The Modulation of Synaptic GABA<sub>A</sub> Receptors in the Thalamus by Eszopiclone and Zolpidem

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ABSTRACT

Eszopiclone (Lunesta; Sepracor, Marlborough, MA) and zolpidem [(N,N,6-trimethyl-2-(4-methylphenyl)-imidazo[1,2-a]pyridine-3-acetamide] are among the most commonly prescribed hypnotics in use in the United States. The thalamus plays a pivotal role in sleep regulation and rhythmicity. Two distinct subtypes of synaptic GABA<sub>A</sub> receptors (GABA<sub>A</sub>-Rs), α<sub>1</sub>β<sub>2</sub>γ<sub>2</sub> and α<sub>3</sub>β<sub>3</sub>γ<sub>2</sub>, are expressed in thalamocortical relay neurons and in interneurons of the RTN (reticular thalamic nucleus), respectively. Thalamocortical neurons also express extrasynaptic GABA<sub>A</sub>-Rs composed of α<sub>2</sub>β<sub>2</sub>δ subunits. In this study, we compared the effects of eszopiclone and zolpidem on miniature inhibitory postsynaptic currents (IPSCs), spontaneous IPSCs, and tonic inhibition in the mouse thalamus. Eszopiclone (0.1–1 μM) slowed the decay phase of IPSCs recorded from RTN neurons, whereas zolpidem was less effective and increased the decay time constant only at ≥0.3 μM. IPSCs of RTN neurons were more sensitive to eszopiclone than zolpidem at all concentrations tested. On the other hand, IPSCs of relay neurons in the ventrobasal nucleus (VB) were more sensitive to zolpidem than eszopiclone. Zolpidem (0.1–1 μM) prolonged the decay of IPSCs from VB neurons, whereas eszopiclone increased the decay time constant only at ≥0.3 μM. Neither of these two hypnotics affected tonic inhibition in relay neurons. Our results demonstrate that eszopiclone has greater efficacy at synaptic GABA<sub>A</sub>-Rs of RTN neurons than in relay neurons, whereas zolpidem exerts bigger effects on relay neurons than RTN neurons. This distinct pattern of activity on thalamic neurons may contribute to some of the observed differences in the clinical effects of these two hypnotics.

Benzodiazepines (BZs) have long been used as anxiolytics, and these drugs have also found widespread use in the treatment of a variety of sleep disorders. The BZs exert their pharmacological effects by binding to specific allosteric BZ sites on GABA<sub>A</sub>-Rs (GABA<sub>A</sub>-Rs) and thereby modulating the function of these receptors. In recent years, awareness of several undesirable effects of the BZs resulted in the development of a new generation of nonbenzodiazepine hypnotics, including the “Z drugs”: zopiclone and zolpidem (Ambien; Sanofi-aventis, Bridgewater, NJ). Zolpidem is an imidazopyrididine, whereas zopiclone is a cyclopyrrolole. The sedative and anxiolytic effects of zopiclone in rodents are produced mainly by eszopiclone (Lunesta; Sepracor, Marlborough, MA), the (S)-enantiomer of zopiclone (Carlson et al., 2001).

Both eszopiclone and zolpidem are believed to promote sleep by interacting with GABA<sub>A</sub>-Rs in a way similar to BZs. Although they are structurally distinct from BZs (Drover, 2004), the Z drugs probably bind to sites on GABA<sub>A</sub>-Rs that are similar to, and overlapping with, the BZ sites. Mammalian GABA<sub>A</sub>-Rs in the central nervous system are pentameric structures consisting of distinct subunits, which can include α<sub>1–6</sub>, β<sub>1–3</sub>, and γ<sub>1–3</sub> or δ (Sieghart and Sperk, 2002). Zolpidem is highly selective for α<sub>1</sub>β<sub>2</sub>γ<sub>2</sub> GABA<sub>A</sub>-Rs (Pritchett and Seeburg, 1990; Wingrove et al., 2002), which have been implicated in the hypnotic actions of the BZs (Rudolph and Möhler, 2004). The selectivity of eszopiclone is not yet fully understood, although the drug has also been shown to act at γ<sub>2</sub>-containing GABA<sub>A</sub>-Rs, including α<sub>1</sub>β<sub>2</sub>γ<sub>2</sub>.

