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Pharmacokinetic–pharmacodynamic modeling of the coexistence of stimulatory and sedative components for midazolam

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Abstract

Midazolam increased the shorter-response rate and decreased the reinforcement rate of a contingency-controlled timing behavior—a differential-reinforcement-of-low-rate 45-s schedule. The responding rate changes observed were immediately interpretable as functions of midazolam concentration during a 3-h session—a period for investigating the onset, peak, and disappearance of midazolam effect—in rats. That the midazolam pharmacokinetic–pharmacodynamic model was a direct application of our alprazolam pharmacokinetic–pharmacodynamic model implies that both drugs exhibit similar pharmacological effects. The two peaks of the shorter-response rate increases produced by midazolam were modeled as a stimulation-sedation model that consisted of two opposing effect-link sigmoidal E_{max} functions. The stimulation-sedation model suggested that midazolam possesses both stimulatory and sedative effects in a continuous but sequential fashion, and hypothesizes the coexistence of stimulation and sedation components for midazolam; this model may help delineate possible mechanisms for rebound side effects and of tolerance in humans. The reinforcement rate was, then, an index for evaluating the deficit in timing performance. © 1998 Elsevier Science B.V.

Keywords: Benzodiazepine; Midazolam; Pharmacokinetic–pharmacodynamic modeling; Sedative effect; Stimulatory effect; Timing performance

1. Introduction

Differential-reinforcement-of-low-rate schedules (e.g., 45-s) produce low rates of responding, as only those responses that occur after a minimum time interval ($\geq$ 45 s) following a previous response are reinforced. Responses that occur before this time elapse are not reinforced, and they reset the timing of the interval. Differential-reinforcement-of-low-rate performance satisfies many of the criteria (i.e., objective, continuous, sensitive and reproducible) proposed as ideal for pharmacodynamic measurement (Dingemanse et al., 1988; Laurijssens and Greenblatt, 1996). In addition, after the drug is administered, one can use reinforced and nonreinforced responses—which generally exhibit decreases and increases, respectively—to evaluate the drug effects. Midazolam is a potent benzodiazepine derivative with sedative, hypnotic, anticonvulsant, muscle-relaxant and anxiolytic activities (Pier et al., 1981). In past research, we found that the differential-reinforcement-of-low-rate 45-s performance largely corresponded to its respective pharmacokinetics after drug administration (Lau and Wang, 1996; Lau et al., 1997a). For example, we used both pharmacokinetics and the effects of midazolam on DRL 45-s performance to clarify that the subcutaneous (s.c.), as opposed to the intraperitoneal (i.p.) route, is the route of choice for the evaluation of midazolam dose-response relations (Lau et al., 1996).

Despite the wide use of midazolam in animal behavioral research (e.g., operant behavior, drug discrimination), pharmacokinetic–pharmacodynamic modeling is rarely performed. We proposed an integrated pharmacokinetic–pharmacodynamic model for alprazolam in order to describe and to predict the time course profiles for serum alprazolam concentration, the reinforcement rate, and the shorter-response (nonreinforced) rate in a previous study (Lau and Heatherington, 1997). The comprehensive pharmacokinetic–pharmacodynamic model also described the interplay between the two rates of responding. We used two sigmoidal E_{max} models having actions opposite in direction to account for the observed shorter-response rate increases and an indirect response model to describe the reinforcement rate changes. The model suggested that

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alprazolam possesses both stimulatory and sedative effects in a continuous but sequential fashion—the stimulatory effect preceded the sedative effect and the former lasted longer than the latter. As a result, the reinforcement rate was an index of the timing performance deficits. Some behavioral effects of benzodiazepines were readily evident as characteristics of the coexistence of both stimulatory and sedative effects for alprazolam proposed by the model; no other mechanism has been proposed for these observations. For example, following chronic benzodiazepine administration, tolerance develops rapidly to the sedative or depressant effect of high doses, but it does not develop to the stimulatory effect of low doses (File and Pellow, 1985; Griffiths and Goudie, 1987; Flaherty et al., 1996). In addition, repeated low-dose administration can even enhance the stimulatory effect (Sansone, 1979). If, indeed, stimulatory and sedative effects were continuous—and exhibited a sigmoidal E_{max} function—but were opposed components, then stimulation would be evident and would become enhanced if tolerance to sedation developed progressively.

One of the aims of the present study was to investigate s.c. midazolam dose-response relations in an ascending and a descending order in 3-h sessions under the differential-reinforcement-of-low-rate 45-s schedule to examine whether the size of the preceding dose affected the response of the subsequent dose when the doses were separated by 3–5 days. We used both reinforced and nonreinforced (i.e., shorter) responses to evaluate the effects of midazolam. The second aim was to characterize midazolam concentration-effect relations in vivo using the abovementioned alprazolam pharmacokinetic–pharmacodynamic model to investigate whether the model was prototypical for benzodiazepines and can be generalized to midazolam. The implications of this model for adverse side effects noted in humans after benzodiazepine administration will be presented in Section 4.

2. Materials and methods

2.1. Pharmacodynamics: differential-reinforcement-of-low-rate 45-s performance

2.1.1. Animals

Four male, albino, virus free Sprague–Dawley rats (HSD, Indianapolis, IN) with a mean initial body weight of 385 g (range 383–388 g; approximately 80 days old) were used. They were housed individually in a temperature-regulated room with a daily cycle of illumination from 7:00 a.m. to 7:00 p.m. They were reduced to 80% of their initial, adult free-feeding body weights by receiving limited daily food rations (5 g for the first day, 10 g for the next 5 days) and were then maintained at their 80% body weights by being given a daily food supplement (range: 14–16 g). Water was continuously available in the living cages. Experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publ. No. 85-23, revised 1985).

2.1.2. Drugs

Midazolam maleate was obtained from Hoffmann-La Roche, (Nutley, NJ) and was dissolved in 0.9% NaCl. Drug doses of midazolam were expressed in terms of the salt and were corrected to midazolam base for calculating the pharmacokinetic parameters.

2.1.3. Apparatus

Four operant Plexiglas chambers were used, and have been described previously (Lau and Wang, 1996). Each chamber, equipped with a response lever and a stainless steel food-pellet receptacle into which 45-mg dustless pellets (BioServ, Frenchtown, NJ) could be delivered, was enclosed in a sound-attenuating shell and was controlled by an IBM-type 486X computer. Session contingencies were programmed and data recorded using QuickBasic.

2.1.4. Procedure

The animals were magazine trained initially for 15 min on a noncontingent random-time schedule. Responses on the lever were shaped by successive approximations and were reinforced when inter-response times were greater than 3 s. The temporal requirement was slowly increased to an inter-response time of 45 s over 10–20 sessions. Once training was complete, a 3-h operant session was conducted at the same time every day. After intersession performance had stabilized, the two drug-administration series began. The two series consisted of midazolam dose-response determinations in an ascending order (vehicle, 0.3, 1, 3 and 10 mg/kg) and in a descending order (10, 3, 1 and 0.3 mg/kg). The injections were separated by 3–5 days. Each drug series was separated by 10 noninjection sessions. All injections were given immediately before the start of a session. Midazolam was administered s.c. in an injection volume of 1 ml/kg body weight.

