Pharmacology Biochemistry and Behavior, Vol. 66, No. 2, pp. 361–369, 2000

0091-3057/00/$–see front matter

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PII S0091-3057(00)00176-3

# Precipitated and Spontaneous Withdrawal Following Administration of Lorazepam But Not Zolpidem

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Received 23 December 1998; Revised 12 October 1999; Accepted 28 October 1999

ELLIOT, E. E. AND J. M. WHITE. *Precipitated and spontaneous withdrawal following administration of lorazepam but not zolpidem.* PHARMACOL BIOCHEM BEHAV **66**(2) 361–369, 2000.—Radiotelemetry was utilized to compare zolpidem and lorazepam tolerance and withdrawal in rats. Locomotor activity, electromyographic activity (EMG), and body temperatures were used to assess the acute drug effects, and as measures of tolerance and withdrawal. Lorazepam, zolpidem, or vehicle was administered for 12 days, and data were recorded daily, immediately, after treatment. Data were also recorded immediately after flumazenil (25 mg/kg, IP) precipitated withdrawal and during 4 days of spontaneous withdrawal. Complete tolerance to the acute effects of lorazepam administration developed within 7 days of treatment and both flumazenil-precipitated and spontaneous withdrawal were observed. In contrast, there was no tolerance to the sedative actions of zolpidem administration after 12 days, but complete tolerance to the hypothermic and muscle relaxant effects was apparent after 8 days of treatment. Despite the presence of tolerance, no evidence of either spontaneous or flumazenil-induced withdrawal was recorded in these rats. In conclusion, this model suggests that as a sedative zolpidem has significant advantages over the classic benzodiazepines. © 2000 Elsevier Science Inc.

Zolpidem Lorazepam Radiotelemetry Tolerance Withdrawal

BENZODIAZEPINES have been used as hypnotics, sedatives, anxiolytics, muscle relaxants, and anticonvulsants since the early 1960s. However, chronic use of benzodiazepines often leads to tolerance and dependence followed by a withdrawal syndrome upon drug cessation. For example, lorazepam tolerance and withdrawal have been widely reported in rodents following lorazepam discontinuation (13,14,21, 27,33,34,36), while comparable findings, especially tolerance to the physiological, behavioral, and subjective effects of lorazepam, have been reported in humans (23). Additionally, as a direct result of withdrawal symptoms, lorazepam cessation has been reported to be problematic in many patients wishing to discontinue therapy (39,47). Thus, the development of alternative compounds possessing benzodiazepine-like actions, without the problems of dependence, would be of considerable value.

Lorazepam is a prototypical benzodiazepine that possesses muscle relaxant, sedative, anticonvulsant and anxiolytic properties. It is believed that this full spectrum of benzodiazepine-like actions arises from its similar affinity for $GABA_A$ receptors containing $\alpha_1$, $\alpha_2$, $\alpha_3$, and $\alpha_5$ subunits. The imidazopyridine zolpidem is a nonbenzodiazepine that dose dependently blocks PTZ isoniazid, and electroshock-induced convulsions, and induces sedation, muscle relaxation, and body temperature in rodents (8,9,43). In humans, zolpidem possesses only mild anticonvulsant, muscle relaxant, and anxiolytic properties, and is used in the short-term treatment of insomnia. Evidence from a Swiss 3-year postmarketing study suggests that zolpidem is a safe and effective agent with a low potential for abuse or dependence (16). However, there have been individual reports of seizures associated with ingestion of high doses (60-100 mg) of zolpidem (5,17). These individuals escalated their dose as a result of tolerance to the hypnotic effect achieved initially at the prescribed therapeutic dose. In contrast to lorazepam, zolpidem has a high selectivity for $GABA_A$ receptors containing the $\alpha_1$ subunit (2). It has been postulated that this selectivity for the $\alpha_1$ subunit may confer a different pharmacological profile from the traditional benzodiazepines (9).

Using animal models, there have been conflicting reports concerning zolpidem's ability to induce tolerance and dependence following chronic administration. In rodents, zolpidem

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has been reported to produce little tolerance or dependence (38,41,42). In contrast, tolerance to the ataxic and sedative effects of zolpidem self-administration in baboons, and then suppression of food intake (a withdrawal effect) following zolpidem cessation, have been reported (19). Further, in a more recent study, these same researchers have reported zolpidem self-injection and flumazenil-precipitated withdrawal in baboons following 35 days of zolpidem administration (49).

The aim of the present study was to compare lorazepam and zolpidem tolerance and dependence based on measures of electromyography (EMG), locomotor activity, and body temperature recorded using radiotelemetry. Lorazepam was selected as a prototypical benzodiazepine for comparison with zolpidem, because short-term administration of this compound has been shown to induce tolerance and dependence in other rat models (21,27,48). Furthermore, lorazepam discontinuation signs have been reported following both spontaneous and flumazenil precipitated withdrawal in humans (4,20).

