Zolpidem Physical Dependence Assessed Across Increasing Doses Under a Once-Daily Dosing Regimen in Baboons

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ABSTRACT

The current study examined behavioral effects and possible development of physical dependence after once-daily doses of zolpidem (0, 1.0, 3.2, 10.0, 32.0 mg/kg intragastrically [i.g.]) in three baboons. Each dose was administered for 17 days and then the dose was increased; the 32.0 mg/kg dose was administered for 27 days. Baboons had access to food pellets for 20 hr/day beginning 15 min after dosing. Each day, baboons were presented with a fine motor task. Observation sessions were conducted 1 hr after dosing on days 1, 10, 12 and 14 of each dose condition and after termination of drug dosing. On days 10 and 14 of each dose condition, vehicle and flumazenil (5 mg/kg i.m.) were administered, respectively. Zolpidem increased the number of pellets obtained by two of three baboons. Vomit and/or retch and grimace (signs believed to be indicative of abdominal discomfort) were observed in one or two baboons during all zolpidem dose conditions (1.0–32.0 mg/kg). Time to complete the fine motor task increased dose-dependently in all three baboons, and incoordination was observed during the task in two baboons at 10.0 and 32.0 mg/kg. Analysis of blood plasma showed that measurable levels of zolpidem were present 24 hr after dosing in all drug conditions. The signs of flumazenil-precipitated withdrawal were summarized on a 9-point scale. Scores ranged from 1 to 5 in the 1.0 mg/kg condition, from 2 to 5 in the 3.2 and 10.0 mg/kg conditions and from 4 to 6 in the 32.0 mg/kg condition. Signs that were considered intermediate in severity were observed. Specifically, tremor, jerk and/or rigidly braced posture was observed in one baboon at 1.0 mg/kg, two baboons at the next two doses and all three baboons at 32.0 mg/kg. Vomit and/or retch also occurred in two baboons at dose conditions above 1.0 mg/kg. Discontinuation of zolpidem dosing after 78 to 79 days resulted in mild withdrawal signs (e.g., number of pellets obtained were lower and number of 1-min intervals increased in which eyes were closed, or in which lying down, head lower than torso posture and/or withdrawn posture were observed) on the first day in two baboons. The peak withdrawal scores were 4 or 5 on days 5 to 10; two baboons vomited and/or retched and all three baboons showed tremor, jerk and/or rigidly braced posture. Thus, zolpidem produced physical dependence under once-daily dosing conditions, and the severity of the withdrawal syndrome can be characterized as intermediate.

Zolpidem is an imidazopyridine hypnotic, which exerts its effects via the benzodiazepine (Bz or \( \alpha \), Langer and Arbilla, 1988) modulatory site on the GABA\(_A\) receptor-chloride ionophore complex (Biggio et al., 1989; Lloyd and Zivkovic, 1988). The distribution of zolpidem binding sites in rodent brain parallels that of the classic Bz-Type 1-selective triazolopyridazone CL 218, 872 (Benavides et al., 1988, 1992; Langer and Arbilla, 1988). Zolpidem preferentially binds GABA\(_A\) receptor isoforms containing the alpha-1 subunit (combined with beta-2, gamma-2) and showed lower affinity for receptor isoforms in which alpha-2, alpha-3 and alpha-5 subunits re-

 \[ \text{ABBREVIATIONS: Bz, benzodiazepine; GABA, } \gamma\text{-aminobutyric acid; i.g., intragastric; p.o., per os.} \]

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Zolpidem did not increase the intake of palatable food in satiated rats (Yerbury and Cooper, 1989), standard food (i.e., rat chow) in food-restricted rats (Sanger and Zivkovic, 1988) or the consumption of hypertonic saline solutions in rehydrating rats (Cooper and Desa, 1988). However, zolpidem did increase consumption of palatable fluid in water-deprived rats, and the increases were similar to those produced by diazepam (Stanhope et al., 1993). The discriminative stimulus effects of zolpidem were distinguishable from those of classic Bz agonists. Rats trained to discriminate chlordiazepoxide from saline did not generalize fully to zolpidem, even when tested at doses that suppressed responding (Depoortere et al., 1986; Sanger et al., 1987); all rats generalized to other Bzs and to some non-Bz ligands. Furthermore, not all rats trained to discriminate zolpidem from saline generalized to chlordiazepoxide or to most other Bzs, even when tested at doses that suppressed responding (Sanger et al., 1987; Sanger and Zivkovic, 1986). Not all lorazepam-trained rats showed full generalization to zolpidem, whereas diazepam- and pentobarbital-trained rats did (Ator and Griffiths, 1992a). However, Rowlett and Woolverton (1997) found that pentobarbital-trained rats did not generalize to zolpidem. Rats trained to discriminate a low and a high dose of midazolam from the no-drug condition generalized to zolpidem by making the low- but not the high-dose response (Sannerud and Ator, 1995). Cross-tolerance between zolpidem and Bz agonists apparently does not develop in mice and rats (Cohen and Sanger, 1994; Cox et al., 1988; Sanger and Zivkovic, 1987). Although tolerance generally develops to the sedative and ataxic effects of Bz agonists, there was only a small degree of tolerance to the rate-decreasing effects of zolpidem on operant behavior after repeated dosing (Sanger and Zivkovic, 1987, 1992). Similarly, there was no evidence of physical dependence as measured by a lack of convulsant activity after flumazenil administration or discontinuation of chronic zolpidem administration in mice and rats (Perrault et al., 1992; VonVoightlander and Lewis, 1991). Taken together, the behavioral data in rodents suggest a unique pharmacological profile for zolpidem when compared with classic Bzs.

In studies in nonhuman primates, Rowlett and Woolverton (1997) found that rhesus monkeys trained to discriminate pentobarbital from saline generalized to zolpidem. Griffiths et al. (1992) found that four of five baboons trained to discriminate lorazepam from the no-drug condition and three of three baboons trained to discriminate pentobarbital from the no-drug condition generalized to zolpidem. Similarly, normal human subjects trained to discriminate pentobarbital from placebo also generalized to zolpidem (Rush et al., 1997). Zolpidem, triazolam and temazepam produced similar behavioral and subject-rated effects in normal human subjects in a double-blind study (Rush and Griffiths, 1996). In other studies in human subjects, zolpidem and triazolam produced similar effects on most measures (e.g., behavioral performance and subject-rated effects) (Evans et al., 1990; Mintzer et al., in press). However, there are differences between zolpidem and triazolam in some performance measures in normal subjects (Mintzer et al., in press) and in subjective effects in subjects with histories of drug abuse (Evans et al., 1990). In baboons, behavioral signs of sedation and myorelaxation decreased with repeated administration of zolpidem, which suggests the development of tolerance (Griffiths et al., 1992; Kaminski et al., 1993). In addition, discontinuation of zolpidem after 2 weeks of i.v. administration in baboons produced a decrease in the number of pellets delivered per day (Griffiths et al., 1992; Kaminski et al., 1993) and produced other elements of a mild withdrawal syndrome (Kaminski et al., 1993), which indicated that the baboons may have become physically dependent on zolpidem. Mild to intermediate withdrawal symptoms (including tremor, jerk, nausea and abdominal pain) have been reported in patients treated for insomnia with high doses of zolpidem (Cavallaro et al., 1993). Thus, the effects of zolpidem appear to be more similar to those of classic Bz agonists in nonhuman primates and humans than they are in the rat.

