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Sleep and Its Modulation by Drugs That Affect $GABA_A$ Receptor Function

Marike Lancel* and Axel Steiger

Insomnia, which can be defined as a discrepancy between the need for sleep and the perceived quantity and/or quality of sleep, constitutes a major medical problem. The most frequently prescribed sleeping pills are agonistic modulators of $GABA_A$ receptors. To examine the changes in sleep that can be induced by a stimulation of $GABA_A$ receptor function, this article reviews the hypnotic properties of the different classes of agonistic modulators of $GABA_A$ receptors—barbiturates, benzodiazepines, zolpidem, zopiclone, and neuro(active) steroids—and of selective $GABA_A$ agonists assessed in various mammalian species, including noninsomniac subjects. Although quantitative differences clearly exist, the agonistic modulators appear to have many actions in common. They are very effective in inducing and maintaining sleep, but, with the possible exception of the neurosteroids, inhibit the dream-associated rapid eye movement (REM) stage of sleep. With regard to the signals in the electroencephalogram during non-REM sleep, all these drugs promote the occurence of spindles, which are characteristic for shallow sleep, and, with the exception of barbiturates, have been shown to depress slow waves, which usually identify deep sleep. Upon chronic usage, all these drugs may produce tolerance and dependence. This occurs to the greatest extent with the barbiturates, and to the least extent with the newer hypnotics zolpidem and zopiclone. The small number of studies on the GABA analogues and the selective $GABA_A$ agonists muscimol and 4,5,6,7-tetrahydroisoxazolopyridin-3-ol (THIP) indicate that these compounds have little effect on sleep initiation, but may increase sleep maintenance and promote deep sleep, without disrupting REM sleep. The hypnotic properties of these $GABA_A$ agonists seem to differ considerably from those of agonistic modulators, and may have beneficial effects in the treatment of disturbances in maintaining sleep and of nonrefreshing sleep.

Keywords: electroencephalograms · GABA agonists · receptors · sleep

1. Introduction

Sleep is one of the most time-consuming behaviors. Humans, for instance, spend up to 30 % of their lives sleeping. Although the precise function of sleep is still debatable, it is a general experience that sleep loss leads to fatigue. Furthermore, numerous studies demonstrated that prolonged wakefulness may have manifold consequences, ranging from an impairment of cognitive and psychomotor performance[1] and a lowering of immune competence[2] to the production of transient emotional lability and psychotic symptomatology.[3] Thus, sleep seems essential to physiological and psychological integrity.

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Sleep disturbances are very common, with estimates varying between 4 and 45 % of the adult population.[4] In addition to specific sleep disorders—such as sleep apnea (cessation of respiration), nocturnal myoclonus (involuntary contraction of a muscle), and narcolepsy (excessive daytime sleepiness)—a lot of people have complaints concerning the quantity or quality of their sleep. These complaints include difficulty in going to sleep, multiple or long-lasting nocturnal awakenings, early morning awakening, and superficial, nonrefreshing sleep. In view of the nightly distress associated with insomnia and its impact on well-being, it is not astonishing that many people suffering from poor sleep use hypnotics.[5] In the early 1900s the first generation of powerful sleeping pills, the barbiturates, was introduced. They are disfavored now because of their toxicity and the rapid development of tolerance and of physical as well as psychological drug-dependency. Barbiturate withdrawal produces a rebound insomnia, a transient worsening of insomnia beyond pretreatment levels. From the 1960s the barbiturates were replaced by the benzodiazepines. Benzodiazepines are much safer than barbiturates. However, chronic use has serious disadvantages: They readily cause tolerance and dependency and rebound

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insomnia can occur upon abrupt discontinuation, particularly of the shorter acting benzodiazepines. A few years ago a third generation of hypnotics, the compounds zolpidem and zopiclone, came on the market. Both compounds seem to have a lower habituation and dependency potential than barbiturates and benzodiazepines.[6] Although these three generations of hypnotics differ with respect to their tolerability profile and dependence potential, they have a striking feature in common: All of these compounds are ligands for one of the multiple allosteric binding sites at the γ-aminobutyric acid $(GABA)_A$ receptor complex and enhance $GABA_A$ receptor function upon binding.

To examine the hypnotic properties of ligands for different binding sites associated with the $GABA_A$ receptor, this article reviews the effects on sleep of drugs that either potentiate or activate $GABA_A$ receptor mediated transmission. Given the vast literature, which precludes a complete review, we restricted ourselves to reports on the acute or short-term effects of peripherally administered substances on polysomnographically recorded sleep in healthy humans without sleep complaints and in the most extensively studied animals, namely, cats and rats. The review begins with a brief description of the structure and regulation of sleep and with an overview of the configuration and function of $GABA_A$ receptors.

2. Sleep Structure and Regulation

In mammals sleep consists of two different stages, rapid eye movement (REM) sleep and non-REM sleep. These states of sleep are usually distinguished from each other by measuring the electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG). The predominant state of sleep, non-REM sleep, is characterized by salient signals in the EEG, particularly spindles and slow waves (Figure 1). Spindles, which constitute the landmark of sleep onset, last 0.5 to 2 s and are composed of sigma frequencies (11 – 16 Hz) with a waxing and waning amplitude. Slow waves are high-amplitude waves in the delta frequency range (1 – 4 Hz). As awakening thresholds rise with the increased occurrence of slow waves,[7] spectral power in the slow wave frequencies (slow wave activity, SWA) is commonly used as an index for non-REM sleep intensity. In general, eye movements are absent and,

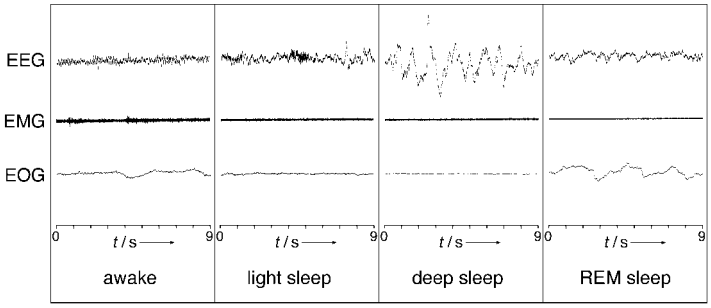

Figure 1. Electroencephalographic (EEG), electromyographic (EMG), and electrooculographic (EOG) recordings in an adult subject during wakefulness, light sleep, deep sleep, and REM sleep. The EEG for the awake state contains alpha waves (8 – 12 Hz), which typically occur during relaxed wakefulness with closed eyes. The EEG of the light sleep sample contains sleep spindles, and that of deep sleep slow waves.

Marike Lancel, born in 1959 in Leiden, The Netherlands, studied physiological psychology at the University of Leiden. In 1991 she completed her PhD in psychology at the University of Basel, Switzerland, based on experiments performed in the CNS department of Ciba Geigy, Basel, on sleep regulation in mammalian species. Thereafter, she was at the Max Planck Institute of Psychiatry in Munich as the head of the animal sleep laboratory and since 1998 also as the head of the research group Pharmacology of Sleep. From 1997 on, she has received a grant from the Deutsche Forschungsgemeinschaft to complete a habilitation. She is a member of the scientific board of the "Deutsche Gesellschaft für Schlafforschung und Schlafmedizin" and of the "European Sleep Research Society". Her main fields of interest are sleep regulatory processes, the influence of hormones on sleep, and the interaction between immune system function and sleep – wake behavior.