Radiotelemetry has a number of advantages over conventional measures of tolerance and dependence. These include measurement of multiple parameters simultaneously in the home cage, continuous recording over a long time period, and minimal handling-related or restraint stress to the animals involved. Furthermore, this technique minimizes the influence of learning, a problem associated with multiple testing when assessing tolerance. Unlike techniques such as the rotarod measure of ataxia or the pull-up test of muscle relaxation, radiotelemetry provides continuous data during "normal" animal activity in the absence of the researcher.

Lorazepam and zolpidem have similar half-lives in rats, 1.5 and 1.3–1.5 h, respectively (12,44), and neither drug has active metabolites, thus accumulation of these compounds following continuous administration is unlikely. Doses of lorazepam and zolpidem were based on a previous study where zolpidem (5 mg/kg) and lorazepam (12.5 mg/kg) decreased locomotor activity to 20 and 26%, respectively, of saline controls, 20 min after administration (unpublished data). Precipitated withdrawal was measured following administration of the benzodiazepine antagonist flumazenil, and spontaneous withdrawal was recorded over the 4 days following cessation of drug administration. Flumazenil has been shown to antagonize the central effects of both zolpidem and lorazepam (15,29,37) and precipitate abrupt withdrawal signs following their discontinuation (26,32,49). The flumazenil dose used was comparable to doses administered to rats, to either precipitate benzodiazepine withdrawal (3) or reverse the depressant effects of benzodiazepines (6), without inducing intrinsic activity in naive animals (31).

## METHOD

### *Subjects*

Male Hooded Wistar rats (Waite Institute, Adelaide) weighing 250–300 g, were singly caged in a temperature- (21 ± 2°C) and humidity-controlled room and maintained on a 12L:12D cycle from 0700 h. All animals were treated in accordance with the principles and guidelines of the University of Adelaide Animal Ethics Committee.

### *Drugs and Drug Preparation*

The general anaesthetic agent was prepared from a mixture of one part Nembutal (sodium pentobarbitone 60 mg/ml) to nine parts Brietal (sodium methohexitone 10 mg/ml). Tribrissen antibiotic (trimethoprim 80 mg/ml and sulfadiazine 400 mg/ml) 0.5 ml/kg/day was administered after surgery. Lorazepam 12.5 mg/kg (gift from Wyeth, Sydney, NSW), and zolpidem 5 mg/kg (gift from Synthélabo Recherché, France) were dissolved in 50% polyethylene glycol 400 (PEG 400) for subcutaneous (SC) injection. Flumazenil 25 mg/kg (gift from Hoffmann–LaRoche, Basel, Switzerland) was suspended in 4% Tween 80 (BDH). All drugs were injected in a 1 ml/kg volume by the SC route, except flumazenil, which was injected intraperitoneally (IP). Lorazepam (0.19 mg/ml) and zolpidem (0.1 mg/ml) were dissolved in 45% 2-Hydroxypropyl-β-cyclodextrin (RBI, Natick, MA), and diluted in tap water for oral administration.

### *Experimental Design*

Radiotransmitters (Data Sciences, St. Paul, MN) were surgically implanted in anaesthetized rats 1 week after habituation in their home cage. The transmitter was placed in the abdomen and the electrodes sutured into the left thigh muscle. Rats were given daily SC injections of Tribrissen antibiotic for 5 days, and were allowed 1 week to recover. To achieve rapid benzodiazepine dependence rats were administered lorazepam (0.19 mg/ml) or zolpidem (0.1 mg/ml) dissolved in β-cyclodextrin in drinking water for 12 days. Additionally, to measure the development of benzodiazepine tolerance on a daily basis, rats were injected once daily with either lorazepam (12.5 mg/kg) or zolpidem (5 mg/kg) SC, followed immediately by daily data collection for 12 days. Control animals were administered vehicle (50% PEG) SC and 1% β-cyclodextrin in drinking water for 12 days. Rat weights and drinking volumes were recorded daily.

The daily drug intake was calculated from the amount consumed in the drinking water, plus the daily SC injection of lorazepam or zolpidem. The oral dose of lorazepam was calculated to provide a total daily intake of approximately 37 mg/kg, when combined with the SC dose. This total daily intake of lorazepam was based on that used by van der Laan et al. (48), who recorded both daytime and nocturnal home cage locomotor activity as a measure of lorazepam withdrawal. The SC zolpidem dose (5 mg/kg) was one that produced an acute sedative action equivalent to that produced by lorazepam (12.5 mg/kg) in an earlier pilot study. The oral dose of zolpidem was calculated to provide a total daily intake of approximately 15 mg/kg, a dose comparable with the lorazepam total daily intake. The flumazenil dose was one that was observed to completely block the sedative and hypothermic effects of lorazepam (12.5 mg/kg, SC) in naive rats in an earlier pilot study.

The time line for treatment and data collection (EMG, activity and temperature) was as follows: days 1–12 inclusive: daily SC injection of either lorazepam (12.5 mg/kg), zolpidem (5 mg/kg) or vehicle followed immediately by data collection (drinking water with added lorazepam was removed during data collection; lorazepam, zolpidem or vehicle was administered in drinking water during non-testing time. Day 13: drinking water with drug or vehicle removed, flumazenil (25 mg/kg) injected IP, followed immediately by data collection. EMG, activity, and temperature data were collected at 10-min intervals for at least 70 min immediately after daily treatment. These parameters were also recorded for 4 days, at 30-min intervals, 24 h after the last dose of lorazepam, zolpidem, or vehicle.