The thalamus plays an important role in sleep regulation, and damage to the thalamus interferes with normal sleep (Steriade and Timofeev, 2003). Both GABAergic interneurons in the reticular nucleus (RTN) of the thalamus and thalamocortical relay neurons in relay nuclei contribute to the generation of the characteristic oscillations seen in the EEG during sleep, including sleep spindles (7–12 Hz) and the slow waves associated with non-REM sleep (1–4 Hz) (McCormick and Bal, 1997; Steriade and Timofeev, 2003).

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ABBREVIATIONS: BZ, benzodiazepine; GABA<sub>A</sub>-R, GABA<sub>A</sub> receptor; RTN, reticular thalamic nucleus; EEG, electroencephalogram; REM, rapid eye movement; IPSC, inhibitory postsynaptic current; mIPSC, miniature IPSC; VB, ventrobasal thalamic nucleus.
A variety of GABA<sub>A</sub>-R subtypes exist in the thalamus; synaptic inhibition in thalamocortical relay neurons involves α<sub>1</sub>β<sub>2</sub>γ<sub>2</sub> receptors, whereas synaptic inhibition in the RTN is mediated by α<sub>3</sub>β<sub>3</sub>γ<sub>2</sub> receptors (Huntsman et al., 1999; Pirker et al., 2000; Jia et al., 2005). In addition, a tonic form of inhibition in thalamocortical relay neurons is generated via persistent activation of extrasynaptic α<sub>3</sub>β<sub>3</sub>δ receptors (Percello et al., 2003; Belelli et al., 2005; Cope et al., 2005; Jia et al., 2005, 2008; Chandra et al., 2006; Bright et al., 2007; Peden et al., 2008). In the present study, we compared the actions of eszopiclone and zolpidem on these three populations of GABA<sub>A</sub>-Rs in the mouse thalamus.

Materials and Methods

Electrophysiological Recordings in Brain Slices. Experiments were performed in accordance with institutional and federal guidelines, using mice between 20 and 60 days old (C57BL/6) by methods we have described previously (Jia et al., 2005). Animals were anesthetized with halothane, and brains were removed and placed in ice-cold slicing solution, which contained: 2.5 mM KCl, 26 mM NaHCO<sub>3</sub>, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 220 mM sucrose, 11 mM glucose, 10 mM MgSO<sub>4</sub> and 0.5 mM CaCl<sub>2</sub> before horizontal slices (300-μm thick) were cut on a microslicer (VT 1000S; Leica, Wetzlar, Germany). Slices were perfused with carbogenated artificial cerebrospinal fluid, and applied by perfusion. Each slice was only treated with one drug. Eszopiclone was kindly provided by Sepracor. Off-line analysis was performed using MiniAnalysis 5.5 (Synaptosoft, Decatur, GA), SigmaPlot 6.0 (SPSS Inc., Chicago, IL), and Excel 2003 (Microsoft, Redmond, WA). Spontaneous IPSCs were detected and analyzed using MiniAnalysis as described previously (Jia et al., 2005). Numeric data are expressed as mean ± S.E.M., except where indicated. The statistical significance of results was assessed using Student’s t test or one-way analysis of variance, and a level of p < 0.05 was considered significant.

Results

Effects of Eszopiclone on IPSCs in RTN Neurons. We began by investigating whether eszopiclone modulates spontaneous IPSCs in RTN neurons. The majority of GABA<sub>A</sub>-Rs in RTN neurons are located at synaptic sites and are composed of α<sub>3</sub>β<sub>3</sub>γ<sub>2</sub> subunits (Huntsman et al., 1999; Pirker et al., 2000; Jia et al., 2005). In our recordings, spontaneous IPSCs are readily observed in RTN neurons, with a mean amplitude of 31 ± 5 pA and weighted decay time constant (τ<sub>W</sub>) of 68 ± 4 ms (n = 21). A low concentration of eszopiclone (0.1 μM) increased τ<sub>W</sub> by 20 ± 9% (p < 0.05, n = 8). At 0.3 μM, eszopiclone increases τ<sub>W</sub> by 39 ± 6% (p < 0.01, n = 8) and amplitude by 23 ± 7% (p < 0.05, n = 8). At 1 μM, eszopiclone significantly increases both τ<sub>W</sub> (59 ± 9%, p < 0.001, n = 8) and amplitude (32 ± 9%, p < 0.05, n = 8; Fig. 1A).

