Current Theories about the Mechanisms of Benzodiazepines and Neuroleptic Drugs

James J. Richter, M.D., Ph.D.*

Progress in research on the molecular mechanisms of action of volatile anesthetics has been reviewed recently.¹,² Volatile hydrocarbons probably cause anesthesia by affecting fluidity, volume, and protein interactions in the lipid phase of synaptic membranes.¹ The molecular mechanisms of volatile anesthetics are therefore distinct from the more specific actions of parenteral agents that bind to receptors and modulate synaptic transmission. The identification of opiate receptors and peptide ligands has inspired much research and new molecular interpretations of pain and the analgesic properties of narcotics. Enkephalins and narcotic receptors have been reviewed for anesthesiologists elsewhere.³ This paper reviews new theories about mechanisms of action of benzodiazepines and neuroleptic drugs in the context of neurotransmitter function. An exhaustive critical analysis of basic research is not intended. My purpose is to outline, for clinical anesthesiologists, new trends in neuropharmacology.

Sherrington⁴,⁵ established that synaptic communication between neurons is the basis for nervous system function. Pharmacologic manipulation of neurophysiology is interpreted as the chemical modulation of axonal conduction or synaptic activity. Synaptic communication depends on the release of a chemical neurotransmitter from the presynaptic ending and the binding of the transmitter to a postsynaptic receptor. These fundamental principles of neuropharmacology are thoroughly discussed in the recent book by Cooper, Bloom, and Roth.⁶ After reviewing data for relevant neurotransmitters, I discuss below theories about the mechanisms of action of benzodiazepine and neuroleptic drugs.

Neurotransmitters

To be identified as a neurotransmitter, a compound should meet five criteria.⁷,⁸

1) The compound should be present (along with precursors and synthetic enzymes) within synaptic endings of the central nervous system.
2) Mechanisms should exist for inactivation (catabolism or reuptake).
3) When the compound is administered exogenously, it should produce the same effects as neural stimulation.
4) Drugs that are known to modulate the synaptic system in question should have similar effects on the postsynaptic changes caused by the exogenous compound and by stimulation of the presynaptic nerve.
5) When the presynaptic nerve is stimulated, the compound should be released from the nerve terminal.

The concept of chemical neurotransmission originated from the observations of Loree of the effects of acetylcholine on isolated heart preparations.⁹ Other early models for chemical neurotransmission were based on observations made in preparations of the peripheral nervous system such as sympathetic ganglia and myoneural junctions. Because of the anatomic complexity of the brain, the five criteria for identifying a neurotransmitter are much more difficult to establish for central nervous system functions. To acknowledge these limitations of experimental technique, neuroscientists use the adjective "putative" when discussing compounds as potential neurotransmitters. Experimental techniques and observations have been inadequate to confirm all criteria for most of the compounds thought to function as neurotransmitters. However, the word "neurotransmitter" is appearing more frequently without the adjective "putative." This is a consequence of the growing confidence among neuro-
chemists of the validity of neurotransmitter concepts. Therefore, the following discussion omits the word "putative."

Barchas et al. have reviewed the biochemistry of neurotransmitters and neuromodulators. They distinguish between neurotransmitters, which carry signals from one cell to another, and neuromodulators, compounds that "amplify or dampen neuronal activity." For some compounds there is strong evidence to suggest that they have either excitatory or inhibitory neurotransmitter properties. The function of acetylcholine as the chemical mediator at the myoneural junction is well known to all anesthesiologists. Cholinergic transmission is also an important phenomenon within the brain. Biogenic amines (catecholamines, serotonin, etc.) have neurotransmitter properties in the peripheral as well as in the central nervous system. The chemistry and pharmacology of these compounds are thoroughly discussed in many standard texts.

Many compounds that are thought to be neurotransmitters are amino acids. Investigators have been unable to establish firmly the five criteria necessary to identify unequivocally an amino acid as a neurotransmitter, because these compounds are ubiquitous in living tissue as components of proteins, and because they interact with carbohydrate metabolism.