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Axel Steiger, born in 1953 in Kaiserslautern, Germany, studied medicine in Heidelberg and Vienna. In 1979 he finished his MD with a thesis on the mechanism of REM sleep induction by γ-hydroxybutyric acid. After one year of work in experimental neurophysiology at the University of Munich he started training in psychiatry at the University of Essen. From 1981 to 1987 he worked as a physician in the Departments of Psychiatry and Neurology of the University of Mainz and was there head of the sleep laboratory. He then moved as senior psychiatrist and director of the sleep laboratory to the University of Freiburg, where he completed a habilitation on sleep-endocrine activity in depressed patients and normal controls. Since 1990 he is senior psychiatrist, member of the executive board, and head of the sleep and hormone group at the Max Planck Institute of Psychiatry in Munich. His main fields of interest are pharmacopsychiatry and sleep-endocrinology in psychiatric patients and normal humans.

except for occassional postural adjustments, the muscles are relaxed during non-REM sleep. In humans, non-REM sleep is subdivided into four stages:[8] Stages 1 and 2 represent shallow sleep, and stages 3 and 4 reflect deep, slow wave sleep (SWS). In contrast to non-REM sleep, REM sleep is an activated brain state and associated with dreams. It is accompanied by typical rapid eye movements, a very low muscle tonus, and EEG signals that are reminiscent of those observed during wakefulness (low-voltage, fast activity in primates and regular theta activity (4–9 Hz) in rodents). In animals such as cats and rats, the transition from non-REM to REM sleep, called pre-REM sleep here, can easily be recognized by the occurrence of long-lasting, high-amplitude spindlelike EEG signals on a background of theta activity.[9] In most studies pre-REM sleep was considered as part of non-REM sleep, but in some investigations it was treated as a separate stage. Non-REM and REM sleep alternate, giving rise to non-REM/REM cycles of about 90 min in adult humans (Figure 2) and of a much shorter duration in smaller species.[10]

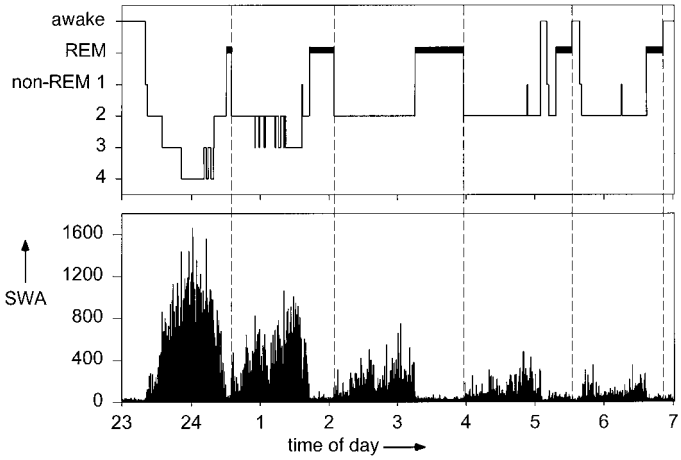


Figure 2. Typical time course of vigilance stages and EEG slow wave activity (μV^2; 0.78–4.29 Hz) from 11 p.m. to 7 a.m. the following day in a young adult. The end of five completed non-REM/REM cycles is indicated by dashed vertical lines.

A vast number of studies showed that sleep is regulated by an interaction between circadian and homeostatic components. Circadian regulatory processes determine the timing of sleep. For instance, humans usually sleep during the night, and rats during the day; cats do not exhibit one predominant sleep phase, but are highly active during dusk and dawn. Furthermore, circadian processes have a major impact on the structure of sleep, including sleep maintenance and the duration of the REM episodes, which tends to increase across the main sleep period (Figure 2).[11] Lesion studies demonstrated that, along with many other diurnally fluctuating parameters, the sleep–wake rhythm is triggered in the suprachiasmatic nuclei of the hypothalamus.[12] In contrast, the intensity of non-REM sleep is primarily a function of prior sleep and wakefulness. This is shown by the monotonic decline of SWS and SWA in the course of sleep[13] (Figure 2) and by the fact that naps decrease[14] whereas extended wakefulness increases SWS and SWA during subsequent sleep.[13b–g, 15] The duration of prior wakefulness also affects sleep propensity, duration, and continuity. These sleep–wake-dependent processes are generally assumed to be mediated by endogenous sleep-promoting substances that accumulate during wakefullness and are eliminated during sleep.[16] In addition to the factors of the time of day and the need for sleep, sleep is markedly affected by age. Polysomnographic studies revealed consistent age-related changes in sleep. In general, the ability to fall asleep is barely affected, but the ability to stay asleep decreases with age, as reflected by an increase in nocturnal awakenings. Furthermore, both SWS and SWA progressively decline during aging, which indicates a decrease in sleep intensity.[17] In view of the fact that sleep quality deteriorates with age, it is not surprising that the regular usage of hypnotics is particularly heavy in the elderly.[5b]

3. Structure of $GABA_A$ Receptors

GABA is one of the most prevalent neurotransmitters in the mammalian central nervous system.[18] As 20–50% of all central synapses use GABA as their neurotransmitter,[19] it controls the activity of a large percentage of the neurons. GABA interacts with two types of membrane-bound receptors, $GABA_A$ and $GABA_B$ receptors, which differ in function as well as in pharmacology.[20] In most brain regions, $GABA_A$ receptors outnumber $GABA_B$ receptors.[21] $GABA_A$ receptors constitute fast-acting ligand-gated anion channels (Figure 3).

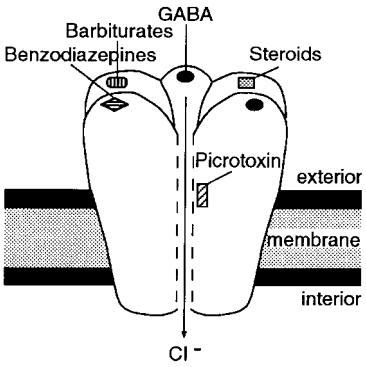
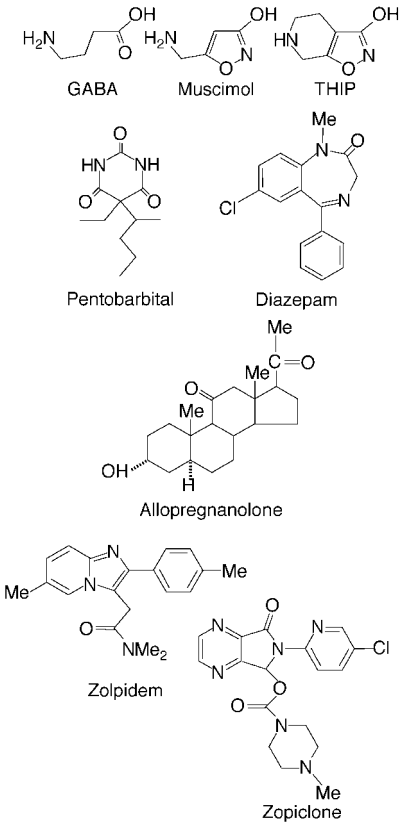


Figure 3. Schematic illustration of the $GABA_A$ receptor complex.