The present study was designed to further characterize the behavioral effects and possible physical-dependence-producing effects of zolpidem in baboons. The effects of a range of zolpidem doses (1.0–32.0 mg/kg), delivered via the i.g. route, were compared with a vehicle condition, using a once-daily dosing regimen. The initial dose of zolpidem used in the current experiments was the lowest dose to occasion drug-lever-appropriate responding (≥80%) in most baboons in a drug discrimination procedure (Griffiths et al., 1992). Specifically, three of five baboons trained to discriminate lorazepam (1.8 mg/kg p.o.) from the no-drug condition responded on the lorazepam-appropriate lever after receiving 1.0 mg/kg p.o. zolpidem. A dose of 3.2 mg/kg p.o. zolpidem occasioned lorazepam-appropriate responding in four of five lorazepam-trained baboons. Thus, it was presumed that the lowest zolpidem dose, 1.0 mg/kg i.g., chosen for the current experiment would be behaviorally active.

The dose was increased by a 0.5 log unit approximately every 17 days. The Bz antagonist, flumazenil, was administered after 2 weeks of each dosing condition to determine whether a precipitated withdrawal syndrome would occur. To aid in evaluation of the dependence-producing effects of once-daily dosing with zolpidem, blood plasma determinations of zolpidem were made with blood samples obtained 24 hr after dosing on days 15, 16 or 17 of each dose condition. After the highest dose condition was completed, the nature and the time course of spontaneous drug withdrawal was evaluated after discontinuation of zolpidem dosing.

**Methods**

**Subjects**

Three adult male baboons (Papio cynocephalus, baboon GZ, and Papio anubis, baboon DI and DS, obtained from Primate Imports, New York, NY) served as subjects. All baboons had previous experience under operant conditioning procedures with food pellet reinforcement. Baboons GZ and DS had served in a study that investigated the physical dependence potential of i.g. triazolam and zaleplon. Baboon GZ also had a 3-year history of i.v. self-administration of various nonsedative drugs (e.g., cocaine, imipramine). Baboon DI had a 9-year history of i.v. self-administration that included sedative/anxiolytics (Griffiths et al., 1991). No experimental drug conditions had been conducted with any of the baboons for 3 to 11 months before the current experiments and feeding generally had been unrestricted. The baboons received one piece of fresh produce and a multi-vitamin daily (generally at about 11:00 A.M.), and had access to 1-g banana-flavored pellets (BIO-Serv, Frenchtown, NJ, for baboons DS and DI, and P. J. Noyes, Lancaster, NH for baboon GZ) for 20 hr each day (see below). Tap water was continuously available, and the amount consumed was recorded daily. Body weights for the three baboons ranged from 24.5 to 38.5 kg before the first experimental condition began.
Intragastric catheters for baboons GZ and DS had been implanted 10 and 17 months, respectively, before the current experiments and were still patent. The i.g. catheter for baboon DI was implanted 5 weeks before the current experiments. Procedures for the i.g. surgery and description of the vest and tether system have been described previously (Lukas et al., 1982). The catheter was implanted surgically into the stomach. The catheter exited in the midscapular region of the back and was protected by a vest and tether system that permitted the baboon free movement inside the cage. Baboons were recovered fully from the surgery and showed normal dietary intake before experimental conditions were initiated. Once the experimental dosing regimen began, the baboons were anesthetized with ketamine HCl (150–300 mg) generally every 15 to 17 days (i.e., in the 3 days after the flumazenil challenge described below) to permit weighing, medical examinations, exit site maintenance and cage washing. Catheter blockage or equipment problems occasionally necessitated unplanned ketamine administration as noted below.

Apparatus

Baboons were housed individually in standard primate cages, which also served as the experimental chambers, in the laboratory. The tether was connected to a customized liquid swivel (ITC Life Science, Woodland Hills, CA), which was attached to the top of the cage. To maintain catheter patency, distilled water was infused slowly (approximately 550 ml/24 hr) into the catheter using a peristaltic pump (model 1201, Harvard Apparatus, South Natick, MA). Cages were equipped with a bench, which ran along one of the side walls, a stainless steel water spout, which was attached to the front of the cage, and an aluminum intelligence panel (45.7 × 63.5 cm), which was mounted on the rear wall. A Lindsley operandum (Gerbrands Corp., Arlington, MA) was mounted in the lower left quadrant of the panel. A pull of the operandum sufficient to operate a microswitch was required for the response. A green, 1.5-cm jewel light was mounted above the operandum. A hopper, for delivery of food pellets, was located in the center of the panel. The back wall of the food hopper was a white Plexiglas panel (5 × 5 cm), which was transilluminated during pellet delivery. The pellet feeder (BRS/LVE, Inc., Laurel, MD or Gerbrands Corp., Arlington, MA), the water bottle and the peristaltic pump were on a grating above the cage. Room ceiling lights were brightly illuminated for 13 hr/day (6:00 A.M. to 7:00 P.M.) and dimly illuminated for the remaining 11 hr/day.

The number of pellets delivered per 30-min interval was collected with IBM compatible personal computers with Med-PC software and instrumentation (Med Associates, Inc., East Fairfield, VT). Behavioral observation data were collected using IBM compatible laptop computers, programmed to provide a screen format and prompts for entering each behavioral sign and posture; the raw data were saved to diskettes. The raisin retrieval task used clear Plexiglas trays onto which six 3-cm cups with 1-cm-high rims were mounted. The distance between the cups permitted each cup to be easily accessible between the bars of the cage when the tray was placed against them at the front of the baboon cage. The raisin retrieval task was timed using a stopwatch.

Procedures

Food reinforcement procedure. During all dosing conditions, the baboons had access to an unlimited number of food pellets each day by completing a fixed number of responses on the Lindsley operandum for each pellet delivery (i.e., a fixed-ratio, FR, schedule of reinforcement). The FR value was adjusted before beginning the experimental conditions so that 1) the number of pellets delivered per day was stable (i.e., no increasing or decreasing trends) for at least 14 days, 2) virtually all pellets delivered per day were consumed (i.e., no more than two to three pellets were found in the pans) and 3) the number of pellets delivered per day was sufficient to maintain body weights in adult baboons of their size and activity level. The pans under the baboons cages were inspected daily for pellets to confirm that the pellets obtained were consumed. The final FR value was 10 responses for each pellet delivery for all baboons. Stable performance under an FR 10 schedule of food reinforcement was established for at least a month before the vehicle base line (see below) began. Pellet availability was correlated with illumination of the jewel light above the Lindsley operandum. During i.g. dosing conditions, pellet availability began 15 min after the i.g. injection and continued for 20 hr; pellet access was terminated 4 hr before drug dosing to facilitate drug absorption.

