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Eszopiclone stimulates the hypothalamo-pituitary-adrenal axis in the rat

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ABSTRACT

Eszopiclone (Lunesta®) is used for the treatment of insomnia. It is the *S* (+)-enantiomer of racemic zopiclone, a cyclopyrrolone with no structural similarity to the hypnotic drugs zolpidem and zaleplon or to the benzodiazepines and barbiturates. Although eszopiclone interacts with the gamma-aminobutyric acid A-type ($GABA_A$) receptor complex, it has a different binding profile than other sedative/hypnotic agents and modulates the receptor complex in a unique manner. Thus, eszopiclone might produce different pharmacological effects compared to other sedative/hypnotic agents. Beside their behavioral properties, sedative/hypnotic drugs affect the hypothalamo-pituitary-adrenal (HPA) axis. In general, low doses of benzodiazepine-type drugs decrease, whereas high doses increase the activity of the HPA axis. Furthermore, benzodiazepines reduce stress-induced increases in HPA axis activity. The goal of the present study was to characterize the effects of eszopiclone on the HPA axis in the rat. Male rats were injected with saline or eszopiclone and trunk blood was collected for the measurement of plasma levels of adrenocorticotropin (ACTH) and corticosterone by radioimmunoassay. The acute administration of eszopiclone produced dose-dependent increases in plasma levels of ACTH and corticosterone, and tolerance developed to these effects after repeated drug administration. Pretreatment with eszopiclone did not affect stress-induced stimulation of the HPA axis. These results show that eszopiclone and the benzodiazepine-type drugs differentially affect the HPA axis.

1. Introduction

Eszopiclone (Lunesta®) is a sedative/hypnotic drug that is used for the treatment of insomnia (Najib, 2006). It is the *S* (+)-enantiomer of racemic zopiclone, a cyclopyrrolone with no structural similarity to the hypnotic drugs zolpidem and zaleplon, or to the benzodiazepines and barbiturates. Eszopiclone, along with zaleplon and zolpidem, is a member of a class of drugs known as nonbenzodiazepine benzodiazepine receptor agonists. These drugs bind to sites on the $GABA_A$ receptor that are similar to or overlap with the sites at which benzodiazepines act (Jia et al., 2009). $GABA_A$ receptors are made up of heterogeneous pentameric clusters of proteins, and five protein subunits and a least 19 subunit isoforms have been described with distinct regional and cellular distribution patterns (Sieghart, 2006). Eszopiclone has been hypothesized to act through the α_1, α_2, α_3 and α_5 subunits of the $GABA_A$ receptor (Hanson et al., 2008; Nutt and Stahl, 2010). Although eszopiclone interacts with the $GABA_A$ receptor complex, it appears to have a different binding profile than benzodiazepine-type drugs and modulates the receptor complex in a different manner (Carlson et al., 2001). For example, the cyclopyrrolone-type sedative/hypnotic drugs do not exhibit full competitive antagonism at the benzodiazepine binding site (Trifiletti and Snyder, 1984). Furthermore, there are differences among that nonbenzodiazepine sedative/hypnotic drugs, as eszopiclone and zolpidem bind to the binding pocket on the binding site in a different manner (Hanson et al., 2008) and produce dissimilar electrophysiological properties (Jia et al., 2009).

The behavioral effects of the nonbenzodiazepine benzodiazepine agonists are qualitatively similar to those produced by benzodiazepines such as diazepam and alprazolam, but there are quantitative differences. For example, the sedative/hypnotic effects of the nonbenzodiazepine drugs occur at lower doses than the anticonvulsant and muscle relaxant effects (Sanger, 2004), they have less of an impact on cognitive function and psychomotor performance, and cause less disruption of normal sleep architecture (Terzano et al., 2003; Hanson et al., 2008). Thus, eszopiclone might produce different pharmacological effects compared to other sedative/hypnotic agents.

