanol and hypnotic steroid hormone modulation of GABA-mediated chloride ion transport in rat brain synaptoneurosomes. Adv Biochem Psychopharmacol 45:247–261, 1988

64. Macdonald RL, Weddle MG, Gross RA: Benzodiazepine, β-carboline, and barbiturate actions on GABA responses. Adv Biochem Psychopharmacol 41:33–49, 1986

65. Mathers DA: Pentobarbital promotes bursts of γ-aminobuturic acid-activated single channel currents in cultured mouse central neurons. Neurosci Lett 60:121–126, 1985

66. Mathers DA: The $GABA_A$ receptor: New insights from single channel recording. Synapse 1:96–101, 1987

67. Holland KD, Canney DJ, Rothman SM, Ferrendelli JA, Covey DF: Physiological modulation of the GABA receptor by convulsant and anticonvulsant barbiturates in cultured rat hippocampal neurons. Brain Res 516:147–150, 1990

68. Braestrup C, Nielsen M, Olsen C: Urinary and brain β-carboline-3-carboxylates as potent inhibitors of brain benzodiazepine receptors. Proc Natl Acad Sci U S A 77:2288–2292, 1980

69. Pena C, Medina J, Noval M, Palandini A, De Roberts E: Isolation

and identification in bovine cerebral cortex of *n*-butyl-β-carboline-3-carboxylate, a potent endogenous benzodiazepine binding inhibitor. Proc Natl Acad Sci U S A 83:4952–4956, 1986

70. Jensen LH, Peterson EN, Beastrup C: Audiogenic seizures in DBA/2 mice discriminate sensitivity between low efficacy benzodiazepine receptor agonists and inverse agonists. Life Sci 33:393–399, 1983

71. Braestrup C, Schmiechen R, Neef G, Nielsen M, Petersen EN: Interaction of convulsant ligands with the benzodiazepine receptor. Science 216:1241–1243, 1982

72. Braestrup C, Nielsen M, Honore T, Jensen LH, Petersen EN: Benzodiazepine receptor ligands with positive and negative efficacy. Neuropharmacology 22:1451–1457, 1983

73. Cepeda C, Tnaaka T, Besselievere R, Potier P, Naquet R, Rossier J: Proconvulsant effects in baboons of β-carboline, a putative endogenous ligand for benzodiazepine receptors. Neurosci Lett 24:53–57, 1981

74. Jensen MS, Lambert JDC: The interaction of the β-carboline derivative DMCM with inhibitory amino acid responses on cultured mouse neurons. Neurosci Lett 40:175–179, 1983

75. Polc P, Ropert N, Wright DM: Ethyl β-carboline-3-carboxylate antagonizes the action of GABA and benzodiazepines in the hippocampus. Brain Res 217:216–220, 1981

76. Skerritt JH, Macdonald RL: Benzodiazepine receptor ligand actions on GABA responses, β-carbolines, purines. Eur J Pharmacol 101:135–141, 1984

77. Olsen R, Wong E, Stauber G, Murakami D, King R, Fischer J: Neurotransmitter Receptors: Mechanisms of Action and Regulation. Edited by Kito S, Segawa T, Kuriyama K, Yamamura H, Olsen R. New York, Plenum, 1984, pp 205–219

78. Study RE, Barker JL: Diazepam and (−)-pentobarbital: Fluctuation analysis reveals different mechanisms for potentiation of γ-aminobutyric acid responses in cultured central neurons. Proc Natl Acad Sci U S A 78:7180–7184, 1981

79. Olsen R, Fischer J, Dunwiddie T: Barbiturate enhancement of γ-aminobutyric acid receptor binding and function as a mechanism of anesthesia, Molecular and Cellular Mechanisms of Anaesthetics. Edited by Roth S, Miller K. New York, Plenum, 1985, pp 165–178

80. Olsen R, Stauber G, King R: Structure and function of the barbiturate modulated benzodiazepine/GABA receptor protein complex, GABAergic Transmission and Anxiety. Edited by Biggio G, Costa E. New York, Raven, 1986, pp 21–32

