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Zolpidem-induced changes in activity, metabolism, and anxiety in rats

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ABSTRACT

Gamma aminobutyric acid (GABA)-A receptor modulators constitute the majority of clinically relevant sedative-hypnotics. Zolpidem (Ambien) is a nonbenzodiazepine GABA-A receptor modulator that binds with high affinity to GABA-A receptors expressing alpha-1 subunits. The present study examined the effects of a new approach to the oral administration of zolpidem on locomotor activity, body weight, food intake, relative food intake, feed efficiency, anxiety, and visceral adiposity in rats. Effects of withdrawal associated with cessation of the drug were also recorded. A daily chronically administered oral 10 mg/kg dose of zolpidem caused a decrease in locomotor activity, an increase in food intake and relative food intake, and a more positive feed efficiency during the drug-administration period. Anxiety and visceral adiposity also increased in animals receiving the drug. During withdrawal of zolpidem, there was a decrease in body weight, food intake, relative food intake, and anxiety, as well as a negative feed efficiency. These results suggest that zolpidem can modulate locomotor activity, metabolism, and anxiety-related behavior. A highly positive feed efficiency and increased visceral adiposity associated with zolpidem intake were unique findings of this study.
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1. Introduction

Benzodiazepines have been widely used clinically as sedative/hypnotics, anxiolytics, and muscle relaxants. However, their long-term use has been questioned because of adverse memory effects, addictive properties and development of tolerance. Benzodiazepines are considered as nonselective gamma-aminobutyric acid (GABA)-A modulators since they show affinity for GABA-A receptors containing alpha-1, alpha-2, alpha-3, or alpha-5 subunits (Möhler et al., 2002). DaSettimo et al. (2007) proposed that the alpha-1 subunit is responsible for sedative action; whereas, the alpha-2 and/or alpha-3 subunits mediate anxiolytic activity and myorelaxation effects. In 2005, Cooper predicted that the alpha-2 and alpha-3 subunits are also responsible for hyperphagia induced by benzodiazepines and, in 2009, Morris et al. concluded, in a study with mutant mice, that the hyperphagic effects are mediated by modulation of the alpha-3 subunit.

Zolpidem (Ambien) is a nonbenzodiazepine drug commonly used for the treatment of insomnia. It has been shown to have observable sedative and hypnotic effects in humans (Nicholson and Pascoe, 1986) and animals (Sanger and Zivkovic, 1988). As a hypnotic drug, it works by slowing activity in the brain to allow sleep, and it is used in the temporary treatment of insomnia characterized by difficulties with sleep initiation (Nicholson and Pascoe, 1986; Wilson and Nutt, 2007). Zolpidem belongs to the imidazopyridine class that is an agonist at the benzodiazepine receptor binding site of the GABA-A receptor complex (Holm and Goa, 2000). The sedative/hypnotic pharmacological effects of zolpidem are selectively mediated by the alpha-1 subunit of the GABA-A receptor complex, resulting in distinct clinical effects (Langtry and Benfield, 1990). An in vivo study utilizing mice demonstrated that the sedative/hypnotic and anticonvulsant activities of zolpidem are due to its action at the alpha-1 GABA-A receptor subunit (Crestani et al., 2000). Furthermore, when tested on recombinant receptors, zolpidem displayed a high potency at the alpha-1 GABA-A receptor subunit (Langer et al., 1992). Crestani et al. (2000) showed that zolpidem had a reduced affinity for the alpha-2 and alpha-3 subunits and a lack of interaction with the alpha-5 subunit.

Owing to its clinical efficacy, safety, and favorable pharmacokinetic and pharmacodynamic profiles, it is apparent why zolpidem is one of the most commonly prescribed hypnotic drugs (Wilson and Nutt, 2007; Salvà and Costa, 1995). However, problems associated with zolpidem use have emerged. Renger et al. (2004) reported that zolpidem can alter sleep architecture in rats and mice. In humans, case reports of zolpidem-induced somnambulism, nocturnal eating, sleep-driving, amnesia, and visual hallucinations have been published (Doane and Dalpiaz, 2008; Sansone and Sansone, 2008). In light of these reports, in 2007, the Food and Drug Administration mandated that the manufacturers of zolpidem provide an educational guideline concerning the potential risks of the drug to each prescription recipient (Sansone and Sansone, 2008).

Turning to the role of zolpidem on food intake, data are inadequate and conflicting. Sanger and Zivkovic (1988) showed that zolpidem did not cause a reliable increase in food intake in rats. In a study by Yerbury and Cooper (1989), zolpidem was given prior to presentation of a palatable diet. No effect on food intake was noted in either presatiated or

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standard conditions. On the other hand, Stanhope et al. (1993) proposed that palatability of food could account for an increase in food consumption by rats receiving zolpidem.