Upon activation by GABA or an appropriate analogue ($GABA_A$ agonist, see Scheme 1 for some examples), the membrane conductance for anions, mainly chloride ions, increases. Owing to the fact that the chloride ion concentration within the neurons is generally low, GABAergic transmission usually produces a slight, short-lasting hyperpolarization and thus reduced excitability of the recipient neuron. In addition to GABA recognition sites, $GABA_A$ receptors are endowed with several other binding sites, including those for barbiturates, benzodiazepines, and anesthetic steroids (see Scheme 1 for examples). The binding of an appropriate ligand induces a conformational change and thereby enhances (agonistic modulators) or attenuates (inverse agonistic modulators) GABA-evoked chloride ion flux



Scheme 1. Chemical structures of various agonists and agonistic modulators of $GABA_A$ receptors.

or inhibits the action of agonistic modulators (antagonistic modulators).

The $GABA_A$ receptor complex is a pentameric glycoprotein composed of combinations of multiple polypeptide subunits. To date, five structurally related families of subunits have been identified (α, β, γ, δ, and ρ), each with its own isoforms; there is 70–80% sequence identity within each family.[20b] The precise combination of protein subunits determines the physiological and pharmacological properties of a $GABA_A$ receptor.[22] Coexpression studies demonstrated that a channel with the properties of native receptors requires the combination of at least one α, one β, and one γ subunit.[23] While most native $GABA_A$ receptors are susceptible to modulation by barbiturates and neurosteroids, many are insensitive to benzodiazepines. Sensitivity to benzodiazepines requires the presence of the $\gamma 2$ subunit.[23a] Furthermore, the pharmacological influence of benzodiazepines depends on the type of α subunit. The benzodiazepine type I receptor, which contains an $\alpha 1$ subunit, displays high-affinity binding for certain benzodiazepine receptor ligands, including the benzodiazepines quazepam and cinolazepam and the imidazopyridines zolpidem and alpidem. In contrast, the benzodiazepine type II receptor, which contains $\alpha 2$- or $\alpha 3$-subunits, has a low affinity for these drugs.[20a,b, 22a] Both receptor types are present throughout the brain, but are distributed unevenly: Type I receptors are selectively enriched in the cerebellum and type II receptors predominate in limbic structures, such as the hippocampus, and also occur in spinal motor neurons.[20b, 22a]

4. Effects of Agonistic Modulators of $GABA_A$ Receptors on Sleep

4.1. Barbiturates

The influence of depressant barbiturates on $GABA_A$ receptor function seems to depend on the dose. At high, anaesthetic concentrations they are able to open $GABA_A$-associated chloride channels directly.[24] At lower concentrations, barbiturates increase the affinity for and enhance the response to GABA, primarily by increasing the average open duration of the chloride channels.[25]

In the rat, acute administration of various doses of pentobarbital has been found to shorten the latency to non-REM sleep, to increase pre-REM sleep, and to decrease REM sleep as a function of dose in comparison to placebo. The latter effect is caused by a prolongation of REM sleep latency and by a decrease in the number of REM sleep episodes.[26a]

In the cat, acute administration of phenobarbital appeared to increase non-REM sleep and to decrease wakefulness as well as REM sleep in a dose-related manner compared with the placebo.[26b] Another study assessed the sleep changes during chronic sodium barbital treatment, which was administered in slowly increasing doses to counteract the effects of drug tolerance. Compared with control recordings, sodium barbital persistently increased non-REM sleep and suppressed REM sleep.[26c] Analysis of REM architecture showed that the barbiturate-induced suppression of REM sleep is entirely due to a decrease in the number of REM episodes.[26c,d]. Studies in which pre-REM and non-REM sleep were distinguished revealed that pentobarbital particularly promotes pre-REM sleep.[26e] Experiments focussed on cortical EEG signals showed that moderate doses of barbiturates evoke bursts of rhythmic waves in the frequency range 6–13 Hz, with a waxing and waning amplitude, in cats as well as in other mammalian species.[26f] These signals closely resemble naturally occurring sleep spindles and appear simultaneously in the thalamus[26f], where spindle oscillations originate.[26g] An in vivo electrophysiological study in cat thalamocortical neurons revealed that pentobarbital-induced anesthesia elicits spindle-related firing, but prevents delta oscillations.[26h]

Various reports on the effects of barbiturates on nocturnal sleep in young, noninsomniac subjects exist. Phenobarbital as well as thiopental, given to a relatively small number of subjects, appeared to decrease the percentage of wakefulness, while slightly increasing SWS, and to prolong REM sleep latency in comparison to placebos or with respect to control recordings.[27a,b] Pentobarbital has been shown to reduce the number of awakenings, to shorten sleep onset latency, and to increase total sleep time and non-REM sleep, without significantly affecting any of its substages. In addition, it decreases the percentage of REM sleep, which is related to a delay of REM latency and to a decrease in both the number and duration of REM episodes.[27c,d] Amobarbital reportedly reduces sleep onset latency, decreases the percentage of stage 1, increases the percentage of stage 2, prolongs REM latency, and decreases the percentage of REM sleep. Furthermore, it reduces intra-sleep restlessness, as reflected by a

decrease in the number of switches to wakefulness, movement time, or to stage 1.[27e] Qualitatively similar effects are produced by heptobarbital[27f] and secobarbital,[27g–i] whereby the secobarbital-induced suppression of REM sleep was found to be caused by a shortening of the REM sleep episodes.

Taken together, depressant barbiturates generally increase the ability to fall and to stay asleep, promote non-REM and/or pre-REM sleep, facilitate the occurrence of sleep spindles, and decrease REM sleep owing to a suppression of REM initiation and, at least in humans, to a shortening of the REM episodes.

