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Keywords (separated by ‘-’)	■■■▲

GABA Receptors and the Pharmacology of Sleep

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Abstract

Current GABAergic sleep-promoting medications were developed pragmatically, without making use of the immense diversity of GABA_A receptors. Pharmacogenetic experiments are leading to an understanding of the circuit mechanisms in the hypothalamus by which zolpidem and similar compounds induce sleep at $\alpha 2\beta\gamma 2$ -type GABA_A receptors. Drugs acting at more selective receptor types, for example, at receptors containing the $\alpha 2$ and/or $\alpha 3$ subunits

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[AU1]

Keyw



1 Introduction

For primary insomniacs, those otherwise healthy people who cannot get to sleep or maintain sleep, cognitive behavioural therapy is recommended as the first route to better sleep (Schroek et al. 2016; Wafford and Ebert 2008). If this fails, sleeping medication is then prescribed. These medications, known as sedatives or hypnotics, are generally controlled substances, requiring a medical doctor's authorization. In this chapter, we look at the sedatives/hypnotics used to treat primary insomnia that work by enhancing inhibitory GABAergic transmission. There have been various reviews on sleep medication and GABA_A receptors (Greenblatt and Roth 2012; Lancel and Steiger 1999; Nutt and Stahl 2010; Wafford and Ebert 2008; Winsky-Sommerer 2009). In spite of the passage of time, these reviews remain excellent resources on drug structures, the history of how these drugs were developed and their clinical effects. The GABA_A receptor drug zolpidem (Ambien) remains the first pharmacological line of attack for treating insomnia (Bertisch et al. 2014; Lancel and Steiger 1999; Mignot 2013; Nutt and Stahl 2010). One survey of about 32,300 adults (healthy and not in hospital) found that 3% of them (about 970 people) had been prescribed zolpidem for insomnia in the preceding month (Bertisch et al. 2014). In fact, in the USA, there were 53.4 million prescriptions for zolpidem (Ambien) in 2010 (Greenblatt and Roth 2012). This amounted in 2006 to \$1.5 billion of sales for the company that then principally marketed zolpidem, Sanofi (Morris 2013).

[AU2]

2 Natural Sleep and Hypnosis/Sedation

Sedatives are substances that depress the central nervous system, producing calmness and relaxation, less anxiety and more sleepiness; and hypnotics are defined as substances which induce sleep, so the words "sedative" and "hypnotic" tend to be used interchangeably (Wafford and Ebert 2008). In the GABA field, there is much work done on selective anxiolytics that do not cause sedation, but an anxiolytic drug is also likely to be sleep promoting if sleep latency was measured in a calm environment – in practice, for humans, it would be unlikely that there would be

an anxiolytic that does not influence sleep propensity, since relaxation is part of the preparation for getting ready to sleep.

Some hypnotic/sedative drugs induce vigilance states partially resembling natural NREM sleep, with a delta to theta ratio in the EEG that could be classified as NREM-like. The power of the EEG at different frequencies reflects the degree of synchronization of neural currents at those frequencies in the neocortex and hippocampus. A sleep-like EEG produced by a sedative/hypnotic often has a high delta to theta frequency power ratio but sometimes with an overall lower power than occurring in NREM sleep, which may represent a less deep sleep. For certain compounds (e.g. propofol, etomidate, barbiturates, inhalational compounds), as drug dosage is increased, sedation deepens into general anaesthesia (Franks 2008; Franks and Lieb 1994; Garcia et al. 2010), and the EEG becomes flat, with periodic bursts of large amplitude spikes, a phenomenon termed “burst suppression” (see for example Fig. 6 in Reynolds et al. 2003).

In this chapter, we take drug-induced sedation/hypnosis to mean a NREM-like activity measured in the EEG, reduced or zero movement with the subject being harder to arouse and with a lower body temperature. Even within this drug-induced sedative/hypnotic state, there are differences between drugs and receptor classes. Sedation is easier to define on human subjects, because verbal responses and visual observation reveal if the person is conscious or in an intermediate state and this can be combined with EEG, heart rate and respiratory recordings (polysomnography).

Sedation as a concept has been used rather loosely in experiments on animals. Researchers studying sedation, especially with regard to benzodiazepine and zolpidem action, have tended to separate drug-induced sedation and drug-induced sleep into separate concepts: e.g. benzodiazepine-induced sedation and benzodiazepine-induced sleep. This has added some confusion to the field (Tobler et al. 2001; Winsky-Sommerer 2009). This distinction came about because many experimental studies have defined an animal as sedated by just one parameter, which is if the animal moves less in the open-field assay following drug treatment or if it becomes ataxic. But often the EEG was not measured in these studies on sedation (Crestani et al. 2000; McKernan et al. 2000; Rudolph et al. 1999). Animals could be quietly awake or have a changed motor function independent of sleep through, e.g. catalepsy or cerebellar interference. When, for example, Purkinje cells in the mouse cerebellum were made selectively sensitive to zolpidem, zolpidem given systemically to these mice caused ataxia and reduced the time they could stay on a rotating rod, but the mice were not sedated (Wulff et al. 2007). An EEG analysis on sedation is more incisive, because reduced or changed movement alone is an insufficient criterion to define sedation, and as part of the EEG scoring, movement is implicitly measured, as the EMG is used to help score the vigilance states.

Although we think that reduced movement alone is not an adequate criterion to define sedation, it has also been argued that there is too much reliance on the EEG to characterize vigilance states, without paying enough attention to the obvious feature of how the animal appears visually (Coenen and van Luijtelaar 1991). There is sometimes a dissociation between the EEG and the behaviour of the

animal, so that “a quantitative analysis of the EEG alone might give misleading information about the effects of a compound on the state of vigilance” (Coenen and van Luijcklaar 1991). The anaesthetic urethane, whose molecular targets are still unknown, produces strong and sustained theta in the EEG (Coenen and van Luijcklaar 1991). A strong delta power component in the EEG does not always mean NREM sleep or sedation either. The EEG can contain a strong delta profile in fear-induced freezing (frontal cortex) (Karalis et al. 2016) or following atropine administration (Qiu et al. 2015) or following THIP and muscimol administration, where mice are awake, but have NREM-like oscillations in their prefrontal cortex (Vyazovskiy et al. 2005, 2007; Winsky-Sommerer et al. 2007), and also awake GABA_A receptor δ subunit knockout mice have strong δ oscillations. In the case of muscimol, the state produced is catalepsy, where the animals do not move because the muscles have no tone, similar to REM sleep, but the mice are not asleep (Vyazovskiy et al. 2007).

3 The Perfect Sleeping Pill?

A good “sleeping pill” should reduce the time to enter NREM sleep (reduce “sleep latency”) and allow only limited awakenings, and most researchers assume that sleep-promoting medication should aim to reproduce the sleep EEG profile and architecture (order and length and EEG power of NREM and REM sleep) found in natural sleep. Other practical considerations are that a sleeping pill compound should occupy its receptor sites quickly and have a short half-life so that it on the one hand increases sleep time but on the other does not give “hangover effects” such as daytime sleepiness or reduced daytime alertness (Nutt and Stahl 2010). Zolpidem meets most of these requirements well. The ideal sleep-promoting compound should also have low addictive potential and not cause rebound insomnia when the drug is withdrawn. Unsurprisingly, no sedative could be yet described as “perfect”. Problems with even the best sleeping medications include “confusional arousal” and ataxia and risk of injury if awakening occurs whilst on the medication (Frey et al. 2011; Mignot 2013) and rebound insomnia if use is discontinued (Mignot 2013) (see Sect. 5 below). There can also be serious side effects if sleep-promoting medication is taken with other medications that suppress neuronal activity, e.g. mixing zolpidem with narcotic pain killers or alcohol. Some people, particularly the elderly, who take zolpidem have more risk of car accidents the next day if they are driving (Booth et al. 2016). In fact, starting around 2006, there are various stories in the media of zolpidem (Ambien)-induced delirium and people acting strangely under the drug’s influence during sleep walking – it is unclear if these aberrant behaviours are really due to zolpidem (see Morris 2013).