Animals were habituated to handling immediately after recovery from surgery. However, during the experiment, han-

dling was restricted to weighing, injecting, and activating and deactivating the radiotransmitters by passing a magnet underneath the rats' abdomens. Data were collected between 1200–1400 h daily, while the rats remained in their home cages. Each cage was placed on top of a receiver that was connected to an IBM-compatible, 486 DX computer. EMG bursts were sampled at 600 HZ for 2 s per minute, every minute for at least 1 h. Each sample was low cut filtered at 50 HZ, to eliminate movement interference, and fully rectified. Consecutive 1-min EMG bursts were then averaged over 10 min to give one data point for every 10 min. All data are expressed as the mean ± SEM of each 10-min time period. Statistics were analyzed using the GraphPad Prism package. Area under the curve (AUC) followed by analysis of variance, and Tukey's tests were used to compare activity, temperature and EMG between treatment groups.

## RESULTS

### *Rat Weights and Drug Intake*

Body weights increased by approximately 9% for all three groups over the 2 weeks of treatment. There were small decreases in body weights (4.55 ± 0.55%) of lorazepam-treated animals during benzodiazepine withdrawal, but no decreases in body weights of either the zolpidem of vehicle-pretreated rats during the equivalent time. There were no significant differences in average fluid consumption over the 12 days of treatment. Rats administered lorazepam consumed an average fluid intake of 12 ml/100 g body weight, compared with 9.2 ml/100 g for zolpidem-administered rats and 9.3 ml/100 g body weight consumed by control animals. Further, the addition of either lorazepam or zolpidem dissolved in β-cyclodextrin did not alter the drinking water intake from predrug administration levels. Total drug intakes ranged from 31 to 44 mg/kg/day (mean 35 mg/kg/day) during lorazepam treatment, and from 13 to 15.1 mg/kg/day (mean 14.2 mg/kg/day) during zolpidem treatment.

### *Acute Effects*

On the first day of acute administration, lorazepam and zolpidem produced moderate decreases in body temperature. This hypothermia was maximal after approximately 70 min (Fig. 1a). Analysis of variance of the area under the curve indicated a significant decrease in body temperature relative to vehicle administered controls, $F(2, 15) = 12.4$, $p < 0.001$. Activity was significantly depressed in both lorazepam- and zolpidem-treated animals compared to controls (Fig. 1b), $F(2, 15) = 14.8$, $p < 0.001$. The similarity in magnitude of activity changes confirms that the doses chosen were approximately equieffective with regard to sedation. As control animals showed a gradual decline in activity over time, the duration of drug effect appeared to be somewhat shorter for this parameter. Lorazepam and zolpidem administration also significantly depressed EMG activity, with lorazepam effecting a larger muscle relaxant action than zolpidem (Fig. 1c), $F(2, 15) = 20.5$, $p < 0.001$. Relatively constant differences in EMG values emerged between experimental and control animals approximately 20 min after drug administration.

### *Tolerance*

Toleranace developed rapidly to the acute hypothermic actions of lorazepam and zolpidem. By the fourth day of treatment there were no significant differences in rat body temperatures 60 min after administration of vehicle, lorazepam, or zolpidem (Fig. 2a). Tolerance to the acute sedative action of lorazepam developed within 4–6 days of administration (Fig. 2b). In contrast, after 8 days of administration, zolpidem induced lower activity levels than those of lorazepam- and vehicle-treated animals ($p < 0.01$). By the 12th and final day of treatment, no tolerance to the sedative effects of zolpidem was recorded. There was no consistent change in activity recorded by vehicle-administered controls. Chronic administration of both zolpidem and lorazepam resulted in tolerance to the acute muscle relaxant actions of these drugs, within 4 and 8 days, respectively (Fig. 2c). Controls administered vehicle recorded no significant changes in muscle relaxation over the 12 days of treatment.

### *Flumazenil Precipitated Withdrawal*

Over the 70 min after flumazenil (25 mg/kg, IP)-precipitated withdrawal, significant decreases in body temperatures were recorded in rats pretreated with lorazepam, but not those pretreated with zolpidem, $F(2, 15) = 10.8$, $p < 0.01$. During those 70 min, immediately after flumazenil administration, attendant increases in activity, $F(2, 15) = 32.0$, $p < 0.001$, and muscle tone, $F(2, 15) = 8.8$, $p < 0.01$, were recorded in lorazepam-pretreated rats (Figs. 3b and c), but not in zolpidem-pretreated rats. This increase in activity was maximal after 50 min, while EMG levels rose rapidly immediately after flumazenil administration. Unlike rats pretreated with lorazepam, there were no significant differences in body temperature, activity, or EMG between zolpidem- and vehicle-treated rats after flumazenil administration. Rats pretreated with vehicle recorded little change in body temperatures, activity, or EMG immediately following flumazenil administration (Fig. 3a–c). Additionally, vehicle-treated animals showed minimal day-to-day variation in these parameters, both before and after flumazenil administration. When compared with data recorded on the days preceding flumazenil administration, a repeated-measures ANOVA indicated no statistically significant effect of flumazenil on body temperature, $F(3, 31) = 1.4$, $p = 0.28$, activity, $F(3, 31) = 1.9$, $p = 0.15$, or muscle tone, $F(3, 31) = 1.7$, $p = 0.20$ (graphs not included).