2.1.5. Data analyses

The inter-response time distributions for the administration of vehicle and those for midazolam were analyzed for 3-h sessions. The first 2 min of data, which were treated as the settling time, were not included in the analysis. Behavioral parameters were derived from the inter-response time distributions: shorter (nonreinforced) responses (inter-response times < 45 s) and reinforced responses (inter-response times ≥ 45 s). Total responses consisted of reinforced and nonreinforced responses. These responses were calculated as rates (responses per min). We have found that the reinforcement rate in the 45–55 s bin, as well as in the ≥ 45 s bin, decreased as a function of dosage for the drugs (e.g., alprazolam, caffeine); the 45–55 s bin function was more sensitive to drug effects than the total reinforce-

ment rate was (≥ 45 s). The 45–55 s bin function also required lower doses to reach the maximum effect than the total reinforcement rate measure did, and it has been used successfully to characterize the acute and chronic alprazolam–caffeine interactions on differential-reinforcement-of-low-rate performance (Lau and Wang, 1996; Lau et al., 1997a,b). Thus, in the present study, we also analyzed the 45–55 s bin to facilitate the comparison with our previous alprazolam results.

2.2. Pharmacokinetics

2.2.1. Animals

Five male, albino, virus-free Sprague–Dawley rats (HSD, Indianapolis, IN) with a mean initial body weight of 382 g (range: 380–386 g; approximately 80 days old) were housed under the conditions and food-limitation regimen used in the pharmacodynamic experiment described above.

2.2.2. Catheterization

Right jugular vein cannulation was performed under sterile conditions and has been described earlier (Lau et al., 1996). The catheter was flushed with 0.9% saline containing 50 units of heparin per ml and was sealed with fishing line when not in use.

2.2.3. Reagents and HPLC

The metabolites of midazolam, α-hydroxymidazolam and 4-hydroxymidazolam, were obtained from Hoffmann-La Roche. Reagents were obtained from standard commercial sources. The serum microsample HPLC method for the determination of midazolam and its metabolites (limit of sensitivity = 10 ng/ml) has been described previously (Ma and Lau, 1996). The capacity factors for 4-hydroxymidazolam, midazolam, and α-hydroxymidazolam are 1.82, 2.76, and 4.09, respectively.

2.2.4. Drug administration and blood sampling

The animals were allowed to recover for at least 2 days from the jugular vein catheterization prior to the drug administration series. The animals initially received an i.v. dose of midazolam (0.75 mg/kg) via the jugular vein catheter, followed by s.c. doses (0.3, 1, 3, and 10 mg/kg) in an ascending order; each separated by 3–5 days. Injections were given in a volume of 1 ml/kg body weight.

Blood samples (100 μl) from the jugular vein catheter were obtained following midazolam administration at 2, 5, 15, 30, 45, 60, 90, 120, 180, and 240 min post-injection. Because the feeding regimen needed to be maintained and the effect of food on midazolam pharmacokinetics avoided, doses were given 4 h prior to the feeding time.

2.2.5. Data analyses

We used SAAM II software system (SAAM II User Guide, 1997) to perform the midazolam pharmacokinetic analysis. The data were described by an open two-compartment model for i.v. midazolam and were fitted to the following equation:

$$C_p = Ae^{\alpha t} + Be^{\beta t}$$

where C_p is the total serum alprazolam concentration at time t; the terms A and B are the extrapolated zero intercepts; and α and β represent the apparent first-order distribution and elimination rate constants, respectively. The half-life ($t_{1/2}$) for the distribution or elimination phase and the volume of distribution for the central compartment (V_c) were calculated by the following equations: $t_{1/2} = 0.693/\alpha$ or β and $V_c = \text{Dose}/(A + B)$. The pharmacokinetic parameters clearance (Cl) and volume of distribution at steady state (V_{ss}) were calculated using noncompartmental methodology. For the s.c. route, the absorption rate constant (k_a) was also determined. The area under the serum midazolam concentration-time curve ($\text{AUC}_{0-\infty}$) and the area under the first moment of the serum midazolam concentration-time curve ($\text{AUMC}_{0-\infty}$) were calculated by the following equation: $\text{AUC} = A/\alpha + B/\beta$; $\text{AUMC} = \text{AUC} = A/\alpha^2 + B/\beta^2$. Total Cl was then defined as $\text{Dose}/\text{AUC}_{0-\infty}$, and V_{ss} as $\text{Dose} \times \text{AUMC}_{0-\infty}/\text{AUC}^2_{0-\infty}$. The values reported as the maximum concentration (C_{max}) and the time at which C_{max} occurred (T_{max}) are the actual observed values. Statistical analyses for the comparison of pharmacokinetic parameters were performed by repeated-measures, one-way analysis of variance (ANOVA). Although the doses used for the s.c. route of administration differed from the dose used for the i.v. route, absolute bioavailability can be calculated by the following formula because there was a linear relation between $\text{AUC}_{0-\infty}$ values and the midazolam doses (Table 1):

$$F = \left[(\text{AUC}_{0-\infty})_{\text{s.c.}} D_{\text{i.v.}}\right] / \left[(\text{AUC}_{0-\infty})_{\text{i.v.}} D_{\text{s.c.}}\right]$$

where $\text{AUC}_{0-\infty\text{s.c.}}$ and $\text{AUC}_{0-\infty\text{i.v.}}$ are the respective AUCs for the s.c. and i.v. routes, and $D_{\text{s.c.}}$ and $D_{\text{i.v.}}$ are the respective doses. Statistical analyses for the comparison of pharmacokinetic parameters were performed by repeated-measures, one-way ANOVA.

2.3. Pharmacokinetic and pharmacodynamic modeling

We performed pharmacokinetic and pharmacodynamic data analyses on mean data (pharmacokinetics, $n = 5$; pharmacodynamics, $n = 4$) using the SAAM II software system. Because pharmacokinetic and pharmacodynamic data were obtained in parallel studies, individual profiles were not used. The between-group design for the pharmacokinetic–pharmacodynamic modeling was chosen to prevent any effect of blood sampling on differential-reinforcement-of-low-rate performance. Consequently, inter- or intra-individual variability cannot be assessed. Assessment of the goodness of fit of each proposed model to experimental data was based on Akaike's Information Criterion, objective function, correlation matrix, residual and weighted residual plots, visual plots, and statistics

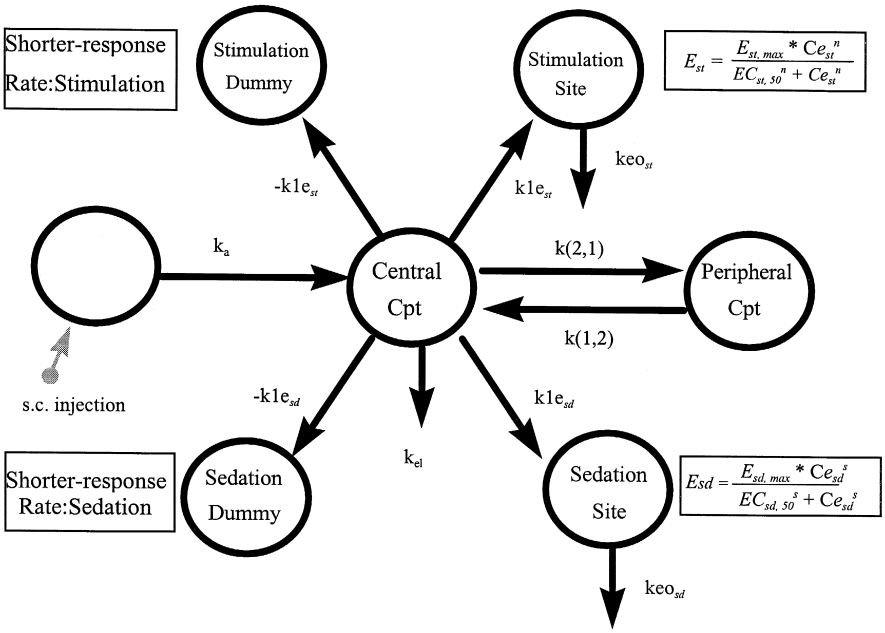

Fig. 1. Diagrammatic representation of the pharmacokinetic–pharmacodynamic model (stimulation-sedation model) used to describe shorter-response rate following s.c. administration of a single dose of midazolam.

associated with the model—S.D., coefficient of variance (C.V.), and total objective function values. The values of the objective function are a measure of how well the calculated values match the data values, whereas the values of Akaike Information Criterion can be used to evaluate model order and perform model discrimination.

