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Acute zolpidem administration produces pharmacodynamic and receptor occupancy changes at similar doses

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Abstract

Zolpidem is chemically unrelated to classical benzodiazepines but has demonstrated relatively high affinity binding to the α_1 $GABA_A$ receptor. To assess pharmacodynamic and neurochemical effects of zolpidem, open-field behavior, pentylenetetrazole-induced seizure threshold and benzodiazepine receptor binding in vitro were evaluated in the same animal following a single dose of zolpidem. Zolpidem (2, 5 and 10 mg/kg), lorazepam (2 mg/kg) or vehicle was administered intraperitoneally in male CD-1 mice. Behavioral activity, assessed by three open-field parameters, was decreased following the two highest doses of zolpidem (5 and 10 mg/kg), and reached significance at the 10 mg/kg dose. Locomotor activity was also decreased significantly by lorazepam as expected. Pentylenetetrazole-induced seizure threshold was increased with the administration of 2 and 10 mg/kg zolpidem as well as with lorazepam. Apparent affinity (K_D) of [^{3}H]flunitrazepam, a non-selective ligand, for the benzodiazepine receptor in cortical membrane preparations was not significantly changed, while receptor number (B_{max}) was decreased at all doses of zolpidem, reaching significance at the 10 mg/kg dose. These results confirm that the behavioral effects of zolpidem are similar to those of classical benzodiazepines. In addition, zolpidem had no significant effect on the affinity of the benzodiazepine receptor for [^{3}H]flunitrazepam, but did decrease the density of receptor binding sites.

Keywords: Behavior; Benzodiazepine; GABA; Zolpidem; Pentylenetetrazole-induced seizures

1. Introduction

Introduced into clinical practice in the United States in 1992, zolpidem is now the most commonly prescribed hypnotic due to its clinical efficacy, safety, ability to be well tolerated and favorable pharmacokinetic profile (Langtry and Benfield, 1990; Rush, 1998). Although structurally unrelated to benzodiazepines, the imidazopyridine zolpidem produces its effects at the benzodiazepine binding site on the $GABA_A$ receptor (Sanger et al., 1994). Zolpidem binds to the benzodiazepine-$GABA_A$ receptor complex with an affinity that depends on α subunit composition (Ruano et al., 1992). Three zolpidem binding sites have been demonstrated in the adult rat brain: a high affinity site on α_1-containing $GABA_A$ receptors (K_i=20 nM), a low affinity site on α_2 and α_3-containing $GABA_A$ receptors (K_i=400 nM) and a very low affinity site on α_5-containing $GABA_A$ receptors (K_i>5000 nM) (Benavides et al., 1993; Langer et al., 1992; McKernan et al., 1991; Mertens et al., 1993; Pritchett and Seeburg, 1990; Ruano et al., 1992). This is in direct contrast to classical benzodiazepines which bind all $GABA_A$ receptors with similar affinity and are, therefore, non-selective (Langer and Arbilla, 1988).

There are few studies to date which have examined the sedative and anticonvulsant effects of acute zolpidem treatment in the same animal. Several investigators have separately established that acute administration of the imidazopyridines produces decreased locomotor activity. Sanger et al. (1986) demonstrated that acute administration of zolpidem decreased locomotor activity in mice at doses ranging from 0.25 to 2 mg/kg. Elliot and White (2001) also demonstrated a sedative effect of zolpidem in rats at doses of 5 and 10 mg/kg. Other investigators have examined both sedative and anticonvulsant effects of

