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Acute and Chronic Effects of Ramelteon in Rhesus Monkeys (*Macaca mulatta*): Dependence Liability Studies

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The acute and chronic effects of ramelteon, an MT_1/MT_2 receptor agonist, were evaluated in rhesus monkeys (*Macaca mulatta*) to assess discriminative stimulus effects in comparison with traditional benzodiazepine receptor agonists and to assess physical dependence potential. Discriminative effects of ramelteon were compared with midazolam in untreated monkeys and in diazepam-dependent monkeys that discriminated flumazenil. Dependence potential of ramelteon after daily 1-year administration (and intermittent discontinuation) was evaluated with standard operant procedures. Ramelteon did not produce benzodiazepine-like discriminative stimulus effects at doses up to 10 mg/kg. Long-term treatment or its discontinuation had no significant effect on spontaneous behavior, operant behavior, body weight, motor activity, or posture. These findings suggest that ramelteon is not likely to have benzodiazepine-like abuse or dependence liability.

Keywords: abuse liability, dependence, benzodiazepine receptor agonist, long term

According to a recent survey (National Sleep Foundation, 2005), approximately 75% of adults in the United States are affected by at least one symptom of a sleep problem, and 54% have at least one symptom related to insomnia. Sedative hypnotics, including traditional benzodiazepine receptor agonists (BZRAs; e.g., temazepam, triazolam) and newer BZRAs (e.g., zolpidem, zaleplon, zopiclone), are the most commonly prescribed treatments. These medications share a common mechanism of action, agonism (i.e., positive modulation) at benzodiazepine receptors on the $GABA_A$ receptor complex, which is related to their hypnotic effects. In vitro studies have demonstrated that midazolam and diazepam, which are traditional BZRAs, bind nonselectively to various benzodiazepine receptor subtypes, whereas newer BZRAs (e.g., zaleplon, zolpidem) bind selectively to the $alpha_1$ subunit of benzodiazepine receptors (e.g., BZ_1 receptors). This specificity is thought to reduce unwanted effects associated with the nonselective inhibitory actions of traditional BZRAs. Despite these pharmacologic differences, studies in humans (Rush, Baker, & Rowlett, 2000) and in nonhuman primates (Ator, 2002; McMahon et al., 2002) have shown that nonselective and BZ_1-selective BZRAs share discriminative stimulus effects. Additionally, both traditional and newer BZRAs are scheduled drugs (Schedule IV) because of their potential for abuse and tolerance (Ator, 2005). Abuse liability with BZRA insomnia medications, particularly with long-term use, remains a significant concern among prescribing physicians.

Ramelteon is a novel, selective MT_1/MT_2 melatonin receptor agonist (Miyamoto et al., 2004; Yukuhiro et al., 2004) that has been recently approved for the treatment of insomnia. In adults with transient and chronic insomnia, ramelteon has been shown to reduce latency to persistent sleep and is well tolerated (Roth, Seiden, et al., 2005; Roth, Stubbs, & Walsh, 2005; Zammit et al., 2005). Ramelteon does not have significant affinity for other receptors, including benzodiazepine and opioid receptors (Kato et al., 2005). Unlike sedative hypnotics, ramelteon specifically targets MT_1/MT_2 receptors, which are primarily located in the suprachiasmatic nucleus—a part of a hypothalamic neural circuit known to be involved in the sleep–wake cycle. By modulating the release of melatonin via the suprachiasmatic nucleus, circadian wake signals are inhibited, resulting in a signal for sleep. It is thought that this highly selective, sleep-circuit specific activity may be underlying the ability of ramelteon to promote the onset of sleep without impairing cognitive function or causing next-day hangover, withdrawal symptoms, or rebound insomnia (Erman, Seiden, & Zammit, 2003; Roth, Seiden, et al., 2005; Roth, Stubbs, & Walsh, 2005; Zammit et al., 2005). Also, this unique mechanism of action may be related to the apparent lack of abuse potential

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reported with ramelteon administration (Griffiths, Suess, & Johnson, 2005).

The purpose of the following three studies was to assess the acute and chronic effects of ramelteon in rhesus monkeys with regard to discriminative stimulus effects and physical dependence potential, and specifically to compare ramelteon with a prototypic BZRA. It was hypothesized that ramelteon would not share behavioral effects with traditional sedative hypnotic benzodiazepines.