### *Spontaneous Withdrawal*

In zolpidem-pretreated rats and vehicle-treated controls, daytime activity levels (expressed as a proportion of total 24-h activity) accounted for approximately 30% of the total activity in any one 24-h period. However, during the first 24-h period after lorazepam cessation (Wd1) daytime activity levels rose to 45% of the total 24-h activity (Fig. 4a), with nighttime activity levels lower than those recorded by controls. This elevated daytime activity was still evident when recording stopped on withdrawal day four (Wd4). During these same 4 days EMG levels in lorazepam-pretreated rats were also higher than those recorded in either vehicle or zolpidem-pretreated rats, and these were still elevated when recording ceased on the fourth withdrawal day (Fig. 4b).

## DISCUSSION

In both lorazepam and zolpidem-pretreated rats some degree of tolerance was associated with chronic drug administration. Complete tolerance to the hypothermic, sedative, and

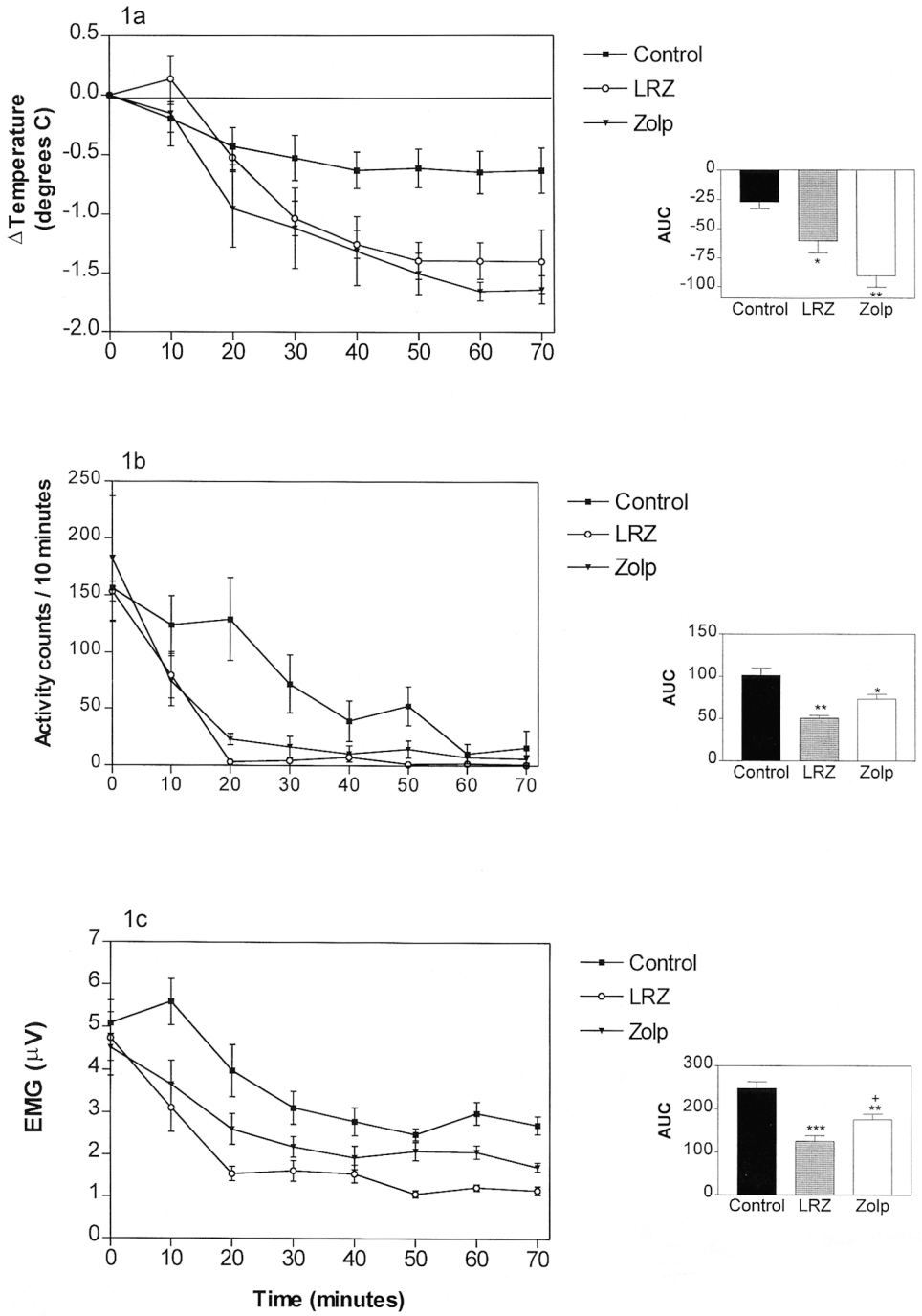


FIG. 1. (a) Acute changes in body temperature after SC administration of vehicle 1 ml/kg, (■ control), lorazepam 12.5 mg/kg (○), or zolpidem 5 mg/kg (▼). ANOVA of area under the curve (expressed as degrees × minutes) over 70 min indicated a significant drug effect. $^{**}p < 0.01$, $^{*}p < 0.05$, compared to vehicle-administered controls. All values are represented as mean ± SEM. (b) Changes in activity after SC administration of vehicle 1 ml/kg, (■ control), lorazepam 12.5 mg/kg (○), or zolpidem 5 mg/kg (▼). ANOVA of area under the curve (expressed as log counts × minutes) over 70 min indicated a significant drug effect. $^{**}p < 0.01$, $^{*}p < 0.05$, compared to vehicle-administered controls. All values are represented as mean ± SEM. (c) Changes in EMG after SC administration of vehicle 1 ml/kg, (■ control), lorazepam 12.5 mg/kg (○), or zolpidem 5 mg/kg (▼). ANOVA of area under the curve (expressed as μV × minutes) over 70 min indicated a significant drug effect. $^{***}p < 0.01$, $^{**}p < 0.01$, compared to vehicle-administered controls. $^{+}p < 0.05$, compared to lorazepam. All values are represented as mean ± SEM.