81. Skerritt JH, Willow M, Johnston GAR: Diazepam enhancement of low affinity GABA binding to rat brain membranes. Neurosci Lett 29:63–66, 1982

82. Bowery N, Price G, Hudson A, Hill D, Wilkin G, Turnbull M: GABA receptor multiplicity. Neuropharmacology 23:219–231, 1984

83. Richards J, Mohler H: Benzodiazepine receptors. Neuropharmacology 23:661–672, 1984

84. Guidotti A, Toffano G, Costa E: An endogenous protein modulates the affinity of the GABA and benzodiazepine receptors in rat brain. Nature 275:553–555, 1978

85. Martin I, Candy J: Facilitation of benzodiazepine binding by sodium chloride and GABA. Neuropharmacology 17:993–998, 1978

86. Tallman J, Thamas J, Gallager D: GABAergic modulation of benzodiazepine binding site sensitivity. Nature 274:383–385, 1978

87. Chweh AY, Ulloque RA, Swinyard EA, Wolf HH: Effect of picrotoxin on benzodiazepine receptor binding. Neurochem Res 10: 871–877, 1985

88. Mohler H, Richards J: Agonist and antagonist benzodiazepine receptor interaction *in vitro*. Nature 294:763–765, 1981

89. Skerritt JH, Johnston GAR: Interactions of some anesthetic, convulsant, and anticonvulsant drugs at GABA-benzodiazepine receptor-ionophore complexes, rat brain synaptosomal membranes. Neurochem Res 8:1351–1362, 1983

90. Braestrup C, Nielsen M: GABA reduces binding of [^{3}Hmethyl β-carboline-3-carboxylate to brain benzodiazepine receptors. Nature 292:472–480, 1981

91. Iadarola MJ, Fanelli RJ, Mcmamara JO, Wilson WA: Comparison of the effects of diphenylbarbituric acid, phenobarbital and secobarbital on GABA-mediated inhibition and benzodiazepine binding. J Pharmacol Exp Ther 232:127–133, 1985

92. Supavilai P, Karobath M: Differential modulation of [^{35}S]TBPS binding by the occupancy of benzodiazepine receptors with its ligands. Eur J Pharmacol 91:145–146, 1983

93. Mehta A, Maharaj K: Characteristics of flunitrazepam binding to primary cultured spinal cord neurones and its modulation by GABAergic drugs. J Neurochem 49:1491–1497, 1987

94. Schwartz R, Skolnick P, Seale T, Paul S: Demonstration of GABA/barbiturate-receptor mediated chloride transport in rat brain synaptoneurosomes: A functional assay of GABA receptor-effector coupling. Adv Biochem Psychopharmacol 41:33–49, 1986

95. Braestrup C, Honore T, Nielsen M, Petersen E, Jensen L: Interaction of convulsive ligands with benzodiazepine receptors. Science 216:1241–1243, 1982

96. Wong EHF, Leeb-Lundberg LMF, Teichberg VI, Olsen RW: γ-Aminobutyric acid activation of $^{36}Cl^-$ flux in rat hippocampal slices and its potentiation by barbiturates. Brain Res 303:267–275, 1984

97. Choi DW, Farb DH, Fischbach GD: Chlordiazepoxide selectively augments GABA action in spinal cord cell cultures. Nature 269:342–344, 1977

98. Costa A, Guiotti A, Mao CC, Suria A: New concepts on the mechanism of action of benzodiazepines. Life Sci 17:167–186, 1975

99. Macdonald R: Benzodiazepines specifically modulate GABA-mediated postsynaptic inhibition in cultured mammalian neurons. Nature 271:564–565, 1978

100. Harrison NL, Majewski MD, Harrington JW, Barker JL: Structure-activity relationships for steroid interaction with γ-aminobutyric $acid_A$ receptor complex. J Pharmacol Exp Ther 241:346–353, 1987

