ORIGINAL INVESTIGATION

The impact of age on the hypnotic effects of eszopiclone and zolpidem in the guinea pig

Mingchu Xi · Michael H. Chase

Abstract

Rational Eszopiclone and zolpidem are hypnotics that differentially affect sleep and waking states in adult animals. Therefore, it was of interest to compare their effects on the states of sleep and wakefulness in aged animals.

Objectives Our objective was to determine the responses to eszopiclone and zolpidem vis-à-vis sleep and waking states in aged guinea pigs and to compare them with the effects of these hypnotics in adult animals.

Methods Aged guinea pigs were prepared to monitor sleep and waking states and to perform a frequency analysis of the EEG. Eszopiclone and zolpidem were administered intraperitoneally (1, 3, and 10 mg/kg).

Results Eszopiclone produced a more rapid and greater increase in NREM sleep as well as longer duration episodes of NREM sleep compared with zolpidem. There was also a significant increase in the latency to REM sleep with eszopiclone, but not with zolpidem. EEG power during NREM sleep increased in the delta band and decreased in the theta band following eszopiclone administration, whereas zolpidem had no effect on any of the frequency bands analyzed.

Conclusions In aged as well as adult guinea pigs, eszopiclone is a more effective hypnotic insofar as it produces a shorter latency to NREM sleep, a greater amount of NREM sleep and EEG delta waves. Differences in the effects produced by eszopiclone and zolpidem as a function of the aging process likely reflect the fact that they bind to different subunits of the $GABA_A$ receptors, which are differentially reactive to the aging process.

Keywords Hypnotics · NREM sleep · EEG power · Aging

Introduction

In humans, old age is characterized by a decrease in nonrapid eye movement (NREM) and REM sleep as well as an increase in sleep latency and in the number of awakenings (Bliwise 2005). Sleep-related disorders, such as insomnia, sleep apnea, and periodic limb movements, occur more frequently in elderly individuals than in adults (Ancoli-Israel et al. 1985; Foley et al. 1995). There is also an increase in sleep disorders in the aged that is reflected by the enhanced use of hypnotics (Mendelson 1987). In addition, the aging brain appears to be more sensitive to the sedative effects of classic hypnotics, such as benzodiazepines and cognitive dysfunctions, which are more prevalent in the elderly, may be aggravated by their use (Hemmeter et al. 2000). In the elderly, the long plasma elimination half lives of the benzodiazepines and their

M. Xi · M. H. Chase
WebSciences International,
1251 Westwood Blvd.,
Los Angeles, CA 90024, USA

M. H. Chase (✉)
Department of Physiology, School of Medicine,
University of California,
Los Angeles, CA 90095, USA
e-mail: mchase@websciences.org

metabolites are increased due to a reduced capacity for oxidative hydroxylation (Lader 1993). Because aged individuals are less able to compensate for impairments caused by these hypnotics, the use of benzodiazepines to treat insomnia, especially in the elderly, has been considered problematic (Hemmeter et al. 2000). However, unlike classic hypnotics, nonbenzodiazepine hypnotics, such as zopiclone (a cyclopyrrolone derivative) whose s-isomer is eszopiclone and zolpidem (an imidazopyridine derivative) have fewer adverse effects (Declerck et al. 1992; Hemmeter et al. 2000; Lader 1992; Lancel 1999; Morin 2006; Noble et al. 1998).

Zopiclone and zolpidem act by potentiating the benzodiazepine-GABA$_A$ receptor complex by binding to different GABA$_A$ receptor subunits. Thus, zopiclone (or eszopiclone) exhibits considerable activity at GABA$_A$ receptors containing $\alpha1$, $\alpha2$, $\alpha3$, and $\alpha5$ subunits (Damgen and Luddens 1999; Davies et al. 2000; Smith et al. 2001). On the other hand, zolpidem displays a very high and selective affinity for $\alpha1$ subunits with significantly less or no affinity for $\alpha2$, $\alpha3$, and $\alpha5$ subunits (Sanna et al. 2002). Certain of the subunits of GABA$_A$ receptors that are activated by the preceding hypnotics display age-related changes in activity (Rissman et al. 2007). Therefore, it was of interest to compare the hypnotic actions of eszopiclone and zolpidem in old age. In addition, data from these studies would establish a foundation for understanding the cellular bases that are responsible for changes in sleep patterns as well as the effectiveness of hypnotics in the elderly, which at present are unknown.

We recently examined the hypnotic effects of eszopiclone and zolpidem in the adult guinea pig (Xi and Chase 2008). We found that eszopiclone, compared with zolpidem, has a pharmacological profile in the adult guinea pig (4–7 months old) that is characterized by a more rapid onset of hypnotic action and an increased amount of time spent in NREM sleep. In addition, eszopiclone produced an increase in EEG delta power, whereas zolpidem had no effect on delta activity. While our previous study and a number of other reports have described the hypnotic effects of eszopiclone (or zopiclone) and zolpidem in adult animals (Chen et al. 2005; Depoortere et al. 1991; Gauthier et al. 1997; Gottesmann et al. 1998; Mailliet et al. 2001; Noguchi et al. 2004; Stutzmann et al. 1992; Xi and Chase 2008), there have been no quantitative studies that have directly compared the effects of eszopiclone and zolpidem on the states of sleep and wakefulness in aged animals or related these effects to those produced in adult animals. Therefore, in the present study, we examined the effects of the intraperitoneal injection of eszopiclone and zolpidem on sleep and waking states, as well as on EEG power, in chronically instrumented, unanesthetized aged guinea pigs. We also compared these data with the effects produced by eszopiclone and zolpidem in adult animals.