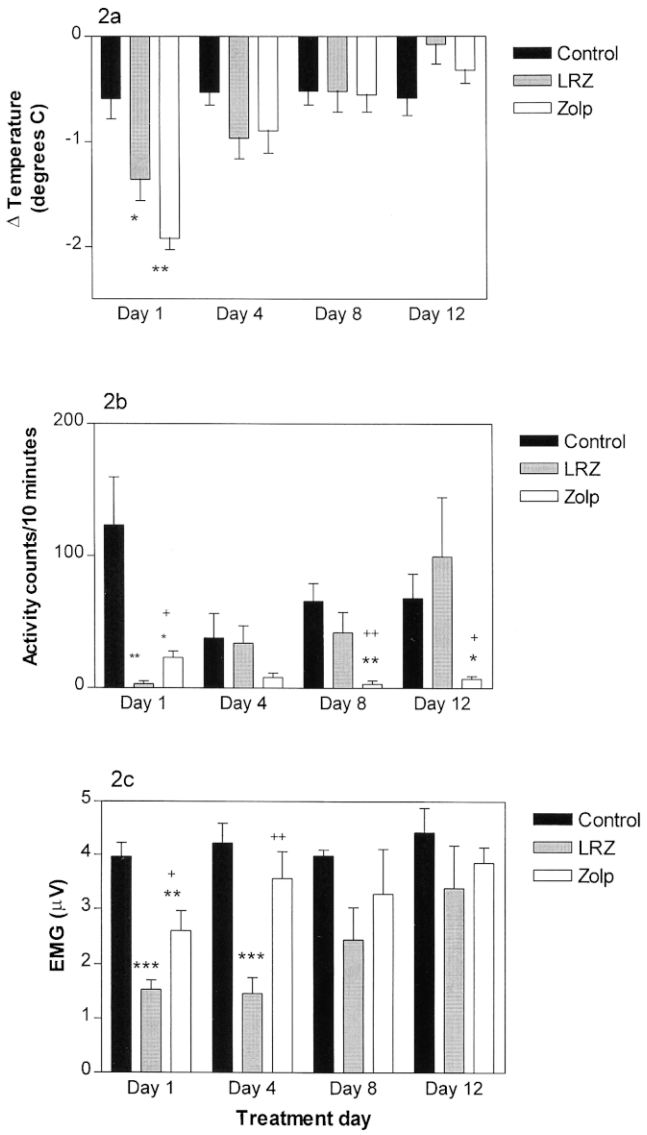


FIG. 2. (a) Acquisition of tolerance to the acute hypothermic effects of lorazepam (12.5 mg/kg) and zolpidem (5 mg/kg). Body temperatures were recorded 60 min after drug administration. $^{**}p < 0.01$, $^{*}p < 0.05$, compared to vehicle-administered controls. All values are represented as mean ± SEM. (b) Acquisition of tolerance to the acute sedative effects of lorazepam (12.5 mg/kg) and zolpidem (5 mg/kg). Activity was recorded 20 min after drug administration. $^{**}p < 0.01$, $^{*}p < 0.05$, compared to vehicle-administered controls. $^{++}p < 0.01$, $^{+}p < 0.05$, compared to lorazepam. All values are represented as mean ± SEM. (c) Acquisition of tolerance to the acute muscle relaxant effects of lorazepam (12.5 mg/kg) and zolpidem (5 mg/kg). EMG was recorded 20 min after drug administration. $^{***}p < 0.001$, $^{**}p < 0.01$, compared to vehicle-administered controls. $^{++}p < 0.01$, $^{+}p < 0.05$, compared to lorazepam. All values are represented as mean ± SEM.

muscle-relaxant actions of lorazepam developed within 1 week of administration. However, the rate of development of tolerance development differed across the measures, with temperature the most rapid and EMG the slowest. These results are in agreement with those of others (13,36), who have reported tolerance to the sedative and hypothermic actions of lorazepam within 3 days of administration. No reports of tolerance to the acute muscle relaxant effects of lorazepam are known to the present authors. However, after 7 days of lorazepam administration in mice, Miller et al. (33) reported a 50% decrease in benzodiazepine receptor binding and a decrease in $GABA_A$ receptor function that was closely correlated with maximal tolerance to rotarod ataxia. Rotarod ataxia has a component of muscle relaxation, and indeed, has been used as a measure of muscle relaxation (9). Thus, these data from Miller et al. (33) suggest that, in rodents, maximal tolerance to the muscle relaxant effects of lorazepam occurs within 7 days of administration, a result in accord with the findings from the present study.