101. Harrison NL, Vicini S, Barker JL: A steroid anesthetic prolongs inhibitory postsynaptic currents in cultured rat hippocampal neurons. J Neurosci 7:604–609, 1987

102. Majewski MD, Harrison NL, Schwartz RD, Barker JL, Paul SM: Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232:1004–1007, 1986

103. Gee KW, Bolger MB, Brinton RE, Coirini H, McEwen BS: Steroid modulation of the chloride ionophore in rat brain: Structure-activity requirements, regional dependence and mechanism of action. J Pharmacol Exp Ther 246:803–812, 1988

104. Im WB, Blakeman DP, Davis JP, Ayer DE: Studies on the mechanism of interactions between anesthetic steroids and γ-aminobutyric $acid_A$ receptors. Mol Pharmacol 37:429–434, 1990

105. Turner DM, Ranson RW, Yang JS-J, Olsen RW: Steroid anesthetics and naturally occurring analogs modulate the γ-aminobutyric acid receptor complex at a site distinct from barbiturates. J Pharmacol Exp Ther 248:960–966, 1989

106. Yau JLW, Balfour DJK, Stevenson IH: Modulation of the $GABA_A$ receptor by barbiturates and pregnane steroids: Differential effects

of the influence of assay temperature. J Pharm Pharmacol 42:175–180, 1990

107. Harrison NL, Simmonds MA: Modulation of the GABA receptor complex by a steroid anaesthetic. Brain Res 323:287–292, 1984

108. Barker JL, Harrison NL, Lange GD, Owen DG: Potentiation of γ-aminobutyric-acid-activated chloride conductance by a steroid anaesthetic in cultured rat spinal neurones. J Physiol (Lond) 386: 485–501, 1987

109. Robertson B: Actions of anaesthetics and avermectin on $GABA_A$ chloride channels in mammalian dorsal root ganglion neurones. Br J Pharmacol 98:167–176, 1989

110. Lambert JJ, Peters JA, Sturgess NC, Hales TG: Steroid modulation of the $GABA_A$ receptor complex: Electrophysiological studies. Ciba Found Symp 153:56–82, 1990

111. Deitrich RA, Dunwiddie TV, Harris RA, Erwin VG: Mechanism of action of ethanol: Initial central nervous system actions. Physiol Rev 41:489–537, 1989

112. Allan AM, Harris RA: Involvement of neuronal chloride channels in ethanol intoxication, tolerance, and dependence, Recent Developments in Alcoholism. Edited by Galanter M. New York, Plenum, 1987, pp 313–325

113. Ticku MK, Kulkarni SK: Molecular interactions of ethanol with GABAergic system and potential of RO15-4513 as an ethanol antagonist. Pharmacol Biochem Behav 30:501–510, 1988

114. Suzdak PD, Glowa JR, Crawley JN, Schwartz RD, Skolnick P, Paul SM: A selective imidazobenzodiazepine antagonist of ethanol in the rat. Science 234:1243–1247, 1986

115. Lister RG, Nutt DJ: Is Ro 14-4513 a specific alcohol antagonist? Trends Neurosci 10:223–225, 1987

116. Hellevuo K, Kiianmaa K, Juhakoski A, Kim C: Intoxicating effects of lorazepam and barbital in rat lines selected for differential sensitivity to ethanol. Psychopharmacology 91:263–267, 1987

117. Marley RJ, Wehner JM: GABA enhancement of flunitrazepam binding in mice selectively bred for differential sensitivity to ethanol. Alcohol Clin Exp Res 7(1):25–32, 1987

118. Marley RJ, Freund RK, Wehner JM: Differential response to flurazepam in long-sleep and short-sleep mice. Pharmacol Biochem Behav 31:453–458, 1988

119. Gallaher EJ, Gionet SE: Initial sensitivity and tolerance to ethanol in mice genetically selected for diazepam sensitivity. Alcohol Clin Exp Res 12:77–80, 1988