4.2. Benzodiazepines

Electrophysiological experiments showed that sedative benzodiazepines are inactive at the $GABA_A$ receptor complex in the absence of GABA, but enhance the frequency of GABA-induced chloride channel openings.[25a, 28] Possibly, benzodiazepines strengthen the coupling between the $GABA_A$ receptor and the chloride ion channels and thereby augment the efficiency of GABA to open the chloride channel.[20b]

Figure 4 presents the typical dose-dependent alterations in sleep structure and EEG power densities within non-REM sleep induced by the benzodiazepine midazolam in the rat. Placebo-controlled studies in the rat showed that acute, systemic administration of the benzodiazepines flurazepam, diazepam, triazolam, and midazolam produces a dose-dependent shortening of sleep onset latency and an increase in non-REM sleep. The promotion of non-REM sleep is most pronounced during the dark period, which is probably due to the small amount of spontaneously occurring sleep. Additionally, these drugs consistently delay the latency to REM sleep and decrease REM sleep time.[29a–i] Reports on the influence of benzodiazepines on REM sleep architecture are contradictive: While diazepam, triazolam, and midazolam have been shown to shorten the duration of the REM sleep episodes in one study,[26a] midazolam was found to mainly decrease the number of REM sleep episodes in another.[29h] Experiments in which pre-REM sleep was distinguished revealed that benzodiazepines markedly increase this stage.[26a] Spectral analysis of the EEG signals following midazolam administration yielded a depression of slow EEG components and an elevation in the spindle and higher frequencies within non-REM sleep in a dose-related manner (Figure 4B).[29f,h] A more detailed analysis showed that midazolam produces a general attenuation of SWA and enhancement of spindle activity during non-REM sleep, without affecting their time course within the non-REM sleep episodes.[29h]

In contrast to the soporiferous effects in rats, benzodiazepines induce vigilance in cats. Administration of nitrazepam has been shown to produce restlessness and to increase wakefulness, at the expense of non-REM and REM sleep, in a dose-dependent fashion.[26b, 29j] The suppression of sleep is generated both by a decrease in the number and duration of the non-REM and REM episodes.[29j] Qualitatively similar effects have been observed after administration of flunitrazepam,[26b, 29k,l] diazepam,[29j,m] triazolam, and midazolam[29l] to cats. It has not been readily explained why cats, similar to rats and humans, respond to barbiturates with sleep, while benzodiazepines lack a hypnotic action in this species. Electrophysiological experiments indicate that barbiturates selectively depress neurons of the brainstem reticular formation, which constitute a major arousal system, whereas benzodiazepines seem primarily to inhibit parts of the limbic system and may thereby enhance both activating and deactivating systems.[26f] Thus, differences in the physiology of species may contribute to their differential reponse—restlessness versus sedation—to benzodiazepines. Spectral analysis of the EEG signals in the cat revealed that diazepam attenuates delta and theta activity and enhances EEG power density in the frequency range of sleep spindles and in the higher frequency bands within non-REM sleep in cats.[29m] Thus, in spite of the species-specific changes in sleep–wake behavior, benzodiazepines seem to induce comparable alterations in the sleep EEG.

Most studies on the effects of the long-acting benzodiazepine nitrazepam on sleep in soundly sleeping subjects report a shortened sleep latency, a reduction in the number of body movements, and an increase in total sleep time and stage 2. Furthermore, nitrazepam produces a delay in the occurrence of the first REM episode and thereby decreases the time of REM sleep.[27e,g, 30]

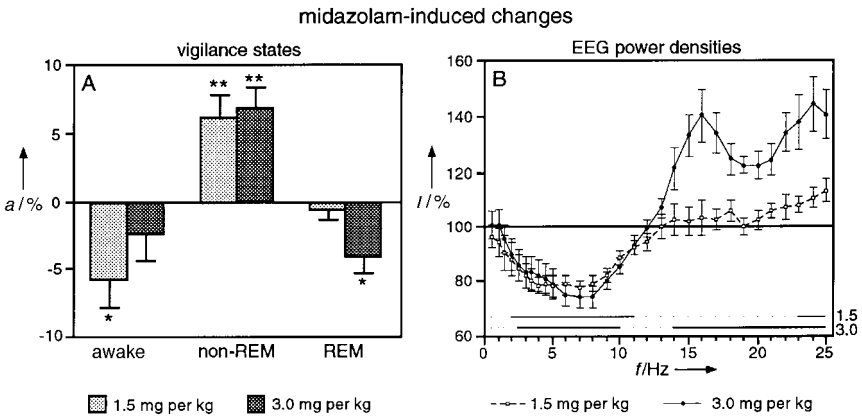


Figure 4. Influence of two doses of midazolam, administered intraperitoneally to rats at the beginning of the light period, on the percentage a of time in each vigilance state (A) and on the average EEG power density within non-REM sleep for the frequencies between 0.5 and 25 Hz (B) during the first 6 h after injection. Values are mean values with standard error of the mean ($n = 8$ for each dose). The vigilance-state data are expressed as deviations from the data with the placebo (percentage of recording time with midazolam minus percentage of recording time with placebo). The EEG data are expressed as a percentage of the corresponding placebo values (for each frequency band an intensity of 100% indicates the average EEG power density within non-REM sleep with placebo). Significant differences from the placebo data are indicated by one ($P < 0.05$) and two asterisks ($P < 0.01$, two-sided, paired t-test) for the vigilance states and with bars at the bottom of the graph for EEG power density ($P < 0.05$, two-sided, paired t-test run on normalized and logarithmically transformed values). The data are reproduced from references [29h, 43d].

Flunitrazepam, another benzodiazepine with a long elimination half-life, has consistently been found to increase total sleep time, which results from a shortened sleep latency, a reduction in the number of awakenings and body movements, and a marked increase in stage 2. In contrast, stage 1, SWS, and REM sleep are decreased, the last effect being due to a prolongation of REM latency and to a reduction in the number of REM sleep episodes.[30a, 31a–d] Flunitrazepam has also been shown to attenuate EEG power density in the lower frequencies (≤ 9 Hz) and to elevate activity in the spindle frequency range within non-REM sleep.[31c,d] Separate analysis of SWA revealed that flunitrazepam decreases mean SWA, while barely affecting the temporal development within and across non-REM sleep episodes.[31e,f] Similar effects on sleep architecture and sleep EEG power have been observed for another long-acting benzodiazepine, flurazepam.[27d, 31b,d, 32a–d] Computer signal analyses demonstrated that flurazepam reduces the amplitude as well as the number of slow waves and increases the occurrence of spindles.[32e–g]

The benzodiazepine bromazepam, which has a mediate elimination half-life, has been shown to reduce the number of body movements, increase stage 2 as well as the spindle rate, decrease stage 1, SWS, and REM sleep, and delay the latency to REM sleep as a function of dose.[30a] One study, in which the influence of a single dose of temazepam on nocturnal sleep was investigated, demonstrated a decrease in the number of awakenings, an increase in the percentage of stage 2, and a lowering of the precentage of wakefulness, stage 1, and SWS. Neither non-REM nor REM sleep latency was affected in this study.[33a] In contrast, when given shortly before daytime sleep following sleep deprivation as well as before night sleep after an early evening nap, temazapam appeared to shorten sleep latency and to delay the latency to REM sleep. Under both conditions, temazepam tended to increase total sleep time and stage 2 and to decrease SWS. Furthermore, spectral analysis of the non-REM sleep-specific EEG showed that temazepam reduced power in the lower frequencies during both conditions and enhanced activity in the spindle frequencies during daytime sleep.[33b]

The intake of the short-acting benzodiazepine midazolam at bedtime reportedly induces a shortening of subjective sleep latency and a reduction in the perceived time in wakefulness and in the number of awakenings.[34a] In a more elaborate study, midazolam was administered shortly before a bedtime that was advanced by four hours to assess its effects at a time of day when sleep propensity is low. Midazolam shortened sleep latency, promoted stage 2, and decreased stage 3, but had little influence on REM sleep. Moreover, midazolam depressed EEG power density in the low-frequency range (≤ 9 Hz) and augmented the activity in the spindle frequency region,[34b] while hardly influencing the time course of SWA and spindle activity within and across non-REM sleep episodes.[34c]