4 **Activating GABAergic Neurons Generally Promotes Sleep: GABA_A Receptor Positive Allosteric Modulators (PAMs) and GABA Agonists Induce Sleep**

γ -Aminobutyric acid (GABA), the main inhibitory transmitter in the mammalian brain, works on the ionotropic (GABA_A) and metabotropic (GABA_B) receptor classes. The natural sleep-promoting circuitry in the brain uses sleep-active GABAergic neurons to inhibit wake-active neurons in wake-promoting circuitry (Chung et al. 2017; Lin et al. 1989; Nitz and Siegel 1996; Sherin et al. 1998; Uygun et al. 2016) (Fig. 1). Hence, it might seem intuitively obvious that enhancing GABAergic transmission throughout the brain with positive allosteric modulators (PAMs) of GABA_A receptors, and thus enhancing network inhibition, will induce sedation. And indeed, with a few interesting exceptions, this is the case.

Most effective hypnotics/sedatives and anaesthetics developed to date enhance GABA's action at GABA_A receptors, working as PAMs at the receptor. Most drugs were developed and marketed without knowledge of their GABA_A receptor targets, and this knowledge was gained often only decades after the drugs were first in use (Franks 2008). PAM compounds do not usually work on the GABA_A receptors by themselves but require GABA to bind to the receptor as well. By causing GABA to prolong the duration of the inhibitory postsynaptic currents through GABA_A receptors, PAMs enhance ongoing GABAergic transmission. These drugs, which have varied molecular structures and come from multiple classes of compound, include amobarbital (barbiturates); compounds acting as agonists at the benzodiazepine site, e.g. zolpidem (an imidazopyridine), zaleplon (a pyrazolopyrimidine) and zopiclone (a cyclopyrrolone); and the benzodiazepines, e.g. diazepam, flurazepam, quazepam, temazepam and triazolam (Bertisch et al. 2014; Gottesmann et al. 1998; Lancel and Steiger 1999; Nutt and Stahl 2010). Two current major intravenous anaesthetics used during hospital operations to induce and maintain general anaesthesia, etomidate and propofol, are also selective PAMs at GABA_A receptors (Franks 2008). At low concentrations, general anaesthetics produce sedation. As the drug concentrations increase, the difference in principle between propofol and zolpidem, why the first can induce and maintain general anaesthesia, whilst the other is only a sleeping pill which cannot induce more than sleep, is that probably propofol prolongs IPSCs more than zolpidem and it is a more potent PAM. In addition, propofol works on all GABA_A receptor types, whereas benzodiazepines and zolpidem are more restricted in which receptors they can activate.

Hans Selye, who discovered and conceptualized the physiological responses to stress, also first observed that the endogenous steroid progesterone could cause sedation (Gunn et al. 2015; Herd et al. 2007; Lancel and Steiger 1999). Decades later it was discovered that reduced versions of progesterone, i.e. some of its metabolites, are potent PAMs of GABA_A receptors (Harrison and Simmonds 1984; Lan et al. 1990; Lancel et al. 1999; Majewska et al. 1986) and these progesterone metabolites (e.g. allopregnanolone) produce behavioural effects, e.g. sedation/sleep and anxiolysis, broadly similar to other PAMs of GABA_A receptors except that steroids work on a wider range of subunit combinations and

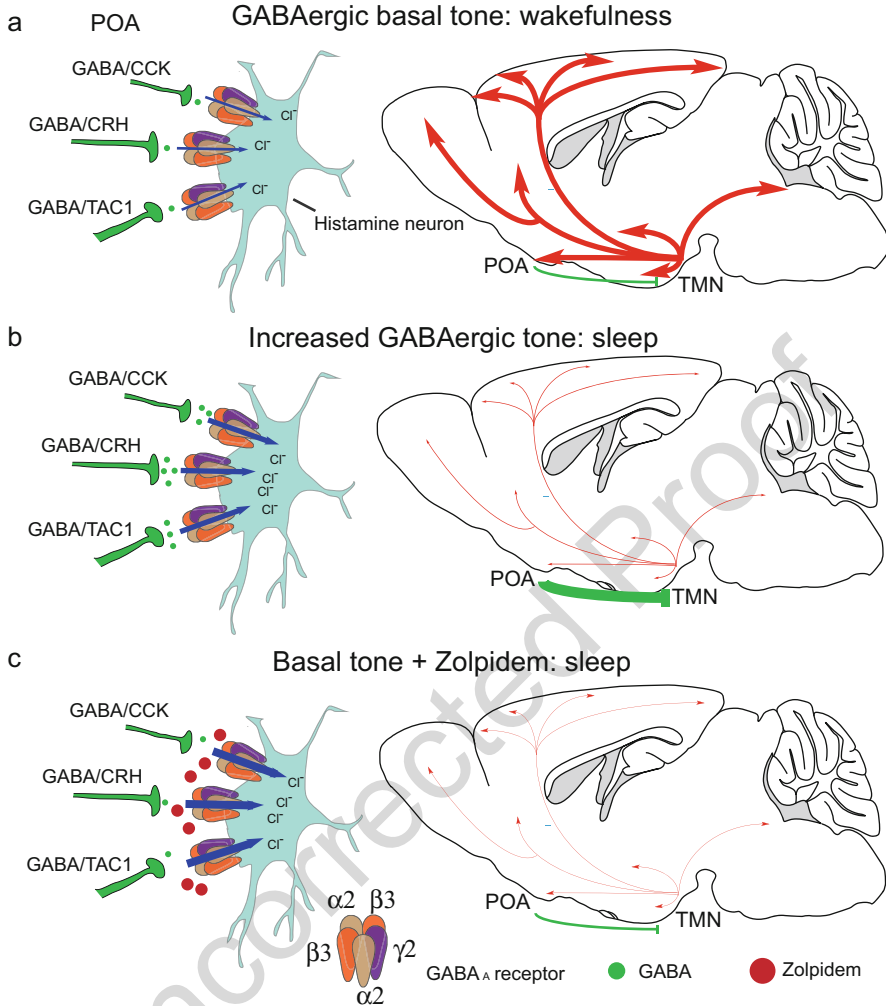


Fig. 1 Schematic of how GABAergic afferents from the preoptic area (POA) of the hypothalamus inhibit wake-promoting histamine neurons and how zolpidem enhances this natural inhibitory pathway to induce sleep. **(a)** During wakefulness, there is a basal GABAergic tone from the POA afferents (green) onto the histamine neurons in the tuberomammillary area (TMN). These wake-active neurons project widely (red arrows) to release histamine to promote aspects of arousal. In addition to GABA, the GABAergic afferents release a range of peptides (e.g. CCK, CRH, TAC1) into the tuberomammillary area (Chung et al. 2017). The GABA_A receptors located postsynaptically on the histamine neurons are probably mixtures of $\alpha 1\beta 2$ and $\alpha 2\beta 2$ receptors. The $\gamma 1$ and ϵ subunits are also expressed in some histamine neurons (May et al. 2013). **(b)** At the onset of NREM sleep and during NREM sleep, the GABAergic afferents have increased activity (thicker green line), hence depressing histaminergic activity (thin red arrows). Similar scenarios would also be happening at other ascending aminergic centres, e.g. the locus coeruleus and dorsal raphe. **(c)** After taking zolpidem, the drug will act as a PAM on the postsynaptic GABA_A receptors to enhance the GABA afferent input onto histamine neurons and inhibit them, effectively mimicking the situation occurring in natural NREM sleep shown in panel (a) (Uygun et al. 2016)