Experiment 1

In the first experiment, a drug discrimination procedure was used to examine whether ramelteon produced discriminative stimulus effects similar to the benzodiazepine midazolam. If ramelteon and midazolam have similar subjective effects, then ramelteon should produce midazolam-like responding in a drug discrimination procedure.

Method

Subjects

Four female, adult rhesus monkeys (*Macaca mulatta*) were housed individually in stainless steel cages on a 14:10-hr light–dark cycle. Each monkey had received a variety of drugs in prior behavioral experiments and ketamine during routine veterinary procedures. Monkeys were maintained at 95% free-feeding weight (range = 3.8–9.4 kg) with a diet comprising primate chow (High Protein Monkey Diet; Harlan Teklad, Madison, Wisconsin), fresh fruit, and peanuts; water was available ad libitum.

The monkeys in all three studies were maintained in accordance with the Institutional Animal Care and Use Committee, the University of Texas Health Science Center at San Antonio, and the 1996 Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, National Academy of Sciences).

Apparatus

During experimental sessions, monkeys were seated in restraining chairs (Model R001; Primate Products, Miami, Florida) located in ventilated, sound-attenuating chambers. Each chamber was equipped with two response levers, stimulus lights, and a food cup. An interface (Med Associates, St. Albans, Vermont) connected the chambers to a computer that controlled and recorded the experimental events. Monkeys responded under a stimulus-shock termination (SST) schedule (Lelas, Gerak, & France, 1999). A brief electric shock (3 mA, 250 ms) to the feet could be delivered from an alternating current shock generator via shoes that contained brass electrodes.

Drugs

Ramelteon (Takeda Pharmaceutical Company Limited, Osaka, Japan) was added to 30% polyethylene glycol 400 that was dissolved in a 5% dextrose solution for a maximum soluble concentration of 2 mg/mL. Midazolam hydrochloride (Roche Pharma, Manati, Puerto Rico) was purchased as a commercially prepared solution (5 mg/mL) and diluted as needed with sterile saline.

Midazolam Discrimination Procedure

Four monkeys previously trained to discriminate 0.32 mg/kg subcutaneous midazolam from vehicle under a fixed-ratio 5 SST schedule (Lelas et al., 1999) participated in training sessions that consisted of two to eight15-min cycles. Each 15-min cycle comprised a 10-min timeout period and a 5-min response period. During the timeout period, the chamber was dark, and lever presses had no programmed consequence. Monkeys received an injection of drug or vehicle during the 1st minute of the 10-min timeout. The start of the response period was signaled by illumination of two stimulus lights, which indicated that the SST schedule was in effect, and a shock was scheduled to occur every 15 s. Five consecutive responses on the correct (injection-appropriate) lever extinguished the stimulus lights and postponed the SST schedule for 30 s. The lever selection (e.g., left after midazolam; right after vehicle) for an individual monkey remained the same throughout the study. At the end of the 30-s timeout period, stimulus lights were illuminated, and the SST schedule was again in effect. An incorrect response reset the response requirement on the correct lever. Response periods ended after four shocks were delivered or after 5 min, whichever occurred first. If the response period ended before 5 min had elapsed, then the time remaining was a timeout period before the next cycle. Training was complete when the following criteria were satisfied during every cycle of two consecutive training sessions or two of three training sessions: (a) $\geq$80% of the responses were correct (injection-appropriate lever) and (b) less than five responses were incorrect (injection-inappropriate lever) before selecting the correct response. Monkeys were required to satisfy these criteria for both midazolam and vehicle training sessions.

Test sessions were conducted to determine whether ramelteon shared discriminative stimulus effects with midazolam. Test sessions were identical to training sessions except (a) ramelteon (0.32, 1.0, 3.2, 5.6, or 10.0 mg/kg, intravenous) was administered instead of midazolam and (b) five responses on either lever postponed the scheduled shock. The discriminative stimulus effects of the training drug—midazolam—were evaluated in a test session in which increasing doses of drug were administered over consecutive cycles with the cumulative dose increasing by 0.5 log units per cycle.

Results

Monkeys trained to discriminate midazolam from vehicle responded predominantly on the vehicle-associated lever when they received either vehicle (see Figure 1, "V," upper panel) or a small dose of midazolam. Following a larger (training) dose of midazolam (0.32 mg/kg), the monkeys responded predominantly on the midazolam lever (see Figure 1, closed circles, upper panel). In contrast, after receiving ramelteon, at doses up to 10.0 mg/kg, monkeys responded predominantly on the vehicle-associated lever (see Figure 1, triangles, upper panel). Midazolam slightly decreased rates of lever pressing, whereas ramelteon had no systematic effect on response rate up to a dose of 10.0 mg/kg (see Figure 1, lower panel).