Materials and methods

Animals and surgical procedures

Experiments were performed on five Hartley guinea pigs (three females and two males; age 28 ± 2 months, body weight 1.10 ± 0.06 kg) obtained from and determined to be in good health by the VA Greater Los Angeles Healthcare System (VAGLAHS). A variety of systems in the guinea pig, and specifically the central nervous system, exhibit age-related changes in animals that are 24 to 36 months old (Abalo et al. 2005; Gabella 2001; Hiller et al. 1992; McDougall and Schuelert 2007; Nozawa et al. 1997; Zhang et al. 2005). Therefore, the guinea pigs that were used in the present study were classified as "aged". Animals, which were housed in a temperature ($22\pm1°C$) and humidity (50–70%) controlled environment with a 12:12-h light/dark cycle (lights on from 600–1800 hours), had ad libitum access to food and water.

The following procedures were carried out in order to monitor the behavioral states of sleep and wakefulness and to determine the effects of intraperitoneally administered eszopiclone and zolpidem. Briefly, under ketamine/xylazine (45/5 mg/kg, im) anesthesia, using sterile surgical procedures, each guinea pig was implanted with electrodes for recording the cortical electroencephalogram (EEG), the electrooculogram (EOG), and the electromyogram (EMG). To record EEG activity, screw electrodes were implanted bilaterally in the calvarium overlying the cortex at the following coordinators (relative to bregma): 2 mm anterior, 2 mm lateral; 5 mm posterior, and 3 mm lateral. EMG electrodes were inserted bilaterally into the dorsal neck muscles. A Winchester plug, connected to these electrodes, was bonded to the calvarium with acrylic cement. Following surgery, antibiotics were administrated both systemically and topically. After recovery from surgery, each guinea pig was adapted to the recording environment on a daily basis for at least 2 weeks.

Procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academic Press, Washington DC, 1996) and approved by the Animal Research Committee of the VAGLAHS, and all efforts were made to minimize the number of animals used in this study.

Drug administration

Experimental sessions were conducted between 1100 and 1600 hours. Eszopiclone (Sepracor Canada Ltd; Windsor,

Canada) and zolpidem (Sanofi-Aventis USA, Bridgewater, NJ) were separately administered, intraperitoneally, at doses of 1, 3, and 10 mg/kg (0.5 ml). The doses of eszopiclone and zolpidem that were used in the present study are within the range of those typically employed when these substances are administered as hypnotics in experimental animals (Chen et al. 2005; Depoortere et al. 1991; Gauthier et al. 1997; Gottesmann et al. 1998; Mailliet et al. 2001; Noguchi et al. 2002, 2004; Stutzmann et al. 1992; Xi and Chase 2008). Vehicle was injected as a control (50 mM acetate buffer solution, pH 4.5, 0.5 ml). Each animal received injections of doses of 1, 3, and 10 mg/kg of both drugs and vehicle in a randomized protocol, which were administrated during the first 10 min of each experimental session. At least 2 days (>2 days) transpired between each injection session in order to make sure that all drug effects were reversible and did not impact responses produced by subsequent drug injections.

Recording and data analysis

The behavioral states of wakefulness, NREM sleep, and REM sleep were scored on the basis of polygraphic records which included EEG, EOG, and EMG data that were monitored and digitized using a Macintosh computer running AxoGraph X software (AxoGraph Scientific, Sydney, Australia); polygraphic data were scored in 30-s epochs according to previously described criteria (Escudero and Vidal 1996; Ibuka 1984; Tobler and Franken 1993). The following dependent variables were determined for each recording session: (1) percentage of time spent in wakefulness, NREM, and REM sleep; (2) latency to the onset of the first episode of NREM and REM sleep; because injections were carried out during wakefulness, there was no measure of the latency to wakefulness; (3) number of episodes of each behavioral state per hour (frequency); and (4) duration of episodes of each behavioral state. EEG power during each state was determined on the basis of a fast Fourier transform analysis of the EEG. Successive 15-s time segments of EEG signals in 1 Hz bins from 0.5–30 Hz over a period of 2 h were analyzed using AxoGraph X software. The relative power (percent of total) in each of the following frequency bands during each state was determined: delta (0.5–4 Hz); theta (4–9 Hz); sigma (9–14 Hz); and beta (14–30 Hz). Experimental data are expressed as means ± SEM. The statistical significance of the difference between sample means was evaluated using completely randomized one-way analysis of variance (ANOVA). After a one-way ANOVA, post hoc comparisons with the Scheffe method were used to determine the individual levels of significant differences between control (vehicle) and eszopiclone, control and zolpidem, and eszopi-

clone and zolpidem. A p value of <0.05 was considered to be statistically significant.

Results

In the present study, intraperitoneal injections of 1, 3, and 10 mg/kg of eszopiclone and 1, 3, and 10 mg/kg of zolpidem, as well as control injections of vehicle were performed in order to determine the effects of eszopiclone and zolpidem on sleep and waking states in the aged guinea pig. Control injection of vehicle was ineffective in modifying the states of sleep or wakefulness. No statistically significant changes in the time spent in sleep or wakefulness were observed during the third and fourth hours following the administration of either eszopiclone or zolpidem compared with vehicle control sessions. Thus, the effects of eszopiclone and zolpidem were limited to changes that occurred during the first 2 h following their administration. The following sections describe and compare, quantitatively, the effects of eszopiclone and zolpidem on the states of wakefulness, NREM, and REM sleep, as well as EEG power, during the first 2 h following the administration of these drugs.

