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GABA

RICHARD W. OLSEN

GABA IS THE MAJOR INHIBITORY NEUROTRANSMITTER IN THE NERVOUS SYSTEM

Several amino acids are found in high concentrations in brain, and some have been established as neurotransmitters. *L-Glutamic acid (glutamate)* is the major neurotransmitter for fast excitatory synaptic transmission, whereas *γ-aminobutyric acid (GABA)* is the major neurotransmitter for fast inhibitory synaptic transmission. Glycine is a secondary rapid inhibitory neurotransmitter, especially in the spinal cord (1,2). Because of the widespread presence and utilization of glutamate and GABA as transmitters, one could say that they are involved in all functions of the central nervous system (CNS), as well as in all diseases. At any point in the CNS, one is either at a cell that uses or responds to glutamate and GABA or no more than one cell removed. Many clinical conditions including psychiatric disorders appear to involve an imbalance in excitation and inhibition, and therapeutics thus involve attempts to restore the balance. The GABA system is the target of a wide range of drugs active on the CNS, including anxiolytics, sedative-hypnotics, general anesthetics, and anticonvulsants (3). See the chapters on GABA in previous editions of this book (1,4).

Since its discovery in the CNS in the early 1950s (5,6), GABA was shown to fulfill the criteria for establishment as a neurotransmitter (Fig. 12.1). It is synthesized by a specific enzyme, L-glutamic acid decarboxylase (GAD), in one step from L-glutamate. Thus, in addition to its role in protein synthesis, in cofactors such as folic acid and in hormones such as thyrotropin-releasing hormone, and its action as a neurotransmitter itself, glutamate must be available in certain nerve endings for biosynthesis of GABA. Much of the glutamate and GABA used as neurotransmitter is derived from glial storage pools of glutamine (2,6). Two genes for GAD have been cloned, and the two forms of the enzyme are proposed to differ in their affinity for the cofactor pyridoxal phosphate and the subcellular localization (7). GABA was shown to be released from electrically stimulated inhibitory nerve cells (8), and a mechanism of rapid removal from the synaptic release site was demonstrated by identification of high-affinity transporter proteins (9,10). The application of GABA and structural analogues to cells innervated by GABAergic neurons produces effects on that target cell identical to those produced by stimulating the inhibitory innervation (11).

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PHYSIOLOGY AND PHARMACOLOGY OF $GABA_A$, $GABA_B$, AND $GABA_C$ RECEPTORS

GABA-mediated synaptic inhibition involves rapid, less than 100-millisecond, inhibitory postsynaptic potentials and slower, more than 100-millisecond, inhibitory postsynaptic potentials. The former were shown by voltage clamp to involve increased chloride ion permeability and to be blocked by the plant convulsant drug picrotoxin, as seen with GABA action in invertebrates, such as crayfish muscle and nerve preparations (12). The rapid chloride current defined a physiologic receptor mechanism termed the *$GABA_A$ receptor,* also pharmacologically defined by the antagonist bicuculline, as well as picrotoxin, and the agonist muscimol (Fig. 12.2). Thus, the $GABA_A$ receptor is a chloride channel regulated by GABA binding, and it is now grouped in the superfamily of ligand-gated ion channel receptors, which includes the well-characterized nicotinic acetylcholine receptor, present at the skeletal neuromuscular junction (13,14).

Chloride channel gating is generally inhibitory on a neuron by virtue of stabilizing the membrane potential near the resting level. However, under conditions of high intracellular chloride, for example, in immature neurons with low capacity to maintain a chloride gradient, increasing chloride permeability can depolarize the membrane potential. This depolarization could be sufficient to fire the cell, and it would be likely to activate certain voltage-gated ion channels, including calcium, that can, in turn, regulate other cellular events. Variable permeability to bicarbonate ions for some subtypes of $GABA_A$ receptor (GABAR) could

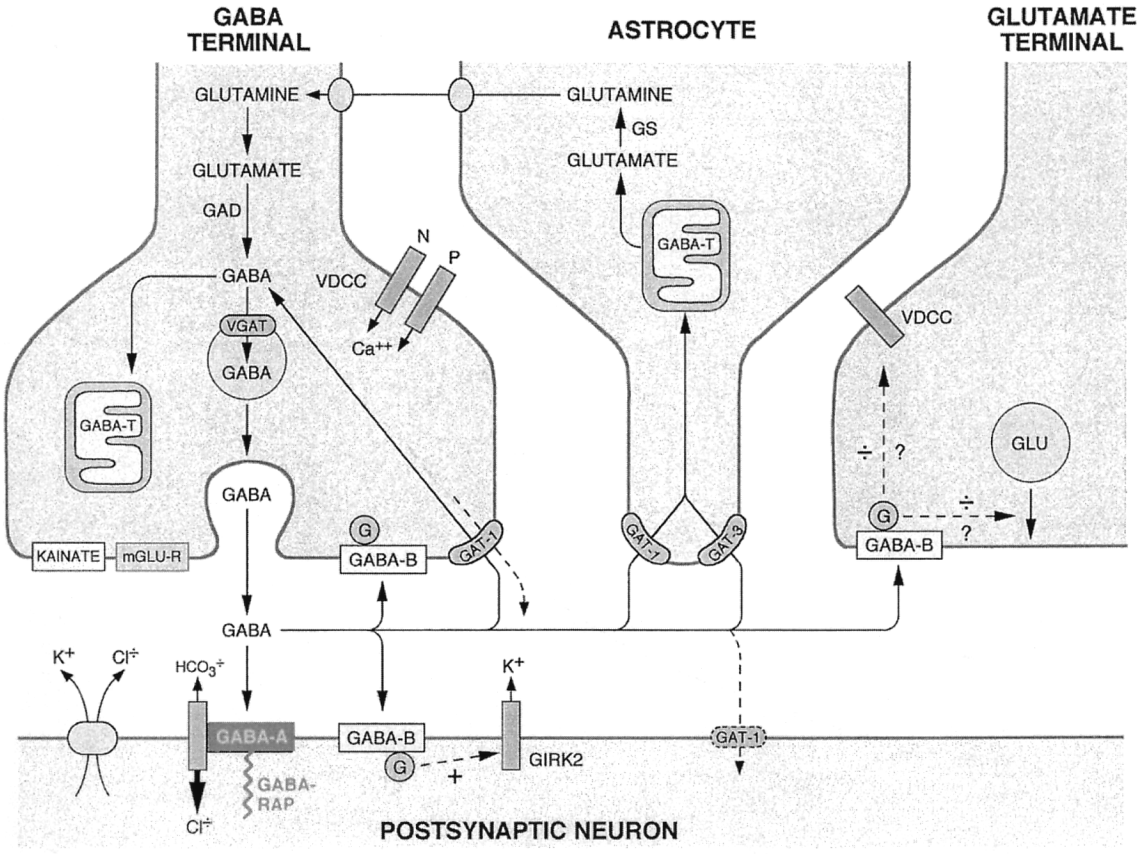

FIGURE 12.1. Schematic GABA synapse. Diagram showing the main features of the GABA synapse. Transporters are indicated by oval symbols, receptors and ion channels by rectangular symbols. **A:** Transporters: GAT-1, GAT-3, plasma membrane GABA transporters; VGAT, vesicular GABA transporter. **B:** Receptors: GABA-A, ionotropic GABA receptor; GABA-B, G-protein–coupled GABA receptor; KAINATE, presynaptic kainate receptor; MGLUR, metabotropic glutamate receptor. **C:** Ion channels: GIRK2, G-protein–coupled inwardly rectifying K^+ channel; VDCC: voltage-dependent calcium channel. **D:** Enzymes: GABA-T, GABA transaminase; GAD, glutamic acid decarboxylase; GS, glutamate synthetase. (Courtesy of O.P. Ottersen; design G. Lothe.)

also play a role in depolarization (15). Such depolarizing GABAR action has been proposed as an important excitatory system in developing brain (16), and it may explain the well-known trophic action of GABA to promote both survival and differentiation during development (17).

