The Role of the GABA<sub>A</sub> 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.§

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<sub>A</sub>-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.

in the gray matter of the mammalian CNS (fig. 1). Not long after its discovery, GABA was shown to be an inhibitory neurotransmitter in the mammalian CNS. The quantification of its action was made possible by early electrophysiologic studies using the crayfish stretch receptor preparation. 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. Bloom and Iversen in 1971 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. 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. 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. Thus, the former bicuculline-sensitive GABA receptors have been termed GABA<sub>A</sub> receptors and the latter potassium channel-modulating receptors are called GABA<sub>B</sub> receptors. It is now clear that, in the human brain, activation of GABA<sub>A</sub> receptors by synaptically released GABA is responsible for the fast inhibitory postsynaptic potential, whereas activation of GABA<sub>B</sub> receptors underlies a much slower and longer lasting inhibition. The two receptors not only are functionally distinct, but they belong to separate classes of receptor families. The GABA<sub>A</sub> receptor is a ligand-gated ion channel, whereas the GABA<sub>B</sub> receptor is a G protein-coupled receptor.

The purpose of the present review is to provide a comprehensive picture of the GABA<sub>A</sub> receptor as a site for anesthetic action. Compounds that impair or enhance GABA<sub>A</sub> 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. 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<sub>A</sub> Receptor Channels

The cloning of the nicotinic acetylcholine, glycine, and the GABA<sub>A</sub> receptors 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. 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.

The initial cloning of the GABA<sub>A</sub> receptor channel provided evidence for two different but related subunits (alpha and beta). Since that time, molecular neuro-
GABA AND ANESTHESIA

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 α1–6, β1–3, γ1–3, 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. GABAergic Agonists and Antagonists

<table>
<thead>
<tr>
<th>GABA&lt;sub&gt;a&lt;/sub&gt; receptor agonists</th>
<th>GABA (γ-aminobutyric acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscimol (5-iminomethyl-3-hydroxy-isoxazole)</td>
<td></td>
</tr>
<tr>
<td>Isovouacine (1,2,5,6-tetrahydroisoconitine acid)</td>
<td></td>
</tr>
<tr>
<td>Isoneptic acid (hexahydroisonicotinic acid)</td>
<td></td>
</tr>
<tr>
<td>THIP hydrochloride (4,5,6,7-tetrahydroisoxazole[5,4,6-</td>
<td>pyridin-3-ol HCl)</td>
</tr>
<tr>
<td>THPO hydrate (4,5,6,7-tetrahydroisoxazole[5,4,6-</td>
<td>pyridin-3-ol hydrate)</td>
</tr>
<tr>
<td>Competitive GABA&lt;sub&gt;a&lt;/sub&gt; receptor antagonists</td>
<td>(&lt;)-Bicuculline methiodide, methyl chloride, methyl bromide</td>
</tr>
<tr>
<td>SR-95531 (2,3-carboxy-2-propyl)-3-amino-6p-(4-</td>
<td></td>
</tr>
<tr>
<td>methoxyphenyl)pyridazinilum bromide)</td>
<td></td>
</tr>
<tr>
<td>Noncompetitive GABA&lt;sub&gt;a&lt;/sub&gt; receptor antagonists-Cl&lt;sup&gt;-&lt;/sup&gt; channel blockers</td>
<td></td>
</tr>
<tr>
<td>Picrotoxin (isolated from the seed of Anamirta occulus)</td>
<td></td>
</tr>
<tr>
<td>(7) TBPS (2-butyl-bicyclo-phosphorothionate)</td>
<td></td>
</tr>
<tr>
<td>(7) Penicillin</td>
<td></td>
</tr>
<tr>
<td>(7) Pentetetazol, metrazole</td>
<td></td>
</tr>
<tr>
<td>Benzodiazepine site</td>
<td></td>
</tr>
<tr>
<td>Agonists (augment the response of GABA)</td>
<td></td>
</tr>
<tr>
<td>CGS 9896</td>
<td></td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td></td>
</tr>
<tr>
<td>Clonazepam</td>
<td></td>
</tr>
<tr>
<td>Fluoritizapam</td>
<td></td>
</tr>
<tr>
<td>Flurazepam</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td></td>
</tr>
<tr>
<td>Midazolam</td>
<td></td>
</tr>
<tr>
<td>Zolpidem</td>
<td></td>
</tr>
<tr>
<td>Inverse agonists (decrease in response to GABA)</td>
<td></td>
</tr>
<tr>
<td>Ro 19-4603</td>
<td></td>
</tr>
<tr>
<td>DMCM (methyl 6,7-dimethoxy-4-ethyl-carboline-3-</td>
<td></td>
</tr>
<tr>
<td>carboxylate)</td>
<td></td>
</tr>
<tr>
<td>β-CCE (ethyl β-carboline-3-carboxylate)</td>
<td></td>
</tr>
<tr>
<td>β-CCM (methyl β-carboline-3-carboxylate)</td>
<td></td>
</tr>
<tr>
<td>Ro 15-4513</td>
<td></td>
</tr>
<tr>
<td>FG 7142 (N-methyl β-carboline-3-carboxamide)</td>
<td></td>
</tr>
<tr>
<td>Antagonists (no effect on their own, but block effect of both</td>
<td></td>
</tr>
<tr>
<td>agonists and antagonists)</td>
<td></td>
</tr>
<tr>
<td>β-CCP (propyl β-carboline-3-carboxylate)</td>
<td></td>
</tr>
<tr>
<td>Flumazenil (Ro 15-1786)</td>
<td></td>
</tr>
<tr>
<td>Ro 14-7437</td>
<td></td>
</tr>
<tr>
<td>Barbtrurate site</td>
<td></td>
</tr>
<tr>
<td>Agonists</td>
<td></td>
</tr>
<tr>
<td>Pentobarbital (5-ethyl-5-(1-methylbutyl)barbitaluric acid)</td>
<td></td>
</tr>
<tr>
<td>Phenoarbital (5-ethyl-5-phenobarbitaluric acid)</td>
<td></td>
</tr>
<tr>
<td>(-)MPPB (11-1-methyl-5-phenyl-5-propylbarbitaluric acid)</td>
<td></td>
</tr>
<tr>
<td>(7) TBPS (2-butyl-bicyclo-phosphorothionate)</td>
<td></td>
</tr>
</tbody>
</table>