The properties of spontaneous IPSCs can be influenced by both pre- and postsynaptic factors because most spontaneous

![Fig. 1](H11021/H11021/H11021) The effects of eszopiclone and zolpidem on IPSCs in RTN neurons. A, typical averaged spontaneous IPSC traces before (black) and during (gray) eszopiclone application. Normalized traces illustrate the difference between them in decay phase. The summarized bar figure shows the dose-dependent effects of eszopiclone (0.1–1 μM) on spontaneous IPSCs. B, typical averaged spontaneous IPSC traces before (black) and during (gray) zolpidem application. Normalized traces illustrate the difference between them in decay phase. At all three concentrations (0.1–1 μM), the amplitude of spontaneous IPSCs is not significantly changed by zolpidem. *, p < 0.05; **, p < 0.01; ***, p < 0.001.
IPSCs are driven by action potentials generated in presynaptic neurons. To isolate the postsynaptic effects of the drugs, we also examined the effects of eszopiclone on miniature IPSCs (mIPSCs), which are isolated by recording in 1 μM tetrodotoxin. Eszopiclone (0.1 μM) increased τ_m of mIPSCs by 14 ± 4% (p < 0.05, n = 8). At 0.3 μM, eszopiclone significantly increased τ_m by 43 ± 5% (p < 0.01, n = 8), and at 1 μM, eszopiclone significantly increased τ_m (63 ± 13%, p < 0.001, n = 8) and amplitude (25 ± 9%, p < 0.05, n = 8).

**Effects of Zolpidem on IPSCs in RTN Neurons.** We then tested the effects of zolpidem on IPSCs in RTN neurons. The amplitude of spontaneous IPSCs of RTN neurons is not significantly changed by zolpidem (0.1–1 μM). However, zolpidem (0.3 μM) did increase the weighted decay time constant by 12 ± 5% (p < 0.05, n = 10), and 1 μM zolpidem significantly increased τ_w by 21 ± 6% (p < 0.01, n = 10; Fig. 1B). Zolpidem had similar effects on mIPSCs. Zolpidem (0.3 μM) increased τ_m by 13 ± 6% (p < 0.05, n = 6), and 1 μM zolpidem significantly increased τ_m by 22 ± 5% (p < 0.01, n = 6).

The prolongation of IPSC decay phase in RTN neurons by zolpidem and eszopiclone is compared in Fig. 2. Eszopiclone has a greater efficacy than zolpidem at all three concentrations (0.1–1 μM) on both spontaneous and miniature IPSCs. The receptors mediating IPSCs in RTN neurons seem to be more sensitive to eszopiclone than to zolpidem.

**Effects of Eszopiclone on IPSCs in VB Neurons.** Synaptic GABAA-Rs in VB neurons are thought to be mainly the α3β3γ2 subtype (Pirker et al., 2000; Jia et al., 2005). The mean time constant of IPSCs recorded from VB relay neurons (16.2 ± 1.2 ms, n = 22) is much smaller than that of RTN neurons, in agreement with previous studies (Zhang et al., 1997; Huntsman and Huguenard, 2000; Schofield and Huguenard, 2007). At low concentrations (0.1 μM), eszopiclone did not change any properties of spontaneous IPSCs in VB neurons. At 0.3 μM, eszopiclone significantly increased τ_m by 12 ± 4% (p < 0.01, n = 9). At 1 μM, eszopiclone produced a small increase in both amplitude (10 ± 4%, p < 0.05, n = 10) and τ_w (20 ± 4%, p < 0.001, n = 10; Fig. 3A). The effects of eszopiclone on τ_w were reversible after washout of the drug (n = 2). The coapplication of 1 μM flumazenil totally blocked the effects of zolpidem (percentage change of τ_w, compared with control, 1%; n = 3 experiments). The effects of zolpidem on τ_w were also slowly reversible after washout of the drug (n = 2). Zolpidem also significantly increases mIPSC amplitude by 11 ± 3% (p < 0.05, n = 6) at 0.3 μM and by 15 ± 4% (p < 0.01, n = 7; Fig. 3B) at 1 μM.

The effects of eszopiclone and zolpidem on IPSCs in VB neurons were compared, as shown in Fig. 4. Eszopiclone is less efficacious than zolpidem at all three concentrations (0.1–1 μM) on both spontaneous and miniature IPSCs. It seems that the receptors mediating IPSCs in VB neurons are more sensitive to zolpidem than to eszopiclone.