Experiment 2

In a second experiment, a drug discrimination procedure was used to examine whether ramelteon modified the discriminative stimulus effects of flumazenil, a benzodiazepine receptor antagonist, in diazepam-treated monkeys. This investigation was designed to examine whether ramelteon and BZRAs share discriminative stimulus effects and, therefore, whether they have common pharmacologic mechanisms (Overton & Winter, 1974). If ramelteon and BZRAs share a common pharmacologic mechanism, then administration of ramelteon should attenuate the discriminative stimulus effects of flumazenil in this study.

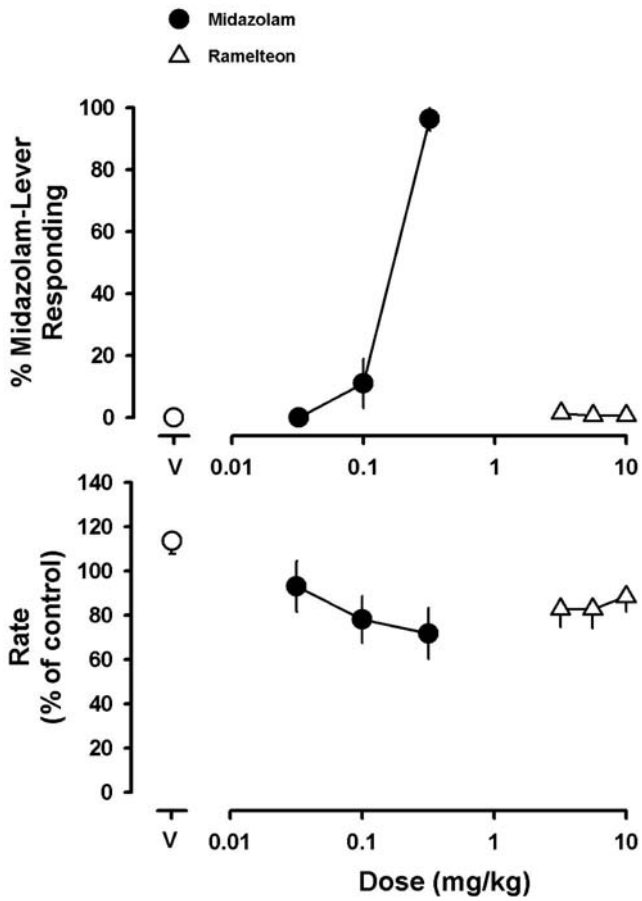


Figure 1. Discriminative stimulus (upper) and rate-altering (lower) effects of midazolam and ramelteon in 4 monkeys trained to discriminate between midazolam and vehicle. A value of 100% indicates that monkeys exclusively pressed the midazolam-associated lever; 0% indicates that the monkeys exclusively pressed the vehicle lever. Ordinates: upper panel, average percentage of responses on the midazolam-associated lever plus or minus one standard error of the mean; lower panel, average rate of responding plus or minus one standard error of the mean, expressed as a percentage of the control (vehicle) response rate. Abscissa: dose in milligrams/kilograms body weight; "V" indicates saline vehicle. Note: Not all monkeys were tested at the 0.32 mg/kg dose.

Method

Subjects and Apparatus

Four (3 male, 1 female) adult rhesus monkeys (*Macaca mulatta*) were used in Experiment 2. This group of monkeys was separate from those used in Experiment 1 and Experiment 3. Housing, feeding, and previous drug exposure were the same as in Experiment 1. The apparatus used was identical to that used in Experiment 1 apart from the shock feature, as responding by monkeys in this experiment was reinforced only with food pellets.

Drugs

Ramelteon (Takeda Pharmaceutical Company Limited, Osaka, Japan) was added to 30% polyethylene glycol 400 that was dissolved in a 5% dextrose solution for a maximum soluble concentration of 2 mg/mL. Flumazenil (F. Hoffmann LaRoche, Basel, Switzerland) was dissolved in a vehicle comprising 40% propylene glycol (Sigma-Aldrich), 50% saline, and 10% ethanol at a final concentration of 1.0 mg/mL. Diazepam tablets (10 mg; Zenith Laboratories, Northvale, New Jersey) were dissolved in fruit punch with Suspending Agent K, mixed in a blender, and administered with a 12-gauge drinking needle attached to a 60-mL plastic syringe. To obtain a dose of 5.6 mg/kg diazepam, a standard concentration of diazepam (1.0 mg/mL) was administered in a volume that was adjusted to individual body weights.