Changes in the percentage of time spent in sleep and waking states following the administration of eszopiclone and zolpidem

Figure 1 presents an overview of the effects of the administration of eszopiclone and zolpidem on the percentage of time spent in sleep and waking states in the aged guinea pig. The following data are the results of an ANOVA analysis that documents the significant effect on the percentage of time spent in NREM sleep and waking states that occurred a conjunction with the administration of eszopiclone and zolpidem (NREM sleep: $df=6,71$, $F=18.35$, $p<0.001$; wakefulness: $df=6,71$, $F=16.33$, $p<0.001$). Compared to vehicle injections, the administration of eszopiclone at 1, 3, and 10 mg/kg resulted in significant increases in the time spent in NREM sleep by 34.0%, 65.7%, and 85.1% (vehicle 26.8±1.6%, $n=12$; 1 mg/kg eszopiclone 35.9±1.7%, $n=12$, $p=0.037$; 3 mg/kg eszopiclone 44.4±1.7%, $n=12$, $p<0.001$; 10 mg/kg eszopiclone 49.6±2.1%, $n=8$, $p<0.001$, post hoc Scheffe method) and decreases in wakefulness by 12.0%, 23.7%, and 30.6% (vehicle 72.2±1.8%, $n=12$; 1 mg/kg eszopiclone 63.5±1.7%, $n=12$, $p=0.064$; 3 mg/kg eszopiclone: 55.1±1.7%, $n=12$, $p<0.001$; 10 mg/kg eszopiclone 50.1±2.2%, $n=8$, $p<0.001$, post hoc Scheffe method), respectively.

Following the injection of zolpidem at 1 mg/kg, there were no significant changes in the percentage of time spent in sleep and waking states. However, the administration of
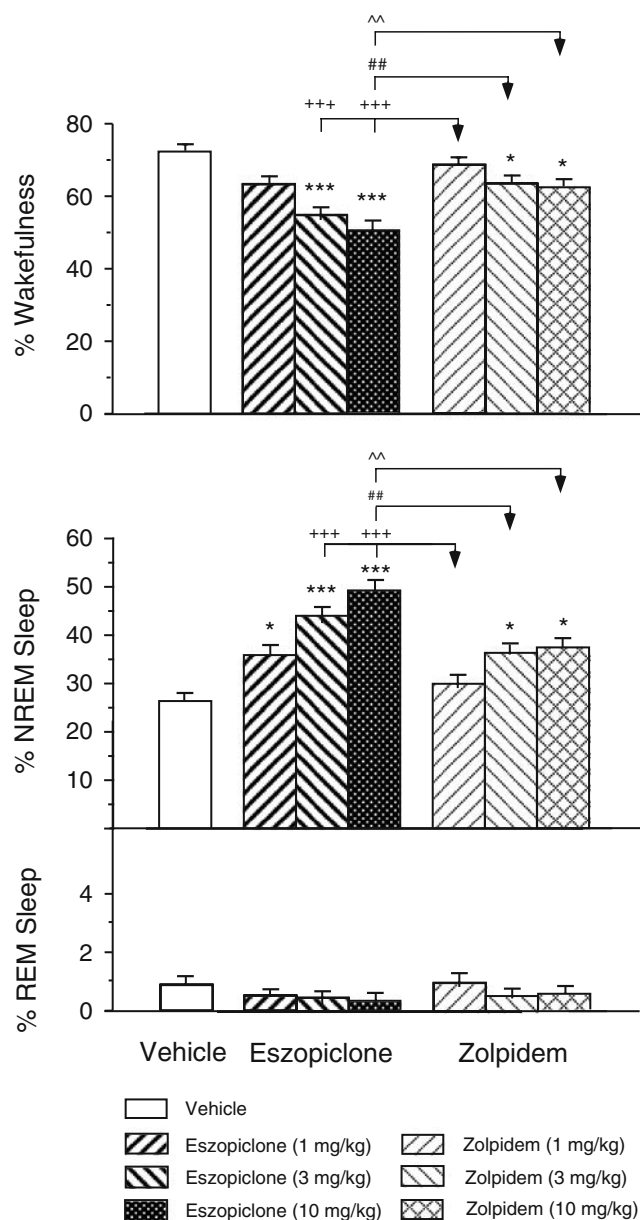
Fig. 1 Effects of eszopiclone and zolpidem on the mean percentage of time spent in wakefulness, NREM sleep, and REM sleep during the first 2-h period following the administration of these drugs at the doses of 1, 3, and 10 mg/kg in the aged guinea pig ($n=5$). Each *bar* represents the mean value; *error bars* indicate the SEM of each population. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs. vehicle control group; +++$p<0.001$ vs. 1 mg/kg zolpidem group; ##$p<0.01$ vs. 3 mg/kg zolpidem group; ^^$p<0.01$ vs. 10 mg/kg zolpidem group by post hoc Scheffe method

zolpidem at 3 and 10 mg/kg significantly increased the percentage of time spent in NREM sleep by 35.4% and 37.7% (3 mg/kg zoipidem 36.4±1.8%, $n=12$, $p=0.022$; 10 mg/kg zoipidem 36.9±1.8%, $n=10$, $p=0.021$, post hoc Scheffe method) and decreased the time spent in wakefulness by 12.5% and 13.4% (3 mg/kg zoipidem 63.2±1.8%,

$n=12$, $p=0.049$; 10 mg/kg zoipidem: 62.5±1.9%, $n=10$, $p=0.039$, post hoc Scheffe method), respectively.

A significant difference in the percentage of NREM sleep and wakefulness was observed on the basis of post hoc analyses following the administration of eszopiclone compared to zolpidem. The administration of eszolpiclone at 3 mg/kg (but not at 1 mg/kg) resulted in an increase in the percentage of time spent in NREM sleep and a decrease in wakefulness that was significantly greater than the time spent in these states after the administration of 1 mg/kg of zolpidem (NREM sleep 3 mg/kg eszopiclone vs. 1 mg/kg zolpidem, $p<0.001$; wakefulness 3 mg/kg eszopiclone vs. 1 mg/kg zolpidem, $p<0.001$, post hoc Scheffe method). The administration of eszolpiclone at 10 mg/kg also resulted in a significant increase in NREM sleep and a decrease in wakefulness compared to the time spent in these states after the administration of 1, 3, or 10 mg/kg of zolpidem (NREM sleep 10 mg/kg eszopiclone vs. 1 mg/kg zolpidem, $p<0.001$, 10 mg/kg eszopiclone vs. 3 mg/kg zolpidem, $p=0.001$; 10 mg/kg eszopiclone vs. 10 mg/kg zolpidem, $p=0.004$; wakefulness 10 mg/kg eszopiclone vs. 1 mg/kg zolpidem, $p<0.001$; 10 mg/kg eszopiclone vs. 3 mg/kg zolpidem, $p=0.002$; 10 mg/kg eszopiclone vs. 10 mg/kg zolpidem, $p=0.008$, post hoc Scheffe method).