Studies on the anxiolytic or anxiogenic effects of zolpidem are inconsistent. Utilizing the elevated plus maze (EPM), Cui et al. (2007) showed that zolpidem had an anxiolytic effect in mice. A study with rats by Davies et al. (1994) indicated that a low dose of zolpidem increased time spent in the open arms of an EPM, but higher doses decreased time spent in the open arms and increased time spent in the closed arms of the EPM. In a study on the acute administration of zolpidem, Gonzalez-Pardo et al. (2006) reported that rats could not be evaluated on the EPM due to the highly sedative effects of the dose employed.

The present research was designed to investigate the effects of zolpidem on locomotor activity, body weight, food intake, relative food intake, feed efficiency, anxiety, and visceral adiposity in rats. These parameters were studied utilizing the following: 1) a new approach to the oral administration of zolpidem in a "treat" (sweet condensed milk) that is consumed completely in a short period of time, 2) chronic rather than acute administration of zolpidem, 3) use of the EPM during the dark phase of the light/dark cycle, and 4) use of the concepts of relative food intake and feed efficiency in addition to body weight and food intake as indicators of metabolism. Effects of withdrawal associated with cessation of the drug were also recorded.

2. Materials and methods

All procedures were approved by the local Institutional Animal Care and Use Committee and followed the National Institute of Health Guide for the Care and Use of Laboratory Animals. The experiment was conducted with 12 male Long Evans rats (Harlan, Inc., Indianapolis, IN), 10 weeks old and weighing approximately 325 g at the beginning of the recorded habituated period. Rats were housed individually in cages equipped with a running wheel and kept on a 12-hour light/12-hour dark cycle under constant room temperature (21 ± 1 °C). The cages were cleaned daily at the beginning of the dark cycle. At this time, the remaining food and water (from the previous day) were removed from each cage and measured. Each rat was then taken from its home cage, placed in a weighing chamber, weighed, and returned to its home cage. The rats were fed a standard laboratory diet, Rodent Diet #5001 (PMW International, LLC, Brentwood, MO) and given 500 μL of a sweet condensed milk "treat" in a small, shallow, glass container for a 15-minute period before new food and water were presented. Within three days, all animals completely consumed the "treat" during the 15-minute period. At the conclusion of the 15-minute period, all containers were removed from the cages. Both food and water were provided ad libitum throughout the experiment.

Following the habituation period, the rats were divided into two groups. Six rats were given 10 mg/kg of zolpidem (Sanofi-Aventis, Bridgewater, NJ) dissolved in distilled water (vehicle) in a volume of 1 ml/kg daily in the condensed milk and were designated as the Z group (experimental group). The 6 control (C) rats received the vehicle with no drug in the condensed milk. The experimental period lasted for 3 weeks, utilizing the same procedures that were employed in the habituation period. A 3-day withdrawal period ensued with the continuation of presentation of condensed milk to both groups.

The individual cages were equipped with a running wheel (MiniMitter, Bend, OR), which continually monitored locomotor activity via a magnetic switch which sent data to a multiplexer. The multiplexer, in turn, relayed information to a computer for storage. Vital View (MiniMitter, Bend, OR) was utilized for recording activity data.

The EPM (Kinder Scientific, Poway, CA) was employed to measure the behavioral response of anxiety for C and Z animals during three different periods of the study (habituation, drug administration, and drug withdrawal). The rat maze overall dimensions were 44″ wide by 44″ deep by 33.5 ″ tall. The arena dimensions were: each arm — 4.25″ wide and 19.75″ long; intersection — 4.25″ by 4.25″; and closed walls — 5.75″ high. The maze was elevated 33.5″ above the floor. Individual rats were exposed to a 5-minute test in each period in the EPM and the responses were recorded and sent to a personal computer for storage. Testing trials, which took place two hours into the dark cycle, occurred 2 days before termination of the habituation period, 2 days before termination of the experimental period, and 2 days after the removal of the drug (withdrawal period). Rats were placed in the center of the EPM and the amount of time spent in the open and closed arms was monitored. For all test sessions, the path of each rat was registered automatically by a computerized image analysis system. After each subject was tested, the EPM was wiped clean with a wet (water) sponge and dried.

