Role of GABA_A receptors in the physiology and pharmacology of sleep

Raphaëlle Winsky-Sommerer

Institute of Pharmacology and Toxicology, University of Zurich, Switzerland

Raphaëlle Winsky-Sommerer

Section for Chronobiology and Sleep Research

Institute of Pharmacology and Toxicology

University of Zurich

Winterthurerstrasse 190

CH-8057 Zurich, Switzerland

E-mail: winsky@pharma.uzh.ch

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Abstract

Most sedative-hypnotics used in insomnia treatment target the γ -aminobutyric acid (GABA)_A receptors. A vast repertoire of GABA_A receptor subtypes was identified and displays specific electrophysiological and functional properties. GABA_A-mediated inhibition traditionally refers to “phasic” inhibition, arising from synaptic GABA_A receptors, which transiently inhibit neurons. However, there is growing evidence that peri/extra-synaptic GABA_A receptors are continuously activated by low GABA concentrations and mediate a “tonic” conductance. This slower type of signaling appears to play a key role in controlling cell excitability. This review aims at summarizing recent knowledge on GABA transmission, including the emergence of tonic conductance, and highlighting the importance of GABA_A receptor heterogeneity. The mechanism of action of sedative-hypnotic drugs and their effects on sleep and the electroencephalogram (EEG) will be reported. Furthermore, studies using genetically-engineered mice will be emphasized, providing insights into the role of GABA_A receptors in mechanisms underlying physiological and pharmacological sleep. Finally, we will address the potential of GABA_A receptors pharmacology for the treatment of insomnia.

1. Introduction

It is a common experience that sleep is critical to recover from tiredness and thereby has potent restorative virtues. Poor quality of sleep, typical for insomnia, has many adverse effects on daily life. Insomnia is highly prevalent. It is defined by complaints of disturbed sleep in the presence of adequate opportunity for sleep. The disturbances can consist of one or several features including difficulty in initiating sleep, maintaining sleep, waking up too early and, non-restorative or poor-quality sleep (Mendelson *et al.*, 2004; Roth, 2007). It has been proposed that insomnia is a hyperarousal disorder which could reflect a deficit in sleep homeostasis (Borbély & Achermann, 2005; Vgontzas, 2005). Pharmacology plays an important role in insomnia therapy, targeting several transmitter and peptide systems, including γ -aminobutyric acid/GABAergic, serotonergic, as well as histaminergic and hypocretin/orexinergic systems (Wafford & Ebert, 2008). Past and current treatments have been dominated by sedative-hypnotic drugs acting at GABA_A receptors, including the traditional benzodiazepines (BZ) and non-BZ drugs (i.e., “Z”-drugs: zolpidem, zaleplon, zopiclone, eszopiclone).

The classical view of neuronal activity relies on the balance between two modes of transmission, excitation mediated by glutamate and inhibition mediated by GABA. GABA, the predominant inhibitory transmitter in the brain, plays a key role in neuronal firing patterns and activity of neuronal networks. In the central nervous system, GABA exerts its effect via ionotropic GABA_A and GABA_c receptors, and metabotropic GABA_B receptors (Barnard *et al.*, 1998; Bowery, 2006). GABA_B receptors are insensitive to drugs which target GABA_A and GABA_c receptors are primarily expressed in retina and optical layers of the superior colliculi. This review will thus focus on GABA_A receptors. Since their first characterization as “benzodiazepine”-receptors in the late 70s (Braestrup *et al.*, 1977; Möhler & Okada, 1977), GABA_A receptors have been shown to be a major site for clinically-relevant drugs, including barbiturates, some general anesthetics, as well as ethanol (Mohler, 2006). A vast repertoire of GABA_A receptor subtypes was identified, underlying the complexity of GABA_A-mediated transmission. Recently, the emergence of tonic inhibition, mediated by GABA_A

receptors located outside the synaptic junction, challenged the traditional view of GABA_A-mediated transmission. This novel type of conductance may provide further insights into the understanding of sleep mechanisms, as well as the effects of sedative-hypnotic drugs on sleep.

The following review will first recapitulate properties of GABA_A receptors underlying the heterogeneity of GABA_A-mediated transmission. Then the knowledge on pharmacological profiles of sedative-hypnotic drugs and their effects on sleep will be summarized. Studies using genetically-engineered mice providing insights into the role of GABA_A receptors in mechanisms underlying sleep will be highlighted.

2. Heterogeneity of GABA_A receptors and GABA_A-mediated transmission

GABA is released from the presynaptic neuron into the synaptic cleft. It exerts its action by binding to two cognate sites on the extracellular surface of GABA_A receptors. The activation of the ligand-gated ion channel results in a very rapid increase in permeability for chloride ions, leading to membrane hyperpolarization and inhibition of excitability (Maconochie *et al.*, 1994).

In addition to the fast inhibitory transmission, there is increasing evidence that GABA_A chloride conductance can depolarize postsynaptic neurons and may have excitatory actions. When combined with other depolarizing inputs within a specific time window, activation of GABA_A receptors may facilitate action potential generation. Therefore, GABAergic inputs at the same synapse may result in different actions depending on intracellular chloride concentrations at the site of GABAergic input, as well as the spatio-temporal summation with other inputs (Gulledge & Stuart, 2003; Marty & Llano, 2005). Furthermore, but beyond the scope of this review, GABA_A receptors not only mediate their action at the level of postsynaptic neurons but also presynaptically (Kullmann *et al.*, 2005).

2.1. Heterogeneity of subunits forming a variety of GABA_A receptors.

GABA_A receptors are pentameric hetero-oligomers, the subunits' assembly forming the central ion channel. Several subunit classes have been identified in mammalian genomes, each of the subunits displaying several isoforms (α 1-6, β 1-3, γ 1-3, δ , ϵ , π , ρ 1-3, and θ ; (Barnard *et al.*, 1998; Whiting *et al.*, 1999). The potential permutations forming pentameric oligomers are considerable. However, not all subunits co-assemble to form GABA_A subtypes. For instance, δ and γ subunits are mutually exclusive (Sieghart *et al.*, 1999). The δ , ϵ and π subunits are believed to substitute for the γ subunit, whereas the θ may replace the β subunit. The subunit composition and stoichiometry of native GABA_A receptors is not yet completely elucidated. The properties of native GABA_A receptors could be recapitulated *in vitro* when α , β and γ subunits were co-expressed (Pritchett *et al.*, 1989). Further available evidence favors GABA_A receptors containing α -, β -subunits, and in the vast majority of cases a γ subunit in a 2:2:1 stoichiometry (Chang *et al.*, 1996; Tretter *et al.*, 1997). A 2:1:2 stoichiometry has also been reported (Backus *et al.*, 1993). The majority of receptors appear to consist of a single α isoform. However, a minority of populations containing α 1 α 2, α 1 α 3, α 1 α 5, α 2 α 3 or α 3 α 5 pairs have been detected (Barnard *et al.*, 1998).

2.2. Specific anatomical expression of GABA_A subtypes.

The assembly of subunits forming the GABA_A receptors also depends on the regional distribution of each subunit in the brain (Wisden *et al.*, 1988; Fritschy & Mohler, 1995; Pirker *et al.*, 2000). Furthermore, the cellular (somato-dendritic *versus* axonal) and subcellular localization of GABA_A receptors on neurons contributes to the complexity of GABA_A receptor heterogeneity (Somogyi *et al.*, 1989; Kullmann *et al.*, 2005; Trigo *et al.*, 2008). While α 1-, α 2-, α 3-containing subtypes are mainly localized synaptically, these receptor subtypes are also found outside the synapse (Table 1) (Somogyi *et al.*, 1989). The δ -containing receptors (i.e., α 4 β x δ , α 1 β x δ , and α 6 β x δ) appear to be predominantly or exclusively peri- and/or extra-synaptic, though morphological evidence for such extrasynaptic

localization is scarce (Nusser *et al.*, 1998; Fritschy & Brunig, 2003). The $\alpha 5$ -containing receptors are mainly extrasynaptic. Nevertheless, there is functional and morphological evidence that a pool of $\alpha 5$ -containing receptors also concentrates at GABAergic synapses located on dendrites (Serwanski *et al.*, 2006; Ali & Thomson, 2008). This segregation between synaptic and extrasynaptic GABA_A receptors is important since they are involved in mediating two distinct forms of GABA_A-transmission (Semyanov *et al.*, 2004; Mody, 2005).