Following the injection of eszopiclone or zolpidem at 1, 3, or 10 mg/kg, there was no significant change in the time spent in REM sleep ($df=6,71$, $F=0.86$, $p=0.527$, ANOVA), although there was a tendency toward a decrease in the REM sleep following the injection of eszopiclone or zolpidem at the higher doses of 3 or 10 mg/kg.

Changes in the latency to NREM and REM sleep following the administration of eszopiclone and zolpidem

Figure 2 presents the effects of eszopiclone and zolpidem on the mean latency to the onset of the first episode of NREM and REM sleep following the administration of these drugs. Significant drug effects occurred as revealed by ANOVA analysis with respect to the latency to both NREM sleep and REM sleep (NREM sleep: $df=6,71$, $F=13.88$, $p<0.001$; REM sleep: $df=6,27$, $F=4.82$, $p=0.002$).

Following the injection of eszopiclone at 1, 3, and 10 mg/kg, the mean latency to NREM sleep was significantly shorter compared with vehicle controls, respectively (1 mg/kg eszopiclone vs. vehicle, $p<0.001$; 3 mg/kg eszopiclone vs. vehicle, $p<0.001$; 10 mg/kg eszopiclone vs. vehicle, $p<0.001$, post hoc Scheffe method). Eszopiclone at 3 and 10 mg/kg also significantly increased the mean latency to REM sleep (3 mg/kg eszopiclone vs. vehicle, $p=0.032$; 10 mg/kg eszopiclone vs. vehicle, $p=0.034$, post hoc Scheffe method).

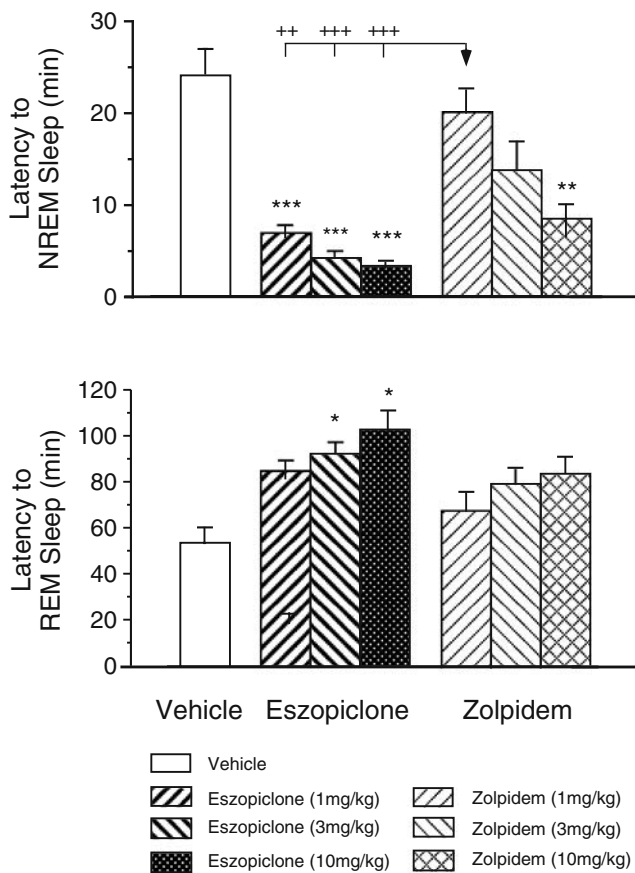There were no significant changes in the mean latency to NREM sleep following the injection of zolpidem at 1 and

Fig. 2 Dosage effects of eszopiclone and zolpidem on the latency to NREM sleep and REM sleep in the aged guinea pig (*n*=5). Each *bar* represents the mean value; *error bars* indicate the SEM of each population. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs. vehicle control group; ++$p<0.01$, +++$p<0.001$ vs. 1 mg/kg zolpidem group by post hoc Scheffe method

3 mg/kg, although there was a tendency toward a decrease in the latency to NREM sleep at 3 mg/kg. However, zolpidem at 10 mg/kg resulted in a significant decrease in the latency to NREM sleep (10 mg/kg zolpidem vs. vehicle, $p=0.001$, post hoc Scheffe method). There was no effect on the latency to REM sleep with any dose of zolpidem.

Following the injection of eszopiclone at 1, 3, and 10 mg/kg, the mean latency to NREM sleep was significantly shorter than that following zolpidem administration at doses of 1 mg/kg, respectively (1 mg/kg eszopiclone vs. 1 mg/kg zolpidem, $p=0.005$; 3 mg/kg eszopiclone vs. 1 mg/kg zolpidem, $p<0.001$; 10 mg/kg eszopiclone vs. 1 mg/kg zolpidem, $p<0.001$, post hoc Scheffe method).

Changes in the duration and frequency of episodes of sleep and wakefulness following the administration of eszopiclone and zolpidem