The slow inhibitory polysynaptic potentials were shown to be insensitive to $GABA_A$ drugs such as bicuculline, but to be activated by β-chlorophenyl GABA (the antispastic drug baclofen) and to be mediated by a G-protein–coupled receptor that increases potassium conductance (18), now called the $GABA_B$ receptor. A further inhibitory GABA response was observed in some cells to be "non-A, non-B," neither bicuculline nor baclofen sensitive and sometimes called $GABA_C$ (19), and generally sensitive to the GABA analogue *cis*-aminocrotonic acid. $GABA_C$–type inhibition was shown to involve a rapid chloride conductance, as with $GABA_A$ receptors; however, it was not only insensitive to bicuculline, but also not modified by other $GABA_A$ drugs, such as benzodiazepines and anesthetics (19). The eventual cloning of a retinal-specific subunit cDNA ρ that produced bicuculline-insensitive GABA chloride channels appeared to account for $GABA_C$ receptors (20). However, because of the structural and functional homology with $GABA_A$ receptors, the International Union of Pharmacology subcommittee on nomenclature recommended that these ρ receptors not be called $GABA_C$ receptors, but rather a subtype of pharmacologically unique $GABA_A$ receptors (21).

$GABA_B$ receptors were shown to mediate presynaptic inhibition on some nerve endings and postsynaptic inhibition on some cell bodies or dendrites. The coupling mechanism depends on the cell location, because several G-protein–coupled effectors can be used, involving negative modulation of adenylate kinase and negative modulation of inositol tris phosphate production. These lead to activation of potassium channels or inhibition of voltage-gated calcium channels (22). Presynaptic inhibition of GABA release

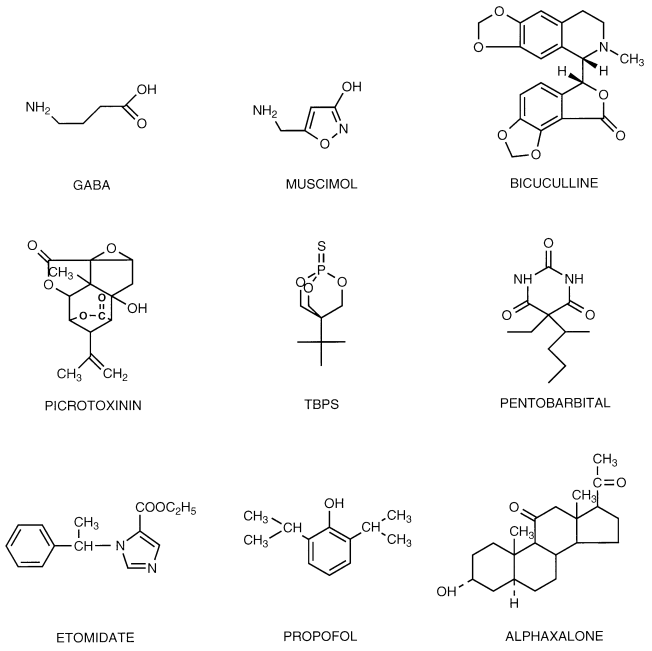

FIGURE 12.2. Chemical structures of $GABA_A$ receptor drugs.

by GABA involves $GABA_B$ autoreceptors, and their activation would be overall excitatory, as opposed to inhibition of glutamate release, which would be overall inhibitory. Considerable effort was therefore expended to determine whether different $GABA_B$ receptors could mediate these very different functions, possibly allowing the development of receptor subtype-specific drugs. Although some classic pharmacology studies supported this hypothesis (18, 22), it was the long-awaited cloning of the $GABA_B$ receptor (23) that established the true situation. The first receptor exists as two splice variants, and additional clones for $GABA_B$ receptor subtype genes have been isolated. Surprisingly, the $GABA_B$ receptors appear to exist as heterodimers, previously unknown for G-protein–coupled receptors. The dimers produce the diverse pharmacologic specificity for the GABA site and the diverse coupling mechanisms observed in nature (24). It seems that the pharmacology of $GABA_B$ receptors is in a very promising infancy.

STRUCTURE AND FUNCTION OF $GABA_A$ RECEPTORS

The GABARs are the major players in CNS function and relevance to psychopharmacology. These receptors, defined by pharmacologists using electrophysiologic and other techniques (14,22), were identified in brain homogenates by radioligand binding (25), and are shown to have the correct specificity for GABA analogues expected from the neuropharmacology (26,27). The GABAR protein (Fig. 12.3) also contains binding sites for benzodiazepines, picrotoxin, barbiturates, and other anesthetics, all of which allosterically interact with each other (28). One or more polypeptides of 45 to 60 kd on sodium dodecylsulfate–polyacrylamide gel

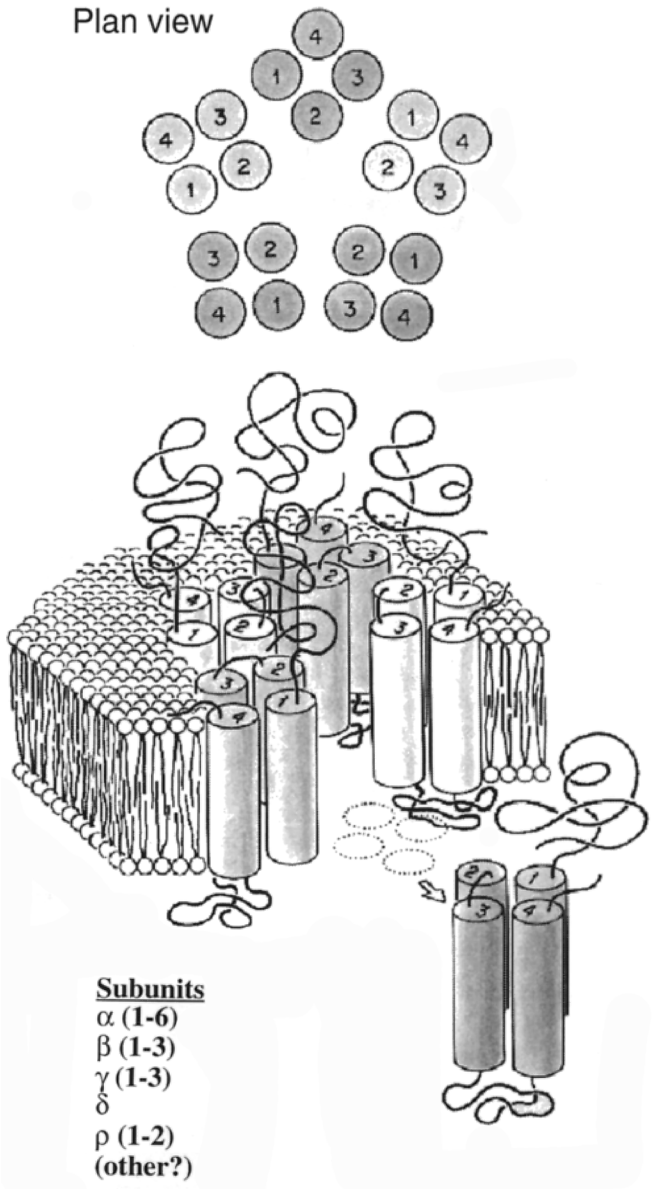

FIGURE 12.3. Schematic $GABA_a$ receptor structure. The chloride channel is shown as a pore in the center of five equivalent subunits, each with four membrane-spanning domains (see the isolated subunit at the **bottom**). Because of the existence of subunit families, many such heteropentamer combinations are possible, each with multiple drug sites. Ligand sites: GABA: agonists (muscimol), antagonists (bicuculline); Benzodiazepine: agonists (flunitrazepam), antagonists (flumazenil), inverse agonists (DMCM); Picrotoxin/Convulsant (TBPS); Barbituate (phenobarbitol); Steroid (alphaxalone, allopregnanolone); Volatile Anesthetic (halothane). (Modified from Olsen RW, Tobin AJ. Molecular biology of $GABA_A$ receptors. *FASEB J* 1990;4:1469–1480, with permission.)