2.3. Two distinct modes of GABA_A-mediated transmission: phasic versus tonic inhibition

GABA_A-mediated transmission traditionally refers to the transient -“phasic”- inhibitory postsynaptic currents (IPSCs) following the activation of synaptic receptors by high concentration (mM range) of GABA released from presynaptic nerve terminals (Maconochie *et al.*, 1994). However, there is growing evidence that extrasynaptic GABA_A receptors are continuously activated by low (<50 μ M) GABA concentration and thereby mediate a persistent -“tonic”- inhibition (Fig. 1). Though the discovery of tonic inhibition is relatively recent, new insights emphasize the importance of this transmission (Semyanov *et al.*, 2004; Cavelier *et al.*, 2005; Farrant & Nusser, 2005; Mody, 2005; Walker & Semyanov, 2008).

Electrophysiological recordings in rodent brain slices demonstrated the presence of GABA_A-mediated tonic inhibition in several regions and specific cell-types, including cerebellar granule cells (Brickley *et al.*, 1996), relay neurons of the ventrobasal thalamic nuclei (Porcello *et al.*, 2003; Belelli *et al.*, 2005; Jia *et al.*, 2005), pyramidal cells of neocortical layers II-III and V (Drasbek & Jensen, 2006; Drasbek *et al.*, 2007; Yamada *et al.*, 2007; Vardya *et al.*, 2008), as well as the hippocampal dentate gyrus granule cells and the hippocampal interneurons of the molecular layer (Glykys *et al.*, 2008). The occurrence of tonic currents correlates with the regional and extrasynaptic distribution of specific GABA_A subtypes (Table 1, Fig.2). The majority of extrasynaptic receptors is believed to contain the δ -subunit. This subunit shows high expression levels in the cerebellum, numerous thalamic

relay nuclei, the outer layers of the cortex (I-III) and the dentate gyrus (Wisden *et al.*, 1988; Fritschy & Mohler, 1995; Peng *et al.*, 2002). The δ subunit is preferentially associated with the $\alpha 4$ subunit (Sur *et al.*, 1999) and the $\alpha 4$ expression pattern parallels that of the δ subunit. Nonetheless, a fraction of $\gamma 2$ -containing receptors located extrasynaptically also contributes to tonic conductance when activated by ambient GABA.

2.4. *GABA_A subtypes display distinct electrophysiological properties.*

The subcellular segregation of specific GABA_A subtypes together with the biophysical properties of GABA_A receptors (i.e., concentration of ligand to induce the half maximal response, rate and extent of activation, desensitization and deactivation of the current) define their respective contribution to phasic or tonic conductance (for review, Vicini & Ortinski, 2004; Farrant & Nusser, 2005). The subunit composition of the receptor has considerable impact on the biophysical properties of the chloride channel. The type of α -subunit determines the kinetics of receptor activation (Verdoorn *et al.*, 1990). δ -containing receptors (i.e., $\alpha 6\beta 3\delta$ and $\alpha 4\beta 3\delta$) require low concentrations of GABA to reach 50% activation of the maximal response ($EC_{50} = \sim 0.3\text{--}0.7 \mu\text{M}$; (Brown *et al.*, 2002; Farrant & Nusser, 2005). In contrast, $\gamma 2$ -containing receptors (e.g. $\alpha 1\beta 3\gamma 2$ or $\alpha 2\beta 3\gamma 2$ combinations) have a lower affinity for GABA and thus higher EC_{50} ($\sim 6\text{--}14 \mu\text{M}$). δ containing receptors display lower rate of desensitization compared to γ -containing receptors, and the rate and extend of desensitization is also dependent on the type of α -subunit present.

Phasic and tonic conductance can be modulated by changes in the number and properties of receptors, as well as GABA release and uptake. Extrasynaptic receptors respond to low, persistent concentration of GABA in the extracellular space, potentially due to spillover from the synapse as well as release from astrocytes (Fig.1) (Walker & Semyanov, 2008). Other amino acids, such as taurine and β -alanine, may also be involved in the generation of tonic currents (Cavelier *et al.*, 2005; Jia *et al.*, 2008; Walker & Semyanov, 2008). Elevated ambient GABA (possibly drug-induced) or actions of

allosteric modulators, such as BZ and Z-drugs, may recruit other extrasynaptic and/or synaptic receptors in a given neuronal circuit which may contribute tonic conductance.

Overall, the affinity for GABA, the rate and extent of desensitization, together with the subcellular segregation of distinct GABA_A subtypes are key parameters underlying their differential activation and their contribution to phasic or tonic conductance.

2.5. Physiological significance of phasic and tonic inhibition.

Phasic inhibition, mediated by the activation of synaptic GABA_A receptors is characterized by IPSC currents that peak and decay within milliseconds (Mody *et al.*, 1994). This transient activation is due to the rapid clearance of GABA from the synaptic cleft by active transporters and simple diffusion (Grabauskas, 2005).

In contrast, the persistent activation of extrasynaptic receptors may result in an increase of membrane conductance (Semyanov *et al.*, 2004; Cavelier *et al.*, 2005; Farrant & Nusser, 2005), thereby reducing the change in membrane voltage elicited by excitatory input currents. Tonic conductance was proposed to decrease spatial and temporal integration of excitatory signals (Pouille & Scanziani, 2001; Cavelier *et al.*, 2005). In cerebellar granule cells, frequency of action potential firing induced by an excitatory input can be altered upon pharmacological blockade of tonic inhibition (Hamann *et al.*, 2002). Tonic inhibition in this cell-type generated a mean inhibitory conductance 3-fold larger than currents produced by high frequency action potentials evoked by vesicular release of GABA. Moreover, it contributed to ~90% of the total inhibition (Hamann *et al.*, 2002). The magnitude of tonic conductance varies among cell-types.

In summary, phasic conductance rapidly and transiently inhibits neuronal excitability. In contrast, the effects of the tonic “background” conductance, a slower type of signaling, reduce the capability of excitatory stimuli to generate action potentials. Thereby, tonic conductance modulates neuronal firing rates and adjusts the excitability of neuronal networks. This property of establishing a “baseline”

excitability level of neuronal networks raises the intriguing possibility that such a mechanism may be involved in sleep and cognition.

2.6 Tonic inhibition is present in brain regions and cell populations relevant for sleep.

The high expression levels of the extrasynaptic $\alpha 4\beta\gamma\delta$ subtype, as well as the presence of GABA_A-mediated tonic currents in the thalamus and cortex (see section 2.3) are of particular interest in the context of sleep physiology (Table 1, Fig. 2). Tonic currents represent 80 % of the total GABA_A-mediated transmission in relay cells of the thalamus, while no tonic currents were observed in the thalamic reticular nucleus (Belelli *et al.*, 2005). The thalamus and cortex form a functional network via integrated recurrent loops, enabling the synchronized activity of large-scale ensembles of neurons which generate specific brain oscillations (Steriade *et al.*, 1986; Jones, 2002; Steriade, 2003). The type of firing of thalamo-cortical neurons *in vivo* is behavioral-state dependent. Low-threshold bursting is associated with non-rapid eye movement (NREM) sleep, while tonic firing is typical for waking. Blocking tonic inhibition favors tonic firing in thalamic slices (Cope *et al.*, 2005). On the other hand, pharmacological enhancement of tonic inhibition hyperpolarized thalamic relay neurons. Modulation of tonic conductance also alters the temporal precision of low-threshold burst firing (Bright *et al.*, 2007). Interestingly, widespread changes in GABA release across the thalamus increased tonic inhibition in the thalamic relay neurons while a local increase in GABA had no effect (Bright & Brickley, 2008). By adjusting the excitability of neuronal circuitries, tonic inhibition may play an important role in modulating the magnitude and frequency of network oscillations characterizing vigilance states and their associated behaviors.

