Mechanisms of Sleep Induction by GABA$_A$ Receptor Agonists

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Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system. GABA$_A$ receptors are pentameric complexes that function as ligand-gated chloride ion channels. Two types of inhibitory neurotransmission are mediated via GABA$_A$ receptors. Phasic inhibition results from the activation of receptors at the synapse by intermittent release of high concentrations of GABA from presynaptic terminals. Tonic inhibition, in contrast, is mediated by the continuous activation of receptors located outside the synaptic cleft by low concentrations of ambient GABA. These "extrasynaptic" receptors have a higher affinity for GABA and slower rates of desensitization and deactivation than do the classical "synaptic" receptors. A variety of subunit families make up GABA$_A$ receptors; a total of 19 distinct subunits have been cloned. This diversity in subunit composition results in substantial anatomical, functional, and pharmacologic heterogeneity. Receptors containing the α1, α2, or α3 subunits with γ2 are usually found at synapses and are sensitive to benzodiazepines and zolpidem, whereas α4 and α6 subunits are often found with δ and play a role in extrasynaptic receptors (in thalamus and dentate), as does the α5 subunit (in CA1). The α4$\beta$$\delta$ receptors are insensitive to benzodiazepines and zolpidem, but show high sensitivity to other sedative-hypnotic drugs, including ethanol and the novel hypnotic drug gaboxadol (THIP). This review will examine the role of heterogeneity of GABA$_A$ receptors, and recent research demonstrating subunit-dependent modulation of receptors by novel pharmacologic agents will be discussed.

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Sleep deficits caused by disorders of sleep are highly prevalent in developed nations, where work stress and shift work contribute significantly to sleeplessness. A recent survey revealed that more than 75% of adult Americans experience 1 or more symptoms of a sleep disorder at least a few nights per week,[1] and sleep problems become even more prevalent in patient populations with comorbid conditions such as elderly adults.[2] Medical illness such as cardiovascular disease, pain, and neurologic disease, as well as polypharmacy, changes in diurnal rhythms, and poor sleep hygiene all contribute to deficits in sleep. The presence of psychiatric illness, including depression, anxiety, bipolar disorder, schizophrenia, and dementia, also increases the risk of insomnia.[3]

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Hypnotics, including the classical benzodiazepines, such as diazepam or chlordiazepoxide, were used for decades to treat patients with sleep deficits, but varying adverse effects and problems with dependence have been associated with these medications. Some of these problems can be attributed to the general depression of the central nervous system (CNS) caused by these medications. The new-generation benzodiazepine receptor agonists (BZRAs) zolpidem, zaleplon, and eszopiclone have fewer side effects but are still associated with next-day sedation and memory impairments. Next-generation γ-aminobutyric acid (GABA) receptor agonists aim to maximize hypnotic effectiveness while minimizing next-day adverse effects. Gaboxadol, or THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol), currently under development for the treatment of insomnia, is a GABA$_A$ receptor agonist that targets extrasynaptic GABA$_A$ receptors, a different subset of receptors than the synaptic receptors modulated by the currently available BZRAs. This review discusses the mechanisms of sleep and how GABA$_A$ receptor agonists and modulators might target various aspects of the sleep circuitry.

## SLEEP AND AROUSAL CENTERS: THE SLEEP SWITCH

Sleep and arousal can be viewed as competing states of the CNS separated by abrupt transitions, but sleep itself is