At the termination of the experiment, all of the animals were sacrificed and each rat was examined for visceral adiposity (inguinal, renal, and mesenteric fats). By use of visual observation, two people previously trained in making comparative adiposity determinations and who were not involved in this experimental procedure, assessed visceral adiposity. A subjective scale of 0 through 4 was employed to quantify the amount of adipose tissue surrounding the viscera (Wideman and Murphy, 2009). Numbers on the scale were: 0 — rat was considered emaciated with no visible fat; 1 — rat was considered normal with some fat deposition, but less than 2; 2 — rat had greater fat deposition than 1, but less than 3; 3 — rat had prominent amounts of fat; and 4 — rat was considered obese with excessive amounts of fat in all three areas studied. Utilizing representative rats in each category, observers of adiposity practiced the scoring method together and then separately until the scoring method for each observer was consistent with those of other observers. In the experiment, observers consistently used the same rating scale and percentage agreement was calculated.

Locomotor activity data were analyzed by an independent samples *t*-test comparing the percentage change in the mean number of wheel revolutions/24 h of C and Z animals from the last day of the habituation period (day 7) to the last day of the experimental period (day 28). Body weight, food intake, relative food intake (food intake divided by body weight × 100), and feed efficiency (change in body weight divided by food intake in the same time period) data from the last day of the habituation period, the last day of the experimental period, and the last day of the withdrawal period were analyzed utilizing a 2 (group: C vs. Z) × 3 (period) repeated measures ANOVA (SPSS). Post-hoc testing employed Bonferroni tests, and a p value of <0.05 was considered significant. The EPM data were analyzed from the maze schedule noted above utilizing the same statistical paradigms. The percentage of agreement between the two observers was calculated utilizing the formula: agreements / agreements + disagreements × 100% = P%. An independent samples *t*-test was employed for statistical analysis of visceral adiposity.

3. Results

Fig. 1 presents the peak number of running wheel revolutions in a 24 h period for one representative C and Z animal throughout the experiment. This figure shows that the C and Z animals were comparable in their peak number of wheel revolutions/day during the habituation period. However, a marked decrease in locomotor activity by Z animals was demonstrated during the experimental period with a return to the habituation level during the withdrawal period.

Fig. 2 illustrates the percentage change in running wheel activity from the last day of the habituation period to the last day of the experimental period comparing all C and Z animals. There was a statistically significant difference between C and Z animals [t (10) = 3.02, $p<0.05$] with a 3.5% drop in mean number of running wheel revolutions/24 h by C animals compared to a 25.9% drop by Z animals.

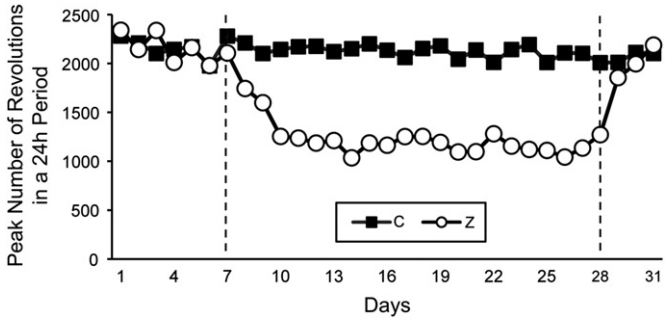

Fig. 1. Peak number of running wheel revolutions each day for one representative C and Z animal throughout the experiment (habituation period = days 1–7, experimental period = days 8–28, and withdrawal period = days 29–31).

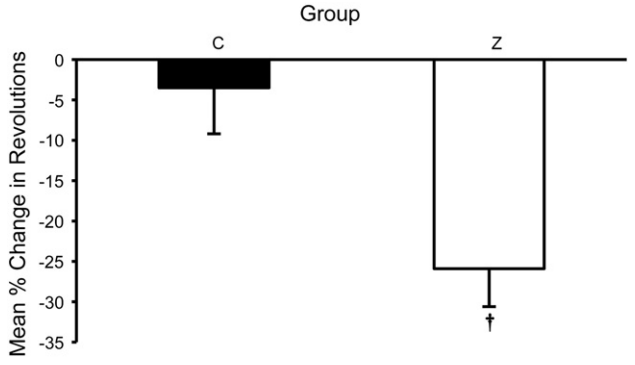

Fig. 2. Mean (+SEM) percentage change in number of wheel revolutions for C and Z rats from the last day of the habituation (H) period to the last day of the experimental (E) period. The † indicates that Z animal dropped significantly more than C animals in running wheel activity.

Fig. 3 presents the mean body weight for the two groups of rats for the last day of the habituation, experimental, and withdrawal periods. The ANOVA indicated a main effect for period [$F(2, 20) = 209.24$, $p<0.001$]; no main effect for group [$F(1, 10) = 0.00$, $p>0.05$]; and a significant interaction between period and group [$F(2, 20) = 12.91$, $p<0.001$]. Statistically, there were significant differences among the three periods. The body weights of both groups of animals were significantly higher during the experimental and withdrawal periods as compared to the habituation period, but there was no significant difference between the two groups during the habituation, experimental, or withdrawal periods. Concerning the interaction, Z animals exhibited a significant decrease in body weight from the end of the experimental period to the end of the withdrawal period.