Flumazenil Discrimination Procedure

Monkeys were treated daily with diazepam for at least 1 year before the start of the study. During the training period, 4 monkeys received 5.6 mg/kg of diazepam per os 3 hr before the start of the training sessions. Monkeys were trained previously to discriminate between subcutaneous injection of vehicle and flumazenil (i.e., left lever flumazenil and right lever vehicle; Gerak & France, 1999). Training sessions comprised two to eight 15-min test cycles, which consisted of a 10-min timeout period followed by a 5-min response period. The chamber was dark, and lever presses had no programmed consequence during the timeout period; the start of the response period was signaled by illumination of two stimulus lights. The visual stimuli indicated that a fixed-ratio 5 schedule of food presentation was in effect. A maximum of 10 food pellets was available during each cycle. When the maximum number of food pellets was delivered in less than 5 min, the remainder of the response period was a timeout. Vehicle or flumazenil (0.32 mg/kg subcutaneous to 3 monkeys and 0.1 mg/kg subcutaneous to 1 monkey) was administered during the 1st minute of the timeout of each cycle. Flumazenil training cycles could be preceded by one to six vehicle injection cycles. Test sessions were conducted after the following training criteria were satisfied: (a) $\geq$80% of the total responses were correct (drug-appropriate lever) and (b) less than five responses were incorrect (injection-inappropriate lever) before selecting the correct response. Before each test session, these criteria had to be satisfied for both flumazenil and vehicle training sessions.

Test sessions were identical to training sessions except that (a) increasing doses of flumazenil were administered across cycles and preceded by vehicle or ramelteon and (b) five responses on either lever resulted in the delivery of food. To determine whether ramelteon modified the discriminative stimulus effects of flumazenil in diazepam-treated monkeys, monkeys received an acute injection of ramelteon (3.2–10.0 mg/kg, intravenous), or an equal volume of vehicle, 15 min prior to flumazenil administration. A cumulative dosing procedure was used in which the dose of flumazenil increased 0.5 log units per cycle, which allowed a complete dose-effect curve to be determined in a single 1.0–1.25 hr session (i.e., four or five 15-min cycles).

Results

Diazepam-treated monkeys trained to discriminate flumazenil from vehicle responded predominantly on the vehicle-associated lever after receiving vehicle (see Figure 2, "V," upper panel) and predominantly on the flumazenil-associated lever when they received 0.32 mg/kg of flumazenil (see Figure 2, closed circles, upper panel). When administered 15 min prior to increasing doses of flumazenil, ramelteon did not attenuate the discriminative stimulus effects of flumazenil. As shown in Figure 2 (upper panel), the flumazenil dose-response curve was not altered by pretreatment with 10.0 mg/kg of ramelteon. Qualitatively similar results were obtained with smaller doses of ramelteon (data not shown). Rates of lever pressing under vehicle control and ramelteon conditions were slightly decreased (see Figure 2, bottom panel).