Once-a-day dosing conditions and flumazenil administration procedures. Injections were administered daily via the catheter at approximately 9:00 or 9:30 A.M. (±15 min), depending on the baboon. The successive dosing conditions were: vehicle, 1.0, 3.2, 19.0 and 32.0 mg/kg zolpidem. Each dose condition lasted 17 days, except that the 32.0 mg/kg condition continued an additional 10 days after the flumazenil challenge before drug dosing was terminated (as described below). On days 10 and 14 of each dose condition, flumazenil vehicle and 5 mg/kg of flumazenil, respectively, were administered i.m. to assess whether a precipitated withdrawal syndrome would occur. The high dose of flumazenil (5 mg/kg) was chosen because 1) it has reliably precipitated withdrawal in baboons treated with chronic high Bz doses (Ator and Griffiths, 1992b; Lamb and Griffiths, 1984, 1985; Lukas and Griffiths, 1984) and 2) it facilitates comparison of the present results with previous studies on Bz dependence in the baboon. Dosing with zolpidem continued for an additional 3 days after flumazenil to permit restabilization and to provide a window of opportunity for conducting the baboon weighing and physical examination (as described above) before the next dosing condition began. After 78 to 79 days of total dosing with zolpidem, drug dosing was terminated.

Observations

Observations were conducted with use of a list of behavioral signs and postures that had been established previously as reliable indicators of the Bz withdrawal syndrome, and provided a sensitive assessment of activity levels, sedation and motor coordination (Ator and Griffiths, 1992b; Lukas and Griffiths, 1982; Sannerud et al., 1992). These behavioral signs and postures are defined in table 1.

The observer (EMW, DMG and research technician MFC) sat at a small table with a laptop computer in front of each baboon’s cage. The list of possible behavioral signs and postures that could be scored were displayed on the computer screen. Frequency counts of behavioral signs were collected in consecutive, 1-min intervals. A computer-generated tone sounded at the beginning of each interval prompting the observer to record each baboon’s posture. In addition, observers could type comments at any time, which were saved in 1-min bins across the observation period; all observers were instructed to include in the comments whether vomiting, or just retching, occurred. They also noted if food-maintained responding, defecation and/or diarhrea occurred during each interval.

Before the experiment began, the behavioral definitions were memorized by all observers and training observations were conducted by pairs of observers. During this period, the baboons were habituated to the observers and observation procedures, and differences among observers in interpretations of the behavioral definitions and other aspects of recording behavior were resolved. The criterion for agreement on occurrence and nonoccurrence of all behaviors and postures between all possible pairs of observers was 90% or greater before experimental conditions were initiated. During the experiment itself, independent observations were conducted by two observers during randomly selected 30-min and 60-min observation sessions; observations included all possible pairs of observers (i.e., EMW and DMG, DMG and MFC, MFC and EMW). The percent reliability was calculated as follows: The occurrence or nonoccurrence of each behavior and posture recorded during each 1-min interval of the observation session was counted for each observer. The agreement between observers for the occurrence of each behavior and posture and the agreement between observers for the non-

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TABLE 1  
Definitions of behavioral signs and postures scored in observation sessions

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<th>Behavioral signs</th>
<th>Definitions</th>
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<tr>
<td>Aggression</td>
<td>Behavior directed at another animal or human consisting of barking, yawning with canines exposed,</td>
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<td>cage shaking, raised eyebrows and staring.</td>
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<tr>
<td>Ataxia&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Incoordination that is characterized by slow, uncertain movements (for example, when reaching, slow</td>
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<td></td>
<td>ly and with jerking movement or may overshoot its mark) and tremor (action and postural); can also include</td>
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<td>swaying or falling over during sitting and locomotion.</td>
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<tr>
<td>Bruxism</td>
<td>Gritting or grinding the teeth.</td>
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<tr>
<td>Eyes closed</td>
<td>Eyelids closed over the eyeballs for at least 2 sec, as in sleeping.</td>
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<tr>
<td>Grooming</td>
<td>Rubbing hair in search of objects (parasites, dirt) and removal of found objects.</td>
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<tr>
<td>Jerk&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Muscle contraction and/or relaxation leading to a quick jerking movement or twitch; jerk may be</td>
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<td></td>
<td>rhythmic and repetitive or shock-like (e.g., clonic or myoclonic jerks); can be independent of</td>
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<tr>
<td>Lip droop</td>
<td>Loss of facial muscle tone evidenced by a characteristic droop of the lower lip.</td>
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<tr>
<td>Lip smack</td>
<td>Rapid movement of the tongue and lips resulting in a smacking sound that usually is directed at</td>
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<td>either another animal or a human.</td>
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<tr>
<td>Locomotion</td>
<td>Gross movement from one location to another with use of the limbs.</td>
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<tr>
<td>Masturbation</td>
<td>Either manipulation of the flaccid penis until it is erect or manipulation of the erect penis,</td>
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<tr>
<td></td>
<td>usually characterized by a stroking movement.</td>
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<tr>
<td>Nose rub</td>
<td>Substantial displacement of the nares externus from midline by either a limb or cage bars.</td>
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<tr>
<td>Nose wipe</td>
<td>Quick, wiping movement across the nares externus with a limb.</td>
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<tr>
<td>Scratch</td>
<td>Rubbing or scraping slightly, as with the fingernails. Note: changes from one spot on the body to</td>
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<td>another defines the beginning of a new scratching bout.</td>
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<tr>
<td>Seizure&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Consists of several components: 1) “Tonic spasm” that is characterized by contraction of muscle</td>
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<td>groups; usually takes the form of extension of first, the back and neck and second, the arms and</td>
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<td></td>
<td>legs. Breathing may be suspended. 2) “Clonic” jerks that are characterized by a mild, repetitive</td>
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<td></td>
<td>tremor with progression to brief, violent, rhythmic flexor spams that involve the body. After tonic</td>
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<td>and clonic phases, animal may lie still for about 5 min.</td>
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<tr>
<td>Tremor&lt;sup&gt;a&lt;/sup&gt;</td>
<td>A more or less regular, rhythmic movement of the limbs, head, or trunk produced by synchronous</td>
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<td></td>
<td>contractions of antagonistic muscles. Rhythmic quality distinguishes it from other involuntary</td>
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<td></td>
<td>movements. Its biphasic character distinguishes it from clonic jerks. It frequently occurs during</td>
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<td></td>
<td>periods of demand on the muscles (action and postural tremors) such as when the animal is reaching</td>
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<td>for an object.</td>
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<tr>
<td>Vomit/retch</td>
<td>Making an effort to vomit (often accompanied by a “gagging” sound) or disorging the stomach</td>
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<td>contents through the mouth; may not expel the vomitus; in such cases, the presence of vomit may</td>
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<td>often be assumed if the animal subsequently engages in chewing and swallowing.</td>
</tr>
<tr>
<td>Wet dog shake</td>
<td>Rapid, side-to-side movement of the head and upper body, resembling the action of a wet dog shaking</td>
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<td>the water off of its fur.</td>
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<tr>
<td>Yawn</td>
<td>Opening the mouth wide with a prolonged, deep inhalation of air.</td>
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<tr>
<th>Postures</th>
<th>Definitions</th>
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<tr>
<td>Normal</td>
<td>Sitting upright; may be holding lightly onto bars; may have feet up.</td>
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<tr>
<td>Rigidly braced</td>
<td>Appears to brace itself by holding onto the cage bars while showing obviously enhanced muscle</td>
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<td></td>
<td>tension, faciculations, and tremors.</td>
</tr>
<tr>
<td>Lying down</td>
<td>Lying down on either the bench or cage floor, appears lethargic, and is unresponsive to normal</td>
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<td>verbal or physical stimuli.</td>
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<tr>
<td>Head lower than torso</td>
<td>Standing on all four limbs with the head held below the waist (but not if level with or higher than</td>
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<tr>
<td></td>
<td>the waist); may be accompanied by vomit and/or retch, but can occur independently; may take the</td>
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<td></td>
<td>form of the back legs on the bench and front legs on the cage floor; animal typically remains in</td>
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<td></td>
<td>posture for prolonged periods; not scored if also reaching for objects or foraging in cage pan.</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>Sitting on either the floor or bench with chin on chest and unresponsive to normal stimuli.</td>
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<tr>
<td>Convulsing</td>
<td>Posture recorded during clonic or tonic-clonic seizure (see above).</td>
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</tbody>
</table>


The occurrence of each behavior and posture was divided by the total number of intervals for that observation session. The overall inter-rater reliability for all behaviors and postures during the experiment was greater than 97%.