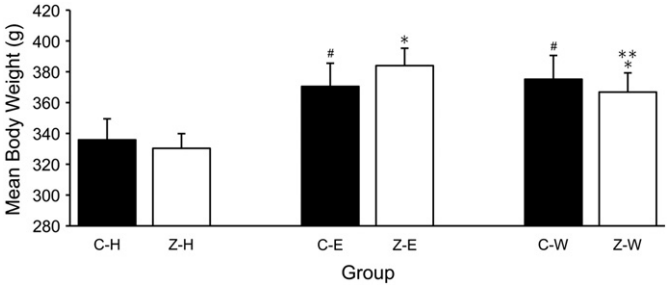

Fig. 3. Mean (+SEM) body weight for C and Z rats for the last day of the H period, the last day of the E period, and the last day of the withdrawal (W) period. The # indicates that the body weights of C animals were significantly higher during the E and W periods as compared to the H period. The * indicates that the body weights of Z animals were significantly higher during the E and W periods as compared to the H period. The ** indicates that Z animals showed a significant decrease in body weight between the E and W periods.

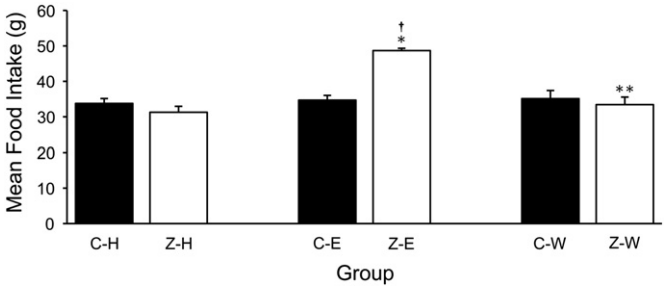

Fig. 4. Mean (+SEM) food intake for C and Z rats on the last day of the H, E, and W periods. The † indicates that Z animals ate significantly more food at the end of the E period compared with C animals. The * indicates that the Z animals ate significantly more food at the end of the E period compared to the end of the H period, and the ** shows that the Z animals ate significantly less food at the end of the W period than at the end of the E period.

Fig. 4 shows the mean food intake for the two groups of rats for the last day of the habituation, experimental, and withdrawal periods. The ANOVA indicated a main effect for period [$F(2, 20) = 33.36$, $p<0.001$]; no main effect for group [$F(1, 10) = 3.86$, $p>0.05$]; and a significant interaction between period and group [$F(2, 20) = 30.36$, $p<0.001$]. Statistically, there were significant differences among the three periods. Food consumption was comparable between the two groups during the habituation period when no zolpidem was administered to the animals. When zolpidem was administered, the Z group consumed significantly more rat chow than the C group. There was no significant difference between the two groups during withdrawal. The C animals showed no significant differences among the three periods; whereas, Z animals demonstrated a significant difference between the habituation and experimental periods, consuming more food during the experimental period. When the drug was removed during the withdrawal period, the Z animals significantly decreased their food intake to the level of that observed in the habituation period.

Fig. 5 presents the mean relative food intake for C and Z animals for the last day of the habituation, experimental, and withdrawal periods. The ANOVA demonstrated a main effect for period [$F(2, 20) = 6.52$, $p<0.01$]; no main effect for group [$F(1, 10) = 2.58$, $p>0.05$]; and a significant interaction between period and group [$F(2, 20) = 9.90$, $p = 0.001$]. Relative food intake was comparable between the two groups during the habituation period. When zolpidem was administered, the Z group had a significantly higher relative food intake than the C group. There was no significant difference between the two groups during withdrawal. The C animals showed no significant differences among the three periods; whereas, Z animals demonstrated a significant difference between the habituation and experimental periods, having a higher relative food intake in the experimental period. When the drug

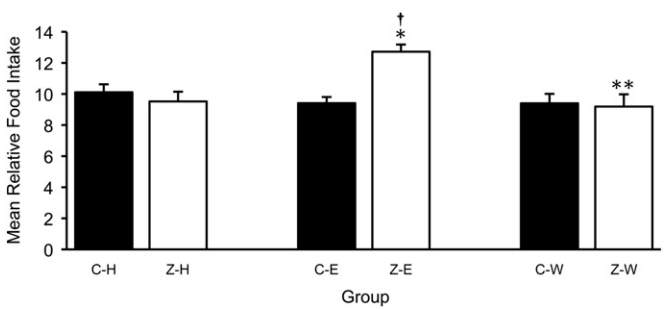

Fig. 5. Mean (+SEM) relative food intake for C and Z rats on the last day of the H, E, and W periods. The † indicates that Z animals had a significantly greater relative food intake value at the end of the E period than C animals. The * indicates that Z animals had a significantly greater relative food intake value at the end of the E period compared to the end of the H period and the ** shows that the Z animals had a significantly lower relative food intake at the end of the W period compared to the end of the E period.

was removed during the withdrawal period, the Z animals significantly decreased their relative food intake to the level of that of the habituation period.