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

the opening of GABA<sub>a</sub> receptor channels. In addition, a number of neurotransmitter systems have been shown to selectively innervate GABAergic interneurons<sup>28,29</sup> 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.<sup>30-33</sup> In rats and mice, THIP produced analgesia, sedation, and loss of righting reflex.<sup>34</sup> 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 somato-sensory event-related potentials in cats.<sup>35</sup> 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<sub>a</sub> receptor agonists. It will be evident from this review that anesthetics can influence GABAergic inhibition by different mechanisms. Some agents directly activate the GABA<sub>a</sub> 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<sub>a</sub> 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<sub>a</sub> receptor/chloride channel complex (and any other proposed site). Discrepancies exist be-

Anesthesiology, V 78, No 4, Apr 1993
between 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<sub>A</sub> Receptor Complex by Anesthetics**

**Barbiturates**

Barbiturates form a large and diverse class of drugs, and an extensive literature concerning their effects on the GABA<sub>A</sub> receptor complex is available. In addition to their effects on GABA<sub>A</sub> receptor activity, barbiturates produce many other effects, including inhibition of glutamate and adenosine receptor activity, as well as release of norepinephrine, serotonin, and acetylcholine. 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. 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<sub>A</sub> agonists muscimol and THIP. This suggests that hypnosis is at least partly mediated by activation of the GABA<sub>A</sub> receptor complex. This theory is strengthened by the finding that pentobarbital-induced loss of righting reflex is decreased by the GABA<sub>A</sub> antagonist bicuculline, demonstrating that blocking GABA<sub>A</sub> receptors is antihypnotic. More evidence implicating the GABA<sub>A</sub> 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, 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. This effect is not modified by benzodiazepine antagonists or inverse agonists, indicating that the anticonvulsant effect of barbiturates does not depend on the benzodiazepine receptor. An interesting corollary is that the GABA<sub>a</sub> blocker picrotoxin has a protective effect against a lethal dose of pentobarbital in animals. However, since picrotoxin is a potent convulsant, this pharmacologic reversal is of no clinical utility. At present, results derived from *in vitro* studies support the possibility that barbiturates produce their sedative-hypnotic effects *via* actions at the GABA<sub>a</sub> receptor complex, but definitive data in humans is lacking and will be difficult to acquire given the proconvulsant effects of GABA<sub>a</sub> receptor blockers.