are not just “dependent” on $\gamma 2$ -subunit-containing GABA_A receptors (Herd et al. 2007) (see Sect. 6, *GABA_A Receptors: A Family of Subunits..*” for details on the subunits). Allopregnanolone ($t_{1/2}$ = just 35 min) was improved to give ganaxolone with better oral bioavailability and a longer half-life. This drug is highly sedative and could, in principle, be used to treat insomnia, as well as epilepsy and anxiety. In fact, allopregnanolone is a potent sedative before anything else, and therefore drug developers have concentrated on reducing its sedative properties so that it can be used for other treatments. The synthetic steroid-like derivative UCI-50027 is more anxiolytic than sedative as assessed by its effect on movement (Hogenkamp et al. 2014). This illustrates the difficulty for drug developers interested in new sleeping medications. The intellectual property space for these drugs is crowded, and there are no strong commercial advantages in having a steroid-based sedative, as opposed to benzodiazepines or zolpidem.

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5 Addiction and Rebound Insomnia

Barbiturates, the original sedatives in use since the beginning of the twentieth century, and benzodiazepines have generally fallen out of use as sleep-promoting agents. Barbiturates are simply dangerous because of the risk of overdose and lethal if taken at too high a dose because they can directly gate the GABA_A receptors and depress breathing and cardiac regulation centres. Hence, barbiturates are now only used in specialist medical applications and until recently on “death row”. Similarly, the benzodiazepine drugs Librium and Valium, household names in the 1960s, were used decades before it was understood that they were PAMs of GABA_A receptors (Mohler 2015; Randall 1961). The benzodiazepine triazolam (Halcion) ran into legal trouble when patients committed criminal offences and had accidents whilst having this drug prescribed for sleep (Mignot 2013). Consequently, in the USA triazolam is prescribed at a lower dose, and in the UK it is not available, having been banned as a “sleeping pill” in 1991. Both classes of drugs are addictive, and tolerance emerges, and rebound insomnia appears when the drugs are withdrawn, so that the patient sleeps even less well than before. Rebound insomnia is an interesting phenomenon and presumably indicates that GABA_A receptors on neurons in the sleep regulatory circuitry have become downregulated with continuous PAM use; without the drug, the relevant neurons are not sufficiently inhibited by endogenous GABAergic transmission. The circuitry and neurons involved are unknown, but it would be good to know what these are as we may then have a more precise understanding of the mechanisms involved. It could well be that PAMs down-modulate GABA_A receptors on neurons that control the homeostatic sleep circuitry – the receptors on these neurons (e.g. orexin and melanin-concentrating hormone neurons) are dynamically modulated by the sleep drive (Toossi et al. 2016).

6 **GABA_A Receptors: A Family of Subunits Assembled to Make Many Subtypes of Receptor (Opportunities to Discover PAMs That Selectively Promote Sleep and Anxiolysis)**

The GABA_A receptor drugs in clinical use have been discovered pragmatically without using knowledge about the molecular structures of the receptors. We will now look at the receptors on which these drugs act, because pharmacologists hope to design more precise drugs based on the structure of the receptors. GABA_A receptors were first cloned in 1987 and are now fairly well understood at the structural level (Levitan et al. 1988; Miller and Aricescu 2014; Mohler 2015; Olsen 2015; Pritchett et al. 1989; Puthenkalam et al. 2016; Schofield et al. 1987; Seeburg et al. 1990; Shivers et al. 1989; Sieghart 2015; Sigel and Steinmann 2012). They are GABA-gated chloride channels, in the same gene superfamily as glycine receptors, nicotinic acetylcholine receptors and serotonin 5-HT₃ receptors. There are six alpha ($\alpha 1$ – $\alpha 6$) subunit genes, three beta ($\beta 1$ – $\beta 3$) subunit genes, three gamma ($\gamma 1$ – $\gamma 3$) subunit genes and one delta (δ) subunit gene; additionally, there are also epsilon (ϵ), theta (θ) and pi (π) subunit genes and three rho (ρ) subunit genes. The subunit genes are differentially expressed throughout the brain and enteric nervous system (Fritschy and Mohler 1995; Moragues et al. 2002; Pirker et al. 2000; Seifi et al. 2014; Sinkkonen et al. 2000; Wisden et al. 1988, 1992). Because the subunits can assemble in different combinations, there are many subtypes of GABA_A receptor. We still do not know precisely how many.

Five subunits are arranged in a ring with the chloride channel at the centre. For the best studied types of GABA_A receptor, the ring usually contains two α subunits, two β subunits and a γ or δ subunit. Two GABA molecules activate the receptor, binding at the α and β subunit interfaces. There are many mechanistic possibilities for PAM drugs to enhance (or sometimes decrease) GABA's action at the receptor complex. The receptor complex contains binding sites for allosteric modulators, which bind within the membrane helices, such as the propofol binding site at the interface between the extracellular domain and transmembrane domain 2 (Franks 2015; Yip et al. 2013), or the benzodiazepine and z-drug binding site between the α and $\gamma 2$ subunit interfaces (Ogris et al. 2004; Puthenkalam et al. 2016). A few drugs work at the GABA binding site – between the α and β subunits – these drugs are known as orthosteric ligands (THIP/gaboxadol and muscimol) and mimic GABA's agonist actions (Puthenkalam et al. 2016).

For current drugs the important target combinations of receptor subunits are $\alpha 1\beta 2$, $\alpha 2\beta 2$, $\alpha 3\beta 2$, $\alpha 4\beta 2$, $\alpha 4\beta \delta$ and $\alpha 5\beta 1/2/3\gamma 2$ receptors. Benzodiazepines and the “z-drugs” (e.g. zolpidem, zopiclone) require an α and $\gamma 2$ subunit in the receptor complex (Cope et al. 2004; Ogris et al. 2004; Pritchett and Seeburg 1990; Pritchett et al. 1989; Seeburg et al. 1990); for these drugs the type of β subunit is less important. Receptors that contain the $\gamma 2$ subunit are enriched in the postsynaptic area but are also present extrasynaptically (Fig. 2). Synaptic locations would also be expected for receptors that contain the $\gamma 1$ or the $\gamma 3$ subunits, but receptors with these subunits have been relatively little studied (Herb et al. 1992) and are