3. Pharmacological profiles of sedative-hypnotic drugs targeting GABA_A receptors and their effects on sleep

The structural complexity of GABA_A receptors, the multiple receptor subtypes with distinct electrophysiological properties (channel kinetics, affinity for GABA, rate of desensitization, phasic *versus* tonic conductance), and specific regional, cellular and subcellular distribution, underlie their functional properties and pharmacological profiles.

Most sedative-hypnotics used in the treatment of insomnia target the GABA_A receptors. It is commonly accepted that anxiolytic, anticonvulsant, myorelaxant, as well as amnestic, sedative and hypnotic actions of these drugs are due to their “general” inhibitory effect on neuronal activity (Rudolph & Mohler, 2004). Understanding how these various sedative-hypnotic drugs enhance phasic and/or tonic GABA_A-mediated transmission is of particular importance.

3.1 Binding sites on the GABA_A receptors & pharmacological profiles of sedative-hypnotic drugs.

GABA binds at the interface between α and β subunits. BZs and Z-drugs act via the so-called BZ binding site located at the interface between α and γ subunits (Sigel & Buhr, 1997), and thereby specifically bind to γ -containing receptors. In contrast, GABA_A agonists bind to the same binding sites as GABA, between the α and β subunits (Amin & Weiss, 1993; Smith & Olsen, 1994). The δ subunit was recently reported to confer high agonist sensitivity (You & Dunn, 2007).

The requirement of the γ subunit for the binding infers that BZs and Z-drugs will act on the majority of GABA_A receptors (Table 1). BZ and Z-drugs are positive allosteric modulators, i.e., they enhance the GABA-induced chloride current. They have little intrinsic activity in the absence of GABA. The binding of BZs and Z-drugs alters the duration of IPSCs, primarily determined by the α subunit present in the GABA_A receptor subtypes they bind to (for review, Möhler *et al.*, 2002).

The specificity of sedative-hypnotic drugs for distinct GABA_A receptor subtypes is usually characterized *in vitro*, using *Xenopus* oocytes or cell systems expressing recombinant GABA_A receptors. Animal models have proved to be a powerful tool to assess *in vivo* the drugs' specificity for the various GABA_A receptor subtypes (discussed in section 4).

3.2. Effects of sedative-hypnotics on sleep physiology.

Vigilance states (i.e., waking, sleep) are characterized by complex patterns of brain activity reflected by the electroencephalogram (EEG). Sleep is divided into two distinct stages: NREM sleep and REM sleep. In humans, NREM sleep is further subdivided into 4 stages 1-4 according to the predominance of slow waves. In stage 2, the frequency of brain waves is reduced and EEG hallmarks called spindles occur. Responsiveness to stimuli decreases as EEG slow waves become more predominant. Thus, stages 3 and 4, also referred to as “deep” sleep or “slow-wave” sleep, are characterized by high amplitude slow waves.

Each vigilance state has its EEG signature. Quantitative EEG analysis allows the extraction of important functional parameters such as slow-wave-activity (SWA, or “delta” activity) in NREM sleep, encompassing components of the EEG signal in the frequency range of approximately 0.5-4.5 Hz. SWA is considered as an indicator of sleep “depth” or “intensity”. The amplitude of slow waves occurring after sleep onset, and reflected by SWA, depends on the amount of time previously spent awake (Borbély *et al.*, 1981). After a night's sleep, slow waves show markedly decreased amplitude in the morning. Interestingly, individuals showing a lower proportion of deep sleep are more likely to self-medicate (Roth, 2007). NREM sleep stage 2 is characterized by spindle activity, defined as power in the frequency range between ~12-15 Hz. The functional significance of slow waves and spindles is still unknown. REM sleep and waking are characterized in animals (i.e., rodents) by a pronounced activity in the theta (6-9 Hz) frequency range.

Ideally, a hypnotic drug should, reduce the latency to sleep onset, reduce waking after sleep onset, enhance aspects of sleep such as NREM sleep duration and SWA, without affecting sleep architecture (i.e., the alternation of NREM-REM sleep cycles). The mechanism of action of drugs traditionally used to treat insomnia, and their effects on sleep, are summarized below. Drugs which recently received considerable attention for their potential to treat insomnia (THIP/gaboxadol, Tiagabine), and other GABA_A modulators studied for their effects on sleep (e.g., alcohol, neurosteroids) are also discussed.

3.2.1. Positive allosteric modulators mainly target synaptic γ 2-containing GABA_A receptors mediating phasic inhibition.

Historically, barbiturates were the first widely prescribed sedatives in the early 1900s. At low concentrations, they act as positive allosteric modulators of GABA_A receptors, at a binding site distinct from that of BZs. At high doses, they directly act at the GABA binding site. Due to their toxicity and development of tolerance and dependency, their prescription and use as hypnotics has been discontinued. Barbiturates markedly alter the EEG in NREM sleep, especially reducing activity <10 Hz. In contrast, frequency components above 10 Hz, encompassing the spindle frequency range are increased. Barbiturates also increase NREM sleep duration and suppress REM sleep (Lancel, 1999).

Classical BZs (e.g., diazepam (valium), flunitrazepam (Rohypnol®), flurazepam (Dalmadorm®), triazolam (Halcion®), midazolam (Dormicum®)) were introduced in the 60s. They were widely prescribed due to their potent anxiolytic and sedative properties. Importantly, BZs lacked the toxicity of barbiturates, as well as the liability of overdose due to their pharmacokinetics (Visser *et al.*, 2003; Mendelson, 2005). BZs act at α 1, α 2, α 3 and α 5 subtypes containing the γ 2 subunit, while α 4 and α 6 subtypes are insensitive to BZs (Table 1) (Möhler *et al.*, 2002). Thus, BZs target the majority of GABA_A receptors, producing sedative, anxiolytic, muscle relaxant, anticonvulsant and

cognition-impairing effects. The slow-elimination of various compounds (e.g., flunitrazepam, flurazepam) was linked to residual effects such as daytime sleepiness and impaired performance. Compounds with a shorter half-life were then developed (e.g. triazolam, midazolam) and seemed to lack residual side-effects (Borbély *et al.*, 1983a). BZs reliably shorten sleep onset latency and increase sleep continuity. REM sleep is typically reduced and REM sleep latency prolonged (Borbély *et al.*, 1991; Mendelson, 2005; Lemmer, 2007). All compounds induce dramatic reduction of the EEG power in the low frequencies (< 10 Hz), encompassing SWA (~ 0.5 -4.5 Hz), while spindle activity is increased (Fig. 3 and 4). These significant changes persisted in the subsequent “drug-free” night (Borbély *et al.*, 1983b; Borbély *et al.*, 1991). The clinical and functional significance of pharmacologically suppressing vigilance states and altering the EEG is uncertain. Interestingly, the typical declining trend of SWA over consecutive NREM sleep episodes is not altered by BZs, suggesting that the homeostatic process of sleep regulation is not disrupted (Borbély & Achermann, 1991). Usage of BZs is curtailed by their side-effects, such as next-day sedation, cognitive impairment and amnesic effects (Fig. 4; for review, Korpi *et al.*, 1997). Their long-term use can also lead to development of tolerance and dependence, as well as rebound symptoms at withdrawal (Roehrs *et al.*, 1990; O'Brien C, 2005; Stewart, 2005). These side effects, together with the need for a more restricted activity profile, have led to the screening of novel compounds also acting at GABA_A BZ-binding site.