120. Suzdak PD, Schwartz RD, Skolnick P, Paul SM: Ethanol stimulates γ-aminobutyric acid receptor-mediated chloride transport in rat brain synaptoneurosomes. Proc Natl Acad Sci U S A 83:4071–4075, 1986

121. Mehta AK, Ticku MK: Ethanol potentiation of GABAergic transmission cultured spinal cord neurons involves gamma-aminobutyric $acid_A$-gated chloride channels. J Pharmacol Exp Ther 246: 558–564, 1988

122. Allan AM, Harris RA: Gamma-aminobutyric acid and ethanol actions: Neurochemical studies of long sleep and short sleep mice. Life Sci 39:2005–2015, 1986

123. Allan AM, Spuhler KP, Harris RA: Gamma-aminobutyric acid-activated chloride channels: relationship to genetic differences in ethanol sensitivity. J Pharmacol Exp Ther 244:866–870, 1988

124. Harris RA, Allan AM: Alcohol intoxication: Ion channels and genetics. FASEB J 3:1689–1695, 1989

125. Harris RA, Allan AM, Daniell LC, Nixon C: Antagonism of ethanol and pentobarbital actions by benzodiazepine inverse agonists. J Pharmacol Exp Ther 247:1012–1017, 1988

126. Nishio M, Narahashi T: Ethanol enhancement of GABA-activated chloride current in rat dorsal root ganglion neurons. Brain Res 518:283–286, 1990

127. Lyon RC, McComb JA, Schreurs J, Goldstein DB: A relationship between alcohol intoxication and the disordering of brain membranes by a series of short-chain alcohols. J Pharmacol Exp Ther 218:669–675, 1981

128. Nakahiro M, Arakawa O, Narahashi T: Ethanol and longer chain alcohols enhance GABA-activated chloride current in rat dorsal root ganglion neurons. FASEB J 4:A1201, 1990

129. Aguayo LG: Ethanol potentiates the $GABA_A$-activated Cl^- current in mouse hippocampal and cortical neurons. Eur J Pharmacol 187:127–130, 1990

130. Moody EJ, Skolnick P: The imidazobenzodiazepine Ro 15-4513 antagonizes methoxyflurane anesthesia. Life Sci 43:1269–1276, 1988

131. Greiner AS, Larach DR: The effect of benzodiazepine receptor antagonism by flumazenil on the MAC of halothane in the rat. ANESTHESIOLOGY 70:644–648, 1989

132. Schwartz AE, Maneksha FR, Kanchuger MS, Sidhu US, Poppers PJ: Flumazenil decreases the minimum alveolar concentration of isoflurane in dogs. ANESTHESIOLOGY 70:764–766, 1989

133. Geller E, Schiff B, Halpern P, Speiser Z, Cohen S: A benzodiazepine receptor antagonist improves emergence of mice from halothane anaesthesia. Neuropharmacology 28:271–274, 1989

134. Moody EJ, Suzdak PD, Paul SM, Skolnick P: Modulation of the benzodiazepine/γ-aminobutyric acid receptor chloride channel complex by inhalation anesthetics. J Neurochem 51:1386–1393, 1988

135. Quinlan JJ, Winter PM, Gallaher EJ, Firestone LL: Halothane enhances GABA-gated chloride flux in mice selectively bred for sensitivity to diazepam (abstr). ANESTHESIOLOGY 75:A582, 1991

136. Longoni B, Olsen RW: Studies on the mechanism of interaction of anesthetics with GABA-A receptors, GABAergic Neurotransmission. Edited by Biggio G, Concas A, Costa E. New York, Raven, 1992, pp 365–378

137. Cheng S-C, Brunner EA: A neurochemical hypothesis for halothane anesthesia. Anesth Analg 54:242–246, 1975

138. Cheng SC, Brunner EA: Inhibition of GABA metabolism in rat brain slices by halothane. ANESTHESIOLOGY 55:26–33, 1981

139. Cheng SC, Brunner EA: Effects of anesthetic agents on synaptosomal GABA disposal. ANESTHESIOLOGY 55:34–40, 1981

140. Scholfield CN: Potentiation of inhibition by general anesthetics in neurones of the olfactory cortex in vitro. Pflugers Arch 383:249–255, 1980