Benzodiazepines have long been used as anxiolytics and hypnotics. Beside their behavioral properties they can affect the hypothalamo-pituitary-adrenal (HPA) axis. In general, low doses of benzodiazepines decrease, whereas high doses increase plasma corticosterone levels in rats (De Souza, 1990). The nonbenzodiazepine benzodiazepine receptor agonists zolpidem and zopiclone also increase plasma levels of ACTH and corticosterone (Mikkelsen et al., 2005). Altering the normal functioning of the HPA axis can have profound pathophysiological consequences by affecting the responses to stressor, disrupting circadian rhythms and inhibiting immune function. The objective of the present study was to characterize the effects of the acute and

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repeated administration of eszopiclone on plasma levels of ACTH and corticosterone in the rat. The effects of eszopiclone on the HPA axis response to restraint stress also were assessed.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (Charles River, Wilmington, MA, USA) were housed two per cage in a humidity- and temperature-controlled (21–22 °C) vivarium on a 12:12 h light–dark cycle (lights on 07:00 h, off 19:00 h) for 10 days prior to drug administration. Food and water were available ad libitum. The rats were handled daily for 5 days prior to drug administration in order to habituate them to the experimental procedure and reduce stress-induced changes in hormone secretion. All injections were given between 10:00 and 11:00 h in order to minimize the effects of circadian rhythms. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Cedars-Sinai Medical Center.

2.2. Acute drug administration

Experiment 1 assessed the effects of the acute administration of eszopiclone on plasma levels of ACTH and corticosterone. Saline or eszopiclone (1.0, 3.0 or 10.0 mg/kg/i.p.) was administered to groups of rats, and they were sacrificed 30, 60, 120 or 180 min after injection. We chose to test doses of eszopiclone that did and did not affect behavior. The dose range was selected based upon the report that 5–10 mg/kg reduced locomotor activity, impaired performance on the rotarod and showed anxiolytic activity in the elevated plus maze, whereas lower doses did not (Carlson et al., 2001). Following decapitation, trunk blood was collected into chilled tubes containing disodium ethylenediaminetetraacetic acid (5.0 mg), sodium azide (0.081 mg) and aprotinin (500 kallikrein units). The blood was immediately centrifuged at 500×*g* for 20 min at 4 °C and aliquots of plasma were frozen on dry ice. The plasma was stored at −70 °C until the subsequent measurement of plasma levels of ACTH and corticosterone by radioimmunoassay (RIA).

2.3. Repeated drug administration

Experiment 2 determined the effects of the repeated administration of eszopiclone on plasma levels of ACTH and corticosterone. On days 1 through 14 the rats were weighed and injected with saline or eszopiclone (3.0 mg/kg/i.p.). On day 15, the rats received either saline or eszopiclone (3.0 mg/kg/i.p.) and were sacrificed 60 min later. The four treatment groups were as follows: repeated saline/acute saline; repeated saline/acute eszopiclone; repeated eszopiclone/acute saline; and repeated eszopiclone/acute eszopiclone. Trunk blood was collected and processed as described above.

2.4. Stress experiment

Experiment 3 characterized the effects of the eszopiclone on stress-induced changes in plasma levels of ACTH and corticosterone. Rats were treated with saline or eszopiclone (3.0 mg/kg/i.p.), and 30 min later they were put into Plexiglas restrainers for 30 min. Separate groups of rats were sacrificed immediately after removal from the restrainers (0) or 30, 60, 120 or 180 min later. An untreated group received neither injections nor restraint stress. Trunk blood was collected and processed as described above.

2.5. RIAs

Plasma levels of ACTH and corticosterone were determined by RIA using anti-ACTH antisera (MP Biomedicals, Solon, OH) and corticosterone antiserum (MP Biomedicals, Solon, OH). The reference standards for the ACTH assay were $ACTH_{1-39}$; and the limits of sensitivity for the two assays were: ACTH, 40.0 pg/ml; and corticosterone, 2.0 ng/ml. As determined by low, medium and high plasma pool replicates, the maximum inter- and intra-assay coefficients of variation for ACTH and corticosterone assays were 19% and 12%, and 14% and 10% respectively. All samples for each hormone for each experiment were analyzed in the same assay. All samples were run in duplicate and analyzed "blindly".

2.6. Data analysis

Because the endocrine data displayed heterogeneity of variance across the experimental groups and the data were not normally distributed, all analyses were conducted on log-transformed raw data values. The acute drug administration data were analyzed by two-way analysis of variance (ANOVA), with treatment and time post-injection the independent variables. Individual time points were then subjected to one-way ANOVA followed where appropriate by Scheffé's post hoc tests. The repeated drug administration data were analyzed by one-way ANOVA followed by Scheffé's post hoc tests. The effects of eszopiclone on restraint stress data were analyzed by two-way ANOVA, followed by *t* tests at each time point. For all comparisons a criterion of $P<.05$ was used for the rejection of the null hypothesis.