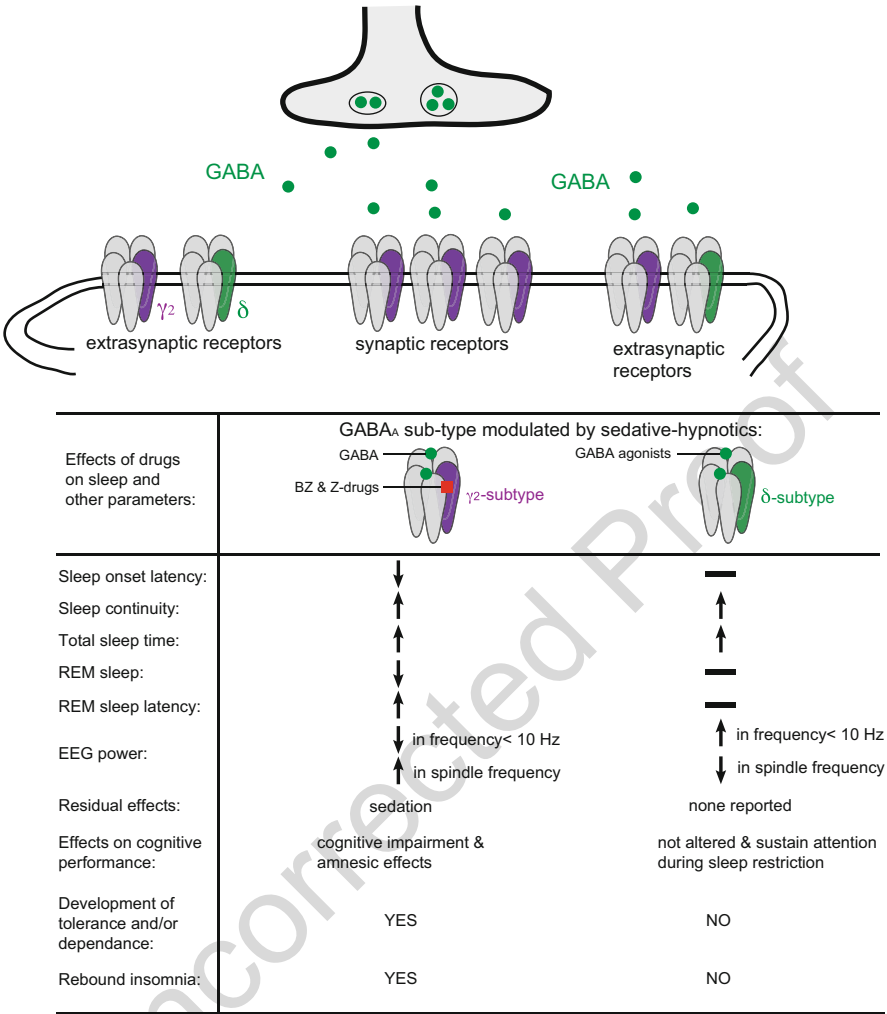


Fig. 2 Summary of how sleep-promoting drugs work on synaptic ($\alpha\beta\gamma_2$ type) and extrasynaptic ($\alpha\beta\gamma_2$ and $\alpha\beta\delta$) GABA_A receptors. The differing types of sleep produced by benzodiazepines (BSz), z-drugs such as zolpidem and GABA agonists such as gaboxadol are summarized in the table. This figure and table have been adapted from (Winsky-Sommerer 2009)

expressed in just a few cell types. Notably the γ_1 gene is expressed in the medial preoptic-hypothalamic area, a sleep regulatory centre (Wisden et al. 1992). The synaptic receptors with the γ_2 subunits convey fast synaptic inhibition (Brickley and Mody 2012). Receptors that contain the δ subunit do not get targeted to the postsynaptic area and are extrasynaptic (Brickley and Mody 2012) (Fig. 2). Activation of extrasynaptic GABA_A receptors by GABA diffusing from the synapse, or being released non-synaptically, produces “tonic inhibition”, which is a sustained

(hence the word “tonic”) activation of GABA_A receptors, with no precise temporal resolution (Brickley and Mody 2012). Agonists (GABA mimetics) such as 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (*THIP* – also known as gaboxadol) act preferentially on δ subunit-containing receptors (Fig. 2). The $\alpha\beta\gamma 2$ -containing GABA_A receptors outside synapses also contribute to tonic inhibition.

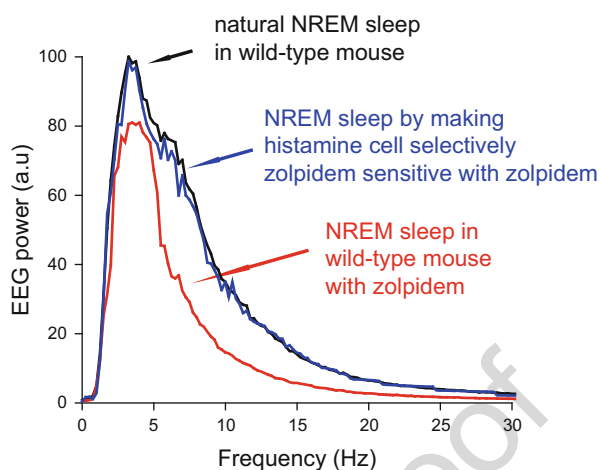
7 Benzodiazepines and “Z-Drug”-Induced Sleep/Sedation: Mechanisms and Circuitry

Benzodiazepines such as diazepam and the z-drugs (e.g. zolpidem) reduce the time to sleep (decrease sleep latency), increase sleep continuity and total sleep time, decrease REM sleep and increase REM sleep latency (Lancel and Steiger 1999; Winsky-Sommerer 2009) (Fig. 2). In terms of the EEG, these drugs induce a NREM-like state, with a higher delta-to-theta ratio, but the power of the EEG in this range is reduced (relative to the power in natural NREM sleep) (Fig. 3). Spindle frequency power (12–14 Hz) is also increased relative to the power of spindles in natural sleep. Lower power in the NREM range induced by these drugs might mean a less deep sleep or a less restorative sleep. Zolpidem-induced sleep has decreased EEG power in the 5–16 HZ range, relative to that in natural sleep, and benzodiazepines depress the EEG power even further (Winsky-Sommerer 2009) (Fig. 3). It is not clear what these changes in power relative to natural sleep really mean for efficacy. It could be perfectly adequate sleep, sufficient to treat insomnia, especially because these drugs are not intended to be taken permanently. For example, the benzodiazepine triazolam substantially depresses NREM EEG power but does not interfere with synaptic plasticity during sleep, whereas zolpidem does actually interfere with plasticity, but the EEG power reduction compared with normal sleep is less (Seibt et al. 2008). This work was done on the *developing* visual cortex of kittens; Seibt et al. conclude: “Our findings demonstrate that alterations in sleep architecture do not necessarily lead to impairments in sleep function. Conversely, hypnotics that produce more “physiological” sleep based on polysomnography may impair critical brain processes, depending on their pharmacology” (Seibt et al. 2008).

Diazepam and the other benzodiazepines work as PAMs on $\alpha 1\beta\gamma 2$, $\alpha 2\beta\gamma 2$, $\alpha 3\beta\gamma 2$ and $\alpha 5\beta\gamma 2$ receptors and do not differentiate between them, whereas zolpidem enhances GABA’s action mainly on three GABA_A receptor subtypes: $\alpha 1\beta\gamma 2$, $\alpha 2\beta\gamma 2$ and $\alpha 3\beta\gamma 2$ (Pritchett and Seeburg 1990; Pritchett et al. 1989); zolpidem has the highest binding affinity at $\alpha 1\beta\gamma 2$ -containing receptors but only by 20-fold, meaning that in practice at normal doses in vivo zolpidem also acts as a PAM at $\alpha 2\beta\gamma 2$ and $\alpha 3\beta\gamma 2$ receptors (Pritchett and Seeburg 1990; Uygun et al. 2016), and zolpidem cannot really be called an “ $\alpha 1$ -selective compound”, even though this term is frequently used. Some benzodiazepines and zolpidem will also, to some extent, enhance GABA’s action at $\alpha\beta\gamma 1$ -type receptors (Khom et al. 2006).