All observation sessions were conducted after completion of the raisin retrieval task (as described below) approximately 60 min after the daily injection. The observers were not blind to the condition in effect. On days 1 and 12 of each dosing condition, 30-min observation sessions were conducted to assess the direct effects of zolpidem during once-daily dosing. To determine whether a precipitated withdrawal syndrome would occur, observation sessions were conducted immediately after i.m. administration of flumazenil vehicle and 5 mg/kg flumazenil, respectively, on days 10 and 14 of each dose condition; these observations were extended to 60 min to encompass the precipitated withdrawal syndrome. The first 30 min of the 60-min observation on day 10 (when flumazenil vehicle was administered) were also included in the analysis of the direct effects of zolpidem. Occasionally, the days of observations were shifted because of equipment problems or catheter blockage in baboon GZ. During the suspending agent vehicle condition, the flumazenil vehicle observation was on day 12; during the 10.0 mg/kg zolpidem condition, observations were on days 11 (flumazenil vehicle), 13 and 15 (flumazenil).

To assess whether a spontaneous withdrawal syndrome would occur, a series of 30-min observation sessions was initiated 24 hr after the last injection of 32.0 mg/kg zolpidem. Observations were conducted daily for the first 15 days; on days 16 to 39, observations were conducted every other day. Observations during drug withdrawal were conducted at approximately the same time of day as previous observations. The variability in number of signs across days and in whether certain behaviors are observed on certain days is to some extent a function of the fact that the 30-min observation represents a relatively small sample of behavior that occurs each day. Thus, the absence of a score for several behaviors should not be interpreted to mean that the behavior was entirely absent, only that it was not observed within the 30-min observation period.

Raisin Retrieval Task

The direct effects of zolpidem on fine motor coordination were assessed daily with a raisin retrieval task; one raisin was placed in each of the six equally spaced cups, and the tray was placed against
the front of the cage. The tray was presented by a research technician (SRB or CDE) for 120 sec or until all six raisins were retrieved, whichever occurred first. Technicians recorded the time (seconds) to retrieve all six raisins, or if less than six raisins were retrieved, the maximum time was recorded. Research technicians also completed a checklist indicating the occurrence or non-occurrence of tremor and incoordination (as defined in table 1), the number of raisins retrieved, the number of raisins dropped and specific comments about behaviors observed during the task (e.g., “severe limb tremors” and “fell off bench while doing task”). Before beginning experimental conditions, all baboons were given repeated trials on the task, and generally retrieved all six raisins in less than 15 sec. The task was presented 1 hr after dosing each day; on days when observations occurred (e.g., days 1, 10, 12 and 14), the task was conducted immediately before the observation session. On days when flumazenil or its vehicle was administered, the raisin retrieval task was repeated immediately after the 60-min observations (i.e., 2 hr after dosing with vehicle and zolpidem) by the person that conducted the observation.

Blood Plasma Drug Level Determinations

On days 15, 16 or 17 of each dosing condition, blood samples were obtained when baboons were anesthetized with ketamine as described above. All blood was drawn at about 9:00 A.M., approximately 24 hr after the injection; the dose for that day was given after blood was drawn and when the baboon was awake from the anesthesia. Blood samples (approximately 10 ml) were injected immediately into a lithium-heparinized tube and centrifuged at 3200 rpm for 12 min. The plasma then was withdrawn and frozen in a polypropylene tube until analysis at Pharmacia and Upjohn, Inc. (Kalamazoo, MI).

Plasma zolpidem levels were assayed according to the method of Salva and Costa (1995) with some modifications. Plasma was thawed and centrifuged to a clear particulate matter. A 2-ml sample of plasma was transferred to a conical tube; 5 ng of an internal standard and 20 ml of 10 N sodium hydroxide were added. The internal standard was a zolpidem analog in which the dimethyl amide functionality was replaced by dimethyl piperazine amide. A 4-ml sample of ethyl acetate was added and the mixture was vortex-mixed. After the phases separated upon standing, the organic layer was removed and transferred to another conical tube. Another 4 ml of ethyl acetate was added and the mixture was vortex-mixed again. After the phases separated upon standing, the organic layer was removed and the two organic layers were combined and dried with a stream of nitrogen. A 150-ml sample of mobile phase was added to the dried material and vortex-mixed. The solution was transferred to a 1.5-ml Eppendorf centrifuge tube and spun at 14,000 rpm for 5 min. A 50-ml sample of the clear supernatant was injected onto an high-performance liquid chromatograph Water's Symmetry C18 column. The high-performance liquid chromatography flow rate was 0.5 ml/min. A Water’s 470 fluorometer detector was used to detect zolpidem and the internal standard at 254 l ex and 390 l em. Water’s Maxima software was used to integrate peaks that co-eluted with zolpidem and the internal standards. Plasma concentrations of zolpidem were calculated knowing the amount of internal standard added to plasma (5 ng), the integrated areas of relevant peaks and the relative response factors determined for equal amounts of zolpidem and internal standard added to and extracted from plasma from baboons treated with vehicle.

Drugs

The present study used zolpidem tartrate, and then zolpidem base (Research Biochemicals International, Natick, MA). The amount of zolpidem base weighed for each dose was adjusted by use of the 1.24 salt/base ratio (Durand et al., 1992) so that the amount of base used (0.807, 2.58, 8.07, 25.8 mg/kg, respectively) would be equivalent to that in the 1.0, 3.2, 10.0, 32.0 mg/kg doses that were calculated based on the salt. Zolpidem base was used for baboon DI at doses of 1.0, 3.2, 10.0 mg/kg; for baboon DS at 3.2 and 10.0 mg/kg; and for baboon GZ at 32.0 mg/kg. All other doses were zolpidem tartrate. The weight of the baboon at the end of each condition was used to calculate the dose for the next condition. Zolpidem stock solutions were mixed with BIO-Serv Agent K (Frenchtown, NJ), 2 g/l blended in distilled water, with a blender. The stock solution was kept in a brown glass bottle under refrigeration for up to 3 days. After mixing on a magnetic stirrer, an appropriate quantity of the stock was drawn up for each baboon’s dose each day. Higher injection volumes were used at higher doses because of problems with catheter blockage after drug injections. The injection volumes were as follows: 10 ml for 1.0 and 3.2 mg/kg, 50 ml for 10 mg/kg and 200 to 500 ml (depending on the weight of the baboon) for 32 mg/kg zolpidem. In the 32 mg/kg condition, the dose for each individual baboon was mixed in an electric blender immediately before administration. Injections were followed by a 10- to 20-ml flush of the BIO-Serv suspending agent suspension. Flumazenil (5 mg/kg, Hoffmann-LaRoche, Basel, Switzerland) was prepared in a vehicle of propylene glycol/ethanol (95%/sterile water (40:10:50 v/v)). The mixture was injected i.m. at a volume of 2 ml immediately after mixing.