2.3.1. Pharmacokinetic analysis

We analyzed mean serum concentration time profiles using compartmental analysis. The distribution and elimination characteristics were determined following the i.v. 0.75 mg/kg midazolam dose. Then, i.v. 0.75 mg/kg and s.c. midazolam profiles (0.3–10 mg/kg) were analyzed simultaneously.

2.3.2. Pharmacodynamic models

2.3.2.1. Shorter-response rate (inter-response time < 45 s): stimulation-sedation model. A multi-compartmental model incorporating two link compartments, representing stimulation and sedation sites, was used to describe the data and has been described previously (Lau and Heatherington, 1997). This effect-link model was based on that proposed by Sheiner et al. (1979) wherein an effect site is

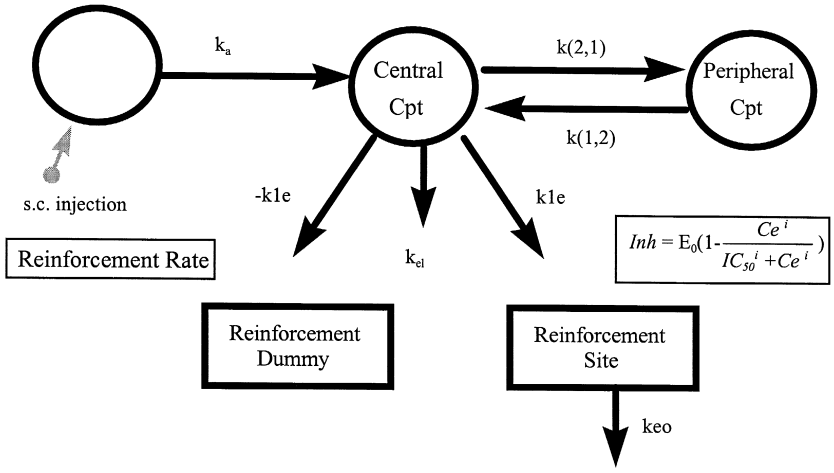

Fig. 2. Diagrammatic representation of the pharmacokinetic–pharmacodynamic model used to describe the reinforcement rate following s.c. administration of a single dose of midazolam.

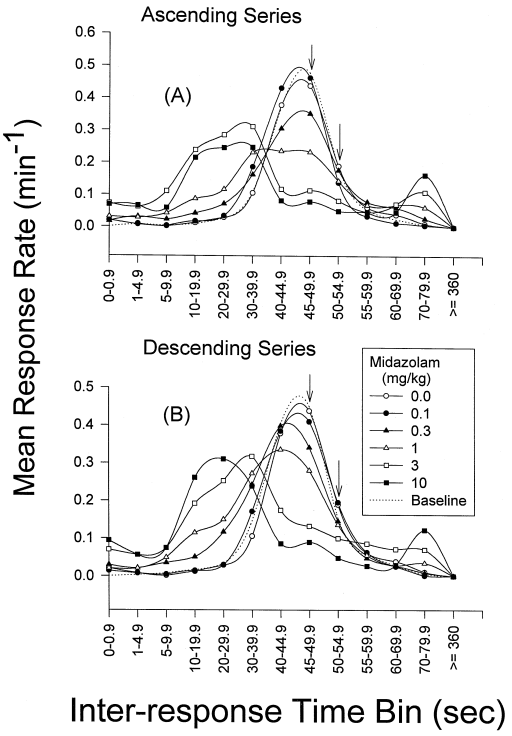

Fig. 3. Mean effects of midazolam on inter-response time distributions for the ascending and descending series during the 3-h session. All responses before and after the first arrow are non-reinforced (< 45 s) and reinforced (≥ 45 s), respectively, and between the two arrows are the 45–55 s bin responses ($n = 4$).

linked to the central compartment via the first order rate constant (kle_{st}), which is very small relative to the other rate constants (Fig. 1). The general assumption is that mass loss via kle_{st} is 'negligible' (Sheiner et al., 1979); however, in order to ensure no loss of mass to the effect site, a 'dummy' compartment was linked to the central compartment via the rate constant $-kle_{st}$. The addition of this compartment did not increase the complexity of the model, as the rate constant was fixed. Drug stimulation site kinetics were defined by the loss rate constant, keo_{st}. Likewise, the sedation model was also incorporated as an effect-link model, such that the sedation site and equivalent dummy compartment were linked to the central compartment via the rate constants kle_{sd} and $-kle_{sd}$, respectively, where subscript sd refers to sedation. The kinetics of the sedation site were defined by the loss rate constant, keo_{sd}.

Stimulatory effect (E_{st}) is described by the sigmoid E_{max} equation, which is expressed in terms of Ce_{st}, such that

$$E_{st} = \frac{E_{st,max} Ce_{st}^{n}}{EC_{st,50}^{n} + Ce_{st}^{n}}$$

where $E_{st,max}$, $EC_{st,50}$ and Ce_{st} are the maximal response, the concentration required to produce 50% maximal response and the stimulation site concentration, respectively, and n is the Hill factor. The sedative effect (E_{sd}), which is opposing and negative, is also described by a sigmoid E_{max} model. This model is expressed in terms of Ce_{sd}, such that

$$E_{sd} = \frac{E_{sd,max} Ce_{sd}^{s}}{EC_{sd,50}^{s} + Ce_{sd}^{s}}$$

where $E_{sd,\,max}$, $EC_{sd,50}$, and Ce_{sd} are the maximal sedative effect, the concentration required to produce 50% sedation,

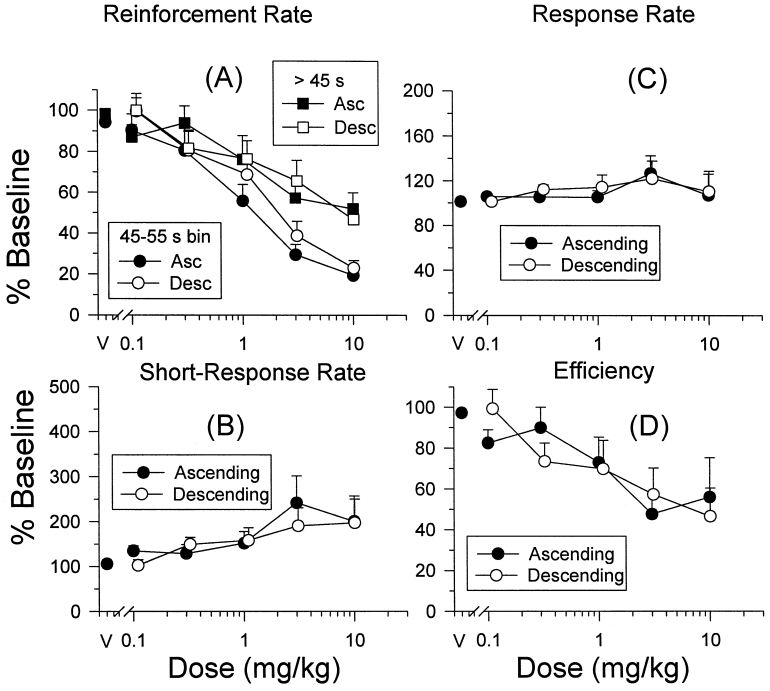

Fig. 4. Mean (S.E.M.) % baseline dose-response curves after midazolam administration (0.3–10 mg/kg) for the ascending and descending dosing series for the 3-h sessions: (A) reinforcement rate in the > 45- and 45–55-s bins; (B) shorter-response rate; (C) total response rate; (D) efficiency.

and the sedation site concentration, respectively, and s is the Hill factor. The overall shorter-response (srr) rate effect (E_{srr}) is the sum of the baseline effect (E_o), the stimulatory effect (E_{st}), and the sedative effect (E_{sd}):

$$E_{srr} = E_o + E_{st} + E_{sd}$$

For each dose (0.3, 1, 3 and 10 mg/kg), the stimulatory parameters (kle_{st}, keo_{st}, Ve_{st}, $E_{st,max}$, $EC_{st,50}$, n) and the sedative parameters (kle_{sd}, keo_{sd}, Ve_{sd}, $E_{sd,max}$, $Ec_{sd,50}$, s) were estimated by simultaneous optimization of the shorter-response rate data.