2.7. Drugs

Eszopiclone (Sepracor, Inc., Marlborough, MA, USA) was dissolved in saline (0.9%) by solubilization in one or two drops 1 N HCl. The pH of the solution was adjusted to 7.0 by the addition of 1 N NaOH. The control subjects were injected with saline (0.9%).

3. Results

3.1. Acute eszopiclone administration studies

The acute administration of eszopiclone produced statistically significant, dose-dependent increases in plasma levels of ACTH (Fig. 1A). Two-way ANOVA showed significant treatment [$F(3,110)=16.23$; $P<0.0001$], time [$F(3,110)=63.68$; $P<0.0001$] and interaction effects [$F(9,110)=6.27$; $P<0.0001$]. Compared to saline-treated controls, ACTH was elevated 30 min after injection with the 1.0. 3.0 and 10.0 mg/kg doses of eszopiclone, and by 120 min the levels were not different than the saline-treated controls. The acute administration of eszopiclone also increased plasma levels of corticosterone (Fig. 1B). There were significant treatment [$F(3,113)=12.84$; $P<0.0001$], time [$F(3,113)=25.98$; $P<0.0001$] and interaction effects [$F(9,113)=5.58$; $P<0.0001$]. Similar to the effects on plasma ACTH, eszopiclone produced a rapid increase in plasma levels of corticosterone. There were no differences among the doses of drug at the 30 min time point, suggesting that the effect was maximal after the lowest dose. Although 120 min after drug administration there was a tendency for the highest dose (10.0 mg/kg) of eszopiclone to increase plasma corticosterone, there were no statistically significant differences compared to saline-treated controls at this time point.

3.2. Repeated eszopiclone administration studies

Groups of rats were treated daily for 14 days with saline or eszopiclone (3.0 mg/kg i.p.), and then on the next day challenged with an acute injection of saline or eszopiclone (3.0 mg/kg i.p.). Acute challenge with eszopiclone produced differential effects on plasma levels of ACTH in the repeated saline- and repeated eszopiclone-treated subjects [$F(3,33)=4.62$; $P=0.0083$]. As found in the first experiment, the acute administration of eszopiclone increased plasma levels of ACTH in the repeated saline-treated rats (Fig. 2A); however, it did not increase plasma ACTH levels in the rats that had received repeated treatment with eszopiclone. A similar effect was found with plasma levels of

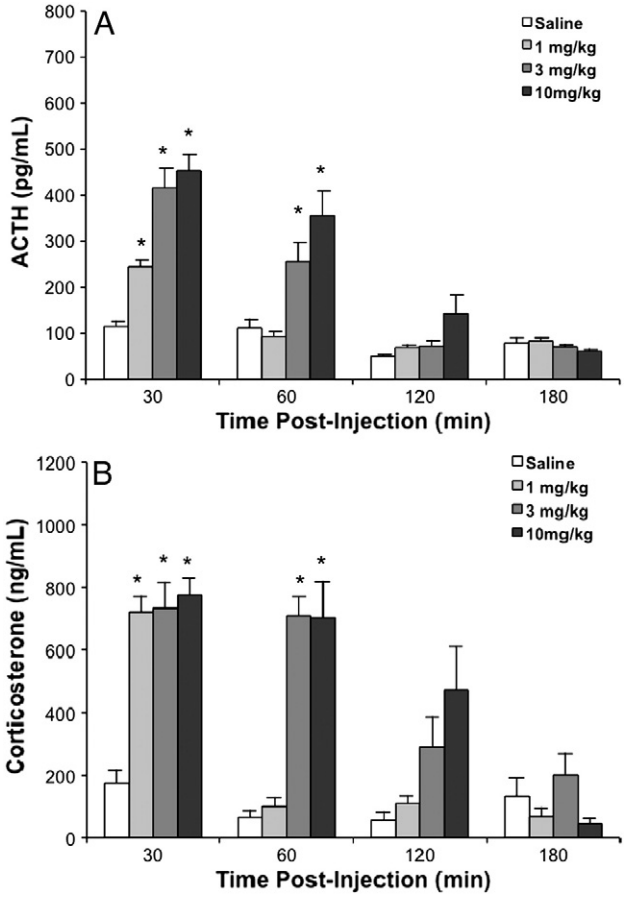

Fig. 1. Time course of the effects of the acute administration of eszopiclone on plasma levels of ACTH (A) and corticosterone (B). Subjects were injected with saline (0.9%) or eszopiclone (1.0, 3.0 or 10.0 mg/kg/i.p.) and sacrificed 30, 60, 120 or 180 min later. Values are expressed as the means + the standard errors of the mean ($n = 6$–9 per group). *P<.05 compared to saline-injected controls at each time point.