141. Nicoll RA: The effects of anaesthetics on synaptic excitation and inhibition in the olfactory bulb. J Physiol (Lond) 223:803–814, 1972

142. Galindo A: Effects of procaine, pentobarbital, and halothane on synaptic transmission in the central nervous system. J Pharmacol Exp Ther 169:185–195, 1969

143. Gage PW, Robertson B: Prolongation of inhibitory postsynaptic currents by pentobarbitone, halothane and ketamine in CA1 pyramidal cells in rat hippocampus. Br J Pharmacol 85:675–681, 1985

144. MacIver MB, Tanelian DL, Mody I: Two mechanisms for anesthetic-induced enhancement of $GABA_A$-mediated neuronal inhibition, Molecular and Cellular Mechanisms of Alcohol and Anesthetics. Edited by Rubin E, Miller KW, Roth SH. New York, The New York Academy of Sciences, 1991, pp 91–96

145. Mody I, Tanelian DL, MacIver MB: Halothane enhances tonic neuronal inhibition by elevating intracellular calcium. Brain Res 538: 319–323, 1991

146. Jones M, Brooks P, Harrison N: Enhancement of GABA-aminobutyric acid-activated chloride currents in cultured rat hippocampal neurones by three volatile anaesthetics. J Physiol (Lond) 449: 279–293, 1992

147. Wakamori M, Ikemoto Y, Akaike N: Effects of two volatile anesthetics and a volatile convulsant on the excitatory and inhibitory amino acid responses in dissociated CNS neurons of the rat. J Neurophysiol 66:2014–2021, 1991

148. Harrison NL, Jones BA, Brooks PA: Three volatile anesthetics enhance GABA-activated chloride currents in cultured rat hippocampal neurons at clinically relevant concentrations (abstr). ANESTHESIOLOGY 75:A1040, 1991

149. Jones MV, Hornberger LA, Harrison NL: Clinical concentrations of three volatile anesthetics prolong GABA-mediated inhibitory postsynaptic currents at synapses between cultured rat hippocampal neurons (abstr). ANESTHESIOLOGY 75:A580, 1991

150. Yeh JZ, Quandt FN, Tanguy J, Nakahiro M, Narahashi T, Brunner EA: General anesthetic action on gamma-aminobutyric acid-activated channels, Molecular and Cellular Mechanisms of Alcohol and Anesthetics. Edited by Rubin E, Miller KW, Roth SH. New York, New York Academy of Sciences, 1991, pp 155–173

151. Nakahiro M, Yeh JZ, Brunner E, Narahashi T: General anesthetics modulate GABA receptor channel complex in rat dorsal root ganglion neurons. FASEB J 3:1850–1854, 1989

152. Peduto VA, Concas A, Santoro G, Biggio G, Gessa GL: Biochemical and electrophysiologic evidence that propofol enhances GABAergic transmission in the rat brain. ANESTHESIOLOGY 75:1000–1009, 1991

153. Concas A, Santoro G, Mascia MP, Serra M, Sanna E, Biggio G: The general anesthetic propofol enhances the function of γ-aminobutyric acid-coupled chloride channel in the rat cerebral cortex. J Neurochem 55:2135–2138, 1990

154. Collins GGS: Effects of the anaesthetic 2,6-diisopropylphenol on synaptic transmission in the rat olfactory cortex slice. Br J Pharmacol 95:939–949, 1988

155. Hales TG, Lambert JJ: Modulation of the $GABA_A$ receptor by propofol: protein or lipid interaction (abstr)? ANESTHESIOLOGY 75: A587, 1991

156. Hales TG, Lambert JJ: The actions of propofol on inhibitory amino acid receptors of bovine adrenomedullary chromaffin cells and rodent central neurones. Br J Pharmacol 104:619–628, 1991