Most studies on the hypnotic effects of triazolam found an increased total sleep time, caused by a shortened sleep onset latency, an increase in stage 2, and a reduction in the duration and number of awakenings. Furthermore, triazolam has little effect on SWS, but seems to decrease REM sleep, which is mainly due to a delay in REM latency.[30f, 31d, 35] Spectral analysis of the EEG within non-REM sleep revealed similar alterations as evoked by other benzodiazepines.[31d–f] Also in accordance with the findings for other benzodiazepines, triazolam has been observed to increase the occurrence of spindles and to decrease the number of slow waves.[35d]

Taken together, the reactivity to benzodiazepines differs between species. In rats and humans benzodiazepine hypnotics increase the ability to fall and to stay asleep and promote non-REM or pre-REM sleep. In the cat, the same drugs induce alertness. Nevertheless, in each of these species they increase the occurrence of spindles and depress slow waves during non-REM sleep and decrease REM sleep, which is related to a suppression of the REM sleep initiation and possibly also to a disruption of its maintenance.

4.3. Zolpidem

In the rat, the imidazopyridine derivative zolpidem, which binds selectively to the benzodiazepine type I receptor, has been demonstrated to shorten the latency to non-REM sleep, to increase non-REM sleep time, to have little effect on pre-REM sleep, and to decrease REM sleep as a function of dose. The last effect is associated with a prolongation of the appearance of the first REM episode and with a transient reduction in the number of REM episodes. The promotion of non-REM sleep and the suppression of REM sleep have been observed during both the light and dark periods.[26a, 29i, 36]

Similar to the effects of benzodiazepines, zolpidem initially induces wakefulness in the cat. Thereafter, it reportedly promotes both non-REM and pre-REM sleep in a dose-related manner.[36a]

The experiments focussed on the effects of zolpidem on night sleep in young, healthy subjects revealed only moderate hypnotic effects. Some reports yielded a dose-dependent, slight increase in total sleep time, which was associated with a shortening of sleep onset latency. Most studies revealed no influence on the number of awakenings or on the time in the non-REM sleep substages, but found a tendential decrease in REM sleep or a slight delay in REM latency.[37a–d] Although zolpidem has minimal effects on time in each of the non-REM sleep stages, spectral analysis of the EEG within non-REM sleep found a marked attenuation of power in the low-frequency bands (≤ 10 Hz) and an enhancement in the frequency range of sleep spindles.[37d] More pronounced effects of zolpidem were observed on sleep during the daytime, when the propensity and need for sleep are relatively low. During such a condition, it appears to increase total sleep time, mainly caused by a decrease in the number of awakenings and an increase in stage 3, and to decrease REM sleep, without significantly affecting the latencies to either non-REM or REM sleep, in a dose-related fashion.[37a] The observed increase in visually scored stage 3 may be related to the selective binding of zolpidem to benzodiazepine type I receptors and their regional distribution. Furthermore, the effects of zolpidem on sleep have been shown to increase with age. The administration of zolpidem to noninsomniac middle-aged subjects prior to bedtime reduces wakefulness and the number of awakenings, decreases stage 1, increases stage 2,

and, without affecting total time in REM sleep, delays the latency to REM sleep as a function of dose.[37a] In geriatric subjects, without sleep complaints, zolpidem has been found to shorten the subjective and objective sleep onset latency, to reduce time in wakefulness after sleep onset, to increase the percentage of stage 2, and to decrease the percentage of stage 1 as well as REM sleep in a dose-dependent fashion.[37e]

Thus, except for the finding that it does not promote pre-REM sleep in the rat, zolpidem affects sleep in a benzodiazepine-like fashion. In cats, zolpidem transiently produces wakefulness, whereas in both rats and humans it is able to induce and maintain non-REM sleep, facilitates spindling, and, though not detectable by visual scoring, depresses slow components in the EEG during non-REM sleep, and initially inhibits the appearance of REM sleep.

4.4. Zopiclone

The cyclopyrrolone derivate zopiclone, which is structurally related to zolpidem, probably binds at a physically distinct domain on the $GABA_A$ receptor that is closely linked to a benzodiazepine binding site.[20b, 38a] Zolpidem administered to rats during the light period decreases wakefulness, caused by a shortening of the latency to and an increase of the time spent in non-REM sleep, as a function of dose. Furthermore, it decreases pre-REM and REM sleep, which is generated by a delay in the latency of both stages and a reduction in the number of pre-REM and REM sleep episodes.[38a,b]

In the cat, zopiclone has been shown to increase wakefulness, at the expense of all sleep stages, particularly non-REM and REM sleep, in a dose-related manner.[38c,d]

Administration of zopiclone to young, soundly sleeping subjects has only minimal effects on nocturnal sleep. It merely seems to decrease stage 1, to increase stage 2, and to prolong the latency to REM sleep.[39a–f] In noninsomniac, middle-aged subjects zopiclone additionally decreases wakefulness and the number of awakenings.[39g] Zopiclone appears to have more pronounced effects in a phase-advanced sleep schedule: When given prior to a bedtime that was advanced by four to six hours, zopiclone has been shown to shorten sleep onset latency and to increase total sleep time owing to an increase in stage 2.[34b, 39h] Although it hardly affects visually scored SWS, spectral analysis of the EEG within non-REM sleep yielded a prominent reduction in power density in the low-frequency range (≤ 10 Hz) and an enhanced activity in the spindle frequencies both during phase-advanced and nocturnal sleep.[34b, 39d] Furthermore, zopiclone induced a general decrease in SWA and an increase in spindle activity, without disrupting the inter- or intraepisodic time course.[34a] Period-crossing analysis showed that zopiclone decreases the number of high-amplitude slow waves and increases the number of low-amplitude slow waves.[39i] The elevation in spindle activity appears to be brought about by an increased spindle density.[39j]

Taken together, the effects of zopiclone on sleep are to a large extent comparable to those evoked by short-acting benzodiazepines. In cats, zopiclone transiently promotes wakefulness, while in humans and rats it is able to induce and consolidate non-REM sleep, facilitates the occurrence of spindles, attenuates low-frequency EEG signals, and suppresses the initiation of REM sleep.