**Binding Studies.** Barbiturates modulate the binding of other ligands to the GABA<sub>a</sub> receptor. The interactions between the binding of GABA, GABA<sub>a</sub> blocking convulsants, barbiturates, and benzodiazepines with respect to their distinct binding sites on the GABA<sub>a</sub> 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. 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.

Barbiturates markedly potentiate the affinity of GABA binding to the GABA<sub>a</sub> receptor in a manner dependent on the presence of chloride ions. Scatchard analysis of binding data indicates that barbiturates appear to increase the number of available GABA receptors and slow the dissociation of GABA from receptors. 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. This very low-affinity GABA binding site also binds the GABA antagonist bicuculline. Barbiturates selectively enhance only agonist binding by decreasing the affinity of bicuculline binding to this very low-affinity GABA binding site.

The binding to GABA<sub>a</sub> receptors of convulsant GABA<sub>a</sub> blockers (e.g., picrotoxin, TBPS) is inhibited by barbiturates. Barbiturates markedly accelerate dissociation of the caged convulsant TBPS from its recognition site. The relative potencies of barbiturates to inhibit this binding correlate well with their ability to enhance GABAergic synaptic transmission.

Barbiturates increase the binding of benzodiazepines to the GABA<sub>a</sub> receptor by increasing their affinity in a chloride-dependent manner. This capacity to increase GABA and benzodiazepine binding correlates well with the anesthetic potencies of the barbiturates studied. 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.

Barbiturates decrease the affinity of benzodiazepine inverse agonists (discussed in more detail later) for the benzodiazepine receptor, thereby selectively enhancing only agonist binding *via* allosteric interaction.

**Chloride Flux Studies.** The development of biochemical assays for GABA-mediated 36Cl<sup>-</sup> flux in various neuronal preparations has provided the basis for analytical studies of GABA<sub>a</sub> receptor function. In the absence of GABA, pentobarbital increases 36Cl<sup>-</sup> uptake into synaptoneurosomes. In addition, pentobarbital markedly potentiates 36Cl<sup>-</sup> uptake induced by GABA and the GABA agonist muscimol. The anesthetic pentobarbital is approximately ten times as potent as the anticonvulsant phenobarbital in this effect, 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<sub>a</sub> receptor antagonists bicuculline and TBPS. The benzodiazepine inverse agonist Ro 15-4513 had no effect on pentobarbital-enhanced GABA-stimulated 36Cl<sup>-</sup> flux.

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<sub>a</sub> antagonist picrotoxin and occurs in various regions of the spinal cord.
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. 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.

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, 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-carboxethoxy-β-carboline (β-CCB, a β-carboline) was extracted from human urine. Although this compound may have been formed during the extraction and identification procedure, another β-carboline, 3-carboxobutoxy-β-carboline (β-CCE), with high affinity for the benzodiazepine receptor subsequently was identified in pig brain. 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. 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.

**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. Benzodiazepine agonists enhance the binding of GABA to the very low-affinity GABA binding sites on the GABA_A receptor complex. The binding of benzodiazepines to the GABA_A receptor complex is enhanced by GABA, barbiturates, and chloride ions. Picrotoxin inhibits basal, GABA-stimulated, and pentobarbital-stimulated [3H] 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. GABA agonists have no effect on the binding of the benzodiazepine antagonist flumazenil, and inhibit the binding of the inverse agonist β-CCB. 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. Barbiturates also inhibit the binding of β-CCB to rat brain membranes. Similar to the effect of barbiturates, benzodiazepine agonists decrease the binding of the caged convulsant TBPS, whereas inverse agonists increase this binding.