electrophoresis were identified in brain homogenates as constituents of the GABAR by photoaffinity labeling with the radioactive benzodiazepine flunitrazepam (29,30), and monoclonal antibodies were developed to the partially purified bovine receptor, which recognized the photolabeled peptides using Western blotting (31).

The GABAR proteins were purified using benzodiazepine affinity chromatography (32), which allowed partial protein sequencing and expression cloning of two receptor genes (13). GABA-activated currents were demonstrated in *Xenopus* oocytes using cDNAs for two polypeptides that contained the partial sequences within their coded sequence, and these were designated α and β. At first, these were thought (incorrectly) to correspond to the two bands seen in the purified protein (32). These two subunits were related to each other and also to the nicotinic acetylcholine receptor family of subunits, a finding indicating a superfamily of receptor polypeptide genes and a likely heteropentameric structure (Fig. 12.3) (13,14). These two cDNAs were used as probes to clone additional family members with more or less sequence homology to the first two. Those with high homology were named with the same Greek letter, whereas those with less homology were given other Greek letters. The current repertoire involves α1 to 6, β1 to 3, γ1 to 3, δ, ϵ, θ, π, and ρ1 to 3 (21). There are also a few splice variants; for example, γ2 exists in two forms differing in an eight-amino acid insert in the intracellular loop that includes a substrate serine for protein kinase C (33). All the subunits are related to each other and have molecular weights of about 50 kd. The purified receptor protein thus actually contains about a dozen subunit polypeptides, of varying amount (6). Hydropathy plots show that they have a long extracellular N-terminal domain, which has glycosylation sites and is believed to carry the GABA binding site. They have four membrane-spanning domains (M1 to M4) of about 25 residues each, a long intracellular loop between M3 and M4, and a short extracellular C-terminal tail. These subunits are arranged as heteropentamers (Fig. 12.3), several of which are common in nature, but whose expression varies with both age and brain region. The different receptor subtypes have biological differences, such as location, affinity for GABA, and channel properties, as well as pharmacologic heterogeneity. Most receptors contain two copies of one type of α subunit, two copies of one type of β subunit, and a γ subunit. Rarely, another subunit (δ, ϵ, θ) can substitute for γ (30,33). The presence of a γ subunit is needed for benzodiazepine sensitivity, and other subunits affect the detailed specificity. For example, the α subunits define the benzodiazepine pharmacology, and some subunits α4 and α6 do not bind classic benzodiazepine agonists; the detailed pharmacology depends on the small differences in polypeptide sequence for the various subunits (6,34–36). Because of the unique location of receptor subtypes, and thus unique functions of the circuits involved, great hope for new drugs of improved pharmacologic profile has been expressed. The GABAR strategy has certainly not been exhausted.

GABA$_A$ RECEPTORS ARE THE SITES OF ACTION OF BENZODIAZEPINES AND BARBITURATES

The actions of several classes of CNS depressant drugs had for some time been suggested to involve enhancement of inhibitory synaptic transmission. In particular, the anxiolytic effects of benzodiazepines were shown probably to result from potentiation of GABA action (37,38). When the benzodiazepine receptors were discovered using radioligand binding to brain homogenates (1,4,39,40), it was quickly determined that the benzodiazepine binding sites were physically present on the GABA$_A$ receptor–chloride channel complex (28,41). The various types of drug binding site on the GABA$_A$ receptor allosterically interact with each other in the test tube. Barbiturates and related sedatives also enhance GABA$_A$ receptor–mediated inhibition, and their pharmacologic spectrum overlaps with that of the benzodiazepines and related substances, such as zolpidem, zopiclone, and abecarnil (Fig. 12.4). The selective actions of benzodiazepines not shown by barbiturates or vice versa are believed to arise from heterogeneity in GABA receptor sensitivity to the drugs, and corresponding heterogeneity in brain regions, circuits, and functions. Further, some GABARs are insensitive to benzodiazepines but not to barbiturates, as well as additional nonoverlapping, nonGABA actions of high doses, especially barbiturates. In addition, the two classes of drugs have a different mechanism of action at the molecular channel level; barbiturates prolong the lifetime of GABA currents, in addition to gating channels directly at high concentrations, whereas benzodiazepines increase the frequency of opening of GABAR channels and do not directly open channels in the absence of GABA (3,42).

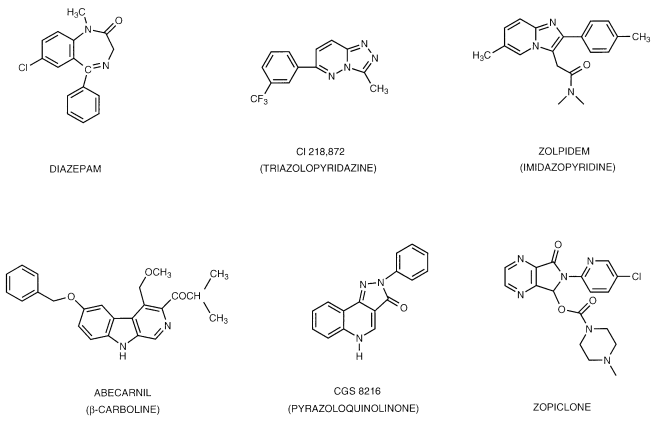

FIGURE 12.4. Chemical structures of drugs active at the benzodiazepine site on the GABA$_A$ receptor–chloride channel complex.

The classical benzodiazepines such as diazepam (Valium) have had a tremendous history in psychopharmacology, reaching tremendous sales, primarily for clinical anxiety (38, 43–45). Other uses of benzodiazepines include sedation, muscle relaxation, and a significant utilization for treatment of panic (1,45). Various structural analogues were developed by numerous firms, with slight variations in pharmacokinetics and other details, and quite a few nonbenzodiazepine structures were discovered that act at the benzodiazepine site on the GABAR to enhance GABA-mediated inhibition (Fig. 12.4) (46). This group includes compounds called β-carbolines, some of which were isolated from biological tissues (47). However, neither the β-carbolines nor any peptides have been demonstrated to act as biological ligands at benzodiazepine receptor sites (45). Surprisingly, some β-carbolines, and indeed, benzodiazepines and other types of chemical structures active on the benzodiazepine site, were found to have the opposite pharmacologic efficacy as classic benzodiazepine ligands such as diazepam; that is, they are anxiogenic and proconvulsant in animals and inhibit GABAR function in cells, while binding to the same sites as agonist benzodiazepine site ligands. These compounds were given the name *inverse agonists* (48,49).