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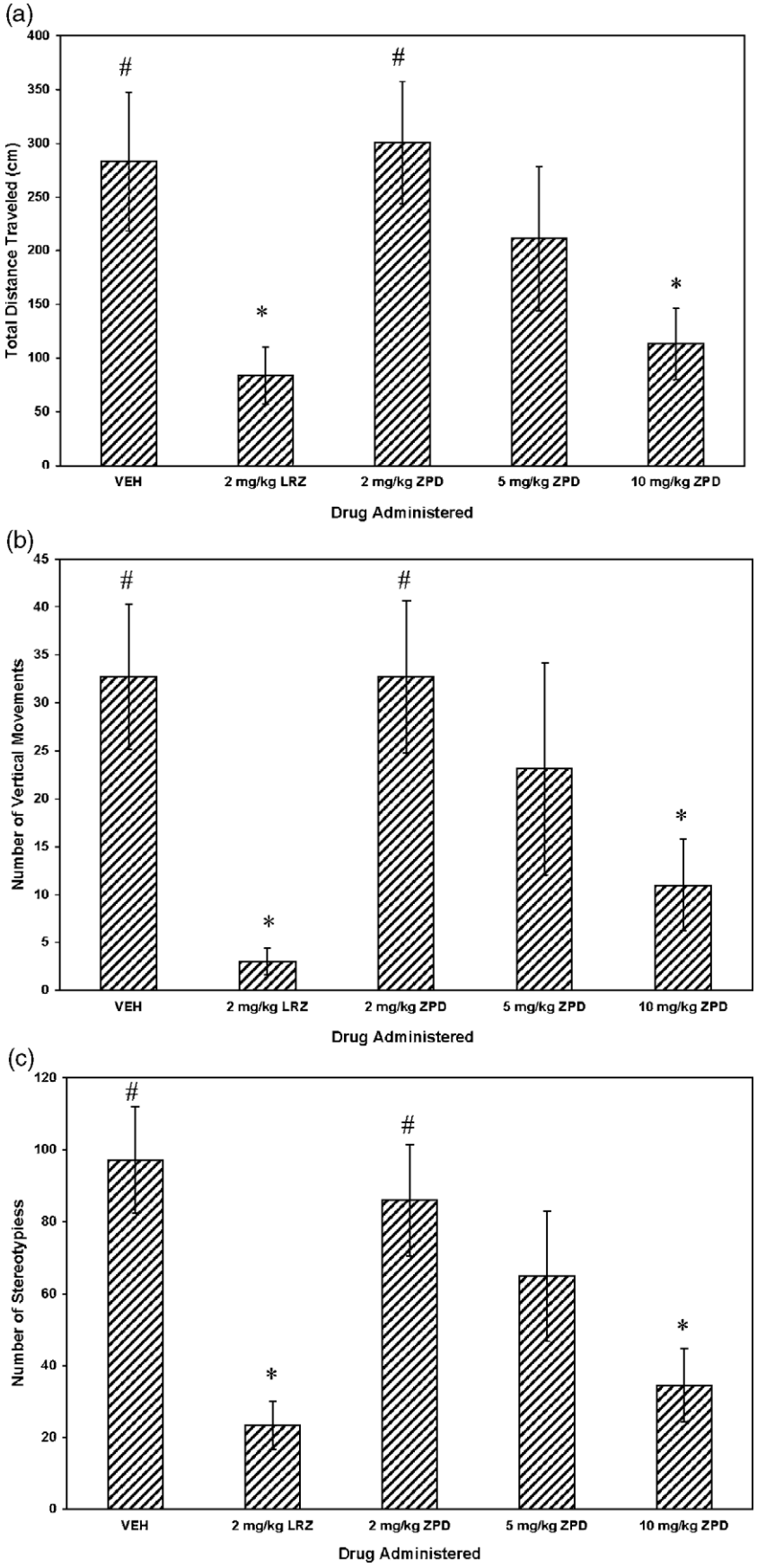
(a)
Total Distance Traveled (cm)
400
350
300
250
200
150
100
50
0
#
#
*
*
VEH
2 mg/kg LRZ
2 mg/kg ZPD
5 mg/kg ZPD
10 mg/kg ZPD
Drug Administered
(b)
Number of Vertical Movements
45
40
35
30
25
20
15
10
5
0
#
#
*
*
VEH
2 mg/kg LRZ
2 mg/kg ZPD
5 mg/kg ZPD
10 mg/kg ZPD
Drug Administered
(c)
Number of Stereotypiess
120
100
80
60
40
20
0
#
#
*
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VEH
2 mg/kg LRZ
2 mg/kg ZPD
5 mg/kg ZPD
10 mg/kg ZPD
Drug Administered

zolpidem in rodents but not in the same animal. For example, Griebel et al. (1999) examined both the locomotor and anticonvulsant effect of zolpidem but in different strains of mice. Locomotion decreased in CD1 mice while latency to pentylenetetrazole seizures was increased in OF1 mice. In a study by Davies et al. (1994), zolpidem greatly decreased locomotor activity but had no effect on pentylenetetrazole-induced seizures. Sanger et al. (1996) looked at these behavioral parameters in CD-1 mice but not in the same animal. They demonstrated a sedative and anticonvulsant effect of zolpidem, confirming earlier studies by Perrault et al. (1990) and Depoortere et al. (1986) which found that sedation occurs at a zolpidem dose much lower than that which produces anticonvulsant and myorelaxant effects respectively.