**Chloride Flux Studies.** Benzodiazepines alone have no effect on 36Cl⁻ flux into synaptoneuroses in the absence of an exogenously applied GABA_A agonists. In the presence of GABA or muscimol, benzodiazepine agonists increase 36Cl⁻ uptake in a concentration-dependent manner. Benzodiazepine agonists shift the concentration-response curve for muscimol-stimulated...
uptake to the left without decreasing $V_{max}$. This increase in $^{36}$Cl\textsuperscript{-} uptake is blocked completely by the benzodiazepine antagonist flumazenil. The benzodiazepine inverse agonist $\beta$-CCE inhibited muscimol-stimulated $^{36}$Cl\textsuperscript{-} flux in rat brain synaptoneurosomes in a competitive fashion, shifting the concentration-response curve to the right. In contrast, in spinal cord neurons, $\beta$-CCE and DCMC act as noncompetitive inhibitors of GABA$\text{A}$. In hippocampal slices, DCMC does not alter muscimol-stimulated $^{36}$Cl\textsuperscript{-} flux. The reasons for these discrepancies may lie in regional differences in the subunit composition of the GABA$\text{A}$ receptor complex, but taken together, the results on $^{36}$Cl\textsuperscript{-} 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.

**Electrophysiologic Studies.** Benzodiazepine agonists potentiate GABA$\text{A}$-induced chloride channel opening in a manner consistent with increasing the association of GABA$\text{A}$ 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. Benzodiazepine agonists also enhance GABAergic synaptic transmission and the response to exogenously applied GABA. 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$\text{A}$ receptor-chloride ion channel complex. At nanomolar to low micromolar concentrations, these steroid derivatives can stimulate $[^{3}H]j$unitrazepam and $[^{3}H]m$uscimol binding and displace the convulsant $[^{35}SJTPS from its binding site in an allosteric manner. Structure-activity relationship data demonstrated that the essential features of the active structures are a 5α- or 5β-reduced pregnane skeleton with a hydroxyl at C3 in the a-position and a ketone group at C20.

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

Most recently, steroid anesthetics (e.g., alphaxalone) and other naturally occurring analogs have been shown to modulate the GABA$\text{A}$ receptor complex at a site distinct from the barbiturates. These studies also suggest the existence of a specific binding site for the steroids in the GABA$\text{A}$ receptor complex.

**Electrophysiologic Studies.** In the rat cuneate nucleus slice, alphaxalone potentiated depolarizing responses to perfused GABA and muscimol but not to glycine. 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. The nonanesthetic 3B-hydroxy-alphaxalone analog was without effect. Higher alphaxalone concentrations, similar to anesthetic concentrations, increased membrane conductance in the absence of exogenous GABA, suggesting alphaxalone increases chloride conductance. 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. 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. In another preparation, bovine chromaffin cells, alphaxalone, and other pregnane steroids directly activated the GABA$\text{A}$ receptor at a site distinct from the barbiturate and benzodiazepine allosteric sites.

In this study, intracellularly applied alphaxalone had no effect on the GABA$\text{A}$ 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$\text{A}$ 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.\textsuperscript{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\textsubscript{A} system has 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 GABA\textsubscript{A}mimetic drugs can increase the actions of ethanol, whereas GABA antagonists reduce them.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{119} These studies implicate the GABA\textsubscript{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\textsubscript{A}-activated chloride channels by measuring uptake of $^{36}$Cl$^-$ into cultured spinal cord neurons or isolated brain membrane vesicles (synaptosomes). Ethanol increases GABA\textsubscript{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.\textsuperscript{46,60,114,116,120,122} 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\textsubscript{A}-activated chloride flux was augmented by both ethanol and benzodiazepines in LS and DS but not SS and DR mice.\textsuperscript{46,123,124} 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 heterogenous stock mice selected for differences in ethanol sleep time.\textsuperscript{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.\textsuperscript{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.\textsuperscript{126} The use of longer chain alcohols (n-butanol-C\textsubscript{4}, n-hexanol-C\textsubscript{6}, and n-octanol-C\textsubscript{8}) for which hypnotic effects increase with chain length\textsuperscript{127} augmented the peak current induced by GABA in a concentration-dependent manner.\textsuperscript{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.\textsuperscript{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. 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. However, in dogs, flumazenil (0.15–0.45 mg/kg) significantly decreased halothane MAC. 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. 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, picotoxin-sensitive fashion. 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 MAC potency. In mice selectively bred for sensitivity to diazepam (DS), halothane enhanced GABA$\text{A}$-gated chloride flux to a much greater extent than in the diazepam resistant (DR) strain. Detailed studies of this nature will be required to sort out the extent to which volatile agents (and other anesthetics) act at GABA$\text{A}$/benzodiazepine/barbiturate receptors. Taken together, these studies indicate that the volatile anesthetics interact with the GABA$\text{A}$ receptor, but the interaction may be more complex than for other GABA$\text{A}$ 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. 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 transmission pool of rat cerebral cortical slices by more than 100%. 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. Further work by Cheng and Brunner studied GABA disposal in synaposomes by measuring the conversion of $[^{1-14}$C]GABA to $^{14}$CO$_2$ and concluded that chloroform, halothane, enflurane, and ether inhibit this process. Although high concentrations of volatile agents appear to increase GABA levels in the brain by blocking breakdown, 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. 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. At 3% halothane, inhibitory postsynaptic current decay time constants were prolonged twofold, whereas rise time and amplitude were not affected.