Fig. 6 gives the mean feed efficiency for C and Z animals for the last day of the habituation, experimental, and withdrawal periods. The ANOVA showed a main effect for period [$F(2, 20) = 18.67$, $p<0.001$]; no main effect for group [$F(1, 10) = 2.23$, $p>0.05$]; and a significant interaction between period and group [$F(2, 20) = 15.29$, $p<0.001$]. Feed efficiency was comparable between the two groups during the habituation period. When zolpidem was administered, the Z group had a significantly more positive feed efficiency than the C group. During the withdrawal period the Z group had a negative feed efficiency, while the C group continued to have a positive feed efficiency. The C animals showed no significant differences among the three periods; whereas, Z animals demonstrated a significant difference between the habituation and experimental periods, with a greater positive feed efficiency present while the drug was administered. During the withdrawal period, the Z animals developed a statistically significant strong negative feed efficiency compared to both the habituation and experimental periods which were positive.

Fig. 7 shows the mean time spent by the animals in the open arms of the EPM on select days of the three periods (see Materials and methods) of the experiment. The ANOVA indicated a marginally significant main effect for period [$F(2, 20) = 3.35$, $p = 0.056$]; a main effect for group [$F(1, 10) = 21.42$, $p = 0.001$] and a significant interaction between period and group [$F(2, 20) = 20.57$, $p<0.001$]. Time spent in the open arms was comparable between the two groups during the habituation period. On the other hand, during the experimental period, C animals spent significantly more time in the open arms than Z animals. During the withdrawal period, however, there was no significant difference between the two groups of animals. The C animals spent significantly more time in the open arms during the experimental period as compared to the habituation period, and significantly less time in the open arms during the withdrawal period as compared to the experimental period. The time spent by the C animals in the open arms during the withdrawal and habituation periods was not significant. The Z animals spent significantly less time in the open arms during the experimental period as compared to the habituation period. There was no significant difference in time spent in the open arms by the Z animals during the withdrawal period as compared to the experimental period. The time spent in the open arms during the withdrawal and habituation periods by the Z animals was marginally significant ($p = 0.078$).

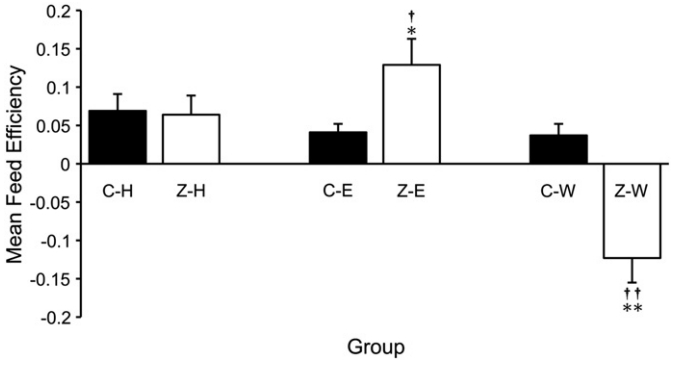

Fig. 6. Mean (+SEM) feed efficiency for C and Z animals on the last day of the H, E, and W periods. The † indicates that Z animals had a significantly more positive feed efficiency at the end of the E period than C animals and the †† shows that Z animals had a significantly more negative feed efficiency at the end of the W period than C animals. The * indicates that Z animals had a significantly greater positive feed efficiency value at the end of the E period compared to the end of the H period and the ** shows that Z animals had a significantly greater negative feed efficiency at the end of the W period compared to the end of the E period.

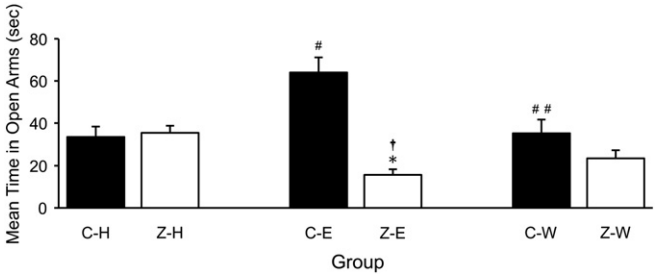

Fig. 7. Mean (+SEM) time spent in the open arms of the elevated plus maze for C and Z rats during H, E, and W periods. The † indicates that Z animals spent significantly less time in the open arms than C animals during the E period. The # indicates that the C animals spent significantly more time in the open arms during the E period compared to the H period. The ## indicates that the C animals spent significantly less time in the open arms during the W period as compared to the E period. The * indicates that the Z animals spent significantly less time in the open arms during the E period as compared to the H period.