2.3.2.2. Reinforcement rate in the 45–55 s bin. The effect-link model was constructed as described above, wherein the effect site is linked to the central compartment via the first order rate constant kle (Fig. 2). Drug effect (E) is described by the classical inhibitory E_{max} model which is expressed in terms of Ce, such that

$$E = E_o\left[1 - \frac{Ce^i}{IC_{50}^i + Ce^i}\right]$$

where E_o, IC_{50}, and Ce are the baseline response (100%), the midazolam concentration required to produce 50% inhibition, and the effect site concentration, respectively, and i is the Hill factor. One set of link parameters (kle, keo) and pharmacodynamic parameters (IC_{50}, i) were estimated upon simultaneous optimization of the reinforcement rate data following the administration of 0.3, 1, 3 and 10 mg/kg midazolam.

2.3.3. Integration of pharmacokinetics and pharmacodynamics

In pharmacokinetic–pharmacodynamic modeling, we first obtained the pharmacokinetic parameters derived from the serum midazolam concentration-time profiles. We then used these pharmacokinetic parameters as constants for estimating the pharmacodynamic parameters for the stimulation-sedation model and for the effect-link model derived from the shorter-response rate-time profiles and the reinforcement rate profiles, respectively. Pharmacokinetic–pharmacodynamic data were fitted simultaneously for all the midazolam doses (0.3–10 mg/kg) for each measure of the differential-reinforcement-of-low-rate performance. Diagrammatic representations of the pharmacokinetic–pharmacodynamic models for the shorter-response rate and the reinforcement rate are shown in Figs. 1 and 2.

3. Results

3.1. Effects of midazolam on differential-reinforcement-of-low-rate performance

Fig. 3A,B show the effects of midazolam on inter-response time distributions for the 3-h sessions. Midazolam shifted the inter-response time distributions in a dose-related fashion; it increased the shorter inter-response times (< 45 s) and decreased the reinforced inter-response times (≥ 45 s). However, the long inter-response times (70–79.9

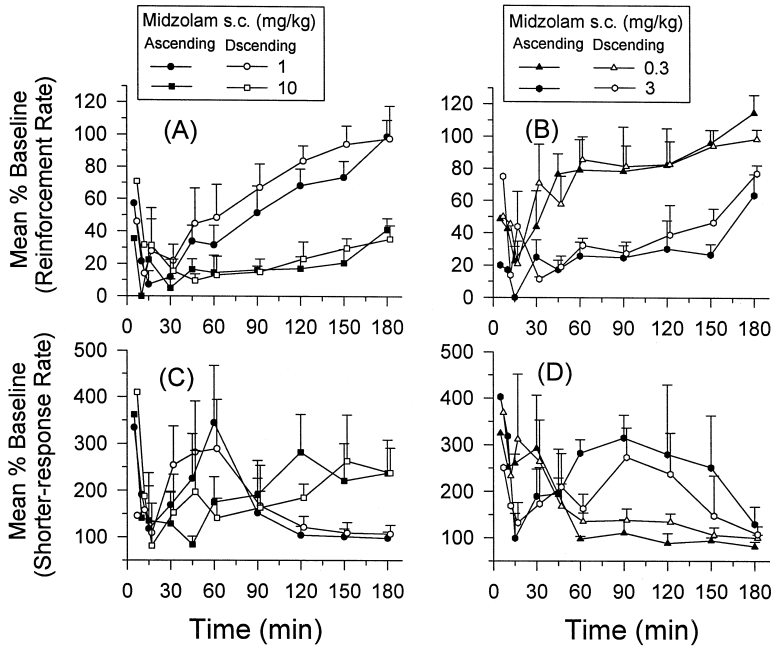

Fig. 5. Response rate–time profiles, expressed as % baseline (S.E.M.) after the four midazolam administration (0.3–10 mg/kg) for the two dosing series: (A) reinforcement rate for 1 and 10 mg/kg; (B) reinforcement rate for 0.3 and 3 mg/kg; (C) shorter-response rate for 1 and 10 mg/kg; (D) shorter-response rate for 0.3 and 3 mg/kg.

s) in the reinforced bins increased with each dose. Following the ascending and descending midazolam dosing series, the inter-response time distributions were generally similar for a given dose; some minor variations were detected.

Fig. 4A–D show an overview of differential-reinforcement-of-low-rate performance for the 3-h session following vehicle and midazolam administration for the ascending and descending series. The decreases in reinforcement rate in the 45–55 s bin, and in bins larger than 45 s, were linear with respect to the midazolam dose (Fig. 4A). As mentioned in Section 2, the 45–55 s bin function was more sensitive to drug effects than the total reinforcement rate was ($\geq$ 45 s). Hereinafter, the reinforcement rate refers to the reinforcement rate in the 45–55 s bin. At higher doses, midazolam increased the shorter-response rate as shown in Fig. 4B. The opposing relation between the reinforcement and shorter-response rates after midazolam administration resulted in a flat total response rate across the doses (Fig. 4C). Consequently, efficiency for midazolam was similar to the reinforcement-rate function (Fig. 4D). On the basis of these four performance indices, we concluded that the effects of midazolam were indifferent for the two dosing series.

The mean percent baseline of reinforcement rate-time profiles were dose and time dependent after midazolam administration (Fig. 5A,B). The decreases in reinforcement rate reached E_{max} between 15–30 min and then returned to the baseline level at the end of the sessions after the administration of the two lower midazolam doses (0.3 and 1 mg/kg), but those for the two higher doses (3 and 10 mg/kg) remained low. As for the shorter-response rate-time profiles, they exhibited different patterns compared to those for the reinforcement rate (Fig. 5C,D). The effects of midazolam on the shorter-response rate increased immediately following drug administration (0.3–10 mg/kg). The rate then decreased to near baseline level except for the lowest dose (0.3 mg/kg). However, the shorter-response rate again increased in a dose-related fashion in terms of both time to and duration of the second peak. The time of the rising and the duration of the second peak were a function of dose. For each dose, the second peak occurred later and lasted longer. Both the reinforcement rate- and shorter-response rate-time profiles were similar for a given midazolam dose for the two dosing series; some minor variations were detected.

3.2. Midazolam pharmacokinetics

Following i.v. administration, midazolam was eliminated according to a biphasic process (Fig. 6A); it was rapidly distributed with a mean distribution half-life ($t_{1/2\alpha}$) of 4.2 min and a mean terminal elimination half-life ($t_{1/2\beta}$) of 39.2 min (Table 1). The V_c, V_{ss} and Cl were 0.8 l/kg, 1.75 l/kg, and 2.34 l/h per kg, respectively.