Using whole-cell patch-clamp recording in hippocampal slices, halothane (1.2 vol%) prolonged the decay time constant of GABA$\text{A}$-mediated spontaneous inhibitory postsynaptic currents by 275%. Intracellular administration of the calcium chelator BAPTA or the calcium-release inhibitor dantrolene to the hippocampal neurons significantly reduced halothane's effect. In extracellular recordings, the halothane-induced depression of hippocampal population spike amplitude was blocked by the GABA$\text{A}$ antagonist bicuculline. Taken together, these findings suggest that a major depressant effect of halothane involves the enhancement of GABA$\text{A}$-mediated inhibition through release of intracellular calcium.

In cultured or acutely dissociated rat dorsal root gan-
glion cells, the volatile anesthetics halothane, enfurane, and isoflurane, at 2 MAC concentrations, enhanced the GABA_α-activated current. 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.

**Propofol (2,6-Diisopropylphenol)**

**Binding and Chloride Flux Studies.** Propofol was found to inhibit [35S]TBPS binding to membrane preparations from rat cerebral cortex in a concentration-dependent fashion. Propofol, in the presence of either alphaxalone or pentobarbital, produced an additive inhibition of [35S]TBPS binding that was greater than that produced by either agent alone, suggesting separate sites of action for these drugs. Propofol also enhanced [3H]GABA binding in the rat cerebral cortex and in cortical membrane preparations. In addition, propofol potentiates muscimol-induced stimulation of Cl^- uptake in membrane vesicle preparations. These findings suggest that propofol may exert its effects by enhancing the function of the GABA_α 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. In bovine chromaffin cells, known to have neuronal-like GABA_α receptors, propofol (1.7 μM) increased the size of GABA-activated currents by 500%. 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. 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 [3H]GABA binding by increasing the number of high-affinity binding sites rather than by altering receptor affinity. Etomidate also has been shown to enhance [3H]-diazepam binding to rat cerebral cortical membranes. This effect on [3H]-diazepam binding was shown to be brain region-specific, occurring in the rat forebrain but not in the cerebellum. There are specific populations of GABA_α receptor subunit complexes, demonstrating regional brain distributions selective for forebrain versus cerebellum. Etomidate may act selectively on such a subpopulation of GABA_α 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. 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. 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. 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_α-gated currents by 185%. 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. In the 1940s, adrenergically induced anesthesia and analgesia were demonstrated in several species, including humans. Subsequently, it has been shown that α2-
adrenergic receptors mediate the sedative/anesthetic effects of these drugs.\textsuperscript{169-171}

**GABA Release Studies.** Noradrenaline and the $\alpha_2$-adrenoreceptor agonist clonidine enhance, in a concentration-dependent manner, the release of endogenous GABA from rat hippocampal synaptosomes.\textsuperscript{172, 173} This effect is blocked by the $\alpha_2$-adrenoreceptor antagonist yohimbine but not by the $\alpha_1$-antagonist prazosin. From these studies, it has been concluded that GABAergic nerve terminals in rat hippocampus possess $\alpha_2$-adrenoreceptors whose activation causes enhancement of GABA release.