157. Yamamura T, Ohtsuka H, Furmido H, Tsutahara S, Kemmotsu O: Does propofol enhance the GABA-mediated synaptic transmission (abstr)? ANESTHESIOLOGY 75:A588, 1991

158. Willow M: A comparison of the actions of pentobarbitone and etomidate on [^{3}H]GABA binding to crude synaptosomal rat brain membranes. Brain Res 220:427–431, 1981

159. Thyagarajan R, Ramanjaneyulu R, Ticku MK: Enhancement of diazepam and γ-aminobutyric acid binding by (+) etomidate and pentobarbital. J Neurosci 41:578–585, 1983

160. Ashton D, Geerts R, Waterkeyn C, Leysen JE: Etomidate stereospecifically stimulates forebrain, but not cerebellar, ^{3}H-diazepam binding. Life Sci 29:2631–2636, 1981

161. Evans RH, Hill RG: The GABA-mimetic action of etomidate. Biophys J 484–487, 1977

162. Ashton D, Wauqier A: Modulation of a GABAergic inhibitory circuit in the in vitro hippocampus by etomidate isomers. Anesth Analg 64:975–980, 1985

163. Proctor WR, Mynlieff M, Dunwiddie TV: Facilitatory action of etomidate and pentobarbital on recurrent inhibition in rat hippocampal pyramidal neurons. J Neurosci 6:3161–3168, 1986

164. Yang J: Etomidate modulation of central $GABA_A$ receptor-gated current (abstr). ANESTHESIOLOGY 75:A578, 1991

165. Bass A: Uber eine Wirkung des Adrenalins auf das Gehirm. Z ges Neurol Psychiat 26:600–601, 1914

166. Feldberg W, Sherwood SL: Injections of drugs into the lateral ventricle of the cat. J Physiol (Lond) 123:148–167, 1954

167. Leimdorfer A, Arana R, Hack MA: Hyperglycemia induced by the action of adrenalin on the central nervous system. Am J Physiol 150:588–595, 1947

168. Leimdorfer A, Metzner WRT: Analgesia and anaesthesia induced by epinephrine. Am J Physiol 157:116–121, 1949

169. Doze VA, Chen B, Maze M: Dexmedetomidine diminishes halothane anesthetic requirements in rats through a postsynaptic α_2-adrenergic receptor. ANESTHESIOLOGY 69:818–823, 1989

170. Drew GM, Gower AJ, Marriott AS: Pharmacological characterization of alpha-adrenoreceptors which mediate clonidine-induced sedation. Br J Pharmacol 61:468P, 1977

171. Drew GM, Gower AJ, Marriott AS: Alpha2 adrenoreceptors mediate clonidine-induced sedation in the rat. Br J Pharmacol 67: 133–141, 1979

172. Pittaluga A, Raiteri M: GABAergic nerve terminals in rat hippocampus possess α_2-adrenoceptors regulating GABA release. Neurosci Lett 76:363–367, 1987

173. Maura G, Pittaluga A, Ulivi M, Raiteri M: Enhancement of endogenous GABA release from rat synaptosomal preparations is mediated by α_2-adrenoceptors pharmacologically different from α_2-autoreceptors. Eur J Pharmacol 157:23–29, 1988

174. Mueller AL, Dunwiddie TV: Anticonvulsant and proconvulsant actions of alpha- and beta-noradrenergic agonists on epileptiform activity in rat hippocampus *in vitro*. Epilepsia 24:46–57, 1983

175. Pang K, Rose GM: Differential effects of norepinephrine on hippocampal complex-spike and θ-neurons. Brain Res 425:146–158, 1987

176. Rose GM, Pang KCH: Differential effect of norepinephrine upon granule cells and interneurons in the dentate gyrus. Brain Res 488:353–356, 1989

177. Doze VA, Cohen GA, Madison DV: Synaptic localization of adrenergic disinhibition in the rat hippocampus. Neuron 6:889–900, 1991