An important inhibitory transmitter in the brain is gamma-aminobutyric acid (GABA). Peripheral and central nervous system tissues contain high concentrations of GABA. Thorough studies, using the peripheral nervous system of the lobster, have established that GABA is the chemical mediator for inhibitory neurotransmission. Subsequent studies suggest that GABA is the major inhibitory neurotransmitter in mammalian brain. The inhibitory neurotransmitter properties of GABA have been deduced from studies of the effects of drugs on the metabolic turnover and receptor binding of GABA in brain tissue. Glutamic acid is decarboxylated by the enzyme glutamic acid decarboxylase (GAD) to produce GABA. Certain convulsive drugs, such as pentylentetrazol, inhibit GAD activity and thus decrease the rate of GABA synthesis. The consequent decreased tissue concentration of GABA results in a decrease in the inhibitory "tone" of upper motor neurons, and results in myoclonic seizure activity. Other convulsive drugs, such as phenoxybenzamine, are GABA antagonists, and directly bind to the GABA receptor on postsynaptic membranes. The antagonistic action of these drugs prevents the normal inhibitory influence of GABA on the upper motor neurons and thereby causes seizure activity. Only a limited number of convulsant drugs affect GABA function in neural tissue. Molecular mechanisms of seizure activity are not thoroughly understood, and are certainly much more complicated than merely decreased efficiency of GABA-mediated transmission. Other convulsants and many anticonvulsants (such as barbiturates and phenytoin) have no effect on the GABA-containing neurons.

Gamma-aminobutyric acid-mediated neurotransmission controls systems other than inhibition of motor activity. In particular, GABA-ergic neurons may influence activity of other neurons that secrete dopamine or serotonin. In some brain areas, increased release of GABA results in decreased release of norepinephrine and increased release of serotonin by adjacent neural cells. Synapses using GABA as a chemical mediator therefore modulate the activity of other neuronal systems that utilize biogenic amines for their neurotransmission. Cheng and Brunner are studying changes in GABA concentrations caused by anesthetic agents. They have suggested that anesthetics can increase concentrations of GABA in critical regions of the brain, and that the increased concentration of this inhibitory neurotransmitter may contribute to the anesthetic effects of certain drugs.

Glycine, the simplest alpha amino acid, may be the major inhibitory transmitter of the spinal cord. The neurotransmitter function of glycine was first suggested by Aprison and Wernan in 1965, when they described unusually high concentrations of this amino acid in the nerve roots and spinal cord of the cat. Within the spinal cord, glycine concentration is particularly high in the ventral gray matter, suggesting that glycine may mediate inhibition of motor neurons originating in the spinal cord. Strychnine (a convulsant) is an antagonist of glycine and binds directly to glycine receptors in the spinal cord. The antagonistic action of strychnine prevents normal inhibitory function of glycine and results in motor seizure activity. Although glycine is not distributed widely in the brain, it may have an important role in inhibitory neural transmission in the brain stem. The evidence suggests that GABA is the major inhibitory neurotransmitter in the brain, and that glycine is the major inhibitory neurotransmitter in the spinal cord and brain stem.

Dopamine is a neurotransmitter that influences the extrapyramidal functions of the basal ganglia. The neurotransmitter functions of dopamine have been characterized in detail. In presynaptic nerve endings, the precursor tyrosine is synthesized (by tyrosine hydroxylase and dopa decarboxylase) into dopamine, which is stored in vesicles. Depolarization of the presynaptic cell causes the release of dopamine that crosses the cleft and binds to receptors on the post-
synaptic membrane. When occupied by neurotransmitter molecules, receptors change membrane permeability and initiate other intracellular events that may result in stimulation or inhibition of the postsynaptic neuron. The neurotransmitter effects are terminated when the molecules diffuse away from the receptor. Figure 1 is a generalized scheme that illustrates neurotransmitter function.

Microscopic localization of specific neurotransmitters is accomplished with the techniques of fluorescence histochemistry. Fuxe and Hokfelt\textsuperscript{19} recently reviewed the neurotransmitter distribution in specific neuroanatomic tracts in the central nervous system.