Data Analysis

A single-subject design was used in which each baboon served as his own control. The direct effects of dosing with zolpidem on pellets per day and on all behaviors recorded during the 30-min observational sessions on days 1 and 12 and in the first 30 min of the 60-min observation on day 10 (when flumazenil vehicle was administered) were judged to be significant based vehicle control z-scores. Control observations were conducted before and after drug dosing; these included days 1, 10 and 12 during the original vehicle base line and the last 3 days of postwithdrawal vehicle observations (days 31, 33, 35, 37 or 39, depending on the baboon). For behaviors that were predicted to increase (e.g., lip droop, ataxia, withdrawn chin on chest posture, lying down, eyes closed), significance was determined if the observed frequency of that behavior or posture exceeded the vehicle control z-score, above which 5% of the scores would be expected to fall for a one-tailed test. For all other behaviors that were predicted to increase or decrease, the z-scores delineated the top 2.5% and the bottom 2.5% for a two-tailed test.

A constellation of behaviors considered to be indicative of a Bztype withdrawal syndrome in the baboon was used to derive a “withdrawal score” for both flumazenil-precipitated withdrawal and spontaneous withdrawal. In deriving the withdrawal score, 1 point was assigned in each of nine categories of behaviors or postures; the maximum possible score was 9. The nine categories of behavioral and postural differences used to determine the withdrawal score were as follows: 1) pellets per day decreased; 2) the time to complete the second raisin retrieval task increased; 3) the number of 1-min intervals increased in which eyes were closed or in which lying down, head lower-than-torso posture and/or a withdrawn chin-on-chest posture were observed; 4) locomotion increased or decreased; 5) self-directed behaviors (nose rub, nose wipe, scratch and wet dog shake) increased; 6) aggressive threat, bruxism or yawning increased; 7) tremor (limb/body), jerk and/or rigidly braced posture increased; 8) vomit and/or retch increased; 9) seizures increased. It is important to note that this is a nonweighted scale; a higher score does not necessarily denote a more severe withdrawal.

Behaviors during the 60-min flumazenil-challenge observations were judged to be significantly increased if they were higher than the frequencies recorded during the following three observation sessions: 1) the flumazenil vehicle during the original suspending agent vehicle condition, 2) flumazenil during the original suspending agent vehicle condition and 3) flumazenil vehicle during the same dosing condition. These three control observations accounted for the effects of the suspending agent vehicle, of the flumazenil alone and of chronic drug on the behavior.

The incidence of spontaneous withdrawal behaviors scored during a 30-min observation session after drug dosing ended was judged as
significantly increased or decreased as follows: For behaviors that were predicted to increase during withdrawal, points were assigned only if a) the observed frequency of that behavior or posture exceeded the vehicle control z-score, above which 5% of the scores would be expected to fall for a one-tailed test. Because locomotion was predicted to increase or decrease during withdrawal (increased activity has been reported during withdrawal from Bzs; Rickels et al., 1990), the z-scores for this behavior delineated the top 2.5% and the bottom 2.5% for a two-tailed test. The vehicle control z-scores were based on the frequencies recorded during a total of six observation sessions; these included the three observation sessions (i.e., days 1, 10, 12) during original suspending agent vehicle base line, and the last 3 days of observations during the postwithdrawal period (i.e., days 31, 33, 35, 37 or 39, depending on the baboon). An additional criterion for inclusion in the withdrawal score was that b) the observed frequency of behavior also had to exceed the frequencies obtained during the three observations during the 32.0 mg/kg condition which preceded termination of zolpidem dosing. This criterion accounted for both time and/or drug-produced changes in the behaviors.

Yanagita and Takahashi (1970) used the presence of or change in specific behaviors to characterize the severity of withdrawal as “mild,” “intermediate” or “severe” in barbiturate-dependent rhesus monkeys, and later to grade withdrawal from different Bzs including chlordiazepoxide, diazepam and triazolam (reviews in Woods et al., 1987). To allow comparison across studies, classifications of withdrawal as mild, intermediate and severe, which were based on those used by Yanagita and colleagues, also were used in the present study. Specifically, behavioral signs that would result in a mild classification included aggression, mild tremor and reduction in pellets delivered per day in the current study. Additional behaviors that have been associated with mild Bz withdrawal in the baboon include increased self-directed behaviors (e.g., nose rubbing, nose wiping, scratching), withdrawn posture (i.e., sitting with chin on chest) and postures that may be associated with abdominal discomfort and nausea (e.g., head-lower-than-torso posture). Signs that resulted in an intermediate classification included aggravated tremor, rigidly braced postures (i.e., muscle rigidity), impaired motor activities and vomit and/or retch. Signs that resulted in a severe classification were the occurrence of seizures and convulsions. Thus, withdrawal would be characterized as intermediate if vomit, retch, tremors, jerks and/or rigidly braced posture was observed and severe if seizure activity was observed regardless of the total number of signs observed.

**Results**

**The Effects of Zolpidem During Once-a-Day Dosing**

**Observations.** All three baboons showed increases in one or more behavioral signs associated with sedation during zolpidem dosing, particularly at the higher doses (10.0, 32.0 mg/kg). Among the specific behaviors listed that could be scored, the sedative/myorelaxant effects of a drug would be detected by increases in ataxia, eyes closed, lip droop, decreases in locomotion and increases in the frequency of resting postures (e.g., lying down and/or the withdrawn chin-on-chest posture). Locomotion was not decreased in any baboon compared with vehicle. Lip droop and ataxia were not observed during vehicle control conditions. Lip droop was observed in two baboons at 3.2 and 10.0 mg/kg, and in all three baboons at 32.0 mg/kg zolpidem. Ataxia was observed in two baboons at the two lower zolpidem doses (1.0, 3.2 mg/kg), and more frequently in all three baboons at the two higher doses (10.0, 32.0 mg/kg). During observation sessions, ataxia was typically recorded during operation of the food lever and during position changes. Severe ataxia was noted during the raisin retrieval task in two baboons; comments written by the observers indicated that baboon GZ was “falling off of bench” during raisin retrieval task on day 22 of 32.0 mg/kg zolpidem, and that baboon DI “fell off the bench twice” while doing the raisin retrieval task on day 11 of 10.0 mg/kg zolpidem. Baboon DS showed less ataxia, but showed an increased incidence of resting postures (i.e., lying down and/or withdrawn chin-on-chest posture) relative to vehicle.