Given this spectrum of efficacy, it would be expected that compounds with true antagonist efficacy would exist, and these were found, for example, Ro15-1788, or flumazenil (50). This compound does not affect GABAR function on its own, but it blocks the actions of both agonists to enhance GABAR function and inverse agonists to inhibit GABAR function. In animals, it also reverses the pharmacologic actions of both agonists and inverse agonists (50). Thus, an antagonist can be used to treat overdose of agonist, or inverse agonist, and it triggers withdrawal in individuals treated on a long-term basis with agonists (45,51). Flumazenil administration to rats after long-term administration of diazepam was found to reverse tolerance rapidly and permanently, and treated animals showed no long-lasting effects but resembled treatment-naive animals (52,53). This finding suggested that benzodiazepine antagonists may be useful in reversing benzodiazepine dependence and also potentially for other GABAR drugs, such as ethanol. This has not proved effective so far, however (43,45).

Certain benzodiazepines have considerable success in the treatment of some types of epilepsy (38). Every emergency medical cart contains injectable benzodiazepine (diazepam, clonazepam, lorazepam) for convulsions and status epilepticus. However, long-term therapy of epilepsy with benzodiazepines is often prevented by the development of tolerance to the anticonvulsant actions, without change in blood levels (54). The development of tolerance to long-term administration of benzodiazepines, and also of withdrawal signs (43, 45,55), is consistent with the development of psychological and physical dependence with these drugs. The potential for abuse with CNS depressant drugs in general and benzodiazepines in particular is well known, as is the interaction with ethanol. This has led to a considerable drop in prescriptions of these agents for routine anxiety. Because the danger of fatal overdose with benzodiazepines is lower than that of ethanol and barbiturates, and because withdrawal symptoms are less dangerous for benzodiazepines than for alcohol, benzodiazepines reached considerable popularity in treatment of alcoholism. However, the two drugs show cross-tolerance and cross-dependence, so substitution of benzodiazepines for ethanol is merely substituting one addiction for another (55).

Conversely, an interesting observation was made with the benzodiazepine partial inverse agonist Ro15-4513. This compound was found to antagonize the behavioral effects of ethanol (49), as well as the *in vitro* action of ethanol to enhance GABAR function (56). (Ethanol and GABA are discussed further later.) Moreover, the action of Ro15-4513 to antagonize ethanol occurred under conditions of assay, such as behavior, tissue, or species, in which Ro15-4513 itself did not exhibit inverse agonist activity or inhibit GABAR function, nor did it reverse the actions of pentobarbital (56–59). Thus, this compound or one like it had potential as an ''alcohol antidote'' in humans, by reducing intoxication and perhaps withdrawal and craving. Unfortunately, the ethical decisions involved in prescribing such a drug were made moot by discovery that Ro15-4513 was tremorigenic and proconvulsant in nonhuman primates, as well as other animals (60).

Understanding the mechanism of tolerance development has been a research topic of high interest, especially for epilepsy treatment, but also because of the relevance to brain plasticity. Whereas long-term administration of benzodiazepines may produce tolerance in part by down-regulation of receptor levels, considerable evidence suggests that receptors are not removed, but rather are altered in some way to produce tolerance (61–64). Besides tolerance development to long-term use of agonist benzodiazepines, sensitization to the actions of inverse agonists is observed; that is, excitatory benzodiazepine receptor ligands become more efficacious (65). This may resemble the kindling process seen with long-term administration of inverse agonists; that is, repeated administration of nonconvulsant doses of inverse agonists eventually leads to convulsions to that dose. This resembles the electrical kindling model of epilepsy, in which repeated electrical stimuli with nonconvulsive amplitude eventually evoke a seizure (66). Thus, long-term administration of benzodiazepine agonists or inverse agonists may shift the set point of the GABAR toward the excitatory or lower functional end of the spectrum (65,67). Dependence on benzodiazepines and alcohol resulting from long-term administration (abuse) may be exacerbated by a kindling-like development of increased severity of withdrawal symptoms, with an increased risk of relapse (68). Another aspect of the tolerance model is the possibility of replacing one type of GABAR subunit with another that still responds to GABA

but not to the chronically administered modulatory drug (69,70).

$GABA_A$ RECEPTORS ARE THE TARGETS OF ALCOHOL, GENERAL ANESTHETICS, AND NEUROSTEROIDS

Alcohols are CNS depressants with a pharmacologic spectrum of action overlapping those of the benzodiazepines and barbiturates, known to act by enhancement of GABAR. Long-chain alcohols have anesthetic activity, as does ethanol at high doses (greater than 100 mM), whereas the intoxicating effects at lower concentrations (10 to 100 mM) have been suggested to involve blockade of *N*-methyl-d-aspartate (NMDA)–type glutamate receptors (71) or enhancement of GABAR (72–74). Because the latter effect varies considerably among, for example, laboratories, preparations, assays, and brain regions, unique ethanol-sensitive subtypes of GABAR were suggested, but they have not been established. Alternatively, and most popular currently, is the hypothesis that ethanol acts on GABAR indirectly to produce important aspects of its pharmacologic actions in cells and in animals (75). For example, ethanol may interact with membrane signaling proteins that regulate GABAR and NMDA receptors.

$GABA_A$ receptor function appears to be modulated by an endogenous substance: not a benzodiazepine-like or a picrotoxin-like peptide, but a barbiturate-like steroid. The neurosteroids are endogenous steroid hormone metabolites that have direct and rapid actions on cells not involving steroid hormone receptors or regulation of gene expression. Progesterone was shown to produce rapid sedative activity, a finding that led to the development of the clinical intravenous steroid anesthetic, alphaxalone. Progesterone has anxiolytic and anticonvulsant activity; discontinuation after long-term administration leads to withdrawal signs that are clearly CNS mediated: these actions are mediated by the progesterone metabolite, produced primarily in the adrenals but to some extent in brain, 3α-hydroxy-5-α-pregnane-20-one (76–78). The neuroactive steroids act principally by binding directly to membrane $GABA_A$ receptors and enhancing their function in a manner resembling the barbiturates (79,80).

Many related steroid compounds have been developed as lead compounds for potential use as antiepileptics, anxiolytics, and sedative-hypnotics (81). Whether these compounds are biologically relevant is uncertain, but this is suggested by considerable evidence. Endogenous steroids reach levels sufficient to modulate $GABA_A$ receptors during conditions of stress and anxiety, and during pregnancy (82,83). These compounds are probably involved in CNS plasticity responses to chronic stress and possibly epileptogenesis, and even drug dependence (84,85). The progesterone metabolite is the endogenous steroid that appears to be the most likely to be biologically relevant, but metabolites of testosterone and cortisone are also active (77,81). Pregnenolone sulfate, a biosynthetic intermediate in the synthesis of all the steroid hormones, present in high levels in the CNS, has weak activity as an antagonist of GABA function, but this appears to involve another mechanism and is unlikely to be biological (85). Neurosteroid action apparently has relevance to alcohol action. GABA-active steroids can substitute for ethanol in discriminative stimulus testing in rats and monkeys, and neurosteroids are synthesized in brain in response to ethanol administration and may mediate some of the pharmacologic actions (86). The neurosteroid-GABA connection potentially may be fruitful for new applications in psychopharmacology. As the endogenous functions of neurosteroids in stress control, seizure protection, attention and learning, and possibly even sleep, become better delineated, additional therapeutic approaches may arise.