**Benzodiazepines**

Benzodiazepines have the following pharmacologic properties:

1) Antianxiety
2) Anticonvulsant
3) Sedation
4) Centrally-mediated muscle relaxation
5) Amnesia

The isolation and properties of drug-specific receptors are being described as the first steps toward understanding molecular mechanisms of neuropharmacology. The descriptions of opiate receptors and endogenous opiate-like ligands (enkephalins) in the brain by Hughes and Kosterlitz\textsuperscript{18} and Pasternak and Snyder\textsuperscript{19} have stimulated research and consequent identification of receptors for a wide variety of drugs that have central nervous system actions. Specific receptors for benzodiazepines were identified in brain tissue in 1977. Mohler and Okada\textsuperscript{20} incubated radioactive diazepam with a variety of subcellular fractions isolated from brain tissue. Only small amounts of triated diazepam were taken up by slices of cerebral cortex. However, the subcellular distribution of triated diazepam bound to membranes showed that there was binding in the fraction containing synaptosomes. At least 70 per cent of binding can be accounted for in the synaptic membrane. The receptor affinity for benzodiazepines was stereospecific, the stronger affinity being for the pharmacologically active isomers.

The regional distribution of benzodiazepine receptors in the rat brain was highest in the cerebral cortex, followed by the hypothalamus, cerebellum, corpus striatum, and medulla. Human brain tissue obtained at autopsy also contained benzodiazepine receptors with stereospecificity and regional distribution similar to those found in the rat brain.\textsuperscript{20} The regional distribution of benzodiazepine receptors parallels the distribution that has been described for GABA receptors. Receptors for benzodiazepine drugs and for GABA are not identical because the compounds do not show competitive binding to the respective receptor sites.\textsuperscript{20} However, the parallel anatomic distributions suggest that benzodiazepines might have some influence on the activity of GABA-containing neurons.
Independently, Braestrup and Squires\textsuperscript{21,22} also identified benzodiazepine receptors in 1977. Their data were consistent with those of Mohler and Okada.\textsuperscript{20} They suggested that the binding site is probably a membrane protein in nerve cells. They further showed that the abilities of various benzodiazepines to bind to receptors correlated well with their pharmacologic potencies as anxiolytic agents. An unusual property of benzodiazepine receptors is their lack of affinity for any of the known neurotransmitters or any of the agonist or antagonist drugs. Braestrup and Squires suggested that there may be unknown endogenous ligands that react with benzodiazepine-specific receptors in brain tissue.

Skolnick \textit{et al.}\textsuperscript{23} have recently published evidence that inosine may be an endogenous ligand of the brain benzodiazepine receptor. Their data show that purines (inosine and hypoxanthine) can function as endogenous ligands and have anticonvulsant properties when injected into cerebral ventricles of experimental animals. Further purification and more precise histochemical location of benzodiazepine receptors will be facilitated by the recent synthesis of a compound (irazepine) that is a noncompetitive, irreversible inhibitor of diazepam binding to receptors.\textsuperscript{24}

The physiologic importance of benzodiazepine receptors is demonstrated in a report by Paul and Skolnick.\textsuperscript{25} They showed that following seizures, produced by either electroshock or injections of pentylentetrazol, the number of specific binding sites for benzodiazepines on cerebral cortex membranes increases. They suggested that the increase of benzodiazepine receptors following experimental seizure activity could enhance the physiologic effect of endogenous ligands (\textit{e.g.}, inosine). These observations may be relevant to the potency and rapid onset of anticonvulsant properties of benzodiazepines in the treatment of status epilepticus.

Benzodiazepines are anticonvulsants for seizures caused by interference with GABA neurotransmission. Inhibitors of GABA synthesis (\textit{e.g.}, allylglycine) cause seizures. Antagonists of GABA such as picrotoxin and bicuculline are convulants. Seizures induced by these mechanisms can be prevented by benzodiazepines. Because phenobarbital and phenytoin are less effective anticonvulsants for seizures produced by these methods, it is suggested that the GABA-ergic mechanism for benzodiazepines is unique and does not extend to mechanisms for other anticonvulsant drugs.\textsuperscript{26}

Benzodiazepines may influence the postsynaptic phenomena at GABA-ergic synapses.\textsuperscript{27} Electrophysiologic studies have shown that benzodiazepines do not have direct GABA agonist activity, but that they do potentiate the effects of GABA. This implies that benzodiazepines can influence GABA transmission only when GABA is present at the synapse. The data suggest that the anticonvulsant property of the drugs results from facilitation of GABA action at receptors on postsynaptic membranes.\textsuperscript{26,27}