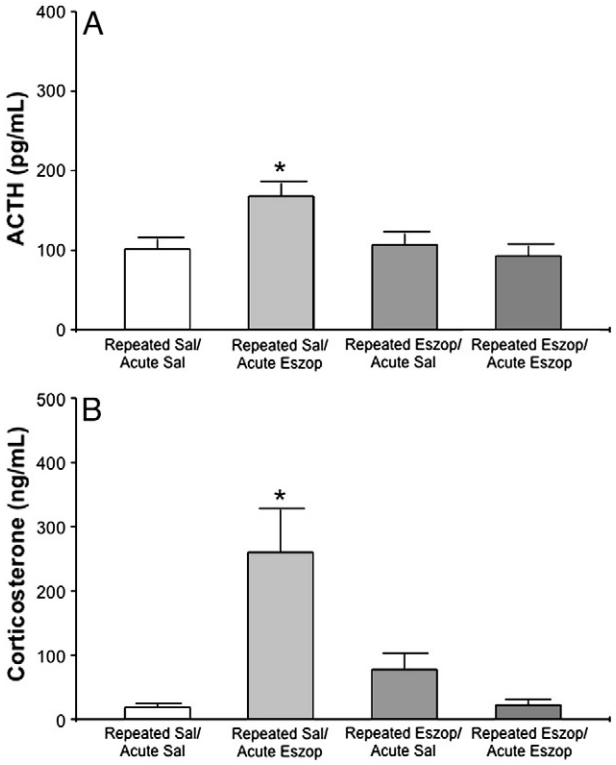

Fig. 2. Effects of the repeated administration of eszopiclone on plasma levels of ACTH (A) and corticosterone (B). Subjects were given daily injections of saline (0.9%) or eszopiclone (3.0 mg/kg/i.p.) for 14 days. On day 15 they were injected with saline or eszopiclone (3.0 mg/kg/i.p.). Values are expressed as the means + the standard errors of the mean ($n = 7$–10 per group). *P<.05 compared to repeated saline/acute saline- and repeated eszopiclone/acute eszopiclone-treated groups.

corticosterone [$F(3,33) = 12.28$; $P<0.0001$]. The acute administration of eszopiclone increased plasma levels of corticosterone in the rats that had received repeated treatment with saline (Fig. 2B); however, it did not increase plasma corticosterone levels in rats that had received repeated treatment with eszopiclone. Thus, tolerance develops to the stimulatory effects of eszopiclone on the HPA axis after repeated administration.

3.3. Effects of eszopiclone on restraint stress

Restraint stress produced high plasma levels of ACTH that rapidly decreased after removal of the rats from the restraint tubes (Fig. 3A). Although there was a significant time effect [$F(1,4) = 202.99$; $P<0.0001$], comparison of the eszopiclone- and saline-treated rats show no treatment [$F(1,83) = 2.56$; $P = 0.1136$] or interaction [$F(4,83) = 1.25$; $P = 0.2944$] effects. With respect to plasma corticosterone levels (Fig. 3B), there was no significant treatment effect [$F(1,83) = 2.04$; $P = 0.1568$], but there were significant time [$F(1,4) = 60.98$; $P<0.0001$] and interaction effects [$F(4,83) = 3.24$; $P = 0.0160$]. These results indicate that pretreatment with eszopiclone does not affect plasma levels of ACTH or corticosterone after restraint stress.