The difference in the dose of zolpidem needed for sedative and anticonvulsive effects found in the above studies may be the result of interstrain or interanimal variability. It is probable that, like classical benzodiazepines, a similar dose of zolpidem will produce both effects in an individual animal. No studies to date have correlated the sedative and anticonvulsant effects of zolpidem. To test this hypothesis, we examined open-field activity, pentylenetetrazole-induced seizure threshold as well as benzodiazepine binding in vitro following acute zolpidem administration in the same CD-1 mice. This will allow us to relate the behavioral effects of zolpidem to any benzodiazepine receptor changes.

2. Materials and methods

2.1. Materials

All experimental procedures employed in the present study were approved by the Tufts University Animal Research Committee and are in accordance with the National Institutes of Health Guide for the Use and Care of Laboratory Animals (NIH Publications No. 80-23, revised 1996). Male CD-1 mice, 6 to 8 weeks of age (Charles River Laboratories, Wilmington, MA), were maintained on a 12 h light/dark cycle and given food and water ad libitum. [^{3}H]flunitrazepam (specific activity 71 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Zolpidem was generously donated by its pharmaceutical manufacturer (Lorex Pharmaceuticals, Chicago, IL).

2.2. Drug administration

Zolpidem (2, 5 or 10 mg/kg) or lorazepam (2 mg/kg) was dissolved in polyethylene glycol (PEG) 400 and injected intraperitoneally in a volume of 0.15 ml. Vehicle-treated mice received PEG 400 alone.

2.3. Open-field activity

Fifteen minutes following drug administration, distance traveled, rears and stereotypy were assessed in 5 min intervals for 20 min in an Omnitech Digiscan apparatus (Columbus, OH) which measures a variety of activity measures using photocell beams. Prior experience indicates that the measures of activity that are most sensitive and replicable are: total horizontal distance, vertical rears, and stereotypy (Fahey et al., 2001, 1998, 1999). Averages of each parameter for the total 20 min test period were computed. Between each test, the interior of the activity chamber was cleaned with 70% ethanol and dried. All testing occurred between 9:00 a.m. and 12:00 p.m. The number of animals per group is as follows: VEH ($n=8$); 2 mg/kg LRZ ($n=7$); 2 mg/kg ZPD ($n=9$); 5 mg/kg ZPD ($n=7$) and 10 mg/kg ZPD ($n=8$).

2.4. Pentylenetetrazole-induced seizure threshold

Immediately following removal from the open-field activity monitor, dorsal tail veins in the mice were cannulated with a 25 g × 3/8″ butterfly needle. Mice were allowed to roam freely while pentylenetetrazole (7.5 mg/ml) was infused at a rate of 0.6 ml/min (dissolved in physiologic saline) until induction of a full tonic–clonic seizure as determined by two trained observers (Fahey et al., 2001, 1998, 1999). Several animals in each group did not seize due to improper cannulation of the tail vein and were omitted from the data analysis of the seizure threshold data only. The number of animals per group is as follows: VEH ($n=5$); 2 mg/kg LRZ ($n=4$); 2 mg/kg ZPD ($n=6$); 5 mg/kg ZPD ($n=6$) and 10 mg/kg ZPD ($n=6$).