Chronic zolpidem administration resulted in tolerance to the hypothermic and muscle relaxant effects, but no tolerance to the sedative actions was recorded. This absence of tolerance to zolpidem-induced sedation is in agreement with the results of Perrault et al. (38), who administered zolpidem (30 mg/kg bidaily) to mice for 10 consecutive days. However, it contrasts directly with the findings of Griffiths et al. (19), who reported tolerance to ataxia and sedation within 5 days in baboons. The reasons for these discrepant findings are unclear, but the species difference in these studies is probably a significant factor. In view of the fact that there have been reports of tolerance to zolpidem in humans (5,17), and that nonhuman primates are genetically closer to humans than rats, baboons may offer a more reliable model for measuring sedative tolerance.

Surprisingly, despite the development of tolerance to the hypothermic and muscle-relaxant effects of zolpidem, there was no evidence of either precipitated or spontaneous withdrawal in the zolpidem treatment group. In contrast, pronounced withdrawal signs were evident in the lorazepam treatment group, both with antagonist administration and as spontaneous withdrawal. While the temperature changes during flumazenil precipitated lorazepam withdrawal were in the same direction as the acute drug effect, activity and EMG changes were in the opposite direction. Although withdrawal is most often expressed as signs and symptoms opposite to the direct effects of the drug (e.g., hyperactivity vs. sedation), this is not true for all withdrawal signs: acute heroin and heroin withdrawal have been reported to produce hypothermia in the same study (46). In the present study, flumazenil precipitated activity and EMG changes that were in the direction opposite to the acute drug effect. In contrast, temperature changes during withdrawal were in the same direction as the drug effect.

This latter result is in contrast to those reported by Gupta et al. (21) and Goudie et al. (18), who reported increases in body temperature during spontaneous lorazepam and chlordiazepoxide withdrawal. Both groups measured body temperatures using a rectal probe, which itself produces an increase in body temperature (45). However, one theoretical account of benzodiazepine dependence would predict a decrease in body temperature during withdrawal. Nutt (35) and Allan et al. (1) have proposed that in benzodiazepine-dependent animals, benzodiazepine agonists elicit actions more like antagonists, accounting for benzodiazepine tolerance, and that benzodiazepine antagonists elicit inverse agonist actions, accounting for precipitated benzodiazepine withdrawal. This "receptor shift" theory would predict decreases in body temperature during benzodiazepine withdrawal, as such changes have been reported after administration of the benzodiazepine inverse agonists FG 7142 and DMCM in naive rats and mice (24,45).

Other signs of withdrawal in lorazepam-treated animals were opposite to the direct effects of the drug, i.e., an increase in both locomotor activity and muscle tone over the 70 min after