The effects on the duration of episodes of sleep and waking states were examined after the injection of eszopiclone and

zolpidem (Fig. 3). ANOVA revealed significant drug effects vis-à-vis the duration of episodes of NREM sleep (df=6,71 F=6.78, $p<0.001$). Following the injection of eszopiclone at 3 and 10 mg/kg, the mean duration of the episodes of NREM sleep was significantly longer than the episodes of NREM that occurred with vehicle (3 mg/kg eszopiclone vs. vehicle $p=0.011$; 10 mg/kg eszopiclone vs. vehicle $p=0.002$, post hoc Scheffe method). Eszopiclone at 3 and 10 mg/kg also resulted in a significant decrease in the mean duration of episodes of wakefulness (3 mg/kg eszopiclone vs. vehicle $p=0.040$; 10 mg/kg eszopiclone vs. vehicle $p=0.034$, post hoc Scheffe method). No significant effects on
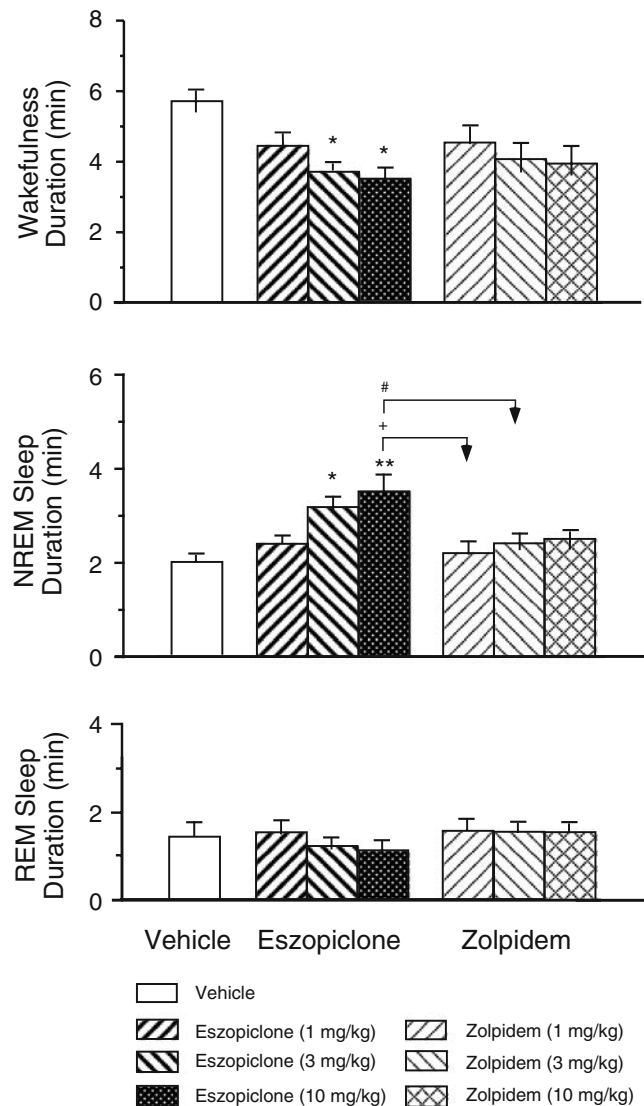

Fig. 3 The mean duration of episodes of wakefulness, NREM sleep and REM sleep following the administration of eszopiclone and zolpidem at the doses of 1, 3, and 10 mg/kg in the aged guinea pig (*n*=5). *$p<0.05$, **$p<0.01$ vs. vehicle control group; +$p<0.05$ vs. 1 mg/kg zolpidem group; #$p<0.05$ vs. 3 mg/kg zolpidem group by post hoc Scheffe method

the preceding parameters were observed following the injection of eszopiclone at 1 mg/kg.

Following the injection of zolpidem at all doses, the mean duration of episodes of NREM sleep and wakefulness was not significantly different from that following the injection of vehicle. However, there was a tendency for an increase in the duration of episodes of NREM sleep and a decrease in the duration of episodes of wakefulness following the administration of zolpidem, but only at 3 or 10 mg/kg.

Post hoc analyses revealed a significant increase in the mean duration of episodes of NREM sleep following the injection of eszopiclone at 10 mg/kg compared to the duration of NREM sleep episodes following the injection of 1 or 3 mg/kg of zolpidem (10 mg/kg eszopiclone vs. 1 mg/kg zolpidem, $p=0.012$; 10 mg/kg eszopiclone vs. 3 mg/kg zolpidem, $p=0.023$, post hoc Scheffe method).

Following the administration of eszopiclone and zolpidem at all doses, the frequency of episodes of NREM sleep, REM sleep, and wakefulness was not significantly different from

that following the injection of vehicle, although there was a tendency for a reduction in the frequency of episodes of REM sleep following the administration of zolpidem at 3 or 10 mg/kg.

Changes in spectral EEG power following the administration of eszopiclone and zolpidem

The effects of eszopiclone and zolpidem on EEG power during NREM sleep are shown in Fig. 4, which consists of plots of relative EEG power density during NREM sleep following the injection of eszopiclone, zolpidem, and vehicle. ANOVA revealed significant drug effects on the relative EEG power in the delta and theta bands during NREM sleep which occurred following the injection of eszopiclone and zolpidem (the delta band: $df=6,71$ $F=6.73$, $p<0.001$; the theta band: $df=6,71$ $F=7.80$, $p<0.001$).