Costa and Guidotti\textsuperscript{28} suggest that in the membranes of certain cells, the lipid portion contains proteins that have specific binding sites for GABA and other proteins with specific binding sites for benzodiazepines. These two binding sites are coupled to a modulator protein and a complex of large molecules that may resemble associated subunits in allosteric enzymes.\textsuperscript{26,28} In this model of an allosteric protein modulator for GABA function, the benzodiazepines have no direct GABA-mimetic action; rather, they increase the efficiency of endogenous GABA in its postsynaptic effects. According to the model, when benzodiazepine receptors are occupied, an allosteric change in the GABA receptor complex occurs that favors increased binding of GABA to its own receptor. The ultimate consequence may be an increased GABA-mimetic effect on postsynaptic membrane function.\textsuperscript{27}

Snyder \textit{et al.}\textsuperscript{89} have suggested that the sedative properties of benzodiazepines could result also from facilitation of GABA-ergic neurotransmission, possibly in the cortex. Snyder and Enna\textsuperscript{14} pointed out that the sedative and anxiolytic properties of benzodiazepines can be distinguished from their muscle relaxation effects. Antianxiety properties are distinguished from sedative properties by different dose–response relationships. Antianxiety doses of benzodiazepines do not influence GABA metabolism, and they do not influence the release of GABA from presynaptic nerve terminals.\textsuperscript{14}

Snyder and Enna reviewed data that show that benzodiazepines have an affinity for glycine receptors in spinal cord. The affinity of benzodiazepines for binding to the glycine receptor correlates with their potency as muscle relaxants and antianxiety agents. Snyder \textit{et al.} suggested that benzodiazepines have glycine-mimetic effects. Antianxiety properties of the benzodiazepines could be attributed to glycine-mimetic action in the brainstem. Muscle relaxation could result from glycine-mimetic effects in the spinal cord, according to Snyder \textit{et al.}\textsuperscript{14,20}

Benzodiazepine mechanisms proposed by Snyder \textit{et al.} are summarized in figure 2. There has been no speculation about the neurochemical mechanisms for drug-induced amnesia, probably because neurochemical models for memory are too vague.

Stein, Beluzzi, and Wise\textsuperscript{30} have an alternate theory for the antianxiety effects of benzodiazepines. They
suggested that the drugs may reduce anxiety by decreasing the activities of 5-hydroxytryptamine and acetylcholine synapses. In their model, GABA-mediated inhibition would decrease 5-hydroxytryptamine-mediated transmission.\textsuperscript{31}

**Pharmacologic Mechanisms of Neuroleptic Drugs**

The "neuroleptic" properties of phenothiazines and butyrophenones are well known to anesthesiologists. The sedation (and state of apparent indifference to the environment), decreased gross motor activity, antiemetic action, and alpha-adrenergic blocking action produced by these drugs are exploited when they are used to supplement other intravenously administered agents to produce general anesthesia. Side effects of neuroleptics on extrapyramidal tract function gave early clues about the mechanisms of action of these drugs.\textsuperscript{32,33} Parkinsonism is a disease of extrapyramidal tract function that is characterized by decreased concentrations of dopamine in the basal ganglia, particularly in the corpus striatum. The development of L-dopa therapy for parkinsonism by Cotzias et al.\textsuperscript{34} is well known, and confirmed the idea that extrapyramidal symptoms can result from decreased availability of dopamine in the basal ganglia. Since neuroleptics can mimic the extrapyramidal tract manifestations of parkinsonism, it was suggested that these drugs might influence the normal operation of dopamine in the basal ganglia, and that the pharmacologic actions of these drugs could be related to the interference with dopamine function as a neurotransmitter.\textsuperscript{32}

Neuroleptics do not change the concentration of dopamine in brain tissue. This observation suggested that neuroleptics might act by blocking dopamine receptors inhibiting the normal neurotransmission mediated by dopamine. This hypothesis is supported by the observation that neuroleptics also block the behavioral effects of apomorphine and of amphetamine.\textsuperscript{32} Apomorphine is a direct-acting dopamine agonist, and amphetamine increases the release of dopamine from presynaptic endings. Dopamine receptors have been identified by experiments that use radioactively labeled compounds. The dopamine receptors show stereospecificity, and the binding affinity for dopamine antagonists correlates well with their potency as antipsychotics.\textsuperscript{34} Creese et al.\textsuperscript{32} summarized the current theory for the antipsychotic action of neuroleptic drugs.