**GABA\textsubscript{A}-R Subtype Selectivity of Eszopiclone and Zolpidem.** The percentage changes by eszopiclone on IPSCs of VB neurons and RTN neurons were compared (Fig. 5, A and B). Both spontaneous and miniature IPSCs in RTN neurons are more sensitive to eszopiclone (at all three concentrations tested) than those in VB neurons. In contrast, IPSCs in RTN neurons are less sensitive to zolpidem than those in VB neurons (Fig. 5, C and D).

**Tonic Currents in VB Neurons Are Insensitive to Eszopiclone and Zolpidem.** In addition to synaptic GABA\textsubscript{A}-Rs, extrasynaptic α3β3δ receptors are also expressed in VB relay neurons (Porcellio et al., 2003; Belelli et al., 2005; Cope et al., 2005; Jia et al., 2005; Chandra et al., 2006; Bright et al., 2007; Jia et al., 2008; Peden et al., 2008). Gaboxadoxol (4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol), an experimental hypnotic drug that is an agonist at α3β3δ receptors, has been shown to enhance tonic inhibition in VB neurons (Belelli et al., 2005; Cope et al., 2005; Jia et al., 2005). We examined whether eszopiclone or zolpidem modulates tonic currents mediated by extrasynaptic GABA\textsubscript{A}-Rs. In fact, neither 1 μM eszopiclone (0.2 ± 1.1 pA, n = 7) nor 1 μM zolpidem (0.5 ± 1.2 pA, n = 7) had any effect on the resting membrane current in VB neurons (Fig. 6).
Discussion

In this study, we compared the pharmacological effects of eszopiclone and zolpidem on synaptic and tonic inhibition in the mouse thalamus. We found that IPSCs recorded from RTN neurons are more sensitive to eszopiclone than zolpidem, whereas IPSCs from VB relay neurons are more sensitive to zolpidem. Tonic inhibition in VB neurons is insensitive to both hypnotics.

The diverse family of GABA<sub>A</sub>-R subunits is extensive and includes six isoforms of the α subunit that show considerable sequence diversity, unlike the β subunit isoforms, which are >90% identical at the amino acid level. This variety of α subunits confers substantial anatomical, functional, and pharmacological heterogeneity upon GABA<sub>A</sub>-Rs. There are discrete patterns of localization of the different α subunits, at the regional (Fritschy and Möhler, 1995; Sperr et al., 1997; Pirker et al., 2000), subcellular, and synaptic levels (Nusser et al., 1996). A great deal of functional diversity exists among the α subunits. For example, the α<sub>1</sub> subunit isoform influences BZ pharmacology (Pritchett et al., 1989; Pritchett and Seeburg, 1990; Hadingham et al., 1993). Two types of BZ binding sites were proposed in the 1980s, and we now know that GABA<sub>A</sub>-Rs with BZ 1 sites contain α<sub>1</sub> subunits, whereas those with BZ 2 sites contain α<sub>2</sub>, α<sub>3</sub>, or α<sub>5</sub> subunits. Zolpidem has a selective high affinity for BZ 1 sites and a very low affinity and extremely low efficacy at receptors that contain α<sub>5</sub> subunits (Pritchett and Seeburg, 1990; Wingrove et al., 2002), whereas the subunit specificity of eszopiclone has not yet been fully described.

Among the GABA<sub>A</sub> receptor α subunits, the α<sub>1</sub> and α<sub>4</sub> subunits are heavily expressed in thalamic relay nuclei that comprise the VB, but both seem to be absent from the RTN, as is the δ subunit. In contrast, α<sub>3</sub> subunits are apparently absent from VB but present in RTN. A variety of experiem-
tal data suggest that GABA<sub>4</sub>-Rs in RTN neurons act as synaptic receptors and are likely to consist almost exclusively of α3, β3, and γ2 subunits (Fritschy and Möhler, 1995; Oh et al., 1995; Gibbs et al., 1996; Huntsman et al., 1999; Browne et al., 2001). In contrast, both synaptic and extrasynaptic GABA<sub>4</sub>-Rs have been identified in thalamic relay neurons. Synaptic GABA<sub>4</sub>-Rs mainly contain α1, β2, and γ2 subunits, whereas extrasynaptic GABA<sub>4</sub>-Rs are composed of α4, β2, and δ subunits (Belelli et al., 2005; Cope et al., 2005; Jia et al., 2005; Chandra et al., 2006).