The absorption of midazolam was rapid after the four s.c. midazolam doses (0.3, 1, 3, and 10 mg/kg) as reflected by the T_{max} values, which were in the range of 2.6–5.8 min (Table 1). Thus, the absorption rate constants (k_a) could not be determined accurately and were not included in Table 1. After reaching C_{max} values for the four doses, midazolam serum concentration rapidly decreased and a slower decay followed as indicated by the $t_{1/2\alpha}$ and the $t_{1/2\beta}$ values, respectively (Fig. 6B and Table 1). The values of α and β did not significantly differ statistically across the four doses. There was a linear relation between $AUC_{0-\infty}$ and the midazolam doses. The bioavailability values for the four s.c. doses ranged from 42–56%. The two hydroxy midazolam metabolites were not detected for both routes of administration.

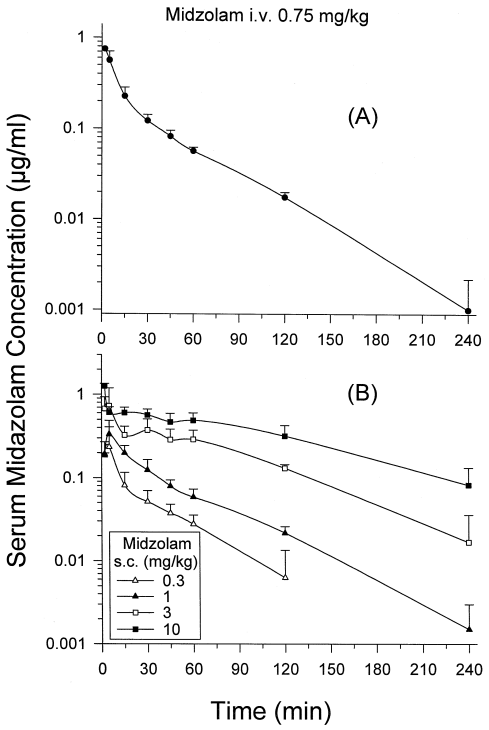

Fig. 6. Mean (S.E.M.) measured serum concentration–time profiles following midazolam administration ($n = 5$): (A) i.v. 0.75 mg/kg; (B) s.c. 0.3–10 mg/kg.

3.3. Pharmacokinetic and pharmacodynamic modeling

Inasmuch as both the shorter-response rate and the reinforcement rate showed minimal differences for the ascending and descending injection series (Figs. 3–5), we used the mean values of the two series to perform the pharmacodynamic analyses. It has been reported that there is no systematic change in the benzodiazepine plasma concentration profile under a chronic dose regimen in humans (Greenblatt and Shader, 1986). Thus, for modeling purposes, the dose-response midazolam pharmacokinetic analysis was conducted only in an ascending order as described in Section 2.

Table 1
Mean pharmacokinetic parameters (±S.D.) for midazolam after two routes of administration in rats ($n = 5$)

	i.v.	s.c.			
Midazolam maleate dose (mg/kg)	0.75	0.3	1.0	3.0	10
Midazolam base dose (mg/kg)	0.55	0.22	0.73	2.21	7.3
V_c (l/kg)	0.80 (0.59)				
V_{ss} (l/kg)	1.75 (0.86)				
Cl (l/h/kg)	2.34 (0.60)				
α (/min)	0.166 (0.07)	0.602 (0.07)	0.349 (0.59)	0.466 (0.65)	0.80 (0.77)
$t_{\alpha 1/2}$ (min)	4.2	1.2	2.0	1.5	0.87
β (/min)	0.018 (0.008)	0.017 (0.006)	0.012 (0.005)	0.009 (0.004)	0.012 (0.004)
$t_{\beta 1/2}$ (min)	39.2	40.0	59.01	77.63	57.8
$AUC_{(0-\infty)}$ ($\mu g \times min/ml$)	15.34 (3.60)	3.26 (0.63)	9.82 (2.12)	33.03 (13.62)	83.81 (42.14)
C_{max} ($\mu g/ml$)		0.24 (0.25)	0.30 (0.24)	0.70 (0.69)	1.07 (0.44)
T_{max} (min)		2.60 (1.34)	5.80 (5.36)	5.20 (5.63)	4.60 (5.81)
F%		56.86 (21.32)	50.26 (14.58)	53.67 (18.37)	42.68 (22.01)

3.3.1. Shorter-response rate (inter-response times < 45 s): stimulation-sedation model

In the stimulation-sedation model, the parameters kle_{st} and kle_{sd} were both fixed at 0.0001/min; however, this numeric value has been shown to be of no consequence (Sheiner et al., 1979). $E_{sd,max}$ was set equal to $-E_{st,max}$ as the sedation was capable of totally negating the stimulatory effect, such that the measured effect was approximately equal to E_o.

Fig. 7 shows the predicted stimulatory effect (E_{st}), the predicted sedative effect (E_{sd}), the predicted shorter-response rate, and the observed shorter-response rate for each dose. The estimated parameters and the associated errors, expressed as C.V.%, are shown in Table 2. Al-

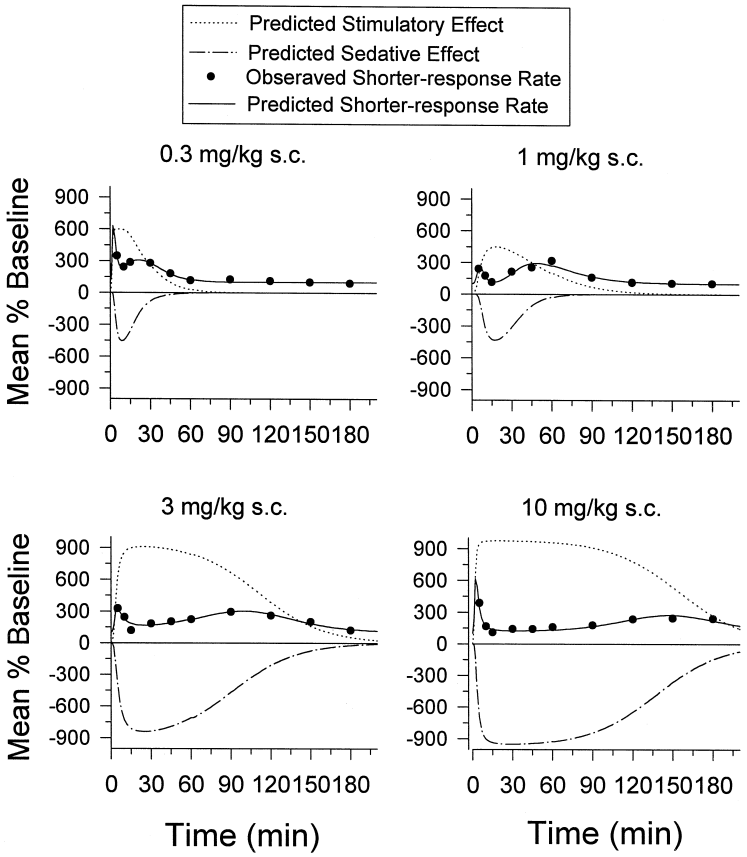

Fig. 7. Measured shorter-response rate, and the predicted stimulatory effect, sedative effect and shorter-response rates vs. time using proposed model following four midazolam doses (0.3–10 mg/kg).