2.5. [^{3}H]flunitrazepam binding

Benzodiazepine binding in vitro was performed in mouse cortical synaptosomes (P_2) as previously described using [^{3}H]flunitrazepam (Miller et al., 1988). Immediately following seizure induction, animals were sacrificed by cervical dislocation and brain regions were dissected apart, weighed, and placed in 30 volumes of 0.32 M sucrose. Tissues were mechanically homogenized, and the homogenate centrifuged at 1000×g for 10 min. Pellets were discarded and supernatants recentrifuged at 48,000×g at 4 °C for 20 min, then resuspended in 50 volumes of 50 mM Tris–HCl buffer (pH 7.4). The washing procedure was repeated 3 times, the pellets were resuspended in Tris–HCl, and stored at −70 °C. At the time of assay, membrane preparations were thawed, resuspended, and washed again. Benzodiazepine receptor binding was determined by addition of brain membranes (0.1 ml, containing approximately 50 μg of protein) to a series of tubes containing various concentrations of

Fig. 1. Effect of acute zolpidem administration on open field activity. Data from a) distance traveled, b) number of rears and c) number of stereotypies was recorded at 5 min intervals for a total of 20 min. Zolpidem (2, 5 or 10 mg/kg) or vehicle was administered intraperitoneally 15 min prior to behavioral assessment. The number of animals per group is as follows: VEH ($n=8$); 2 mg/kg LRZ ($n=7$); 2 mg/kg ZPD ($n=9$); 5 mg/kg ZPD ($n=7$) and 10 mg/kg ZPD ($n=8$). Averages for each parameter were computed for the entire test period. Bars represent mean response±S.E.M. Significant differences from vehicle or LRZ are indicated by * or # ($p<0.05$) respectively as determined by analysis of variance or Kruskal–Wallis analysis of variance on ranks followed by Dunnett's or Dunn's posthoc tests.

[^{3}H]flunitrazepam ranging from 0.1 to 10 nM in the presence (non-specific binding) or absence (total binding) of a saturating concentration of 10^{-5} M flumazenil. Tris–HCl buffer was added to all tubes to achieve a final volume of 0.5 ml. After incubation for 45 min at 4 °C, membranes were vacuum filtered using a Brandel Cell Harvester (M48R) onto Whatman GF/B filters and washed with ice-cold buffer. Filters were counted by conventional scintillation spectrometry. Standard analyses of binding data using nonlinear regression was used to determine the number of binding sites (B_{max}) and the apparent affinity (K_D) using Graphpad Prism v 4.0 (Graphpad Software, San Diego, CA). Synaptosomes were not made from the cortical tissue of animals that died following seizure and, thus, they were omitted from the data analysis of the binding data only. Animals that did not seize were included in the analysis of the binding data. The number of animals per group is as follows: VEH ($n=7$); 2 mg/kg ZPD ($n=8$); 5 mg/kg ZPD ($n=6$) and 10 mg/kg ZPD ($n=6$).

2.6. Data analysis

Data from binding studies were analyzed using the GraphPad PRISM software version 4.0 (GraphPad Software, San Diego, CA). Comparisons between groups were performed using analysis of variance or Kruskal–Wallis analysis of variance on ranks with Dunnett's or Dunn's posthoc tests respectively. Data presented are mean ± S.E.M.

3. Results

3.1. Open-field activity

As expected, lorazepam significantly decreased locomotor activity compared to vehicle control ($p<0.05$). A significant decrease in total distance traveled, total number of rears and total number of stereotypies was observed following treatment with 10 mg/kg zolpidem ($p<0.05$). There was no significant difference between the lower two doses of zolpidem and vehicle-treated animals on any of the three behavioral parameters examined (Fig. 1), although distance traveled in the 5 mg/kg group was lower than the vehicle group. The lowest dose of zolpidem was significantly different from lorazepam on all of the open field parameters ($p<0.05$). Analysis of the data by 5 min increments determined that there was no significant effect of the 2 or 5 mg/kg dose of zolpidem compared to vehicle at any interval during the monitoring period on any open field parameter (data not shown). The highest zolpidem dose (10 mg/kg) significantly decreased all three parameters compared to vehicle during the initial three 5 min intervals. During the last 5 min of monitoring (16–20 min), this dose of zolpidem (10 mg/kg) increased activity back to its first interval level (0–5 min), which was not different from vehicle treated animals during the same monitoring period (16–20 min).

3.2. Pentylenetetrazole-induced seizure threshold

There was a significant increase ($p<0.05$) in the amount of pentylenetetrazole needed to induce a tonic–clonic seizure following administration of both 2 and 10 mg/kg zolpidem as well as lorazepam when compared to vehicle controls (Fig. 2). Seizure threshold of the two lowest doses of zolpidem was significantly different from lorazepam ($p<0.05$).