a progressive process with several distinct, identifiable phases. Sleep can be subdivided into 2 physiologically distinct states: rapid eye movement (REM) and non-REM (NREM) sleep. These states, which vary in length and alternate throughout the night, occur in cycles of approximately 90 to 110 minutes. NREM sleep can be further subdivided into stages (1–4), which are successive stages of deepening sleep. Stages 3 and 4 of NREM sleep comprise the period known as slow-wave sleep (SWS), and these physiologically defined periods can be easily identified with electroencephalography. The control centers of these mutually exclusive states of sleep and arousal can be localized to anatomically distinct structures in the basal forebrain, hypothalamus, midbrain, and brain stem. The transitions between sleep and arousal are triggered by activity in subcortical structures, but the ultimate effects are experienced in the cerebral cortex. Anatomical correlates of sleep and arousal were first posited by von Economo in the early decades of the 20th century.[4] Following an outbreak of viral encephalitis that left patients with disturbances of sleep or arousal, von Economo correlated sleep/wake behavior of individuals with neuroanatomical lesions found upon postmortem examination. Based on his observations, he hypothesized that sleep centers must be localized to the basal forebrain and anterior hypothalamus, whereas arousal centers were associated with midbrain and posterior hypothalamic structures. Recent investigations have substantiated von Economo's hypothesis and have helped to elucidate mechanisms for the control of sleep and arousal.

A number of techniques have been used to identify neuroanatomical regions involved in sleep or arousal. Many investigators rely on measuring the activity of a region indirectly, using immunologic methods to measure the expression of specific neuronal proteins that are regulated by activity. The expression of *c-fos*, for example, increases rapidly and dramatically in neurons that are firing actively and can be easily assessed by immunostaining tissues from various regions of the CNS. *C-fos* staining has been used as a measure of the activity of a specific region of the CNS. In sleeping animals, the expression of *c-fos*–related protein is very low in brain stem regions that are implicated in arousal, whereas *c-fos* expression is high in the hypothalamic ventrolateral preoptic area (VLPO), which is active during sleep.[5]

Electrophysiologic recordings can provide even more direct methods to identify cell types and regions involved in sleep or arousal. Investigators can either record from individual neurons or use field recordings to identify groups of neurons responsible for arousal and sleep within different regions of the brain during behavioral experiments. Lesioning of specific brain regions through chemical or surgical methods can further isolate cell groups to provide increased specificity in locating sleep and arousal regions within the brain. Using these techniques, a number

Figure 1. Ascending Arousal Pathways[a]
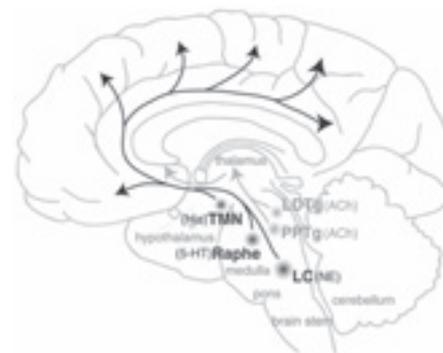
of regions have been correlated with arousal (Figure 1). In the brain stem, the serotonergic neurons of the raphe nuclei, the noradrenergic neurons of the locus coeruleus (LC), and the cholinergic neurons of the laterodorsal tegmentum (LDT) and pedunculopontine tegmentum (PPT) are active during arousal and inactive during sleep.[6] In the hypothalamus, the histaminergic/GABAergic neurons of the tuberomammillary nucleus (TMN) are associated with arousal.[7] Lesions to the neurons of the TMN, however, do not seem to affect spontaneous wakefulness or sleep.[8] The neurons of the LC, raphe nuclei, and TMN project to, and stimulate arousal in, the cerebral cortex, whereas the neurons of the LDT and PPT project to the thalamus.[6,9,10]

Another group of arousal neurons are found in perifornical hypothalamic areas (PeF). These neurons express the excitatory neuropeptide transmitter orexin, which is also known as hypocretin.[11] Defects in orexin expression result in narcolepsy and cataplexy.[12,13] Although these neurons project to the cerebral cortex, hippocampus, and thalamus, they also project to the other arousal centers in the brain stem and hypothalamus. The latter observation suggests that these neurons reinforce the arousal system.[6]