4. Discussion

The acute administration of eszopiclone produced dose-dependent increases in plasma levels of ACTH and corticosterone, and tolerance developed to these effects after repeated drug administration. In addition, treatment with eszopiclone did not affect stress-induced stimulation of the HPA axis. The stimulation of the HPA axis was present 30 min after drug administration, but it was short-lived; plasma levels of ACTH and corticosterone were not significantly different than saline-treated controls 120 min after drug administration. It is interesting to note that the increase in plasma corticosterone after the lowest dose of eszopiclone (1.0 mg/kg) was greater than that produced by 30 min of restraint stress.

Eszopiclone also affects behavior in rats. For example, the acute administration of eszopiclone alters locomotor activity, disrupts rotarod performance and shows anxiolytic activity in the elevated plus maze (Carlson et al., 2001). However, these behavioral effects occur at higher doses (e.g., 5.0–10.0 mg/kg) than those required to stimulate the HPA axis (e.g., 1.0 mg/kg) in the present study. Thus, doses of eszopiclone that are not active in some behavioral tests stimulate the HPA axis in the rat.

Studies examining the effects benzodiazepines on the HPA axis have yielded conflicting results. Under basal conditions, depending on the dose, species and sex, benzodiazepines inhibit or stimulate or have no effect on the HPA axis (Pohorecky et al., 1988; Kalogeras et al., 1990; De Boer et al., 1991; Owens et al., 1991; Wilson et al., 1996; Mikkelsen et al., 2005). Furthermore, both benzodiazepine agonists and inverse agonists can stimulate the HPA axis (Eisenberg and Johnson, 1989), and these effects are blocked by the benzodiazepine receptor antagonist flumazenil (De Boer et al., 1991). In general, low doses of benzodiazepines decrease, whereas high doses increase plasma corticosterone levels in rats (De Souza, 1990). Mikkelsen et al. (2005) found that the nonbenzodiazepine benzodiazepine receptor agonists zolpidem and zopiclone produced dose-dependent increases in plasma levels of corticosterone, and low dose decreases in plasma corticosterone levels were not found. Similarly, in the present study the acute administration

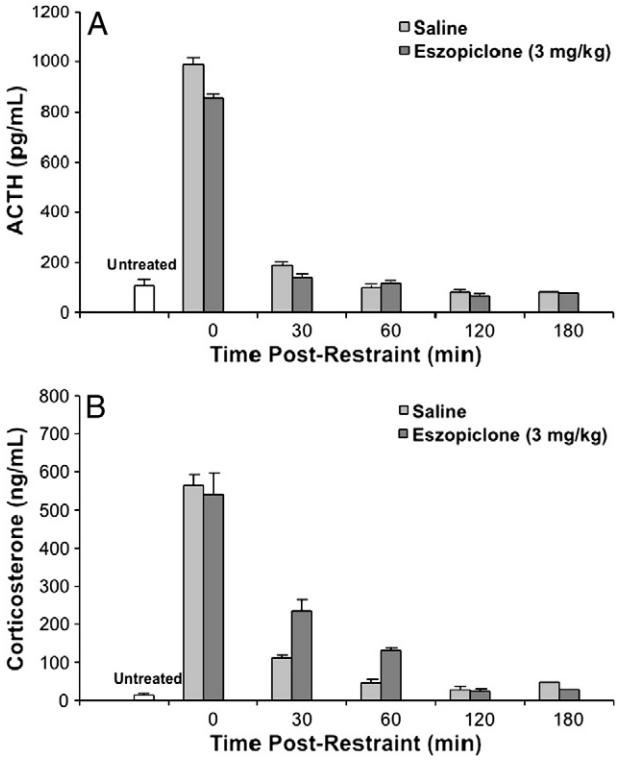

Fig. 3. Effects of pretreatment with eszopiclone on plasma levels of ACTH (A) and corticosterone (B) after restraint stress. Rats were pretreated with saline (0.9%) or eszopiclone (3.0 mg/kg/i.p.), and 30 min later they were placed into Plexiglas restraint tubes for 30 min. Groups of rats were sacrificed immediately after removal from the tubes (0) or 30, 60, 120 or 180 min later. The untreated group received neither injections nor restraint stress. Values are expressed as the means + the standard errors of the mean ($n = 8$–10 per group).

of eszopiclone produced only stimulatory effects on the HPA axis. Thus, there is a fundamental difference in the dose–response effect of the benzodiazepines and the nonbenzodiazepine benzodiazepine receptor agonists on the HPA axis.