4.5. Neuroactive Steroids

Various steroid hormones modulate $GABA_A$-mediated transmission, probably through unique steroid binding sites that are distinct from those for barbiturates and benzodiazepines.[20a,b, 40] Particularly the ring A reduced metabolites of progesterone, 3α-hydroxy-5α-dihydroprogesterone (allopregnanolone) and 3α-hydroxy-5β-dihydroprogesterone (pregnanolone), and of deoxycorticosterone (DOC), tetrahydro-DOC (THDOC), are potent naturally occurring agonistic modulators of $GABA_A$ receptors. At low nanomolar concentrations these steroids augment GABA-induced $GABA_A$ receptor currents by increasing both the frequency and duration of chloride channel opening. Similar to barbiturates, at high concentrations they are able to directly activate $GABA_A$ receptors in the absence of GABA.[41]

Already in the 1940s progesterone was found to exert anesthetic actions, in that peripheral administration of high doses of progesterone causes the loss of the righting reflex in rats.[42a] Progesterone has recently been shown to influence sleep in the rat.[42b] It shortens the latency to non-REM sleep, decreases wakefulness due to a selective promotion of pre-REMS (Figure 5A), and increases the duration of the non-REM episodes as a function of dose. At a high dose, progesterone suppresses REMS by a prolongation of REM latency and a reduction in the number of REM episodes. Spectral analysis of the EEG within non-REM sleep yielded an attenuation of low-frequency (≤ 7 Hz) activity and enhancements in the spindle and higher frequency bands (Figure 5B). In young, healthy subjects, a single oral dose of 300 mg of micronized progesterone at bedtime was found to increase stage 2, to tendentially decrease stage 4 and REM sleep, and to depress EEG activity in the lower frequencies, while augmenting the activity in the frequencies above 15 Hz within non-REM sleep.[42c] In both studies progesterone produced an elevation in the levels of allopregnanolone and, to a lesser extent, of pregnanolone in the brain and/or plasma.

The observations that progesterone evokes benzodiazepine-like changes in sleep and that the temporal development of the alterations in sleep and in the concentrations of allopregnanolone and pregnanolone parallel strongly suggest that progesterone affects sleep through its bioconversion into neuroactive metabolites. In line with this postulate, the $GABA_A$ receptor blocker picrotoxin appeared effectively to attenuate most of the changes in sleep evoked by progesterone in the rat.[42d] Further support for this notion comes from studies on the influence of exogenous pregnanolone and allopregnanolone. In the rat, administration of various doses of pregnanolone and a synthetic analogue, CCD-3693, during the dark period rapidly promotes non-REM sleep in a dose-related manner, without disrupting REM sleep.[29i] Unfortunately, pre-REM sleep was not distinguished in this study. The administration of allopregnanolone at the beginning of the light period was found to shorten non-REM sleep latency and to significantly increase pre-REM sleep, without interfering with REM sleep (Figure 5C), as a function of dose. Moreover,

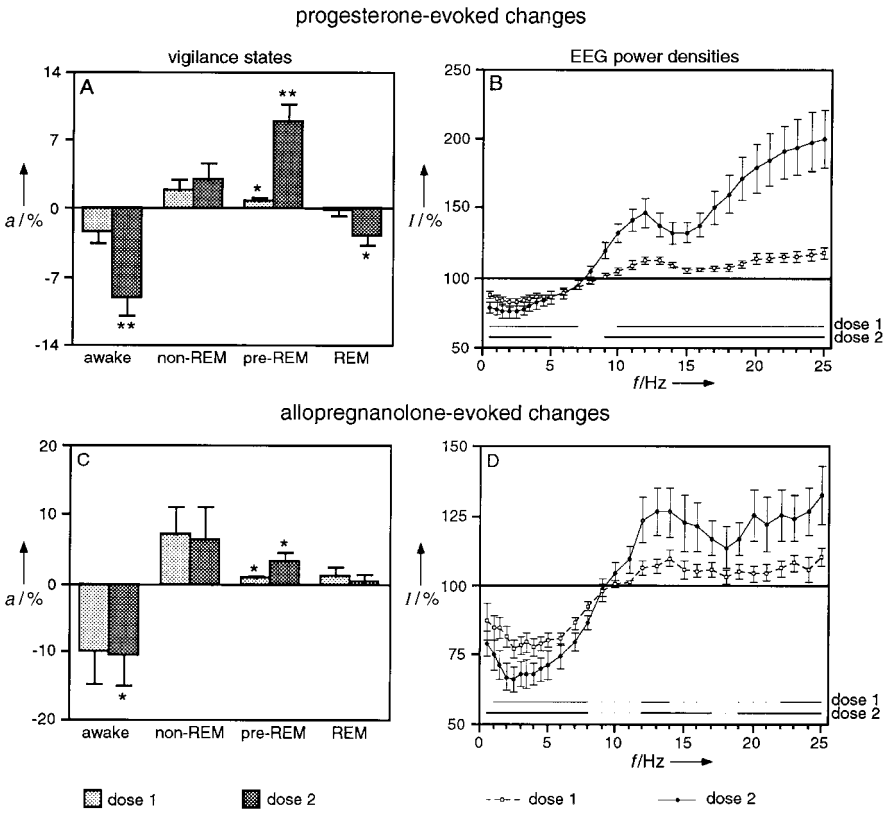


Figure 5. Influence of two doses of progesterone (A, B) and of allopregnanolone (C, D), administered intraperitoneally to rats at the beginning of the light period, on percentage *a* of time in each vigilance state and on average EEG power densities within non-REM sleep during the first 12 h after administration of progesterone and during the first 2 h after administration of allopregnanolone. Values are mean values with standard errors of the mean ($n = 8$). See the legend to Figure 4 for more information. Both substances were dissolved in 35% hydroxypropyl-β-cyclodextrin solution, and, to attain a slower release, allopregnanolone was thereafter mixed with corn oil. The doses employed were 90 (dose 1) and 180 mg per kg (dose 2) of progesterone and 7.5 (dose 1) and 15 mg per kg (dose 2) of allopregnanolone. The data are reproduced from references [42b,e].

it depressed slow frequency components (≤ 8 Hz) and augmented EEG power density in the spindle and higher frequency bands (Figure 5D).[42e] Intriguingly, this study showed that doses of allopregnanolone and progesterone that produce comparable elevations in the brain levels of allopregnanolone have quantitatively and qualitatively similar effects on sleep. Pregnanolone has also been demonstrated to increase sleep propensity in humans. Short polygraphic recordings made during resting conditions at 30-minute intervals revealed that pregnanolone, administered early in the morning to young healthy subjects, increases the number of sleep attempts and the sleep time compared with predosing recordings.[42f]

Much less attention has been given to the soporific effects of THDOC. One study investigated the effects of THDOC, administered during the light period, on sleep in rats and found a dose-dependent decrease of non-REM sleep latency and an increase in non-REM sleep time.[42g] As pre-REM sleep was not separated from non-REM sleep, it is unknown whether THDOC particularly promotes pre-REM sleep. Studies on the influence of the THDOC precursor DOC on sleep in humans did not find alterations.[42h,i] One possible explanation for the lack of effect is that DOC may not be able to enter the brain.

Although the given findings await confirmation and need to be extended, they strongly suggest that steroidogenic agonistic modulators of $GABA_A$ receptors evoke a sleep profile reminiscent of that induced by short-acting benzodiazepines. Similar to benzodiazepines, these steroids appear to be able to increase the ability to fall and to stay asleep, to promote non-REM or pre-REM sleep, to enhance the occurrence of spindles, and to attenuate slow EEG waves during non-REM sleep. In contrast to benzodiazepines, they do not seem to inhibit the initiation of REM sleep. However, since a high dose of progesterone has been demonstrated to clearly interfere with REM sleep in the rat, it can not be excluded that higher doses of its neuroactive metabolites exert a similar effect.