In contrast to the several regions involved in arousal, only a small number are involved with sleep. The most prominent sleep-associated nucleus is the VLPO of the hypothalamus.[6] The neurons of the VLPO are active during sleep and quiescent during arousal, and lesions to the cat and rat VLPO abolish sleep.[14–16] The neurons of the VLPO express the inhibitory transmitters GABA and galanin[17,18] and project to and inhibit the arousal nuclei (TMN, LC, LDT, PPT, PeF, and raphe nuclei; Figure 2).[6] The arousal and sleep centers are reciprocally innervated by inhibitory connections, and the neurons in VLPO can likewise be inhibited by the arousal nuclei (TMN, LC, and raphe nuclei).[6,18–20]

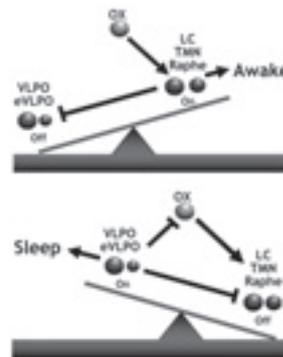Figure 2. VLPO Axons Innervate the Entire Ascending Arousal System[a]

Figure 3. The Sleep Switch[a]

These observations led Saper et al. to propose the following "sleep-switch" model (Figure 3).[6,10,20] The sleep nucleus (VLPO) and the arousal nuclei (TMN, LC, and raphe nuclei) are mutually inhibitory. When the inhibitory drive on the VLPO is high, the VLPO is silenced, reducing the inhibition on the arousal centers, which results in continued arousal. When the inhibitory drive on the VLPO is eased, the arousal centers are inhibited and sleep ensues. This switch between arousal and sleep can be almost immediate and can be demonstrated physiologically and behaviorally. The switch is not always stable, however, and under certain conditions can rapidly alternate between arousal and sleep states, as seen, for example, in patients suffering from narcolepsy. Although orexin neurons project to the VLPO, neurons in the VLPO do not express receptors for orexin and are thus not inhibited by PeF. In this model, the orexin neurons of the PeF stabilize the switch by providing additional excitatory drive to the LC, TMN, and raphe nuclei during wakefulness. Loss of orexin neurons would therefore be expected to cause narcolepsy, and this is indeed the case. Postmortem analysis of patients with narcolepsy has revealed a selective and massive loss of orexin-producing PeF cells, lending support to this model.[21]

The sleep and arousal centers therefore constitute a switching mechanism that can ultimately trigger sleep or arousal in the thalamus and cerebral cortex. The stages of sleep, which can be identified during electroencephalography or electrophysiologic recordings, measure characteristic patterns of activity in the thalamus and cerebral cortex. Slow-wave sleep, occurring during stages 3 and 4, is dominated by 2 characteristic patterns of activity. Delta waves are low-frequency (0.5–4 Hz), high-amplitude oscillations, whereas spindle/sigma waves are higher-frequency (7–14 Hz) oscillations that are superimposed on the delta waves.[22] Both rhythms originate in the thalamus. Thalamic neurons that project to the cortex also send axon collaterals to the thalamic reticular nucleus (nRT), where GABAergic neurons of the nRT in turn serve to inhibit the activity of the thalamocortical projection neurons. Activation of these GABAergic nRT cells can initiate both delta and spindle waves, which then entrain the activity of the cortex via the projection neurons. Once established in the cortex, these "slow-wave" rhythms can be maintained independent of the thalamus. The ascending arousal systems can abolish these sleep rhythms by either lowering the threshold of activation of cortical neurons directly or by activating high-frequency tonic firing in the thalamic projection cells, thereby suppressing the slow rhythmic activity in the thalamus.[22]

In summary, sleep and arousal are behavioral states that are dependent on activity in lower regions of the CNS, which then trigger sleep or arousal in the thalamus and/or cerebral cortex. In the awake state, the arousal centers of the brain stem are active, and this results in depolarization of thalamocortical projection neurons, suppressing SWS activity in corticothalamic circuits. When the inhibitory drive on the VLPO is decreased, VLPO neurons become active and inhibit the arousal circuits, allowing for hyperpolarization of the thalamic projection neurons, which is essential for the generation of slow-wave rhythms, thus enabling SWS activity in corticothalamic circuits to predominate.