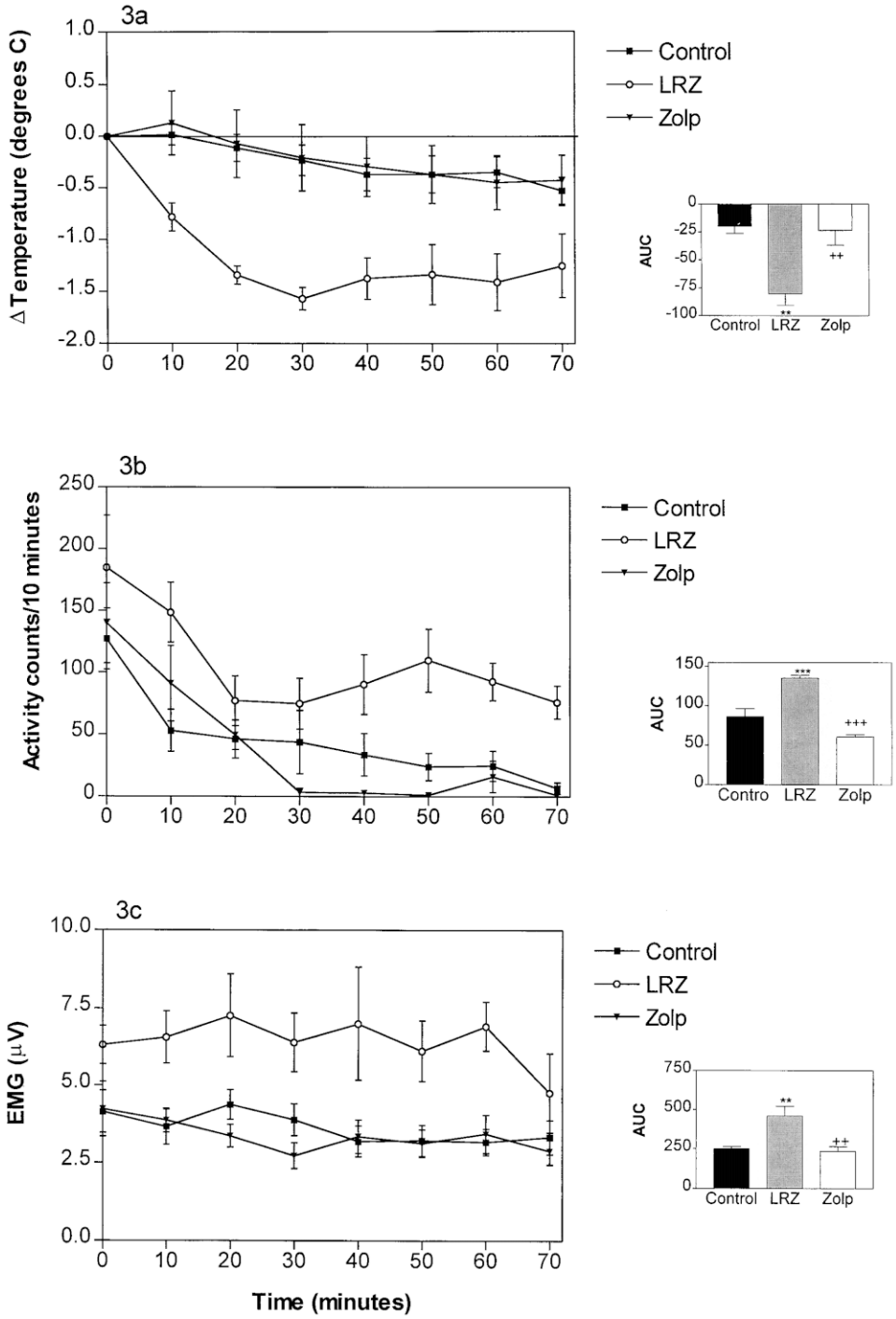


FIG. 3. (a) Changes in body temperature after IP administration of flumazenil (25 mg/kg) following 12 days of treatment with vehicle 1% β-CD, (■ control), lorazepam 35 mg/kg (○), or zolpidem 15 mg/kg (▼). ANOVA of area under the curve (expressed as degrees × minutes) over 70 min indicated a significant withdrawal effect. $^{**}p < 0.01$, compared to vehicle-administered controls. $^{++}p < 0.01$, compared to lorazepam. All values are represented as mean ± SEM. (b) Changes in activity after IP administration of flumazenil (25 mg/kg) following 12 days of treatment with vehicle 1% β-CD, (■ control), lorazepam 35 mg/kg (○), or zolpidem 15 mg/kg (▼). ANOVA of area under the curve (expressed as log counts × minutes) over 70 min indicated a significant withdrawal effect. $^{***}p < 0.001$, compared to vehicle-administered controls. $^{+++}p < 0.001$, compared to lorazepam. All values are represented as mean ± SEM. (c) Changes in EMG after IP administration of flumazenil (25 mg/kg) following 12 days of treatment with vehicle 1% β-CD, (■ control), lorazepam 35 mg/kg (○), or zolpidem 15 mg/kg (▼). ANOVA of area under the curve (expressed as μV × minutes) over 70 min indicated a significant withdrawal effect. $^{**}p < 0.01$, compared to vehicle-administered controls. $^{++}p < 0.01$, compared to lorazepam. All values are represented as mean ± SEM.