Eszopiclone, at a dose of 3 or 10 mg/kg, significantly increased EEG power in the delta band (vehicle 38.6±0.6 %, $n=12$; 3 mg/kg eszopiclone 41.8±0.4 %, $n=12$, $p=0.026$;
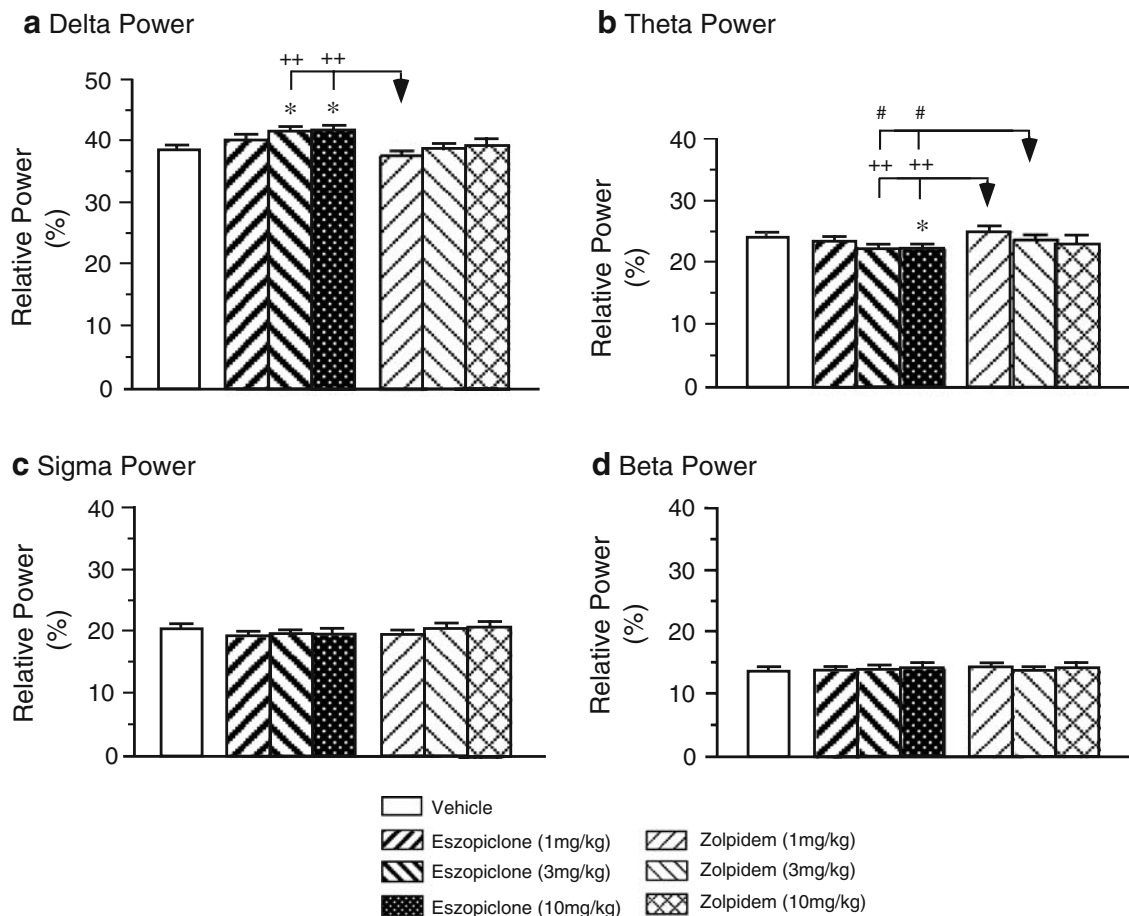

Fig. 4 Effects of eszopiclone and zolpidem on the relative power of EEG of the frontal cortex in the delta, theta, sigma, and beta frequency bands during NREM sleep in the aged guinea pig ($n=5$). *$p<0.05$ vs. the vehicle control group; +$p<0.05$, ++$p<0.01$ vs. 1 mg/kg zolpidem group; #$p<0.05$ vs. 3 mg/kg zolpidem group by post hoc Scheffe method

10 mg/kg eszopiclone 42.1±0.5 %, n=8, p=0.030, post hoc Scheffe method) and reduced EEG power in the theta band (vehicle: 23.8±0.3 %, n=12; 3 mg/kg eszopiclone 22.2±0.3%, n=12, p=0.085; 10 mg/ kg eszopiclone 21.8± 0.3%, n=8, p=0.037, post hoc Scheffe method). EEG power in the sigma and beta bands were not significantly altered following the administration of eszopiclone at 1, 3, or 10 mg/kg. However, there were no significant changes in EEG power in any of the frequency bands analyzed during NREM sleep following zolpidem administration at any dose.

Comparisons between eszopiclone and zolpidem injections with post hoc analyses revealed that eszopiclone administration at 3 or 10 mg/kg significantly increased the relative power of the EEG in the delta band compared to that after zolpidem administration at 1 mg/kg (3 mg/kg eszopiclone vs. 1 mg/kg zolpidem, p=0.003; 10 mg/kg eszopiclone vs. 1 mg/kg zolpidem, p=0.004, post hoc Scheffe method). Eszopiclone administration at 3 or 10 mg/kg also significantly reduced the relative power of the EEG in the theta band compared to that after zolpidem administration at both doses (3 mg/kg eszopiclone vs. 1 mg/kg zolpidem, p=0.004; 10 mg/kg eszopiclone vs. 1 mg/kg zolpidem, p=0.002; 3 mg/kg eszopiclone vs. 3 mg/kg zolpidem, p=0.049; 10 mg/kg eszopiclone vs. 3 mg/kg zolpidem, p=0.021, post hoc Scheffe method).

EEG power during REM sleep was examined following the administration of any doses of eszopiclone and zolpidem. Neither eszopiclone nor zolpidem resulted in any significant change in EEG power in any of the frequency bands analyzed during REM sleep.

Discussion

In the following sections, we discuss the effects of eszopiclone and zolpidem on the states of sleep and wakefulness in the aged guinea pig. These data are also compared with data obtained from our previous study in the adult guinea pig (Xi and Chase 2008) and reports of the effectiveness of these hypnotics in adult animals of other species. The differential binding properties of eszopiclone and zolpidem vis-à-vis specific subunits of the GABA$_A$ receptors and the effect of age on the activity of GABA$_A$ receptors are suggested to be responsible partially for the changes in the effects of these hypnotics in old age.

Hypnotic effects of eszopiclone in the aged guinea pig

In aged guinea pigs, the administration of eszopiclone resulted in significant changes in NREM sleep at doses of 1, 3, and 10 mg/kg that consisted of an increase in the amount of NREM sleep. This increase was accompanied by

a significant decrease in wakefulness. Eszopiclone also significantly reduced the latency to NREM sleep and increased the latency to REM sleep. Furthermore, there was a significant increase in the duration of episodes of NREM sleep and a decrease in the duration of episodes of wakefulness following the administration of eszopiclone at 3 and 10 mg/kg. Finally, no significant effects on the time spent in REM sleep were observed following the administration of eszopiclone, although there was a tendency for eszopiclone to reduce the amount of REM sleep.