GABA RECEPTORS: FUNCTIONS AND DISTRIBUTION

GABA_A receptors, the predominant inhibitory receptors of the CNS, are a family of ligand-gated transmembrane

Figure 4. The Site of Action of Hypnotic Drugs Is Determined by GABA_A Receptor Subunits[a]
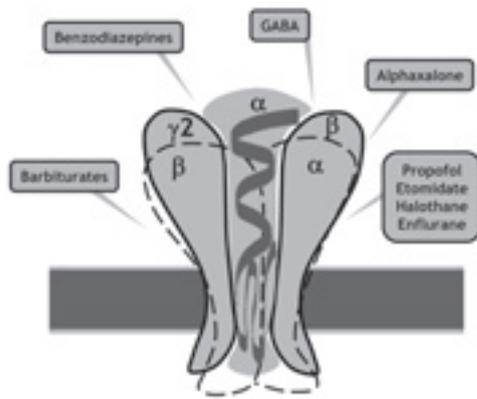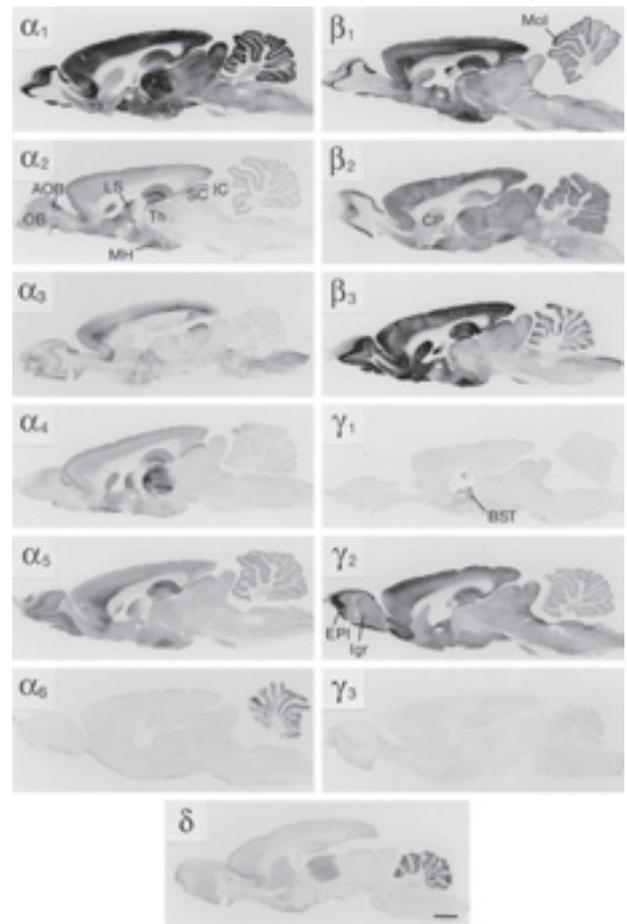

[a]Reproduced with permission from Rudolph and Mohler.[24]
Abbreviation: GABA = γ-aminobutyric acid.

Figure 5. Neuroanatomical Expression of GABA_A Receptor Subunits[a]

[a]Reproduced with permission from Pirker et al.[27]
Abbreviations: AOB = accessory olfactory bulb, BST = bed nucleus of the stria terminalis, CP = caudatoputamen (striatum), EPl = external plexiform layer of the olfactory bulb, GABA = γ-aminobutyric acid, IC = inferior colliculus, Igr = internal granular layer of the olfactory bulb, LS = lateral septal nucleus, MH = medial hypothalamic nuclei, Mol = molecular layer of the cerebellum/dentate gyrus, OB = olfactory bulb, SC = superior colliculus, Th = thalamus.