**Electrophysiologic Studies.** In the rat hippocampal slice preparation, exogenously applied norepinephrine and $\alpha$-adrenergic agonists slowed or stopped spontaneous interictal discharges, suggesting potential enhancement of inhibition.\textsuperscript{174} Other electrophysiologic studies in rat hippocampus have demonstrated that norepinephrine can inhibit complex spike (pyramidal) neurons through an $\alpha_1$ receptor while exciting interneurons by activation of an $\alpha_2$ receptor.\textsuperscript{175, 176} Recently, it has been shown that adrenergic agonists excite interneurons and thereby increase the frequency of spontaneous GABA\textsubscript{A} receptor-mediated inhibitory postsynaptic potentials (IPSPs),\textsuperscript{177} suggesting that $\alpha$-adrenergic agents increase GABAergic inhibition by presynaptically increasing endogenous GABA release.

**Discussion**

**GABA\textsubscript{A} Receptor Involvement in Anesthesia**

There is now compelling evidence implicating anesthetic actions at the GABA\textsubscript{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\textsubscript{A} receptor and 2) a prolongation or augmentation of the Cl\textsuperscript{-} 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\textsubscript{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\textsubscript{A} complex is evident from several interdependent findings. First, GABA\textsubscript{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\textsubscript{A} receptor agonists that act selectively on GABA\textsubscript{A} receptors. Third, and most compelling, evidence from molecular, cellular, and intact animal levels all support an important action for anesthetics on the GABA\textsubscript{A} receptor complex. The extent to which actions on the GABA\textsubscript{A} complex contribute to the overall phenomenon of clinical anesthesia remains to be determined.

This problem has several compounding components. Increased GABA\textsubscript{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\textsuperscript{178, 179} and on postsynaptic ionic conductances contributing to the resting membrane potential.\textsuperscript{180, 181} In other words, anesthetic effects on excitatory synaptic currents can add to direct effects on the GABA\textsubscript{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\textsubscript{A} postsynaptic inhibitory synaptic potentials in these same neurons.\textsuperscript{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 decreased GABA\textsubscript{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<sub>A</sub> inhibition versus actions on excitatory synaptic responses. Several studies have addressed this by using simple invertebrate neuromuscular preparations or by selectively eliminating the GABA<sub>A</sub> system using specific antagonists in the hippocampal slice preparation. The latter approach indicated that effects on the GABA<sub>A</sub> 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<sub>A</sub> inhibition was blocked totally by bicuculline. Similar studies will provide a basis for determining the fractional contribution of enhanced GABA<sub>A</sub> inhibition to the depressant effects for each class of agents. At present, it is likely that actions at the GABA<sub>A</sub> 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 and ketamine have been shown to block GABAergic inhibition; however, GABAergic effects of these agents have not been researched well to date.

**Sensitivity of the GABA<sub>A</sub> Receptor Complex to Anesthetics**

As pointed out above, the GABA<sub>A</sub> 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<sub>A</sub> 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<sub>A</sub> 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<sup>2+</sup> 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. 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<sub>A</sub> 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.

---

*Anesthesiology, V 78, No 4, Apr 1993*
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 agentspecific 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 anesthetics193–195 and most often emphasize the common physicochemical 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 multistate 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 anesthetics.

References


22. Chen QX, Stelzer A, Kay AR, Wong RKS: GABA_A receptor function is regulated by phosphorylation in acutely dissociated guinea pig hippocampal neurons. J Physiol (Lond) 420:207–221, 1990
32. Kjaer M, Nielsen H: The analgesic effects of the GABA-agonist


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


96. Wong EH, Leeb-Lundberg LMF, Teichberg VI, Olsen RW: \( \gamma \)-Aminobutyric acid activation of \( ^{36} \)Cl flux in rat hippocampal slices and its potentiation by barbiturates. Brain Res 353:267–275, 1984


106. You JW, Balfour D, Johnston IH: Modulation of the GABA receptor by barbiturates and pregnane steroids: Differential effects

Anesthesiology, V 78, No 4, Apr 1993


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


Anesthesiology, V 78, No 4, Apr 1993