3.3. Benzodiazepine binding in vitro

There was no significant difference in K_D values for [^{3}H]flunitrazepam following zolpidem administration at the two lowest doses (Fig. 3), although apparent affinity was

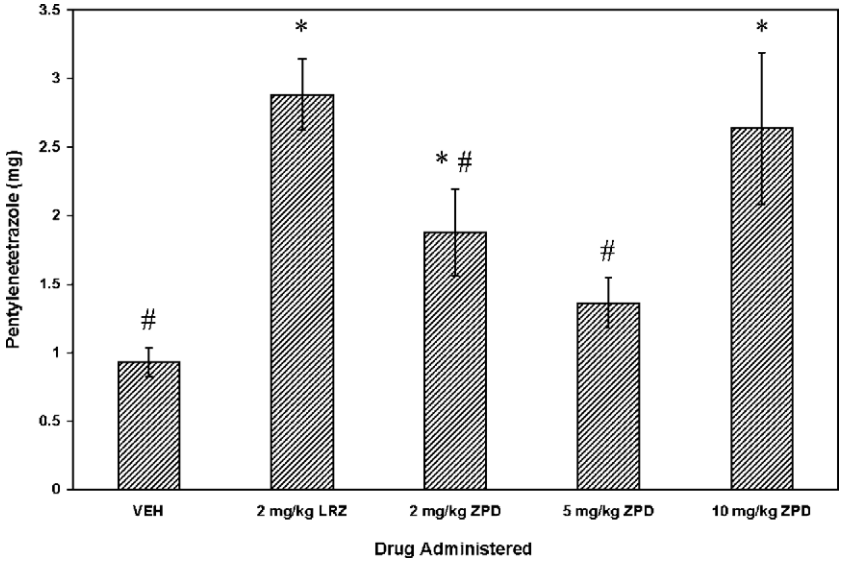

Fig. 2. Effect of acute zolpidem administration on seizure protection. Zolpidem (2, 5 or 10 mg/kg) or vehicle was administered intraperitoneally 15 min prior to behavioral assessment. Immediately following removal from the open-field activity monitor, pentylenetetrazole (7.5 mg/ml) was infused via the tail vein at a rate of 0.6 ml/min until induction of a full tonic–clonic seizure. The number of animals per group is as follows: VEH ($n=5$); 2 mg/kg LRZ ($n=4$); 2 mg/kg ZPD ($n=6$); 5 mg/kg ZPD ($n=6$) and 10 mg/kg ZPD ($n=6$). Bars represent mean response ± S.E.M. Significant differences from vehicle or LRZ are indicated by * or # ($p<0.05$) respectively as determined by analysis of variance or Kruskal–Wallis analysis of variance on ranks followed by Dunnett's or Dunn's posthoc tests.