The generation following the BZs, known as “Z”-drugs, was launched in the 80s. This new class of drugs includes imidazopyridines (zolpidem, Stilnox®, Ambien®, generics available), cyclopyrrilones (zopiclone; eszopiclone, *S*-enantiomer of zopiclone, Lunesta®), pirazolopyrimidines (zaleplon, Sonata®). Indiplon (a derivative of zaleplon) and eszopiclone are currently under review by the US Food and Drug administration for their potential in treating insomnia. Z-drugs are structurally different from the BZs. However, they act on the same binding site. They display high potency at $\alpha 1$ -containing receptors, medium potency at $\alpha 2$ and $\alpha 3$ -containing receptors and lack of interaction at the $\alpha 5$ -subtype (Dämgen & Lüddens, 1999). Overall, Z-drugs show similar actions on sleep as BZs, including the shortening of latency to sleep and a dose-dependent reduction of REM sleep (Fig. 4)

(Brunner *et al.*, 1991; Lader, 1992). Strikingly, Z-drugs share the “spectral EEG signature” of BZs, i.e., consistent reduction of low EEG components (<10 Hz) comprising the SWA range, and an increase in EEG power in the spindle frequency range during NREM sleep (Trachsel *et al.*, 1990; Brunner *et al.*, 1991; Aeschbach *et al.*, 1994). The dynamic pattern of SWA and spindle frequency activity within NREM sleep episodes were also shown to be preserved after administration of Z-drugs (Aeschbach *et al.*, 1994).

These findings suggest that drugs acting at the BZ’s binding site do not interfere with the homeostatic aspect of sleep regulation, but rather with EEG generating mechanisms (Borbély *et al.*, 1991). Overall, while Z-drug hypnotics represent effective sleeping aids, concerns remain regarding their side-effects. In particular, amnesia and risk of tolerance and dependence occur when these compounds are used over an extended period of time (George, 2001).

3.2.2. *Effects of drugs modulating tonic inhibition.*

Recent studies emphasize the potential role of extrasynaptic receptors in the modulation of sleep. A selective GABA_A agonist, THIP (4, 5, 6, 7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol; gaboxadol) was recently investigated for its potential to treat insomnia. Moreover, several compounds studied for their sedative-hypnotic actions have been characterized as endogenous (e.g., neuroactive steroids) or exogenous (e.g tiagabine, alcohol) modulators of GABA_A-tonic conductance.

THIP (gaboxadol) is a relatively old compound. It is a derivative of muscimol sharing a similar structure with GABA (Krogsgaard-Larsen *et al.*, 1977). In a *Xenopus* oocyte recombinant expressing system, THIP acts as a low-potency agonist at $\alpha 1\beta 3\gamma 2$ subtype and is a partial agonist at $\alpha 4\beta 3\gamma 2$ (Brown *et al.*, 2002; Krogsgaard-Larsen *et al.*, 2004). However, it is a full agonist at $\alpha 4\beta 3\delta$ receptors and generates currents larger than those produced by GABA (Brown *et al.*, 2002; Storustovu & Ebert, 2006). The presence of δ subunit was shown to be a strong determinant of the increased pharmacological activity of agonist compounds, while the presence of γ subunit precludes the response

to an agonist (Storustovu & Ebert, 2006). The exquisite selectivity of THIP is used as a tool to identify tonic currents in electrophysiological studies. THIP effects on sleep differ substantially from those induced by allosteric modulators (Fig. 3 and 4). Several studies in human subjects reported a decrease of wakefulness after sleep onset and/or an increase in total sleep time, while REM sleep was not altered. THIP also induced an increase in EEG power encompassing SWA, and a selective decrease in the spindle frequencies during NREM sleep (Faulhaber *et al.*, 1997; Lancel *et al.*, 2001; Mathias *et al.*, 2001a; Mathias *et al.*, 2005; Walsh *et al.*, 2007; Lankford *et al.*, 2008). This compound was discontinued in March 2007 while it was in Phase III clinical trial. Nonetheless, THIP is a powerful tool to investigate the role of the unique GABA_A receptor populations composed of δ -containing receptors (discussed in Section 4).

Another compound, tiagabine, has been recently investigated for its potential as a hypnotic. Tiagabine is a GABA uptake inhibitor launched initially as an anticonvulsant in treatment of epilepsy. It specifically inhibits the GABA transporter GAT-1 (Fig. 1). Such pharmacological manipulation may sustain synaptically-released GABA levels in the synaptic cleft, thereby increasing GABA_A-mediated inhibition as well as activation of GABA_B receptors. The effects of tiagabine on sleep are similar to those evoked by selective GABA_A agonists. Indeed, tiagabine elevates EEG power density in frequencies <10 Hz during NREM sleep, including the SWA range (Mathias *et al.*, 2001b), and increases sleep continuity and time spent in NREM sleep stage 3-4 (Mathias *et al.*, 2001b; Walsh *et al.*, 2005; Roth *et al.*, 2006; Walsh *et al.*, 2006a; Walsh *et al.*, 2006b).

Neuroactive steroids represent a major group of endogenous modulators of GABA_A (Belelli & Lambert, 2005). Extrasynaptic $\alpha 4\beta\delta$ receptors are sensitive to positive allosteric modulation by neurosteroids of the pregnane class (Mihalek *et al.*, 1999; Adkins *et al.*, 2001; Brown *et al.*, 2002; Wohlfarth *et al.*, 2002). Hence, endogenous neurosteroids can selectively enhance GABA_A-mediated tonic conductance (Stell *et al.*, 2003; Herd *et al.*, 2007). The scarce studies addressing the effects of

neuroactive steroids on sleep-wake behavior suggest that different compounds exert distinct effects on sleep (for review, Lancel, 1999; Steiger, 2007).

Alcohol is also a potential allosteric modulator of extrasynaptic GABA_A receptors. Its use to counteract difficulties to sleep is fairly common (Johnson *et al.*, 1998). δ -GABA_A receptors may be sensitive to low ethanol concentrations (Sundstrom-Poromaa *et al.*, 2002; Wallner *et al.*, 2003; Wei *et al.*, 2004). However, the demonstration of the selective action of ethanol on the δ subtype awaits further confirmation since recent studies found no effects of alcohol on these receptors (Borghese *et al.*, 2006; Korpi *et al.*, 2007). Ethanol increases EEG power in the low 0.25-1 Hz frequency range in NREM sleep, while EEG power in the spindle frequency range is decreased (Dijk *et al.*, 1992). The increase in frequencies below 1 Hz is interesting since they comprise slow (<1 Hz) oscillations, an EEG hallmark of sleep. Slow oscillations in thalamo-cortical neurons correspond to the slow membrane potential oscillation consisting of up and down states (Steriade *et al.*, 1993a; Steriade *et al.*, 1993b; 1993c). The effects of ethanol differ from those of BZs, Z-drugs and selective GABA_A agonists.

Overall, allosteric modulators acting at γ 2-containing GABA_A receptors reduce sleep latency and sleep fragmentation. They alter sleep architecture, promoting NREM sleep while suppressing REM sleep. Furthermore, nearly all BZs and Z-drug compounds reduce SWA and increase spindle occurrence. In contrast, drugs modulating extrasynaptic δ -containing receptors do not alter sleep latency, may increase sleep continuity depending on the dose administered, and increase EEG power in the SWA range (Fig.4). These diverse effects on sleep are due to the pharmacokinetics and the neuronal networks targeted by sedative-hypnotic drugs. This raises the interesting possibility that activation of synaptic GABA_A receptors by allosteric modulators plays a role in mechanisms underlying induction/consolidation of NREM sleep and spindles, while suppressing mechanisms

triggering REM sleep and processes underlying slow wave generation. On the other hand, extrasynaptic receptors mediating tonic inhibition may be involved in EEG synchronization.