In addition to the behavioral signs of sedation recorded during the observation sessions, chronic zolpidem produced some signs that may be associated with abdominal discomfort (i.e., head-lower-than-torso posture, vomit and/or retch and grimace) in two baboons. Vomit and/or retch and grimace were not observed during the initial vehicle base-line observations or during the postwithdrawal observations (i.e., baseline levels were recovered). During observation sessions, baboon DI showed grimacing and/or retching at 1.0, 3.2 and 32.0 mg/kg zolpidem, and baboon DS vomited at 32.0 mg/kg zolpidem. Grimacing and retching typically were accompanied by head-lower-than-torso posture in baboon DI. Vomiting was observed outside of the observation period in baboon DS; DS vomited on day 16 of 3.2 mg/kg zolpidem, and on days 9, 21 and 22 of 32.0 mg/kg zolpidem.

The frequency of locomotion, aggression, bruxism, lip smacks, nose wipes, nose rubs, wet-dog shakes, grooming, tremor, jerk and yawns were not systematically altered in the three baboons by zolpidem administration. Rigidly braced posture or convulsing were never observed during zolpidem dosing.

**Raisin retrieval task.** Figure 1 shows the effects of zolpidem on the time (seconds) to complete the raisin retrieval task for the first 14 days of each dose. The task was conducted approximately 1 hr after dosing with vehicle or zolpidem. During the vehicle base line, the time to complete the task (i.e., retrieve all six raisins) was relatively stable for each baboon. The time to complete the raisin retrieval task increased in a dose-dependent manner. As dose increased, the time to complete the task also became more variable for each baboon. Time to complete the task clearly increased above vehicle in all three baboons at the 10.0 and 32.0 mg/kg doses.

In addition to taking longer to complete the raisin retrieval task, baboons showed incoordination and tremors during the task (data not shown). Incoordination was variable across days and dosing conditions, but tended to increase dose-dependently in two of three baboons. At the lowest zolpidem dose (1.0 mg/kg), incoordination was observed for 7, 2 and 0 of 16 total test days in GZ, DS and DI, respectively. Incoordination was observed during the 1.0 mg/kg condition for 11 of 14 total test days in baboon GZ (The task was not conducted on days 2, 3 and 9 because baboon GZ had to be anesthetized to unblock the catheter.) and for 11 and 2 of 16 total test days in baboons DS and DI, respectively. During the 32.0 mg/kg condition, incoordination was observed for 23 of 23 total test days in baboon GZ (Days 8 and 9 were lost because of the need to unblock the catheter) and 3 and 19 of 25 total test days in baboons DS and DI, respectively. No instances of tremor or incoordination were observed during the initial vehicle condition. Tremor occasionally was observed during dosing: Baboon DI showed tremor at 10.0 and 32.0 mg/kg zolpidem, and baboon DS showed tremor on days 5 and 11 at 32.0 mg/kg.
Food-maintained behavior. Figure 2 shows the number of pellets delivered per day during the first 14 days of each dosing condition. Evaluation of the 30-min bins of food-maintained responding showed that during vehicle conditions, pellets were consumed during multiple feeding bouts throughout the day; the total pellets per day ranged between 108 and 277. During zolpidem dosing, the number of pellets per day gradually increased in a dose-dependent manner in two (GZ and DS) of the three baboons; by the end of the 32.0 mg/kg dosing condition, body weights had increased from 31.3 to 32.7 kg in baboon GZ and from 38.4 to 41.8 kg in baboon DS. The pellet data in 30-min bins indicated that baboons GZ and DS began responding immediately after pellets became available and that most of the pellets per day were obtained in the first 2 to 4 hr of the 20-hr period of pellet availability. For baboon DI, there were relatively small changes in the number and distribution of pellets obtained during the 20-hr period of pellet availability during zolpidem dosing conditions; at low doses (1.0, 3.2 mg/kg) pellets were slightly increased above vehicle on some days and body weight was increased by 0.5 kg. However, when compared with the 3.2 mg/kg condition, pellet intake during the 10.0 and 32.0 mg/kg conditions returned to lower levels (but generally within the vehicle base-line range); weight for baboon DI decreased from 24.5 kg in the 3.2 mg/kg condition to 23.4 kg by the end of the 32.0 mg/kg condition. Daily inspection of the pans and food hoppers indicated that virtually all pellets delivered were consumed (i.e., no more than two to three pellets were found in the pan under the cage).

Flumazenil-Precipitated Withdrawal

The effects of the Bz antagonist flumazenil on behaviors relevant to a Bz withdrawal syndrome are summarized with withdrawal scores (fig. 3). Flumazenil administration after 2
weeks of daily administration of the lowest dose (1.0 mg/kg) of zolpidem increased one or more signs when compared with control observations (see “Data Analysis”); withdrawal scores ranged from 1 to 5. In the 3.2 and 10.0 mg/kg conditions, peak withdrawal scores ranged from 2 to 5. The highest withdrawal scores were at the 32.0 mg/kg condition; peak withdrawal scores were 6, 6 and 4 for baboons GZ, DS and DI, respectively.

Flumazenil did not reduce the number of pellets delivered for that day below the range of pellets for the control conditions (see also last day shown in each condition in fig. 2). The raisin retrieval task that was presented 1 hr after flumazenil administration was refused by baboon DS at doses of 3.2 mg/kg zolpidem and higher, but there were no effects in this task after flumazenil for the other two baboons. The number of baboons showing increases in the number of 1-min intervals spent with eyes closed, or in certain postures (i.e. lying down, withdrawn with chin on chest posture, and/or in the head-lower-than-torso posture, which is often associated with nausea and vomiting) were dose-dependently increased: one of three baboons showed an increase in these postures at 1.0 mg/kg zolpidem, as did two of three baboons at 3.2 and three of three at 10.0 and 32.0 mg/kg. Aggressive behaviors (i.e., aggressive threats, yawning and bruxism) increased in baboon GZ in all dose conditions, and in all three baboons at the highest zolpidem dose condition (32.0 mg/kg). All three baboons showed changes in locomotion, which were unsystematically related to dose. Two baboons (DS, DI) showed higher self-directed behaviors (i.e., nose rubbing, nose wiping, scratching and wet dog shakes) after flumazenil in all zolpidem dose conditions. The third baboon (GZ) showed increased self-directed behaviors at 1.0 and 32.0 mg/kg zolpidem, but not at other doses. Baboons GSZ and DI showed increased tremor, jerk and/or rigidly braced posture after flumazenil in three of the four zolpidem dose conditions, but baboon DS did so only at the highest dose (32.0 mg/kg).

In summary, intermediate withdrawal signs (i.e., tremor, jerk and/or rigidly braced posture) were observed in only 1 baboon after flumazenil at the 1.0 mg/kg zolpidem condition. The other two baboons showed mild signs of withdrawal (e.g., increased self-directed behaviors). At the 3.2 mg/kg zolpidem condition, intermediate withdrawal signs were observed in two of three baboons; tremor, jerk and/or rigidly braced posture was increased for both baboons, and vomit and/or retch was also increased in the baboon that had showed tremor and/or jerk at the previous dose condition. At 10.0 mg/kg zolpidem, mild to intermediate withdrawal signs were also observed; one baboon each showed increased self-directed behavior, aggression and tremor, jerk and/or rigidly braced posture. At 32.0 mg/kg zolpidem, intermediate withdrawal signs were observed in all three baboons; vomit and/or retch was increased in two baboons, and tremor, jerk and/or rigidly braced posture was increased in all three baboons. Thus, the
number of signs and the severity of the signs observed during flumazenil-precipitated withdrawal tended to increase across the zolpidem dosing conditions.