The GABA_A receptor targets for benzodiazepines and zolpidem are widespread, and so these drugs will influence nearly all aspects of brain function and do cause

Fig. 3 Schematic of power spectra of zolpidem-evoked NREM sleep. EEG power in the 5–16 Hz range evoked by zolpidem in NREM sleep is less than that found in natural NREM sleep, whereas selectively enhancing GABA_A receptors with zolpidem on histaminergic neurons produces a type of NREM sleep resembling natural NREM in terms of EEG power. This is not actual data but redrawn from (Uygun et al. 2016)



many side effects. An important advance for potentially improving drug selectivity at GABA_A receptors came with the discovery that one particular histidine (H) in the extracellular domain of the alpha subunits, H101, determined if benzodiazepines could bind to the receptor complex (Wieland et al. 1992). This discovery was made because it was noticed that two of the alpha subunits, $\alpha 4$ and $\alpha 6$, formed benzodiazepine-insensitive GABA_A receptors when expressed as recombinant $\alpha 4\beta 2$ or $\alpha 6\beta 2$ subunits combinations (Luddens et al. 1990; Wisden et al. 1991) and that this was because the $\alpha 4$ and $\alpha 6$ subunits contained an arginine R101 and not H101 at their presumed benzodiazepine binding sites (Wieland et al. 1992). When this residue in $\alpha 1$, $\alpha 2$, $\alpha 3$ or $\alpha 5$ was mutated to an R, benzodiazepines could no longer potentiate the GABA response at recombinant $\alpha(H101R)\beta 2$ receptors (Wieland et al. 1992). This mutation was exploited to make a series of knock-in mice where the $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$ subunit genes were systematically mutated in the codon encoding the key histidine H101, so that R101 was incorporated into the subunit instead. These “ $\alpha H101R$ ” mice were used to behaviourally dissect the roles of the different GABA_A receptor subtypes in responding to benzodiazepines and z-drugs such as zolpidem (Mohler 2015; Rudolph et al. 1999), whereby particular GABA_A receptor subtypes had lost the ability to be modulated by diazepam (a pharmacogenetic loss of function experiment). For example, it was found that benzodiazepines do not reduce anxiety in $\alpha 2H101R$ mice (Low et al. 2000) and that $\alpha 1H101R$ do longer get sedated by benzodiazepines or zolpidem (the mice keep moving after drug injection) (Crestani et al. 2000; McKernan et al. 2000; Rudolph et al. 1999). Thus $\alpha\beta\gamma 2$ -type GABA_A receptors with $\alpha 2$ receptors were suggested to contribute to the anxiety-reducing effects of benzodiazepines; those receptors with $\alpha 1$ subunits contribute to the sedative effects of zolpidem and benzodiazepines (Rudolph et al. 1999). In recent experiments, all four lines of $\alpha H101R$ point mutant mice have been bred together to generate either HRRR (order is $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$), RHRR, RRHR or RRRH mice (a pharmacogenetic restriction of function method), so that GABA_A receptors with only one of the α subunits are now enhanced by

diazepam (Ralvenius et al. 2015). With this series of mouse lines, it was found that GABA_A receptors with the $\alpha 5$ subunit, and not the $\alpha 2$ subunit, are the main receptors regulating anxiolysis in response to diazepam (Behlke et al. 2016). In the original, and even new, experiments with “ $\alpha 1H101R$ ” and HRRR mice on “sedation”, the EEG was not measured. But even in the new HRRR experiments, $\alpha 1$ -containing GABA_A receptors seem to be the only target for diazepam to reduce movement and hence cause “sedation” (Ralvenius et al. 2015).

In a separate series of studies from those on sedation (reduced movement) discussed above, the single H to R point mutant α subunit mice were used to explore the effects of diazepam and zolpidem on the EEG and sleep latency. For diazepam, the $\alpha 1H101R$ mutation did not alter the sleep latency or sleep length, so $\alpha 1$ -containing receptors are not involved in diazepam’s hypnotic actions (Tobler et al. 2001). Similarly, $\alpha 3$ H to R point mutant mice had unchanged hypnotic responses to diazepam (Kopp et al. 2003). Instead, it seemed to be the $\alpha 2$ subunit that was partially required (Kopp et al. 2004a). Similarly, the $\alpha 1$ -containing GABA receptors are not responsible for zolpidem’s ability to promote sleep. In mice with $\alpha 1$ subunits made insensitive to zolpidem by the H101R mutation, zolpidem reduces latency to NREM sleep and prolongs NREM sleep time as well as it does in wild-type mice (Kopp et al. 2004b). Thus, although this seems to contradict the sedation data mentioned above (based on inhibiting movement), where zolpidem acts through the $\alpha 1$ -type receptors to induce “sedation”, zolpidem’s sleep-promoting effects must come from enhancing GABA’s actions at GABA_A receptors with $\alpha 2$ and/or $\alpha 3$ subunits (Kopp et al. 2004b). [Note: there has been quite a lot of work done to find $\alpha 2$ - and $\alpha 3$ -selective anxiolytic PAMs, but it would be surprising if such drugs were not also sedatives]. However, the $\alpha 1H101R$ mice show that $\alpha 1$ -containing receptors are needed for zolpidem to produce its characteristic decrease in the EEG power (frequencies between 5 and 16 Hz of zolpidem-induced NREM sleep relative to that found in natural NREM sleep (Kopp et al. 2004b). Zolpidem might induce NREM sleep in part by potentiating histamine neurons in the posterior hypothalamus (see Sect. 11). In the end, it is still curious why there is a disconnect between the $\alpha 1$ -type GABA_A receptors as sedative promoting and the $\alpha 2$ -type receptors as sleep promoting. With hindsight, both types of receptor are involved in promoting different subcomponents of sleep.

The reticular thalamus and layer VI of the neocortex are enriched in their expression of $\alpha 1\beta 2\gamma 2$ - and $\alpha 3\beta 2\gamma 2$ -type receptors (Wisden et al. 1988, 1992). These sites are expected to control thalamocortical oscillations such as the δ oscillation in NREM sleep. However, the global $\alpha 3$ knockout mouse had no obvious sleep-wake phenotype, although possibly another GABA_A receptor subtype was upregulated on the reticular thalamic neurons, as the $\alpha 3$ KO cells still had IPSCs (Winsky-Sommerer et al. 2008). The $\alpha 3$ H to R (loss of function for diazepam) mice were studied to provide more information. However, the effects of diazepam given to these mice on sleep and EEG were no different to mice with diazepam-sensitive $\alpha 3$ subunits (Kopp et al. 2003). Given the recent discovery of the excitatory GABA projection from the lateral hypothalamus to the reticular thalamus, one might speculate that some of the reticular thalamic $\alpha 3\beta \gamma 2$ receptors would contribute to

net excitation and wakefulness, whereas $\alpha 3$ -containing receptors on, e.g. the locus coeruleus, could contribute to sedation (see Sect. 11).

8 Wake-Promoting GABAergic Pathways: Zolpidem Can Promote Transient Arousal from a Small Subset of Patients with Disorders of Consciousness

Some GABAergic pathways, especially some originating in the hypothalamus and brainstem, are actually wake promoting (Chung et al. 2017; Herrera et al. 2016; Venner et al. 2016), and so enhancing the synaptic transmission at their projection sites selectively would be expected to promote wakefulness. For example, some lateral hypothalamic GABAergic neurons project to the GABAergic reticular thalamus neurons (Herrera et al. 2016). Selectively activating these lateral hypothalamic GABAergic neurons produces wakefulness and even emergence from general anaesthesia (Herrera et al. 2016). Thus, selectively enhancing this feedforward inhibition with a GABA_A receptor PAM would probably produce wakefulness. But in practice, current sleep-promoting drugs work all over the brain; this wake-promoting effect of the PAM at this synapse is probably swamped by the net inhibitory effect at most other synapses where PAMs enhance GABAergic inhibition. In cats and juvenile ferrets, benzodiazepines and zolpidem can promote arousal (Hsu et al. 2009; Lancel and Steiger 1999). There are some spectacular findings that zolpidem can promote transient arousal from rare types of disorders of consciousness (coma) – but the circuit mechanisms remain unclear (Chatelle et al. 2014; Williams et al. 2013). Perhaps in some types of disorders of consciousness, selective pathways, such as the lateral hypothalamic-reticular thalamus route mentioned above, are stimulated by zolpidem, and that this is possible because the normal sleep-promoting GABAergic pathways are damaged. Only 5% of patients with disorders of consciousness respond to zolpidem by transiently awakening, and the effect is small when done double-blind and placebo controlled (Thonnard et al. 2013).