Fig. 8 shows the mean time spent by the animals in the closed arms of the EPM on select days of the three periods of the experiment. The ANOVA showed a main effect for period [$F(2, 20) = 28.54$, $p<0.001$]; a main effect for group [$F(1, 10) = 16.85$, $p<0.01$] and a significant interaction between period and group [$F(2, 20) = 23.01$, $p<0.001$]. Time spent in the closed arms was comparable between the two groups during the habituation period. Statistically, there was a significant difference between the two groups in the amount of time that the animals spent in the closed arms during the experimental period. Zolpidem increased the amount of time that the Z rats stayed in the closed arms as compared to the C rats. During the withdrawal period, both groups were comparable in time spent in the closed arms of the maze. The time spent in the closed arms during the habituation and experimental periods by the C rats was not significant; however, they spent statistically less time in the closed arms during the withdrawal period as compared to the habituation period. The Z animals spent statistically more time in the closed arms during the experimental period as compared to the habituation period. The time that the Z animals spent in the closed arm during drug withdrawal was significantly less compared to the time spent in the closed arms during the habituation and experimental periods.

Fig. 9 illustrates relative visceral adiposity. The percentage agreement between the two observers rating adiposity was 97.2%. A *t*-test indicated that there was a significant difference between the two groups [$t(10) = -8.17$, $p<0.001$]. The Z rats accumulated significantly more fat in their body than did the C rats.

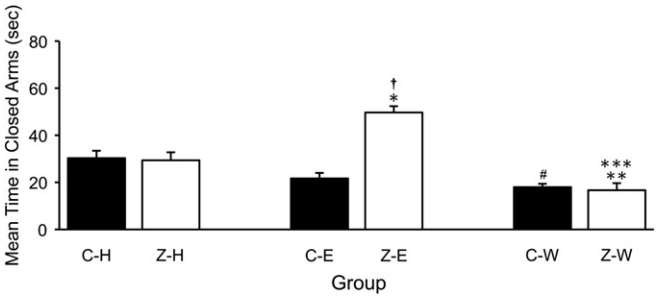

Fig. 8. Mean (+SEM) time spent in the closed arms of the elevated plus maze for C and Z rats during H, E, and W periods. The † indicates that Z animals spent significantly more time in the closed arms than C animals during the E period. The # indicates that C animals spent significantly less time in the closed arms during the W period as compared to the H period. The * indicates that Z animals spent significantly more time in the closed arms during the E period compared to the H period. The ** shows that Z animals spent significantly less time in the closed arms during the W period compared to the H period and the *** indicates that the Z animals spent significantly less time in the closed arms during the W period as compared to the E period.

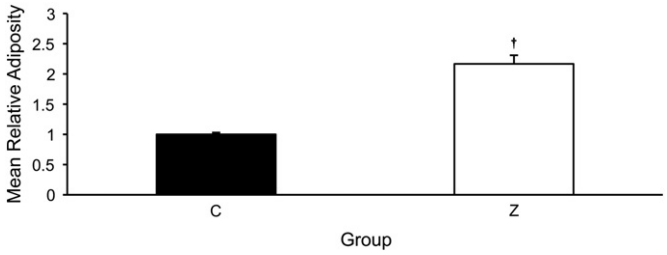

Fig. 9. Mean (+SEM) relative visceral adiposity of C and Z rats at the conclusion of the experiment. The † indicates that Z animals had significantly more adiposity than C animals.

4. Discussion

It has been demonstrated that zolpidem has strong sedative and hypnotic effects in humans due to its high affinity for the alpha-1 subunit of the GABA-A receptor which is believed to be responsible for these effects (Langtry and Benfield, 1990). In the present experiment, zolpidem produced a significant decrease in peak number of wheel revolutions (Fig. 1) and mean % change in revolutions (Fig. 2) in Z animals from the habituation period to the experimental period, as compared to C animals. Other researchers have found a decrease in locomotor activity, measured via radiotelemetry, 20 min after subcutaneous administration of 2.5, 5, and 10 mg/kg doses of zolpidem (Elliot and White, 2001). In their study the authors indicated that the decrease in activity is an indication of the sedative-inducing effect of zolpidem. In that same study, however, they also demonstrated that zolpidem induced muscle relaxation as measured by electromyographic activity. Thus, one could consider locomotor activity as a nonspecific measure that can be influenced by multiple behavioral effects including sedative, muscle relaxant, and ataxic effects. It is important to note that during the period of drug administration, Z animals showed no evidence of the development of tolerance to zolpidem since decreased locomotor activity continued throughout the experimental period (Fig. 1).