Table 2
Pharmacokinetic and pharmacodynamic parameters (C.V.%) estimated by simultaneous modeling of serum concentration, reinforcement rate and shorter-response rate following administration of midazolam (i.v. 0.75, s.c. 0.3–10 mg/kg)

Parameter	Units	Dose (mg/kg)				
		s.c. 0.3	s.c. 1	s.c. 3	s.c. 10	i.v. 0.75
Shorter-response rate parameters: stimulatory effect						
keo_{st}	/min	0.389 (213.3)	0.090 (11.7)	0.069 (27.3)	0.102 (154)	
E_{max}	%	600 (439.9)	635 (23.8)	957 (275.7)	981 (96.3)	
$EC_{st,50}$	μg/ml	0.032 (107.5)	0.112 (18.2)	0.112 (18.2)	0.112 (18.2)	
n		4.44 (62.4)	2.19 (16.8)	1.97 (13.7)	1.91 (80.1)	
Ve_{st}	ml	0.047 (213.3)	0.204 (11.7)	0.268 (27.3)	0.181 (154.1)	
Shorter-response rate parameters: sedative effect						
keo_{sd}	/min	0.137 (172.2)	0.100 (9.2)	0.066 (23.8)	0.051 (46.7)	
E_{max}	%	−600 (439.9)	−635 (23.8)	−957 (275.7)	−981 (96.36)	
$EC_{sd,50}$	μg/ml	0.056 (55.4)	0.147 (9.4)	0.177 (117.1)	0.216 (30.1)	
s		4.16 (75.6)	4.47 (11.8)	1.97 (39.9)	1.92 (21.4)	
Ve_{sd}	ml	0.135 (172.2)	0.184 (9)	0.281 (23.8)	0.364 (46.7)	
Reinforcement rate parameters						
keo	/min	0.082 (36.5)	0.084 (15.4)	0.062 (29)	0.026 (23)	
E_o	%	100	100	100	100	
IC_{50}	μg/ml	0.033 (15.1)	0.059 (6.8)	0.059 (6.8)	0.059 (6.8)	
i		1.71 (29.8)	1.38 (13.4)	0.738 (6.6)	0.738 (6.6)	
Ve	ml	0.224 (36.61)	0.221 (14.93)	0.300 (29.33)	0.715 (21.54)	
Pharmacokinetic parameters						
ka	/min	0.467 (94.86)	0.132 (15.15)	0.101 (28.71)	0.101 (28.71)	N/A
kel	/min	0.099 (4.04)	0.099 (4.04)	0.099 (4.04)	0.099 (4.04)	0.059 (3.39)
k(2,1)	/min	0.206 (33.50)	0.206 (33.50)	0.206 (33.50)	0.206 (33.50)	0.043 (20.93)
k(1,2)	/min	0.075 (24.00)	0.075 (24.00)	0.075 (24.00)	0.075 (24.00)	0.037 (13.51)
V	l/kg					0.62
Total objective function value = 0.923						

Ve_{st}, Ve_{sd} and Ve are the volume of distribution of the stimulation, sedation, and reinforcement rate sites, respectively.

though the value of $E_{st,max}$ increased with each midazolam dose, it did not increase linearly: 600, 635, 957, and 981% for 0.3, 1, 3, and 10 mg/kg, respectively. The values of $EC_{st,50}$ and *n*, which describe the stimulatory effect following the three higher midazolam doses (1–10 mg/kg), changed very little across dose but differed from those for the lowest dose (0.3 mg/kg). For the sedative effect, the $EC_{sd,50}$ values progressively increased with each midazolam dose, whereas values of *s* for the two lower doses (0.3 and 1 mg/kg) were approximately two times greater than those for the two higher doses (3 and 10 mg/kg). The half-lives of equilibration for stimulation and for sedation ($t_{1/2}keo_{st} = \ln 2/keo_{st}$; $t_{1/2}keo_{sd} = \ln 2/keo_{sd}$) ranged from 1.82–10.0 min and from 5.06–13.59 min, respectively, for the four midazolam doses.

The difference in values for the two reference concentrations ($EC_{st,50}$ and $EC_{sd,50}$) for the two opposing effects accounted for the two peaks observed in the shorter-response rate. For the four doses, the smaller $EC_{st,50}$ values in comparison to the $EC_{sd,50}$ values indicated that the onset of the stimulatory effect preceded that of the sedative effect. Thus, the first peak of the shorter-response rate emerged (Fig. 7). The disappearance of the first peak occurred immediately following the onset of the maximum sedative effect. The time the shorter-response rate remained at the baseline level and the emergence and duration of the second peak were dose dependent.

3.3.2. Reinforcement rate in the 45–55 s bin

In the effect-link model for the reinforcement rate, the parameter kle was fixed at 0.0001/min as in the case of

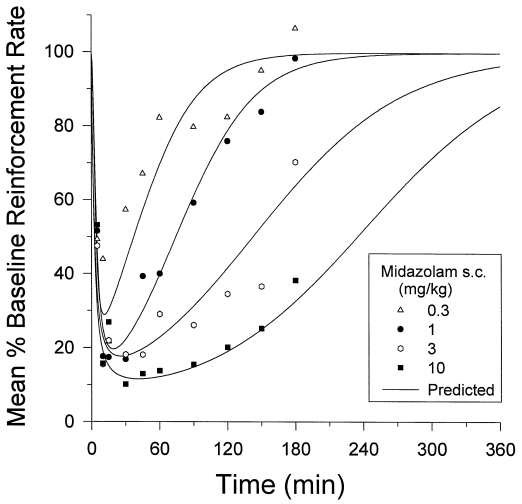

Fig. 8. Measured reinforcement rate and predicted effect vs. time using proposed model following the four midazolam doses (0.3–10 mg/kg).

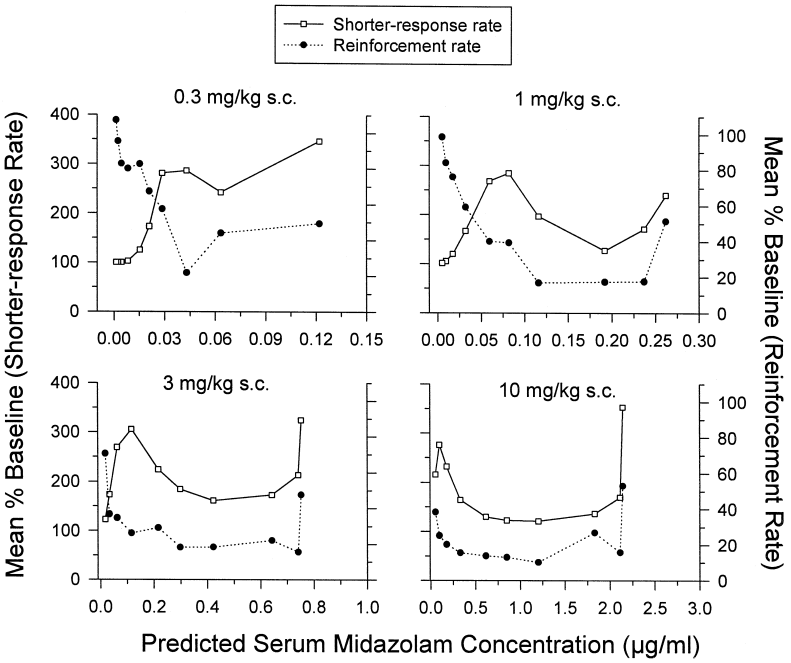

Fig. 9. Measured mean % baseline shorter-response and reinforcement rates vs. model predicted serum concentration in the central compartment following 0.3–10 mg/kg midazolam administration.

the stimulation-sedation model. For the four midazolam doses, the effect-link model described the effects of alprazolam on reinforcement rate well in terms of the recovery of the disruptive performance to the baseline and the delay in attaining the maximum effect (Fig. 8). The reference concentrations, IC_{50}, were similar across the four doses: 0.059 μg/ml for the three higher doses (1–10 mg/kg) and 0.033 μg/ml for the 0.3 mg/kg dose. The half-life of equilibration ranged from 8.25–26.65 min.