chloride channels, which are targets of many sedatives, hypnotics, anesthetics, and anxiolytics. Activation of GABA_A receptors by GABA or receptor agonists opens the intrinsic chloride selective channel that triggers the influx of chloride ions, which hyperpolarizes the neuron, rendering the neuron less capable of responding to excitatory neurotransmitters.[23,24] GABA_A receptors exist as pentameric protein complexes, assembled from a combination of at least 19 subunits from 7 distinct gene families (α, β, γ, δ, ε, θ, and π) with different subunits serving various roles. Some of the subunits express drug-specific binding and modulatory sites; for example, the interface between the α and β subunits contains the binding site for GABA, whereas the α and γ subunits modulate the action of benzodiazepines (Figure 4).[24,25] Specific subunits are also associated with localization and anchoring functions not directly related to agonist binding. The γ subunit, for example, has been shown to be responsible for the clustering of GABA_A receptors and for tethering receptors to postsynaptic complexes.[26] Although GABA_A receptors are ubiquitously expressed throughout the CNS, individual subunits have regional specific expression. Pirker et al.[27] identified patterns of expression of the various subunits in the rat CNS (Figure 5). Some subunits, such as α1, are expressed at high levels in many regions, whereas others, such as α4 or δ, have much lower levels and more restricted patterns of expression. Agonist specificity, anatomical localization, receptor kinetics, and other receptor characteristics are thus dictated by the subunit composition of a GABA_A receptor.

In addition to their neuroanatomically distinct expression patterns, GABA_A receptor subtypes are segregated to distinct regions on neuronal cell membranes relative to their localization to the synapse; receptors are classified as either synaptic or extrasynaptic (Figure 6).[28,29] Synaptic GABA_A receptors are responsible for modulating benzo-

diazepine sensitivity and typically contain α1, α2, or α3 and the γ2 subunit.[30–32] These GABA_A receptors are activated during fast synaptic transmission in a phasic manner by GABA released from the presynaptic terminal. These fast inhibitory responses, known as inhibitory postsynaptic potentials, transiently hyperpolarize postsynaptic neurons, inhibiting spontaneous firing and attenuating the effects of synaptic excitation. Synaptic GABA_A receptors rapidly desensitize in the presence of an agonist and have a low affinity for GABA, thus becoming unresponsive to low ($< 1\mu M$) concentrations of GABA as it diffuses away from the synaptic cleft.[33,34]

Extrasynaptic GABA_A receptors are found outside of the synaptic cleft and are functionally and compositionally distinct from their synaptic counterparts (Figure 6).[28,29,34]

Figure 6. Synaptic and Nonsynaptic Receptors[a]
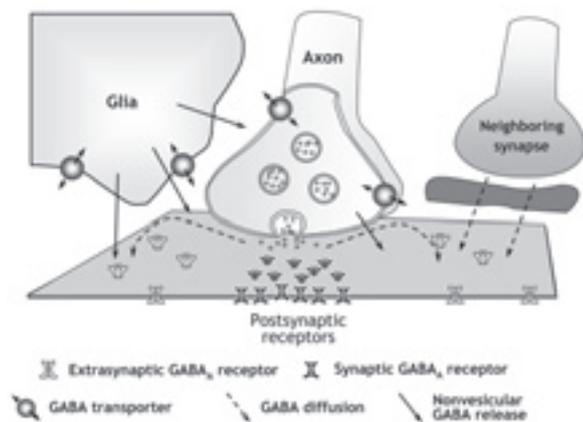
[a]Adapted with permission from Semyanov et al.[29]
Abbreviation: GABA = γ-aminobutyric acid.