Compared with data obtained from our previous study in adult guinea pigs (Xi and Chase 2008), it is interesting to note that the increase in NREM sleep and the reduction in wakefulness following the administration of eszopiclone in the aged guinea pig were smaller than were the changes that occurred when equivalent doses were administered in adult animals (Table 1). In addition, eszopiclone produced a significant increase in the duration of episodes of NREM sleep at 1 and 3 mg/kg in the adult guinea pig, but there was an increase in the duration of NREM sleep episodes only at a dose of 3 mg/kg in the aged guinea pig. However, eszopiclone (1 and 3 mg/kg) resulted in a significant, and comparable, decrease in the mean latency to NREM sleep in both aged and adult animals.

Our previous study in the adult guinea pig demonstrated that there is a significant increase in EEG power in the delta band (0.5–4.0 Hz) and a decrease in EEG power in the theta band (4.0–9.0 Hz) during NREM sleep following the administration of eszopiclone at either 1 or 3 mg/kg (Xi and Chase 2008). In contrast, the present data revealed that there were no significant changes in EEG power in any of the frequency bands analyzed during NREM sleep following eszopiclone administration at 1 mg/kg in aged animals. However, eszopiclone at 3 mg/kg did produce a significant increase in EEG power in the delta band during NREM sleep in the aged animals. There were no significant changes in either the time spent in REM sleep or EEG power during REM sleep following the injection of eszopiclone at either dose in aged guinea pigs, which is similar to the results in the adult animal (Xi and Chase 2008).

The preceding data indicate that in aged guinea pigs, the effects of eszopiclone on sleep and waking states (an increase in NREM and a decrease in wakefulness) and in the power spectra of the EEG during NREM sleep (an increase in delta power) were significant but not quite as strong as the effects produced in adult animals. Zopiclone (presumably eszopiclone) exhibits considerable binding not only to α1 subunits of the GABA$_A$ receptor, but also to GABA$_A$ receptors containing α2, α3, and α5 subunits (Damgen and Luddens 1999; Davies et al. 2000; Smith et al. 2001). A recent study has demonstrated that there is a significant decrease in the expression of α3 and α5

Table 1 Hypnotic effects of eszopiclone and zolpidem in adult and aged guinea pigs

	Eszopiclone				Zolpidem			
	1mg/kg		3mg/kg		1mg/kg		3mg/kg	
	Adult[a]	Aged	Adult[a]	Aged	Adult[a]	Aged	Adult[a]	Aged
Wakefulness	25%↓***	n.c.	34%↓***	24%↓***	n.c.	n.c.	13%↓*	13%↓*
NREM Sleep	60%↑***	34%↑*	81%↑***	66%↑***	n.c.	n.c.	32%↑*	35%↑*
NREM latency	68%↓***	70%↓***	85%↓***	82%↓***	n.c.	n.c.	n.c.	n.c.
REM Latency	82%↑*	n.c.	117%↑**	70%↑*	n.c.	n.c.	n.c.	n.c.
Wakefulness Duration	n.c.	n.c.	35%↓*	36%↓*	n.c.	n.c.	n.c.	n.c.
NREM Sleep Duration	35%↑*	n.c.	65%↑***	60%↑*	n.c.	n.c.	n.c.	n.c.
REM Sleep Frequency	n.c.	n.c.	50%↓*	n.c.	n.c.	n.c.	n.c.	n.c.
Delta Power	10%↑*	n.c.	13%↑**	8%↑*	n.c.	n.c.	n.c.	n.c.
Theta Power	7%↓**	n.c	8%↓**	n.c.	n.c.	n.c.	n.c	n.c

An increase (↑) or decrease (↓) in the percentage of changes in sleep and waking states compared with control (vehicle) data are presented at 1 and 3 mg/kg for eszopiclone and zolpidem in adult and aged guinea pigs. Data regarding the percentage of time spent in REM sleep, the duration of episodes of REM sleep, the frequency of episodes of NREM sleep and wakefulness, EEG power in sigma and beta bands following the administration of eszopiclone or zolpidem at both doses, which were not significantly different from those of vehicle-injected controls, are not included in this table. *$P<0.05$; **$P<0.01$; ***$P<0.001$. Post hoc comparisons with the Scheffe method were used to determine the levels of significant differences between drugs and control (vehicle)

n.c. no significant change

[a] Xi and Chase (2008)

subunits of $GABA_A$ receptors in aged rats compared with young adult animals (Yu et al. 2006). Therefore, we suggest that a decline in the expression of one or more of these subunits of $GABA_A$ receptors in aged animals may provide an explanation for the relatively "weaker" effects of eszopiclone in aged guinea pigs compared to adult guinea pigs.

While no reports have compared the effects of eszopiclone or zopiclone on sleep and waking states either in aged guinea pigs or in aged animals of other species, the present results are generally consistent with findings from separate studies in animals and in humans. In adult rats, zopiclone, administered intraperitoneally in doses from 2.5 to 10 mg/kg, was found to increase NREM sleep, decrease wakefulness, decrease the latency to NREM sleep, and increase the latency to REM sleep (Stutzmann et al. 1992); a decrease in the total time spent in REM sleep was also reported (Gutierrez et al. 1997). However, in another study, zopiclone also significantly increased NREM sleep but had no effect on REM sleep in the adult rat (Noguchi et al. 2004). Spectral analyses in adult rabbits revealed that zopiclone, at a dose of 2 mg/kg, significantly increased power in the delta frequency band and decreased power in the theta frequency band (Noguchi et al. 2002). In humans, zopiclone has been shown to have a positive effect on sleep (e.g., a reduction in sleep latency and an increase in total NREM sleep time) with few adverse responses in healthy elderly subjects (Hemmeter et al. 2000). Zopiclone also significantly shortened sleep latency and

increased total sleep time in elderly patients with insomnia (Scharf et al. 2005). In young healthy subjects, Zopiclone has been reported to produce a minor increase in EEG delta power, a significant decrease in EEG theta power and an increase in EEG sigma power (Kim et al. 1993). However, other studies have reported that zopiclone decreases the power density in other frequency ranges <10 Hz and enhances the power density in the range of 12–15 Hz in adult healthy subjects (Trachsel et al. 1990; Wright et al. 1986).