Fig. 5. GABA<sub>4</sub>-Rs subtype selectivity of eszopiclone and zolpidem. A, summarized figure shows the averaged potentiation ratio of spontaneous IPSC decay time constant in RTN and VB neurons by eszopiclone. Spontaneous IPSCs of RTN neurons are more sensitive to eszopiclone in comparison with those of VB neurons at all three concentrations (0.1–1 μM). B, likewise, miniature IPSCs of RTN neurons are more sensitive to eszopiclone in comparison with those of VB neurons. C, in contrast, spontaneous IPSCs in VB neurons are more sensitive to zolpidem compared with those from RTN neurons. D, miniature IPSCs in VB neurons are also more sensitive to zolpidem compared with those from RTN neurons. * p < 0.05; ** p < 0.01; *** p < 0.001.

Fig. 6. Eszopiclone and zolpidem have no effect on tonic currents. A, typical recording illustrates that 1 μM eszopiclone failed to change the holding current in a VB neuron. B, exemplar trace shows that 1 μM zolpidem also failed to change the holding current in another VB neuron. C, gabazine, a GABA<sub>4</sub>-R antagonist, induced a current shift (~20 pA) corresponding to the tonic background inhibitory currents mediated by extrasynaptic GABA<sub>4</sub>-Rs.
In the present study, we tested the actions of eszopiclone and zolpidem on three populations of GABA<sub>A</sub>-Rs in the thalamus: synaptic GABA<sub>A</sub>-Rs in RTN neurons (α3β3γ2 subtype) and synaptic (α1β2γ2) and extrasynaptic GABA<sub>A</sub>-Rs in VB neurons (α4β2γ2). Our results demonstrate that RTN synaptic GABA<sub>A</sub>-Rs are more sensitive to eszopiclone and that VB synaptic GABA<sub>A</sub>-Rs are more sensitive to zolpidem. Unlike synaptic GABA<sub>A</sub>-Rs, extrasynaptic GABA<sub>A</sub>-Rs are insensitive to both hypnotics. These data are in agreement with previous reports that GABA<sub>A</sub>-Rs that contain α1 subunits have a high affinity for zolpidem and that there is no BZ site in GABA<sub>A</sub>-Rs that contain α4 subunits (Knoflach et al., 1996; Whittemore et al., 1996; Wingrove et al., 2002). Our results also suggest that eszopiclone has a greater efficacy at α3β3γ2 than at α1β2γ2 GABA<sub>A</sub>-Rs (see Fig. 2). Although eszopiclone and zolpidem show quite distinct patterns of preference for the two subtypes of GABA<sub>A</sub>-Rs, both drugs modulate the activity of GABA<sub>A</sub>-Rs through binding to BZ sites.

The primary clinical indication for eszopiclone and zolpidem is in the treatment of insomnia. Both drugs induce and maintain sleep and increase sleep duration, with reduced residual (“morning after”) effects and less rebound insomnia compared with the BZs. Zolpidem induces EEG rhythms that are different from those seen with BZs, whereas the effects of eszopiclone on the EEG during sleep resemble those of BZs more closely (Drover, 2004; Sanger, 2004). Eszopiclone prolonged non-REM sleep and elicited a significant increase in total REM sleep, whereas zolpidem did not (Drover, 2004; Sanger, 2004). In addition to its hypnotic effects, eszopiclone has been found to have anxiolytic effects that may be beneficial in certain classes of patient. In clinical studies, significant improvements in both sleep quality and depressive symptoms have been observed when patients with insomnia and coexisting major depressive disorder were treated with eszopiclone and flunitrazepam simultaneously (Fava et al., 2006; Krytal et al., 2007).

The origin of these distinctions between the clinical pharmacology of the two drugs is not clear. Zolpidem is known for its relative specificity for receptors containing GABA<sub>A</sub>-Rs. It is interesting that a recent study of the two subtypes of GABA<sub>A</sub>-Rs (see Fig. 2). Although eszopiclone and zolpidem have distinct selectivity on synaptic GABA<sub>A</sub>-Rs that contain α1 or α3 subunits in the thalamus, whereas both of them lack activity at α4-containing extrasynaptic GABA<sub>A</sub>-Rs. Further work is needed to explore the drug sensitivity and roles of GABA<sub>A</sub>-Rs that contain α2 or α5 subunits in the brain.

References


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