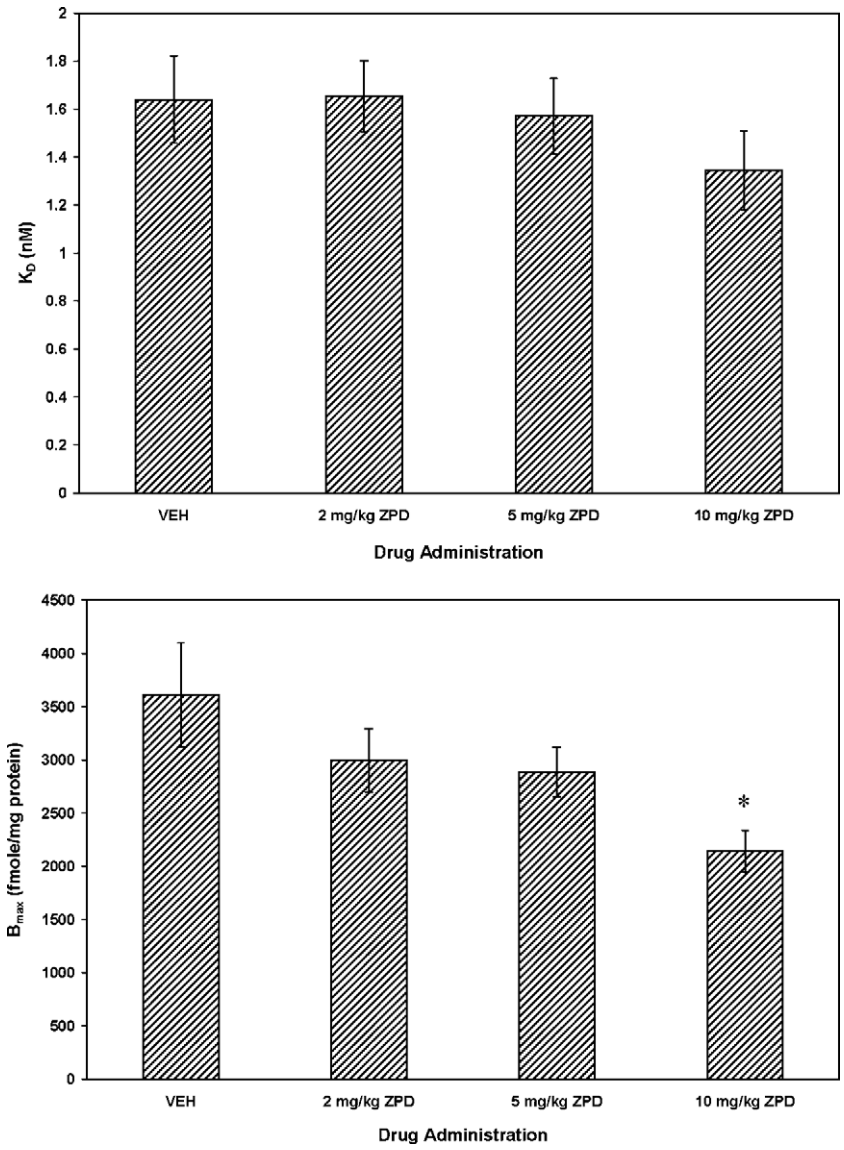

Fig. 3. Benzodiazepine receptor binding in cortex following acute zolpidem administration. Zolpidem (2, 5 or 10 mg/kg) or vehicle was administered intraperitoneally 40 min prior to sacrifice. The number of animals per group is as follows: VEH ($n=7$); 2 mg/kg ZPD ($n=8$); 5 mg/kg ZPD ($n=6$) and 10 mg/kg ZPD ($n=6$). Benzodiazepine binding was performed in cortical synaptosomal membranes (P_2) using [^{3}H]flunitrazepam. Bars represent mean values ± S.E.M. Significant differences from vehicle are indicated by * ($p<0.05$) as determined by analysis of variance followed by Dunnett's posthoc tests.

changed in the 10 mg/kg group. A similar comparison demonstrated a decrease in the B_{max} of [^{3}H]flunitrazepam binding to the cerebral cortex at all doses of zolpidem, reaching significance at 10 mg/kg zolpidem when compared to vehicle-treated animals ($p<0.05$).

4. Discussion

In the current study, we have presented data from open-field activity, pentylenetetrazole-induced seizure threshold and benzodiazepine binding in vitro following acute zolpidem administration in the same CD-1 mice. As expected, lorazepam and zolpidem significantly decreased all three open-field parameters at the highest dose administered. There was a dose-dependent decrease in total distance traveled, number of vertical movements and number of stereotypes with zolpidem, although the lowest two doses were not different statistically from vehicle treated animals. The two highest doses of zolpidem were as efficacious in producing sedation as the classical benzodiazepine lorazepam. These results confirm the sedative effect seen in a number of prior studies of acute zolpidem administration (Davies, 1994; Depoortere, 1986; Elliot, 2001; Griebel, 1999; Perrault, 1990; Sanger, 1986, 1996).

Lorazepam and zolpidem increased pentylenetetrazole-induced seizure threshold, which paralleled alterations in open-field activity. The highest dose of zolpidem appeared to have similar efficacy as an anticonvulsive compared to lorazepam. These data confirm Griebel et al. (1999) who found an increased latency to pentylenetetrazole seizures in OF1 mice.