Our laboratory utilized a new approach to the oral administration of zolpidem. The drug was placed in a sweet condensed milk "treat" that was consumed completely in a short period of time. Oral drug self-administration protocols using rewarding substances (e.g. sucrose, alcohol) are well-established in the rat. However, studies employing this methodology generally place the drug in rewarding substances that are incorporated into food or liquid that will be consumed by the rat over time. Thus, gram or milliliter quantities of the food or liquid will be ingested. This could influence the total caloric intake of the animal. In our study, the total volume of treat consumed is in microliter volumes rather than in milliliter volumes; thus, having a relatively insignificant effect on caloric intake. Also, in other studies, animals may not consume an exact amount of the drug at one critically delimited time period. In our presentation of the drug, the "treat" is totally consumed within 15 min (usually within 2–3 min). Our use of the 10 mg/kg dose in this study has been employed by other researchers (Morairty et al., 2008; Renger et al., 2004; Stanhope et al., 1993). Furthermore, in some studies, animals are food or liquid restricted to enhance ingestion of the drug-containing substance which could introduce an unwanted variable (e.g., stress) into the paradigm. In our study, animals were provided with food and water ad libitum.

Both C and Z animals gained weight throughout the experimental period of 21 days and both groups weighed significantly more at the end of the experimental period as compared to the end of the habituation period, with no significant difference between the two groups in either period (Fig. 3). On the other hand, the present experiment revealed that zolpidem increased food intake from the end of the habituation period to the end of the experimental period and that the Z animals ate significantly more food than the C animals during the experimental period (Fig. 4). Previous studies on the effect of zolpidem on food intake have reported conflicting results. For example, Sanger and Zivkovic (1988) showed that zolpidem did not cause a reliable increase in food intake in rats. These researchers had food available for rats for only 4 h each weekday and food was freely available from Friday evening until Sunday afternoon. When stable baselines of food intake had been established, the rats were injected intraperitoneally with 2.5, 5.0, or 10 mg/kg doses of zolpidem on different days in mixed order 30 min before standard laboratory chow was placed in the cage. In their report, results were presented for the first 2 h of the 4 h period of food availability. In a study by Yerbury and Cooper (1989), nondeprived animals were given intraperitoneal injections of 0.3–3 mg/kg of zolpidem 15 min prior to presentation of a palatable diet. A 30 min intake test of the diet ensued. The tests were conducted once under standard and once under presatiated conditions and there was no effect noted on food intake in either condition. In another food-related study, Stanhope et al. (1993) proposed that palatability of food could account for an increase in food consumption by rats receiving zolpidem. They postulated that it may be more difficult to repress consummatory behavior in rats when a highly palatable liquid (3% D-glucose and 0.1% sodium saccharine w/v in water) was used with zolpidem administration. In their study, access to drinking water was restricted to 90 min per day and, during the 20 min testing session, the subjects were removed from their home cage and had access to "palatable" fluids. Intraperitoneal injections of either 3.0 or 10.0 mg/kg of zolpidem were given 15 min prior to the testing procedure and there was one test session for each rat. It is important to note that differences in experimental procedures may account for the discrepancies obtained between our results concerning the effects of zolpidem on food intake and the results presented in the above studies. Conditions in our study involved oral administration of the drug in a "treat" at the same time daily throughout a 21 day experimental period and provision of standard laboratory rat chow and water ad libitum. These conditions more closely simulate the circumstances under which humans subsist when taking the drug.

In addition to absolute food intake, the present study provides determinations of relative food intake (Fig. 4) and feed efficiency (Fig. 5), which are indicators of the functioning of metabolic processes occurring in mammalian organisms. While receiving the drug, there was a significantly higher relative food intake by Z animals compared to C animals and greater positive feed efficiency in Z animals. The changes observed in these two measures reflect an altered state of metabolism, which may account for the increased adiposity observed in the Z animals in the present experiment (Fig. 9). Additionally, the increased adiposity observed in Z animals may be related to the decrease in locomotor activity by these animals during the experimental period.