3.3.3. Predicted serum midazolam concentration and its relation to shorter-response and reinforcement rates

The interplay between the shorter-response rate and the reinforcement rate becomes visible when the two rates are plotted against the predicted serum midazolam concentration as shown in Fig. 9. The first peak of the shorter-response rate occurred in the beginning of the session. This peak was associated with the higher predicted serum midazolam concentration for a given dose. However, the second

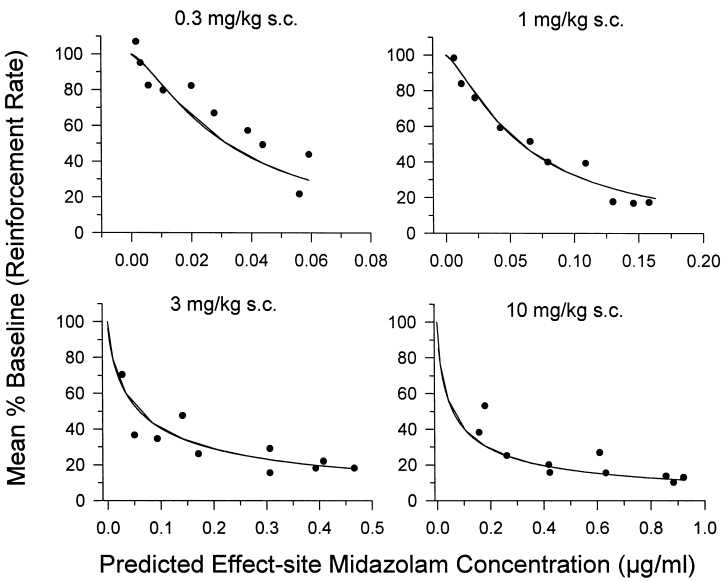

Fig. 10. Measured mean % baseline reinforcement rate vs. model predicted effect-site concentration following 0.3–10 mg/kg midazolam administration.

peak of the shorter-response rate occurred at similar predicted serum midazolam concentrations (0.082, 0.116, and 0.096 μg/ml for 1, 3, and 10 mg/kg, respectively) except for the lowest dose (0.3 mg/kg); for that dose, it occurred at a lower serum midazolam concentration (0.043 μg/ml). At these four concentrations, the reinforcement rate was also similar and ranged from 22–39% of the baseline level. The decreases in reinforcement rate correlated well with the predicted serum midazolam concentration except for an initial lag observed for the four doses. Take together, the reinforcement rate generally remained low until the appearance of the second peak, after which it rapidly and progressively recovered in a dose-related fashion. However, owing to the limited session length, the rates remained low for the two higher doses.

When the rates were plotted against predicted midazolam concentrations at the effect sites for the four doses, Fig. 10 shows each delay in decrease in reinforcement rate in the beginning of the session (i.e., Figs. 8 and 9) was no longer apparent.

4. Discussion

The two measures of differential-reinforcement-of-low-rate 45-s performance, the shorter-response rate and the reinforcement rate, exhibited time- and dose-related changes which were readily interpretable as functions of serum midazolam concentration during 3-h sessions. The stimulation-sedation model and effect-link model presented describe and predict the shorter-response and reinforcement rate changes, respectively. We used one measure of differential-reinforcement-of-low-rate 45-s performance—the reinforcement rate—to investigate its relation to pharmacokinetics in our early work (Lau and Wang, 1996; Lau et al., 1996). The previous alprazolam study presented the first attempt to characterize the effects of alprazolam on both the shorter-response and reinforcement rates of this contingency-controlled differential-reinforcement-of-low-rate 45-s performance in pharmacokinetic–pharmacodynamic modeling (Lau and Heatherington, 1997). Modeling benzodiazepine effects with either an increasing function such as an EEG measure (Mandema et al., 1991; Mandema et al., 1992a) or a decreasing function such as the reinforcement rate is a simple procedure. However, modeling the effects of alprazolam on shorter-response rate became a complex task because no simple function can account for the two observed peaks. This type of biphasic concentration-effect relation also was found for other drugs such as heptabarbital, thiopental, clonidine and oxybutynin (Ebling et al., 1991; Mandema and Danhof, 1990; Paalzow and Edlund, 1979; Venitz et al., 1990). The stimulation-sedation model described the effects of alprazolam on the shorter-response rate by using two effect-link, sigmoidal E_{max} models representing different hypothetical sites but having actions opposite in direction (Fig. 1). This leads us to propose the coexistence of stimulation and sedation components for alprazolam. The detailed behavioral analysis and pharmacokinetic–pharmacodynamic modeling presented in this study reveal that midazolam is similar to alprazolam in this respect. Like alprazolam (a triazolobenzodiazepine), midazolam (an imidazobenzodiazepine) exhibits the pharmacological profile of a 'full' agonist (Pier et al., 1981).

The terminal half-life for both midazolam and alprazolam was similar (40 min); however, the values of V_c, V_{ss}, and Cl for alprazolam were approximately two times greater than those for midazolam after i.v. administration (Lau et al., 1997a; Table 1). The other notable difference between the two drugs is the bioavailability after s.c. administration; the availability for alprazolam was complete, but that for midazolam was only 40–50% (Table 1; Lau et al., 1997a). We have found that there was a distinct difference in oral midazolam bioavailability between food-limited and free-feeding rats; the bioavailability for the food-limited rats was markedly low (4.6%) as opposed to the 45% value reported for free-feeding rats (Lau et al., 1996; Mandema et al., 1991). Nevertheless, midazolam pharmacokinetic parameters for both routes of administration (i.v. and s.c.) reported in this study corresponded to those values reported for food-limited rats (Lau et al., 1996). Midazolam AUC values exhibited a linear function of the dose (Table 1); this finding corresponded to the results reported for the oral route in humans (Bornemann et al., 1985).

Like alprazolam, midazolam decreased the reinforcement rate and increased the shorter-response rate in a dose- and time-related fashion (Figs. 4 and 5). It is best to determine a drug dose-response relation under conditions that a preceding dose produces no residual effect on the succeeding dose. By using differential-reinforcement-of-low-rate 45-s performance, we have found that no mutual interference (e.g., tolerance) occurred between doses for midazolam, alprazolam, and caffeine when these doses were separated by 3–5 days (Lau et al., 1996, 1997a,b). The effects of midazolam on the two rates of differential-reinforcement-of-low-rate 45-s performance were similar regardless of the dosing series (Figs. 4 and 5). This implies that the same midazolam dose-response relation could be obtained if the doses were given in a random order. However, tolerance developed rapidly following chronic midazolam administration in rats (Boisse et al., 1990; Tang et al., 1988).

The pharmacodynamic parameters for the three pharmacodynamic models (stimulation, sedation, and reinforcement rate) were similar across midazolam doses except for the lowest dose, 0.3 mg/kg. For the higher doses (1–10 mg/kg), the reference concentrations ($EC_{st,50}$, $EC_{sd,50}$, and IC_{50}) were greater than those for the 0.3 mg/kg dose but with a smaller Hill factor. These values suggested that the duration of action for the 0.3 mg/kg dose was much shorter than that for each of the higher doses.