Enhancement of $GABA_A$ receptor-mediated inhibition is currently the major candidate molecular mechanism for a generalized theory of general anesthesia. Everyone agrees that the anesthetic action of the steroid alphaxalone occurs by enhancement of GABAR (84,85), and many investigators believe that the actions of high-dose ethanol and other alcohols as anesthetics probably do also (75,87). Further, the sedative-hypnotic effects, and possibly anesthetic effects, of barbiturates and related drugs are considered to act through GABAR (88). Anesthetics are now believed to have a greater effect on membrane ion channels than on many other biological systems and to affect synaptic transmission more potently than nerve conduction. Ligand-gated ion channels, especially receptors for glutamate, glycine, and GABA, are most sensitive (89). All general anesthetics enhance GABA function at anesthetic concentrations (36,75). The ketamine-phencyclidine category of dissociative anesthetics enhances some GABA synapses, but these agents probably inhibit NMDA receptors more potently; further, they produce a different sort of anesthesia (90).

The *Meyer-Overton hypothesis* shows a high correlation for many drugs with respect to potency as a general anesthetic and partition in an oil-water biphasic system. The Meyer-Overton correlation has been found wanting, because of the existence of compounds with identical lipid solubility (oil-water partition coefficient), boiling point, and dipole moment, such as halogenated cyclobutane isomers, that differ in anesthetic potency: only the anesthetic isomers enhance $GABA_A$ receptors (91). Volatile anesthetics and alcohols (87), as well as intravenous agents such as barbiturates, propofol, neuroactive steroids, and etomidate, are all able at anesthetic concentrations to modulate $GABA_A$ receptor binding assays *in vitro* as well as to enhance $GABA_A$ receptor function in cells (36,88).

$GABA_A$ RECEPTORS ARE THE TARGETS OF MANY CNS EXCITANTS

Many naturally occurring and synthetic convulsive agents are blockers of GABA-mediated inhibition (46). The prototypic $GABA_A$ channel blocker picrotoxinin (Fig. 12.2) is isolated from plants of the moonseed family, Menispermaceae, and its close relatives tutin and coriamyrtin, from the New Zealand tutu plant *Coriaria arborea* (92), known as a loco weed, which causes occasional poisonings in cows and even in people. A major category of synthetic potent neurotoxic chemicals (93), comprising the cage convulsants, was discovered to consist of noncompetitive $GABA_A$ receptor antagonists acting at the picrotoxinin site (93–95). One of these drugs, *t*-butyl bicylcophosphorothionate (Fig. 12.2), is a major research tool used to assay GABA receptors by radioligand binding (96). Synthetic butyrolactones with depressant and excitatory actions have also been described for the picrotoxinin site (97). In addition, this drug target appears to be the site of action of the experimental convulsant pentylenetetrazol (PTZ) and numerous polychlorinated hydrocarbon insecticides, including dieldrin, α-endosulfan, and lindane (93). The monoterpenoid thujone is the active constituent of oil of wormwood, the major ingredient of the famous green liqueur, absinthe, outlawed in about 1910. Absinthe was reputed to have hallucinogenic action and to be an inspiration for *fin de siècle* French artists and poets (92). Oil of wormwood has a history as a medicinal herb for treating intestinal worms and killing insects, and thujone is known to cause convulsions in high doses; thujone was demonstrated to be a $GABA_A$ receptor channel blocker like picrotoxinin (98). It remains anecdotal whether thujone/wormwood/absinthe produces psychic actions additional to those of the ethanol in the liqueur.

GABA-blocking agents thus have potential pharmacologic utility as excitants. Although at one time listed in the *Merck Index* and in pharmacology textbooks as a "barbiturate overdose antidote," picrotoxin is too dangerous as a convulsant to attempt to find an appropriate dose in the clinic. PTZ and related agents are known to show anxiogenesis in low doses, but also proabsence seizures. An alerting, attention-activation mechanism may figure to promote learning and memory in certain tasks, that is, nootropism. Partial inverse agonists at the benzodiazepine site, such as Ro15-4513, have been considered as candidates for memory enhancement (38,99), as well as for actions as antagonists and possible anticraving, antiwithdrawal agents for the treatment of addiction to benzodiazepines, alcohol, and many other drugs of abuse, as discussed earlier (1,60,69).

GENETIC ENGINEERING AND PSYCHOPHARMACOLOGY

Gene targeting and transgenic mice have demonstrated several important roles for GABA in the CNS. Knockouts of both GAD67 and $GABA_A$ receptor subunit β3 lead to cleft palate and early neonatal lethality (100–102). GAD65 knockout mice show increased anxiety, increased sensitivity to benzodiazepines, and impaired developmental plasticity in the cortex (103,104). Epilepsy results from knockout of GAD65, GABAR β3, and GABAR δ subunit. Other phenotypic deficits include motor incoordination, movement disorders, cognitive defects, and other CNS circuitry problems resulting from lack of inhibitory synaptic transmission. In particular, the GABAR β3 subunit is implicated in the human genetic disease Angelman syndrome, associated with mutation in maternal chromosome 15q and typified by severe mental retardation, epilepsy, motor incoordination, and sleep disorder (105). Mice targeted for this subunit have a phenotype remarkably similar to Angelman syndrome, especially the epilepsy, but also including the cognitive, motor, and sleep impairment (106).

The γ2 subunit knockout shows early neonatal lethality (107), without cleft palate, involving impaired clustering of $GABA_A$ receptors at synapses (108). Even heterozygotes, with presumably a partial deficit of γ2-containing GABAR, have impaired synapses and overanxious and paranoid behavior (109). Because GABARs are important drug targets, some GABAR subunit knockout mice have impaired sensitivity to drugs, such as decreased response to benzodiazepines in γ2 homozygous knockouts (107). Increased response to benzodiazepines is seen in γ2 heterozygous knockouts or in γ2L null mutants (109, 110). Reduced sensitivity to anesthetics was seen in β3 but not α6 knockouts (102), and reduced sensitivity to neuroactive steroids is observed in the δ subunit knockout (111). This finding may be interesting in light of the apparent biological role of the neurosteroids in normal CNS. Gene targeting in mice also has been employed to "knock in" a mutation of the α1 subunit H101N, which prevents benzodiazepine binding to GABAR containing this subunit (112). The resulting animals have greatly impaired sensitivity to the sedative but not the anxiolytic actions of the benzodiazepines, whereas anticonvulsant activity is partially reduced. This finding indicates that the subtypes of GABAR containing the α1 subunit and the brain circuits in which they function are the substrates for benzodiazepine-stimulated sedation, whereas other GABARs, containing α2, α3, and α5, with α2 the most abundant and the major candidate, subserve specifically the role of GABARs in anxiety pathways sensitive to benzodiazepine therapy. (The observations of Rudolph et al., 1999 (112) were verified by McKernan et al., 2000 (113) for the role of the α1 subunit in the sedative actions of benzodiazepines, and extended by Low et al., 2000 (114) for the role of the α2 subunit in the anxiolytic actions of benzodiazepines.) Thus, new biotechnology applied to drug development is continuing to make new advances in psychopharmacology based on this now relatively "old" or at least well-known neurotransmitter system, GABA.