The appetite-stimulating properties of the benzodiazepines have been elucidated (Berridge and Pecina, 1995; Cooper and Estall, 1985; Wise and Dawson, 1974). However, studies examining appetite-stimulation by zolpidem have produced conflicting results (Cooper and Yerbury, 1988; Davies et al., 1994; Sanger and Zivkovic, 1988; Stanhope et al., 1993; Yerbury and Cooper, 1989). Research has been conducted to ascertain the receptor pharmacology underlying observed hyperphagia induced by the benzodiazepines and zolpidem. For example, the benzodiazepines are known to nonselectively affect the alpha-1, alpha-2, and alpha-3 subunits (Möhler et al., 2002). On the other hand, zolpidem has been shown to selectively modulate the alpha-1 subunit (Langer et al., 1992; Langtry and Benfield, 1990) and to a much lesser extent the alpha-2 and alpha-3 subunits (Crestani et al., 2000). Cooper (2005) predicted that the alpha-2 and alpha-3 subunits were responsible for hyperphagia induced by benzodiazepines and, in a study with mutant mice, Morris et al. (2009) concluded that the hyperphagic effects are mediated by modulation of the alpha-3 subunit. Since the benzodiazepines nonselectively modulate the alpha-3 subunit, humans or animals treated with the benzodiazepines could manifest increased food intake, weight gain, and adiposity. A more modest effect

of zolpidem would be anticipated if the 10 mg/kg dose of drug was not sufficient to modulate alpha subunit-containing receptors maximally. Although hyperphagia developed in animals in our study, more research needs to be conducted before firm conclusions about receptor pharmacology can be made for zolpidem-induced hyperphagia.

Turning to the effects observed on anxiety, studies on the anxiolytic or anxiogenic effects of zolpidem are inconsistent. In humans, a frequent adverse effect of the use of zolpidem that has been reported is anxiety-induction (Krystal et al., 2008). In a study in mice, utilizing the EPM, Cui et al. (2007) showed that a 5 mg/kg dose of zolpidem injected intraperitoneally 15 min before testing had an anxiolytic effect. Utilizing the acute administration of zolpidem, Gonzalez-Pardo et al. (2006) reported that, following a 2 mg/kg intraperitoneal injection of zolpidem, rats could not be evaluated on the EPM due to the highly sedative effects of the dose. In our study utilizing an oral 10 mg/kg dose of zolpidem, the anxiety response was increased with chronic drug administration since the Z animals spent less time in the open arms of the EPM during drug administration (Fig. 7) compared with C animals as well as compared to the habituation period (an anxiogenic effect). It should also be recalled that the animals receiving zolpidem spent significantly more time in the closed arms of the maze during the period of drug administration (Fig. 8) compared with C animals as well as compared to the habituation and the withdrawal periods. This is another indication of the anxiogenic effect of the drug. The fact that zolpidem has an affinity (even though reduced) for the alpha-2 subunit of the GABA-A receptor may account, in part, for the anxiety-related effects of the drug in the Z animals (Crestani et al., 2000; DaSettimo et al., 2007). Our results are similar to those observed in a study by Davies et al. (1994) utilizing rats in which they report decreased time spent in the open arms and increased time spent in the closed arms in doses greater than 0.05 mg/kg. In addition, it is interesting to note in our study that the effects of zolpidem on time spent in the open arms of the maze were still apparent in the withdrawal period of the Z animals (Fig. 7). Factors which may have influenced our results include: 1) testing in the dark phase of the light/dark cycle; 2) age of the animals; and 3) time period between test sessions. Hogg (1996) published a review concerning the validity and variability of the elevated plus-maze as an animal model of anxiety and presented the results of a survey provided to researchers utilizing the EPM. One important aspect of the questionnaire was lighting levels in the test room where experiments with the EPM were conducted. The majority of the responders indicated that their studies were carried out in the light. Conducting tests in the dark may have significantly different effects than conducting those same tests in the light. For example, Andrade et al. (2003) have demonstrated that rats tested at different ages and different times in the light/dark cycle produced different results in the EPM. These investigators showed that animals at 2 months of age tested in the dark cycle spent less time in the open arms as compared to animals tested at 3 months of age in the dark cycle. The authors point out that reexposure to the EPM has usually occurred at short intervals and that longer intervals (e.g., one month) had not been previously described. They proposed that the increase was due to a developmental stage in which rats show "a robust circadian rest–activity rhythm". Thus, the results of our study showing an increase in time spent in the open arms of the maze by the C rats during the experimental period compared to the habituation period may be due to the age of the rats as well as the long time interval (21 days) between test sessions. On the other hand, the decrease seen in time spent in the open arms of the maze by the C animals between the experimental period and withdrawal period tests may not be due to age variations, but rather to adaptation to the open arms because of the short interval of time (4 days) between test sessions.

In conclusion, the present experiment provides a new method of chronic administration of zolpidem to animals. Significant findings related to the effects of zolpidem on locomotor activity, body weight, food intake, relative food intake, feed efficiency, anxiety, and visceral adiposity may have implications that should be considered when prescribing this drug for use in humans. It is important to note that other doses of zolpidem may augment or decrease the effects of this drug on the parameters examined in the present study. Further research could elucidate the significance of these factors as applied to human usage.

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