4. Dissection of the role of GABA_A receptors in sleep and sleep regulation: insights from animal models and pharmacology

Deciphering the sedative-hypnotic properties of drugs modulating either synaptic or extrasynaptic GABA_A receptors relates to the functional analysis of the multiple GABA_A subtypes. The combination of pharmacological approaches with genetically-engineered mice (knock-in and knock-out (KO) mice) proved to be a powerful approach to investigate the function of distinct GABA_A subtypes in mediating the effects of drugs. It certainly emphasized the importance of GABA_A receptor heterogeneity.

4.1. Which GABA_A subtypes mediate the sedative-hypnotic effects of allosteric modulators acting at mainly synaptic GABA_A receptors?

Great progress was achieved in identifying the GABA_A receptor subtypes involved in the effects of BZs and Z-drugs using genetically engineered mice. A point mutation (“knock-in”) in the $\alpha 1$, $\alpha 2$, $\alpha 3$, or $\alpha 5$ subunits was introduced to prevent the binding of diazepam at the BZ binding site ($\alpha 4$ and $\alpha 6$ subtypes being insensitive to BZs). Such point mutation preserves the physiological functions of the receptor, and consists of replacing the conserved histidine residue, prerequisite to BZ binding, by an arginine residue ($\alpha 1$ (H101R), $\alpha 2$ (H101R), $\alpha 3$ (H126R) and $\alpha 5$ (H105R)) (Rudolph & Mohler, 2004). Studies using these knock-in mice showed that the $\alpha 1$ subtype mediates both sedative (Rudolph *et al.*, 1999; Crestani *et al.*, 2000b; McKernan *et al.*, 2000) and amnesic actions of the BZs (Rudolph *et al.*, 1999). On the other hand, muscle relaxant effects are mediated by $\alpha 2$ subtype and at high dose by $\alpha 3$ receptors (Crestani *et al.*, 2001).

In contrast, characterizing which subtype(s) underlie(s) the hypnotic properties of BZs proved to be complex. The $\alpha 1$ subtype was expected to fulfill this role since it mediates the sedative actions of

diazepam. However, $\alpha 1$ -point mutated (H101R) mice displayed a similar response to diazepam (3 mg/kg) compared to control mice (Tobler *et al.*, 2001). In particular, action of diazepam on sleep latency, amount of sleep and suppression of REM sleep does not require the $\alpha 1$ subtype. Strikingly, the reduction of SWA induced by diazepam was even more pronounced in $\alpha 1$ (H101R) mice. Sleep continuity was enhanced by diazepam only in $\alpha 1$ (H101R) mice, suggesting the involvement of other GABA_A subtypes. The $\alpha 3$ -subtype does not appear to mediate the effects of diazepam on sleep architecture, as shown by studies on $\alpha 3$ (H126R) mice and $\alpha 1$ (H101R)/ $\alpha 3$ (H126R) double mutant mice (Kopp *et al.*, 2003; Kopp *et al.*, 2004b). In $\alpha 2$ (H101R) mice, the diazepam-induced decrease of SWA activity in NREM sleep was reduced compared to control mice, as well as the enhancement of theta activity in REM sleep (Kopp *et al.*, 2004a). These findings suggest that $\alpha 2$ -containing receptors, in particular those expressed in the hypothalamic and pontine ascending pathways, are involved in the generation of NREM sleep. The $\alpha 2$ -subtype is also localized in the brainstem-septo-hippocampal systems involved in the generation of theta oscillations (Table 1 & Fig. 2) (Wisden *et al.*, 1992; Fritschy & Mohler, 1995; Vertes & Kocsis, 1997; Jones, 2005; Luppi *et al.*, 2006). Overall, these data indicate that the sedative and hypnotic actions of diazepam are dissociated and mediated by different neuronal circuits.

Whereas diazepam and other BZs have a high affinity for $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$ -subtypes, zolpidem has a 10-fold higher affinity for $\alpha 1$ - than for $\alpha 2$ - and $\alpha 3$ -subtypes and does not recognize the $\alpha 5$ -subtype (Dämgen & Lüddens, 1999). Knock-in mice confirmed *in vivo* its preferential affinity for the $\alpha 1$ -subtype. Sedative effects of zolpidem are mediated through the $\alpha 1$ -subtype (Crestani *et al.*, 2000a). The *in vivo* contribution of the $\gamma 2$ subunit to the actions of zolpidem was also confirmed in point mutated $\gamma 2$ (F77I) mice (Cope *et al.*, 2004). Regarding the hypnotic actions of zolpidem, $\alpha 1$ (H101R) mice did not show the marked reduction in SWA observed in control mice (Kopp *et al.*, 2004b). In contrast, the drug induced an increase in NREM sleep and suppression of REM sleep in both

genotypes. These data suggest that zolpidem-promoting effects on sleep are mediated by $\alpha 2$ and/or $\alpha 3$ subtypes, whereas its effects on the sleep EEG are mediated by the $\alpha 1$ subtype.

4.2. Further characterization of drugs acting at extrasynaptic receptors on sleep and the sleep EEG

The full GABA_A agonist THIP is a powerful compound to assess the involvement of tonic conductance in sleep and further investigate its effects on the sleep EEG. We used a knock-out mice model (δ -subunit KO) to assess whether the effects of THIP on sleep and the sleep EEG are mediated *in vivo* by the extrasynaptic δ -subtype. After THIP administration, δ -KO mice lacked the massive alterations of the EEG and vigilance states observed in wild-type control mice, demonstrating that the effects of THIP on vigilance states are mediated *in vivo* by the extrasynaptic δ -containing receptors (Winsky-Sommerer *et al.*, 2007). In control mice, THIP did not shorten sleep latency but rather reduced the amount of NREM sleep. At higher doses, REM sleep was suppressed (Vyazovskiy *et al.*, 2005; Winsky-Sommerer *et al.*, 2007; Alexandre *et al.*, 2008). Strikingly, THIP consistently induced an abnormal EEG pattern (i.e., recurrent spike wave events, Fig. 3), resulting in a massive increase of EEG power in both waking and NREM sleep in the low frequencies encompassing the SWA range (Vyazovskiy *et al.*, 2005; Winsky-Sommerer *et al.*, 2007; Alexandre *et al.*, 2008). This increase in power may be misleading. One should be aware that the recurrence of spike-wave events, at a frequency of approximately 0.5-1 Hz, as well as their asymmetric shape and high amplitude of the waves, largely contributed to the increase in SWA (Vyazovskiy *et al.*, 2005). An abnormal EEG pattern was observed in the rat, also there contributing to the increase in SWA (Lancel & Faulhaber, 1996; Lancel, 1997).

High amplitude EEG spike-waves seem to be a common feature of drugs enhancing GABA_A-mediated tonic conductance (Fig. 3). Muscimol, another selective GABA_A agonist showing very high affinity for δ -containing receptors (Quirk *et al.*, 1995; Huh *et al.*, 1996; Mihalek *et al.*, 1999), induced such EEG patterns in several species (Pedley *et al.*, 1979; Fariello *et al.*, 1981; Peeters *et al.*, 1989;

Lancel *et al.*, 1996; Lancel *et al.*, 1997; Vyazovskiy *et al.*, 2007). Tiagabine was reported to elicit similar alterations of the EEG (Walton *et al.*, 1994; Coenen *et al.*, 1995; Lancel *et al.*, 1998). It is tempting to draw parallels with spike-wave events induced by the anesthetic etomidate (Reynolds *et al.*, 2003) or the neuromodulator γ -hydroxybutyrate (Kaupmann *et al.*, 2003; Meerlo *et al.*, 2004; Vienne & Tafti, 2008). Etomidate is a potent modulator of extrasynaptic GABA_A receptors (Belelli *et al.*, 2005). In contrast, the effects of exogenous γ -hydroxybutyrate on sleep result from activation of the GABA_B receptors which also mediate a slower type of transmission (Bowery, 2006; Vienne & Tafti, 2008).