There was a positive relationship between the increase in EEG power of the delta frequency band during NREM sleep and the increase in the duration of NREM episodes or the amount of NREM sleep following the administration of eszopiclone. The magnitude of EEG slow wave activity in the delta band is considered to be an index of sleep intensity (Borbely and Achermann 2000; Tobler and Borbely 1986). This is particularly interesting with respect to the use of eszopiclone as a pharmacological tool to improve the quality of sleep in old age, especially when one considers the fact that there is a reduction in delta wave activity in aged humans (Mann and Roschke 2004).

Hypnotic effects of zolpidem in the aged guinea pig

In the aged guinea pig, significant changes in sleep and waking states occurred when zolpidem was administered but only at a dose of 3 or 10 mg/kg. At these doses, it

increased the percentage of time spent in NREM sleep and decreased the amount of wakefulness. There were no significant effects on the time spent in REM sleep with either dose of zolpidem.

It is interesting to note that the changes in the percentage of time spent in NREM sleep following the administration of zolpidem at 1 and 3 mg/kg in the aged guinea pig were similar to those that occurred when the same doses were administrated to adult guinea pigs (Xi and Chase 2008; Table 1). In addition, there were no significant changes in EEG power in any of the frequency bands analyzed during NREM sleep following zolpidem administration in the aged guinea pig, which mirrors our findings in the adult guinea pig (Xi and Chase 2008). Therefore, the present data indicate that there are no marked differences in the effects of zolpidem on sleep and waking states (increase in NREM and decrease in wakefulness) and in the power spectra of the EEG during NREM sleep in aged guinea pigs compared with adult guinea pigs.

Zolpidem is known to exert its hypnotic effects by acting primarily on α1 subunits of GABA$_A$ receptors (Sanna et al. 2002). A number of anatomical studies have shown that there is no prominent change in the expression of α1 subunits of the GABA$_A$ receptor in aged rats compared with adult animals (Gutierrez et al. 1997; Mhatre and Ticku 1992; Yu et al. 2006). Therefore, this may explain the lack of marked differences in the effects of zolpidem in aged and adult guinea pigs. However, additional research is needed to unravel bases of the differences in the effects produced by eszopiclone and zolpidem vis-à-vis changes in the subunits of GABA$_A$ receptors that are targeted by these hypnotics in aged animals.

Our results with zolpidem in the aged guinea pig are generally similar to data of previous studies in adult animals of other species. For example, studies in adult rats have shown that zolpidem, administered at doses of 3 or 10 mg/kg, increased NREM sleep and the mean duration of episodes of NREM sleep during the light period and dark period, respectively (Chen et al. 2005; Depoortere et al. 1991; Mailliet et al. 2001). Another study in the adult rat reported that the latency to NREM was reduced following the administration of zolpidem at 2.5, 5, and 7.5 mg/kg, and the latency to REM sleep was only increased at the two higher doses. In addition, the amount of REM sleep decreased at the highest dose (Gottesmann et al. 1998).

In normal elderly human subjects, zolpidem, in doses between 5 and 20 mg, shortens the latency to NREM sleep and increases the total time spent in NREM sleep (Fairweather et al. 1992; Scharf et al. 1991). In elderly patients with insomnia, zolpidem has been reported to shorten sleep latency and increase total sleep time (Ancoli-Israel et al. 1999; Walsh et al. 2008).

Differences between hypnotic effects of eszopiclone and zolpidem in the aged guinea pig

While the administration of both eszopiclone and zolpidem in aged guinea pigs produced hypnotic effects in the present study, our quantitative analyses reveal significant differences in the effects of these two hypnotics. Firstly, the increase in the percentage of time spent in NREM sleep and the decrease in the time spent in wakefulness following the administration of eszopiclone at 3 or 10 mg/kg were significantly greater than the time spent in NREM sleep and wakefulness after zolpidem administration (which was ineffective at a dose of 1 mg/kg). Secondly, eszopiclone at 1 and 3 mg/kg induced a significant decrease in the mean latency to NREM sleep that was significantly shorter than that which followed the administration of zolpidem at 1 mg/kg. Thirdly, eszopiclone at 3 or 10 mg/kg resulted in a significant increase in the mean duration of episodes of NREM sleep, whereas zolpidem did not produce any significant change in the mean duration of episodes of NREM sleep at any doses. Finally, there was a significant increase in EEG power in the delta band and a decrease in EEG power in the theta band during NREM sleep following the administration of eszopiclone at 3 or 10 mg/kg. In contrast, there were no significant changes in EEG power in any of the frequency bands analyzed during NREM sleep following zolpidem administration at any dose. Therefore, the present data suggest that eszopiclone has a greater effect on NREM sleep and the generation of NREM delta activity than does zolpidem in the aged guinea pig, which is similar to the results obtained in the adult guinea pig (Xi and Chase 2008).

In summary, the present results demonstrate that in the aged guinea pig, eszopiclone, compared with zolpidem, has a more rapid onset of hypnotic action and results in an increased amount in NREM sleep. In addition, eszopiclone produces an increase in EEG delta power, whereas zolpidem has no effect on delta activity. Compared with previous data obtained in the adult guinea pig, the effects of eszopiclone on sleep and waking states and in the power spectra of the EEG during NREM sleep in the aged guinea pig were "weaker" than those in the adult guinea pig, although the effects of zolpidem were similar in aged and adult guinea pigs (Xi and Chase 2008). We, therefore, suggest that in addition to the potential differences in pharmacokinetics and pharmacodynamics of these two drugs (Drover 2004), the relative binding properties of eszopiclone and zolpidem, and the reduction in the expression of specific subunits of GABA$_A$ receptors in aged animals may account for the changes in the relative degree of effectiveness of eszopiclone and zolpidem on the states of sleep and wakefulness as well as the power spectra of the EEG in the aged guinea pig.

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