178. Richards CD: Actions of general anaesthetics on synaptic transmission in the CNS. Br J Anaesth 55:201–207, 1983

179. MacIver MB, Roth SH: Inhalation anaesthetics exhibit pathway-specific and differential actions on hippocampal synaptic responses in vitro. Br J Anaesth 60:680–691, 1988

180. MacIver MB, Kendig JJ: Anesthetic effects on resting membrane potential are voltage-dependent and agent-specific. ANESTHESIOLOGY 74:83–89, 1991

181. Judge SE: Effect of general anaesthetics on synaptic ion channels. Br J Anaesth 55:191–200, 1983

182. MacIver MB, Kendig JJ: Enflurane-induced burst discharge of hippocampal CA1 neurones is blocked by the NMDA receptor antagonist APV. Br J Anaesth 63:296–305, 1989

183. Richter J, Landau EM, Cohen S: Anesthetic and convulsant ethers act on different sites at the crab neuromuscular junction in vitro. Nature 266:70–71, 1977

184. Landau EM, Richter J, Cohen S: Differential solubilities in subregions of the membrane: A nonsteric mechanism of drug specificity. J Med Chem 22:325–327, 1979

185. Deisz RA, Lux HD: Diphenylhydantoin prolongs postsynaptic inhibition and iontophoretic GABA action on the crayfish stretch receptor. Neurosci Lett 5:199–202, 1977

186. Cohen G, Doze V, Madison D: Opioid inhibition of GABA release from presynaptic terminals of rat hippocampal interneurons. Neuron 9:325–335, 1992

187. McCormick DA, Wang Z: Serotonin and noradrenalin excite GABAergic neurones of the guinea-pig and cat nucleus reticularis thalami. J Physiol (Lond) 442:235–255, 1991

188. Ropert N, Guy N: Serotonin facilitates GABAergic transmission in the CA1 region of rat hippocampus in vitro. J Physiol (Lond) 441: 121–136, 1991

189. Andreasen M, Lambert JDC: Noradrenaline receptors participate in the regulation of GABAergic inhibition in area CA1 of the rat hippocampus. J Physiol (Lond) 439:649–669, 1991

190. Luddens H, Wisden W: Function and pharmacology of multiple $GABA_A$ receptor subunits. TIPS 12:49–51, 1991

191. Krnjevic K: Cellular mechanisms of anesthesia. New York, The New York Academy of Sciences, 1991, pp 1–16

192. MacIver MB, Roth SH: Anesthetics produce differential actions on membrane responses of the crayfish stretch receptor neuron. Eur J Pharmacol 141:67–77, 1987

193. Ueda I, Hirakawa M, Arakawa K, Kamaya H: Do anesthetics fluidize membranes? ANESTHESIOLOGY 64:67–71, 1986

194. Trudell JR: Role of membrane fluidity in anesthetic action, Drug and Anesthetic Effects on Membrane Structure and Function. Edited by Aloia RC, Curtain CC. New York, Wiley-Liss, 1991, pp 1–14

195. Roth SH, Miller KW: Inside the "black box," Molecular and Cellular Mechanisms of Anesthetics. Edited by Roth SH, Miller KW. New York, Plenum, 1986, pp 261–267

196. Seeman P: The membrane actions of anesthetics and tranquilizers. Pharmacol Rev 24:583–655, 1972

197. Roth SH: Physical mechanisms of anesthesia. Annu Rev Pharmacol Toxicol 19:159–178, 1979

198. MacIver MB, Roth SH: Barbiturate effects on hippocampal excitatory synaptic responses are selective and pathway specific. Can J Physiol Pharmacol 65:385–394, 1987

199. Huang LY, Barker JL: Pentobarbital: Stereospecific actions of the (+) and (−) isomers revealed on cultured mammalian neurons. Science 207:195–197, 1980

200. Franks NP, Lieb WR: Stereospecific effects of inhalational general anesthetic optical isomers on nerve ion channels. Science 254:427–430, 1991