This drug-induced spike-wave activity was reported to be accompanied by a sedated behavioral state (Walton *et al.*, 1994; Meerlo *et al.*, 2004; Vyazovskiy *et al.*, 2005; Vyazovskiy *et al.*, 2007; Winsky-Sommerer *et al.*, 2007; Alexandre *et al.*, 2008; Vienne & Tafti, 2008). During the catalepsy-like state induced by muscimol, spike-waves occur synchronously in the frontal and parietal EEGs (Vyazovskiy *et al.*, 2007). This synchronous occurrence of high amplitude waves across cortical areas induced by muscimol is consistent with recent data suggesting an important role of inhibitory neurons in the large-scale synchronization of the slow <1 Hz oscillation in membrane potential undergone by cortical neurons during sleep (Volgushev *et al.*, 2006).

Overall, drug-induced increase of GABA_A tonic conductance resulted in dramatic EEG alterations. This “excessive” conductance is inherent to the high sensitivity of GABA_A agonists compared to GABA (Storustovu & Ebert, 2006). It was shown that relatively minor changes in chloride concentrations within the postsynaptic neurons can lead to a switch from hyperpolarizing to depolarizing activity. Thus, the effects of drugs acting at extrasynaptic receptors (THIP, muscimol, tiagabine, etomidate), especially in the thalamo-cortical networks, may result in quite profound alterations in properties of the networks. Furthermore, these findings suggest that tonic inhibition can easily be shifted outside the homeostatic range. Thereby, the dose-window of drugs acting directly at extrasynaptic GABA_A receptors will be inevitably narrow. In the same line, GABA is a high affinity but low-efficacy agonist at extrasynaptic δ -containing receptors emphasizing the impact of

endogenous modulators of tonic inhibition. For instance, neuroactive steroids may have profound effects by enhancing the efficacy of GABA (Stell *et al.*, 2003; Belelli & Lambert, 2005). In the opposite situation, loss of tonic conductance in thalamo-cortical networks might induce seizure activity if such conductance is critical to limit increased excitability. δ -KO mice showed some seizures episodes (Olsen *et al.*, 1997), suggesting an increased excitability or impaired inhibitory timing within the thalamus (Porcello *et al.*, 2003).

General anesthesia and sleep depress consciousness. They may share common neurophysiological elements (Tung & Mendelson, 2004; Franks, 2008). Although anesthetics are beyond the scope of this review, it is noteworthy that a number of them (e.g. etomidate, propofol, isoflurane) have been recently described as potent modulators of extrasynaptic GABA_A δ -containing receptors (Bonin & Orser, 2008). The use of genetically-engineered mice has also played a critical role in distinguishing the role of specific GABA_A subtypes in mediating their sedative, anesthetic and amnesic actions (Rudolph & Mohler, 2004).

4.3. Evaluate the role of GABA_A receptors expressed in specific neuronal networks

Knock-out mice lacking distinct GABA_A subunits exhibit compensatory changes. For instance, α 1-KO mice show major alterations in the expression of several GABA_A subtypes to compensate for the loss of expression of the α 1 gene (Sur *et al.*, 2001; Kralic *et al.*, 2006). These compensatory changes may thereby preclude the dissection of each subtype's contribution to an observed sleep phenotype.

However, such potential caveats uncovered adaptative mechanisms. For instance, studies in α 6-KO mice showed that the loss of tonic inhibition due to the lack of extrasynaptic α 6 β δ receptors was compensated for, underlining the importance of tonic inhibition in the cerebellar granule cells. The compensatory mechanism was identified as an increased expression of the TASK-1 potassium channel

which also inhibits cellular excitability in a continuous “tonic” manner (Brickley *et al.*, 2001). The opposite studies were recently performed, analyzing the consequences of the loss of TASK-1 potassium conductance. TASK-1 KO mice showed an increased impact of the GABA_A receptor activation. Moreover, TASK-1 female KO mice were more affected by THIP compared to controls, while males showed similar responses in both genotypes. These findings suggest that a sex-dependent regulation of extrasynaptic GABA_A receptors is involved in the changes induced by TASK-1 deletion (Linden *et al.*, 2008).

The role of $\beta 3$ and $\alpha 3$ subtypes in sleep regulation was particularly interesting to address due to their expression in the reticular nucleus of the thalamus (nRT; Fig. 2). As previously mentioned, typical oscillations in the sleep EEG are closely associated with specific patterns of neuronal activity arising from the thalamo-cortical networks. Hence, the progressive hyperpolarization of thalamocortical neurons occurring during the progression from waking to deep sleep results in fluctuations in the membrane potential, which are initially in the frequency range of sleep spindles and then of slow waves (Steriade, 2003). The nRT plays a pivotal role in the thalamo-cortical circuitry by providing the main inhibitory input onto the thalamic relay nuclei (Huguenard & Prince, 1994; Cox *et al.*, 1996). Only 10 % of mice with a disruption of the $\beta 3$ -subunit survive (Homanics *et al.*, 1997). They display symptoms reminiscent of the human Angelman syndrome, including hyperactivity, poor motor coordination, repetitive stereotypical behavior, seizures and EEG abnormalities (DeLorey *et al.*, 1998). In thalamic slices from $\beta 3$ -KO mice, GABA_A inhibition was disrupted in the nRT and oscillatory synchrony dramatically intensified (Huntsman *et al.*, 1999). A sleep study showed that consistent with the disrupted inhibitory transmission in the nRT, the usual increase of EEG power in the spindle frequency range at the transition of NREM sleep to REM sleep was reduced in $\beta 3$ -KO mice. Moreover, SWA during NREM sleep was significantly higher in the $\beta 3$ -KO mice (Wisor *et al.*, 2002). These findings are however difficult to interpret since the study is performed on a “survivor” population where compensatory mechanisms are likely to occur.

The $\alpha 3$ subtype displays a restricted distribution and is predominantly expressed in several neuronal populations crucial for sleep (Fig. 2, Table 1). It is strategically located in the nRT, the cortical layer VI, as well as in the monoaminergic and serotonergic neurons of the brainstem and the cholinergic neurons of the basal forebrain. In $\alpha 3$ -KO mice, no compensation of the $\alpha 3$ -GABA_A receptors by other α subtypes seemed to occur in the nRT (Studer *et al.*, 2006). Baseline sleep recordings did not reveal any major differences between $\alpha 3$ -KO mice and wild-type controls (Winsky-Sommerer *et al.*, 2008). Consistent with the results in $\beta 3$ -KO mice, we observed in $\alpha 3$ -KO mice a reduced increase in power in the spindle frequency band (10–15 Hz) at the transition between NREM–REM sleep. Furthermore, sleep pressure did not uncover differences in sleep regulation between the genotypes. The puzzling lack of sleep phenotype suggested compensatory mechanisms. Synaptic GABA_A clustering plays an important role for the function of inhibitory synapses. Strikingly, despite the disruption of postsynaptic GABA_A receptor clusters in $\alpha 3$ -KO mice, GABA_A-mediated inhibition is retained in the thalamic reticular nucleus (Winsky-Sommerer *et al.*, 2008). These findings indicate that other GABA_A receptor subtypes are present. Since the ϵ subunit is coexpressed with $\alpha 3$, the compensatory mechanism could be due to this subtype (Moragues *et al.*, 2000). The structure (subunit partner) and function of native GABA_A receptors containing the ϵ subunit are unknown. This subtype shows a very discrete distribution and in particular in regions involved in sleep (Fig. 2, Table 1). It is predominantly expressed in the locus coeruleus and the hypothalamus, where 70 % of orexin neurons and 25 % of histaminergic neurons express the ϵ subunit mRNA (Sinkkonen *et al.*, 2000; Sergeeva *et al.*, 2005). It is also localized in the cholinergic and monoaminergic neurons, as well as the hippocampus (Whiting *et al.*, 1997). Interestingly, the ϵ gene is clustered on the same chromosome (Xq28) as the $\alpha 3$ and θ genes (Whiting *et al.*, 1999). The recombinant ϵ subtype has peculiar electrophysiological properties: it shows a high sensitivity to GABA, and seems to be insensitive to BZs, which is expected if it substitutes the γ subunit (Ranna *et al.*, 2006). Presence of the ϵ subunit also reduced neurosteroid and anesthetic modulation (Davies *et al.*, 1997; Belelli *et al.*, 2002). Moreover, some findings are suggestive of spontaneous channel opening (Neelands *et al.*, 1999;