Depoortere et al. (1986) and Sanger et al. (1996) also demonstrated an increased latency to seizure in mice treated acutely with zolpidem. However, both of these studies found that the dose of zolpidem needed to produce sedation was much lower than that needed to produce its anticonvulsive effects. In the present study, the doses needed to produce each of these effects were the same. We found no selective effect of zolpidem for sedation over seizure protection. However, an anticonvulsant effect of zolpidem was observed at 2 mg/kg which was likely due to one animal that needed twice the average dose of pentylenetetrazole to seize. In addition, unlike the highest zolpidem dose, this dose was not as efficacious at preventing seizures as the classical benzodiazepine lorazepam. While pentylenetetrazole was used in all three studies, we measured the amount of pentylenetetrazole that was needed to induce a tonic–clonic seizure in a freely moving mouse with continuous i.v. infusion. Most other pentylenetetrazole studies use a subcutaneous injection (125 mg/kg) and measure latency to seizure. In addition, as was stated in the introduction, previous studies did not examine both locomotor activity and seizure protection in the same animal as we did in the present study. Our results directly contradict Davies et al. (1994) who found no anticonvulsant effect of acute zolpidem administration.

The differential effects of ligands which act at the benzodiazepine receptor are postulated to be mediated by specific receptor subtypes which differ in their subunit composition and regional distribution in the brain (McKernan et al., 2000; Rudolph et al., 1999). In these studies which employed α_1 H101R mice, the sedative action of diazepam was attributed to the α_1 subtype of the GABA receptor. In addition, the anticonvusant effect of the classical benzodiazepine was partially attributed to the same receptor subtype. Crestani et al. (2000) used the same animal model, but not the same individual animal, to demonstrate that the sedative and anticonvulsant actions of zolpidem (60 mg/kg) were exclusively mediated by the α_1 subtype of GABA receptor. The results of the present study, which demonstrate a sedative and an anticonvulsant effect of the α_1 selective ligand zolpidem at the same dose in the same animal, provide additional evidence that these two behavioral effects may be mediated via the same subtype of $GABA_A$ receptor.

Acute administration of zolpidem at 10 mg/kg produced a significant decrease in the number of [^{3}H]flunitrazepam binding sites in the cerebral cortex. There was no significant effect of any dose of zolpidem, however, on the affinity of [^{3}H]flunitrazepam for the benzodiazepine receptor. The present K_D and B_{max} (1.64 nM and 3612.38 fmol/mg respectively) are in line with published values from saturation binding experiments in mouse brain (Dalezios and Matsokis, 1998; Lopes et al., 2004; Tehrani and Barnes, 1997). Chronic administration of benzodiazepines is known to result in receptor downregulation manifested as a decrease in receptor density, but it was unknown whether a change in the number of or apparent affinity to total central benzodiazepine receptors would occur so quickly after administration of a single dose of zolpidem. While this decrease in binding sites could be a spurious reduction in binding due to competition by residual zolpidem, this is unlikely due to the number of washes performed in the synaptosomal preparation. In addition, the apparent affinity of the receptor was not changed. Downregulation of the benzodiazepine receptor following acute zolpidem treatment suggests the degradation of the receptor since [^{3}H]flunitrazepam can penetrate membranes and would detect internalized receptors as well. It is also possible that the decrease in benzodiazepine binding sites is selective for the α_1 $GABA_A$ receptor due to substitution of the α_1 subunit by one of the other α subunits. Binding assays using other benzodiazepine radioligands such as [^{3}H]muscimol or [^{35}S]TBPS would help to determine if the number of other benzodiazepine sites remained unchanged. Since the saturation binding studies were completed using [^{3}H]flunitrazepam which binds equally well to both subtypes of benzodiazepine receptors, it is not possible to state which receptor subtype was downregulated. However, based on the knowledge that zolpidem selectively binds to α_1 $GABA_A$ receptors and the relative abundance of these receptors in the cortex, it is hypothesized that the majority of downregulated receptors are α_1 $GABA_A$. The change in receptor number did not alter the affinity of [^{3}H]flunitrazepam for the remaining receptors, which was to be expected since classical benzodiazepines bind to all $GABA_A$ receptor subtypes with equal affinity (Braestrup et al., 1977; Möhler and Okada, 1977).

The present results confirm that the selective α_1 $GABA_A$ receptor ligand zolpidem exhibits sedative and anticonvulsant effects similar to those of classical benzodiazepines at a clinically relevant dose. In addition, these novel results from the same animal demonstrate that the behavioral effects of acute exposure to zolpidem are coupled to a significant downregulation of benzodiazepine receptors in the brain without changing affinity for the remaining receptors.

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