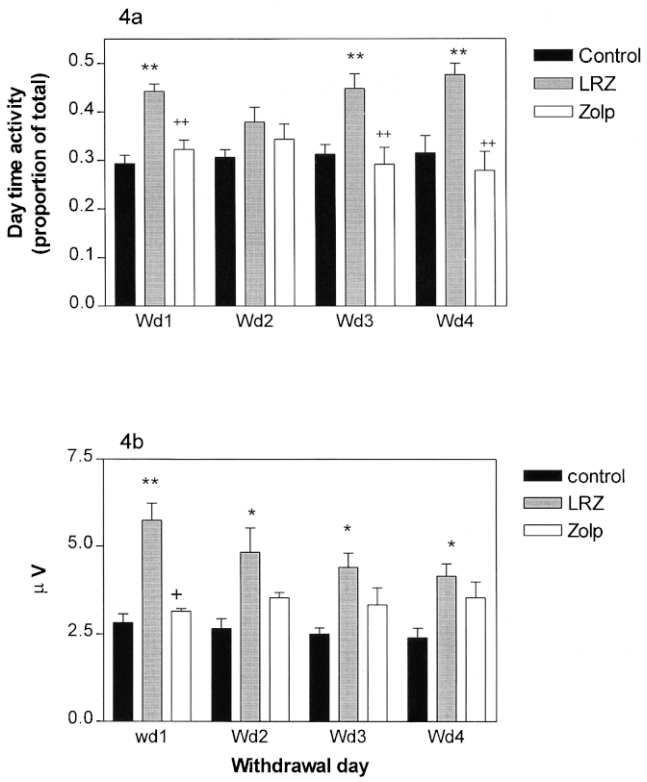


FIG. 4. (a) Daytime activity, as a proportion of total daily activity, over the 4 days after lorazepam, zolpidem, and vehicle discontinuation. $^{**}p < 0.01$, compared to vehicle-treated controls. $^{++}p < 0.01$, compared to lorazepam. All values are represented as mean ± SEM. (b) EMG levels of rats (between 0900 and 1200 h) over the 4 days after lorazepam, zolpidem, and vehicle discontinuation. $^{*}p < 0.01$, compared to vehicle-treated controls. $^{+}p < 0.05$, compared to lorazepam. All values are represented as mean ± SEM, $n = 6$ per group.

flumazenil administration. This increase in locomotor activity was similar in magnitude to that reported by van der Laan et al. (48) after induction of lorazepam dependence in rats. van der Laan and co-workers reported increases in both home cage and open-field activity during spontaneous withdrawal. Thus, it appears that the precipitated withdrawal activity data quantified using radiotelemetry are similar to those reported during spontaneous withdrawal quantified using open field activity (30,34).

Flumazenil's effects on activity and muscle tone in these benzodiazepine-dependent rats were also consistent with some benzodiazepine inverse agonists-like actions. When given to human volunteers, FG 7142 was reported to increase muscle tone, anxiety, and movement, so much so that in one case the effects had to be reversed with lormetazepam (11). Thus, the precipitated withdrawal signs observed in the present study may well be due to inverse agonist-like actions induced by flumazenil as a result of benzodiazepine receptor changes associated with lorazepam tolerance and dependence.

While there have been some reports of flumazenil possessing weak benzodiazepine agonist properties, the literature provides conflicting evidence. In behavioral studies, flumazenil administration (4–20 mg/kg) has been reported to increase head dipping, both alone and in combination with chlordiazepoxide, on the holeboard test (15). However, on the murine plus-maze test, flumazenil (10 and 40 mg/kg) was reported to have no effect when administered alone, and to antagonize diazepam-induced head dipping (7). Similarly, in drug discrimination paradigms, rats have been reported to both reliably discriminate flumazenil from saline (10) and to produce saline appropriate responding (22). Nevertheless, while flumazenil may possess weak benzodiazepine agonist-like actions in some paradigms, there appeared to be little evidence of agonist effects in the present study. On day 13, control animals recorded no significant changes in body temperature, activity, or EMG over the 70 min immediately following flumazenil (25 mg/kg) administration. Moreover, when these data were compared to those recorded after administration of vehicle (on the days immediately preceding flumazenil administration) within-subject comparisons revealed no statistically significant differences on any measures, suggesting little intrinsic activity of flumazenil.

One of the advantages of this radiotelemetry model is that both spontaneous and precipitated benzodiazepine withdrawal could be assessed in the same animals. The recording of activity, temperature, and EMG data over the 4 days following lorazepam cessation demonstrated that withdrawal occurred in the absence of flumazenil. Flumazenil has been reported to have an elimination half-life of 16 min from rat brain after IP administration of 10 mg/kg (28). Hence, it is unlikely that it would be pharmacologically active in the animals in this study 20 h after administration.

Lorazepam-pretreated rats experienced episodic increases in muscle tone throughout the 4-day recording period. The magnitude of these episodic changes in EMG was similar to those recorded after flumazenil administration during the first 24-h period, but then declined slowly over the following 3 days. There were no significant changes in EMG levels in zolpidem-pretreated rats. Increases in locomotor activity were confined to daytime, when basal activity levels were low, with evening activity decreased over baseline levels. van der Laan (48) reported that lorazepam withdrawn rats exhibited increases in daytime and moderate decreases in nocturnal locomotor activity for at least 1 week after lorazepam cessation. This increased day time activity may be akin to the rebound sleep disturbances reported by humans after cessation of short to intermediate-acting benzodiazepines (25,40).

The main aim of this study was to compare lorazepam and zolpidem with regard to their ability to induce tolerance and dependence following chronic administration. The lorazepam and zolpidem dosing regime, daily injections combined with drug in the drinking water for 12 days, produced tolerance and, in the case of lorazepam, dependence. In contrast, there was no evidence of zolpidem withdrawal signs, despite the development of tolerance to the muscle relaxant and hypothermic actions of this drug. These data suggest that zolpidem has significant advantages over the classic benzodiazepines when used as a sedative. However, while these data are consistent with the results from other studies (38,41,42), they contrast with the results of studies in baboons (19,49) and from some reports in humans (5,20). This would suggest that future research should be directed to the conditions under which dependence does and does not develop following zolpidem administration. Species differences, dosing regime, duration of administration, and other factors need to be examined in future studies.

ACKNOWLEDGEMENTS

The authors are grateful for gifts of compounds from Recherche and Hoffmann-LaRoche. Animals used in this study were treated in accordance with the guide for care and use of laboratory animal resources, National Institutes of Health (NIH) publication 1985.

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