**Spontaneous Withdrawal**

Dosing with zolpidem ended after the 32.0 mg/kg dosing condition. At the time drug was discontinued, the total number of days of dosing with zolpidem (1.0–32.0 mg/kg) was 78 days for baboons DS and DI and 79 days for baboon GZ. The effects of termination of chronic zolpidem dosing on signs during observation sessions, food-maintained behavior, and time to complete the raisin retrieval task are summarized by withdrawal scores. Figure 4 shows the spontaneous withdrawal scores across the first 15 days after zolpidem dosing ended. Baboons GZ and DS had scores of 2 on the first day, but baboon DI first showed withdrawal signs on day 2 (score of 1). The peak scores ranged from 4 to 5 across the three baboons, and the peak score first occurred by 5 to 10 days after dosing ended. By the 15th day of this condition, scores ranged from 3 to 4.

Withdrawal from zolpidem did not disrupt performance of the raisin retrieval task. In fact, the duration to complete the task rapidly returned to vehicle control levels in all three baboons after the discontinuation of zolpidem dosing. In addition, baboons generally completed the task without signs of tremor or incoordination. During the observation sessions, there was an increased number of 1-min intervals with postural changes (i.e., lying down, head-lower-than-torso posture, withdrawn chin-on-chest posture) and/or eyes closed. Locomotion was relatively unaffected, but all baboons showed increased frequency of self-directed behaviors (i.e., nose wipe, nose rub, scratch and/or wet-dog shakes), and aggressive behaviors (i.e., aggressive threats, yawns, bruxism). The number of times these contributed to the withdrawal score varied across baboons, and did not show a pattern across days. All three baboons showed tremor, jerk and/or rigidly braced posture; but for baboons GZ and DS, it was on 5 and 7, respectively of the first 15 days and for DI, it was only on 4 of those days. Vomit and/or retch was scored on 2 or 3 of the first 10 days of withdrawal for baboons DS and DI. Seizures were not observed in any of the baboons.

As also shown in figure 5, pellets/day decreased below base-line vehicle control levels for at least the first 15 days in two baboons (GZ, DS), and then increased toward the vehicle control levels. Weight loss was also evident after termination of zolpidem dosing in baboon GZ (from 32.7 to 30.5 kg), and DS (from 41.8 to 39.2 kg) when compared with weights during the 32.0 mg/kg zolpidem condition that preceded the withdrawal condition; these body weights were similar to those obtained during the initial vehicle baseline. The number of pellets delivered per day and body weight for baboon DI after termination of zolpidem dosing were similar to those obtained during vehicle control and during the 32.0 mg/kg zolpidem dosing conditions.

**Levels of Drug in Blood Plasma**

Figure 6 presents plasma levels for zolpidem 24 hr after dosing with each dose of zolpidem. Zolpidem was detected 24 hr after drug administration in all three baboons at even the lowest doses. Zolpidem levels in plasma generally increased dose-dependently. Plasma levels ranged from 0.66 to 2.87 ng/ml at 1.0 mg/kg zolpidem and from 1.36 to 4.09 ng/ml at 3.2 mg/kg zolpidem. Plasma levels were increased substantially in two baboons (DS and DI, 19.4 and 17.4 ng/ml, respectively), and were slightly increased in the third baboon (GZ, 4.23 ng/ml) at 10.0 mg/kg zolpidem. At 32.0 mg/kg zolpidem, plasma levels were higher for baboon GZ (11.2 ng/ml), similar to the 10.0 mg/kg dose for baboon DS (19.6 ng/ml) and reduced in baboon DI (3.41 ng/ml). Chromatograms showing the relative peak areas for each dose indicated a lack of proportionality for zolpidem for baboon DI for
Discussion

The current results demonstrated that zolpidem produced behavioral signs of sedation, myorelaxation and motor impairment under once-daily dosing conditions in baboons. Zolpidem has produced motor impairment in rodents (Sanger et al., 1987, 1996) and humans (Dingemanse et al., 1992) and of high doses of Bz agonists (e.g., chlordiazepoxide, diazepam, lorazepam, midazolam and triazolam) (Ator et al., 1996; Griffiths et al., 1981, 1991; Sannerud et al., 1989). Thus, the sedative effects of zolpidem in the current study were similar to the sedative effects of Bz agonists and were consistent with the sedative-hypnotic profile of zolpidem in rodents (Depoortere et al., 1986; Sanger et al., 1987; Sanger and Zivkovic, 1988) and humans (Balkin et al., 1992; Langtry and Benfield, 1990).

Benzodiazepine agonists typically increase consummatory behaviors in rats and nonhuman primates, including the baboon (Cooper and Moores, 1985; Foltin et al., 1989; Randall et al., 1960; Weerts et al., 1993). In the current study, zolpidem increased food-maintained behavior; and this increase was progressive in two of the three baboons across the weeks in which the i.g. dose was increased every 17 days. Because virtually all pellets delivered were consumed by the baboons, this increase also represents an increase in food intake. Similar effects on food intake were observed with the triazolo-benzodiazepine hypnotic triazolam (Ator et al., 1996) and the pyrazolopyrimidine zaleplon in baboons with the same once-daily dosing procedure (Ator NA, Weerts EM, Kaminski BJ, Kautz MA and Griffiths RR, unpublished observations). The finding that zolpidem increased food-maintained behavior and food intake in the current experiment is consistent with previous studies in baboons in which zolpidem was delivered via the i.v. route (Griffiths et al., 1992; Kaminski et al., 1993), but contrasts with studies in rats that showed that zolpidem did not alter consummatory behaviors (Cooper and Desa, 1988; Sanger and Zivkovic, 1988; Yerbury and Cooper, 1989; cf., Stanhope et al., 1993, who found increases in drinking palatable fluids in water-deprived rats).

Although zolpidem increased food-maintained behavior in baboons, zolpidem also produced signs suggestive of abdominal discomfort, including vomiting, in two of three baboons. These signs were not observed during vehicle control conditions and have not been observed during dosing with other sedative-hypnotics (e.g., triazolam) using this dosing procedure in baboons (Ator et al., 1996). Evans et al. (1990) reported that zolpidem (15.0, 30.0 and 45.0 mg) (but not triazolam, 0.25, 0.5, 0.75 mg) increased human subject ratings of “queasy/sick to stomach,” and also produced emesis (5 of 15 subjects vomited after the highest zolpidem dose, and 4 of 15 subjects vomited after the two lower doses.) In a post-marketing survey in insomniac patients (n = 1972) treated with zolpidem (Ganzoni et al., 1995), there were 33 reports (1.7%) of gastrointestinal distress that included nausea, vomiting, abdominal pain and/or diarrhea.