1) Neuroleptic drugs block the dopamine receptor. This results in an increased firing rate and increased metabolism of presynaptic dopamine.
2) Neuroleptics also affect presynaptic dopamine receptors that further increase the activation of tyrosine hydroxylase, which results in increased synthesis of dopamine.

3) Neuroleptic drugs increase the amount of dopamine released from electrically stimulated brain slices.

They point out that increasing the concentrations of dopamine by presynaptic actions of neuroleptic drugs is unlikely to be responsible for antipsychotic effects, because other drugs that release dopamine actually mimic or exacerbate symptoms of schizophrenia. Therefore, the current concept is that neuroleptics work by actions on the postsynaptic receptor sites that decrease the neurotransmitter function of dopamine. Creese et al.32 suggest a two-state model for the molecular site of action of neuroleptics. The model is consistent with the demonstration that there are two distinct but interrelated binding sites on postsynaptic membranes.

1) There is a saturable site that has the greatest affinity for radiolabeled dopamine.
2) The second saturable site has its greatest affinity for binding of radiolabeled haloperidol.

According to the model, dopamine agonists bind to the first site and antagonists bind to the second site. The two sites are different aspects of a single receptor that exists in an equilibrium between the two states. The binding of antagonists to the second site converts the receptor to the antagonist state and leaves few agonist sites available to the endogenous neurotransmitter. Lysergic acid diethylamide (LSD) has mixed agonist–antagonist properties in this model system. Affinities of drugs for the haloperidol site (site 2 above) correlate very well with antipsychotic potencies of neuroleptic drugs. This model for antipsychotic action assumes that extrapyramidal side effects result from blockade of dopamine receptors in the corpus striatum and that the antipsychotic properties result from blockade of dopamine receptors in other brain regions.

Seeman et al.34 have reviewed the research that has characterized dopamine receptors. They discuss the presence of high- and low-affinity binding sites for agonist compounds (dopamine or apomorphine). These different binding sites could represent pre- and postsynaptic sites.34

Balsara et al.,36 have studied the effects of drugs on serotonin-containing neurons and their relationships to the neuroleptic effects of haloperidol. Their data suggest that the neuroleptic effect depends upon the balance between dopaminergic and serotonergic neural pathways, and that the serotonin-secreting synapses have an inhibitory action on dopamine neurons.

Joseph, Firth and Waddington38 have very recently proposed a model for the dopaminergic mechanisms and cognitive deficits in schizophrenia. They point out that the biochemical changes associated with schizophrenia are not likely to be confined to imbalances in one neurotransmitter system. Their model proposes a complex interrelationship between neural pathways that control the attention-focusing actions of the patients. Their hypothesis is that schizophrenia results from a breakdown in the mechanisms that control conscious attention.36 Experimental work with neuroleptic drugs has focused mainly on the antipsychotic and extrapyramidal actions, because these are the properties most often encountered. The sedative and other neuroleptic actions may result from modulation of dopaminergic pathways.

Summary

The data reviewed above are the basis for the models of drug action proposed by neuropharmacologists. Mechanisms of benzodiazepine action are currently attributed to increased inhibitory neurotransmission. Costa and Guidotti39 proposed that anticonvulsant and sedative properties are a consequence of enhanced inhibitory neurotransmission mediated by GABA. Muscle relaxation may result from glycine-mimetic actions of benzodiazepines in the spinal cord. Anti-anxiety mechanisms may be effects of both glycine- and GABA-mediated inhibition of specific neuronal pathways in the brainstem and brain (see fig. 2). Antipsychotic and extrapyramidal effects of neuroleptic drugs may be a consequence of their action at dopaminergic receptors.

References

8. Oja SS, Kontro P: Neurotransmitter actions of taurine in the