Although benzodiazepines commonly are prescribed for chronic use, single dosing of benzodiazepine is also used in anesthesia, and in hypnotic and anxiolytic therapies. In single-dose pharmacodynamic studies, a delay is often observed between drug serum concentrations and drug effect. This so-called hysteresis is generally dealt with by assuming a hypothetical effect compartment exists in a linked model (Sheiner et al., 1979), although other models (e.g., indirect response models) have also been applied (Dayneka et al., 1993; Jusko and Ko, 1994). The decreases in reinforcement rate produced by alprazolam were best described by an indirect response model instead of an effect-link model in terms of description for individual doses (Lau and Heatherington, 1997). In the present study, we used the effect-link model to describe the delay in effect of midazolam on reinforcement rate for the four doses; no difference was found when we used the indirect response model. In humans, the delay of the onset of the effect has been observed with alprazolam and has been attributed to a distributional delay (Smith et al., 1984). However, no hysteresis was observed between midazolam blood concentrations and EEG effect in rats (Mandema et al., 1992a). For the four midazolam doses, the IC_{50} for the reinforcement rate was in the range of 0.033–0.059 μg/ml, which was similar to that reported for rats by aperiodic EEG analysis (Mandema et al., 1991, 1992a,b).

The sigmoidal E_{max} model, as used here, is appealing for pharmacodynamic analysis owing to its mathematical similarity to receptor binding and is used widely for benzodiazepines in human and animal research (Laurijssens and Greenblatt, 1996; Mandema et al., 1992a,b). Although biphasic drug effects can be modeled by using two opposing E_{max} functions (Lau and Heatherington, 1997; Paalzow and Edlund, 1979; Venitz et al., 1990), alternative approaches are available if difficulties arise in the estimation of these pharmacodynamic parameters such as E_{max} (Ebling et al., 1991; Venitz, 1995). In our previous study of the pharmacodynamic characterization of stimulatory and sedative effects of alprazolam, we estimated the pharmacodynamic parameters (i.e., $EC_{st,50}$, $EC_{sd,50}$, n, s, and keo) by fixing $E_{st,max}$ at an experimentally reasonable value; namely, the maximum observed value of the shorter-response rate + S.E.M. (Lau and Heatherington, 1997). In the present study, $E_{st,max}$ for the four midazolam doses (0.3–10 mg/kg) were estimated by simultaneous optimization of the shorter-response rate data. $E_{st,max}$ increased somewhat with midazolam dose rather than remaining constant across dose (Table 2). During model formulation, we also used the same approach as mentioned above for alprazolam to evaluate the outcome of $EC_{st,50}$, $EC_{sd,50}$, n, s and keo when $E_{st,max}$ was set at 600% (i.e., 386% $\pm$ 240%; Fig. 5C,D). These values were similar to those reported in Table 2 when $E_{st,max}$ was not fixed, but the description and statistics were inferior as reflected by the Akaike Information Criterion. That the use of the two estimation procedures led to similar outcomes implies that the maximum effect of midazolam on the shorter-response rate had been reached with the doses used in the present study.

Benzodiazepines, like many drugs, often exhibit a dose-related, biphasic effect on behavior in animals. At lower doses, benzodiazepines increase response rates for operant or schedule-controlled behavior (File and Pellow, 1985; Griffiths and Goudie, 1987; Burke et al., 1994), as well as for spontaneous activity (Lopez et al., 1988); i.e., they produce a 'stimulatory' effect. Conversely, at higher doses, they typically decrease these rates. 'Sedation' was observed as the maximum effect following high-dose benzodiazepine administration (e.g., 3 mg/kg s.c. midazolam) with animals maintaining a crouched position without movement (Lau et al., 1996). Benzodiazepines exert their effects through the GABA-benzodiazepine receptor complex (Haefely et al., 1985), and thus it is not surprising that the behavioral endpoints observed correspond to the benzodiazepine receptor in vivo binding for the respective doses; lower and higher doses of alprazolam increased and decreased benzodiazepine receptor numbers, respectively, although this was not observed for other benzodiazepines (Kaplan et al., 1990; Lopez et al., 1988; Miller et al., 1987; Burke et al., 1994). However, whether or not the stimulatory and the sedative effects of benzodiazepines in different tests reflect a common underlying mechanism is not at present clear, especially if inferences are mainly based on time-course data collapsed into a single point rather than on detailed temporal changes.

Inasmuch as the pharmacological response often can be predicted from the respective pharmacokinetics (Lau et al., 1997a; Lau and Wang, 1996), we chose to investigate the low–high-dose behavioral effects from a different approach. We hypothesized that the low and the high dose effects resulted from the low and the high concentration effects and accounted for the observed stimulatory and sedative effects, respectively. Thus, following the administration of a high dose, both kinds of effects may be detected as follows: although a high benzodiazepine dose can produce a predominantly sedative effect, it is also true that as the benzodiazepine undergoes absorption and disposition, a stimulatory effect would emerge whenever the plasma benzodiazepine concentrations reached a level comparable to that produced by a low dose. Indeed, for the four midazolam doses, the $EC_{st,50}$ values (0.032–0.112 μg/ml) and the $EC_{sd,50}$ values (0.056–0.216 μg/ml) corresponded to the low and the high concentration effects, respectively. This reveals that the stimulatory effect becomes visible only at lower serum midazolam concentrations, whereas the sedative effect is associated with higher concentrations. This is coincident with the ascending and descending limbs of the midazolam pharmacokinetic profile, considering distributional effects to hypothetical compartments following s.c administration. The reinforcement rate had the lowest reference concentration (Table 2) and was used as an index for evaluating the timing perfor-

mance on the differential-reinforcement-of-low-rate 45-s schedule. This rate is sensitive to both the stimulatory and sedative effects, as it only recovered rapidly to the baseline level during the disappearance phase of the shorter-response rate (Fig. 9).

By using an i.v. alprazolam dose (1.25 mg/kg), we identified that the two peaks of the shorter-response rate produced by s.c. alprazolam occurred at the transition phases, i.e., the onset and offset of the maximum reinforcement rate (Lau and Heatherington, 1997). Owing to the rapidity of the i.v. route, the onset of the transition phase was lacking so that the first peak did not occur for the i.v. alprazolam dose. Furthermore, the second peak of the shorter-response rate emerged after the i.v. alprazolam dose and was route independent in terms of time, magnitude, and duration of the peak. Although an i.v. midazolam dose was not given in the present study, the fact that the appearance of the two peaks in the shorter-response rate could be modeled by the same functions as used for alprazolam suggested that rats had similar responses to midazolam and alprazolam with respect to the shorter-response rate. Caffeine, a psychomotor stimulant, produced only a single peak of the shorter-response rate (Lau et al., 1997a).

Benzodiazepines are widely used for their therapeutic effects in humans. But they also are associated with a variety of adverse side effects, which have been increasingly recognized in recent years—e.g., early-morning insomnia, daytime anxiety, tension, or panic (Vgontzas et al., 1995). The second peak in differential-reinforcement-of-low-rate performance resembles many aspects of the rebound period in humans: behaviorally, it is the transient rebound (i.e., stimulatory or agitated) phase for returning to the baseline; pharmacokinetically, it is associated with decreasing benzodiazepine concentrations. Thus, the stimulation-sedation model may serve as a laboratory model for studying human rebound agitated behavior and of tolerance. Consequently, it can help in delineating the possible mechanisms involved in this rebound phase. Furthermore, the model can serve as a useful screening function for drug development because it dissociates the behavioral components of stimulation and sedation in differential-reinforcement-of-low-rate performance. For example, both midazolam and alprazolam have similar pharmacological effects and exhibit both stimulatory and sedative components. Other agents, such as novel anxiolytic or hypnotic drugs, may exhibit primarily one or the other of the two components. Finally, we have demonstrated that the two measures of differential-reinforcement-of-low-rate performance—the reinforcement and shorter-response rates—are valid, clinically relevant pharmacodynamic measures.

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