There was little evidence of tolerance to the behavioral effects of zolpidem during once-daily dosing. In fact, the hyperphagic effects and motor deficits produced by zolpidem...
progressively increased across the dosing conditions until drug was discontinued. Tolerance to the sedative and ataxic effects of Bz agonists in baboons typically occurs within the first few days of repeated dosing (Lamb and Griffiths, 1985; Lukas and Griffiths, 1982; Sannerud et al., 1989). However, the ataxic effects of daily i.v. zolpidem (3.2 or 5.6 mg/kg) in baboons did diminish with repeated dosing (Griffiths et al., 1992; Kaminski et al., 1993). Aside from route of administration, there were many dosing parameters that could account for the apparent lack of tolerance in the present study. In particular, progressively increasing the zolpidem dose every 17 days in the present study may have masked the development of tolerance. A lack of tolerance to effects of triazolam (Ator et al., 1986) and zaleplon (Ator NA, Weerts EM, Kaminski BJ, Kautz MA and Griffiths RR, unpublished observations) also was found using a similar dosing regimen and set of behavioral assessments in baboons. It is possible that tolerance may have developed if longer cycles (i.e., more than 17 days) of dosing conditions were used. However, tolerance was not evident even after 27 days of the 32.0 mg/kg dose condition. The lack of tolerance to the sedative effects of zolpidem in the current study is consistent with the results of studies in rats (Perrault et al., 1992; Sanger and Zivkovic, 1987, 1992; Sanger et al., 1994) and with the finding from clinical studies that no tolerance to the hypnotic effect of zolpidem was found in humans with insomnia (review in Salva and Costa, 1995).

The mean elimination half-life of zolpidem in humans after single oral doses (5–20 mg p.o.) has ranged across studies from 1.5 to 3.2 hr (review in Salva and Costa, 1995). Although elimination half-lives in rats and cynomolgus monkeys have been shorter than in humans (Durand et al., 1992), it is reasonable to assume that the elimination half-life in adult male baboons might be more similar to that in humans (Fridman and Popova, 1988). If the elimination half-life in baboons is assumed to be within the range of those reported above for humans it seems likely that, given the large total doses administered (e.g., approximately 30 mg at the 1.0 mg/kg dose), some drug still would be present 24 hr after dosing. In human volunteers, mean peak plasma concentrations of 200 ng/ml were reached by 2.3 hr on day 15 of repeated zolpidem dosing (20 mg/kg/day p.o., for 15 days), which declined to about 10 ng/ml after 12 hr and to less than 5 ng/ml after 24 hr (Durand et al., 1992; Thénot et al., 1988). Similar low concentrations of zolpidem in blood plasma were obtained 24 hr after dosing with 1.0 and 3.2 mg/kg in the current study in baboons. In the current study, levels of zolpidem in plasma were dose dependent across the 1.0 to the 10.0 mg/kg condition, but there was some indication that baboons may have metabolized the 32.0 mg/kg dose differently (J. Althaus, personal communication). Previous studies have not reported an accumulation of zolpidem in plasma with chronic dosing in rats (5.0 mg/kg−1 i.p., once daily, for 7 or 28 days) (Trenque et al., 1994) or human volunteers (20 mg/day p.o. for 15 days) (Thénot et al., 1988); and the results of the study in humans suggested that zolpidem neither induced nor inhibited its own metabolism.

The behavioral signs that are characteristic of Bz withdrawal are similar to the behavioral signs that are characteristic of withdrawal from barbiturates in humans and laboratory animals (Henningfield and Ator, 1986; Woods et al., 1987). The behavioral signs of Bz withdrawal have been well characterized in the baboon (Ator and Griffiths, 1992b; Lukas and Griffiths, 1982; Lamb and Griffiths, 1984; Sannerud et al., 1989). The current study graded withdrawal as mild, intermediate or severe based on the characterization of barbiturate and Bz withdrawal in rhesus monkeys by Yanagita and colleagues (Yanagita and Takahashi, 1970, Woods et al., 1987, see “Methods”). Administration of the Bz antagonist flumazenil during once-daily dosing with zolpidem precipitated a mild-to-intermediate withdrawal syndrome (as evidenced by increased vomit and/or retch, tremor, jerks and/or rigidly braced posture), which peaked at the highest dose of zolpidem (32.0 mg/kg). As few as 2 weeks of zolpidem administration were sufficient to produce some signs of physical dependence, and the number of signs observed and apparent severity of physical dependence increased in a dose- and time-related manner. Severe withdrawal signs (e.g., seizures) were not observed in any baboon. The current findings are consistent with those of Schoch and colleagues (as reviewed in Stephens and Sanger, 1996) in which the partial inverse agonist sarmazenil precipitated a withdrawal syndrome in squirrel monkeys treated with high doses of zolpidem. The mild-to-intermediate precipitated withdrawal syndrome was similar to that observed in studies with other Bz agonists given p.o. or i.g. in baboons and in which agonist dose and/or duration of dosing increased (Ator and Griffiths, 1992b; Lukas and Griffiths, 1984; Sannerud et al., 1989).

A mild-to-intermediate withdrawal syndrome also was observed in all baboons when chronic zolpidem dosing was discontinued abruptly. Mild signs of withdrawal (e.g., reduced food maintained behavior, increased postural changes, aggressive behaviors and self-directed behaviors) were apparent within the first few days after zolpidem was discontinued, followed by intermediate withdrawal signs (e.g., tremor, jerk and/or rigidly braced posture and vomit and/or retch) within 5 to 7 days after zolpidem was discontinued. Seizure, the most severe sign generally seen during Bz withdrawal, was not observed in any baboon. The time-limited suppression of food-maintained behavior after discontinuation of zolpidem dosing in the present study is similar to that reported previously (Griffiths et al., 1992; Kaminski et al., 1993), and is consistent with that observed in baboons during withdrawal from classic Bz agonists and the β-carboline abecarnil (Lamb and Griffiths, 1984; Lukas and Griffiths, 1982; Sannerud et al., 1992). In humans, signs of withdrawal after abrupt discontinuation of Bzs typically include nausea, vomiting and loss of appetite (Rickels et al., 1990). It is interesting that the one baboon for which pellets per day did not decrease during withdrawal was the one baboon for which pellets per day had not increased progressively across the entire period of dosing. Research to investigate this phenomenon is in progress.

Chronic Bz administration reliably produces physical dependence in a variety of species (for review see Woods et al., 1987, 1992). Previous studies in rodents concluded that there was no evidence of zolpidem physical dependence after chronic administration (Perrault et al., 1992; VonVoithlander and Lewis, 1991). The development of physical dependence in the present study differs with the findings of those studies, but is consistent with the observations that zolpidem physical dependence appeared to develop after high doses of zolpidem in squirrel monkeys (Schoch and colleagues as cited in Stephens and Sanger, 1996) and under conditions of re-
peated i.m. or i.v. administration or self-administration in the baboon (Griffiths et al., 1992; Kaminski et al., 1993). The difference in the dependence-producing effects of zolpidem between rats and monkeys adds to the contrasting profile for zolpidem in drug discrimination studies with rats and monkeys. These differences between the rodent studies and the monkey studies may yet be determined to be a function of the behavioral assays used, the dosing parameters selected or differential pharmacokinetics of zolpidem. Another possibility is that the binding characteristics of zolpidem in primate brain may be different from those reported for rodent brain (Abadie et al., 1996; Schmid et al., 1995; cf, Dennis et al., 1988).

In conclusion, the current experiments in baboons showed that the effects of zolpidem were similar to those of Bz agonists triazolam (Ator et al., 1996) and zaleplon (Ator NA, Weerts EM, Kaminski BJ, Kautz MA and Griffiths RR, unpublished observations) studied under similar procedures in baboons. Zolpidem produced several behavioral effects in ba-

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