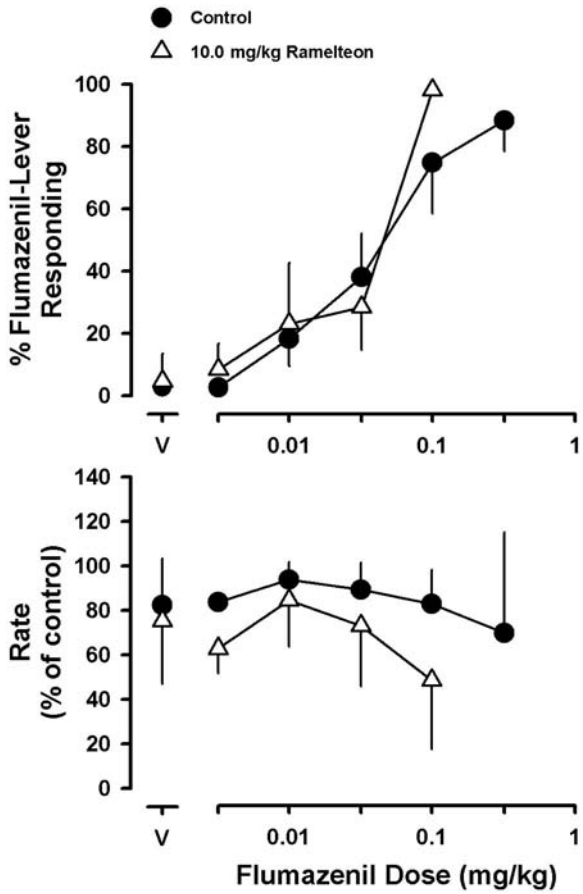


Figure 2. Discriminative stimulus (upper) and rate-altering (lower) effects of flumazenil alone and in combination with 10.0 mg/kg of ramelteon in 4 diazepam-treated monkeys trained to discriminate between flumazenil and vehicle. "V" indicates saline vehicle. See Figure 1 for additional details.

Experiment 3

In a third study, we evaluated monkeys with long-term (1-year) exposure to ramelteon for signs of primary physical dependence through clinical behavior, operant behavior, and plasma concentration assessments. If dependence develops to ramelteon, then temporary discontinuation of daily treatment should be reflected by changes in behavior or body weight.

Method

Subjects and Apparatus

Four (3 male, 1 female) adult rhesus monkeys (*Macaca mulatta*) were used in Experiment 3. This group of monkeys was separate from those used in Experiment 1 and Experiment 2. Housing, feeding, and previous drug exposure were the same as in Experiment 1. The apparatus used during the operant task was identical to that used in Experiments 1 and 2.

Surgical Procedure

Monkeys had a chronic, indwelling intragastric catheter (SIL-C70 Custom, 2.41 mm outer diameter and 1.32 mm inner diameter; Instech Solomon, Plymouth Meeting, Pennsylvania) implanted under aseptic conditions and halothane anesthesia. The catheter was secured to the stomach wall and then was tunneled to the midscapular region where it exited. The monkeys wore a mesh jacket (Lormir Biomedical, New York) equipped with a pocket, which stored the distal end of the catheter. Catheter patency was evaluated periodically by radiography immediately after intragastric infusion of a radio-opaque dye.

Drugs

Methyl cellulose (Dow Chemical, Midland, Michigan) was dissolved in injection-grade water (B-Baun) to make a 0.5% (wt/vol) vehicle solution. Ramelteon (Takeda Pharmaceutical Company Limited, Osaka, Japan) was added to the vehicle solution to make a 20 mg/mL suspension.

Physical Dependence Assessment

To evaluate whether physical dependence to ramelteon develops following daily treatment, monkeys received 10 mg/kg/day of ramelteon for 1 year. Ramelteon was administered 15 min prior to the daily behavioral sessions via the indwelling intragastric catheter. During the first 5 days of Weeks 14, 27, and 40, ramelteon treatment was temporarily discontinued and replaced with a vehicle infusion of equal volume. Monkeys received ramelteon on Days 6 and 7 of these weeks. Ramelteon treatment was terminated at the start of Week 53. Three types of assessments were conducted: pharmacokinetic, clinical, and operant behavior.

Pharmacokinetic. Ramelteon plasma concentrations were measured during Week 2 of treatment. Blood samples (2 ml) were obtained by acute stick of the saphenous vein at the following time points: immediately before, and 0.25, 0.5, 1, 3, 6, and 24 hr after drug administration. Blood samples were also taken every day during the 5-day drug-free period, including the day before and the day after discontinuation. These specimens were collected approximately 1 hr after drug or vehicle administration.

Clinical. Once weekly during ramelteon treatment and once daily during vehicle administration (i.e., Weeks 14, 27, 40, and 53) monkeys were observed for any of 33 clinical signs for 10 min: flips, shaking the cage, opening and closing the mouth, teeth grinding, rubbing lips on bars, yawning, vocalization, lip smacking, nose rubbing, scratching, biting fingernails, wet dog shake, grooming, locomotion, drinking, jerks, directed jerks, tremors, sitting upright, standing, lying down, rigidly braced, head lower than torso, baring teeth, salivation, nose wipe, eyes closed (>2 s), retching, vomiting, fetal position, rigid abdomen, abdominal defense, and uncooperativity. Observation began 25 min before the operant sessions on vehicle days or immediately preceding ramelteon treatment. The presence or absence of clinical signs was recorded every 15 s. Monkeys were also tested for their sensitivity to tactile stimulation, and their respiratory frequency was quantified by counting chest movement for 1 min. Technicians recorded any other unusual behavior.