This subgroup of GABA$_A$ receptors is insensitive to benzo-diazepines, and functional receptors are primarily composed of the α4 or α6 subunits in combination with the δ subunit. Extrasynaptic GABA$_A$ receptors have a high affinity for GABA and desensitize very slowly, maximizing sensitivity to low concentrations of GABA (ambient levels of 0.1–0.5 μM GABA exist within the CNS) as it diffuses away from the synaptic cleft and from nonsynaptic release sites.[34] Unlike synaptic GABA$_A$ receptors that rapidly and transiently inhibit neuronal excitation, the activation of extrasynaptic receptors has sustained effects on neurons. This tonic activation of extrasynaptic receptors decreases input resistance and therefore increases the excitatory current required for action-potential generation, thus altering firing rates and patterns of activation, thereby altering neuronal networks and long-term computational processes.[28]

GABA$_A$ receptors have historically been the main targets for commercially useful hypnotics. Benzodiazepines, the most widely used class of hypnotics, are high-affinity allosteric modulators of GABA$_A$ receptors and act indirectly by potentiating GABA activation of these receptors. Benzodiazepines bind to synaptic GABA$_A$ receptors containing α1, α2, α3, or α5 subunits with comparable affinity.[35] These subunits commonly associate with the γ2 subunit, which is responsible for targeting functional, assembled receptors to the synapse that can be modulated by benzodiazepines. Mutations to the α1 subunit eliminate the sedative, anticonvulsant, and amnestic actions of benzodiazepines, but do not affect the anxiolytic or myorelaxant properties.[30] These results suggest that benzodiazepines mediate a variety of activities through a number of different GABA$_A$ receptor subtypes. This absence of selectivity in GABA$_A$ receptor modulation is further reflected in the widespread anatomical distribution of these receptors

sensitive to modulation by benzodiazepines (Figure 5).[27] Because benzodiazepines are physically addictive, produce a wide range of nonsedative effects, and induce tolerance when taken for prolonged periods, new classes of hypnotic GABA$_A$ receptor agonists have been developed in attempts to create drugs with a more specific action. These compounds can induce sleep with a better safety profile and may treat patients with sleep disorders more effectively.

Zolpidem, zaleplon, and eszopiclone are allosteric non-benzodiazepine BZRAs, which, in contrast to benzodiazepines, have a shorter duration of action and fewer residual next-day effects. All 3 of these nonbenzodiazepine BZRA hypnotics bind to GABA$_A$ receptors expressing the α1 subunit; however, zolpidem shows greater selectivity for α1-containing receptors when compared with zopiclone, triazolam, or midazolam. GABA$_A$ receptors containing α1 subunits may mediate sedative effects of many GABA$_A$ receptor agonists, but they have also been shown to produce memory impairments.[36] Although BZRAs are more selective than benzodiazepines, these hypnotics do not specifically target centers involved in sleep or arousal, because α1-containing receptors are found at synaptic sites throughout the nervous system (Figure 5).[27]

Gaboxadol, or THIP, is a direct GABA$_A$ receptor agonist and exhibits a very different mechanism of action compared with the benzodiazepines and the BZRAs.[37] Gaboxadol is a potent agonist of GABA$_A$ receptors that specifically targets extrasynaptic GABA$_A$ receptors.[38] Extrasynaptic GABA$_A$ receptors commonly contain α4 and δ subunits and are expressed to a much lower extent and in more limited areas of the CNS, suggesting that gaboxadol has a more regional-specific effect in the CNS (Figure 5).[27] The activation of extrasynaptic receptors, enhancing the tonic inhibition of neurons, may provide a more effective means of quieting the CNS and inducing sleep.

To fully comprehend how these different groups of hypnotics induce sleep, the role of GABA$_A$ receptors within specific neuronal pathways involved in sleep and arousal will be discussed next.

MECHANISMS OF SLEEP INDUCTION BY GABA$_A$ RECEPTOR AGONISTS

Several neuroanatomical structures that are involved in the control of sleep and arousal have already been described. It is known that sleep can be triggered by activating sleep areas, suppressing arousal areas, or enabling slow-wave activity in corticothalamic circuitry. Although the mechanisms by which the various hypnotics induce sleep have yet to be completely elucidated, some intelligent hypotheses can be proposed based on knowledge of the central sleep/arousal circuitry and the molecular mechanisms of these agents.