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REFERENCES

1. Paul SM. GABA and glycine. In: Bloom FE, Kupfer DJ, eds. *Psychopharmacology: the fourth generation of progress.* New York: Raven, 1995:87–94.
2. Olsen RW, DeLorey TM. GABA and glycine. In: Siegel GJ, Agranoff BW, Albers RW, et al., eds. *Basic neurochemistry,* sixth ed. New York: Lippincott Williams & Wilkins, 1999:335–346.
3. Macdonald RL, Olsen RW. $GABA_A$ receptor channels. *Annu Rev Neurosci* 1994;17: 569–602.
4. Enna SJ, Möhler H. γ-Aminobutyric acid (GABA) receptors and their association with benzodiazepine recognition sites. In: Meltzer HY, ed. *Psychopharmacology: the third generation of progress.* New York: Raven, 1987:265–272.
5. Roberts E. Disinhibition as an organizing principle in the nervous system: the role of the GABA system. In: Roberts E, Chase TN, Tower DB, eds. *GABA in nervous system function.* New York: Raven, 1976:515–539.
6. Martin DL, Olsen RW, eds. *GABA in the nervous system: the view at 50 years.* Philadelphia: Lippincott Williams & Wilkins, 2000.
7. Erlander MC, Tobin AJ. The structural and functional heterogeneity of glutamic acid decarboxylase: a review. *Neurochem Res* 1991;16:215–226.
8. Otsuka J, Iversen LL, Hall ZW, et al. Release of γ-aminobutyric acid from inhibitory nerves of lobster. *Proc Natl Acad Sci USA* 1966;56:1110–1115.
9. Guastella J, Nelson N, Nelson H, et al. Cloning and experession of a rat brain GABA transporter. *Science* 1990;249:1303–1306.
10. Borden LA. GABA transporter heterogeneity: pharmacology and cellular locations. *Neurochem Int* 1996;29:335–356.
11. Krnjevic K. Chemical nature of synaptic transmission in vertebrates. *Physiol Rev* 1974;54:418–540.
12. Takeuchi A. Studies of inhibitory effects of GABA in invertebrate nervous systems. In: Roberts E, Chase TN, Tower DB, eds. *GABA in nervous system function.* New York: Raven, 1976: 255–267.
13. Schofield PR, Darlison MG, Fujita N, et al. Sequence and functional expression of the $GABA_A$ receptor shows a ligand gated receptor superfamily. *Nature* 1987;328:221–227.
14. Olsen RW, Tobin AJ. Molecular biology of $GABA_A$ receptors. *FASEB J* 1990;4:1469–1480.
15. Rivera C, Volpio J, Payne JA, et al. The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 1999;397:251–254.
16. Ben-Ari Y, Khazipov R, Leinekugel X, et al. $GABA_A$, NMDA and AMPA receptors: a developmentally regulated ''menage a trois.'' *Trends Neurosci* 1997;20:523–529.
17. Belhage B, Hansen GH, Elster L, et al. Effects of γ-aminobutyric acid (GABA) on synaptogenesis and synaptic function. *Perspect Dev Neurobiol* 1998;5:235–246.
18. Bowery NG. $GABA_B$ receptor pharmacology. *Annu Rev Pharmacol Toxicol* 1993;33:109–117.
19. Johnston GAR. Molecular biology, pharmacology, and physiology of $GABA_C$ receptors. In: Enna SJ, Bowery NG, eds. *The GABA receptors,* second ed. Totowa, NJ: Humana, 1997: 297–323.
20. Shimada S, Cutting G, Uhl GR. γ-Aminobutyric acid A or C receptors? GABA ρ1 receptor RNA induces bicuculline-, barbiturate-, and benzodiazepine-insensitive GABA responses in *Xenopus* oocytes. *Mol Pharmacol* 1992;41:683–687.
21. Barnard EA, Skolnick P, Olsen RW, et al. Sub-types of γ-aminobutyric $acid_A$ receptors: classification on the basis of subunit structure and receptor function. Int Union Pharmacol XV. *Pharmacol Rev* 1998;50:291–313.
22. Enna SJ, Bowery NG, eds. *The GABA receptors,* second ed. Totowa, NJ: Humana, 1997.
23. Kaupmann K, Huggel K, Heid J, et al. Expression cloning of $GABA_B$ receptors uncovers similarity to metabotropic glutamate receptors. *Nature* 1997;386:239–246.
24. Marshall FH, Jones KA, Kaupmann K, et al. $GABA_B$ receptors: the first 7TM heterodimers. *Trends Pharmacol Sci* 1999;20: 396–399.
25. Zukin SR, Young AB, Snyder SH. γ-Aminobutyric acid binding to receptor sites in rat central nervous system. *Proc Natl Acad Sci USA* 1974;71:4802–4807.
26. Krogsgaard-Larsen P, Johnston GAR. Structure-activity studies on the inhibition of GABA binding to rat brain membranes by muscimol and related compounds. *J Neurochem* 1978;30: 1377–1382.
27. Olsen RW, Venter JC, eds. *Benzodiazepine/GABA receptors and chloride channels: structural and functional properties, receptor biochemistry and methodology,* vol 5. New York: Alan R. Liss, 1986.
28. Olsen RW. Drug interactions at the GABA receptor-ionophore complex. *Annu Rev Pharmacol Toxicol* 1982;22:245–277.
29. Möhler H, Battersby MK, Richards JG. Benzodiazepine receptor protein identified and visualized in brain tissue by a photoaffinity label. *Proc Natl Acad Sci USA* 1980;77:1666–1670.
30. Sieghart W. Structure and pharmacology of $GABA_A$ receptor subtypes. *Pharmacol Rev* 1995;47:181–234.
31. Schoch P, Richards JG, Haring P, et al. Co-localization of $GABA_A$ receptor and benzodiazepine receptors in the brain shown by monoclonal antibodies. *Nature* 1985;314:168–171.
32. Sigel E, Stephenson FA, Mamalaki C, et al. A γ-aminobutryric acid/benzodiazepine receptor complex from bovine cerebral cortex: purification and partial characterization. *J Biol Chem* 1983; 258:6965–6971.
33. McKernan RM, Whiting PJ. Which $GABA_A$ receptor subtypes really occur in the Brain? *Trends Neurosci* 1996;19:139–143.
34. Lüddens H, Korpi ER, Seeburg PH. $GABA_A$/benzodiazepine receptor heterogeneity: neurophysiological implications. *Neuropharmacology* 1995;34:245–254.
35. Sigel E, Buhr A. The benzodiazepine binding site of $GABA_A$ receptors. *Trends Pharmacol Sci* 1997;18:425–429.
36. Olsen RW. The molecular mechanism of action of general anesthetics: structural aspects of interactions with $GABA_A$ receptors. *Toxicol Lett* 1998;100–101:193–201.
37. Costa E, Greengard P, eds. Mechanism of action of benzodiazepines. *Adv Biochem Psychopharmacol* 1975;14:318.
38. Haefely W. Allosteric modulation of the $GABA_A$ receptor channel: a mechanism for interaction with a multitude of central nervous system functions. In: Möhler H, DaPrada M, eds. *The challenge of neuropharmacology.* Basel: Editiones Roche, 1994: 15–39.
39. Squires RF, Braestrup C. Benzodiazepine receptors in rat brain. *Nature* 1977;266:732–734.
40. Möhler H, Okada T. Benzodiazepine receptor: demonstration in the central nervous system. *Science* 1977;198:849–851.
41. Tallman J, Thomas J, Gallager D. GABAergic modulation of benzodiazepine binding site sensitivity. *Nature* 1978;274: 383–385.
42. Study RE, Barker JL. Diazepam and (−)-pentobarbital: fluctuation analysis reveals different mechanisms for potentiation of GABA responses in cultured central neurons. *Proc Natl Acad Sci USA* 1981;78:7180–7184.