5. Effects of $GABA_A$ Receptor Agonists on Sleep

$GABA_A$ agonists, such as muscimol and 4,5,6,7-tetrahydroisoxazolo-pyridin-3-ol (THIP), are structural analogues of GABA that interact directly with the GABA binding site on the $GABA_A$ receptor complex and thereby increase the membrane conductance for chloride ions.

In the rat, peripheral administration of muscimol during the light period exerts little effect on sleep latency, but increases non-REM sleep and, to a lesser extent, REM sleep as a function of dose (Figure 6A) and tends to increase the duration of the non-REM and REM episodes. EEG spectral analysis revealed that muscimol enhances low-frequency activity within non-REM sleep (Figure 6B). The mean elevation of SWA appeared to be associated with an overall increase of SWA within the non-REM sleep episodes.[29h] The partial agonist THIP, administered during the light period, has also been demonstrated to increase non-REMS (Figure 6C), to tendentially lengthen the duration of the non-REM episodes, and to augment slow frequency components in the EEG within non-REM sleep (Figure 6D).[43a] Qualitatively similar, but more pronounced effects of THIP were observed when administered during the dark period.[43b] In young, noninsomniac subjects a single oral dose of THIP, given half an hour before bedtime, increased the sleep efficiency index (ratio of total sleep time to time in bed) and time in SWS in comparison to a placebo (Figure 7A). Moreover, it enhanced SWA and attenuated EEG activity in the frequency range of sleep spindles within non-REM sleep (Figures 7B, C).[43c] These findings suggest that at least the $GABA_A$ agonists muscimol and THIP are able to increase the maintenance and intensity of non-REM sleep, without disrupting REM sleep.

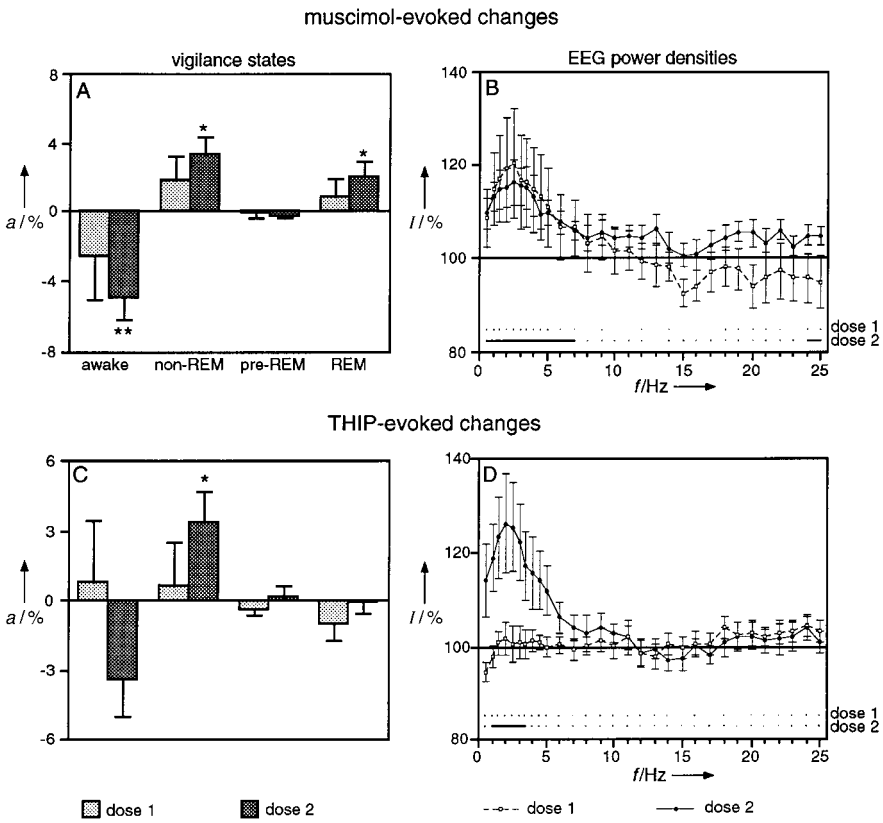


Figure 6. Effects of two doses of muscimol (A, B) and of THIP (C, D), administered intraperitoneally to rats at the beginning of the light period, on percentage *a* of time in each vigilance state and on average EEG power densities within non-REM sleep during the first 6 h after injection. Values are mean values with standard errors of the mean ($n = 8$ for each substance). See the legend to Figure 4 for more information. The doses employed were 0.2 (dose 1) and 0.4 mg per kg (dose 2) of muscimol and 2 (dose 1) and 4 mg per kg (dose 2) of THIP. The data are reproduced from references [29h, 43a].

Since $GABA_A$ agonists and benzodiazepines mimic and potentiate the action of endogenous GABA at the $GABA_A$ receptor, one would expect that $GABA_A$ agonists have effects similar to benzodiazepines and that their effects should be enhanced by benzodiazepines. With regard to their influence on sleep parameters, this is clearly not the case. While muscimol and THIP particularly promote deep non-REM sleep, benzodiazepines increase sleep propensity, promote a state with the EEG characteristics of shallow sleep, and suppress SWS and REM sleep. Moreover, studies in which benzodiazepines and muscimol were coadministered to rats found that these compounds do not augment each other's effects on sleep architecture,[29c,e, 43d] but instead even attenuate each other's effects on the EEG signals within non-REM sleep.[43d] These findings, which are at variance with the classical view, indicate that the influence of agonists and agonistic modulators of $GABA_A$ receptors on electrical brain activity differs substantially. This notion is supported by observations made in studies on non-convulsive, absence-epilepsy (petit-mal) and on drug discrimination. Whereas $GABA_A$ agonists trigger or exacerbate absence-epilepsy in epilepsy-prone animals,[44a] various agonistic modulators suppress spontaneous as well as $GABA_A$ agonist induced absence-epileptic sei zures.[29g, 44b] Rats trained to discriminate benzodiazepines from saline react the same to other benzodiazepines as well as to barbiturates, allopregnanolone, and THDOC[45a,b], but not to muscimol or THIP.[45c]

A possible explanation for the divergent effects of $GABA_A$ agonists and benzodiazepines is that these agents activate different $GABA_A$ receptor subtypes. Whereas muscimol and THIP are likely to act on all $GABA_A$ receptors, the responsiveness to benzodiazepines depends on the precise receptor composition. If this postulate were true, one might expect that the massive activation by GABA analogues dominates the response and masks the modulation evoked by benzodiazepines. As outlined above, this has neither been observed in sleep investigations nor in absence-epilepsy studies. One may also postulate that the hypnotic effects of benzodiazepines are not mediated by