The locus of action of a hypnotic can be inferred from the location of its receptors in the CNS. The exact locus of action of benzodiazepines is difficult to determine because they bind promiscuously to many GABA$_A$ receptor subtypes, which are ubiquitously expressed within the nervous system.[27] The α1-specific BZRAs (zolpidem, zaleplon, and eszopiclone) are more specific, yet even the GABA$_A$ receptor α1 subunit to which these hypnotics bind is expressed widely within the CNS at high levels,[27] suggesting these hypnotics may exert their effects in multiple regions.

In comparison, the more restricted expression of the extrasynaptic receptors suggests more specific effects of gaboxadol on sleep compared with benzodiazepines or α1-specific hypnotics. Gaboxadol binds to and preferentially activates GABA$_A$ receptors containing α4 or α6 and δ subunits. These subunits are expressed in sleep and arousal centers, and, thus, gaboxadol may induce sleep by either activating sleep centers or inhibiting arousal centers. Alpha4 and δ subunits are highly expressed in the nuclei of the thalamus and, to a lesser extent, the cerebral cortex.[27] It is thus likely that gaboxadol induces sleep by altering corticothalamic activity. It is not known whether α4 and δ subunits are present in the VLPO, but since the effects of gaboxadol are normally inhibitory, it is likely that the activation of VLPO neurons by gaboxadol is indirect, presumably by relief of inhibition from other inputs.

In rats and healthy young humans, gaboxadol increases NREM and SWS, but does not affect REM sleep.[39,40] In comparison with younger individuals, the VLPO is significantly smaller in elderly humans.[41] This reduction in the size of the VLPO may be associated with altered sleep patterns in older individuals. However, although SWS diminishes with age in humans and in rats,[42–45] gaboxadol can promote SWS in elderly humans and rats.[40,46]

As discussed above, delta waves, the signature activity of SWS, which originate within corticothalamic circuits, are enhanced by gaboxadol.[47] Gaboxadol may therefore induce SWS/delta activity in the cerebral cortex by altering the activity of thalamic projection neurons. Thalamocortical neurons become progressively hyperpolarized during the transition from arousal to SWS.[48] Because thalamocortical neurons are also hyperpolarized by gaboxadol, but not by benzodiazepines, this suggests that gaboxadol may inhibit these cells and induce sleep through mechanisms similar to what occurs through natural mechanisms.[49,50] Gaboxadol may also induce delta wave activity by altering cortical network activity directly,[51] since it has also been reported to induce a tonic current in the mouse cerebral cortex.

CONCLUSION

GABA$_A$ receptors are the molecular targets for almost all of the hypnotics currently approved for the treatment of insomnia. These receptors are expressed throughout the CNS, but individual subunits show anatomical and subcellular specificity. Synaptic GABA$_A$ receptors containing α1 subunits have been the historical target of hypnotics such as benzodiazepines and BZRAs. Receptors containing benzodiazepine-sensitive subunits are ubiquitously expressed throughout the CNS, possibly responsible for the presence of residual next-day sleepiness and adverse cognitive effects. Extrasynaptic GABA$_A$ receptors containing α4 and δ GABA$_A$ receptor subunits show much more regional specificity. Gaboxadol targets this subset of receptors, which are selectively expressed in several regions of the brain that are important for sleep and arousal. These extrasynaptic GABA$_A$ receptors are currently being studied as targets for the treatment of insomnia.

Drug names: chlordiazepoxide (Librium and others), diazepam (Diastat and others), enflurane (Ethrane), eszopiclone (Lunesta), etomidate (Amidate), triazolam (Halcion and others), zaleplon (Sonata), zolpidem (Ambien).

Disclosure of off-label usage: The author has determined that, to the best of his knowledge, gaboxadol, diazepam, chlordiazepoxide, and midazolam are not approved by the U.S. Food and Drug Administration for the treatment of insomnia.

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