Operant behavior. Daily sessions were 30–120 min in duration and consisted of two to eight 15-min cycles. Each cycle began with a 10-min timeout period during which the chamber was dark, and lever presses had no programmed consequence. The timeout period was followed by a 2-min response period during which a green light was illuminated, and the monkeys could receive food by pressing a lever 10 times (FR10). This period was followed by a 1-min timeout period, then a final 2-min response period when a red light was illuminated, and monkeys could delay scheduled shock by responding under an FR10 schedule on the same lever that was active in the food component. The rate of responding (responses/seconds) was recorded for all components during all cycles. During a typical week, the number of cycles conducted per day was as follows: Day 1 = 8 cycles, Day 2 = 4 cycles, Day 3 = 2 cycles, Day 4 = 4 cycles, Day 5 = 8 cycles, Day 6 = 4 cycles, and Day 7 = 2 cycles.

Data Analyses

Effects of ramelteon discontinuation on body weight and directly observable signs were each analyzed separately by a two-factor analysis of variance (ANOVA; NCSS, Kaysville, Utah) with discontinuation (two levels: before and during discontinuation) and discontinuation period (four levels) as within-subjects (repeated) factors. Effects of ramelteon discontinuation on rates of operant responding to obtain food and to avoid shock were analyzed by a three-factor ANOVA with discontinuation, discontinuation period, and cycle (two levels: food and shock) as within-subjects factors. These analyses were performed on values that, for each monkey, were averaged across the 5 discontinuation days, in comparison with the average of five control values that most recently preceded ramelteon discontinuation. In addition, possible differences among the 5 discontinuation days were examined by performing a three-factor ANOVA (with day [five levels], discontinuation period, and cycle as within-subject factors) on operant response rate values.

Possible effects of drug treatment on body weight and rate of operant responding were analyzed by ANOVA with period (three levels: before the start of drug administration, after the start of drug administration, and before the first discontinuation) as the within-subjects factor. In the analysis of operant responding, cycle was an additional within-subjects factor. These analyses were performed on averages of the last five values obtained before the start of drug administration, the first five values after the start of drug administration, and the five values most recently preceding the first ramelteon discontinuation. When appropriate, ANOVAs were followed by Tukey–Kramer multiple-comparison tests. An alpha level of .05 was used for all statistical tests.

Results

Pharmacokinetic

The highest plasma concentrations (T_{max}) of ramelteon were attained between 0.25 and 3 hr after ramelteon administration. The maximal plasma concentration (C_{max}) was markedly different between 1 female monkey (454.0 ng/mL) and the 3 male monkeys (164.0, 57.5, and 144.0 ng/mL). Similarly, a considerable difference in area under the curve (AUC) was noted between 1 female monkey (3,035.3 ng × h/mL) and the 3 male monkeys (351.7, 254.6, and 382.9 ng × h/mL). The plasma concentrations declined to less than the lower limit of quantification (0.5 ng/mL) during the 5 days of drug discontinuation (data not shown).

Clinical

Ramelteon treatment did not significantly affect body weight over the course of the study—main effect of period: $F(2, 6) = 0.14$, $p = .88$—such that the average body weight before treatment ($M = 8.1$ kg, $SEM = 0.6$), at the beginning of treatment ($M = 8.2$ kg, $SEM = 0.5$), and before the first discontinuation period after prolonged treatment ($M = 8.1$ kg, $SEM = 0.6$), were similar.

Ramelteon discontinuation also did not significantly affect body weight during any of the discontinuation periods (see Table 1): main effect of discontinuation, $F(1, 3) = 0.11$, $p = .76$; main effect of discontinuation period, $F(3, 9) = 1.09$, $p = .40$; and interaction, $F(3, 9) = 0.97$, $p = .45$. During ramelteon treatment and discontinuation, the individual body weights averaged across periods ranged from 6.4 to 8.7 kg ($M = 8.1$, $SEM = 0.6$).

Among the 33 signs that were scored, 10 were never observed in the course of this study. Discontinuation of ramelteon treatment did not significantly affect any of the 23 (except flips) observed clinical signs (shaking the cage, opening and closing the mouth, teeth grinding, rubbing lips on bars, yawning, vocalization, lip smacking, nose rubbing, scratching, biting fingernails, wet dog shake, grooming, locomotion, drinking, jerks, directed jerks, tremors, sitting upright, standing, lying down, rigidly braced, and head lower than torso): main effect of discontinuation, $F(1, 3) \leq 7.54$, $p > .05$; main effect of discontinuation period, $F(3, 9) \leq 3.16$, $p > .05$; and interaction of discontinuation and discontinuation period, $F(3, 9) \leq 1.65$, $p > .05$. The analysis of flips did not show significant main effect of discontinuation, $F(1, 3) = 0.13$, $p = .74$, or discontinuation periods, $F(3, 9) = 0.13$, $p = .74$, but showed a significant interaction between discontinuation and discontinuation period, $F(3, 9) = 4.50$, $p = .034$. Tukey–Kramer multiple-comparison tests showed that this interaction resulted from a significantly higher incidence of flips before the first discontinuation period than during most of the subsequent control and discontinuation periods.