9 Extrasynaptic GABA_A Receptors, δ Subunits and Sleep

An important class of GABA_A receptors exists exclusively outside the synapse and contains δ subunits (Brickley and Mody 2012). Delta-containing receptors are involved in tonic inhibition, sensing extrasynaptic GABA. This type of inhibition may be used physiologically as a gain control system. In the forebrain, the δ subunit is paired primarily with the $\alpha 4$ and $\alpha 1$ subunits (Fig. 4). In thalamic relay neurons, which have some of the highest expression of $\alpha 4$ and δ subunits in the brain and which also co-express the $\alpha 1$ and $\beta 2$ subunits, the receptor could be $\alpha 4\beta 2\delta$ or $\alpha 1\alpha 4\beta 2\delta$ (Shivers et al. 1989; Wisden et al. 1991, 1992). The $\alpha 1\beta\delta$ subunit combination also exists in the neocortex and hippocampus on selective GABAergic

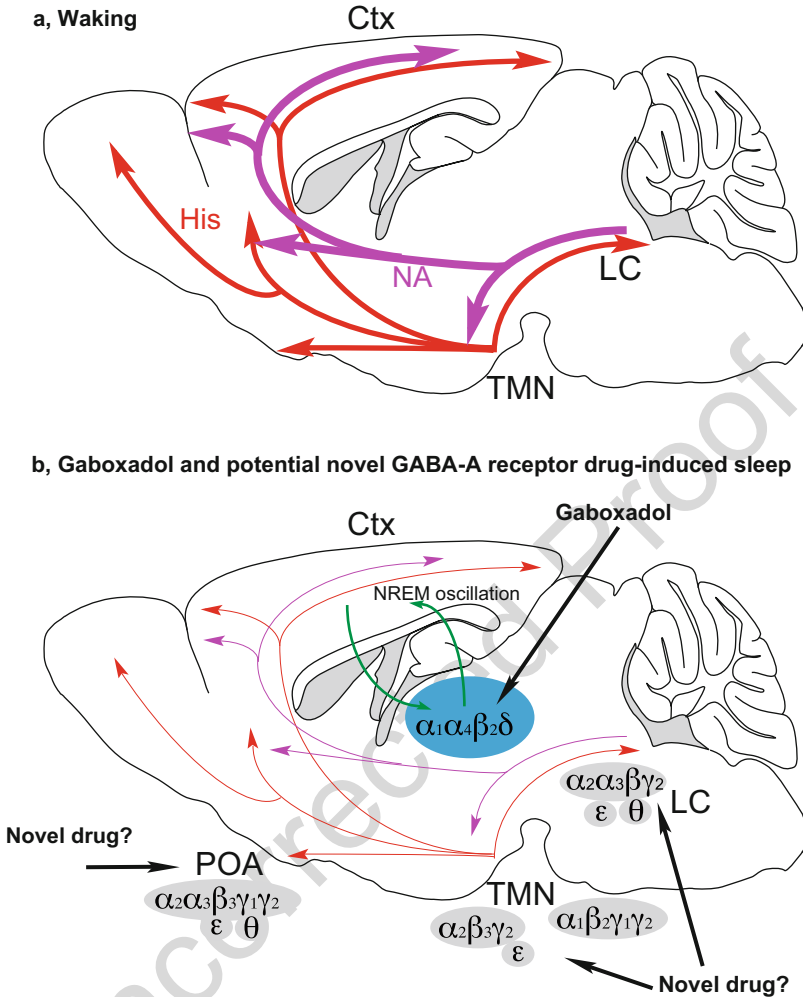


Fig. 4 Possible novel GABA_A receptor targets in the hypothalamus and ascending arousal neurons. **(a)** Wakefulness is sustained in part by ascending aminergic neurons such as the histaminergic neurons in the tuberomammillary nucleus (TMN) and the noradrenergic neurons in the locus coeruleus (LC). These release histamine (His) and noradrenaline (NA) widely throughout the neocortex (Ctx) and other regions. **(b)** Gaboxadol activates $\alpha_1\alpha_4\beta_2\delta$ receptors in the thalamus (T) to induce NREM sleep by promoting δ -type oscillations. Speculative novel targets for yet undiscovered sleep-promoting drugs that work via GABA_A receptors could be the ascending arousal neurons and neurons on the preoptic area (POA) of the hypothalamus. These areas express various GABA_A receptor subunit genes such as the γ_1 , ϵ and θ subunit genes. The predominant α subunit genes expressed in these areas tend to be the α_2 and α_3 subunit genes. The receptor subunit combinations in these areas, apart from the obvious one of $\alpha_1\beta_3\gamma_2$ and $\alpha_2\beta_3\gamma_2$ in histamine neurons, have not been clearly elucidated. Some drugs could work to suppress wakefulness by dampening down the histamine and noradrenaline systems. Other drugs might interfere with wake-promoting GABAergic projections (not shown) in the POA area

interneuron types, especially the parvalbumin types (Ferando and Mody 2015; Milenkovic et al. 2013).

Gaboxadol (or THIP) is a direct GABA mimetic ligand at δ -containing receptors. Mice with genetically deleted δ subunits are unresponsive to muscimol and THIP (Winsky-Sommerer et al. 2007). The δ -containing receptors are insensitive to benzodiazepines, but their GABA-activated currents are potentiated by steroids and propofol, and this depends on the level of extrasynaptic GABA (Houston et al. 2012). It was suggested that GABA acting at δ -containing GABA_A receptors at thalamic extrasynaptic GABA_A receptors on thalamic relay cells might induce slow wave activity – the delta oscillations of NREM sleep (Wafford and Ebert 2006) (Fig. 4), although δ receptors are certainly dispensable for this, as global δ knockout mice have normal sleep-wake cycles and vigilance states (Winsky-Sommerer et al. 2007). But THIP does increase the tonic conductance on mouse thalamic relay neurons, and this could in theory promote delta NREM-like oscillations (Belelli et al. 2005), although clearly there are other δ -containing GABA_A receptors in the mouse forebrain (perhaps on GABA interneurons) where THIP acts to counteract sleep and instead promotes agitation and actually delays sleep.