Maksay *et al.*, 2003; Wagner *et al.*, 2005), which would produce a tonic inhibition comparable to the one mediated by δ -containing receptors. Due to its expression in neuronal populations crucial for sleep and peculiar electrophysiological characteristics, it will be interesting to investigate the role of the ε -subtype in sleep. These findings show that the adaptative changes taking place are sophisticated enough to sustain thalamo-cortical function underlying complex behaviors and related brain activity in $\alpha 3$ -KO mice. The recent description of the imidazopyridine TP003 compound, which appears to be selective at the $\alpha 3$ -containing receptors, may provide an alternative approach to investigate the role of the $\alpha 3$ subtype in sleep (Dias *et al.*, 2005).

Mice lacking the $\gamma 2$ subunit die within the first 2 postnatal weeks. The number of BZ sites is reduced by 90 % in these mice, confirming that $\gamma 2$ is necessary for BZ binding (Gunther *et al.*, 1995). A sleep study in $\gamma 2$ -heterozygous mice did not reveal any difference in vigilance states or EEG hallmarks in these mice compared to their wild-type controls, suggesting the occurrence of compensatory mechanisms (I. Tobler, unpublished results).

The use of knock-in and knock-out mice has provided considerable insights into the physiological, pharmacological and behavioral role of individual GABA_A receptor subtypes, as well as the neurophysiological mechanisms underlying sleep. These studies emphasized the necessity to consider GABA_A receptor-mediated transmission as a highly flexible process. This also revealed potent homeostatic mechanisms maintaining balance between excitation and inhibition in the CNS (e.g. $\alpha 1$ -KO, $\alpha 3$ -KO, $\alpha 6$ -KO). New models allowing temporally and/or spatially controlled deletion of genes in mice will help refine the precision of analysis. Notably, a novel approach restoring drug sensitivity in chosen cell types was recently developed and may prove to be invaluable to investigate the role of specific GABA_A subtypes and neuronal circuits (Wulff *et al.*, 2007). Moreover, drugs that specifically target defined GABA_A receptors are expected to provide new prospects for insomnia therapy and be used as tools to study sleep.

5. GABA_A receptors pharmacology: future prospects for the treatment of insomnia

BZs and Z-drugs are the predominant hypnotics used in the treatment of insomnia. Meanwhile, multiple modes of GABA-mediated transmission have been characterized, i.e., the tonic conductance mediated by the extrasynaptic GABA_A receptors, as well as GABA_A subtype-dependent properties of phasic IPSCs. These distinct modes are localized in defined neuronal networks, in a cell-specific manner, often operating in parallel in a given structure. These findings suggest the value to develop drugs based on the specificity for GABA_A subtypes in distinct neuronal circuits. Moreover, since the tonic conductance may have profound effects on neuronal excitability and thereby network activity, further characterizing its physiological role in sleep is of importance.

Three GABA_A allosteric modulators are currently in clinical development: Indiplon-IR and -MR (Immediate/Modified Release; developed by Neurocrine Biosciences) are awaiting approval from the US Food and Drug Administration. Indiplon is a derivative of zaleplon and shows a similar pharmacological profile, including selectivity for the GABA_A $\alpha 1$ subtype (Neubauer, 2005; Marrs, 2008). The EVT-201 compound (developed by Evotec) and Adiplon (NG2-73; developed by Neurogen) are in phase II clinical trials. They both have the characteristic to be partial agonists at the BZ binding site. Interestingly, NG2-73 may modulate a more restricted set of neuronal networks involved in sleep, if its selectivity for the $\alpha 3$ subtype is confirmed. Only brief descriptions of their effects are available, and to date, no results have been published.

The GABA_A δ subtype has emerged as an important pharmacological target for insomnia. Novel compounds selectively acting as allosteric modulators of the δ subtype have been described very recently (Wafford *et al.*, 2008). These compounds potentiate tonic currents mediated by the δ subtype. They may provide useful tools to assess *in vivo* the impact of tonic conductance in sleep and the benefits of its increase in a manner which, in contrast to selective agonists such as THIP or muscimol, may prove to stay within a homeostatic range. Further characterization of their actions on neurophysiology will provide important insights to predict the potential therapeutic effects of this class of drugs. Drugs effective in insomnia treatment should not impair daytime performance. Thus, in

addition to sleep, these novel compounds will also be useful to assess the impact of tonic conductance on cognition.

In the context of drugs targeting the GABA δ subtype, the interactions between neurosteroids and extrasynaptic receptors should be taken into consideration. They may predict a gender-specific drug responsiveness to the drug (Linden *et al.*, 2008). Several findings indicate that neurosteroids exert a subtle regulation of extrasynaptic GABA_A receptors (Herd *et al.*, 2007). Fluctuations of circulating neurosteroids accompanying the oestrous cycle, gestation, or stress, induce profound and rapid alterations of the GABA_A-mediated tonic conductance (Maguire *et al.*, 2005; Maguire & Mody, 2007; , 2008).

GABA_A receptors are subjected to a highly dynamic regulation, including multiple mechanisms such as phosphorylation, post-translational modifications, and regulation of receptor trafficking (Leidenheimer *et al.*, 1991; Fritschy & Brunig, 2003; Luscher & Keller, 2004). In this line, agonist activation of GABA_A receptors as well as chronic BZ treatment have been shown to increase GABA_A receptor endocytosis (Tehrani & Barnes, 1991; Calkin & Barnes, 1994). Epigenetic mechanisms also modulate GABA_A receptor expression in response to stressful stimuli. These emerging regulatory mechanisms could be relevant in the pathophysiology of insomnia.

6. Conclusions.

The heterogeneity of GABA_A receptors, the emergence of tonic conductance, as well as the potent adaptive mechanisms taking place in genetically-engineered mice, provide attractive avenues to further investigate the role of GABA_A receptors in the homeostasis of sleep. Understanding the mechanisms underlying physiological sleep may provide an optimal basis to develop novel GABA_A subtype-selective compounds to treat sleep disorders, as well as to characterize their effects on the EEG and performance in human subjects. Because of the central role of GABAergic transmission on

regulation of neuronal networks and complex behaviors, it would be of interest to investigate whether GABA_A receptors function is altered in insomnia and/or undergo dynamic changes to maintain homeostasis in this sleep disorder.

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Abbreviations

ACh: acetylcholine; ADO: adenosine; BZ: benzodiazepine; DA: dopamine; DpMe: deep mesencephalic reticular nucleus; DR: dorsal raphe nucleus; EEG: electroencephalogram; GABA: γ -aminobutyric acid; Glu: glutamate; Hcrt: hypocretins/orexins; His: histamine; IPSCs: inhibitory postsynaptic currents; KO: knockout; LC: locus coeruleus; LDTg: laterodorsal tegmental nucleus; MCH: melanin concentrating hormone; NA: noradrenaline; n.d: non determined; NREM sleep: non rapid-eye movement sleep; nRT: reticular nucleus of the thalamus; PeF: perifornical nucleus. PH: posterior hypothalamus area; PPTg: pedunculo pontine tegmental nucleus; SubC: subcoeruleus nucleus; SWA: slow-wave activity; TM: tuberomammillary nuclei; VLPAG: ventrolateral periaqueductal gray; VLPO: ventrolateral preoptic area; 5-HT: serotonin.