The results of the present study also demonstrate that tolerance develops to the stimulatory effects of eszopiclone on the HPA axis after repeated administration. This is in contrast to the lack of evidence of tolerance to the hypnotic effects of eszopiclone in humans (Krystal et al., 2003; Sanger, 2004; Zammit et al., 2004; Roth et al., 2005). In the rat benzodiazepine withdrawal is accompanied by increases in plasma levels of ACTH and corticosterone (Eisenberg, 1987; Eisenberg and Johnson, 1989; Owens et al., 1991). The finding that plasma levels of ACTH and corticosterone were not elevated in the rats that received repeated eszopiclone and acute saline suggests that they were not undergoing withdrawal 24 h after the last eszopiclone injection. It is possible that withdrawal induced increases in plasma ACTH and corticosterone might have been observed at earlier or later time points; however, clinical studies have found no evidence of rebound insomnia after long-term treatment with eszopiclone (Zammit et al., 2004; Roth et al., 2005).

In the present study acute treatment with eszopiclone had no effect on the increase in plasma levels of ACTH and corticosterone produced by restraint stress. Benzodiazepines reduce stress-induced activation of the HPA axis (Grandison, 1983; Pohorecky et al., 1988; De Souza, 1990); however, Lahti and Barshun (1975) found that low doses of benzodiazepines reduced, whereas higher doses increased stress-induced activation of the HPA axis. Because the dose of eszopiclone used in the stress study (3.0 mg/kg) stimulated that HPA axis when given alone (e.g., Fig. 1A and B), it is possible that lower doses might have reduced the HPA axis response to stress. It has been reported that the corticosterone response to stress is not reduced after chronic treatment with the benzodiazepine diazepam (Lahti and Barshun, 1975; Mazurkiewicz-Kwilecki and Baddoo, 1986), and this effect has been found to be sex dependent (Wilson et al., 1996).

The mechanism underlying the effects of eszopiclone and benzodiazepines on the HPA axis is not clear. The regulation of the HPA axis is very complex and involves numerous hierarchical circuits (Herman et al., 2003). CRH is a major physiological regulator of ACTH secretion from the anterior pituitary, and cell bodies of CRH neurons are found in the paraventricular nucleus of the hypothalamus. Studies have shown the benzodiazepines decrease central levels of CRH (Owens et al., 1991) and inhibit serotonin-induced CRH release in vitro (Kalogeras et al., 1990). However, zolpidem, zopiclone and diazepam all increase Fos expression in the nuclei in an area overlapping with the CRH containing subportion of the paraventricular nucleus (Mikkelsen et al., 2005). Mikkelsen et al. (2005) have hypothesized that these drugs act on at least two distinct $GABA_A$ receptors with at least two different α subunits. One is within the paraventricular nucleus and mediates the inhibitory effects on the HPA axis, whereas the other is outside of the nucleus, and disinhibits a local GABAergic input into paraventricular nucleus. Specifically, GABAergic projections from the medial amygdaloid nucleus and the lateral septum might be involved in the activation of the HPA axis (Cullinan et al., 2008). There are differences in the binding profiles (Hanson et al., 2008) and the electrophysiological properties of eszopiclone and zolpidem in thalamic neurons (Jia et al., 2009). However, the effects of eszopiclone on the HPA axis found in the present study are very similar to those of zolpidem, a selective α_1 subunit agonist (Mikkelsen et al., 2005). These results suggest that the stimulatory effects of eszopiclone on the HPA axis are mediated by the α_1 subunits.

In summary, the acute administration of eszopiclone stimulates the HPA axis in the rat, and tolerance develops to these effects after repeated drug administration. Pretreatment with eszopiclone does not affect stress-induced stimulation of the HPA axis. Although eszopiclone has a unique binding profile and modulates the $GABA_A$ receptor complex in a different manner compared to the benzodiazepines and other nonbenzodiazepine benzodiazepine receptor agonists, a common feature is that all of these drugs can stimulate the HPA axis in the rat. The benzodiazepines can inhibit the HPA axis at low doses, and reduce stress-induced activation of the HPA axis, whereas this is not the case with the nonbenzodiazepine benzodiazepine receptor agonists such as eszopiclone. The clinical relevance of this finding is that although the nonbenzodiazepine benzodiazepine receptor might be effective hypnotic agents, they might have limited utility in the treatment of anxiety disorders.

Acknowledgments

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