In mice, THIP definitely would not be regarded as a sleep-promoting drug (Alexandre et al. 2008; Winsky-Sommerer et al. 2007) – in fact, quite the opposite. A similar acting drug, muscimol, in mice promoted catalepsy and delayed sleep onset (Vyazovskiy et al. 2007). But there are big species differences in the way this drug works. Studies on rats suggested THIP would be a good drug to promote sleep (Lancel and Langebartels 2000). In rats, THIP increased the amount and depth of NREM sleep (Lancel and Langebartels 2000). Based on the results in rats, THIP/gaboxadol went into human clinical trials to test if the drug promoted sleep (the drug had already been used many times before in human trials for treating tardive dyskinesia and cancer pain, and many patients had previously reported sleep as a side effect). It was generally well tolerated and seemed to promote sleep as well as zolpidem (Fig. 2). In contrast to zolpidem, gaboxadol enhances delta power in NREM sleep in humans (Dijk et al. 2010; Lundahl et al. 2007, 2012). Also in contrast to zolpidem, gaboxadol caused no rebound insomnia on drug withdrawal (Hajak et al. 2009). But in 2007 gaboxadol failed in Phase III for sleep studies, although it is not quite clear why. Studies with the drug's efficacy in sleep on primary insomnia outpatients gave conflicting results. Some groups found at 15 mg/kg it promotes sleep, decreasing latency and increased the feeling of wakefulness after sleep in studies conducted on patients using sleep diaries ($N = 742$) (Hajak et al. 2009). As the size of the samples were scaled up, other studies on humans found that gaboxadol (THIP) at 10 mg/kg had no effect on sleep (Roth et al. 2010). At 15 mg/kg, it failed to reduce sleep latency consistently in clinical trials with, for example, one patient group reporting sleep-promoting effects, another not (Roth et al. 2010). Some inconsistent effects were reported for the prolongation of sleep duration, with the drug having effects in females but not males. Some people complained of nausea and dizziness and some severe adverse effects as the dose was increased (Lundahl et al. 2007). This could be because another target of THIP/

gaboxadol is the $\alpha 6\beta\delta$ receptor, which is expressed on cerebellar granule cells where it generates a tonic inhibitory GABA-activated current (Brickley et al. 2001; Luddens et al. 1990; Shivers et al. 1989; Wisden et al. 1992). Thus, on cerebellar granule cells, THIP is likely to stimulate the tonic conductance mediated by extrasynaptic GABA and acutely modulating gain control of motor systems and the vestibular reflex – hence dizziness. An improved sleep-promoting drug for humans might need to target only $\alpha 4\beta\delta$ -type receptors – these are not expressed in the cerebellum. Gaboxadol is not sold in the USA and has no FDA approval. It is now unlikely to ever appear as a sleep-promoting drug commercially. A PAM, DS-2, that works selectively at δ -containing GABA_A receptors (Wafford et al. 2009) does not seem to have been tested for its effects on sleep promotion. Strangely, THIP/gaboxadol still seems like it would be an excellent sleep-promoting drug for some. Hamilton Morris' compelling account in Harper's Magazine of private gaboxadol ingestion (which took place in 2013 after clinical trials had ceased) is recommended reading (Morris 2013) .

10 GABA_B Receptors and Sleep Promotion

GABA_B receptors are dimeric G protein-coupled receptors that produce slow metabotropic inhibition and are essential to prevent over excitation and the emergence of seizures (Gassmann and Bettler 2012; Pin and Bettler 2016). Functionally, the slow GABA_B-mediated inhibition is quite similar to the tonic conductance inhibition arising from δ -type GABA_A receptors. GABA_B receptors are located extrasynaptically, often on presynaptic terminals of, e.g. glutamate or GABA neurons. Activating GABA_B receptors opens K⁺ channels or closes voltage-gated Ca²⁺ channels, reducing transmitter release from terminals. At the cellular level, activation of GABA_B receptors is inhibitory, but at the network level, activating these receptors can produce either net excitatory or inhibitory effects. GABA_B receptors acting presynaptically to decrease GABA release could be excitatory or inhibitory at the network level, depending on whether the GABAergic neuron is inhibiting another GABAergic terminal or a glutamatergic excitatory terminal.

Compared with GABA_A receptors, there is no substantial receptor diversity for GABA_B receptors. All GABA_B receptors are made from two subunits, GABA_{B1} and GABA_{B2}. There are two subtypes of GABA_B receptor because of two splice versions of the GABA_{B1} gene, GABA_{B1a} and GABA_{B1b}, that pair with GABA_{B2}. Modulator proteins or auxiliary subunits (e.g. potassium channel tetramerization (KCTD) proteins KCTD8, KCTD12, KCTD12b and KCTD16), which are expressed cell type selectively, may increase the functional diversity of GABA_B receptors and allow more flexibility for developing drugs (Pin and Bettler 2016). Given that the two GABA_B receptor subunits are widely expressed in the brain forming receptors that contribute to every aspect of brain function, it seems unlikely that conventional specific GABA_B receptor agonists will selectively interfere with sleep and not have profound side effects. Indeed, GABA_B receptor agonists cannot be used for treating primary insomnia.

Drugs active at GABA_B receptors do promote sleep/sedation, but some of the sedation could result from off-target effects. The best established GABA_B agonist is baclofen. But in mice with GABA_B receptors deleted, baclofen still promoted a delayed NREM sleep (Vienne et al. 2010). In mice and rats, the drug GHB (γ -hydroxybutyric acid, the sodium salt is called sodium oxybate) promotes sedation: hypo-locomotion, NREM sleep with increased delta power in the EEG and decreased body temperature (Kaupmann et al. 2003; Vienne et al. 2012; Wisor et al. 2006) – the drug also increases delta power during wakefulness (Vienne et al. 2012). The drug needs to be used at a high dose to get these effects (50–150 mg/kg) (Vienne et al. 2010) or 300 mg/kg (Wisor et al. 2006). In mice with deleted GABA_B receptors, GHB no longer induces sedation but still binds with high affinity to neuronal membranes (Kaupmann et al. 2003). Thus, GHB causes sedation by activating GABA_B receptors. GHB has a low affinity at these receptors – typically 3 mM GHB is needed to activate endogenous GABA_B receptor responses on neurons (Connelly et al. 2013). The function and identity of the high-affinity GHB binding site remain a mystery – GHB does not activate $\alpha\beta\delta$ GABA_A receptors, so these are not the target (Connelly et al. 2013). In spite of its unclear pharmacology, GHB can help reduce the symptoms of patients suffering from narcolepsy with cataplexy (Black et al. 2014). In mice, baclofen similarly reduces the symptoms of narcolepsy with cataplexy, in part by promoting high delta power NREM sleep (Black et al. 2014); again, it is unclear if this is due to GABA_B receptor activation or due to an off-target effect.

GABA_B receptor inhibition is embedded throughout the sleep-wake circuitry. Mice with genetically deleted GABA_B receptors (global knockouts) are unhealthy and lose weight and have seizures and disrupted sleep, with sleep being fragmented and with reduced power across all EEG frequencies (Vienne et al. 2010). If we try to look more selectively, mice with GABA_B receptors selectively removed from wake-promoting orexin neurons in the mouse lateral hypothalamus have a strongly fragmented sleep-wake pattern, with many more transitions between wake and NREM sleep, and upregulation of GABA_A receptors on these neurons (Matsuki et al. 2009). The overall amount of sleep and wake in these mice was unaffected by the GABA_B receptor deletion – the increased fragmentation was due to more transitions between all vigilance states but no sign of cataplexy – there were no abnormal transitions from wake to REM sleep, for example (Matsuki et al. 2009). But in contrast, selectively deleting GABA_B receptors from histaminergic neurons does not alter any parameter of the sleep-wake cycle, so GABA_B receptor modulation of the histamine system is dispensable (Zecharia et al. 2012).

11 GABA_A Receptors Expressed on Nodal Points of the Sleep-Wake Circuitry as Drug Targets for Sleep?

Some GABA_A receptors are highly expressed in nodal points of circuitries that control vigilance states. So, activating GABA_A receptors on these nodes will influence activity in many other parts of the brain. Injecting barbiturates, muscimol

(GABA_A receptor agonist) or propofol into a small area of the rat brain stem, the mesopontine tegmental area, induces anaesthesia, presumably because some key hub neurons (whose identity is unknown) that have far-reaching projections have been inhibited (Abulafia et al. 2009; Minert and Devor 2016). Similarly, injecting GABA agonist drugs (muscimol) into the posterior hypothalamus of awake cats produces NREM sleep, presumably because of the inhibition of wake-promoting histamine neurons, as these are hub neurons that promote wakefulness and influence many brain areas simultaneously (Lin et al. 1989; Nelson et al. 2002; Uygun et al. 2016) (Fig. 1).