43. Usdin E, Skolnick P, Tallman JF, et al., eds. *Pharmacology of benzodiazepines.* London: MacMillan, 1982.
44. Zorumski CF, Isenberg KE. Insights into the structure and function of GABA-benzodiazepine receptors: ion channels and psychiatry. *Am J Psychiatry* 1991;148:162–173.
45. Biggio G, Sanna E, Serra M, Costa E, eds. $GABA_A$ Receptors and anxiety. *Adv Biochem Psychopharmacol* 1995;48:459.
46. Olsen RW, Gordey M. $GABA_A$ receptor chloride ion channels. In: Endo M, Kurachi Y, Mishina M, eds. *Handbook of experimental pharmacology,* vol 147. Heidelberg: Springer, 2000: 497–515.
47. Braestrup C, Nielsen M, Olsen CE. Urinary and brain beta-carboline-3-carboxylates as potent inhibitors of brain benzodiazepine receptors. *Proc Natl Acad Sci USA* 1980;77:2288–2292.
48. Braestrup C, Schmiechen R, Neff G, et al. Interaction of convulsive ligands with benzodiazepine receptors. *Science* 1982;216: 1241–1243.
49. Bonetti EP, Burkhard WP, Gabl M, et al. Ro15-4513:Partial inverse agonist at the BZR and interaction with ethanol. *Pharmacol Biochem Behav* 1989;31:733–749.
50. Hunkeler W, Möhler H, Pieri L, et al. Selective antagonists of benzodiazepines. *Nature* 1981;290:414–516.
51. File SE, Pellow S. Do the intrinsic actions of benzodiazepine receptor antagonists imply the existence of an endogenous ligand for benzodiazepine receptors? *Adv Biochem Psychopharmacol* 1986;41:187–202.
52. Gonsalves SF, Gallager DW. Persistent reversal of tolerance to anticonvulsant effects and GABAergic subsensitivity by a single exposure to benzodiazepine antagonist during chronic benzodiazepine administration. *J Pharmacol Exp Ther* 1987;244:79–83.
53. Tietz EI, Zeng XJ, Chen S, et al. Antagonist-induced reversal of functional and structural measures of hippocampal benzodiazepine tolerance. *J Pharmacol Exp Ther* 1999;291:932–942.
54. Olsen RW. Antiepileptic actions of benzodiazepines. In: Faingold CL, Fromm GH, eds. Drugs for control of epilepsy: actions on neuronal networks involved in seizure disorders. Boca Raton, FL: CRC, 1991:463–476.
55. Trimble MR, ed. *Benzodiazepines divided: a multidisciplinary review.* New York: Wiley, 1983.
56. Suzdak PD, Glowa JR, Crawley JN, et al. A selective imidazobenzodiazepine antagonist of ethanol in the rat. *Science* 1986; 234:1243–1247.
57. Hoffman PL, Tabakoff B, Szabo G, et al. Effect of an imidazobenzodiazepine, Ro15-4513, on the incoordination and hypothermia produced by ethanol and pentobarbital. *Life Sci* 1987; 41:611–619.
58. Kulkarni SK, Ticku MK. Ro15-4513 but not FG7142 preferentially reverses anticonvulsant effects of ethanol against bicuculline and picrotoxin-induced convulsions in rats. *Pharmacol Biochem Behav* 1989;32:233–240.
59. Nutt DJ, Lister RG. Antagonizing the anticonvulsant effect of ethanol using drugs acting at the benzodiazepine/GABA receptor complex. *Pharmacol Biochem Behav* 1989;31:751–755.
60. Lister RG, Nutt DJ. Is Ro 15-4513 a specific alcohol antagonist? *Trends Neurosci* 1987;10:223–225.
61. Gallager DW, Laskoski JM, Gonsalves SF, et al. Chronic benzodiazepine treatment decreases postsynaptic GABA sensitivity. *Nature* 1984;308:74–77.
62. Tietz EI, Chiu TH, Rosenberg HC. Regional GABA/benzodiazepine/chloride channel coupling after acute and chronic benzodiazepine treatment. *Eur J Pharmacol* 1989;167:57–65.
63. Miller LG, Greenblatt DJ, Barnhill JG, et al. Chronic benzodiazepine administration. I. Tolerance is associated with benzodiazepine receptor down-regulation and decreased GABA receptor function. *J Pharmacol Exp Ther* 1988;246:170–177.
64. Poisbeau P, Williams SR, Mody I. Silent $GABA_A$ synapses during flurazepam withdrawal are region-specific in the hippocampal formation. *J Neurosci* 1997;17:3467–3475.
65. Little HJ, Nutt DJ, Taylor SC. Bidirectional effects of chronic treatment with agonists and inverse agonists at the benzodiazepine receptor. *Brain Res Bull* 1987;19:371–378.
66. Goddard GV. Development of epileptic seizures through brain stimulation at low intensity. *Nature* 1967;214:1020–1021.
67. Stephens DN, Schneider HH, Weidmann R, et al. Decreased sensitivity to benzodiazepine receptor agonists and increased sensitivity to inverse agonists following chronic treatments: evidence for separate mechanisms. *Adv Biochem Psychopharmacol* 1988;45:337–345.
68. Kokka N, Sapp DW, Taylor AN, et al. The kindling model of alcohol dependence: similar persistent reduction in seizure threshold to pentylenetetrazol in animals receiving chronic ethanol or chronic pentylenetetrazol. *Alcohol Clin Exp Res* 1993; 17:525–531.
69. Costa E, Auta J, Caruncho H, et al. A search for a new anticonvulsant and anxiolytic benzodiazepine devoid of side effects and tolerance liability. *Adv Biochem Psychopharmacol* 1995;48: 75–92.
70. Holt RA, Bateson AN, Martin IL. Chronic treatment with diazepam or abecarnil differentially affects the expression of $GABA_A$ receptor subunit mRNAs in the rat cortex. *Neuropharmacology* 1996;35:1457–1463.
71. Lovinger DM, White G, Weight FF. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science* 1989; 243:1721–1724.
72. Suzdak PD, Schwartz RD, Skolnick P, et al. Ethanol stimulates GABA receptor-mediated chloride transport in rat brain synaptoneurosomes. *Proc Natl Acad Sci USA* 1986;83:4071–4075.
73. Allan AM, Harris RA. Acute and chronic ethanol treatments alter GABA receptor-operated chloride channels. *Pharmacol Biochem Behav* 1987;27:665–670.
74. Mehta AK, Ticku MK. Ethanol potentiation of GABAergic transmission in cultured spinal cord neurons involves $GABA_A$-gated chloride channels. *J Pharmacol Exp Ther* 1988;246: 558–564.
75. Mihic SJ, Harris RA. Alcohol actions at the $GABA_A$ receptor/chloride channel complex. In: Dietrich RA, Erwin VG, eds. *Pharmacological effects of ethanol on the nervous system.* Boca Raton, FL: CRC, 1996:51–72.
76. Majewska MD, Harrison NL, Schwartz RD, et al. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* 1986;232:1004–1007.
77. Simmonds MA. Modulation of the $GABA_A$ receptor by steroids. *Semin Neurosci* 1991;3:231–239.
78. Smith SS, Gong QH, Hsu FC, et al. $GABA_A$ receptor $\alpha 4$ subunit suppression prevents withdrawal properties of an endogenous steroid. *Nature* 1998;392:926–930.
79. Harrison NL, Vicini S, Barker JL. A steroid anesthetic prolongs inhibitory postsynaptic currents in cultured rat hippocampal neurons. *J Neurosci* 1987;7:604–609.
80. Lambert JJ, Belelli D, Hill-Venning C, et al. Neurosteroids and $GABA_A$ receptor function. *Trends Pharmacol Sci* 1995;16: 295–303.
81. Gee KW, McCauley L, Lan NC. A putative receptor for neurosteroids on the $GABA_A$ receptor complex: the pharmacological properties and therapeutic potential of epalons. *Crit Rev Neurobiol* 1995;9:207–277.
82. Barbaccia ML, Roscetti G, Trabucchi M, et al. Time-dependent changes in rat brain neuroactive steroid concentrations and $GABA_A$ receptor function after acute stress. *Neuroendocrinology* 1996;63:166–172.
83. Concas A, Mostallino MC, Porcu P, et al. Role of brain allopregnanolone in the plasticity of $GABA_A$ receptor in rat brain