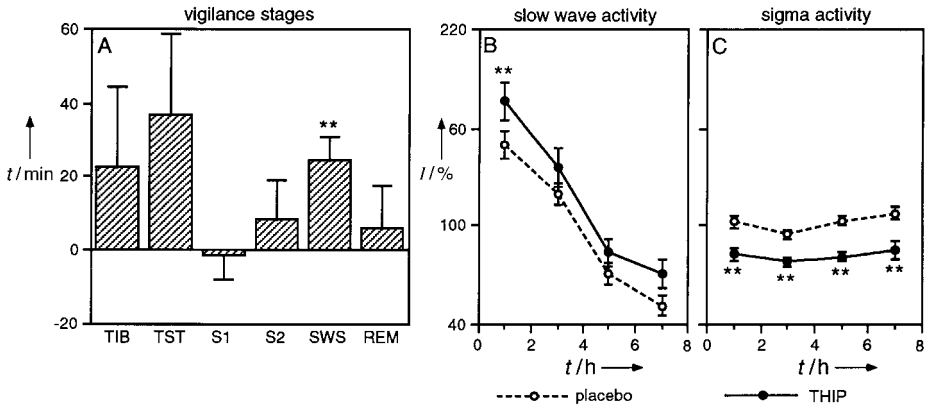


Figure 7. Influence of 20 mg of THIP, given orally shortly before bedtime to young healthy subjects, on visually scored sleep parameters (A) and on average slow wave activity (B) and sigma activity (C) within non-REM sleep for two-hour intervals. Values are mean values with standard errors of the mean ($n = 10$). The sleep states are expressed as deviations from the data with the placebo (time after administration of THIP [min] minus time after administration of the placebo [min]). Average slow wave activity (0.8 – 4.3 Hz) and sigma activity (12.5 – 14.8 Hz), which are plotted in the middle of the two-hour intervals, are expressed as percentage of the average power densities within non-REM sleep during the entire night with placebo. TIB = total time in bed; TST = total sleep time; S1 = stage 1; S2 = stage 2; SWS = slow wave sleep; REM = rapid eye movement sleep. Significant differences between the treatments are indicated by two asterisks ($P < 0.01$; Wilcoxon matched pairs signed rank test for vigilance states, and two-sided, paired *t*-test for EEG power densities). The data are reproduced from reference [43c].

$GABA_A$ receptors. This is not very likely since other agonistic modulators of $GABA_A$ receptors exert comparable effects on sleep as benzodiazepines, and flumazenil, an antagonist of the benzodiazepine binding site, has been shown to totally antagonize nearly all benzodiazepine-induced sleep alterations.[31c] Sleep-related processes are associated with the liberation of GABA in specific brain regions. Thus, an alternative and obvious explanation for the different effects of $GABA_A$ agonists and benzodiazepines is that the former stimulate $GABA_A$ receptors throughout the brain, while benzodiazepines potentiate the action of selectively released GABA on $GABA_A$ receptors. Furthermore, GABA and GABA analogues may exert differential net effects on $GABA_A$ receptor function under in vivo conditions. The action of liberated GABA is temporally and spatially confined by rapid uptake into neurons and glial cells.[46] In contrast, both muscimol and THIP are poor substrates for uptake mechanisms and may therefore produce more tonic hyperpolarizations than synaptically released GABA, which is likely to have a considerable impact on neural electric activity. In line with the postulate that the distinct effects of muscimol and THIP are caused by an unphysiological tonic activation of $GABA_A$ receptors, the GABA re-uptake inhibitor tiagabine has recently been shown to facilitate absence-epileptic seizures[47a] and to enhance low-frequency components in the EEG within non-REM sleep in the rat.[47b]

Taken together, the few available studies on the hypnotic effects of muscimol and THIP suggest that these $GABA_A$ agonists have minimal effects on sleep initiation, but are able to increase sleep maintenance and to promote deep sleep, while having no disrupting influence on REM sleep. Moreover, in humans THIP selectively depresses EEG power density in the spindle frequencies. It is at present unclear whether this is due to a reduction in spindle rate, amplitude, and/or duration.

6. Summary and Outlook

The reviewed data demonstrate a clear link between the hypnotic properties of drugs and their action at the $GABA_A$ receptor complex. However, depending on the site of binding, various substances exert different effects on sleep.

The previously and currently marketed sleeping pills that act at the $GABA_A$ receptor—barbiturates, benzodiazepines, zolpidem, and zopiclone—produce an agonistic modulation and thereby effectually increase the ability to fall and to stay asleep. A common disadvantage of these drugs is the lacking capacity to induce physiological sleep. The most natural method to promote sleep is sleep deprivation. As described in Section 2, sleep deprivation is consistently followed by an increase in deep sleep, as reflected by an increase in SWS and SWA. Benzodiazepines, zolpidem, and zopiclone modulate sleep in the opposite fashion, as they suppress SWS and/or SWA. In addition, all these substances as well as barbiturates facilitate the occurrence of sleep spindles, which are a sign of shallow sleep. Furthermore, these drugs decrease REM sleep.

Beside changing the natural sleep pattern, the aforementioned substances exert other relevant side effects. Barbiturates are now obsolete in the treatment of insomnia, owing to their toxicity. Accidental or suicidal poisoning with barbiturates may be lethal. Benzodiazepines are much safer than barbiturates and were for a long time termed "lucky pills", until the risk of dependency and addiction became evident. Nowadays the prescription of benzodiazepines is restricted in many countries. In Germany, for example, benzodiazepines are the only class of drugs for which an official usage guideline was released by an expert group on behalf of the federal government. One major point is the recommendation that benzodiazepines should not be prescribed long-term. Since insomonia is frequently chronic, doctors and patients have the problem that they must discontinue successful treatment and undergo the risk of a relapse. The newer hypnotics zolpidem and zopiclone, which exert qualitatively similar effects on sleep as benzodiazepines, were claimed to have a lower tolerance and dependency potential. However, recent observations suggest that particularly patients with a history of substance abuse as well as patients with psychiatric disorders are at risk for abuse of zolpidem and zopiclone.[48a]

The finding that certain naturally occurring steroid metabolites are agonistic modulators of $GABA_A$ receptors gives rise to the question of whether such substances are putative hypnotics. The results reviewed here indicates that the effects of such neurosteroids on sleep largely resemble those of benzodiazepines. The only difference is that they may not suppress REM sleep. Research in mice indicates that tolerance develops rapidly. Similar to the case of the benzodiazepine temazepam, the sedative response to an acute dose of the neuroactive steroid minaxolone is lost in mice after treatment with the drug for seven days.[48b] Thus, endogenous neurosteroids may play a role in physiological sleep–wake regulation, but they seem unsuitable as sleeping pills.

In contrast to agonistic modulators of $GABA_A$ receptors, the $GABA_A$ agonists muscimol and THIP enhance SWA and exert minimal effects on REM sleep. Although only few studies have been done with these compounds, it is evident that they mimic the effects of a physiological increase in the need for sleep as evoked by sleep deprivation. Possibly, $GABA_A$ agonists may have prospects in the treatment of sleep that is too light and nonrefreshing. As SWS and SWA decrease during normal aging, especially the elderly may benefit from compounds with such a working profile. Further research is necesarry to delineate the effects of $GABA_A$ agonists in insomniacs. Moreover, the effects of long-term treatment and drug withdrawal need to be investigated.

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