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Table 1. Characteristics of the main GABA_A receptor subtypes.

Subunit composition	Expression in specific cell types	Subcellular distribution	GABA _A -mediated transmission	Ligands relevant in sleep
$\alpha 1\beta 2\gamma 2$	-Cortex, hippocampus : principal cells & interneurons -Thalamus: relay neurons -Pallidum, substantia nigra: GABAergic neurons -Cerebellum: Purkinje cells, stellate, basket cells & granule cells	synaptic, extrasynaptic	phasic / tonic	BZ, Z-drugs
$\alpha 2\beta 3\gamma 2$	-Hippocampus: principal cells -Striatum: spiny stellate neurons -Inferior olivary neurons -Motoneurons	synaptic, extrasynaptic	Phasic / tonic	BZ, Z-drugs
$\alpha 3\beta x\gamma 2$	-Cortex: principal cells in layers V-VI -Basal forebrain cholinergic neurons -Thalamus: reticular thalamic neurons -Brainstem: serotonergic (dorsal raphe) & noradrenergic (locus coeruleus) neurons -Cerebellum: Golgi cells -Inferior olivary neurons	synaptic, extrasynaptic	phasic/ tonic	BZ, Z-drugs
$\alpha 4\beta 2\gamma 2$ $\alpha 4\beta x\delta$	-Hippocampus: dentate gyrus granule cells -Thalamus: relay neurons -Hippocampus: dentate gyrus granule cells -Thalamus: relay neurons	synaptic*, extrasynaptic* extrasynaptic*	phasic / tonic tonic	Insensitive: BZ- & Z-drugs Insensitive: BZ- & Z-drugs Selective GABA _A agonists, Neurosteroids, Ethanol?
$\alpha 5\beta x\gamma 2$	-Hippocampus: granule cells -Spinal trigeminal neurons	synaptic, extrasynaptic	phasic / tonic	BZ
$\alpha 6\beta x\gamma 2$ $\alpha 6\beta x\delta$	Cerebellum: granule cells Cerebellum: granule cells	synaptic extrasynaptic	phasic tonic	Insensitive: BZ- & Z-drugs Insensitive: BZ- & Z-drugs Ethanol?
ϵ -subtype	-Septum: GnRH neurons -Basal forebrain: cholinergic cells -Hypothalamus: hypocretinergic, histaminergic, oxytocinergic, dopaminergic neurons -Brainstem: dopaminergic (substantia nigra), serotonergic (raphe nuclei) & noradrenergic (locus coeruleus) neurons	n.d	tonic	Insensitive: BZ
θ -subtype	-Hippocampus: dentate gyrus granule cells -Basal forebrain: cholinergic cells -Hypothalamus: dopaminergic neurons -Brainstem: serotonergic & noradrenergic (locus coeruleus) neurons	n.d	n.d	n.d

GnRH: gonadotropin-releasing hormone; n.d: not determined. x: subunit isoform undetermined. ?: matter of debate. *: scarce neuromorphological evidence. For the regional and cellular expression of GABA_A subtypes, see (Bonnert *et al.*, 1999; Moragues *et al.*, 2000; Möhler *et al.*, 2002; Moragues *et al.*, 2002; Fritschy & Brunig, 2003).

Figure legends

Figure 1. Scheme of a GABAergic synapse and diversity of GABA-mediated transmission.

GABA released in the synaptic cleft exerts its action via presynaptic and postsynaptic GABA_A as well as GABA_B receptors. Binding of GABA at synaptic GABA_A receptors results in inhibitory postsynaptic currents (IPSCs) which transiently inhibit neurons for 10-100 ms. In contrast, activation of extrasynaptic GABA_A receptors mediates tonic currents, playing a key role in adjusting membrane conductance. GABA transporters (GAT-1, GAT-3) can operate in a bidirectional way (i.e., remove GABA from the extracellular space or provide a source of GABA), thereby potentially modulating tonic inhibition. Synaptic and extrasynaptic receptors, which underlie these two forms of transmission, differ in their structural composition, subcellular localization, as well as electrophysiological characteristics, resulting in distinct pharmacological and functional properties.

Figure 2. Expression of GABA_A subtypes in major structures and neurotransmitter systems involved in the regulation of vigilance states.

In blue, predominant GABA_A subtype; In black, other subtype(s) present. Red stars indicate regions where tonic currents were identified by electrophysiology. The activity of the major neuronal networks involved in waking, NREM and REM sleep is reviewed elsewhere (Jones, 2005; Luppi *et al.*, 2006). ACh, acetylcholine. ADO, adenosine. DA, dopamine. DpMe, deep mesencephalic reticular nucleus. DR, dorsal raphe nucleus. Glu, glutamate. Hcrt, hypocretins/orexins. His, histamine. LC, locus coeruleus. LDTg, laterodorsal tegmental nucleus. MCH, melanin concentrating hormone. NA, noradrenaline. n.d.: non determined. nRt, reticular nucleus of the thalamus. PeF, perifornical nucleus. PH, posterior hypothalamus area. PPTg, pedunculopontine tegmental nucleus. SubC, subcoeruleus nucleus. TM, tuberomammillary nuclei. VLPAg, ventrolateral periaqueductal gray. VLPO, ventrolateral preoptic area. 5-HT, serotonin.

Figure 3. Representative examples of 12-sec electroencephalogram traces (frontal EEG) and EEG power spectra, during waking and NREM sleep, following administration of vehicle or sedative-hypnotic drugs in mice.

Benzodiazepines and Z-drugs (e.g., diazepam 3 mg/kg and zolpidem 5 mg/kg, respectively; in blue) do not induce visually observable changes at the level of the raw EEG compared to the placebo condition (i.e., vehicle; in black). In contrast, after administration of a low dose of two GABA_A agonists (muscimol (2 mg/kg) or THIP (4 mg/kg); in red), the EEG pattern is characterized, both during waking and NREM sleep, by recurring spike-wave events. Muscimol and THIP both act as superagonists at the δ -subtype. By enhancing tonic conductance, both drugs dramatically altered the brain electrical activity. Spectral analysis reveals profound alterations induced by sedative-hypnotic drugs in the EEG activity. GABA_A allosteric modulators (e.g. diazepam) and GABA_A agonists (e.g., THIP) induce opposite changes on the NREM sleep EEG spectra, illustrated for the first 90 minutes after drug administration. The slow-wave activity increase induced by THIP, both in the waking and NREM sleep spectra, is largely due to the recurring spike-events observed at the raw EEG level. Gray bars indicate the slow-wave activity frequency range (~0.5-4. Hz) and the spindle frequency range (~10-15 Hz).

Figure 4. Overview of the effects induced by sedative-hypnotic drugs on sleep and other parameters, depending which GABA_A subtypes the drugs target.

Blue and red indicate γ 2- and δ -subtype, respectively. Freq: frequency.

Effects of drugs on sleep and other parameters:	GABA _A subtype modulated by sedative-hypnotics:
	GABA BZ & Z-drugs γ_2 -subtype	GABA agonists δ -subtype
Sleep onset latency	↓	not altered
Sleep continuity	↑	↑
Total sleep time	↑	↑
REM sleep	↓	not altered
REM sleep latency	↑	not altered
EEG power	↓ in freq. <10 Hz ↑ in spindle freq.	↑ in freq. <10 Hz ↓ in spindle freq.
Residual effects	sedation	none reported
Effects on cognitive performance	cognitive impairment & amnesic effects	not altered & sustain attention during sleep restriction
Development of tolerance and/or dependance	✓	?
Rebound insomnia	✓	?