Table 1
Body Weight (in Kilograms) During Repeated Ramelteon Treatment and Discontinuation Periods

	Ramelteon treatment ($n = 4$)		Discontinuation ($n = 4$)	
Period	*M*	*SEM*	*M*	*SEM*
1	8.1	0.6	8.2	0.6
2	7.8	0.5	7.8	0.5
3	8.2	0.6	8.1	0.6
4	8.3	0.8	8.3	0.8

Discontinuation did not significantly affect respiratory rate during any of the discontinuation periods: main effect of discontinuation, $F(1, 3) = 1.70$, $p = .28$; main effect of discontinuation period, $F(3, 9) = 1.12$, $p = .39$; and interaction of discontinuation and discontinuation period, $F(3, 9) = 1.76$, $p = .22$. During ramelteon treatment and discontinuation, individual respiratory rates averaged across periods ranged from 24.0 to 34.3 respirations per minute ($M = 29.2$, $SEM = 2.7$).

Operant Behavior

Ramelteon treatment did not significantly affect responding to obtain food or to avoid shock: main effect of period, $F(2, 6) = 1.05$, $p = .41$; main effect of cycle, $F(1, 3) = 11.76$, $p < .05$; and interaction, $F(2, 6) = 1.00$, $p = .42$. The mean (standard error of the mean) response rates to obtain food and to avoid shock were 1.07 (0.39) and 2.52 (0.37) responses per second, respectively, before ramelteon treatment; 0.96 (0.34) and 2.35 (0.25) at the beginning of treatment; and 1.49 (0.18) and 2.50 (0.11) before the first discontinuation period, after prolonged treatment.

Ramelteon discontinuation did not significantly affect responding to obtain food or to avoid shock during any of the discontinuation periods (see Table 2): main effect of discontinuation, $F(1, 3) = 2.16$, $p = .24$; main effect of discontinuation period, $F(3, 9) = 0.27$, $p = .84$; and interaction, $F(3, 9) = 1.27$, $p = .34$. Responding to avoid shock ($M = 2.74$ responses per second) occurred at a significantly higher rate than responding to obtain food ($M = 1.28$ responses per second): main effect of cycle, $F(1,$

Table 2
Response Rate (Responses per Second) During Repeated Ramelteon Treatment and Discontinuation Periods

	Responding to obtain food ($n = 4$)				Responding to avoid shock ($n = 4$)			
	Ramelteon treatment		Discontinuation		Ramelteon treatment		Discontinuation	
Period	*M*	*SEM*	*M*	*SEM*	*M*	*SEM*	*M*	*SEM*
1	1.49	0.18	1.53	0.16	2.50	0.11	2.52	0.10
2	1.32	0.17	1.45	0.17	2.36	0.13	2.74	0.22
3	1.01	0.23	1.21	0.29	3.07	0.28	3.14	0.24
4	1.05	0.26	1.21	0.28	2.51	0.15	3.14	0.24

3) = 327.00, $p < .001$. Interactions with other factors were not significant ($p > .05$). Response rate did not differ significantly across the five discontinuation days: main effect of day, $F(4, 12) = 0.94$, $p = .47$; main effect of discontinuation period, $F(3, 9) = 0.23$, $p = .87$; and main effect of cycle, $F(1, 3) = 120.72$, $p < .005$. Interactions were not significant ($p > .05$).

General Discussion

In the current experiments, ramelteon did not share discriminative stimulus effects with a traditional BZRA in two different conditions. During the midazolam discrimination task, ramelteon did not substitute for midazolam, even at doses as high as 10 mg/kg. Additionally, ramelteon did not attenuate the flumazenil discriminative stimulus effects in diazepam-dependent monkeys at doses up to 10 mg/kg. BZRAs substitute for midazolam in the former procedure, and they attenuate the effects of flumazenil in the latter procedure (McMahon, Gerak, & France, 2001). The considerable difference between ramelteon and prototypic benzodiazepines highlights the importance of these results. These findings suggest that ramelteon does not likely share subjective effects with benzodiazepines in humans and, thus, should not be expected to share abuse liability with BZRAs (Ator & Griffiths, 2003; Overton & Winter, 1974).