during pregnancy and after delivery. *Proc Natl Acad Sci USA* 1998;95:13284–13289.
84. Paul SM, Purdy RH. Neuroactive steroids. *FASEB J* 1992;6: 2311–2322.
85. Olsen RW, Sapp DW. Neuroactive steroid modulation of $GABA_A$ receptors. *Adv Biochem Psychopharmacol* 1995;48: 57–74.
86. Morrow AL, Janis GC, VanDoren MJ, et al. Neurosteroids mediate pharmacological actions of ethanol: a new mechanism of ethanol action? *Alcohol Clin Exp Res* 1999;23:1933–1940.
87. Narahashi T, Arakawa O, Nakahiro M, et al. Effects of alcohols on ion channels of cultured neurons. *Ann NY Acad Sci* 1991; 625:26–36.
88. Olsen RW, Fischer JB, Dunwiddie TV. Barbiturate enhancement of GABA receptor binding and function as a mechanism of anesthesia. In: Roth S, Miller K, eds. *Molecular and cellular mechanisms of anaesthetics.* New York: Plenum, 1986:165–177.
89. Franks NP, Lieb WR. Which molecular targets are most relevant to general anesthesia? *Toxicol Lett* 1998;100–101:1–8.
90. Murray TF. Basic pharmacology of ketamine. In: Bowdle TA, Horita A, Kharasch ED, eds. *The Pharmacologic basis of anesthesiology.* New York: Churchill Livingstone, 1994:337–355.
91. Mihic SJ, McQuilken SJ, Eger EI, et al. Potentiation of γ-aminobutyric acid type A receptor-mediated chloride currents by novel halogenated compounds correlates with their abilities to induce general anesthesia. *Mol Pharmacol* 1994;46:851–857.
92. Olsen RW. Absinthe and GABA receptors. *Proc Natl Acad Sci USA* 2000;97:4417–4418.
93. Casida JE, Palmer CJ. 2,6,7-Trioxabicyclo[2.2.2]octanes: chemistry, toxicology, and action at the GABA-gated chloride channel. *Adv Biochem Psychopharmacol* 1988;45:109–123.
94. Bowery NG, Collins JF, Hill RG. Bicyclic phosphorus esters that are potent convulsants and GABA antagonists. *Nature* 1976;261:601–603.
95. Ticku MK, Olsen RW. Cage convulsants inhibit picrotoxinin binding. *Neuropharmacology* 1979;18:315–318.
96. Squires RF, Casida JE, Richardson M, et al. [^{35}S]*t*-Butyl bicyclophosphorothionate binds with high affinity to brain-specific sites coupled to $GABA_A$ and ion recognition sites. *Mol Pharmacol* 1983;23:326–336.
97. Holland KD, Ferrendelli JA, Covey DF, et al. Physiological regulation of the picrotoxin receptor by γ-butyrolactone and γ-thiobutyrolactone in cultured hippocampal neurons. *J Neurosci* 1990;10:1719–1727.
98. Höld KM, Sirisoma SI, Ikeda T, et al. α-Thujone (the active component of absinthe): $GABA_A$ receptor modulation and metabolic detoxification. *Proc Natl Acad Sci USA* 2000;97: 3826–3831.
99. Venault P, Chapouthier G, Prado de Carvalho L, et al. Benzodiazepine impairs and β-carboline enhances performance in learning and memory tasks. *Nature* 1986;321:864–866.
100. Asada H, Kawamura Y, Maruyama K, et al. Cleft palate and decreased brain GABA in mice lacking the 67-kDa isoform of glutamic acid decarboxylase. *Proc Natl Acad Sci USA* 1997;94: 6496–6499.
101. Kash SF, Johnson RS, Tecott LH, et al. Epilepsy in mice deficient in the 65-kDa isoform of glutamic acid decarboxylase. *Proc Natl Acad Sci USA* 1997;94:14060–14065.
102. Olsen RW, Homanics GE. Function of $GABA_A$ receptors: insights from mutant and knockout mice. In: Martin DL, Olsen RW, eds. GABA in the nervous system: the view at 50 years. Philadelphia: Lippincott Williams & Wilkins, 2000:81–96.
103. Hensch TK, Fagiolini M, Mataga N, et al. Local GABA circuit control of experience-dependent plasticity in developing visual cortex. *Science* 1998;282:1504–1508.
104. Kash SF, Tecott LH., Hodge C, Baekkeskov S. Increased anxiety and altered responses to anxiolytics in mice deficient in the 65-kDa isoform of glutamic acid decarboxylase. *Proc Natl Acad Sci USA* 1999;96:1698–1703.
105. Williams CA, Angelman H, Clayton-Smith J, et al. Angelman syndrome: consensus for diagnostic criteria. *Am J Med Genet* 1995;56:237–238.
106. DeLorey TM, Handforth A, Homanics GE, et al. Mice lacking the β3 subunit of the $GABA_A$ receptor have the epilepsy phenotype and many of the behavioral characteristics of Angelman syndrome. *J Neurosci* 1998;18:8505–8514.
107. Günther U, Benson J, Benke D, et al. Benzodiazepine-insensitive mice generated by targeted disruption of the γ2 subunit gene of $GABA_A$ receptors. *Proc Natl Acad Sci USA* 1995;92: 7749–7753.
108. Essrich C, Fritschy JM, Lorez M, et al. Postsynaptic clustering of major $GABA_A$ receptor subtypes requires the γ2 subunit and gephyrin. *Nat Neurosci* 1998;1:563–571.
109. Crestani F, Lorez M, Baer K, et al. Decreased $GABA_A$ receptor clustering results in enhanced anxiety and a bias for threat cues. *Nat Neurosci* 1999;2:833–839.
110. Quinlan JJ, Firestone LL, Homanics GE. Mice lacking the long splice variant of the γ2 subunit of the $GABA_A$ receptor are more sensitive to benzodiazepines. *Pharmacol Biochem Behav* 2000; 66:371–374.
111. Mihalek RM, Banerjee PK, Korpi ER, et al. Attenuated sensitivity to neuroactive steroids in $GABA_A$ receptor δ subunit knockout mice. *Proc Natl Acad Sci USA* 1999;96:12905–12910.
112. Rudolph U, Crestani F, Benke D, et al. Benzodiazepine actions mediated by specific $GABA_A$ receptor subtypes. *Nature* 1999; 401:796–800.
113. McKernan RM, Rosahl TW, Reynolds DS, et al. Sedative but not anxiolytic properties of benzodiazepines are deiated by the $GABA_A$ receptor α1 subtype. *Nature Neurosci* 2000;3:587–592.
114. Low K, Crestani F, Keist R, et al. Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* 2000; 290:131–134.

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