We saw previously that based on global $\alpha 2\text{H}101\text{R}$ mice, zolpidem is most likely acting to promote sleep through $\alpha 2\beta\gamma 2$ and/or $\alpha 3\beta\gamma 2$ GABA_A receptors (Kopp et al. 2004b). The $\alpha 2$ subunits are expressed in hypothalamic areas (Wisden et al. 1992), such as on histaminergic neurons (Fritschy and Mohler 1995; Sergeeva et al. 2005). By using a genetic $\gamma 2$ swap method, whereby zolpidem-sensitive $\gamma 2\text{F}77$ subunits are swapped cell type selectively into a $\gamma 2\text{F}77\text{I}$ mouse, which is insensitive to zolpidem (Cope et al. 2004), selectively augmenting the active GABA input onto hypothalamic histamine neurons by systemic zolpidem administration decreased NREM sleep latency and enhanced sleep time but notably without reducing the power of the oscillations in the 5–16 HZ range of the EEG, so more resembling natural NREM sleep (Uygun et al. 2016) (Fig. 3). This suggests that zolpidem could in part induce sleep by enhancing GABA's actions on histamine neurons.

Some of the most obscure (or, at least, functionally less well studied) and therefore potentially interesting GABA_A receptor subunits are significantly expressed in areas of the brain that regulate homeostatic functions such as sleep. In particular, the $\gamma 1$, ϵ and θ subunits have enriched expression in preoptic-hypothalamic and some brain stem areas (May et al. 2013; Moragues et al. 2002; Sergeeva et al. 2005; Sinkkonen et al. 2000; Wisden et al. 1992) (Fig. 4). A significant feature is that these hypothalamic and brainstem areas frequently use $\alpha 2$ and $\alpha 3$ subunit expression. Triazolam is effective as a PAM at $\alpha 1\beta\gamma 1$ receptors, as to some extent is zolpidem (Khom et al. 2006). This work could be developed further to look for, e.g. $\alpha 2\beta\gamma 1$ -selective ligands. Similarly, a drug selectively working at GABA_A receptors containing $\alpha 3$, ϵ and θ subunits would be interesting to investigate for its sleep-promoting properties. Expressing recombinant ϵ and θ subunits with the $\alpha 3$ and $\beta 1$ subunit reduces sensitivity to many PAMs (propofol, etomidate, pregnenolone and flurazepam) but increases GABA and gaboxadol sensitivity 100-fold (Ranna et al. 2006). The θ -containing receptors could still be potentiated by etomidate (Ranna et al. 2006). It is possible that the ϵ subunit could function as a β -like subunit (Jones and Henderson 2007). Compared with the $\alpha\beta\gamma 2$ and $\alpha 1/4\beta\delta$ class of GABA_A receptors, not enough work has been done on the ϵ and θ subunits to give us a full understanding of the GABA_A receptors to which they contribute.

In rodents, GABA_A receptors assembled from $\alpha 2$, $\alpha 3$, ϵ and θ subunits could be targets for drugs that promote sleep or anxiolysis (Belujon et al. 2009) (Fig. 4). Similar to the histaminergic and other ascending aminergic groups, noradrenergic neurons in the locus coeruleus (LC) project widely throughout the brain and promote

arousal and enhanced cognitive function. Adrenergic LC neurons fire selectively during wakefulness, and thus inhibiting their activity will enhance anxiolysis and sleep. In particular, although lesions of the LC do not influence the amounts of wakefulness, rats with LC lesions do go to sleep more quickly in a novel environment (Gompf et al. 2010). A similar anxiolytic effect was found with selective deletions of $\gamma 2$ -containing GABA_A receptors from histaminergic neurons – no change in the overall sleep-wake cycle was produced, but the mice went to sleep more quickly in a novel environment (Zecharia et al. 2012). Thus, a (novel) drug that reduces LC or histamine function by enhancing GABA_A receptors on these neurons would be anxiolytic and sleep promoting (Fig. 4). The $\alpha 2$ and $\alpha 3$ subunits are found in separate clusters on the cell bodies and dendrites of rat noradrenergic LC neurons (Corteen et al. 2011). These neurons also express the unusual ϵ and θ subunits (Belujon et al. 2009; Sinkkonen et al. 2000), and histamine neurons also express the ϵ gene (Sergeeva et al. 2005). So, receptors assembled from either $\alpha 2$, ϵ and θ or $\alpha 3$, ϵ and θ might be interesting as potential targets for sleep- or anxiolytic-promoting drugs (Fig. 4). A caveat is that in humans, but not rodents, noradrenergic neurons express the $\gamma 2$ subunit (Hellsten et al. 2010). This would mean that human LC neurons would be targets for PAMs that selectively modulate $\alpha 2\beta\gamma 2$ - and $\alpha 3\beta\gamma 2$ -type receptors and these $\alpha 2$ - or $\alpha 3$ -preferring drugs would probably be sedative too (Hellsten et al. 2010). It is unclear what the functional contribution of ϵ and θ subunit contributions would be if the $\gamma 2$ subunit is also present on LC neurons – presumably a complex mix of receptors is present. The native subunit compositions of ϵ - and θ -containing receptors in human LC neurons and human hypothalamic neurons would need to be established – e.g. $\alpha 2\beta 3\theta\epsilon$ or $\alpha 2\theta\epsilon$ or $\alpha 2\beta 3\gamma 2$ combinations. Again, given the differences in the way different species respond to some drugs (see in particular the section above on gaboxadol – Sect. 9), we would need to be cautious in using mice as a system to work up new sleep-promoting drugs.

12 Perspectives

The most “natural” way to induce sleep is sleep deprivation, even if this is not a practical therapy and has frequently been used as a form of torture. The drive to sleep increases in proportion to the amount of time spent awake, until the drive becomes so overwhelming that sleep is unpreventable. Nevertheless, the type of sleep that emerges through this route of sleep deprivation is physiological and is termed recovery sleep. This recovery sleep represents the process of sleep homeostasis: catching up on lost sleep with a longer and deeper sleep (as defined by enhanced EEG δ power for the first stage of NREM sleep) (Landolt et al. 2000). The circuitry controlling sleep homeostasis is not understood in detail, although it does require the preoptic hypothalamus (Zhang et al. 2015), but the mechanisms underlying sleep homeostasis could be one future route to inducing a natural sleep artificially. Current GABAergic medications, such as zolpidem, all discovered without any appreciation of how sleep-promoting circuitry works, do not induce a natural type of recovery sleep and thus are likely to use different mechanisms from

natural sleep (Landolt et al. 2000; Wisor et al. 2006). It seems we were on a new threshold for GABAergic sleep medication with gaboxadol, which actually enhances NREM-type δ power in humans, but the drug failed in clinical trials. On the other hand, the $\alpha 2$ adrenergic agonist dexmedetomidine seems to use the same circuitry in the lateral preoptic hypothalamus to that activated in recovery sleep, albeit with marked hypothermia and changes in blood pressure, and so compounds based on this drug mechanism, targeting the recovery sleep aspect, might be further developed to promote sedation (Zhang et al. 2015). Not enough is known about sleep circuitry to understand if more precise medications will be possible. In the meantime, hypnotic drugs such as zolpidem will continue to be widely used.

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