In support of pharmacologic reports that have indicated that ramelteon selectively targets melatonin MT_1/MT_2 receptors with no affinity for benzodiazepine receptors (Kato et al., 2005), the inability of ramelteon to attenuate the effects of flumazenil also suggests that ramelteon does not share similar pharmacologic mechanisms with BZRAs (Overton & Winter, 1974). Additionally, these data may indicate that ramelteon does not produce benzodiazepine-like physical dependence in humans.

Further support for a lack of physical dependence was provided in Experiment 3. Monkeys treated daily with a large dose of ramelteon for 1 year showed no indication of any abnormal clinical behavior, pharmacokinetics, or alterations in operant behavior related to intermittent discontinuation of long-term ramelteon treatment. Body weight, respiration, and pharmacokinetic parameters were not significantly affected by a large dose of ramelteon. Ramelteon exposure ($AUC_{0-24\ hr}$ and C_{max}) in Experiment 3 ranged from 254.6 ng × h/mL to 3,035.3 ng × h/mL and from 57.5 ng/mL to 454.0 ng/mL, respectively. This is more than 36 times greater than the clinical $AUC_{0\text{-}inf}$ (7 ng × hr/mL) and 11 times greater than the C_{max} (5.7 ng/mL) anticipated in patients receiving ramelteon at the recommended dose of 8 mg (Stubbs & Karim, 2003; Takeda Pharmaceutical Company Limited, 2005). Moreover, the maximum dose studied in the current experiments, and the dose that was administered daily for 1 year (i.e., 10 mg/kg), was more than 300-fold larger than minimally effective doses of ramelteon for shortening the latency and increasing the total duration of sleep in monkeys (Yukuhiro et al., 2004). Finally, observed clinical behavior (with the exception of flips) and ongoing operant behavior (i.e., responding to avoid shock or obtain food) were not affected by ramelteon treatment or its discontinuation. These data indicate that long-term treatment with ramelteon does not result in physical dependence or clinical or behavioral alterations indicative of withdrawal.

Research examining discriminative stimulus effects of other compounds that act on melatonin receptors, specifically melatonin and agomelatine (a melatonin receptor agonist and serotonin antagonist), as compared with BZRAs, have yielded mixed results; however, antagonism studies as well as self-administration studies have suggested that benzodiazepine receptors do not play a role in the actions of melatonin receptor agonists. Previous studies have demonstrated that the discriminative stimulus effects of BZRAs are qualitatively similar across a broad range of conditions (Ator, 2002, 2005; Lelas et al., 1999; McMahon et al., 2001). In one discrimination study, neither melatonin nor agomelatine substituted for diazepam in rats (Wiley, Dance, & Balster, 1998). However, a study by Levesque and Locke (1994) showed that in rats trained to discriminate melatonin from saline, triazolam produced melatonin-appropriate responding in all rats tested, whereas flurazepam only substituted for melatonin in 2 of the 6 rats tested. In this same study, the benzodiazepine receptor antagonist flumazenil selectively blocked melatonin-appropriate responding produced by triazolam but failed to completely block responding produced by melatonin. This finding is consistent with a study in which flumazenil antagonized the sedative and anticonvulsive effects of diazepam but failed to reverse similar effects of melatonin (Green, Nutt, & Cowen, 1982). Together, these antagonist studies suggest that melatonin is not achieving its discriminative stimulus effect through benzodiazepine-like actions. If melatonin agonists acted through the same mechanism as BZRAs, then it would be expected that these agonists would have similar behavioral effects in preclinical assays that are predictive of abuse. However, in a study by

Wiley et al. (1998), agomelatine was not self-administered in rhesus monkeys. Taken together with results from the current drug discrimination experiments, these studies suggest that ramelteon is not likely to share abuse liability with BZRAs.

These findings are potentially important for the treatment of sleep. Given that ramelteon did not substitute for BZRAs in these experiments and did not affect ongoing operant or clinical behavior, it should be expected that any subjective side effects of ramelteon in humans are not like the subjective effects of selective or nonselective BZRAs and that ramelteon is not likely to produce physical dependence even after daily, long-term treatment.

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